

**ABORTIFACIENT ACTIVITIES AND
BIOCHEMICAL ASSESSMENT OF ETHANOLIC
EXTRACT OF *Citrullus colocynthis* PODS IN
PREGNANT FEMALE WISTAR RATS**

BY

**ADETUNJI, JULIANA BUNMI
(02/55EH129)**

APRIL, 2018

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**Being a Thesis Submitted to the Department of Biochemistry,
Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.
In Partial Fulfillment of the Requirements for the Award of the
Degree of Doctor of Philosophy (Ph.D.) in Biochemistry**

APRIL, 2018

APPROVAL PAGE

This Thesis has been read and approved having met the requirements of the Department of Biochemistry and Postgraduate School, University of Ilorin, Ilorin, Nigeria, for the award of Doctor of Philosophy (Ph.D.) Degree in Biochemistry.

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External Examiner

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Date

DEDICATION

This thesis is dedicated to God Almighty.

DECLARATION

I, ADETUNJI Juliana Bunmi, declares that this thesis entitled “ Abortifacient Activities and Biochemical Assessment of Ethanolic Extract of *Citrullus colocynthis* pods on Pregnant Female Wistar Rats” is my research work. It has neither been presented nor accepted in any previous application for higher degree. All contributions have been duly acknowledged. The research work has been ethically approved by the University of Ilorin Ethical Review Committee.

ADETUNJI J. B.

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TABLE OF CONTENT

	Page
TITLE PAGE	i
APPROVAL PAGE	iv
DEDICATION	v
DECLARATION	vi
ACKNOWLEDGEMENT	vii
LIST OF FIGURES	xxiii
LIST OF TABLES	xxx
LIST OF PLATES	xxxi
LIST OF ABBREVIATIONS	xxxii
ABSTRACT	xxxv
CHAPTER ONE: INTRODUCTION	
1.1 Introduction	1
1.2 Statement of Research Problem	3
1.3 Justification of the Study	3
1.4 Aim and Objectives of the Study	4
1.5 Research Hypothesis	5
CHAPTER TWO: LITERATURE REVIEW	
2.1 Literature Review	6
2.2 Medicinal Plants	6
2.3 Abortion	7
2.3.1 Prevalence of Abortion	8
2.3.2 Cost Implications of Abortion	8

2.3.3	Types of Abortion	8
2.3.3.1	Induced Abortion	9
2.3.3.2	Spontaneous Abortion	9
2.3.3.3	Medical Abortion	10
2.3.3.4	Herbal Abortion	10
2.3.4	Signs and Symptoms of Abortion	11
2.3.5	Complications of Abortion	11
2.4	Abortion Therapeutic Drugs	12
2.4.1	Mifepristone	12
2.4.1.1	Mode of Action of Mifepristone	14
2.5	<i>Citrullus colocynthis</i> (L) Schrad (Cucurbitaceae)	14
2.5.1	Classification of <i>Citrullus colocynthis</i>	14
2.5.2	<i>Citrullus colocynthis</i> and its Pharmacological Importance	15
2.6	Phytochemicals	18
2.6.1	Classes of Phytochemicals	18
2.6.1.1	Alkaloids	18
2.6.1.2	Saponins	18
2.6.1.3	Tannins	19
2.6.1.4	Phenols	19
2.6.1.5	Glycosides	19
2.6.1.6	Flavonoids	20
2.7	Abortifacient Indices	20
2.7.1	Abortifacient Activities	20
2.8	Female Reproductive Hormones	21
2.8.1	Follicle Stimulating Hormone (FSH)	21
2.8.1.1	Distribution	22

2.8.1.2	Role of Follicle Stimulating Hormone	22
2.8.1.3	Clinical Significance of Follicle Stimulating Hormone	22
2.8.2	Luteinizing Hormone (LH)	22
2.8.2.1	Distribution	23
2.8.2.2	Role of Luteinizing Hormones	23
2.8.2.3	Clinical Significance of Luteinizing Hormones	23
2.8.3	Progesterone	26
2.8.3.1	Distribution	28
2.8.3.2	Roles of Progesterone	28
2.8.3.3	Clinical Significance of Progesterone	28
2.8.4	Oestrogen	29
2.8.4.1	Distribution	29
2.8.4.2	Roles of Oestrogen Hormone	29
2.8.4.3	Clinical Significance of Oestrogen Hormone	29
2.8.5	Prolactin	29
2.8.5.1	Distribution	30
2.8.5.2	Roles of Prolactin	30
2.8.5.3	Clinical Significance of Prolactin	31
2.9	Haematology	31
2.9.1	Red Blood Cell (RBC)	31
2.9.2	White Blood Cell (WBC)	31
2.9.3	Thrombocytes (Platelets)	32
2.10	Antioxidants	32
2.10.1	Superoxide Dismutases (SOD)	35
2.10.2	Catalases	35
2.11	Tissues Studied	37

2.11.1	Liver	37
2.11.1.1	Structure of the Liver	38
2.11.1.2	Functions of the Liver	41
2.11.2	Kidney	43
2.11.2.1	Structure of the Kidney	43
2.11.2.2	Functions of the Kidney	46
2.11.3	Uterus	47
2.11.3.1	Structure of the Uterus	48
2.11.3.2	Functions of the Uterus	51
2.11.4	Ovary	51
2.11.4.1	Structure of the Ovary	52
2.11.4.2	Functions of the Ovary	54
2.12	Serum	54
2.13	Enzyme Studied	55
2.13.1	Alkaline Phosphatase (EC. 3.1.3.1)	55
2.13.1.1	Distribution	55
2.13.1.2	Activators and Inhibitors	55
2.13.1.3	Isoenzymes	55
2.13.1.4	Clinical application	56
2.13.2	Aspartate Aminotransferase (AST) (EC 2.6.1.1)	57
2.13.2.1	Distribution	57
2.13.2.2	Activators and Inhibitors	57

2.13.2.3	Isoenzymes	57
2.13.2.4	Clinical Application	58
2.13.3	Alanine Aminotransferase (ALT) EC. 2.6.1.2	58
2.13.3.1	Distribution	58
2.13.3.2	Activators and Inhibitors	59
2.13.3.3	Isoenzymes	59
2.13.3.4	Clinical Application	59
2.14	Tissues Function Test	60
2.14.1	Liver Function Indices	60
2.14.1.1	Protein	61
2.14.1.2	Albumin	62
2.14.1.3	Bilirubin	63
2.14.2	Kidney Function Indices	64
2.14.2.1	Urea	64
2.14.2.2	Uric Acid	65
2.14.2.3	Creatinine	66
2.14.3	Serum Electrolytes	67
2.14.3.1	Potassium ion (K^+)	67
2.14.3.2	Sodium ion (Na^+)	68

2.14.3.3	Calcium ion (Ca^{2+})	68
2.14.3.4	Phosphate ion (PO_4^{2-})	70
2.14.3.5	Chloride ion (Cl^-)	71
2.15	Glucose	71
2.16	Lipid Profile	72
2.16.1	Total Cholesterol	72
2.16.2	Triacylglycerol	73
2.16.3	High- Density Lipoprotein Cholesterol (HDL-C)	74
2.16.4	Low-Density Lipoprotein Cholesterol (LDL-C)	75
2.16.5	Atherogenic Index	75
2.17	Organ-Body Weight Ratio	75

CHAPTER THREE: MATERIALS AND METHODS

3.1	Materials	77
3.1.1	Plant Material and Authentication	77
3.1.2	Assay Kits and Drug	77
3.1.3	Animals	77
3.2	Methods	78
3.2.1	Preparation of Plant Extract	78
3.2.2	Determinations of Secondary Metabolites	78

3.2.3	Gas Chromatography – Flame Ionization Detection (GC-FID)	80
3.2.4	Animal Grouping and Extract Administration	80
3.2.5	Determination of Abortifacient Activity	80
3.2.6	Biochemical Assessment	82
3.2.7	Preparation of Serum and Tissue Homogenate	83
3.2.8	Haematological Analysis	84
3.2.9	Determination of Reproductive Hormones	84
3.2.9.1	Prolactin Hormone (PRL)	84
3.2.9.2	Progesterone Hormone	85
3.2.9.3	Oestrogen Hormone	87
3.2.9.4	Luteinizing Hormone (LH)	88
3.2.9.5	Follicle Stimulating Hormone (FSH)	89
3.2.10	Marker Enzymes Activities	90
3.2.10.1	Determination of Alkaline Phosphatase Activity	90
3.2.10.2	Determination of Aspartate Aminotransferase Activity	91
3.2.10.3	Determination of Alanine Aminotransferase Activity	92
3.2.11	Determination of Liver Function Indices	93
3.2.11.1	Determination of Serum Protein Concentration	93
3.2.11.2	Determination of Serum Albumin	94
3.2.11.3	Determination of Serum Bilirubin Concentration	95
3.2.12	Determination of Some Kidney Function Indices	96
3.2.12.1	Determination of Urea Concentration	96

3.2.12.2	Determination of Creatinine Concentration	97
3.2.12.3	Determination of Serum Uric Acid	97
3.2.12.4	Determination Serum Sodium ion Concentration	98
3.2.12.5	Determination of Serum Potassium ion	99
3.2.12.6	Determination of Serum Calcium ion Concentration	100
3.2.12.7	Determination of Serum Phosphate ion Concentration	101
3.2.12.8	Determination of Serum Chloride ion Concentration	101
3.2.13	Determination of Serum Glucose Concentration	102
3.2.14	Antioxidant Enzymes Assay	103
3.2.14.1	Determination of Catalase Activity	103
3.2.14.2	Determination of Superoxide Dismutase (SOD) Activity	105
3.2.15	Determination of Lipid Peroxidation	106
3.2.16	Determination of Lipid Profile	107
3.2.16.1	Total Cholesterol	107
3.2.16.2	High - Density Lipoprotein Cholesterol Concentration (HDL-C)	108
3.2.16.3	Low-Density Lipoprotein Cholesterol Concentration (LDL-C)	108
3.2.16.4	Triacylglycerols	109
3.2.16.5	Atherogenic Index	110
3.2.17	Chromosomal Aberration Evaluation	110
3.2.18	Organ-Body Weight Ratio	111
3.2.19	Statistical Analysis	111

CHAPTER FOUR: RESULTS

4.1	Chemical report of Ethanolic Extract of <i>Citrullus colocythis</i> Pods	112
4.1.1	Secondary Metabolites Screening of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods	112
4.1.2	Mineral Constituents of <i>Citrullus colocynthis</i> Pods	116
4.3	Abortifacient Study	120
4.3.1	Abortifacient Parameters	120
4.3.2	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on the Hormonal Profile	123
4.3.2.1	Progesterone Hormone	123
4.3.2.2	Oestrogen Hormone	123
4.3.2.3	Prolactin Hormone	126
4.3.2.4	Luteinizing Hormone	126
4.3.2.5	Follicle Stimulating Hormone	129
4.4	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Amino Transferases Activity in the Serum and Tissues of Pregnant Wistar Rats	131
4.4.1	Aspartate Aminotransferase Activity	131
4.4.2	Alanine Aminotransferase Activity	131

4.4.3	Alkaline phosphate activity	134
4. 5	Biochemical Assessments	136
4.5.1	Organ- Body Weight Ratios	136
4.5.2	Effect of Twenty-one Days Administration of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Marker Enzymes in Serum and Tissues of Pregnant Wistar Rats	138
4.5.2.1	Aspartate Aminotransferase Activity	138
4.5.2.2	Alanine Aminotransferase Activity	138
4.5.2.3	Alkaline Phosphatase Activity	141
4.5.3	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Liver Functions Indices of Pregnant Female Wistar Rats	143
4.5.4	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> on Glucose Concentration	148
4.5.5	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Kidney Functions Indices of Pregnant Female Wistar Rats	150
4.5.6	Effect of Administration of Ethanolic Extract of <i>Citrullus colocynthis</i> on Lipid Peroxidation	159
4.5.7	Effect of Administration of Ethanolic Extract of <i>Citrullus colocynthis</i> on some Antioxidant Enzymes	161

4.5.8	Effect of Twenty-one Days Administration of Ethanolic Extract Of <i>Citrullus colocynthis</i> Pods on Pregnant Female Wistar Rats Hormonal Profile	164
4.5.9	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum Lipid Profile of Pregnant Female Wistar Rats	170
4.5.10	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Chromosomal Aberration of Pregnant Female Wistar Rats Bone Marrow	176
4.5.11	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Pregnant Female Wistar Rats Haematological Parameters	181

CHAPTER FIVE: DISCUSSION

5.1	Abortifacient Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods	191
5.1.1	Secondary Metabolites	191
5.1.2	Abortifacient parameters	192
5.1.3	Reproductive hormone	194
5.1.3.1	Follicle Stimulating Hormone	194
5.1.3.2	Luteinizing Hormone	195
5.1.3.3	Progesterone	195
5.1.3.4	Oestrogen	196
5.1.3.5	Prolactin	197

5.2	Biochemical Assessment	197
5.2.1	Organ- Body Weight Ratio	197
5.2.2	Haematological Parameters	198
5.2.3	Serum Lipid Profile	199
5.2.4	Tissues Marker Enzymes	200
5.2.4.1	Alkaline Phosphatase (ALP)	201
5.2.4.2	Aminotransferases	202
5.2.4.2.1	Aspartate Aminotransferase	202
5.2.4.2.2	Alanine Aminotransferase	203
5.2.5	Liver Function Indices	203
5.2.6	Kidney Function Indices	204
5.2.7	Chromosomal Aberration	206
5.2.8	Histology of some Tissues after Extract Administration	206

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATION

6.1	Summary	208
6.2	Conclusion	209
6.3	Recommendation	209

REFERENCES

APPENDIX

LIST OF FIGURES

Figure	Title	Page
2.1	Hormonal Regulation during Reproductive Cycle	25
2.2	Chemical Structure of Progesterone	27
2.3	Induction of Abortion through Oxidative Stress	34
2.4	Detoxification Pathway for Reactive Oxygen Species	35
2.5	Structure of the Liver	40
2.6:	Mammalian kidney	45
2.7	Female reproductive organ	50
2.8	Structure of the Ovary	53
4.1	Different Spectra of Compounds Present in <i>Citrullus colocynthis</i> Pod in GC-FID Analyser	 115
4.2	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum Progesterone Level in Pregnant Rats	 124
4.3	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum Oestrogen Level in Pregnant Rats	 125
4.4	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum Prolactin Level in Pregnant Rats	 127
4.5	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum	

	Luteinizing Hormone Level in Pregnant Rats	128
4.6	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum	
	Follicle Stimulating Hormone Level in Pregnant Rats	130
4.7	Specific Activity of Aspartate Aminotransferase in Pregnant Rats	
	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Nine Days	132
4.8	Specific Activity of Alanine Aminotransferase in Pregnant Rats	
	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Nine Days	133
4.9	Specific Activity of Alkaline Phosphatase in Pregnant Rats	
	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Nine Days.	135
4.10	Specific Activity of Aspartate Aminotransferase in Pregnant Rats	
	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Twenty One Days	139
4.11	Specific Activity of Alanine Aminotransferase in Pregnant Rats	
	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Twenty One Days.	140
4.12	Specific Activity of Alkaline Phosphatase in Pregnant Rats	

	Serum and Tissues Following the Administration of Ethanolic	
	Extract of <i>Citrullus colocynthis</i> Pods for Twenty One Days	142
4.13	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum rotein Level in Pregnant Wistar Rats	144
4.14	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Albumin Level in Pregnant Wistar Rats	145
4.15	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Direct Bilirubin Level in Pregnant Wistar Rats	146
4.16	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Total Bilirubin Level in Pregnant Wistar Rats	147
4.17	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> on Serum	
	Glucose Level in Pregnant Wistar Rats	149
4.18	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Uric Acid Level in Pregnant Wistar Rats.	151
4.19	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Creatinine Level in Pregnant Wistar Rats.	152
4.20	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Urea Level in Pregnant Wistar Rats	153
4.21	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	

	Plasma Potassium ion Level in Pregnant Wistar Rats.	154
4.22	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Plasma Sodium ion Level in Pregnant Wistar Rats.	155
4.23	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Plasma Phosphorus ion Level in Pregnant Wistar Rats	156
4.24	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Plasma Chloride ion Level in Pregnant Wistar Rats	157
4.25	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Plasma Calcium ion Level in Pregnant Wistar Rats	158
4.26	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Malonyldialdehyde Activity in Serum of Pregnant Wistar Rats	160
4.27	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Serum	
	Superoxide Dismutase Activity in Pregnant Wistar Rats	162
4.28	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on	
	Serum Catalase Activity in Pregnant Wistar Rats	163
4.29	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>Colocynthis</i> Pods for Twenty One Days on the Progesterone	
	Level of Pregnant Wistar Rats	165
4.30	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	

	<i>colocynthis</i> Pods for Twenty One Days on the Oestrogen	
	Level of Pregnant Wistar Rats	166
4.31	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>colocynthis</i> Pods for Twenty One Days on the Oestrogen	
	Level of Pregnant Wistar Rats	167
4.32	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>colocynthis</i> Pods for Twenty One Days on the Luteinizing	
	Hormone Level of Pregnant Wistar Rats	168
4.33	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>colocynthis</i> Pods for Twenty One Days on the Follicle	
	Stimulating Hormone Level of Pregnant Wistar Rats	169
4.34	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>Colocynthis</i> Pods on Serum Triacylglycerol Level of	
	Pregnant Wistar Rats	171
4.35	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>Colocynthis</i> Pods on Serum Total Cholesterol of Pregnant	
	Wistar Rats	172
4.36	Effect of Administration of Ethanolic Extract of <i>Citrullus</i>	
	<i>Colocynthis</i> Pods on Serum Low Density Lipoprotein	

	Cholesterol Level of Pregnant Wistar Rats	173
4.37	Effect of Administration of Ethanolic Extract of <i>Citrullus Colocynthis</i> Pods on Serum High Hensity Lipoprotein	
	Cholesterol of Pregnant Wistar Rats	174
4.38	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Atherogenic Index of Pregnant Wistar Rats	175
4.39	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on MCA count of Pregnant Wistar Rats	177
4.40	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Chromosomal Aberration Count of Pregnant Wistar Rats	178
4.41	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on Mitotic Index Level of Pregnant Wistar Rats	179
4.42	Effect of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods on TNMA of Pregnant Wistar Rats	180
4.43	Cross Section of the Kidney	183
4.44	Cross Section of the Liver	185
4.45	Cross Section of the Ovary	187
4.46	Cross Section of the Uterus	189
4.47	Standard Curve for H ₂ O ₂	236

4.48	Standard Curve for Aspartate Aminotransferase	237
4.49	Standard Curve for Alanine Aminotransferase	238
4.50	Standard Curve for Protein	239
4.51	Standard Curve for Prolatin	240
4.52	Standard Curve for Progesterone	241
4.53	Standard Curve for Oestrogen	242
4.54	Standard Curve for Follicle Stimulating Hormone	243

LIST OF TABLES

Table	Title	Page
4.1	Secondary Metabolites Screening Result of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods	113
4.2	Other Chemical Constituents of Ethanolic Extract of <i>Citrullus colocynthis</i> Pods	114
4.3	Mineral Constituents of <i>Citrullus colocynthis</i> Pods	117
4.4	Abortifacient Activities of the Ethanolic Extract of <i>Citrullus colocynthis</i> Pods Administered to Pregnant Female Rats	122
4.5	Organ-Body Weight Ratio of Wistar Rats Treated with Ethanolic Extract of <i>Citrullus colocynthis</i> Pods	137
4.6	Haematological Parameters on Pregnant Female Wistar Rats Treated Orally with <i>Citrullus colocynthis</i> Pods Extract	182

LIST OF PLATES

Plate	Title	Page
2.1	<i>Citrullus colocynthis</i> Pods	17
4.1	A and B showing difference in vaginal smear of unmated and mated rats	118
4.3	C, D and E Showing Resorption Level in Animals	119

ABBREVIATIONS

PR	Progesterone Receptors
WHO	World Health Organization
LMP	Last Menstrual Period
PGs	Prostaglandins
CC	<i>Citrullus colocynthis</i>
PAL	Phenylalanine ammonia lyase
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
E2	Oestrogen
PRL	Prolactin
GnRH	Gonadotropin-releasing hormone
CL	Corpus luteum
GH	Growth hormone
CNS	Central nervous system
RBC	Red blood cell
WBC	white blood cell
SOD	Superoxide dismutase
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALPI	Intestinal alkaline phosphatase
ALPP	Placental alkaline phosphatase
ALPL	Liver alkaline phosphatase
GFR	Glomerular filtration rate
SIADH	syndrome of inappropriate secretion of antidiuretic hormone

PTH	Parathyroid hormone
VDDR	Vitamin D dependent rickets
VDRR	Vitamin D resistant rickets
ATP	Adenosine triphosphate
2, 3-DPG	2, 3-diphosphoglycerate
TAG	Triacylglycerol
VLDL	Very low density lipoprotein
LDL	Low density lipoprotein
HDL	High density lipoprotein
GC-FID	Gas Chromatography – Flame Ionization Detection
PCV	Packed cell volume
Hb	Haemoglobin
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
TMB	Tetramethylbenzidine
HRP	Horserash peroxidase
PNPP	Para-nitrophenyl phosphate
BCG	Bromocresol green
POD	Peroxidase
TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid
MDA	Malondialdehyde
TCA	Trichloroacetate
GPO	Glycerophosphate oxidase
EDTA	Ethylene diamine tetra acetic acid

ABSTRACT

The use of abortifacient herbs is now gaining acceptance over abortifacient drugs due to adverse effects and high cost. *Citrullus colocynthis* pods (Bitter Apple in English), have been claimed to be used as an abortifacient in Nigeria without scientific evidence to substantiate it. This study was therefore carried out to evaluate the abortifacient activities of ethanolic extract of *Citrullus colocynthis* pods (EECCP) on pregnant female Wistar rats. The specific objectives were to: (i) qualitatively and quantitatively determine the secondary metabolites and mineral elements in EECCP; (ii) evaluate the abortifacient activity of EECCP on the pregnant female Wistar rats; and (iii) assess the biochemical changes on the pregnant Wistar rats after administration of EECCP.

The secondary metabolites in EECCP were screened using standard methods. Sixty pregnant female rats ($165.27 \pm 7.13\text{g}$) were randomized into six groups (A-F). Group A (control) received orally 0.5 ml of olive oil, Group B received 1.84mg/kg body weight (bwt) of Mifepristone (reference drug) while those in Groups C, D, E and F received orally 100, 200, 400 and 800 mg/kg bwt of EECCP once daily for 9 days post pregnancy after which abortifacient parameters, biochemical changes, chromosomal aberration, and histological evaluation were done. Data were subjected to analysis of variance and Duncan Multiple Range Test at $p < 0.05$.

The results revealed that:

- i. EECCP contained alkaloids, saponins, tannins, phlobatannins, glycosides, phenolics, steroids, terpenes, flavonoids, iron, sodium, copper, chromium, potassium, manganese, zinc, nickel, magnesium and calcium with alkaloids (5.2mg/ml) and calcium (27.17mg/l) being the highest;
- ii. the number of live foetuses, survival ratio, corpora lutea and implantation index were significantly reduced ($p < 0.05$) by EECCP whereas the number of dead foetus, rats that aborted, resorption index, pre- and post-implantation increased significantly;

- iii. EECCP significantly reduced ($p < 0.05$) the levels of progesterone, oestrogen, prolactin, luteinizing hormone and follicle stimulating hormone;
- iv. the decrease in liver and kidney aminotransferases activity by EECCP was accompanied by corresponding increase in the serum enzymes;
- v. EECCP significantly reduced ($p < 0.05$) albumin, total bilirubin, uric acid, total protein, urea, creatinine, glucose, phosphorus, high density lipoprotein cholesterol in the liver, kidney and serum whereas chloride, total cholesterol, low density lipoprotein cholesterol and atherogenic index increased significantly;
- vi. EECCP produced increase ($p < 0.05$) in metaphase chromosomal aberration and decreased the mitotic index;
- vii. EECCP significantly increased malondialdehyde concentrations but reduced significantly ($p < 0.05$) the catalase and superoxide dismutase activities;
- viii. EECCP significantly reduced the white blood cells, platelet count, mean corpuscular haemoglobin and mean corpuscular volume; and
- ix. there were degeneration of corpus luteum, loss of both the theca external layers and oocytes in the ovary, erosion of endometrium, rupture of blood vessels, shrinkage of nucleus and sinusoidal spaces in the liver as well as vacuolation of the parenchyma in the kidney.

The study concluded that EECCP possesses abortifacient activities at the least toxic dose of 400mg/kg body weight. Therefore, EECCP should be further explored for development of abortifacient drug.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction

In recent time, fertility has been controlled or enhanced by women in developing countries through the use of traditional medicine. Increase in population has been a major world problem with its unavoidable and unfavorable consequences such as unemployment, poverty, environmental degradation of natural resources (Luhadia *et al.*, 2015). Presently, the world population crosses eight billion and growing endlessly day by day. Consequently, fertility regulation in human becomes a subject of worldwide concern. It is therefore, obvious that women or school-age girls who are confronted with undesired pregnancies tend to end the pregnancy through the process of abortion. Numerous conventional herbal medicines are locally used to thwart ovulation or insemination, as an anti-implantation and some to stimulate uterine flow or uterine contractions in order to promote labour (Ritchie, 2001). For the past fifty years various plants had been evaluated as potent herbs used to regulate fertility and were later on approved by both national and international organizations (Purohit and Daradka, 1999).

Herbal drugs and traditional remedies commonly referred to as alternative medicine are relatively the most popular therapeutic approach to tackle these condition (abortion) in Nigeria due to the belief from the local communities that being natural, they are non-toxic and safe for consumption. Hence, women in Nigeria, India, and Tanzania and some other countries have resulted in using this herbal remedies for abortion because it is an illegal act in these countries (Mali *et al.*, 2006; Fakeye *et al.*, 2009; Shweta *et al.*, 2011; Jagar, 2015). Though there are new ways to drug discovery

and development, some of which are combinatorial chemistry and computer-based molecular modeling design, these cannot substitute the significant importance of natural products (Heinrich and Bremner, 2006; Thirumal *et al.*, 2012; Gavamukulya *et al.*, 2015).

Majority of the synthetic drugs such as mifepristone used as treatments of last resort of terminal diseases cause severe side effects that are not tolerable whereas the active constituents present in herbs may prevent the adverse effect pose by the synthetic drugs (Valko *et al.*, 2007). It is significant to note that medicinal significant of plants rely on the secondary metabolites which are accountable for the production of distinct physiological action on a human. Some important secondary metabolites are alkaloids, tannins, flavonoids and phenolic compounds (Nakashima *et al.*, 2010). Since the medicinal plant has been generally accepted by people especially in developing countries to be a better cure for most disease conditions, researchers have taken it upon themselves to ascertain the viability of these plants for their efficacy, safety and possible toxic effects that might be associated with it. Some of the herbal medicines used which have been studied previously are *Achyranthes aspera* (Workineh *et al.*, 2006) Unripe papaya (Shende *et al.*, 2013), the root of *Gossypium* (Anwasha, 2015) and the seeds from wild *Momordica charantia* (Quiroz *et al.*, 2014).

C. colocynthis is commonly known as Bitter Apple or Bitter Cucumber in English, “Bara” in Yoruba, “Kabawa” in Hausa and “Ogbe” in Ibo. It belongs to Cucurbitaceae family (Ziyyat *et al.*, 1997). In folkloric medicine, *Citrullus colocynthis* is extensively used by the rural populace as a purgative, an anti-diabetic, an anti-neoplastic, an anti-rheumatic, and an anti-allergic agent (Tannin-Spitz, 2007). Leaves of *C. colocynthis* have anti-inflammatory (Rajamanickam *et al.*, 2010), antidiabetic (Gurudeeban and Ramanathan, 2010), antioxidant and anesthetic

(Ramanathan *et al.*, 2010) effects. *Citrullus colocynthis* is also used in folk medicine against dermatological, gynecological and pulmonary infections (Medenine *et al.*, 2006).

Aqueous extract of leaves of *Citrullus Colocynthis* have local anaesthetic effect (Ramanathan *et al.*, 2001). Moreover, ethanolic extract of its pods is used in folkloric medicine as an abortifacient agent. Thus, aim and objective of this study was to evaluate the abortifacient activities and biochemical assessment of ethanolic extract of *Citrullus colocynthis* pods in pregnant female Wistar rats.

1.2 Statement of Research Problem

Inadequate health facilities in the local clinics and the high cost of the synthetic drugs contribute to the challenges of using herbal concoction by rural dwellers to wash the uterus in abortion crises.

Also, individuals with threatening pregnancy resulted in carrying out induced abortion with herbal concoction to save their lives.

Consequently, the growth in population in Nigeria which currently affects the economy may be challenged with legal abortion which may not be favourable to some people.

1.3 Justification of the Study

Some synthetic drugs have been used over time in controlling population growth one of which is Mifepristone (RU 486) which is an antiprogesterone a derivative of norethindrone. This drug possesses a high binding capacity and progesterone antagonist activity. The abortifacient action of Mifepristone is a result of its interaction with uterine hormone receptors (Marions *et al.*, 2004). Due to the cost

implication of this synthetic drug, preference has been given to *Citrullus colocynthis* pod as an abortifacient agent used in some rural areas in Nigeria. There have been claims in folklore medicine of Nigeria that the pod of *Citrullus colocynthis* can be used to wash the uterus or serves as an abortifacient. But recently, this claim was scientifically carried out by Walid *et al.* (2007) on the short and long-term effect of *Citrullus colocynthis* on reproduction in Jordan but the secondary metabolites were not screened for, also the effect of the extract on reproductive hormones and its detrimental effect were not investigated. This study was therefore aimed at addressing the abortifacient activities and biochemical assessment of the ethanolic extract of *Citrullus colocynthis* pods in pregnant female Wistar rats.

1.4 Aim and Objectives of the Study

The Aim of the Study

The aim of this study was to evaluate the Abortifacient Activities and Biochemical Assessment of Ethanolic Extract of *Citrullus colocynthis* Pods in Pregnant Female Wistar Rats.

Specific objectives of the study were to:

- Ø Qualitatively and quantitatively determine the Secondary metabolites and mineral elements in ethanolic extract of *Citrullus colocynthis* pods
- Ø To evaluate the abortifacient activities of the extract on pregnant female Wistar rats
- Ø Assess the biochemical changes in the pregnant female Wistar rats after administration of ethanolic extract of *Citrullus colocynthis* pods

- Ø Determine effects of the extract on some hematological parameters in pregnant female Wistar rats
- Ø evaluate the DNA damage effect of the extract on pregnant female Wistar rats.
- Ø Examine the histology of the uterus, ovary, kidney and liver tissues

1.5 Research Hypothesis

Null Hypothesis, there is no significant differences between mifepristone and *Citrullus colocynthis* in the induction of abortion

Alternative Hypothesis, there is a significant difference between mifepristone and ethanolic extract of *Citrullus colocynthis* pods in the induction of abortion and biochemical assessment in pregnant Wistar rats.

CHAPTER TWO

2.1 LITRATURE REVIEW

2.2 Medicinal Plants

Medicinal plants are any plants containing one or two actives components that are useful for therapeutic purposes or even serve as precursors for the development or production of useful drugs (Sofowora, 1982). Plant can be referred to as medicinal plant when ethnobotanically reported or scientifically authenticated have been carried out on its biological activity (Elujoba, 1997). These plants can then become a useful source of medicines in developing countries for the treatment of diseases (Bako *et al.*, 2005). World Health Organization (WHO, 2001), claimed that close to 80% of the world population quest for medicinal plants to cure or prevent diseases, this rate is on the high side in African countries. The WHO (2002) estimated that up to 90% of developing countries population depends on the use of medicinal herbs to help meet their primary health care needs although (Jimoh, 2006) claimed that the use of traditional medicine is not limited to the developing countries alone.

Iwu *et al.* (1999) reported that the active constituents obtained from medicinal plants have primary benefits which are quite safer than synthetic drugs with intense therapeutic benefits and also affordable. In the last two centuries, scientist have been investigating into the chemical and biological properties of plants and this have led to the development of modern synthetic drugs as therapeutic agents by the emergence of the active ingredients found in the medicinal plant (Roja and Rao, 2000). The roots, barks, leaves, stems and seeds of medicinal plants have been used to formulate concoction for different ailments (Ogbulie *et al.*, 2004). In developing countries, herbal remedies have been used by women to wash their uterus after a miscarriage

and to control their fertility by preventing implantation, stimulate contractions or promoting uterine flow and induction of labour (Ritchie, 2001). In recent times, women in developing countries have resulted in ending unwanted pregnancies through the use of herbs by a process called abortion.

2.3 Abortion

Abortion is the extinction of a pregnancy when the foetus is not feasible (Annas and Elias, 2007). Since prehistoric times, an abortion is often carried out using herbal medicines, sharp tools, and physical trauma, forcefully or through other traditional methods (Joffe, 2009). An abortion could be spontaneous due to complications in pregnancy or induced either in human or in some other species. Spontaneous abortion in most instances is referred to as miscarriages while the term abortion is mostly used for an induced abortion. In human, therapeutic abortion is often carried out to preserve the health of female animals. It has been estimated that forty two million abortions take place annually, out of which twenty-two million are safe while twenty million are unsafe worldwide (Shah and Ahman, 2009). The unsafe abortion is a major cause of maternal mortality and morbidity.

Sometimes, maternal death could results from safe abortions, while unsafe abortions caused about 70,000 deaths and 5 million disabilities per year ((Shah and Ahman, 2009). The accessibility of safe abortions is determined only by the legality of the process used. It had been reported that only 40% of women in the world today have free access to therapeutic and elective abortion within gestational limits. Plant products for medicinal purposes have been used since primordial time by humans. Some of these medicinal plants possess abortifacient activities which have been use

traditionally in folk medicine (de Almeida *et al.*, 2011). Abortion is said to be performed either in the first or second trimester.

2.3.1 Prevalence of Abortion

It was reported that worldwide an estimate of about 23 – 53 million of induced abortions are performed yearly (WHO, 2002). 19 million out of the unsafe abortion were carried out in developing countries yearly with about 5.5 million done in Africa inclusive of Nigeria leading to over 36,000 maternal deaths. A number of researchers have reported that an estimate of 1 million abortions is carried out in Nigeria annually (Okonofua, 1997). The findings of Henshaw *et al.* (1998) gave an estimation of 610,000 abortions performed yearly. The abortion rate ranges from 10-13 per 1000 women in the north (age 15-44) and 25 per 100 women in the south.

2.3.2 Cost Implication of Abortion

The cost implication of abortion using the modern day technology is in relation to pregnancy gestational age and this ranges from \$6-13 for pregnancy below 8 weeks to \$ 25 -63 for early second trimester pregnancy. These costs led to the non-physicians methods engage in by women with unwanted pregnancy to perform abortion.

2.3.3 Types of Abortion

Abortions are basically classified base on the process by which the pregnancies are terminated which could be induced, spontaneous, medical and herbal.

2.3.3.1 Induced Abortion

Pregnancy can be terminated deliberately due to individual's health issue or in the case of an abnormal foetus. Ideal and precise procedures could be chosen due to it legality, regional availability and doctor-patient favourite. The manner of approach used depends solely on the gestational period of embryo or foetus (Menikoff, 2001). The process involved in an induced abortion could be therapeutic or elective. A therapeutic abortion could be carried out to:

- ✓ Reduce maternal mortality through pregnancy complications
- ✓ Safeguard maternal physical or mental health
- ✓ Thwart the birth of a child with inborn disorders
- ✓ Selectively decrease the foetus number to manage health risks associated with multiple pregnancies.

2.3.3.2 Spontaneous Abortion

Spontaneous abortion can also be referred to as a miscarriage, this is an expulsion of an embryo or foetus caused by accidental trauma or natural prior to the 22nd week of gestation. The pressure inflicted by by this abortion type dropped sharply after the 10th week of the last menstrual period (LMP) (Nilsson and Lars, 1990). Spontaneous abortion which occurs in the first trimester is often caused by embryo or foetus chromosomal abnormalities (Stopper, 2009), it results in at least 50% of early pregnancy losses sampled (Jauniaux *et al*, 1999).

Other causes of spontaneous abortion include vascular diseases, diabetes, hormonal problems, infections and uterus abnormalities (Stoppler, 2009). Maternal age and the

history of a patient on previous spontaneous abortions are the two leading causative factors associated with the leading risk of spontaneous abortion (Jauniaux *et al.*, 1999).

2.3.3.3 Medical Abortion

Medical abortions are mostly carried out effectively during the first trimester of pregnancy with the help of pharmaceutical drugs. Drugs like mifepristone or methotrexate along side prostaglandin (either misoprostol or gemeprost) are the frequently used combined regimens (Spitz *et al.*, 1998). The complications include: gastrointestinal discomfort, abdominal pain, excessive vaginal bleeding, headache, dizziness, and fatigue, incomplete abortion or continuing pregnancy requiring surgical evacuation.

2.3.3.4 Herbal Abortion

Over the years, herbs with abortifacient potency have been used in folkloric medicine. The use of herbal concoction in this approach can cause serious and lethal side effect which can lead to multiple organ failures, this is not recommended by physicians (Cingard and Laborde, 2003).

The importance of plants as a source of anti-fertility (post- and pre-coital contraception, anti-implantation and Abortifacient) drugs had been reported by numerous researchers (Keshri *et al.*, 2003; Yakubu *et al.*, 2009, 2010; Muhammad *et al.*, 2015).

The World Health Organization has also suggested that effective and locally available plants can be used as substitutes for drugs, due to eruption in population which is now a foremost cause of poverty in most developing countries. They thereafter introduced

population control programme, which includes research of the active principles of traditional medicinal plants (Chauhan *et al.*, 2008). It is therefore necessary for researchers to look into some medicinal plants with potent abortifacient properties. Abortifacient agents gotten from indigenous medicinal plants would be pivotal to the developing countries since they will be cost-effective when compared with the available synthetic drugs. Though, about 45.5% of the populace considered that the use of herbal drugs in the termination of pregnancy is a better option and this are people dwelling mainly in the rural areas with believe that it is free of any side effects (Somia *et al.*, 2015).

2.3.4 Signs and Symptoms of Abortion

The features associated with abortions are

- Vaginal discharge
- Abdominal pain
- Fever
- Vaginal bleeding
- Abdominal swelling
- Anaemia
- Diarrhoea
- Shock
- Vomiting

2.3.5 Complications of Abortion

Some disorders result from the diagnosis carried out on women who carried out induced abortions (Okonofua *et al.*, 1999; Okpani and Okpani (2000)). These include:

- Vagina burn
- Abscess of abdominal pelvic
- Ectopic gestation
- Cervical fibroid
- Acute renal failure
- Post abortal sepsis
- Retained products
- Visceral perforation
- Genital laceration
- Infertility

2.4 Abortion Therapeutic Drugs

These are approved contraceptives drugs that are synthesized to either soften or open the cervix or to induce abortion. The drugs work in different ways by blocking the effect of the endocrine hormones responsible for maintaining pregnancy thereby causing contraction of the uterus and they include mifepristone, methotrexate or misoprostol.

2.4.1 Mifepristone

Mifepristone (RU-486) is the only antiprogesterone that is widely used clinically to induce abortion. It does this by entering into the blood cells and inhibit the binding of

progesterone to its receptor thereby increasing the uterine contractility and sensitizes the myometrium to prostaglandins.

Mifepristone and its metabolites antagonized the binding site of progesterone to its receptor. Mifepristone is a 19 metabolites lacking steroid substitute attached to the p-(dimethylamino) phenyl group while hydrophobic 17 α -substituent increases the binding capacity to the progesterone receptor (PR). The substitution of glycine at point 722 in the hormone-binding area of human PR is vital for mifepristone binding (Benhamou *et al.*, 1992). Moreover, mifepristone interacts with receptors which are freely bound in the nucleus in like manner with progesterone on entering the target cell. Hence, mifepristone binds at a faster rate to the PR and binds the PR receptor at a rate that is 2.5–5 times that of progesterone.

The antagonistic action of mifepristone is seen as nearly pure to progesterone, but a slight agonistic action had been reported (Terakawa *et al.*, 2000). It was suggested that the transcriptionally active complex of mifepristone on some genes is in the absence of progesterone (Spitz and Bardin, 1993). Mifepristone failure in terminating a pregnancy has been reported in about 1% of the women. The inability of mifepristone in terminating pregnancy might be due to a genetic variation in the progesterone receptor (Gao, 1998).

Mifepristone also can bind to the glucocorticoid receptor just the same way it binds to PR, with three times binding affinity of dexamethasone. It does bind to the androgen receptor at a lesser rate but does not bind to oestrogen or mineralocorticoid receptor. Thus, combined collection of mifepristone and its metabolites induced the biological actions that results (Heikinheimo *et al.*, 2003). Mifepristone reverses or reduces the action of progesterone in pregnancy to cause an adverse effect. Mifepristone sensitizes the myometrium to prostaglandins (PGs) as well as increases uterine

contractility which is often suppressed in pregnancy at early stage. Therefore, uterine contractions and spontaneous abortion are results of luteolysis before the luteoplacental shift, and a successive decrease in plasma progesterone.

2.4.1.1 Mode of Action of Mifepristone

Progesterone often carried out its biological function via entering into the cell and binding to exact proteins, PR, which are positioned in the nucleus of the cell to get the receptor activated. The receptors also have domain for DNA binding. Spontaneous changes in the conformation of PR is induced by progesterone and other progestins, which then transforms the receptor to one that binds DNA from non-DNA binding. This change is connected with heat shock proteins loss and dimerization of the receptor molecules. Gene transcription rate then increases with activated receptor dimer leading to the production of progesterone effects at cellular and tissue stage crucial for pregnancy continuation. Mifepristone causes dramatic change in the conformation of the receptor which is linked to loss of heat shock protein and dimerization through binding to the inactive progesterone receptor. Hence, when this occurred the DNA bound receptors are transcriptionally inactive and suppressing the effect of progestin leading to pregnancy termination (Spitz and Agranat, 1995).

2.5 *Citrullus colocynthis* (L) Schrad (Cucurbitaceae)

2.5.1 Classification of *Citrullus colocynthis*

Kingdom:	Plantae
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	<i>Citrullus</i>
Species:	<i>C. colocynthis</i>

2.5.2 *Citrullus colocynthis* and its Pharmacological Importance

C. colocynthis is commonly known as Bitter Apple or Bitter Cucumber in English, “Bara” in Yoruba, “Kabawa” in Hausa and “Ogbe” in Ibo (Plate 2.1). It belongs to Cucurbitaceae family (Ziyyat *et al.*, 1997). It is mostly grown in Arabia, Southern India, and Tropical Africa (Neyab, *et al.* 2006) and often cultivated together with cassava in Nigeria (Agahiu *et al.*, 2011). Leaves are variable in size ranging from 3.8 to 6.3 cm in length and 2.5 cm in width. The leaves show a deltoid margin, pale green colour above and ashy colour beneath, scabrid on both surface, 5-7 lobed and are similar to that seen in watermelon because they are of the same family. It produces bitter flavoured fruits about a size of cantaloupe and seeds rich in oil and protein. Compounds found in it include glycosides containing Estrols classified as A, B, K, L, and E cucurbitacins, flavonoids, and alkaloids (Abdel-Hassan *et al.*, 2000; Chen *et al.*, 2005; Yoshikawa *et al.*, 2007). The fruits are extensively used medicinally for stomach pains, because of glucosides such as colocynthin present which are effective cathartic and laxative (Dan *et al.*, 1998). Numerous plant secondary metabolites like cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids, phenolic compounds have been previously reported from this plant (Galal *et al.*, 1997).

The essential amino acid contents of the seed protein have been reported to be adequate (FAO-WHO, 1973). The seeds are reported to contain a higher amount of arginine, tryptophan and the sulfur-containing amino acids (Schafferman *et al.*, 1998). Some pharmacological potential of *Citrullus colocynthis* have been reported in literature, some of which includes anti-inflammatory activities (Belsem *et al.*, 2011), hypoglycemic (Agarwal *et al.*, 2012), Antioxidant, anti-inflammatory/ analgesic or anti-proliferative (Saba and Oridupa, 2010), hypolipidemic (Rahbar and Nabipour,

2010), anti-inflammatory and analgesic activities (Marzouk *et al.*, 2011), anti-alopecia (Dhanotia *et al.*, 2011)



Plate 2.1: *Citrullus colocynthis* pods

2.6 Phytochemicals

These are non-nutrients, chemical compounds or biologically active components of plants. They are referred to as phytochemical or phytoconstituent or secondary metabolites, they are used as defense or protector for plants against microbial infections or invasion by pests and to prolong individual health due to their medicinal advantages (Liu, 2004; Doughari *et al.*, 2009).

2.6.1 Classes of Phytochemicals

2.6.1.1 Alkaloids

They represent the most prevalent secondary metabolites; their main components are largely made of ammonia which is derived from amino acid nitrogen bases with diverse radicals replacing one or more hydrogen atoms in the peptide ring. Majority of alkaloids are freely or readily soluble in alcohol or organic solvent but slightly soluble in water. Alkaloids solutions are extremely bitter. The nitrogenous compounds present in it, function as a defense mechanism against herbivores and pathogens attack on plants, they are often exploited as pharmaceuticals ingredients, stimulants, and narcotics, because of their powerful biological activities. A large quantity of most of this plant metabolite (alkaloids) is in the seeds.

2.6.1.2 Saponins

It is gotten from *Saponaria vaccaria*, meaning a plant that overflows in saponins. Therefore it possesses a 'foamlike' action in water. The hydrolysis of alkaloid produces an aglycone called sapogenin. The sapogenin is of two types: steroidal and triterpenoids. Usually, most sapogenins have their hydroxyl group attached to C-3. *Saponaria vaccaria*, contain glycosides which include sapogenin senegin and quillajic acid known to be toxic.

2.6.1.3 Tannins

They are most widely dispersed in plant flora and are phenolics with high molecular weight. Tannins can be found in all parts of plants like stem, bark, root and outer membrane tissue and are highly water and alcohol soluble. It has a characteristic feature of changing things to leather and is acidic in its action which is an attribute for the survival of phenolics or carboxylic functional group (Kar, 2007). Tannins may form complexes between macromolecules and secondary metabolites such as proteins, carbohydrates, alkaloids, and gelatin. Tannins are of two types, hydrolyzable tannins, and condensed tannins. It has also been used in the formulations of tannin-rich plants used to treat diseases such as vaginal discharge (leucorrhoea), bowel infections (diarrhea) and allergies (rhinorrhea).

2.6.1.4 Phenols

Phenols or polyphenolics are chemical components liable for the natural pigments of fruits. Phenylalanine serves as a precursor for the synthesis of phenolics by the action of phenylalanine ammonia lyase (PAL) enzyme. Phenolics serve numerous vital functions in plants and act as an important defense to plant against the attack of pathogens and herbivore predators, hence they are useful for pathogenic infections management of human (Puupponen- Pimiä *et al.*, 2008).

Phenols also symbolize a crowd of natural antioxidants used mostly as nutraceuticals, they are commonly seen in fruits like apples, green-tea, red-wine etc. with the potentials to fight cancer cells and avert heart ailments to a considerable extent and are assumed to be an anti-inflammatory agent.

2.6.1.5 Glycosides

They undergo neutral reactions which may be hydrolyzed freely with ferments or mineral acids to components such as glucose and aglycone or genin present in

glycosides (Kar, 2007; Firn, 2010). The alcohols like glycerol and phenols represent the aglycone component. Glycosides are usually classified based on their sugar component, aglycone chemical nature or their pharmacological actions.

2.6.1.6 Flavonoids

Flavonoids are the fundamental class of polyphenols broadly distributed within plant flora. Structurally, flavonoids consist of many benzene rings in their composition; they have been supported to be scavengers of free radicals or strong antioxidants (Kar, 2007). Flavans is the precursor for the synthesis of flavonoids, which are numerous in existence in nature and a few serves as pigments for colouration in higher plants. The most common flavonoids are Quercetin, quercitrin, kaempferol and are almost 70% abundant in plants.

2.7 Abortifacient Indices

The abortifacient activity was evaluated using some biological indices used to ascertain abortion.

2.7.1 Abortifacient Activities

Thses are the biological parameters monitored in the pregnant female wistar rats to explore the abortifacient activities of ethanolic extract of *Citrullus colocynthis* pods which include:

- a. Counting of life foetus physically
- b. Counting of the dead foetus physically
- c. Weight of foetus
- d. Computing the survival ratio (%):- It is the ratio of life foetus to that of dead foetus.
- e. Number of rats that aborted
- f. Percentage (%) of rats that aborted:- It is the ratio of rats that aborted to the number of rats used

- g. Number of implantation sites
- h. Number of *Corpora lutea*
- i. Implantation index (%) = (Total number of implantation sites/ number of *Corpora lutea*) x 100
- j. Resorption index (%) = (Total number of resorption sites/ total number of implantation sites) x 100
- k. Pre-implantation loss (%) = (Number of *Corpora lutea* – number of implantation/ number of *Corpora lutea*) x 100
- l. Post-implantation loss (%) = (Number of implantations – number of life fetus/ number of implantation) x 100

2.8 Female Reproductive Hormones

Hormones are chemical substances synthesized in a miniature amount by the endocrine tissue and carried in the blood to other tissue where they act as messengers to regulate the function of the target organ (Nelson and Cox, 2000). Female reproductive hormones in pregnant female albino rats were measured in the course of this study, to determine their concentration in order to provide useful information on any adverse effect on pregnancy and the mechanism of action of plant extract; since the hormonal imbalance in female might lead to abortion (Sizonenko, 1978). The concentration of female reproductive hormones determined includes: follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estrogen (E2) and prolactin (PRL).

2.8.1 Follicle Stimulating Hormone (FSH)

FSH is a glycoprotein (carbohydrate containing protein) gonadotropin which is secreted through the gland (anterior pituitary) in response to the release of gonadotropin-releasing hormone (GnRH) by the hypothalamus (Figure 2.1).

2.8.1.1 Distribution

Follicle stimulating hormone (FSH) is mainly synthesized and secreted by gonadotrophs of the anterior pituitary gland (Grover *et al.*, 2005). It is a hormone found in humans and other animals.

2.8.1.2 Role of Follicle Stimulating Hormone

FSH helps to stimulate maturation of cells and it controls the developmental, growth, puberty stage, and reproductive processes of the human body. FSH triggers initiation of follicular growth, specifically affecting granulosa cells in females (Fowler *et al.*, 2003). It is primarily responsible for stimulating the growth of the ovarian follicle at the follicular phase (Figure 2.1) such as the development of the egg and provides support for the cells surrounding the egg that produce the hormones needed during pregnancy and the fluid around the egg. Follicle stimulating hormone (FSH) and Luteinizing hormones (LH) act synergistically in reproduction.

2.8.1.3 Clinical Significance of Follicle Stimulating Hormone

High serum FSH concentration is commonly observed in a female who is at menopause. The high levels of FSH indicate that the regular restricting response from the gonad is absent, resulting in increased production of pituitary FSH. It is an abnormal situation for high FSH levels occur during the reproductive years, this can be associated with conditions of sub-fertility and infertility. Hypogonadism can cause a diminished secretion of FSH which in females leads to the cessation of reproductive cycles (Radu *et al.*, 2010).

2.8.2 Luteinizing Hormone (LH)

LH is a lutropin which is a heterodimeric glycoprotein secreted in the anterior pituitary gland.

2.8.2.1 Distribution

Luteinizing hormone is found in theca cells of the ovary. It is usually secreted from cells in the anterior pituitary called gonadotrophs.

2.8.2.2 Role of Luteinizing Hormones

A sharp increase of LH leads to LH surge which stimulates ovulation and corpus luteum development in females. It also initiates the changing of the residual follicle to corpus luteum which is responsible for the production of progesterone hormone that prepares the endometrium for implantation (Figure 2.1). LH is responsible for the maintenance of luteal function in the early weeks of pregnancy at the luteal phase (Figure 2.1). It renders helps to theca cells of the ovary that supply androgens and hormonal precursors for estrogen production of (Louvet *et al.*, 1975).

2.8.2.3 Clinical Significance of Luteinizing Hormones

The pulses of gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus control and regulate the LH release by the pituitary gland. The released pulses are subjected to oestrogen feedback received from the gonads. LH levels are also high at menopause just like that of FSH but are normally low during childhood. Elevation in LH level is mostly observed in individuals with polycystic ovary syndrome but unusual to see LH levels above the normal reproductive range. Elevated physiologic LH concentrations are seen during the LH surge occurrence; classically last 48hours (Nielsen *et al.*, 2001). A Perpetual high LH level is a situation that restricted feedback from the gonad is lacking, resulting in the pituitary release of both LH and FSH.

The decrease in the secretion of LH can result in hypogonadism that leads to amenorrhea commonly observed in females. Adequate LH and FSH levels in children exhibiting some signs of pubertal changes may indicate a moderate form of puberty with no fundamental or discernable cause or may just be a normal discrepancy of puberty (Webster, 2006). Normal or below normal levels of LH and FSH can be

noticed in delayed puberty, this is predictable for a youth within this age range. Some drugs such as anticonvulsants, clomiphene, and naloxone can cause LH to increase, while digoxin, oral contraceptives, and hormone treatment may decrease LH levels (Webster, 2006).

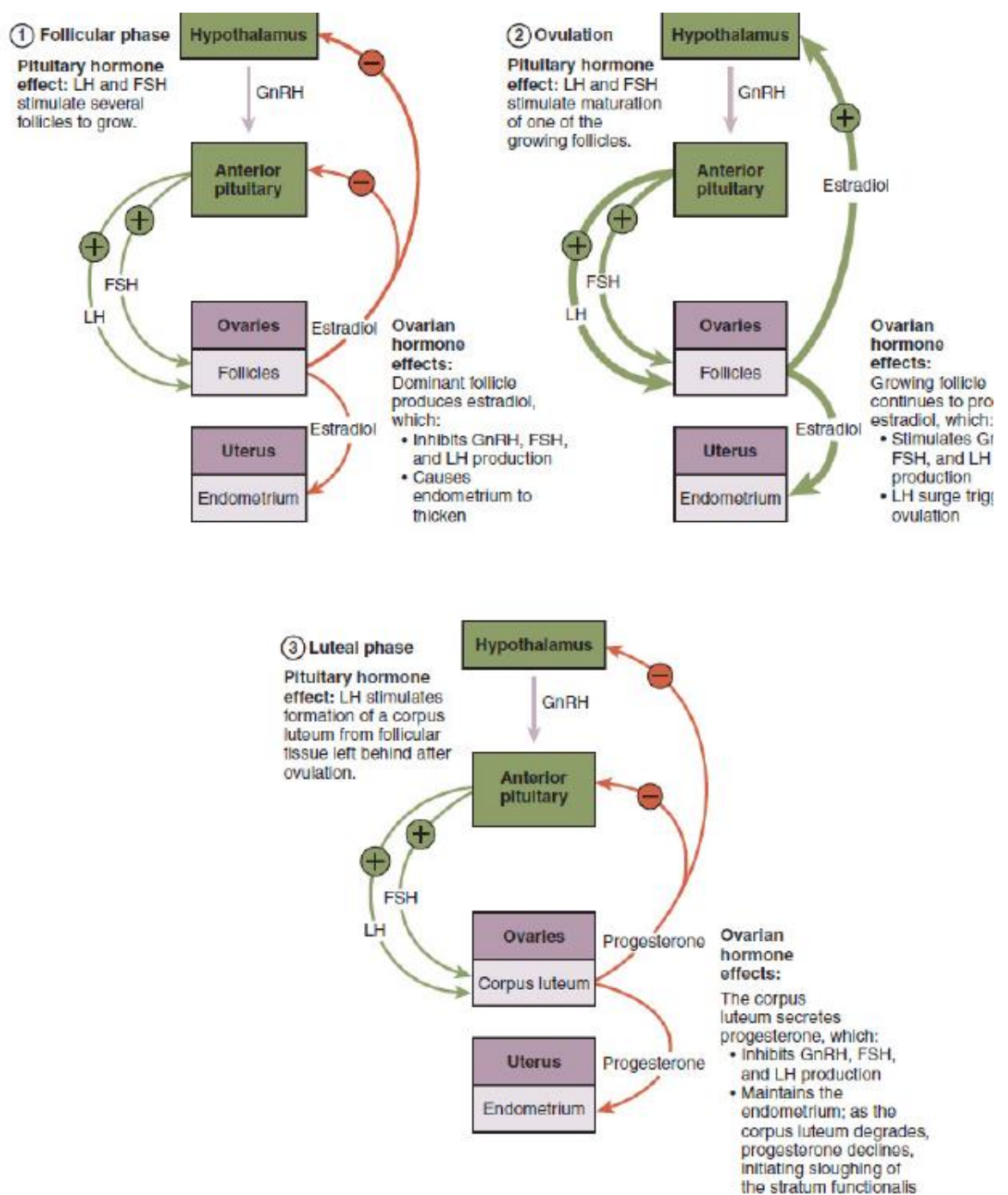


Figure 2.1 : Hormonal Regulation during Reproductive Cycle

(Source from: <https://cnx.org/contents/nMy6SWSQ@5/Anatomy-and-Physiology-of-the-Female-Reproductive-System>)

2.8.3 Progesterone

Progesterone can be referred to as P4 or pregn-4-ene-3, 20-dione a steroid hormone having twenty-one carbon unit (Figure 2.2), the hormone participate in the female menstrual cycle, pregnancy (support conception) and embryogenesis (development or formation of an embryo) of animals (Goodson *et al.*, 2007). Progesterone is primarily produced by the granulosa-lutein cells of the corpus luteum (CL) at the luteal phase of the menstrual cycle and the syncytiotrophoblast of the placenta during pregnancy. Progesterone is a progestogens group of hormones naturally available in human.



2.8.3.1 Distribution

Production of progesterone usually takes place in the ovaries after ovulation during pregnancy has taken place in the corpus luteum and in the placenta. The adipose tissue is sometimes responsible for the storage of progesterone (Goodson *et al.*, 2007).

2.8.3.2 Roles of Progesterone

Progesterone, sometimes known as “hormones of pregnancy” is an important hormone that performs many functions in the development of the fetus. These include:

- It prepares the uterus for implantation and but decreases in concentrations if pregnancy is not initiated resulting in a discharge (menstruation) in humans (Kastner *et al.*, 1990).
- In implantation and gestation there is a decrease in maternal immune response caused by progesterone at the onset of pregnancy (Elizabeth, 2008).
- It decreases uterine smooth muscle contraction.
- In addition, lactation is inhibited by progesterone in pregnancy. Therefore, a drop in progesterone levels after delivery helps to trigger milk production in human (Elizabeth, 2008).

2.8.3.3 Clinical Significance of Progesterone

The drastic reduction in progesterone levels during the pre-ovulation stage in the menstrual cycle increases shortly the following ovulation and in the luteal stage in women (Elizabeth, 2008). Progesterone concentrations is <2ng/ml before ovulation and > 5ng/ml immediately after ovulation. Progesterone levels are maintained initially at lutea levels if pregnancy occurs. The onset of the luteal can cause a placental shift and a rise in progesterone begins which may reach 200-200ng/ml at delivery (term). Progesterone thereafter becomes reduced after delivery and during lactation (Elizabeth, 2008).

2.8.4 Oestrogen

Oestrogen is a hormone that is synthesized locally in extragonadal area or sites and mostly do not enter the circulation but exert it intracrine, autocrine, paracrine, and juxtacrine property by acting directly in the cells of production or on the neighbouring cells (Harada *et al.*, 1999). Oestrogen is a steroid derivative of cholesterol known to have numerous protective properties especially on the foetus during pregnancy

2.8.4.1 Distribution

Oestrogen hormone are produced in a variety of tissues, not only in those customarily known to be involved in that process, such as gonads; ovaries and testes or placenta, but can also be found in brain, adipose tissue, breast, skin, blood vessels, bone, and cartilage (Simpson, 2003).

2.8.4.2 Roles of Oestrogen Hormone

Oestrogen is an important female reproductive hormone in the menstrual cycle that affects several organs. The growth in follicles during the follicular phase stimulates the production of oestrogen which helps to grow the endometrium or uterine lining in readiness for implantation.

2.8.4.3 Clinical Significance of Oestrogen Hormone

Oestrogen levels in the first half of preparative phase are low and are generally less than 50 pg/ml. Seven days before ovulation oestrogen levels begin to increase rapidly, getting to the climax about 200 to 300 pg/ml. The elevation correlates directly with the follicular growth.

2.8.5 Prolactin

Prolactin is a multifunctional hormone whose receptors are expressed in nearly all organs of the human body which in turn enables it to influence multiple physiological processes including endocrine, metabolic, immune system functions and cardiovascular properties. In contrast with the majority of the pituitary hormones,

Prolactin is functioned mainly at physiological condition (Freeman *et al.*, 2000), it is estimated that it has about 300 different biological actions in vertebrates (Bole-Feysot *et al.*, 1998). Prolactin is in the family of hormones grouped as I of the helix bundle protein hormones (Horseman and Yu-Lee, 1994), it includes growth hormone (GH) and placental lactogen. Genes that encode this group evolved from a universal ancestral gene by multiplication approximately 400 million years ago (Cooke *et al.*, 1981).

2.8.5.1 Distribution

Prolactin is abundant in brain structures like the cerebral cortex, hippocampus, amygdala, brain stem, cerebellum and spinal cord (Devito, 1988; Seroogy *et al.*, 1988). While a significant proportion of brain prolactin is of pituitary origin, entering the central nervous system (CNS) via the choroid plexus, the hypothalamus is also capable of synthesizing an identical variant of the hormone (Devito, 1988). PRL gene expression have also been established in diverse regions of the brain, decidua, myometrium, lacrimal gland, thymus, spleen, circulating lymphocytes, lymphoid cells of bone marrow, mammary epithelial cells, skin fibroblasts, and sweat glands in contribution to the anterior pituitary gland secretion (Ben-Jonathan *et al.*, 1996).

PRL can be distributed to several fluid compartments such as serum, cerebrospinal fluid, amniotic fluid, tears, milk, follicular fluid, and sweat. Immune dysfunction and death result mostly due to neutralizing circulating PRL with anti-PRL antibodies (Nagy and Berczi, 1991) suggesting that extra-pituitary PRL is imperative and, in some conditions, can compensate for pituitary PRL.

2.8.5.2 Roles of Prolactin

Prolactin genome in human is a single gene, located in the chromosome that encodes for prolactin (Owerbach *et al.*, 1981). Transcription in prolactin is regulated by two independent promoter regions which regulate: directs pituitary-specific expression

and extra pituitary expression (Berwaer *et al.*, 1994). Several factors influence PRL gene expression, they include estrogen, dopamine, and thyroid hormones (Lamberts and Macleod, 1990).

2.8.5.3 Clinical Significance of Prolactin

The reference range of prolactin is between 3ng/ml to 25ng/ml in females. However, the elevation or decrease in prolactin in the serum from the upper or lower threshold can result to hyperprolactinemia or hypoprolactinemia which have been associated with ovarian dysfunction (Corona *et al.*, 2009; Schwarzler *et al.*, 1997).

2.9 Haematology

Haematology is the study or process of estimating the number and morphology of the constituents of blood predominantly; platelets (thrombocytes), red blood cells (RBC) and white blood cells (WBC) with their significant results in disease monitoring or diagnosis (Merck, 2012). Haematological reports are of great importance to biological and physiological interest in understanding the affiliation of blood to its environment (Ovuru and Ekweozor, 2004).

2.9.1 Red Blood Cell (RBC)

Red blood cells known as erythrocytes contribute mainly to distribute oxygen to all tissues or organs or all part of the body system starting in the lungs via the pulmonary capillaries, removing carbon dioxide from the tissues back to the lungs via systemic capillaries and guarantee that the pH of the body is within an appropriate range (Jagger *et al.*, 2001). To meet the metabolic needs of tissues, the proper amount of nutrients and oxygen ought to be available and delivered to the tissues consistently (Sprague and Ellsworth, 2012).

2.9.2 White Blood Cell (WBC)

WBCs are cells of the immune system derived from the blood sample through physical appearance after centrifugation. The entire leukocytes are synthesized and

are formed from a multipotent cell in the bone marrow referred to as hematopoietic stem cell. WBC can be best grouped as myeloid leukocytes and lymphocytes (Maton *et al.*, 1993).

2.9.3 Thrombocytes (Platelets)

Platelets are one of the components of blood responsible for the stoppage of bleeding or hemorrhage through clumping and clogging of the blood vessel injuries (Laki, 1972). The greatest diameter of platelet is 2-3 μm (Paulus, 1975).

Damages caused to haematological constituents can lead to abnormalities that can affect the morphology, metabolism of erythrocytes, function, leukocytes, and platelets (Comazzi *et al.*, 2004). Anaemia affects red blood cells leading to a decrease in the number of red blood cells with signs like fatigue, shortness of breath and pale skin. Cases of anaemia includes iron deficiency anemia, autoimmune hemolytic anemia, anemia of chronic diseases, a synthetic anemia, deadly anemia (B_{12} deficiency), thalassemia, sickle cell anemia and polycythemia vera (Richard *et al.*, 2008).

2.10 Antioxidants

Antioxidants are substances or agents that protect the cells against detrimental effects of free radicals which are reactive oxygen species like singlet oxygen, hydroxyl radicals, superoxide, peroxynitrite and peroxy radicals that lead to oxidative stress (OS) a foremost cause of cellular damage (Mattson and Cheng, 2006). Natural occurring antioxidants which are endogenous synthesized play a vital position in prevention and maintenance of chronic as well as degenerative diseases, like atherosclerosis, carcinogenesis, cardiac or cerebral ischemia, gestational diabetics, neurodegenerative disorders, rheumatic disorder, ageing as well as DNA damage (Uddin *et al.*, 2008; Jayasri *et al.*, 2009). Antioxidants implement its function via mopping up of 'free-oxygen species' leading to an elevation in literally 'stable

species'. Free radicals are unsteady oxygen species, which ensnare electrons from substances in the immediate surroundings of the body system. The radicals, if not mopped up immediately, leads to abnormalities and disease condition (Figure 2.3) due to the disruption caused to crucial biomolecules such as lipids, proteins present in all membranes and mitochondria (Uddin *et al.*, 2008).

Consequently, accumulations of this free radicals are involved in some disease disorders such as tumour, ulcerogenesis, inflammation, gastrointestinal, infertility, asthma, shock, rheumatoid arthritis, atherosclerosis, diabetes, cystic fibrosis, cardiovascular diseases, neurodegenerative abnormalities (e.g. parkinson, Alzheimer's diseases), and mainly early senescence (Chen *et al.*, 2006; Uddin *et al.*, 2008). Interactions that exist between networks of antioxidants enzymes such as superoxide dismutases, catalases, peroxidases etc. protect the cells from oxidative stress (Sies, 1997). Normally, all superoxide released through oxidative phosphorylation is transformed to hydrogen peroxide and finally to water. A detoxification pathway that involves the interaction of several enzymes with superoxide dismutases (SOD) stepping up the first reaction which is then acted upon by catalases and various peroxidases in order to remove hydrogen peroxide.

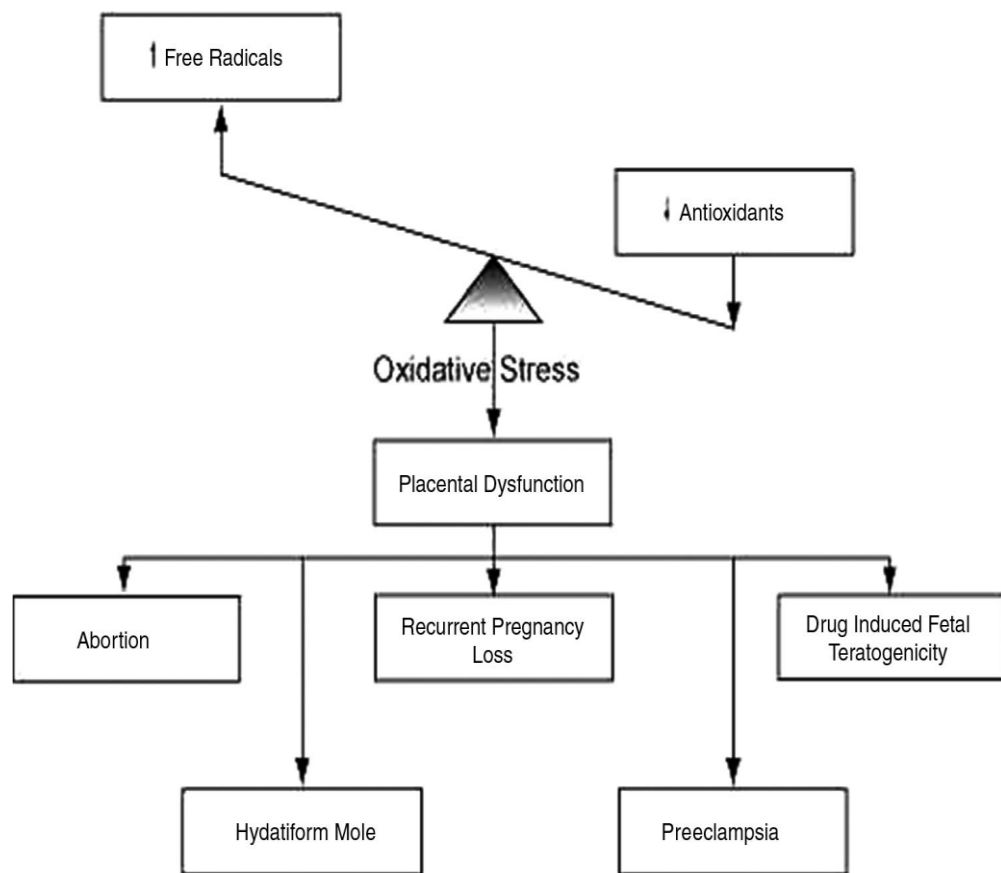


Figure 2.3: Induction of Abortion through Oxidative Stress (Source: Sajal *et al.*, 2007)

2.10.1 Superoxide Dismutases (SOD)

Superoxide dismutases act as an enzyme which catalyzes the degradation of superoxide anion to give hydrogen peroxide and oxygen (Zelko *et al.*, 2002). SODs are enzymes that are found in roughly every aerobic cells and extracellular fluids (Johnson and Giulivi, 2005).

2.10.2 Catalases

They are enzymes which catalyze the conversion of hydrogen peroxide molecule to water and oxygen (Figure 2.4), with the help of iron or manganese cofactor (Chelikani *et al.*, 2004). They are protein localized in the peroxisomes of most eukaryotic cells (del Río *et al.*, 1992).

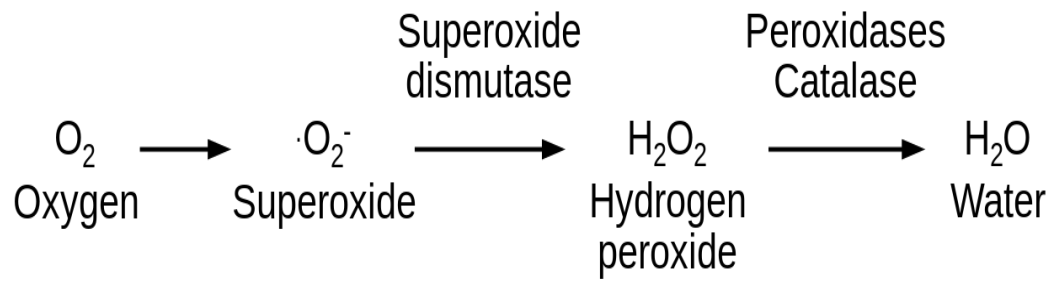


Figure 2.4: Detoxification pathway for reactive oxygen species (Source: Tunca *et al.*, 2008)

2.11 Tissues Studied

Biochemical as well as physiological changes in various organs can be evaluated in animals to ascertain the unwanted effect of chemical agents, plant extract (*Citrullus colocynthis*) and its constituent on the biological system. The organs studied in this work included liver, kidney, uterus, and ovary. The liver and kidney were selected based on their involvement in the absorption, metabolism, distribution, storage, and excretion of waste product. Moreover, the choice of uterus and ovaries was based on the roles they play in reproductive processes.

2.11.1 Liver

The liver is the largest glandular organ of the body system. It is often known to be a “great chemical factory” of the body. It helps to create, detoxify, regulates, and stores most substances used in the gastrointestinal system used for most important digestive functions (Wingerd, 1994). It is a large, reddish-brown organ with two lobes (Ramalingam, 1997). The liver weighs about 1.6-1.8kg in a 70kg man while it weighs about 6.8-7.9kg in a 200g rat (Seelay *et al*; 1998).

In the mammal, the liver is a relatively soft and a resilient mass. It is included amongst the accessory organs of the alimentary system. It is located immediately beneath the diaphragm in the abdominal cavity (Singh, 1999). The liver was therefore chosen to see if the extract had effect on the tissues.

2.11.1.1 Structure of the Liver

The liver is a large, soft, flabby, wedged-shaped and brown organ (Figure 2.5) in the body which secretes and purifies the blood (Wu, 2002). It is enclosed in a thin layer of fibrous connective tissue, which extend through its anterior to divide into two main sections called the lobes. The lobe is divided into two parts the large right lobe and the smaller left lobe (Wingerd, 1994). The lobe is further divided into three smaller lobes (Moore and Dalley 1999). The upper surface of the liver is convex and it is covered with visceral peritoneum while the lower surface is concave and bare (Moore and Dally, 1998). Each lobe is further divided into numerous liver lobules which are the structural and functional subunits of the organ (Wingerd, 1994) which contain columns hepatocyte that radiate from a central vein. Between hepatocyte, columns are endothelium-lined channels, called sinusoids, which carry blood that drain into the central vein (Wingerd, 1994). The lobules are demarcated by connective tissues containing bile duct, the lymphatic vessel, nerve and blood vessels (Luiz *et al.*, 1998).

The hepatocyte is the fundamental structural component of the liver. The hepatocytes in the liver lobules form cellular plates, which are directed from the periphery of the lobule to its center. The space between these plates contains capillaries and the liver sinusoids (Luiz *et al.*, 1998). These sinusoids are formed between the hepatic plates and then into the central vein, these hepatic cells are exposed to portal venous blood continuously (Guyton and Hall, 2000).

A branch of the coelic axis distributes 25% of the total blood flow in the hepatic artery while portal vein drains nearly all the content of gastrointestinal tract and spleen (Kumar and Clark, 2002). The vessels enter the liver using the hepatic portal while the blood from these branches distribute to each segment and pass on to the

sinusoids using the portal tracts. The blood then leaves the sinusoids by flowing into hepatic vein branches joined to three key branches prior to the inferior vena cava (Kumar and Clark, 2002). Sinusoids are deficient in basement membrane and are freely bounded by specialized epithelial cells and phagocytic cells.

The liver upper diaphragmatic surface is smooth while the posterior surface is deeply ridged to house the inferior vena cava. The falciform ligament connects the liver with the anterior abdominal wall and the diaphragm (Singh, 1996). The visceral (inferior) surface is concave and heads towards the inferior or posterior part of the vena cava. The gallbladder and the hepatic portal are positioned on this surface (Singh, 1996).

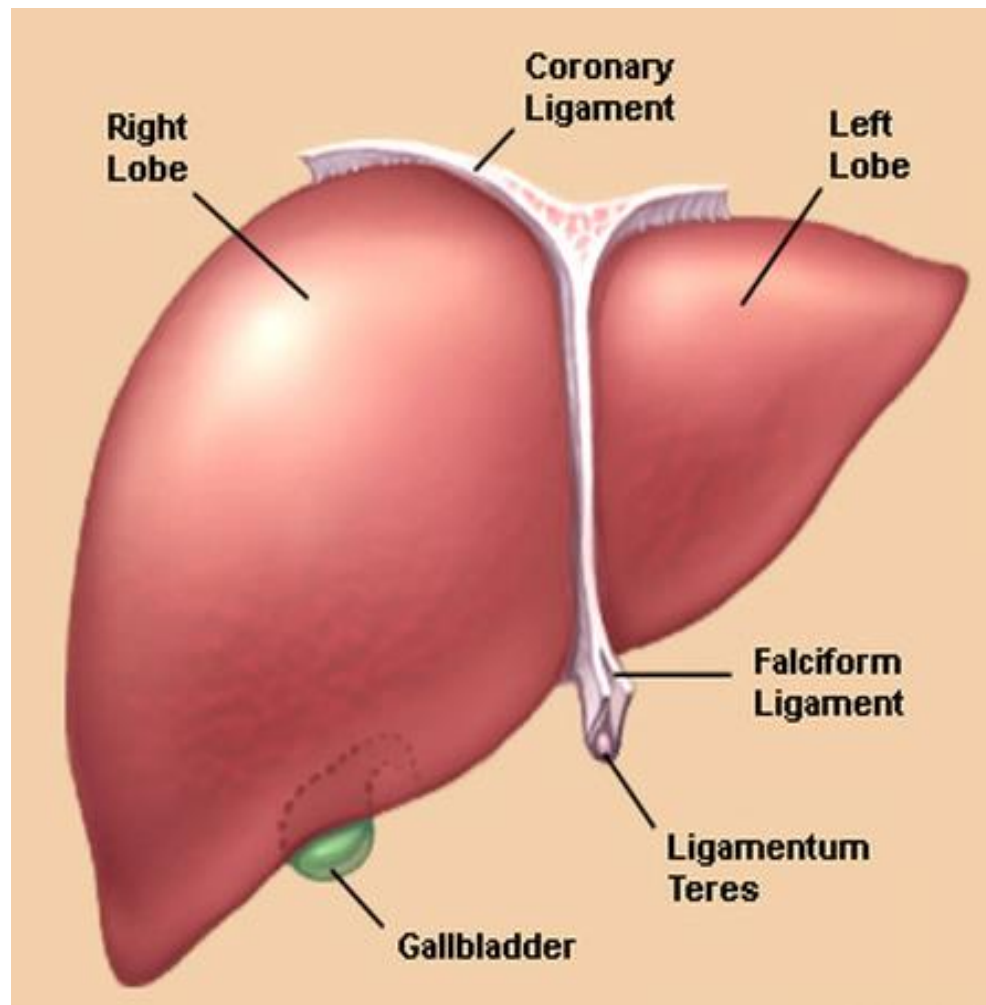


Figure2.5: Structure of the Liver (Brunicards *et al.*, 2010)

2.11.1.2 Functions of the Liver

The complexity and diverse enzymatic content as well as the unique structure, and also because it receives venous blood from the intestine, the liver has a wider range of function than any other organ in the body (Fox, 1996). These functions include:

- a. **Carbohydrate Metabolism:** The liver plays an important duty in maintaining glucose concentration in the blood through converting excess glucose to glycogen for storage or use by other tissues. The process is mediated by the hormone insulin secreted by the pancreas (Ramalingam, 1992). The glycogen formed is stored in the liver and converted to glucose as soon as the blood glucose levels fall below normal ($90\text{mg}/100\text{mc}^3$ blood). This process is stimulated by glycogen, another pancreatic hormone. When the level of glycogen is depleted in the liver, the hypothalamus immediately detects the low glucose level in the blood and then stimulates the release of various hormones which will cause the liver to convert amino acid and glycerol into glucose (Ramalingam, 1997).
- b. **Protein Metabolism:** Almost all the blood protein is synthesized and secreted by the liver with exception of antibodies. The liver releases the required amount of amino acid into the general circulation of the body system. The excess amino acid, which cannot be stored, is then broken down into nitrogen-containing amino parts like urea and ammonia known as deamination. The liver manufactures plasma protein which includes albumin, fibrinogen which is essential for clotting (Ramalingam, 1997).
- c. **Bile Secretion:** The liver serves the most important metabolic and digestive function. The bile acids are the foremost digestive chemical that the liver synthesized. It is secreted during meal and travels via the hepatic duct stem to the small intestine and then help to digest fat into smaller molecules by a process called emulsification. The

bile acids are stored in the gall bladder and secreted into the duodenum when needed (Ramalingam, 1997).

- d. Lipid Metabolism:** Triglycerides are derived from endogenous synthesis and are stored in the liver. Cholesterol and other lipids are synthesized and secreted in the liver, the cholesterol and other compound being esterified while vitamin D hydroxylation occurs. Bile salt derived from cholesterol is secreted into the biliary tract. The conversion of carbohydrate to triglycerides for secretion in the blood as Very Low Density Lipoprotein (VLDL) and storage as fat occurs in the liver. So also does the conversion of fatty acids released from adipose tissue during starvation, to triglycerides for storage, or utilized in ketogenesis for energy production (Nsirim, 1999).
- e. Storage:** The liver is designed in a way that it stores important substance like glucose (in the form of glycogen), fat-soluble vitamin (A, D, E and K) folate, cobalamine (B₁₂) and mineral like copper and iron that are essential for body metabolism (Singh, 1996).
- f. Hormones Modification:** Liver is an organ that modifies and inactivates hormones leading to chronic liver diseases as a result of the hormonal imbalance. For example, the liver metabolized and inactivated oestrogen. Thus the liver exerts a homeostatic effect on the activities of hormone (Ramalingam, 1997). Angiotensinogen, is a hormone synthesized by the liver liable for elevating blood pressure when activated by rennin an enzyme the kidney released at the instance of blood pressure (Rajani *et al.*, 2009).
- g. Drug Metabolism:** The liver is an organ responsible for modification and degradation of virtually all drugs. Most importantly, drugs taken orally are absorbed by the gut and transported through the portal to the liver. Drugs sometimes undergo first-pass metabolism, in the liver through a process that modifies, activates or inactivate the drugs before they enter the systemic circulation.

- h. Detoxification:** The liver helps in converting amino acids to urea, after which they are then excreted in the urine via the kidneys. In event of severe liver diseases, ammonia accumulates in the blood due to decrease blood clearance and inability to form urea. Elevated ammonia concentrations can be toxic particularly to the brain, and can play severe role in the development of hepatic encephalopathy. It detoxifies toxins and drugs before their excretion in the urine (Nsirim, 1999).
- i. Purification, transformation and clearance:** The liver eliminates dangerous substances like toxins and ammonia away from the blood by breaking it down and transforming the toxins to less dangerous compounds (Nsirim, 1999).
- j. Immunology:** Kupffer cells, (specialized macrophages) are involved in the immunological activities performed by reticuloendothelial system. Thus the liver contributes to the body immune system (Nsirim, 1999).

2.11.2 Kidney

The kidneys are paired organs which primary function is the production of urine. Kidneys can be found in all most every animal, including vertebrates and other invertebrates.

2.11.2.1 Structure of the Kidney

The kidneys are bean-shaped organs located below the rib cage on each side of the spine (Figure 2.6). The two kidneys filter about 120 to 150 quarts of blood every day each to create 1 to 2 quarts of urine which are mainly extra fluid and waste products. The bladder receives urine flowing from the kidneys via the ureters which are tiny tubes of muscle located on both sides of the bladder. The urine is mostly stored in the bladder which then caused relaxation to bladder wall muscles. When the bladder is

filled enough, it sends signals to the brain that there is a need to offload. In the process of excretion, the bladder empties the urine by a tube called the urethra, attached to the base of the bladder. Kidneys are made up of about a million nephrons each known as filtering units which filters minute quantity of blood. Nephrons have a filter, called glomerulus, and a tubule. It works in a two-step process, filtered fluid passing through the tubule by transporting needed minerals back into the bloodstream and evacuating wastes (Glodney *et al.*, 2005). Kidneys are very important for day to day activities of the body system because they keep blood composition stable and allow the body to function normally (Cotran *et al.*, 2005).

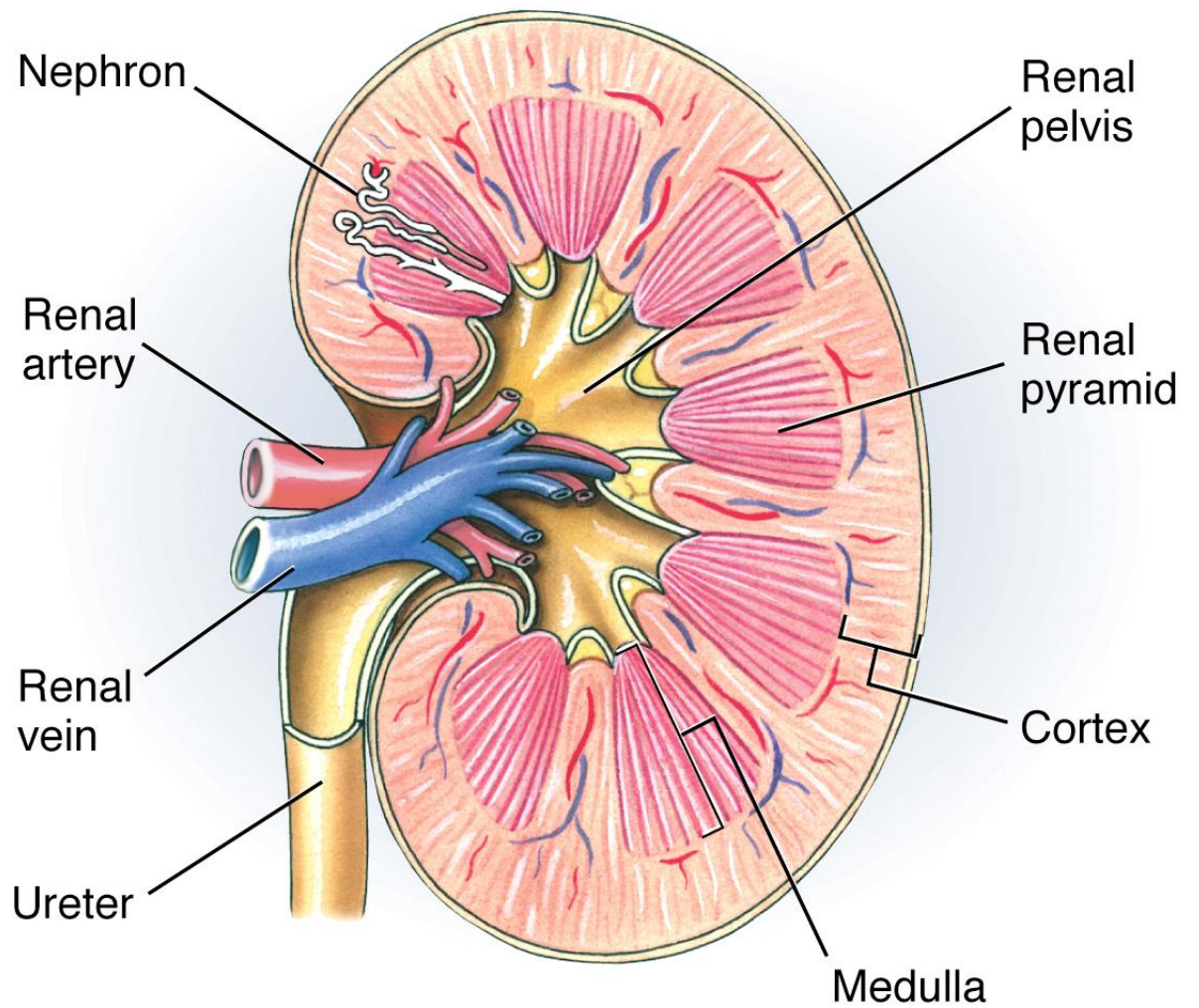


Figure 2.6: Mammalian kidney (Source: Petal, 2009)

2.11.2.2 Functions of the Kidney

- a. Excretion:** The key task of the kidney is the elimination of by-products and excess fluids in the body system. The by-products and excess fluid are mostly removed in the urine from the nephron. Urine production is a highly intricate process of excretion and reabsorption. This process is essential to maintain a steady balance of body chemicals constituents. The urine then flows to the bladder through tubes called 'ureters' (Bruce, 2004).

The bladder stores urine as a waste product until it is finally released through urination. Wastes in the blood are derived from the normal degradation of active tissues, such as muscles, and food products. The Malpighian bodies or Renal Corpuscles is made up of the "Glomerulus and Bowman's capsule", proximal tubule, loop of Henle and distal convoluted tubules which passes into a collecting duct (Weinstein, 1994).

- b. Filtration and Reabsorption:** Urine formation is gotten from a simple mechanism like filtration, reabsorption, and secretion respectively. Filtration takes place at the glomerulus (renal corpuscle) via a route which filtered from the blood waste and large proteins as an ultra-filtrate that finally becomes urine. It generates up to 180 liters filtrate per day (Thomas, 2005; Stephen *et al.*, 2006).

Reabsorption involves the transport of plasma components (such as glucose, amino acids, lactate, ketone bodies and other sugars) in the filtrate back to the blood, this allows for the generation of approximately 2litres of urine (Garabed *et al.*, 1997).

- c. Production of Hormones:** Rennin regulates blood pressure and activates a hormonal mechanism which controls and regulates electrolytes balance and blood pressure. An instant drop in blood pressure causes the kidneys to release rennin

enzyme which will regulate the condition. This enzyme converts blood protein to angiotensin a hormone that induces the release of another hormone called aldosterone from the adrenal gland. It is a hormone that is responsible for the reabsorption of sodium and water back to the blood, leading to rising blood-volume and blood-pressure. These reactions occurring in the kidneys to synthesize aldosterone hormone assist to regulate the level of salt in the blood.

- d. Regulation of body's pH balance:** The kidney controls the body's acid-base balance by adjusting and maintaining body pH balance. Excess hydrogen ions in the blood caused the blood to be too acidic, the kidney will then transport these ions to the urine but bacteria causing many serious degenerative disorders thrive in an acidic environment. Kidneys then help to keep the blood plasma at a neutral pH 7.4 through regulation of the body's pH, the kidneys assist to maintain a surrounding that prevents diseases and promotes proper functioning of all biological systems in the body (Maton, 1993).

They are essential and have homeostatic functions like regulation of electrolytes, maintenance of pH balance, so also maintaining salt and water balance. The kidney also plays a vital role in the intermediary metabolism particularly in amino acid degradation and gluconeogenesis (Le, 2013).

2.11.3 Uterus

The uterus is a special and vital female reproductive organ present in nearly all mammals with human inclusive. Mostly, the cervix opens directly to the vagina at one end and connects to the fallopian tubes depending on the species at the other end. The fetus develops and also grows within the uterus during gestation while it developed completely in placental mammals like humans.

2.11.3.1 Structure of the Uterus

The uterus consists of uterine body and uterine cervix which are coated with tunica mucosa. The cervix of the uterus extends into the vagina (Figure 2.7). It is positioned inside the pelvis via endopelvic fascia (ligaments) condensations. The ligaments are pubocervical, transverse, cervical, cardinal, and uterosacral ligaments. Bundles of nerves network of arteries, veins, and broad ligament are found attached to the ligaments (Maton *et al.*, 1993).

The uterus (womb) is resident within the pelvis directly to dorsal urinary bladder and ventral to rectum. In human, the uterus is pear-shaped and up to three inches (7.6 cm) long. Uterus is automatically divided to four parts: fundus, corpus, cervix and internal oxidative stress.

Uterus is sandwich at the centre of pelvic cavity located in the frontal plane because of the placement of ligamentum latum uteri. Located at the top right corner opposite the cervix is the fundus but do not exceed the linea terminalis. The uterus vaginal portion does not lengthen below interspinal line. The uterus often moves around and therefore, it is not static with pressure exerted on it by the filled bladder or full rectum anteriorly, though moves upwards if both are full but pushes downwards in an increased intra-abdominal pressure. The musculo-fibrous muscles apparatus consisting of suspensory and sustentacular part conferred movement on the uterus. In a normal situation the suspensory parts keeps uterus in anteflexion and anteversion in about 90 percent of women while it continues "floating" in the pelvis. These situations that "tipped," the uterus is referred to as retroverted, where women might be having pains during sexual intercourse, menstruation, urinary tract infections, complexity in conceiving, and using tampons respectively.

The uterus generally is composed of smooth muscle, the myometrium whose innermost layer is the junctional zone and is thickened in adenomyosis. Its parametrium is in loose or free connective tissue as seen in the uterus environment. The perimetrium is the peritoneum layer that covers the fundus, ventral and dorsal aspect of the uterus which is principally upheld by the pelvic diaphragm, perineal body, and urogenital diaphragm.

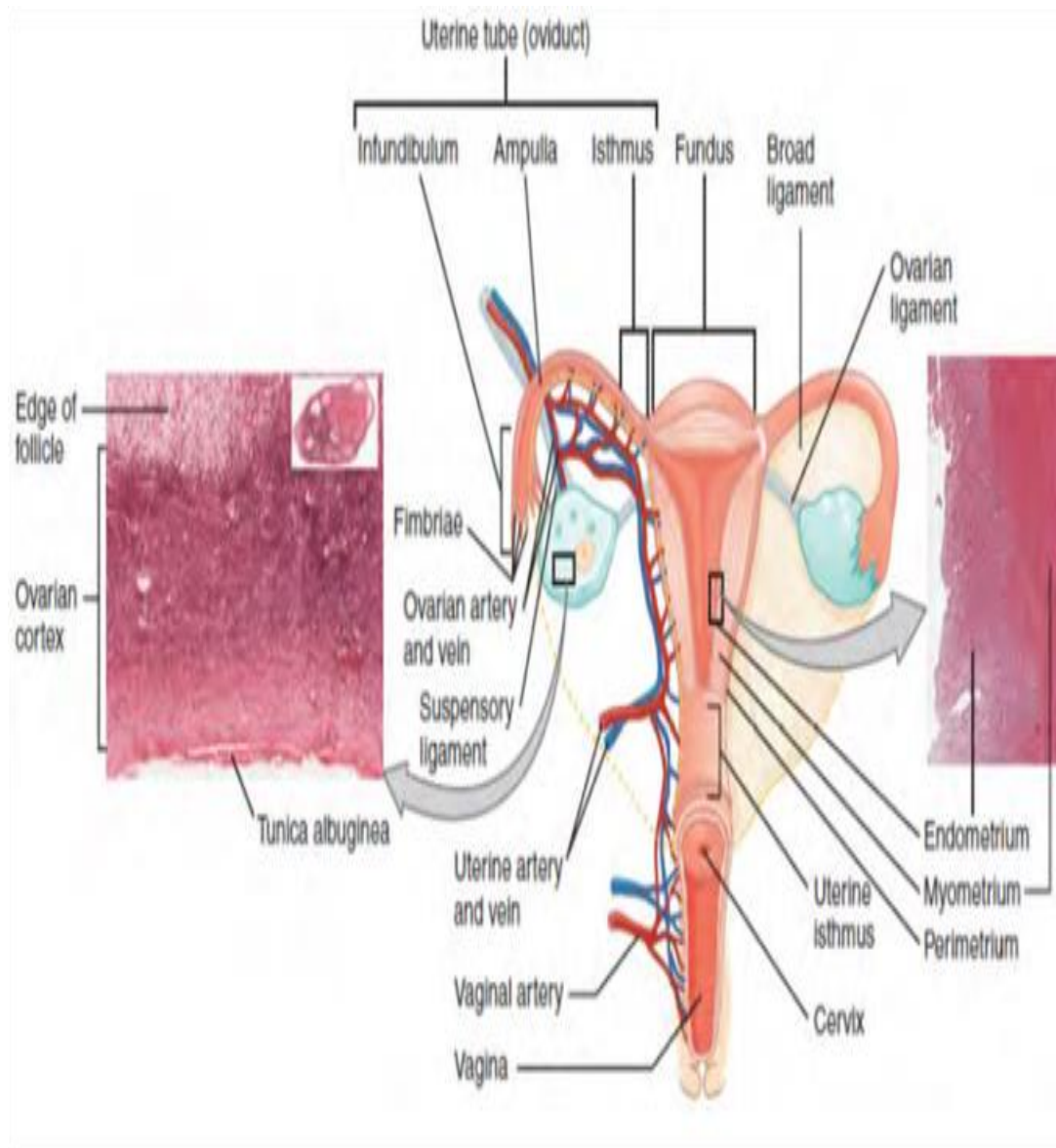


Figure 2.7: Female Reproductive organ (Source: www.boundless.com)

2.11.3.2 Functions of the Uterus

- It plays an important role by directing blood course to pelvis and external genitalia, together with ovaries, vagina, clitoris and labia during sexual response.
- Uterus admit fertilized ovum via the utero-tubal junction from the fallopian tube. It is then implanted into the endometrium, and receives it nourishment from blood vessels which develop solely for this purpose. The ovum fertilized then becomes an embryo, clings to the wall of the uterus and develops into a foetus along with it placenta until childbirth (Maton *et al.*, 1993).
- The uterus is responsible for uterine orgasm to occur (Benjamin and Sherman, 2008).
- Uterus provides structural integrity to uphold the bladder, pelvic bones, bowel and organs. The uterus in conjunction with the bladder is kept in their original or natural position over the pelvic bone and arrangement of bowel behind the uterus (Cotran *et al.*, 2005).

2.11.4 Ovary

The site of gamete or egg (oocyte) creation during fertilization is ovaries. The developing egg cells (oocyte) develop in the available space provided by the ovarian follicles. The follicle consists of various cells types at a different range in accordance with their stages of maturation that can be determined via their sizes. At the complete maturation of oocyte, the pituitary gland secret an LH surge that stimulates follicle breakage and oocyte is then released from the ovaries.

The release and development process of the oocyte is referred to as ovulation. The follicles released are then transformed into corpus luteum and releases progesterone needed for uterus implantation of the embryo. Consequently, the ovaries take turns to

release eggs on a monthly basis. However, eggs are released at random but if there are cases in which one of the ovaries is absent or dysfunction the other ovary would sustain the eggs release.

Ovaries are responsible for the secretion of estrogen, progesterone, and testosterone respectively. The secondary sexual characteristics of females at puberty, maturation, and maintenance of mature reproductive organs are often a crucial responsibility of oestrogen. Progesterone at most times prepares the uterus for pregnancy as well as mammary glands for lactation. Joint actions of the two female reproductive hormones help to promote menstrual cycle changes in the endometrium.

2.11.4.1 Structure of the Ovary

In humans, the pelvic cavity housed the ovaries on either side of the uterus and attaches to fibrous cord known as an ovarian ligament. The ovaries are connected to body system (wall) through the suspensory ligament. Mesovarium serves as a portion of the broad ligament of the uterus that shields the ovary. Thus, the ovary is an organ in the human body fixed to the peritoneum, therefore, making it the only intraperitoneal organ.

The ovary has two parts (extremities) which are tubal extremity which attaches to the end of fallopian tube via the infundibulo pelvic and uterine extremity, it attaches to the uterus through the ovarian ligament and it points downward. (Figure 2.8).

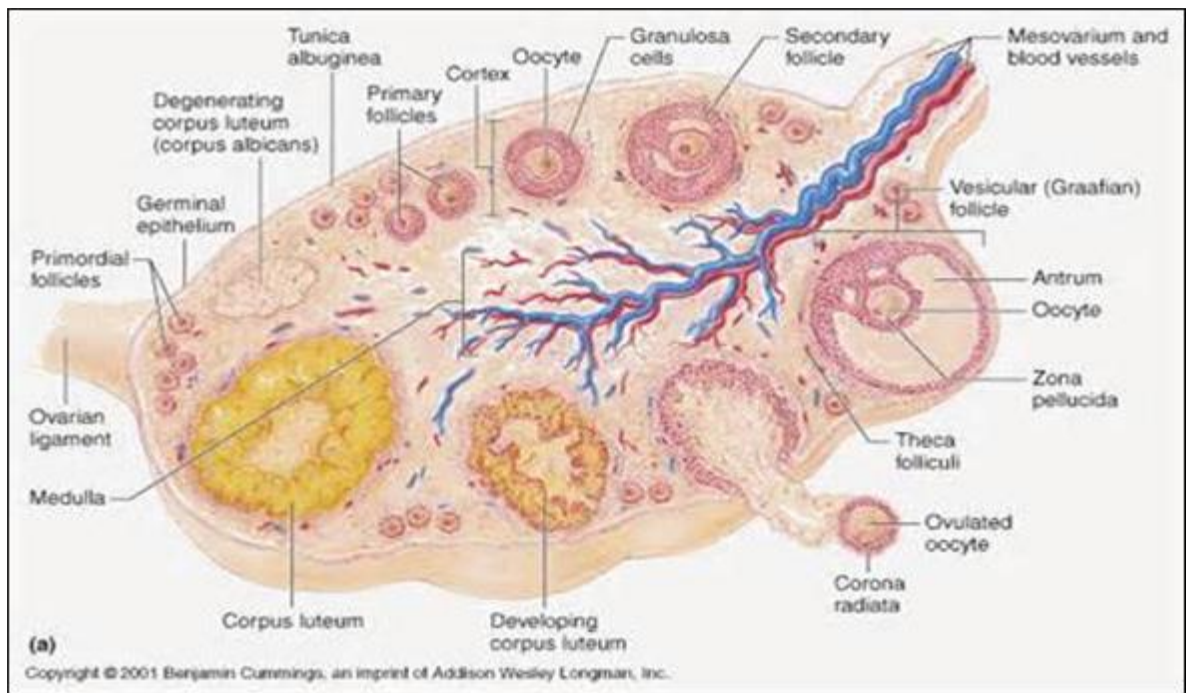


Figure 2.8: Structure of the Ovary

2.11.4.2 Functions of the Ovary

- Ovaries secrete some reproductive hormones like oestrogen, progesterone with testosterone. Oestrogen is liable for secondary sexual character of females at puberty.

2.12 Serum

The fluid portion of the blood obtained after clotting or centrifugation of blood is allowed to clot in the absence of anticoagulant is the serum (Murray *et al*; 2000). When the fibrinogen in the blood is converted to fibrins (clot), the yellowish liquid on top of the clotted blood in a tube is serum or a clear yellowish fluid that is left in the blood after fibrinogen, prothrombin, and other clotting factors must have been removed through clot formation. It is an extracellular fluid responsible for the maintenance of the internal environment of the cell, which is homeostasis (Ganong, 2001).

The serum contains a large number of ions, inorganic molecules and is also found to contain plasma proteins such as albumin and globulin (Ganong, 2001). Blood serum is one of the largest sources of biomarkers, whether for diagnostics or therapeutics. Serum consists of the entire proteins not used in blood clotting, antigens, coagulation, electrolytes, hormones, antibodies, and every exogenous substance (Wang *et al.*, 2002). The serum also has higher serotonin content because of the breakdown of platelet during clotting.

Alterations of tissue composition might significantly affect the levels of serum enzymes. The nature of the enzyme freed as a result of injury will depend on its subcellular localizations and used in numerous diagnostic tests (Martin, 2007).

2.13 Enzyme Studied

The biochemical marker enzymes studied in this work are alkaline phosphatase (ALP) (EC. 3.1.3.1), alanine amino transferase (ALT) also known as glutamate pyruvate transaminase (GPT) (EC.2.6.1.2) and aspartate amino transferase (AST) referred to as glutamate oxaloacetate transaminase (GOT) (EC. 2.6.1.1).

2.13.1 Alkaline Phosphatase (EC. 3.1.3.1)

Alkaline phosphatase (ALP) is in the class of phosphomonoesterases which hydrolyze *in vitro* activity of phosphate esters at an optimum pH of 10 (Walker *et al.*, 1990).

2.13.1.1 Distribution

It is broadly found in mammals, fish, amphibians, reptiles, birds, and bacteria (Yora and Sakagishi, 1986). In animals mostly humans, alkaline phosphatase is located within most tissues in the whole body, however, it is principally concentrated or abundant in the liver, kidney, bile duct, bone, reticuloendothelial tissue and the placenta (Walker *et al.*, 1990).

2.13.1.2 Activators and Inhibitors

The activity of ALP is always activated by cofactors like divalent metallic ions, amino alcohols or 2-amino-2-methyl-1-propanol, diethanolamine, and TRIS buffer. On the other hand, the activity of the enzyme is inhibited by inorganic phosphate (Pi), Be^{2+} , monoethanolamine, chelators of divalent metal like EDTA, oxalate, citrate, cysteine, histidine acid, a buffer of neutral pH, aromatic amino acids, urea and iodoacetamide (Hinsch, 1957).

2.13.1.3 Isoenzymes

In human and most mammals the predominant alkaline phosphatase isoenzymes present are: intestinal ALP, tissues ALP, placental ALP (Walker *et al.*, 1990).

2.13.1.4 Clinical application

High ALP levels can indicate blocked bile duct. Elevation in ALP in the serum could suggest there is an active bone formation taking place because ALP is a byproduct of osteoblast as seen in Paget's disease of bone. Decreased ALP concentrations are not common compared to its elevated levels. Seminoma is a condition that results from elevated placental alkaline phosphatase (Berk and Korenblat, 2007).

Reduced levels of alkaline phosphatase can be seen in diseases such as hypophosphatasia (an autosomal recessive disease condition), postmenopausal estrogen-receiving women therapy due to osteoporosis. Adults with recent heart surgery, malnourished individuals with magnesium deficiency cases, hypothyroidism or severe anemic individuals, cretinism, severe episode of enteritis, pernicious, children with achondroplasia and aplastic anemia, chronic myelogenous leukemia are also known to decrease alkaline phosphatase levels. In addition, oral contraceptives medication or drugs have been established to reduce the activity of alkaline phosphatase (Schiele *et al.*, 1998).

The activity of ALP in normal adult serum is gotten mainly from hepatic and reticuloendothelial sources. The growth of bone in children causes an elevation in serum ALP activity from 2 to 5 times that observed in an adult. Placental ALP is elevated 2 to 3-fold in the second and third trimesters of normal pregnancy. ALP intestinal isoenzyme causes serum enrichment which can lead to a postprandial elevation in persons with blood groups B or O and those who also secrete ABO blood group substance (Walker *et al.*, 1990).

Sustenance of elevated ALP concentrations is linked with liver or bone disorders or both. A collection of primary and secondary hepatic affairs might also be linked with

increased serum ALP activity. Hence, enzyme production increased in response to cholestasis, serum ALP activity also provides a sensitive pointer to obstructive and space-occupying lesions of the liver (Walker *et al.*, 1990).

2.13.2 Aspartate Aminotransferase (AST) (EC 2.6.1.1)

AST is a key enzyme in the flow of carbon between the organic acids and amino acids pools (Michiel and Huub, 1991). AST catalyses the reversible transamination reaction between L-aspartate and alpha (α)-ketoglutarate and requires a pyridoxal phosphate prosthetic group.

2.13.2.1 Distribution

It has been widely spread throughout animal tissues, with the highest concentration in cardiac muscle, liver, and skeletal muscle while kidney, pancreas, lungs, and spleen also contain considerable quantities of the enzyme. It is also found mainly in the cytosol and mitochondria cells of these organs (Boyd, 1983).

2.13.2.2 Activators and Inhibitors

The activities of AST are allosterically inhibited by its product, oxaloacetate (Boyd, 1983). The activator of AST is pyridoxal phosphate, which act as a cofactor in the presence of magnesium (Nelson and Cox, 2000).

2.13.2.3 Isoenzymes

AST has two types of isoenzymes: isoenzyme I, which is cationic and isoenzymes II which is an anionic. Isoenzyme I has a much greater affinity for AST than isoenzyme II (Boyd, 1983).

2.13.2.4 Clinical Application

AST is very useful in the differential diagnosis of jaundice. It is also released from the cardiac muscle thereby resulting in elevated activity in the serum. AST is found in cytoplasm and also mitochondria cells. During minor tissue injury, the predominant form of AST is that gotten from the cytoplasm, with little amount released from the mitochondria. Severe tissue damage results in more of the mitochondrial enzymes being released. Elevated levels of aminotransferases are indicative of myocardial infarction, hepatopathies, muscular dystrophy and damage to internal organs. Increased levels of AST however are generally a result of liver diseases associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. Following a myocardial infarction, serum levels of AST are elevated and reach a peak by 48 to 60 hours after onset (Henry, 1974).

2.13.3 Alanine Aminotransferase (ALT) EC. 2.6.1.2

ALT also referred to as glutamate pyruvate transaminase (GPT) is a vital enzyme present in the intermediary metabolism involving glucose and protein catalyzing the reversible transamination between alanine and 2-oxoglutarate to form Pyruvate and glutamate. The reaction requires pyridoxal phosphate as a prosthetic group.

2.13.3.1 Distribution

ALT is located in the serum and some other body tissues, but is often connected with the liver. ALT occurs normally in most human tissues, especially the liver, heart and skeletal muscle. It is also present in the kidney, pancreas and erythrocytes (Schumann and Klauke, 2003).

2.13.3.2 Activators and Inhibitors

The activity of ALT is stimulated by high concentration of aldehyde, oxoacids and ketones. It is inhibited by Pyruvate which is one of the reaction products (George *et al.*, 1994).

2.13.3.3 Isoenzymes

The isoenzymes of ALT show variation in the rate of reaction with substrates. The mitochondrial fraction of the enzyme shows higher sensitivity to inhibition by accumulated Pyruvate (George *et al.*, 1994). The pH sensitivity and the immunological properties of the cytoplasmic and mitochondrial fraction of ALT are the same, but they can be distinguished by the fact that they have different electrophoretic mobilities (Reitman and Frankel, 1957). Two forms of ALT have been identified, ALT 1 and ALT 2, encoded by separate genes. The cellular and tissue distribution of the different ALT proteins have not been characterized in humans, and their relative contribution to serum is unknown (Lindblom *et al.*, 2007; Miyazaki *et al.*, 2009).

2.13.3.4 Clinical Application

The enzyme is usually investigated clinically as the component of liver function indices to diagnose or screen the liver status. Increase in serum ALT activity is widely used as a substitute marker for tissue damage (Miyazaki *et al.*, 2009). ALT concentration assay has been used as marker for liver injury in people and in preclinical toxicity studies (Yang *et al.*, 2009).

A significant elevation in the activity of ALT frequently recommends availability of other medical related conditions like viral hepatitis, liver damage, bile ducts problems,

infectious mononucleosis, congestive heart failure, or myopathy. However, high concentrations of ALT do not authenticate medical problem exists. Instability in the activity of ALT is normal during the day and an increase in reaction to strenuous physical exercise (excessive work) involved in during the course of the day (Giboney, 2005).

Elevated ALT activity observes in the blood can be a probable underlying cause to be investigated further through the measurement of other enzymes. For instance, elevated ALT level in the serum can result due to liver- cell damage which can be distinguished from biliary duct problems by measuring alkaline phosphatase. Hence, myopathy-related ALT levels can be ruled out by measuring the levels of creatinine kinase enzymes. A Zileuton drug is one of the several drugs reported to increase ALT levels (Yang *et al.*, 2009).

2.14 Tissues Function Test

They are myriad of test or indices carried out in relation to specific organ which can be used to assess impairment or functional capacity of the organ.

2.14.1 Liver Function Indices

A biochemical test that can be used to monitor or assess problems with various metabolic processes and signifying the illness or diseases causing the problems is referred to as function test (Martin *et al.*, 1983). The concentration of proteins, bilirubin with albumin present in the serum can be used to establish the state of the liver and can be used to ascertain several types of liver damage (Naganna, 1989; Yakubu *et al.*, 2003).

2.14.1.1 Protein

Protein is organic compounds which consist of various amino acids assembled in a linear chain and folded into a globular form and joined together by peptide bonds existing between the carboxyl and amino groups of adjacent amino acid residues (Ridley, 2006). Proteins have the highest percentage abundance in the serum. Proteins serve as the building blocks for all cells and body tissues and represent basic components of enzymes, several hormones, antibodies and clotting agents respectively. Proteins can sometimes assist in the transport of hormones, vitamins, minerals, lipids and other materials. Also, they help to balance osmotic pressure of the blood and tissues. Proteins play a critical function in maintaining acid-base balance in the blood. Furthermore, proteins which are present in the serum serves as a reservoir of energy for tissues and muscle (Adkin *et al.*, 2005).

The major component of serum proteins can be grouped into two groups, albumin, and globulins. Other included fibrinogen, regulatory lipoprotein, transferrin and prothrombin. The total serum protein is generally classified as a measure of both albumin and globulin, since other components are in minute quantity (Jacob *et al.*, 2005).

In chronic conditions such as adrenal cortical hypofunction, collagen vascular disease, liver dysfunction, hypersensitivity states, sarcoidosis, dehydration, respiratory distress, hemolysis, cryoglobulinemia, alcoholism, and leukemia there is the tendency for the total protein to be elevated. Whereas, decreased protein concentrations may be seen in cases of malnutrition, malabsorption, liver disease, diarrhea, severe burns, hormones imbalances, loss through the urine in severe kidney diseases, low albumin, low globulins and pregnancy (Jacob *et al.*, 2005).

2.14.1.2 **Albumin**

Albumin is any protein soluble in water and concentrated salt solutions which can experience heat coagulation (Lichenstein *et al.*, 1994). Albumin is almost half the body total protein present in the serum and about 30-40% of the body's total albumin pool is found in the intravascular compartment while the leftover is referred to as extravascular and is located in the interstitial spaces like the muscles and skin. Albumin is also present in minute quantity in virtually all the body tissue fluids such as sweat, tears, gastric juice and bile (Walker *et al.*, 1990).

Serum albumin produced by the liver is the most abundant protein found in the blood plasma and it is about 60% abundant in human. Reduction in this protein might serve as a pointer to liver disease. The decrease can also be linked to kidney disease, due to the presence of albumin in the urine (Berk and Korenblat, 2007). It has been reported that malnutrition or a low protein diet may be attributed to decrease albumin levels (Berk and Korenblat, 2007). Hypoalbuminemia is a disease condition caused by a low concentration of serum albumin which could be caused by liver disease, late pregnancy, nephrotic syndrome, malabsorption, burns, protein-losing enteropathy, malnutrition, artifact, genetic defect or variations and malignancy. Hyperalbuminemia results from the consequence of dehydration notice in the body system. In some instances deficiency of vitamin A (retinol) albumin concentration can be elevated.

The available albumins present in the serum are vital keys in regulating and controlling a volume of blood through maintenance of osmotic pressure of the blood compartment. Albumin is a transporter for molecules with low water solubility like bilirubin (Berk and Korenblat, 2007).

2.14.1.3 Bilirubin

Bilirubin is a yellowish by-product from the catabolism of normal heme. The bulk of bilirubin is produced from the catabolism of hemoglobin and erythrocytes under normal or abnormal destruction (intra or extravascular hemolysis) within mononuclear phagocytes. About 20% of bilirubin is derived from degradation of many hepatic hemoproteins like myoglobin, cytochrome P450 including excess heme from ineffective erythropoiesis in the bone marrow (Chernecky and Berger, 2008). This is responsible for the yellow color seen in bruises, urine, and the yellow discoloration of jaundice. Bilirubin is eliminated through the bile and urine, but an indication of elevated level may indicate exposure to some disease conditions. The expression of the effective antioxidant activity of bilirubin has led to the hypothesis that it plays a physiological role known as a cellular antioxidant (Liu *et al.*, 2008).

Bilirubin is transported through the bloodstream in two forms: indirect (unconjugated) bilirubin which is not soluble in water but moves via the bloodstream to the liver, where it is converted to a soluble form (direct or conjugated) formed from indirect bilirubin (Chernecky and Berger, 2008).

Normal levels of bilirubin an excretory product found in the body reflect the balance between production and excretion (Chernecky and Berger, 2008). Some disease condition might be caused by high levels of bilirubin in the blood plasma, these diseases include, infected gallbladder, Gilbert's syndrome, hepatitis, cirrhosis, gallstone, cancer of the pancreas, sickle cell disease. Drugs such as antibiotics, some birth control pills, indomethacin, diazepam etc can also increase blood bilirubin levels while low concentrations of blood bilirubin can be caused by vitamin C, Phenobarbital, and theophylline (Chernecky and Berger, 2008).

2.14.2 Kidney Function Indices

The series of test performed to estimate the concentration of the indicators in the serum or which otherwise might have been absorbed by the nephron are referred to as kidney function test (Guyton and Hall, 2000). Such parameters of the kidney included urea, creatinine, uric acid and serum electrolytes.

2.14.2.1 Urea

Urea a carbamide is an organic compound having two amine group linked by a carbonyl functional group. Urea is the main nitrogen-containing molecule present in the urine of mammals. It is solid, colorless and odorless substances (although the given off ammonia in the presence of water, has a strong odor) and highly soluble in water and non-toxic.

Urea is produced by the liver through the urea cycle as a waste product of the digestion of protein. Urea production rate increases by high diet or during increased catabolism from starvation, sepsis and tissue damage (Mayne, 1998).

Urea is normally reabsorbed by the inner medullary collecting ducts of the nephrons, thus causing an elevation in osmolarity of the medullary interstitium around the thin ascending limb of the loop of Henle thereby causing water to be reabsorbed. Through urea transporter 2 actions, a number of the reabsorbed urea will ultimately return back into the thin ascending limb of the tubule, via the collecting ducts, and finally into the excreted urine. This process is mostly controlled by anti-diuretic hormone that allows the body to create hyper osmotic urine of higher amount of dissolved substances than obtained in the blood plasma. It is therefore an important act of preventing the loss of water, maintaining blood pressure as well as maintaining the concentration of sodium ion in the blood plasma (Walter, 2009).

Normally kidney expels large amount of urea and in a normal renal condition. The total amount of urea excreted is often affected by glomerulus filtration, which in turn relied on renal functions (Mayne, 1998).

Low serum urea concentration indicates reduced protein catabolism, malnutrition, cachexia, inadequate dietary protein intake (Waring *et al.*, 2008), ethanol, carbohydrate-rich, and protein poor diets and impaired synthetic function in patients with established liver disease (Waring *et al.*, 2008).

2.14.2.2 Uric Acid

Uric acid is composed of some elements such as nitrogen, carbon, oxygen, and hydrogen, it has a structural formula $C_5H_4N_4O_3$. It is formed from catabolism of purine nucleotides and serves as a weak acid under physiologic conditions. pH below 5.75 may indicate that the leading form of non-ionized uric acids is present in the urine (Walker *et al.*, 1990).

Xanthine oxidase serves as a precursor for uric acid synthesis from xanthine and hypoxanthine, which in turn are derived from purine. It is toxic to tissues compare to either xanthine or hypoxanthine. It is released in hypoxic disease conditions (Baillie *et al.*, 2007).

Uric acids fluctuation might be caused by some factors, such as exercise, diet, drugs, and state of hydration. Serum uric acid is the result of the relationship that exists between four major processes that take place: dietary purine intake, endogenous purine metabolism, urinary uric acid excretion and intestinal uricolysis (Walker *et al.*, 1990).

Hyperuricemia a disorder that results from elevated serum uric acid due to high intake of purine-rich foods or impaired excretion of uric acid by the kidneys. It is usually associated with various conditions including, gout, Lesch-Nyhan syndrome, cardiovascular diseases etc. (Nyhan, 2005; Heinig and Johnson, 2006). The decrease in it serum values have been linked with multiple sclerosis (Toncev *et al.*, 2002).

2.14.2.3 Creatinine

Creatinine is the by-product of the catabolism of creatine phosphate in the muscle and is generally produced at a moderate rate in the body (Coca *et al.*, 2007). Creatinine is a chemical that is spontaneously formed as a cyclic derivative of creatine which is usually excreted out of the blood via the kidneys. Malfunctioning of the kidney might lead to a rise in creatinine concentration in the blood (Coca *et al.*, 2007). Therefore, the concentration of creatinine in the blood and urine may be used to estimate creatinine clearance, which reflects the glomerular filtration rate (GFR).

Clinically the GFR is important in the measurement of renal function. Creatinine level in men seems to be higher because of their greater skeletal muscle mass composition when compared to women. Therefore, it has been established that vegetarians have lower creatinine levels (Coca *et al.*, 2007).

Higher levels of creatinine in an indication of severe tubular necrosis, dehydration, diabetic nephropathy, eclampsia, kidney failure, muscular dystrophy, preeclampsia, pyelonephritis, and reduced kidney blood flow. The lower level may indicate myasthenia gravis and muscular dystrophy and myasthenia gravis (Bazari, 2007; Coca *et al.*, 2007).

2.14.3 Serum Electrolytes

Molecules (ions) that are positively or negatively charged found in the extracellular fluids of the body cells like blood plasma are referred to as electrolytes. The electrolytes are used to assess renal integrity, endocrine integrity, and pH balance. These constitute some extensive renal metabolic profiles.

The major electrolytes present in the blood plasma are potassium ion (K^+), sodium ion (Na^+), calcium ion (Ca^{2+}), magnesium ion (Mg^{2+}), chloride ion (Cl^-), phosphate ion (PO_4^{2-}), bicarbonate ion (HCO_3^{2-}) and sulphate ion (SO_4^{2-}).

2.14.3.1 Potassium ion (K^+)

Potassium is an inorganic metallic ion and the body system is rich in this cation. The intracellular space contains majority of this cation (potassium) while a minute quantity is available in the extracellular space (Walker *et al.*, 1990). Potassium is an electrolyte used as a key pointer to renal disorders (Henry, 2001). The kidney is responsible for the filtration of potassium and sodium. Nevertheless, sodium is reabsorbed in the distal convoluted tubule sodium and potassium is secreted. A decrease in renal filtration and secretion will lead to increase in plasma potassium which can then result in renal failure (Henry, 2001). Aldosterone enhances potassium (K^+) secretion in the portion of the distal tubules and the collecting ducts; the effect is however with control of Na^+-K^+ exchange (Guyton and Hall, 2000).

Hyperkalemia is often caused by hemolytic anemia, diabetes, Addison's disorder and toxic compounds resulting in deadly and life-threatening complication of renal failure (Henry, 2001). Its symptoms are frequent low serum potassium in the plasma, diarrhea, vomiting, thiazide diuretics, Cushing's disease, intravenous fluids and syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (Henry, 2001).

2.14.3.2 Sodium ion (Na^+)

Sodium is the principal extracellular cation in the body. It is directly linked to the osmotic pressure of the plasma or serum and it affects the osmolarity of the blood, thereby influencing blood volume and pressure, retention or loss of interstitial fluid (Henry, 2001). The most crucial organ that regulates sodium and potassium ions in the node is the kidneys (Philip, 1994).

The decrease in sodium ions in the plasma is known as dehydration while an increase in its retention leads to edema (Henry, 2001). Water is known to accompany sodium by diffusion but Hypernatremia, promote increased sodium ion without a corresponding gain in water in conditions like diabetes, Cushing's disease, and hyperaldosteronism. Some situations like congestive heart failure, liver cirrhosis result in retention of sodium and an equivalent amount of water by the kidney (Henry, 2001). The condition can result in hypertension or edema, but not an elevated serum sodium concentration. Low serum level of sodium may result from Addison's disease, excessive diuretic therapy, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), burns, diarrhea, vomiting and cystic fibrosis a condition known as Hyponatremia (Henry, 2001).

2.14.3.3 Calcium ion (Ca^{2+})

Calcium is also one of the important ions in the body system which about 98% of the available 1.2kg of calcium in human (adult) skeleton is in the form of hydroxyapatite (Walker *et al.*, 1990). The hydroxyapatite is a lattice-like crystal made up of calcium, phosphorus, and hydroxide. Also, the excess calcium is present in various tissue especially skeletal muscles while the extracellular fluid takes about 50% of the excess (Walker *et al.*, 1990). The calcium in the body system is complex with some other

molecules; protein bound to 45% of the calcium in the blood, the ionized calcium is 45% while 10% are complex to anions in an undissociated salts form. Most physiological active calcium is the ionized calcium and is regulated by a hormone referred to as parathyroid hormone (PTH) through a negative feedback inhibition (Henry, 2001).

In clinical diagnosis calcium and phosphorus are always measured together because both are linked to parathyroid disorders and bone abnormality. PTH leads to resorption of calcium and phosphorus from the bone. Although, PHT promotes reabsorption of calcium in both renal and intestinal mucosa cells whereas phosphorus is excreted (Walker *et al.*, 1990; Henry, 2001). Hence, an increase in serum calcium with the corresponding decrease in phosphorus is seen in hyperparathyroidism condition (Walker *et al.*, 1990; Henry, 2001) while vice versa is obtainable in hypoparathyroidism. Both calcium and phosphorus are low in conditions that result in vitamin D dependent rickets (VDDR), although, calcium is normal and phosphorus is low in vitamin D resistant rickets (VDRR).

Low total calcium might be caused by disease conditions like Hypoparathyroidism, renal failure, hypoalbuminemia, hypovitaminosis D and other conditions while hypoparathyroidism (and alkalosis) only result in low ionized calcium. Conversely, hyperparathyroidism, multiple myeloma, malignancies (those that secrete parathyroid hormone-related protein), antacids, hyperproteinemia, dehydration and hypervitaminosis D cause elevated total calcium, only hyperparathyroidism, malignancy and acidosis cause elevated ionized calcium (Walker *et al.*, 1990; Henry, 2001).

2.14.3.4 Phosphate ion (PO_4^{2-})

Phosphorus is an intracellular anion in mammals that is widely distributed due to its availability. 85% of the total phosphorus in a man is found in the hydroxyapatite phase of the skeleton while the soft tissues contain the other 15%. The extracellular fluid space contains an inorganic phosphate that reflects a minute percentage of total body phosphorus. A number of the phosphate ions present within the body system are in organic form but form complexes with macromolecules like carbohydrates, protein, and lipids (Walker *et al.*, 1990). Phosphorus is a component of the cellular cytoplasm and mitochondria structure and involves in numerous enzymatic processes such as glucose degradation (glycolysis), synthesis of membrane lipids and also oxidative phosphorylation, resulting in the formation of adenosine triphosphate (ATP) as an energy source. Furthermore, the phosphorus influences the oxygen-carrying capacity of hemoglobin through regulation and monitoring of 2, 3-diphosphoglycerate (2, 3-DPG) synthesized (Walker *et al.*, 1990).

The kidney serves as a significant organ that regulates phosphate homeostasis including major clinical perturbations caused by renal phosphate wasting (Walker *et al.*, 1990). Several factors such as hormonal and non-hormonal modify the reabsorption of phosphate. Hyper- and hypophosphatemia are disorders of phosphate homeostasis caused by cellular shifts in phosphate ions. The three key conditions that can cause phosphate dysfunction are dietary intake, gastrointestinal and renal status. Therefore, serum inorganic phosphate is a small portion of body phosphate; alterations in the serum level can take place when body phosphate is low, normal or high (Walker *et al.*, 1990).

2.14.3.5 Chloride ion (Cl⁻)

Chloride is an inorganic anionic halogen that is exclusively distributed around extracellular fluid (ECF) compartment (Walker *et al.*, 1990). The kidneys also assist in the maintenance of the total body chloride balance. Virtually all or parts of the chloride filtered by the glomerulus are reabsorbed through both an active and passive transport movement via the tubules of each nephron. The potentials of the nephrons to reabsorbed chloride keep its concentration in the serum within a slight range (Walker *et al.*, 1990).

The serum chloride concentration can be elevated through an accumulation of surplus chloride to the ECF sections or elimination of water via this compartment. The ECF help to reduce serum chloride ion concentration or add water to this compartment (Walker *et al.*, 1990).

2.15 Glucose

Glucose is a carbohydrate that serves as a vital fuel found in the body system. In the fed state, the majority of the circulating glucose comes from the diet while the glucose use as energy in the fasting state is derived from gluconeogenesis and glycogenolysis (Walker *et al.*, 1990).

Glucose and its related transporters play an important role in energy metabolism during pregnancy for both maternal as well as fetal development. During pregnancy, the glucose concentration in blood circulation is usually within normal range as an increase above normal range will lead to consequent gestational diabetes seen in many cases (Emin *et al.*, 2001). Nevertheless, a tangible transport or movement of glucose from mother to embryo through the placenta is critical to the survival and growth of the embryo in utero, since the metabolism of glucose by the embryo for oxidative

metabolism is minute (Emin *et al.*, 2001). The development of early embryo cannot utilize glucose as an energy source until there is compartmentation (Pantaleon and Kaye, 1998). Blastocyte formation changes the situation drastically when glucose is preferred to oxidized pyruvate as a substrate during embryonic fuel metabolism, this is an alternation signaled at by glucose transport protein (Pantaleon and Kaye, 1998). Overall, glucose transportation can take place via sodium-coupled mechanism or through facilitated diffusion through a concentration gradient. Genes responsible for the transportation of glucose have been designated as GLUT 1 to GLUT 9, base on the way they were identified (Carayannopoulos *et al.*, 2000; Phay *et al.*, 2000).

2.16 Lipid Profile

Lipids of diagnostic importance that are often assessed or monitor in disease conditions include total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and very low-density lipoproteins (Nelson and Cox, 2007).

2.16.1 Total Cholesterol

The total cholesterol is an unsaturated alcohol of the steroid compounds family. It is essential for the normal function of all animal cells and is a fundamental element of their cell membranes (Walker *et al.*, 1990). Cholesterol an important component in the synthesis of bile acids from cholic acid, steroids hormones as well as vitamin A, D, E and K which are fat-soluble vitamins. A vital steroid synthesized by animals in large quantity is cholesterol while a small amount is synthesized by other eukaryotes, such as plants and fungi. It is nearly totally absent amid prokaryotes including bacteria (Pearson *et al.*, 2003). Although, elevated concentration of cholesterol in the serum is a marker of diseases such as heart diseases. Cholesterol is excreted through

the liver by the bile duct to the digestive tract. Usually, up to 50% of cholesterol excreted is absorbed by the small intestine back into the bloodstream (Pearson *et al.*, 2003).

Cholesterol plays a very crucial role in pregnancy because is a substrate for steroid hormones synthesis such as luteinizing, follicle stimulating hormones, progesterone, estrogen, testosterone etc. These hormones initiate pregnancy through ovulation, luteal phase formation and maintained pregnancy during the gestation period. Although cholesterol is essential for the synthesis of steroid hormones, increase in free cholesterol in circulation is related to the high risk of cardiovascular diseases due to its deposition at the arteries which impair blood flow (Sladek *et al.*, 1997). It has been reported that endothelium-derived dilators contribute to uterine artery vasodilation, vascular remodeling and preservation of placental blood flow (Sladek *et al.*, 1997). Inadequate placental blood flow impairs fetal growth and development and in turn may programme the fetus to develop disorders in adulthood in which uterine blood flow has been reduced (Simmons *et al.*, 2001).

2.16.2 Triacylglycerol

Triacylglycerol (TAG) also referred to as triglyceride, it is a derivative of glycerol and three fatty acids. It is the key component of vegetable oil and animal fats (Nelson and Cox, 2000). It constitutes a vital part of very low density lipoprotein (VLDL) and chylomicrons and serves as vital energy sources and transporters of dietary fat. The energy (9kcal/g or 38kJ/g) obtained from it is twice that of carbohydrates and proteins (Nelson and Cox, 2000).

In humans, high level of TAG in the blood stream has been linked to atherosclerosis one of the leading cause of deadly heart disease and stroke (Murray *et al.*, 2000). The

TAG is a crucial fuel for the fetus during pregnancy. Their concentrations are elevated as a result of excess production of TAG-rich very low-density lipoprotein (VLDL) cholesterol (McGladdery and Friohlich, 2001). Also, total and low-density lipoprotein (LDL) cholesterol can be elevated in pregnancy (Choi and Pai, 2000) and decreased concentrations of high-density lipoprotein (HDL) cholesterol (McGladdery and Friohlich, 2001). TAG concentration increases gradually with a maturation of fetus during pregnancy but is significantly elevated during the second trimester (Choi and Pai, 2000). TAG and cholesterol concentrations return back to a normal range before pregnancy 20 to 24 weeks postpartum (Choi and Pai, 2000), with an express decrease in the TAG and total cholesterol in lactating mothers in comparison with non-lactating mothers (Qureshi *et al.*, 1999).

High TAG concentrations can result in acute pancreatitis during pregnancy (Schaefer *et al.*, 1988) and are linked with preeclampsia (Hubel *et al.*, 1996). Furthermore, it could lead to implications of maternal health, maternal TAG concentration correspond to the birth weight of offspring which is independent and free from maternal pre-pregnant Body Mass Index (BMI), maternal weight gain, gender, fasting glucose and gestation age (Sherman *et al.*, 2001).

2.16.3 High- Density Lipoprotein Cholesterol (HDL-C)

This is otherwise referred to as α -lipoprotein which is made up of 13% TAG, 46% phospholipids (PL), 6% cholesterol, 29% cholesterol esters (CE) and 6% fatty acids (FAs) (Nelson and Cox, 2000). HDL-C is the least lipoprotein but highest in density of 1.063-1.20mmol/L. It helps in the removal of cellular cholesterol away from the body cells and tissues for detoxification in the liver and thus referred to as ‘good cholesterol’ (Murray *et al.*, 2000). Elevated concentration of HDL-C correlates inversely with coronary heart disease (Philip, 1995).

2.16.4 Low-Density Lipoprotein Cholesterol (LDL-C)

This is referred to as β - lipoprotein and has a density of 1.006-1.063g/ml. They are prime carriers of cholesterol to other tissues and are therefore referred to as ‘bad cholesterol’ since surplus LDL-C in the blood and tissues such as the myocardial can cause atherosclerosis and ultimately stroke (Stryer, 1995).

Three lipoproteins are liable for transporting cholesterol this include high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein (Enas, 1999). During pregnancy, the levels of these lipoproteins would be altered. It was reported that the concentrations of LDL particularly VLDL1 increased with a consequent decrease in HDL levels at late pregnancy. Both low HDL-C and raised VLDL1 concentration are associated with insulin insensitivity (Naveed *et al.*, 1997). These alterations are also correlated to the risk of coronary artery diseases and its related cases during pregnancy (Naveed *et al.*, 1997).

2.16.5 Atherogenic Index

This is the ratio of LDL-C to HDL-C. It has been used as an indicator of cardiovascular disease (Panagiotakos *et al.*, 2003).

2.17 Organ-Body Weight Ratio

This is the ratio of organ weight to entire body weight of the animals. Organs weight varies considerably and relied on their location and function in the body system. The weight of an organ depends on the number of functions performed by that organ (George *et al.*, 1994).

The organ-body weight ratio can be expressed as

$$\text{Organ-body weight ratio} = \frac{\text{Weight of the organ}}{\text{Weight of animal}} \times 100$$

Alteration in the organ body weight ratio may be an indication of cell limitation or inflammation since the cells are the unit components of the organs. The constriction in the organ may occur as a result of the loss of fluid from the organ due to damage, while inflammation may suggest an increase in organ body weight ratio (Moore and Dalley, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material and Authentication

The *Citrullus colocynthis* pod was obtained from Nigerian Sored Product Research Institute, Ilorin. The authentication of the *Citrullus colocynthis* pod was done at the Plant Biology Department of the University of Ilorin, Ilorin, Nigeria by a taxonomist. The voucher specimen was deposited with voucher number (UIH 001/1160) in the Herbarium of the Plant Biology Department.

3.1.2 Assay Kits and Drug

Alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, albumin, glucose, uric acid, creatinine, urea, high density lipoprotein cholesterol, total cholesterol and triacylglycerol were product of Randox diagnostic kit, Randox Laboratories Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom. Para-nitrophenyl phosphate was a product of Sigma-Aldrich Chemical, United Kingdom. Progesterone, oestrogen, prolactin, follicle stimulating hormone and luteinizing hormone were products of Monobind Inc. USA. Mifepristone is a product of West Coast Pharmaceutical Ltd. Gujarat India. All other reagents were of analytical grade and prepared in accordance with a standard.

3.1.3 Animals

Sixty (60) female albino rats weighing $165.27 \pm 7.13\text{g}$ and matured male albino rats weighing $179.46 \pm 12.18\text{g}$ were obtained from the Institute for Advanced and Medical Research and Training (IAMRAT), University of Ibadan, Ibadan, Oyo State. The

animals were acclimatized under suitable laboratory conditions and were maintained on rat pellets and water *ad libitum*. The study was then carried out subsequent to approval given by the Ethical Committee on the use and care of Experimental Animals with reference number UIL/UERC/LSC/2015/023.

3.2 Methods

3.2.1 Preparation of Plant Extract

The *Citrullus colocynthis* seeds were separated from pod and the pod was oven dried at 40⁰ and then pulverized into a fine powder using Euro premium blender and stored in a dark air-tight container until when needed. 200 g of the powder was extracted with 1L of 80% ethanol and was allowed to percolate in ethanol solvent for a period of 72hrs shaking at intervals. It was filtered using Whatman filter paper No. 1, and the pure filtrate obtained was concentrated under pressure using a rotary evaporator at 40°C after which it was finally concentrated in a water bath at the same temperature to obtain a brownish slurry residue which weighed 19.30g giving percentage yield of 9.65%. It was then reconstituted to a required dose of 100, 200, 400 and 800mg/kg body weight with olive oil.

3.2.2 Determinations of Secondary Metabolites

The preliminary phytochemical assessment of the ethanolic extract of *Citrullus colocynthis* pod (EECCP) was determined with the method described by Harborne (1973), Odebiyi and Sofowora (1978), Trease and Evans (1989), Sofowora (1993), Awe and Sodipo, (2001) and other authors as follows:

Alkaloids: 1ml (1 % v/v HCl) was mixed with 3ml extract in a test tube and heated for 20 minutes. The mixture was then cooled and filtered, 1ml of the filtrate was taken and 2drops of Mayer's reagent was added. A creamy precipitate was observed which confirmed the presence of alkaloids in the extract (Harborne, 1973).

Tannins: 1ml of fresh 10 % w/v ethanolic KOH was measured in a test tube and 1ml of the extract was mixed with it. A dirty white precipitate was observed and it revealed that tannins was present (Odebiyi and Sofowora, 1978).

Anthraquinones: 3ml of the extract was mixed with 10ml of benzene shaken and the mixture was thereafter filtered, the filtrate was collected in a test tube and 5ml of 10 % v/v NH_3 solution was mixed with the filtrate and a reddish coloration confirmed that anthraquinones was presence (Trease and Evans, 1989).

Saponins: 2ml of the extract was measured in a test tube and vigorously shaken for exactly 2 minutes then warmed. The persistent frothing or foaming of the extract indicated the presence of saponins (Wall *et al.*, 1954).

Cardenolides: 1ml of the extract was mixed with 2ml of glacial acetic acid in a test tube and a drop of 5 % w/v FeCl_3 solution. Thereafter, 2 ml concentrated H_2SO_4 was added. Brown ring observed at the interface confirmed that cardenolides are present (Trease and Evans, 1989).

Phenols: 1ml of the extract in the test tube was mixed with two drops of 5% w/v FeCl_3 . A greenish precipitate confirmed that phenolic was present (Awe and Sodipo, 2001).

Cardiac Glycosides: 1ml of the extract was measured and mixed with 2ml of Chloroform and concentrated H_2SO_4 carefully. The reddish-brown colour was observed at the interface which indicated the presence of cardiac glycoside (Sofowora, 1993).

Flavonoids: 3ml of the extract was mixed with 1ml of 10 % w/v NaOH until an intense yellow colour was observed in the mixture which confirmed that flavonoid was present (Awe and Sodipo, 2001).

Steroids: 1ml of the extract was measured in a test tube and 5drops of concentrated H₂SO₄ were added gently by the side. Red colour seen indicates the presence of steroid (Trease and Evans, 1989).

3.2.3 Gas Chromatography – Flame Ionization Detection (GC-FID)

The GC-FID analysis of the plant extract was carried out on a Hewlett Packard Series II using a programmed temperature, 220 °C for the injector, 250 °C for the oven and 280°C for the detector while the holding time was 2mins. Hydrogen gas serves as the combusting gas and Nitrogen as the carrier gas.

3.2.4 Animal Grouping and Extract Administration

The experimental protocol and procedures used were approved by the Animal Ethics Committee of University of Ilorin, Ilorin, Kwara State, Nigeria; and it conforms to the Guidelines of Care and Use of Animals in Research and Teaching (published by the Animal Ethics Committee of University of Ilorin, Ilorin, Kwara State, Nigeria with reference number UIL/UERC/LSC/2015/023). Sixty (60) female Wistar rats weighing $165.27 \pm 7.13\text{g}$ were randomly divided into six groups (A – F) of 10 rats each was used for the experiment. All animals were housed in clean cages kept in an airy room condition and maintained on standard pellet and water *ad libitum*.

3.2.5 Determination of Abortifacient Activity

The method described by Ochiogu *et al.* (2006) was adopted. The female and male albino rats were paired overnight in cages that provide free access to food and water in ratio 2:1. The vaginal smear was collected by carefully inserting a cotton-tipped swab moistened with normal saline and rolled on a labeled clean glass slide and

observed for the presence of protein coagulates (vaginal plug and spermatozoa) under the microscope (Plate 4.1). This day marked the onset of pregnancy and recorded as day zero. The pregnant rats were selected and randomized into six groups (A – F) with five rats each. The animals were administered as follows:

Group A: 0.5ml of olive oil (Control)

Group B: 0.5ml of Mifepristone (1.84mg/kg body weight)

Group C: 0.5ml of 100mg/kg body weight EECCP

Group D: 0.5ml of 200mg/kg body weight EECCP

Group E: 0.5ml of 400mg/kg body weight EECCP

Group F: 0.5ml of 800mg/kg body weight EECCP

Administration was done from the 6th day of pregnancy till the 14th day. On day 15th the rats were sacrificed under light ether anaesthesia. Blood was collected into a clean bottle, the ovaries, uterus, liver and kidney were removed and the following parameters were observed.

- i. Number of live foetus
- ii. Number of dead foetus
- iii. % survival ratio
- iv. Number of rats that aborted
- v. Number of rats with vaginal bleeding
- vi. Percentage (%) of rats that aborted
- vii. Number of implantation
- viii. Number of *Corpora lutea*
- ix. Number of resorption site

- x. Implantation index (%)
- xi. Resorption index (%)
- xii. Pre-implantation loss (%)
- xiii. Post-implantation loss (%)
- xiv. Female reproductive hormonal profile
- xv. Marker enzymes of tissue damage

3.2.6 Biochemical Assessment

The pregnant rats were selected and randomized into six groups (A – F) with five rats each. The animals were administered as follows:

Group A: 0.5ml of olive oil (Control)

Group B: 0.5ml of Mifepristone (1.84mg/kg body weight)

Group C: 0.5ml of 100mg/kg body weight EECCP

Group D: 0.5ml of 200mg/kg body weight EECCP

Group E: 0.5ml of 400mg/kg body weight EECCP

Group F: 0.5ml of 800mg/kg body weight EECCP

Administration was done for a period of 21 days after which rats were laparatomized under light ether anaesthesia on the 22nd day and the blood collected in an EDTA, fluoride and plain bottle, liver and kidney were removed and the following parameters were determined:

- i. Heamatological parameters
- ii. Marker enzymes of tissue damage
- iii. Liver and kidney function indices

- iv. Lipid profile
- v. Hormonal profile
- vi. Lipid peroxidation assay
- vii. Antioxidant enzyme assay
- viii. DNA damage assay
- ix. Histological study

3.2.7 Preparation of Serum and Tissue Homogenate

At the end of the experiment on the fifteen and twenty-second days respectively, the rats were anesthetized in a glass jar containing cotton wool soaked with diethyl ether. The unconscious rats were quickly removed and sacrificed by cervical dislocation. The blood was obtained from the rats by heart puncture technique, into an EDTA bottle and sterilized plain bottles. The blood in the plain bottles was then centrifuged to collect serum. Serum was prepared by centrifugation for 10mins at 3000 rpm in a bench centrifuge.

The clear serum was collected with Pasteur pipette and kept frozen until required (Ngaha, 1982) for the enzyme assays. The animals were dissected and ovary, uterus, liver, and kidney were removed cleaned of blood, weighed separately and dropped in ice-cold 0.25M sucrose solution to maintain the tissue integrity. The tissues were then cut into pieces and then homogenized using Teflon homogenizer in an ice-cold 0.25M sucrose solution (1.5w/v). Homogenate was further centrifuged at $1398 \times g$ for 15minutes to decant the supernatant, which was thereafter transferred into the specimen bottles and kept frozen overnight for the biochemical analysis (Yakubu, 2006).

3.2.8 Haematological Analysis

The blood samples collected in EDTA containers were analyzed for Haemoglobin concentration (Hb), packed cell volume (PCV), red blood count (RBC), white blood count (WBC), platelets count, using automated haematology analyser (Sysmex K2X1: SYSMEX corporation, Japan). The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were then calculated using the formular of Wintrobe (1967) and Diem and Clenter (1970).

3.2.9 Determination of Reproductive Hormones

Serum concentrations of prolactin, progesterone, oestrogen, luteinizing and follicle stimulating hormones were determined

3.2.9.1 Prolactin Hormone (PRL)

The serum prolactin hormones was quantitatively determined using enzyme linked immunoenzymometric assay kit which adopted the principle of Tietz (1995).

➤ Principle

This was based on the interaction that occurs between streptavidin and biotinylated monoclonal anti-PRL antibody on the coated well. Upon addition of the later to the labeled enzyme antibody and serum with the native antigen, a soluble complex formation occurs within antibodies and the native antigen which was deposited to the well with no competition or steric hindrance.

The use of numerous serum of known antigen values generate a dose response curve where the antigen concentration of the unknown serum sample was obtained (Figure 53).

➤ **Procedure**

25µl of the serum and control were pipetted into the streptavidin coated well, 100µl of prolactin biotin reagent (composed of biotinylated monoclonal mouse IgG in buffer, dye, and preservative) were mixed in the microplate wells and swirl gently for 30seconds to mix and then covered. The mixtures in the wells were incubated at room temperature for 30 minutes. The contents of the microplate well were decanted and rinsed thrice with 300µl of wash buffer (surfactant in buffered saline). 100µl of the prolactin enzyme reagent solution (an enzyme (HRP) labeled antibody, in buffer and dye) was further mixed to the content in all wells and incubate at room temperature for 30 minutes. The contents of the microplate well were further decanted and rinsed thrice with 350µl of wash buffer (surfactant in buffered saline). 100µl of working substrate solution of tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂ in a buffer) were also added to all wells and incubate for 15minutes at room temperature. Finally, 50 µl of stop solution (1N Hydrochloric acid (HCl)) was added to the mixture and gently mixed for 20 seconds. The absorbance was then read at 450nm in the microplate reader.

➤ **Calculation**

The serum prolactin concentration of the unknown sample was extrapolated from the dose-response curve (Figure 53).

3.2.9.2 Progesterone Hormone

The concentration of serum Progesterone was quantitatively determined by microplate Enzyme Immunoassay kit which adopted the principle of Tietz, (1999).

➤ **Principle**

This is based on the competitive reaction between the enzyme-antigen conjugate and a serum containing the native antigen for the antibody binding site in biotinylated

antibody when added. Simultaneously, a reaction takes place amid the antibody biotin and the immobilized streptavidin present on the microwell. Separation of the antibody bound fraction after decantation or aspiration then occurred where enzyme activity in the antibody bound fraction is inversely proportional to the native antigen level.

The concentration of antigen in an unknown serum sample can be obtained from the generated dose response curve (Figure 54).

➤ **Procedure**

25µl of reference serum, control or sample was pipetted into the streptavidin coated well and 50µl of 17α-OH progesterone enzyme reagent (containing 17α-OH progesterone- HRP, dye, and steroid conjugate buffer) was added to the wells and microplate was swirl gently for 20seconds to mix, immediately, 50µl of 17α-OH progesterone biotin reagent (containing anti- 17α-OH progesterone biotinylated IgG conjugate buffer and dye) was added to all wells and swirl gently for another 20 seconds to mix properly and was allowed to incubate at room temperature for 60 minutes. The microplate well content was decanted and rinsed thrice with 350µl of wash buffer. 100µl of substrate solution (tetramethylbenzidine and H₂O₂ in a buffer) was added to all wells and incubated for 20 minutes at room temperature. Finally, 50µl of 0.5M H₂SO₄ was added as stop solution to content in all wells and mixed gently for 20 seconds. The absorbance was read at 450nm in the microplate reader.

➤ **Calculation**

The serum progesterone concentration of the unknown sample was extrapolated from the dose-response curve (Figure 54) by correlating the absorbance of the sample at 450nm with the corresponding absorbance on the dose-response curve.

3.2.9.3 Oestrogen Hormone

Serum oestrogen was quantitatively determined by microplate Enzyme Immunoassay kit which adopted the principle of Tietz (1995).

➤ Principle

This is based on the interaction between biotinylated antibody and serum containing antigen. Immediately after short incubation, a competitive reaction occurs when the enzyme conjugate is added between the antigen of the serum and enzyme conjugate for the available antibody sites. Subsequently, a reaction occurs between the biotin-antibody and streptavidin immobilized on the microwell.

➤ Procedure

25µl of serum reference, control or samples was pipetted into streptavidin coated well and 50µl of estradiol-biotin reagent (containing anti-estradiol biotinylated purified rabbit IgG conjugate in buffer and dye), mixed to microplate wells and swirl gently for 25seconds to mix appropriately and then covered to incubate at room temperature for 30minutes. 50µl of estradiol enzyme reagent (Estradiol-HRP conjugate in protein stabilizing matrix and dye) was then added to the mixture in the wells and microplate swirl gently for 25 minutes to mix again and covered. It was set to incubate at room temperature for another 90minutes. The microplate well contents were decanted and rinsed thrice with 350µl of wash buffer (surfactant in buffered saline). 100µl of the substrate solution (TMB and H₂O₂) was then added to all wells and then incubated for 20 minutes. Finally, 50 µl of stop solution (H₂SO₄) was added and gently mixed for 20 seconds. The absorbance was then read at 450 nm in the microplate reader.

➤ **Calculation**

The serum oestrogen concentration of the unknown sample was extrapolated from the dose-response curve by correlating the absorbance of the sample at 450nm with the corresponding absorbance on the dose-response curve (figure 55).

3.2.9.4 Luteinizing Hormone (LH)

The luteinizing hormone concentration in the serum was quantitatively determined using enzyme immunoassay kit which adopted the principle of Tietz (1995).

➤ **Principle**

This works based on the immobilization occurring at microplate well surface through a non-competitive reaction that occurs between streptavidin and biotinylated monoclonal anti-LH antibody, the enzyme-labeled antibody and a serum antigen with no steric hindrance, in order to form a sandwich soluble complex.

Concurrently, the soluble complex formed is deposited on the well and the antibody-bound fraction is then detached from unbound antigen with decantation or aspiration. The use of numerous serum of known antigen values generate a dose response curve where the antigen concentration of an unknown serum sample can be obtained (Figure 56).

➤ **Procedure**

50µl of reference serum, control or sample were pipetted into the assigned coated well (streptavidin coated plate) and 100µl of LH-Enzyme reagent (contains a purified antibody, biotinylated monoclonal mouse IgG in buffer and dye) was mixed to the microplate well then swirl gently for 25 seconds, covered and incubated at room temperature for 60 minutes. The contents of the microplate well were decanted and rinsed thrice with 350µl of wash buffer (surfactant in buffered saline). 100µl of the working substrate solution (TMB and H₂O₂) was then added to all wells and then

incubated for 15 minutes. Finally, 50µl of stop solution (1N HCl) was added and gently mixed for 20 seconds. The absorbance was read at 450nm in the microplate reader.

➤ **Calculation**

The serum LH concentration of the unknown sample was extrapolated from the dose-response curve by correlating the absorbance of the sample at 450nm with the corresponding absorbance on the dose-response curve.

3.2.9.5 Follicle Stimulating Hormone (FSH)

The serum follicle stimulating hormone was quantitatively determined using enzyme linked immunoenzymometric assay kit which adopted the principle of Tietz, (1995).

➤ **Principle**

This was based on a non-competitive reaction between native antigen, streptavidin coated on microplate well as well as exogenously biotinylated monoclonal anti-FSH antibody to form a soluble sandwich complex at the surface of the microplate well which is the result of the interaction between the native antigen and the antibodies.

After the complex stability is attained, the unbound antigen is then separated from antibody-bound fraction through decantation or aspiration. The use of numerous serum of known antigen values generate a dose response curve where the antigen concentration of an unknown serum sample can be obtained (Figure 57).

➤ **Procedure**

50µl of control or specimen was pipetted into streptavidin coated microplate well and 100µl of FSH-Enzyme reagent solution (containing antibody-enzyme, biotinylated monoclonal mouse IgG in buffer and dye) was mixed in all microplate wells and swirl gently for 30 seconds to mix, covered and incubated at room temperature for 60 minutes. Contents of the microplate well were decanted and rinsed thrice with 350µl

of wash buffer. 100µl of the substrate (TMB and H₂O₂) was mixed with the content in all wells and incubated for 15 minutes. Finally, 50µl of stop solution (1N HCl) was added to the mixture and mixed for 20 seconds. The absorbance was read at 450nm in the microplate reader.

➤ **Calculation**

The serum FSH concentration of the unknown sample was extrapolated from the dose-response curve (calibration curve) for follicle stimulating hormone (figure 56).

3.2.10 Marker Enzymes Activities

3.2.10.1 Determination of Alkaline Phosphatase Activity

The procedure described by Wright *et al* (1972b) was used to determine alkaline phosphatase activity.

➤ **Principle**

The amount of phosphate ester split within a given period of time is a measure of the phosphatase activity. Para-nitrophenyl phosphate (PNPP) was hydrolyzed to para-nitrophenol and phosphoric acid at a pH of 10.1. The para-nitrophenol confers a yellowish colour on reaction mixture and its intensity is read spectrophotometrically at 400 nm.

➤ **Procedure**

The method as described by Wright *et al* (1972b) was used to assay for alkaline phosphatase activity. The assay mixture consisted of 0.2ml of sample, 2.2ml Carbonate buffer (0.1M) and 0.1ml MgSO₄·7H₂O (0.1M) was pipetted into a well labeled test tubes assigned. The mixture was then incubated at 37°C for 10minutes. Then 0.5ml P-nitrophenyl phosphate (10mM) was added to the mixture and then incubated for another 30 minutes at 37°C. The reaction was then terminated by adding

2ml NaOH (1N) solution to the mixture. The absorbance was read at 400 nm. The Activity of the Enzyme was calculated using the expression below:

$$\text{Enzyme activity (nM/min/ml)} = \frac{\Delta AB/\text{min} \times 1000 \times TV \times F}{9.9 \times SV \times L}$$

Where:

$\Delta AB/\text{min}$ = Change in absorbance of reaction mixture per minute

TV = Total volume of mixture.

F = Total dilution factor

SV = Enzyme source volume

L = Light path length (1cm)

9.9 = Extinction co-efficient of 1 μm of p-nitrophenol in an alkaline solution of 1 ml and 1 cm path length

1000 = factor introduced for enzyme activity to be expressed in nM/min/mg protein.

The specific activity for alkaline phosphatase was calculated thus:

$$\text{Specific enzyme activity (nM/min/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.2.10.2 Determination of Aspartate Aminotransferase Activity

The procedure described by Reitman and Frankel (1957) was used to assay for the activity of aspartate aminotransferase.

➤ Principle

The enzyme catalyzes the reversible reaction involving alpha (α)-ketoglutarate and L-aspartate to form L-glutamate and oxaloacetate. Aspartate amino transferase was read by monitoring the concentration of oxaloacetate hydrazone formed by 2, 4-dinitrophenyl hydrazine.

➤ Procedure

Activity of aspartate amino transferase was determined by the method of Reitman and Frankel (1957). 0.5ml Substrate (2mM α -oxoglutarate, 100mM of L-aspartate and 100mM, pH 7.4 of phosphate buffer) and 0.1ml of sample or distilled water was pipetted into the assigned test tubes and incubated for 30min at 37°C in a water bath. 0.5ml of 2, 4-dinitrophenylhydrazine (2mM) was mixed to the reaction mixtures; it was then kept for 20min at 25°C. Finally, the reaction was terminated by addition of 5.0ml of NaOH (0.4 N) and absorbance of the sample was read after 5 minutes at 546nm against the blank.

The enzyme activity was obtained from the calibration curve (Figure 52).

$$\text{Specific enzyme activity (nM/min/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.2.10.3 Determination of Alanine Aminotransferase Activity

The method described by Reitman and Frankel (1957) was used to assay for the activity of alanine aminotransferase.

➤ Principle

The level of alanine aminotransferase activity was measured via monitoring the concentration of pyruvate hydrazone formed by 2, 4-dinitrophenyl hydrazine.

➤ Procedure

This was performed using the protocol described by Reitman and Frankel (1957). 0.5ml Substrate (2mmol/L α -oxoglutarate, 200mmol/L of L-alanine and 100mmol/L, pH 7.4 of phosphate buffer) and 0.1ml of the sample or distilled water were pipetted into well-labeled test tubes and incubated for 30 min at 37°C in a water bath. 0.5ml of 2, 4-dinitrophenylhydrazine (2mM) was added to the reaction mixture and mixed. It was then left undisturbed for 20 min at 25°C. Finally, the reaction was terminated by

addition of 5.0ml of NaOH (0.4 N) and the absorbance of the sample was read after 5 minutes at 546nm against the blank.

The enzyme activity was obtained from the calibration curve (Figure 53).

$$\text{Specific enzyme activity (nM/min/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.2.11 Determination of Liver Function Indices

3.2.11.1 Determination of Serum Protein Concentration

The protein concentration in the serum of experimental animals was assayed, colorimetrically using Biuret method as described by Gornall *et al* (1949).

➤ Principle

The principle is based on the formation of purple coloured complex of cupric ion with peptide bond; the intensity is based on the number of peptide bonds present in the solution.

➤ Procedure

1.0ml of sample and 4.0ml of Biuret Reagent ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{NaK}_2\text{C}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) were pipetted into the assigned test tube and mixed carefully by shaking and left undisturbed at room temperature for 30 minutes for colour development. The blank was constituted by using 1.0 ml of distilled water instead of the sample. The absorbance was read against blank at 540 nm. The protein concentration present in the sample was calculated from the calibration curve for bovine serum albumin (Figure 50). The curve was arrived at by plotting the absorbance against different concentration of bovine serum albumin used. Protein concentration of the sample was ascertained from the calibration curve of bovine serum albumin, using this expression:

$$\text{Protein concentration (mg/ml)} = C_s \times F$$

Where: C_s = Protein concentration obtained from the calibration curve

F = dilution factor

3.2.11.2 Determination of Serum Albumin

The albumin content was determined based on the colorimetric method described by Doumas *et al* (1971).

➤ Principle

This works based on the binding of albumin to 3,3',5,5'-tetrabromo- cresol sulphonephthalein (bromocresol green, BCG) which leads to a change in colour. The albumin-BCG-complex formed is then measured depending on the intensity of the colour.

➤ Procedure

0.01ml of the serum sample or standard or distilled water was pipetted into the assigned test tube and 3.0ml of bromocresol green (75mmol/L Succinate buffer; pH 4.2, 0.15mmol/L bromocresol green) were added to the test tube. The mixture was incubated for 5 minutes at 25°C. The absorbance of the sample (Abs_{sample}) and standard (Abs_{standard}) against the reagent blank was then taken at 630nm.

➤ Calculation:

$$\text{Albumin Concentration (g/dl)} = \frac{Abs_{\text{sample}} \times \text{Concentration of standard}}{Abs_{\text{standard}}}$$

3.2.11.3 Determination of Serum Bilirubin Concentration

The bilirubin present was determined based on colorimetric method described by Sherlock (1951).

➤ Principle

The method is based on the interaction which occurs between bilirubin and diazotized sulphanilic acid in an alkaline medium to form a blue complex. The complex in the presence of caffeine, releases albumin-bound bilirubin through which the total bilirubin can be determined.

➤ Procedure for Total Bilirubin

200µl of the sample or distilled water and 200µl Reagent 1 (29mmol/L Sulphanilic acid and Hydrochloric acid) was pipetted into the assigned test tube, a drop of 50µl Sodium Nitrite and 1000µl of reagent 3 (0.26mol/L Caffeine and 0.52mol/L Sodium benzoate) was added to the reaction mixture. At 25°C the mixture was incubated for 10min. Subsequently, 1000µl of Reagent 4 (0.93mol/L Tartrate and 1.9N of NaOH) was pipetted and added to the mixture and incubated for 15min at 25 °C. The absorbance of the sample against blank was read at 578nm.

➤ Calculation

Total bilirubin (mg/dl) = $10.8 \times \text{Abs}_{\text{TB}}(578\text{nm})$

➤ Procedure for Direct Bilirubin

200µl of sample or blank was pipetted into a well-labeled test tube and 200µl Reagent 1 (29mmol/L Sulphanilic acid and Hydrochloric acid), a drop of 50µl Sodium Nitrite, 2000µl of 0.9% NaCl solution was added to the test tube, mixed together and then incubated at 25°C for 10min. The absorbance of the sample against blank was read at 546nm wavelength.

➤ **Calculation**

$$\text{Direct bilirubin (mg/dl)} = 14.4 \times \text{Abs}_{\text{SDB}} (546\text{nm})$$

3.2.12 Determination of Some Kidney Function Indices

3.2.12.1 Determination of Urea Concentration

The urea concentration was estimated according to the colorimetric method of Veniami and Varkirtzi (1970).

➤ **Principle**

- Urea when heated in a strongly acidic condition with a reagent such as diacetyl monoxime, forms a yellow compound via condensation. The reaction occurs in the presence of polyvalent ions like ferric ions and thiosemicarbazide. The red colour intensity observed is proportional to the sample urea concentration.

➤ **Procedure**

10µl of sample or standard and 100µl of reagent 1 solution (116mmol/L of EDTA, 6mmol/L, and Urease 1g/L) was pipetted into a well-labeled test tube. The mixture was then incubated for 10 min at 37°C. 2500µl of 120mmol/L of diluted Phenol and Reagent 3 (27mmol/L of diluted Sodium hypochlorite and 0.14N and NaOH) was pipetted into the mixture, mix thoroughly and incubated at 37°C for 15minutes. The absorbance of the sample and standard against blank was read at the wavelength of 578nm.

➤ **Calculation**

$$\text{Urea Concentration (mmol/l)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

$$\text{Abs}_{\text{standard}}$$

Where Abs = absorbance of sample or standard

3.2.12.2 Determination of Creatinine Concentration

Creatinine concentration was estimated according to colorimetric method described by Bartels and Bormer (1972).

➤ Principle

This method is based on the reaction between creatinine and picric acids which form a coloured complex in an alkaline solution. The amount of the complex formed and creatinine concentration measured at 490nm are directly proportional to one another.

➤ Procedure

0.1ml of sample or standard or distilled water was pipetted into a well labeled test tube and 1.0ml working reagent (35mmol/L Picric acid and 0.32mol/L NaOH) was added. The samples were mixed and the absorbance Abs_1 of the standard and sample were read after 30 seconds. Specifically, 2 minutes after the absorbance Abs_2 of the standard and sample were read again at 490nm.

➤ Calculation

$$Abs_2 - Abs_1 = \Delta A_{\text{sample}} \text{ or } \Delta A_{\text{standard}}$$

$$\text{Concentration of creatinine (mg/dl)} = (\Delta A_{\text{sample}} / \Delta A_{\text{standard}}) \times 2$$

3.2.12.3 Determination of Serum Uric Acid

The concentration of serum uric acid in the serum was determined in accordance to the method described by Krieg *et al* (1986).

➤ Principle

The principle for the determination of uric acid was based on the conversion of uric acid to allantoin and hydrogen peroxide via the action of peroxidase enzyme

which oxidizes 3,5-dichloro-2-hydrobenzenesulfonic acid and 4-aminophenazone to form a red violet quinoneimine. The intensity is proportional to uric acid concentration

➤ **Procedure**

0.02ml of sample, standard or distilled water and 1.0ml of working reagent (0.25mmol/L 4-Aminophenazone, peroxidase, and uricase) was pipetted into an assigned test tube. The mixture was incubated at 25°C for 15minutes and absorbance of sample and standard was then read against blank at 520nm.

➤ **Calculation**

$$\text{Uric acid Concentration (mmol/l)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.12.4 Determination Serum Sodium ion Concentration

The flame photometric method by Tietz (1995) was adopted for the determination of sodium ion level in the plasma/serum of the animal.

➤ **Principle**

The sodium solution under carefully controlled conditions was sprayed (aspirated) into a burner, dissociate to give neutral atoms in the ground state. Some of these are excited in the flame, move to the higher energy and emitted at 590nm. This light then passes through a suitable filter into a photo-sensitive element and the amount of current produced is proportional to the amount of sodium present in the solution.

➤ **Procedure**

0.1 ml of the test sample or sodium standard or distilled water was pipetted into a well labeled test tube and 9.9ml of deionized distilled water was then added and mixed thoroughly. The absorbance was read at 590nm against the reagent blank.

➤ **Calculations**

$$\text{Sodium ion concentration (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

3.2.12.5 Determination of Serum Potassium ion

The flame photometric method by Tietz (1995) was adopted for the determination of potassium ion level in the plasma/serum of the animal.

➤ **Principle**

The potassium solution under carefully controlled conditions was sprayed (aspirated) onto a burner dissociates to give neutral atoms in the ground state. Some of these atoms are excited in the flame, moved to the higher energy state and emitted at 770nm. This light then passes through a suitable filter into a photo-sensitive element and the amount of current produced is proportional to the amount of potassium present in the solution.

➤ **Procedure**

0.1ml of the test sample or potassium standard or distilled water was pipetted into a well labeled test tube and 9.9ml of deionised distilled water was then added and mixed thoroughly. The absorbance was read at 770nm against the reagent blank.

➤ **Calculations**

$$\text{Potassium ion concentration (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.12.6 Determination of Serum Calcium ion Concentration

Serum calcium ion concentration was determined using the colorimetric method described by Biggs and Moorehead (1974).

➤ **Principle**

This principle works on the formation of a violet colour from the reaction of calcium ion with o-cresolphthalein complex in an alkaline medium. The colour intensity is proportional to the calcium concentration.

➤ **Procedure**

25 µl of sample or calcium standard or distilled water was pipetted into the assigned test tube and 1000µl of working reagent (0.16mmol/L O-Cresolphthalein complex, 6.89mmol/L β-Hydroxyquinoline, 60mmol/L Hydrochloric acid and 3.5mol/L 2-amino-2-methyl-propane-1-ol at pH 10.7) was added to it and mix well. The mixture was incubated for 5 minutes at 25⁰C. The absorbance of standard and sample is then read at 578nm against the reagent blank.

➤ **Calculations**

$$\text{Concentration of calcium (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.12.7 Determination of Serum Phosphate ion Concentration

The amount of serum phosphate ion concentration was determined using the colourimetric method described by Tietz (1990).

➤ Principle

The principle was based on the phosphate ion reaction with ammonium molybdate in the presence of sulphuric acid which led to the formation phosphomolybdate complex.

➤ Procedure

10µl of the test sample or distilled water or standard was pipetted into an assigned test tube and 1000µl of the working reagent (0.36mol/L sulphuric acid, 154mmol/L sodium chloride, 3.5mmol/L ammonium molybdate) was added. The content was mixed thoroughly; incubated for 5 minutes at 37°C. The absorbance of the sample and standard were read at 340nm against the reagent blank.

➤ Calculations

$$\text{Concentration of phosphate ion (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.12.8 Determination of Serum Chloride ion Concentration

This was carried out using the methodology described by Tietz (1986) for the determination of the concentration of chloride ion in the serum of the animals.

➤ Principle

Chloride ion in the sample reacts with mercuric thiocyanate by displacing thiocyanate ion. Ferric ion then reacts with the free thiocyanate ions to form a coloured complex which absorbs light at 500nm. Increase in the absorbance is proportional to chloride ion concentration in the sample.

➤ **Procedure**

10µl of the test sample or distilled water or standard was pipetted into an assigned test tube and 1000µl of the working reagent (2.0mmol/L mercuric thiocyanate, 20.0mmol/L ferric nitrate and 29.0mmol/L nitric acid) was added. The content was mix thoroughly and the absorbance of standard or sample was read at 500nm against the reagent blank.

➤ **Calculations**

$$\text{Concentration of chloride ion (mmol/L)} = \frac{\Delta\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\Delta\text{Abs}_{\text{standard}}}$$

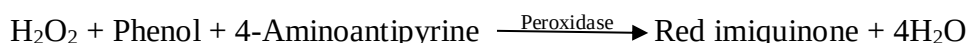
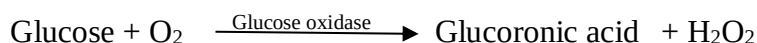
Where ΔAbs = change in absorbance of sample or standard

3.2.13 Determination of Serum Glucose Concentration

The procedure described by Trinder (1969) was used for serum glucose determinations.

➤ **Principle**

This principle works based on the conversion of glucose into gluconate in the presence of glucose oxidase enzyme. The hydrogen peroxide (H_2O_2) produced in the reaction is degraded by peroxidase (POD) to give water and oxygen. The hydrogen peroxide with phenol and 4-aminoantipyrine give a coloured product.. The increase in absorbance correlates to glucose concentration in the sample.



➤ **Procedure**

10µl of sample or standard or distilled water was pipetted into the assigned test tube and 1000µl of working reagent (120.0mmol/L Phosphate buffer (pH 7.4), 10.0mmol/L phenol, 0.3mmol/L 4-aminoantipyrine, glucose oxidase and peroxidase) were mix

with it. The mixture was incubated at 37°C for 10 minutes. The sample and standard absorbance against reagent blank was read at 505nm.

➤ **Calculation**

$$\text{Concentration of glucose (mg/dl)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.14 Antioxidant Enzymes Assay

3.2.14.1 Determination of Catalase Activity

Catalase activity was measured according to the protocol described by (Clairborne, 1995).

➤ **Principle**

In this method dichromate in acetate is reduced to chromic acetate and perchromic acid (an unstable intermediate) when heated with H₂O₂. The chromic acetate formed is to be colorimetrically measured within 570-610nm. Hence, dichromate do not absorb within this region, therefore, existence of the substance in the assay mixture does not obstruct the colorimetric determination of chromic acetate. Catalase preparation thus split H₂O₂ at different time interval and dichromate/acetic acid mixture when added stops the reaction at a particular time and then heat. After heating the reaction mixture, the remaining H₂O₂ is then determined by measuring the absorbance of chromic acetate formed spectrophotometrically.

➤ **Procedure for Spectrophotometrically Determination of H₂O₂**

10 to 100 μmoles of H₂O₂ solution was measured into test tubes. Thereafter, 2ml of dichromate/acetate was then mixed to each test tube. It then produced instantaneously an unstable blue compound known as perchromic acid. Further heating for 10 minutes in a boiling water bath caused a colour changed from an unstable blue to a stable green compound due to formation of chromic acetate. The reaction mixture was cooled at room temperature and make up to 3ml after which the absorbance was read spectrophotometrically at 570nm. The graph concentration of standard against absorbance was then plotted (Figure 49).

➤ **Determination of Catalase Activity of Samples**

The assay mixture consists of 4ml of 0.2M H₂O₂ solution and 5ml phosphate buffer, pH 7. Thereafter, 1ml of the diluted sample was added speedily to the reaction mixture at room temperature and swirl the flask gently. At the 60 seconds interval, 1ml of the reaction mixture was measured and added into a test tube containing 2ml dichromate/acetic acid reagent. Heat the mixture for 10 minutes in a boiling water bath to decompose and turn the blue precipitate to green solution. The absorbance was then read and the concentration of hydrogen peroxide contents of the sample was then estimated from the graph plotted above.

➤ **Calculation**

The concentration of catalase was extrapolated from the graph plotted for H₂O₂ determination (figure 49) to get the remaining H₂O₂

$$\text{H}_2\text{O}_2 \text{ consumed} = 800\mu\text{moles} - \text{H}_2\text{O}_2 \text{ remaining}$$

$$\text{Catalase activity (unit/mg protein)} = \frac{\Delta \text{Abs/min} \times 1000}{43.6 \times \text{mg protein/ml of reaction mixture}}$$

43.6 x mg protein/ml of reaction mixture

3.2.14.2 Determination of Superoxide Dismutase (SOD) Activity

The activity of superoxide dismutase (SOD) was determined by the protocol described by (Misra and Fridovich, 1972).

➤ Principle

The principle was based on the formation of superoxide anion generated via the xanthine oxidase reaction which caused oxidation of epinephrine (adrenaline) to adrenochrome leading to an increasing concentration of epinephrine anion in the reaction mixture. The inhibition of the autooxidation of epinephrine at pH 10.2 by superoxide dismutase is the basis for the simple assay of the enzyme.

➤ Procedure

0.2ml was taken from the diluted sample and mixed with 2.5ml of 0.05M carbonate buffer (pH 10.2). The reaction was initiated by adding 0.3ml of freshly prepared 0.3mM epinephrine to the reaction mixture. The increase in absorbance was read every 30 seconds for 150 seconds at wavelength 480nm.

➤ Calculation

$$\text{Increase in absorbance} = \frac{\text{Abs}_f - \text{Abs}_i}{2.5}$$

Where Abs_i = Absorbance after 30 seconds

Abs_f = Absorbance after 150 seconds

$$\% \text{ Inhibition} = \frac{100 \times \text{Increase in absorbance of substrate}}{\text{Increase in absorbance of blank}}$$

1 unit of SOD activity is given as the concentration of the enzyme (mg protein/ml) in the serum that can cause 50% inhibition of epinephrine oxidation.

3.2.15 Determination of Lipid Peroxidation

The procedure described by Rice- Evans *et al.* (1986) was used in determining the thiobarbituric acid reactive substances (TBARS) generated during lipid peroxidation.

➤ Principle

This is based on the reaction that occurs between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA) a product of lipid peroxidation. A pinkish colour complex is formed which is extractable by organic solvent like butanol when heated in an acidic pH and was estimated at 532nm.

➤ Procedure

0.4ml aliquot amount of serum was mixed with 1.6ml Tris-KCl buffer and 0.5ml of 30% TCA. Thereafter, 0.5ml of 0.75% TBA was mixed with the reaction mixture; it was positioned in a boiling water bath for 45 minutes. The reaction mixture was cooled in ice and then centrifuged at 3000g. The clear supernatant was withdrawn into the cuvette and absorbance of the sample was read against the blank at 532nm.

➤ Calculation

The method of Adam-Vizi and Seregi (1982) was used to calculate the MDA concentrations in units/mg protein.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{mixture volume}}{E_{532\text{nm}} \times \text{volume of sample} \times \text{mg protein}}$$

3.2.16 Determination of Lipid Profile

Serum concentrations of total cholesterol, triacylglycerol, high-density lipoprotein cholesterol were determined. The low density lipoprotein and atherogenic index were then calculated.

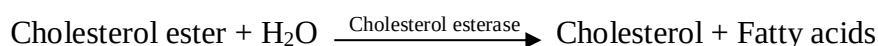
3.2.16.1 Total Cholesterol

The total cholesterol concentration was determined based on “CHOD-PAP” method in accordance with procedure described by Roeschlau *et al* (1974).

➤ Principle

The reaction is based on the hydrolysis and oxidation of cholesterol ester and cholesterol. In the presence of 4-aminoantipyrine, there is the formation of a red complex quinoneimine. The absorbance of the reaction mixture gives the concentration of cholesterol in the sample.

➤ The principle is summarized by the reactions:



➤ Procedure

10 µl of sample, standard or distilled water was pipetted into a well-labeled test tube. 1000 µl of the working reagent (80mmol/L Pipes Buffer; pH6, 0.24mmol/L 4-Aminoantipyrine, 6.0mmol/L phenol, peroxidase, cholesterol esterase and cholesterol oxidase) was then added. The contents were mixed thoroughly and left undisturbed at 25 °C for 10 minutes and sample and standard absorbance was read at 546nm against the blank.

➤ **Calculations**

$$\text{Concentration of cholesterol (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{Concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.16.2 High - Density Lipoprotein Cholesterol Concentration (HDL-C)

HDL-C was determined using the procedure described by Hainline *et al.* (1980).

➤ **Principle**

The principle works by precipitating LDL, VLDL and chylomicron fractions that occurred on addition of phosphotungstic acid in the presence of magnesium ions to the sample. The supernatant containing the HDL fraction after centrifugation the cholesterol, was then measured spectrophotometrically at 546nm.

➤ **Procedure**

100 µl of serum, standard or distilled water was pipetted into an assigned test tube and 1000 µl of the working reagent (0.55mmol/L phosphotungstic acid and 25.0mmol/L magnesium chloride) was added. The mixture was incubated for 10 minutes at 25°C and absorbance of standard or sample was read at 546nm against the blank within 60 minutes.

3.2.16.3 Low-Density Lipoprotein Cholesterol Concentration (LDL-C)

LDL-C was determined using the Friedewald formular (Friedewald *et al.*, 1972).

➤ **Calculation**

LDLcholesterol concentration in the sample was calculated as:

$$\text{LDL cholesterol (mmol/L)} = \text{Total cholesterol} - (\text{Triglycerols} + \text{HDL-cholesterol})$$

2.2

Where 2.2 – coverage factor

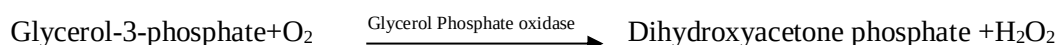
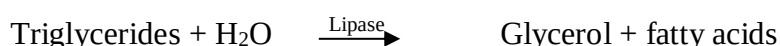
3.2.16.4 Triacylglycerols

Triacylglycerols was determined using the glycerol phosphate oxidase reaction method described by Fossati and Prencipe (1982).

➤ Principle

This is based on the hydrolysis of triacylglycerol which occurs through the action of lipase to form glycerol and fatty acids. The glycerol formed undergoes a phosphorylation reaction at C-3 by adenosine-5-triphosphate catalyzed by glycerol kinase to yield glycerol-3-phosphate and adenosine-5-diphosphate. Glycerophosphate oxidase (GPO) then catalyzed the conversion of Glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide which then reacts with 4-aminophenazone and 4-chlorophenol in a reaction catalyzed by peroxidase to yield a red colored quinoneimine dye.

The principle is illustrated by the following reactions:



➤ Procedure

10µl serum or standard was pipetted into an assigned test tube and 1000 µl of the working reagent (containing 0.5mmol/L 4-aminophenazone, 1.0mmol/L ATP, lipases, glycerol-kinase, glycerol-3-phosphate oxidase, peroxidase, 5.5mmol/L 4-chlorophenol, 17.5mmol/L magnesium ion and 40mmol/L pipes buffer; pH 7.6) was

then added. It was mixed thoroughly and incubated at 25°C for 10 minutes after which the absorbance of standard or sample was read at 546nm against the blank within 60minutes.

➤ **Calculations**

$$\text{Concentration of triacylglycerol (mmol/L)} = \frac{\text{Abs}_{\text{sample}} \times \text{concentration of standard}}{\text{Abs}_{\text{standard}}}$$

Where Abs = absorbance of sample or standard

3.2.16.5 Atherogenic Index

The atherogenic index was computed using the expression given by Panagiotakos *et Al.* (2003) as LDL-C/HDL-C.

3.2.17 Chromosomal Aberration Evaluation

The method described by Bakare (2009), was used for Chromosomal aberration analysis of the bone marrow. The femurs of rats were surgically removed and kept in normal saline. Thereafter, the epiphyses cut off and the bone marrow was flushed with 2.2 % Sodium citrate into a petri dish and then transferred to an Eppendorf tube. It was centrifuged at 2000rpm and the supernatant was removed. To the pellet 0.5ml of 0.075M KCl was added and left at 25 °C for 30 minutes before centrifuging at 2000 rpm for 5minutes. The supernatant was removed again and the 1ml of freshly prepared chilled Carnoy's fixative was gently mixed and allowed to stand for 10 minutes and centrifuged at 2000 rpm for 5minutes. The fixing and centrifuging were done thrice. 3 drops of the final suspension of pellet and fixative were administered onto grease free, pre-cleaned glass slides. The slides were air dried and stained with 5% Giemsa stain for 20 minutes.

The slides were made permanent by dipping them in xylene and then drops from Canada balsam were added and covered with slips, metaphase spreads were observed at a magnification of 1000x.

3.2.18 Organ-Body Weight Ratio

Organ-body weight ratio for liver, kidney, uterus and ovary were determined using the expression:

$$\frac{\text{Weight of the organ}}{\text{Weight of the animal}} \times 100$$

3.2.19 Statistical Analysis

Values were expressed as mean $\pm$ SEM. Statistical analysis was conducted using the SPSS software (version 20). In order to compare mean values, the collected data were subjected to one-way ANOVA analysis, followed by Duncan Multiple Range test for comparisons. The significance differences between the means were determined at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical report of Ethanolic Extract of *Citrullus colocynthis* Pods

4.1.1 Secondary Metabolites Screening of Ethanolic Extract of *Citrullus colocynthis* Pods

The phytochemical screening of ethanolic extract of *Citrullus colocynthis* pod revealed the presence of alkaloids, saponins, tannins, glycosides, phenols, and flavonoids in appreciable amount while phlobatannin, steroids, and terpenes were present in minute quantity; it was also observed that anthraquinones, cardenolides, and chalcones were totally absent (Table 4.1). In terms of quantity, it was observed that alkaloids had 0.52%, phenols 0.45%, saponins 0.14%, glycosides and flavonoids 0.12% while tannins and phlobatannins were 0.03 and 0.02% in abundance (Table 4.1).

It was also observed that the extract contained some other organic chemical constituent like spinasterol, isovitexin, corilagin, catechin, cucurbitacin E, B and S, quercetin, gallocatechin, myricetin, gallic acids, Cucurbitacin S, Gastrodin, Colocynthoside B, 3.0 Cafeoylquinic acid, Colocynthoside A (Figure 4.1) at different concentrations but gallic acid and catechin were the most abundant with 34.47% and 25.67% (Table 4.2).

Table 4.1: Secondary Metabolites Screening Result of Ethanolic Extract of***Citrullus colocynthis* Pod**

Parameters	Observation	Concentration (%)
Alkaloids	+++	0.52±0.03
Saponins	++	0.14±0.00
Tannins	++	0.03±0.00
Phlobatannins	+	0.02±0.01
Glycosides	++	0.12±0.00
Phenols	+++	0.45±0.01
Anthraquinones	-	-
Cardenolids	-	-
Steroids	+	0.00±0.00
Terpenes	+	0.00±0.00
Flavonoids	+	0.12±0.01
Chalcones	-	-

Values are mean ± SEM of three determinations

Table 4.2: Other Chemical Constituents of Ethanolic Extract of *Citrullus colocynthis* Pods

Component	Conc. (mg/g)	% abundance
Spinastrol	1.28	3.27
Isovitexin	0.67	1.71
Corilagin	0.36	0.92
Catechin	10.04	25.67
Cucurbitacin E	0.87	2.22
Quercetin	0.98	2.51
Gallo catechin	3.13	8.00
Myricetin	3.41	8.72
Cucurbitacin B	3.65	9.33
Gallic acid	13.48	34.47
Cucurbitasin S	0.03	0.08
Gastrodin	0.84	2.15
Colocynthoside B	0.10	0.26
3.0 Cafeoylquininate	0.16	0.41
Colocynthoside A	0.11	0.28

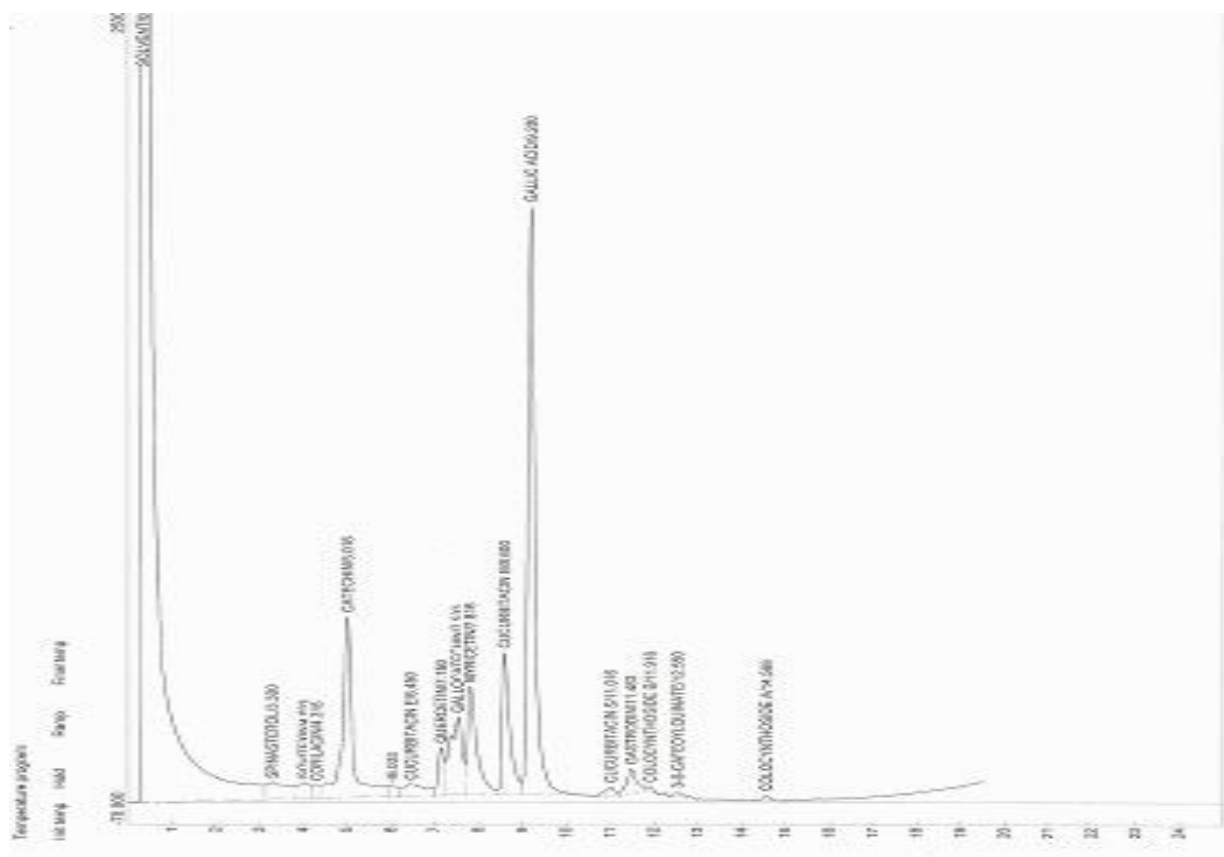


Figure 4.1 Different Spectra of Compounds Present in *Citrullus colocynthis* Pod in GC-FID Analyser

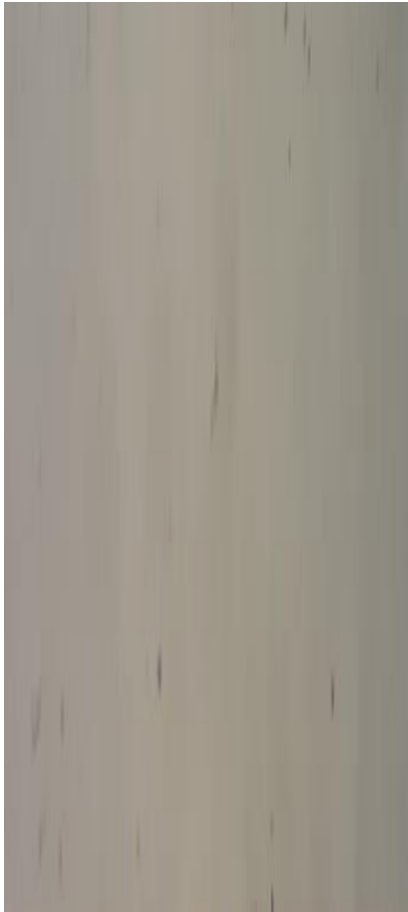
4.1.2 Mineral Constituents of *Citrullus colocynthis* Pods

The mineral analysis of the *Citrullus colocynthis* pod showed the following minerals were present iron, sodium, copper, chromium, potassium, cadmium, manganese, zinc, nickel, magnesium and calcium with calcium being the most abundant (Table 4.3).

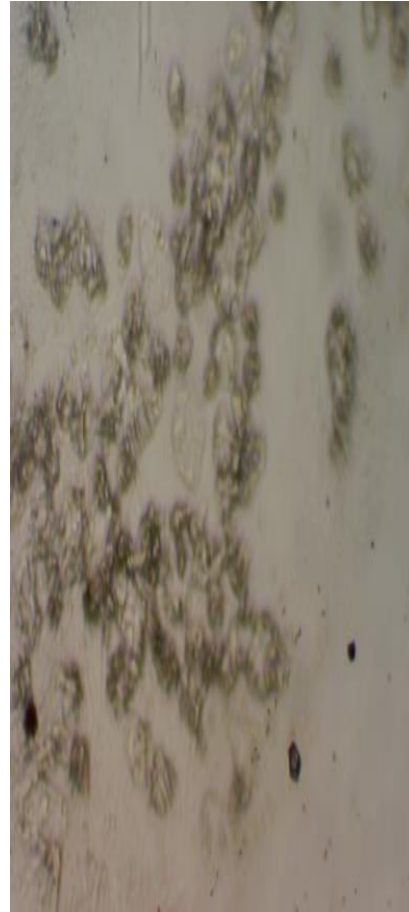
Table 4.3: Mineral Constituents of *Citrullus colocynthis* Pods

Minerals	Values (mg/l)
Iron	12.067 $\pm$ 0.689
Sodium	5.250 $\pm$ 0.000
Copper	0.142 $\pm$ 0.001
Chromium	0.187 $\pm$ 0.013
Potassium	17.867 $\pm$ 0.361
Cadium	0.003 $\pm$ 0.001
Manganese	3.130 $\pm$ 0.040
Zinc	0.195 $\pm$ 0.002
Nickel	0.056 $\pm$ 0.002
Magnesium	7.600 $\pm$ 0.100
Calcium	27.167 $\pm$ 0.593

Values are mean $\pm$ SEM of three determinations



A



B

Plate 4.1: A and B showing difference in vaginal smear of unmated and mated rats



C



D



E

Plate 4.3: C, D and E Showing Resorption Level in Animals

4.3 Abortifacient Study

4.3.1 Abortifacient Parameters

The administration of the ethanolic extract of *Citrullus colocynthis* pods caused a decrease in final weight gain of the maternal rats especially at 800mg/kg bwt extract and mifepristone group in when compared with the control. A sharp decrease was noticed in the percentage weight gain of the other groups (Table 4.4). The extract also caused a significant ($p<0.05$) reduction in the number of life fetuses significantly in all the groups treated when compared with control (Table 4.4). Similarly, no life foetus was recorded in the pregnant rats treated with mifepristone and 800 mg/kg body weight of the extract of *Citrullus colocynthis*. All the extract doses significantly increased ($p<0.05$) the number of dead foetus when compared with control as there was no dead foetus in the mifepristone-treated group. The survival ratio was calculated to be 81.48%, 66.66% and 18.75% for 100, 200 and 400mg/kg body weight of extract, 0% for the mifepristone and 800mg/kg body weight treated groups respectively, as compared with control which was (100%). In the group administered with mifepristone and 800 mg/kg body weight extract there were 100% aborted rats, whereas 20, 40 and 80% was observed in groups administered with 100, 200 and 400mg/kg body weight of extract when compared to control (Table 4.4).

The number of corpora lutea decreased significantly ($p<0.05$) with an increased dose of extract as shown in table 4.4. It was shown that a significant ($p<0.05$) decrease resulted in the computed implantation index in extract treated and mifepristone treated groups in comparison with control, except the group treated with 100mg/kg body weight extract which did not show a much significant ($p>0.05$) different. Furthermore, there was an increase in the resorption index for all extract

treated and mifepristone groups when compared with the control but the highest and least percentage was observed in 400mg/kg body weight extract and mifepristone group. The pre-implantation loss and post-implantation loss increased significantly ($p < 0.05$) in all the extract treated groups and are dose dependent as compared with control. However, mifepristone and 800mg/kg body weight treated group showed 100% post-implantation loss as a measure of abortion when compared with 26.95% observed in the control (Table 4.4).

Table 4.4: Abortifacient Activities of the Ethanolic Extract of *Citrullus colocynthis***Administered to Pregnant Female Rats**

Parameters	Control	Mifepristone	100mg/kg bwt CC	200mg/kg bwt CC	400mg/kg bwt CC	800mg/kg bwt CC
Maternal initial weight(g)	162.96±3.01 ^a	161.38±2.57 ^a	165.32±2.09 ^a _b	162.88±2.56 ^a	164.14±3.20 ^a	167.24±1.35 ^b
Maternal final weight (g)	195.16±4.85 ^a	176.90±1.65 ^b	195.72±4.67 ^a	193.40±4.49 ^a	194.26±2.06 ^a	185.50±2.59 ^c
Percentage gain in weight (%)	19.76	9.62	18.39	18.74	18.35	10.92
No. of life fetus (g)	5.80 ± 0.67 ^a	0.00 ± 0.00 ^b	2.20 ± 0.32 ^c	1.60 ± 0.24 ^b	0.60 ± 0.40 ^b	0.00 ± 0.00 ^b
No. of dead fetus (g)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	2.70 ± 0.45 ^b	2.40 ± 0.51 ^b	3.20 ± 0.37 ^c	3.60 ± 0.40 ^c
Weight of fetus (g)	2.44± 0.21 ^a	0.00 ± 0.00 ^b	1.98 ± 0.09 ^a	1.42 ± 0.36 ^b	0.44 ± 0.07 ^c	0.00 ± 0.00 ^b
Survival ratio of fetus (%)	100	0	81.48	66.66	18.75	0
No. of rats that aborted	0	5	1	2	4	5
Percentage aborted (%)	0	100	20	40	80	100
No. of implantation site	7.94 ± 0.55 ^a	1.00 ± 0.32 ^b	7.33 ± 0.55 ^a	5.60 ± 0.5 ^c	2.30 ± 0.56 ^d	1.25 ± 0.49 ^b
No. of resorption site	0.00 ± 0.00 ^a	0.20 ± 0.40 ^a	4.51 ± 0.81 ^b	3.97 ± 0.73 ^c	2.20 ± 0.40 ^d	0.80 ± 0.58 ^a
No. of <i>Corpora lutea</i>	8.90 ± 1.36 ^a	2.01± 1.07 ^b	8.60 ± 1.29 ^a	7.20 ± 1.24 ^a	3.11 ± 1.64 ^b	1.85± 0.86 ^b
Implantation index (%)	89.21	46.62	85.23	77.78	73.95	67.76
Resorption index (%)	0	20	61.53	70.89	95.65	64.00
Pre-implantation loss (%)	10.79	50.25	14.77	22.22	26.05	32.43
Post-implantation loss (%)	26.95	100	69.99	71.43	73.91	100

Mean (n=5 determinations) ± SEM. **b-d** Test values for each parameter are significantly different from the control (p<0.05)

4.3.2 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on the Hormonal Profile

4.3.2.1 Progesterone Hormone

The administration of ethanolic extract of *Citrullus colocynthis* pods at doses 100, 200, 400, 800 mg/kg body weight and mifepristone as reference drug caused significant ($p < 0.05$) decrease in concentration of serum progesterone in all groups when compared with the control (Figure 4.2). The 100, 200, 400, 800mg/kg body weight and mifepristone decreased the progesterone by 9.9%, 20.12%, 38.82%, 55.28% and 58.94% respectively.

4.3.2.2 Oestrogen Hormone

Administration of ethanolic extract of *Citrullus colocynthis* pod significantly decreased ($p < 0.05$) serum oestrogen hormone concentration in pregnant rats in a dose dependent manner as well as mifepristone treated group when compared with the control (Figure 4.3). Mifepristone treated group recorded the greatest reduction of 72.73%.

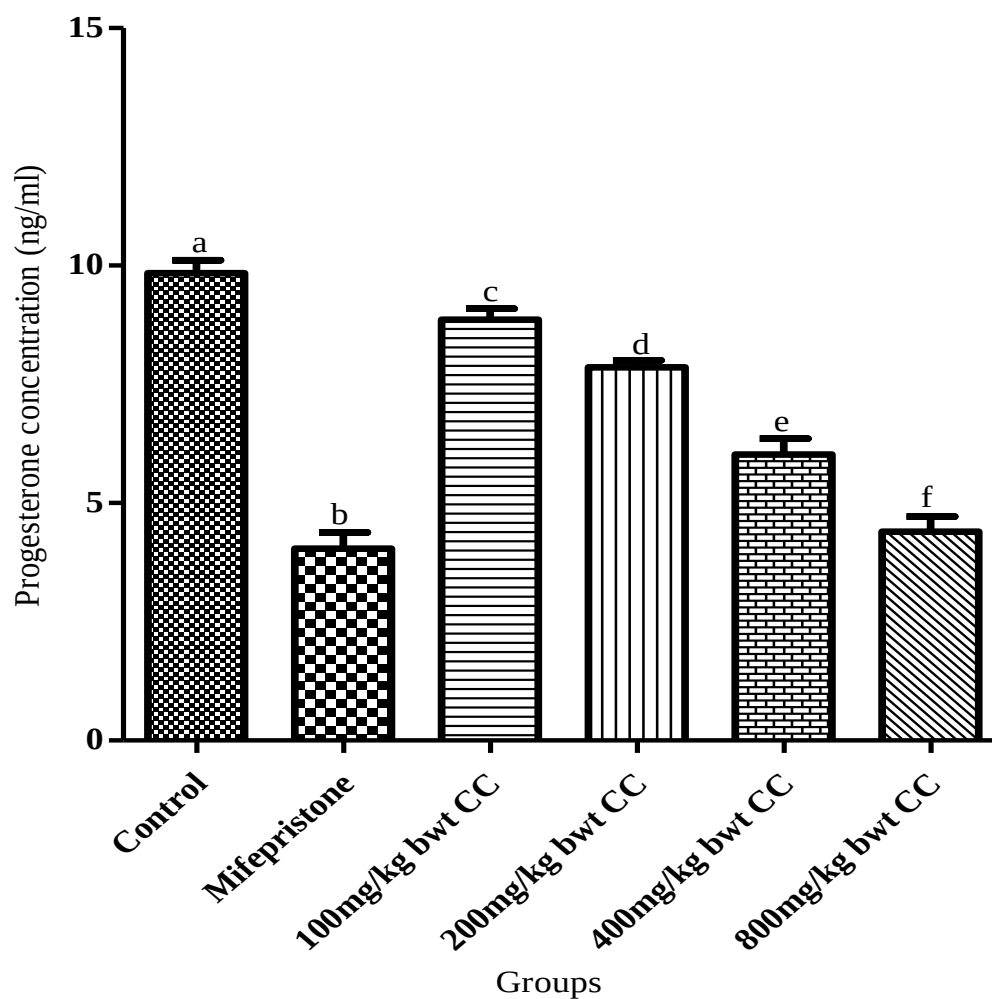


Figure 4.2: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Progesterone Level in Pregnant Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from control.

CC: *Citrullus colocynthis*

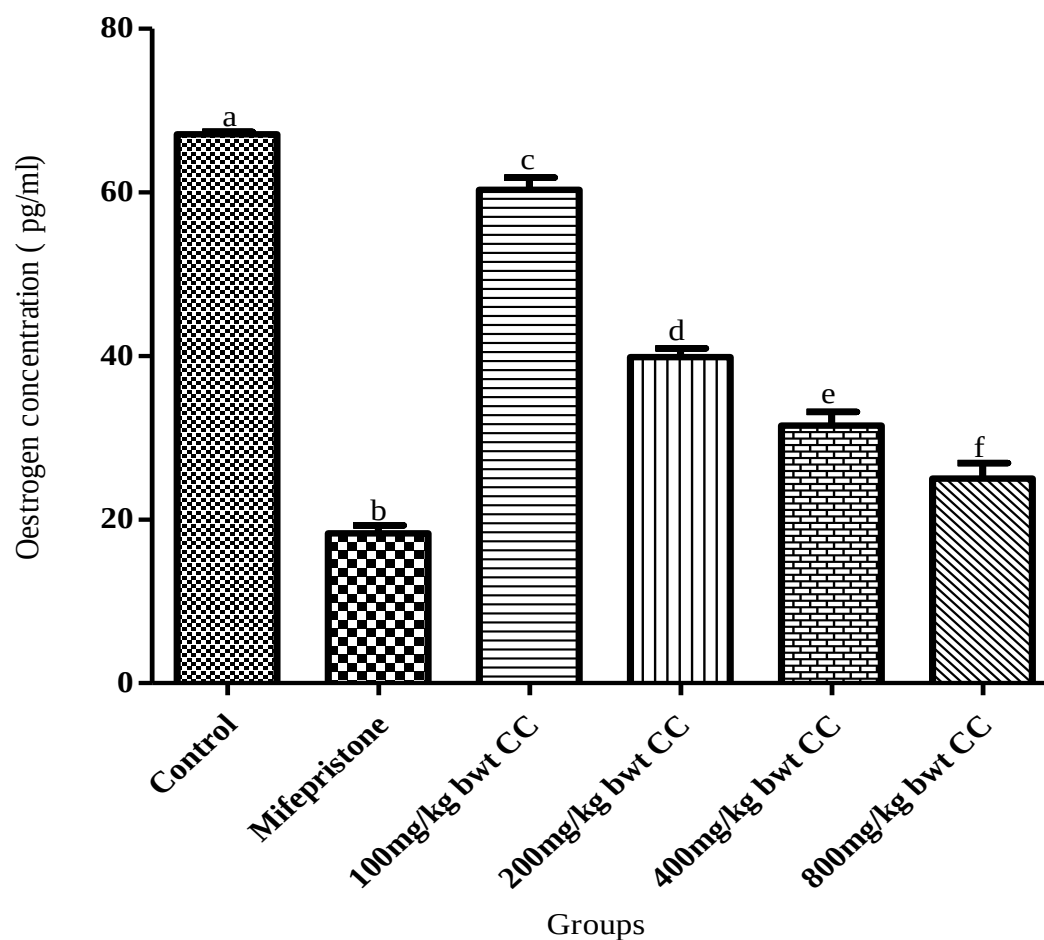


Figure 4.3: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Oestrogen Level in Pregnant Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from control.

CC: *Citrullus colocynthis*

4.3.2.3 Prolactin Hormone

Administration of ethanolic extract of *Citrullus colocynthis* pod at all doses explores and mifepristone significantly ($p < 0.05$) decreased prolactin hormone level when compared with control (Figure 4.4). 100, 200, 400, 800mg/kg body weight extract and mifepristone decreased the prolactin concentration by 20.56%, 32.22%, 37.50%, 71.39% and 54.72% respectively.

4.3.2.4 Luteinizing Hormone

Administration of ethanolic extract of *Citrullus colocynthis* pod at 100, 200, 400, 800mg/kg body weight, and mifepristone, caused a significant ($p < 0.05$) decrease in serum luteinizing hormone concentrations when compared with control (Figure 4.5). The reduction was 3.13%, 18.30%, 28.57%, 43.75% and 47.77% respectively for all the groups.

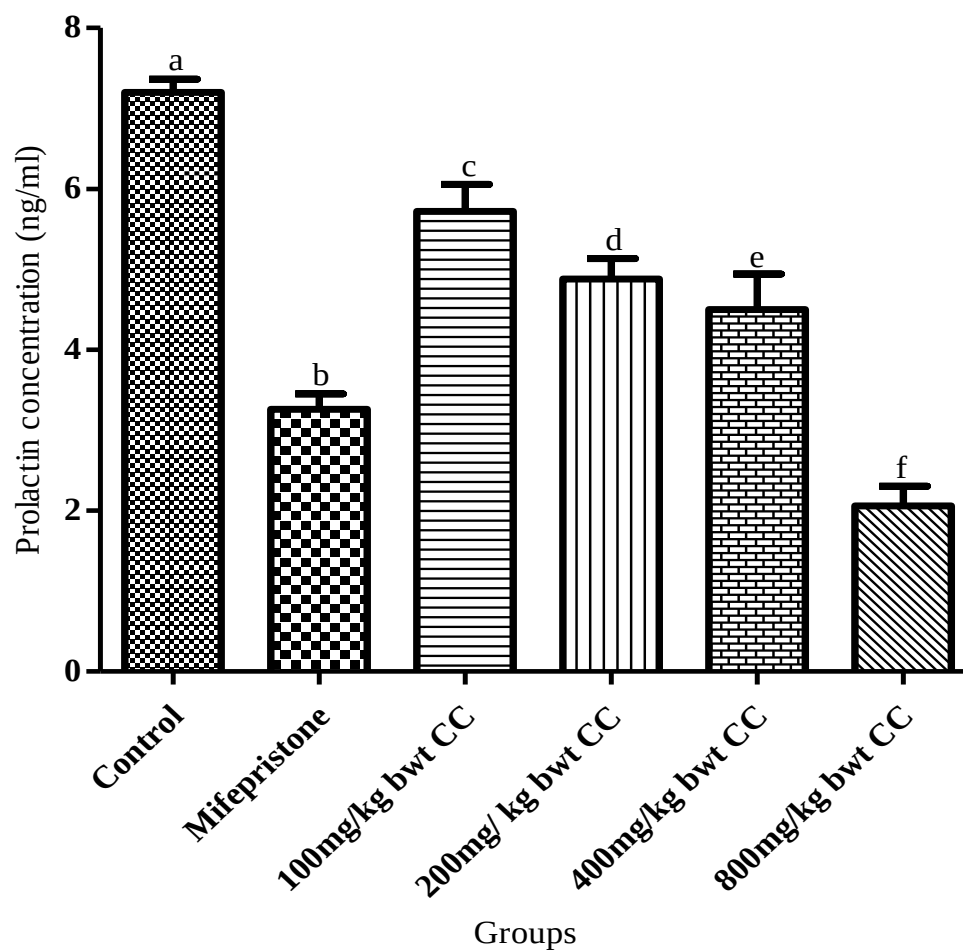


Figure 4.4: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Prolactin Level in Pregnant Rats

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from control.

CC: *Citrullus colocynthis*

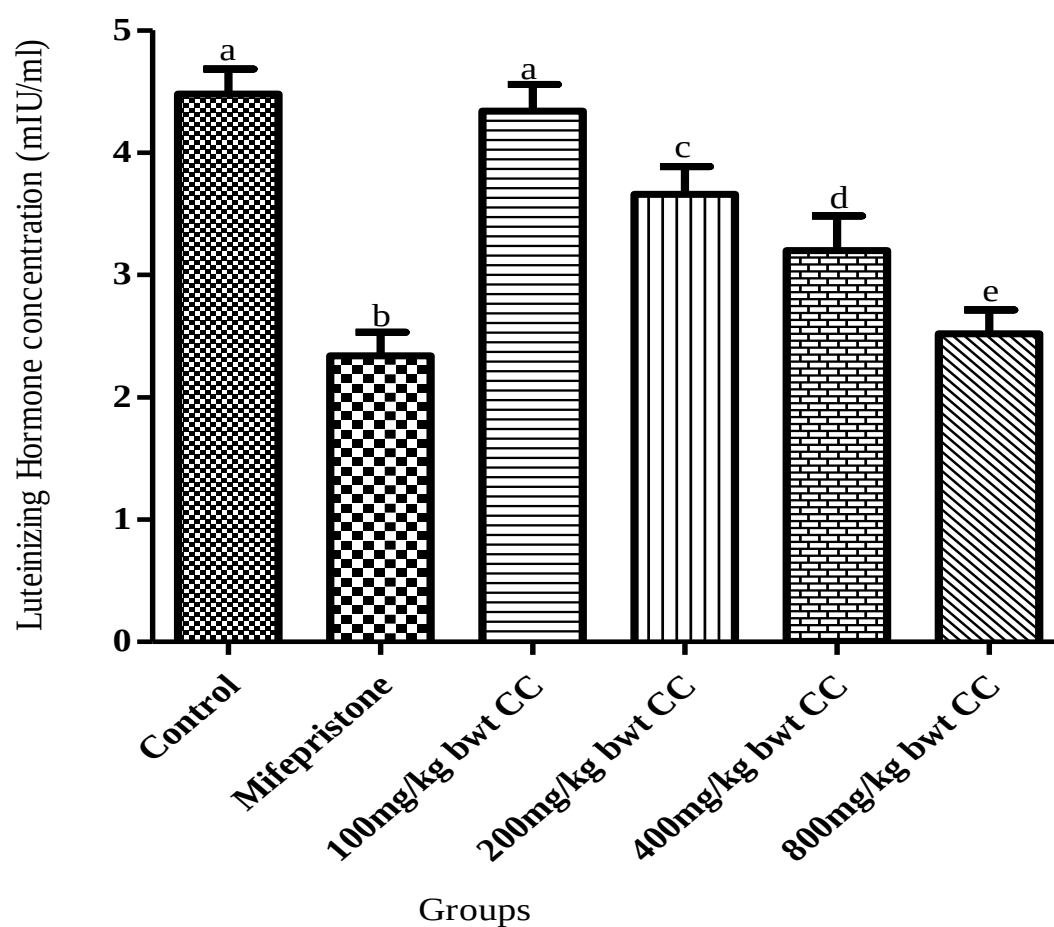


Figure 4.5: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Luteinizing Hormone Level in Pregnant Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from control.

CC: *Citrullus colocynthis*

4.3.2.5 Follicle Stimulating Hormone

There was a significant reduction ($p < 0.05$) of serum follicle stimulating hormone concentrations of all extract treated groups and mifepristone when compared with the control (Figure 4.6). The decrease was 14.29%, 27.73%, 24.37%, 36.97% and 46.22% respectively with an increase in dose and mifepristone treated group.

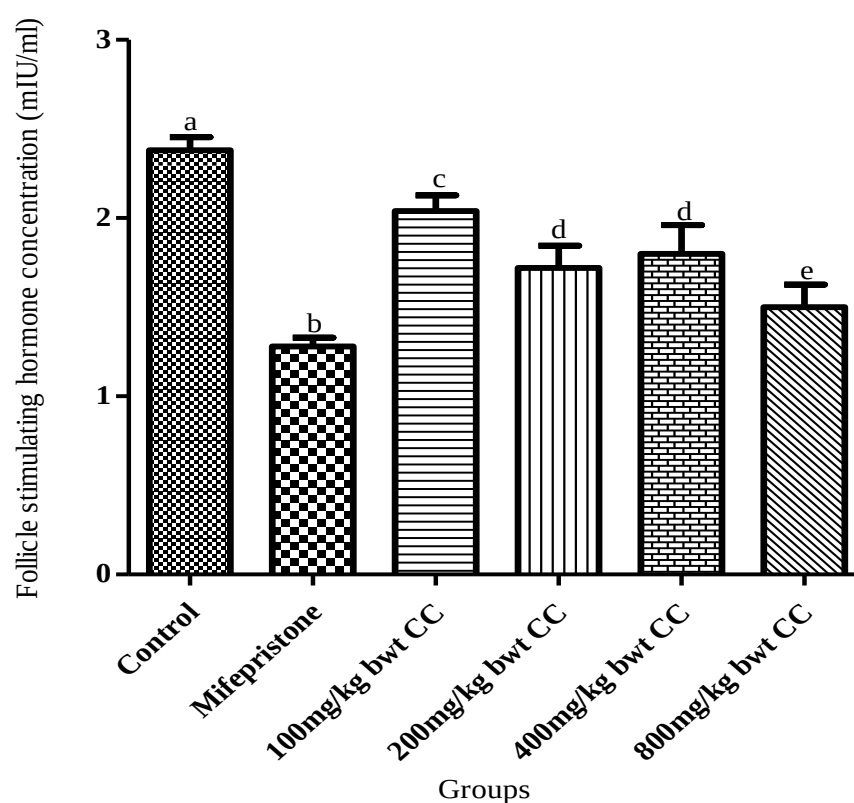


Figure 4.6: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Follicle Stimulating Hormone Level in Pregnant Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from control.

CC: *Citrullus colocynthis*

4.4 Effect of Ethanolic Extract of *Citrullus colocynthis* Pod on Amino Transferases Activity in the Serum and Tissues of Pregnant Wistar Rats

4.4.1 Aspartate Aminotransferase Activity

Figure 4.7, reveals that different doses of the ethanolic extract of *Citrullus colocynthis* pods administered and mifepristone caused a significant ($p<0.05$) increase in the activity of serum aspartate amino transferase in all the groups treated when compared with control. In contrast, significant ($p<0.05$) reduction was noticed in the activity of aspartate amino transferase in both the liver and the kidney of pregnant female rats administered with the same dose of ethanolic extract of *Citrullus colocynthis* pods when compared with control (Figure 4.7).

4.4.2 Alanine Aminotransferase Activity

Administration of 400, 800mg/kg body weight *Citrullus colocynthis* ethanolic pod extract and mifepristone caused significant increase ($p<0.05$) in serum alanine amino transferase activity of all pregnant female rats when compared to control, whereas rats treated with 100 and 200mg/kg body weight groups does not show any significant ($p<0.05$) difference from the control (Figure 4.8). Furthermore, significant ($p<0.05$) reduction was observed in the activity of alanine aminotransferase in both liver and kidney of pregnant female rats administered with various doses of ethanolic extract of *Citrullus colocynthis* pods when compared with control (Figure 4.8).

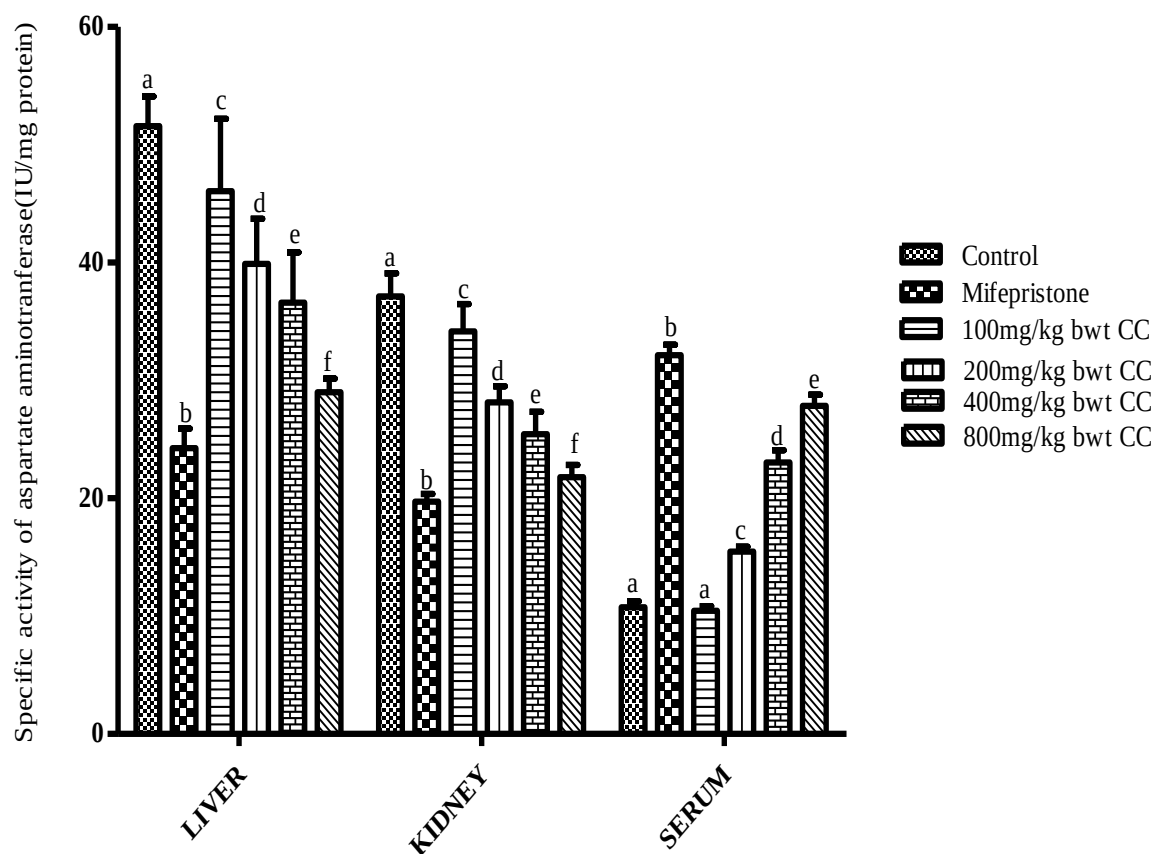


Figure 4.7: Specific Activity of Aspartate Aminotransferase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pod for Nine Days
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

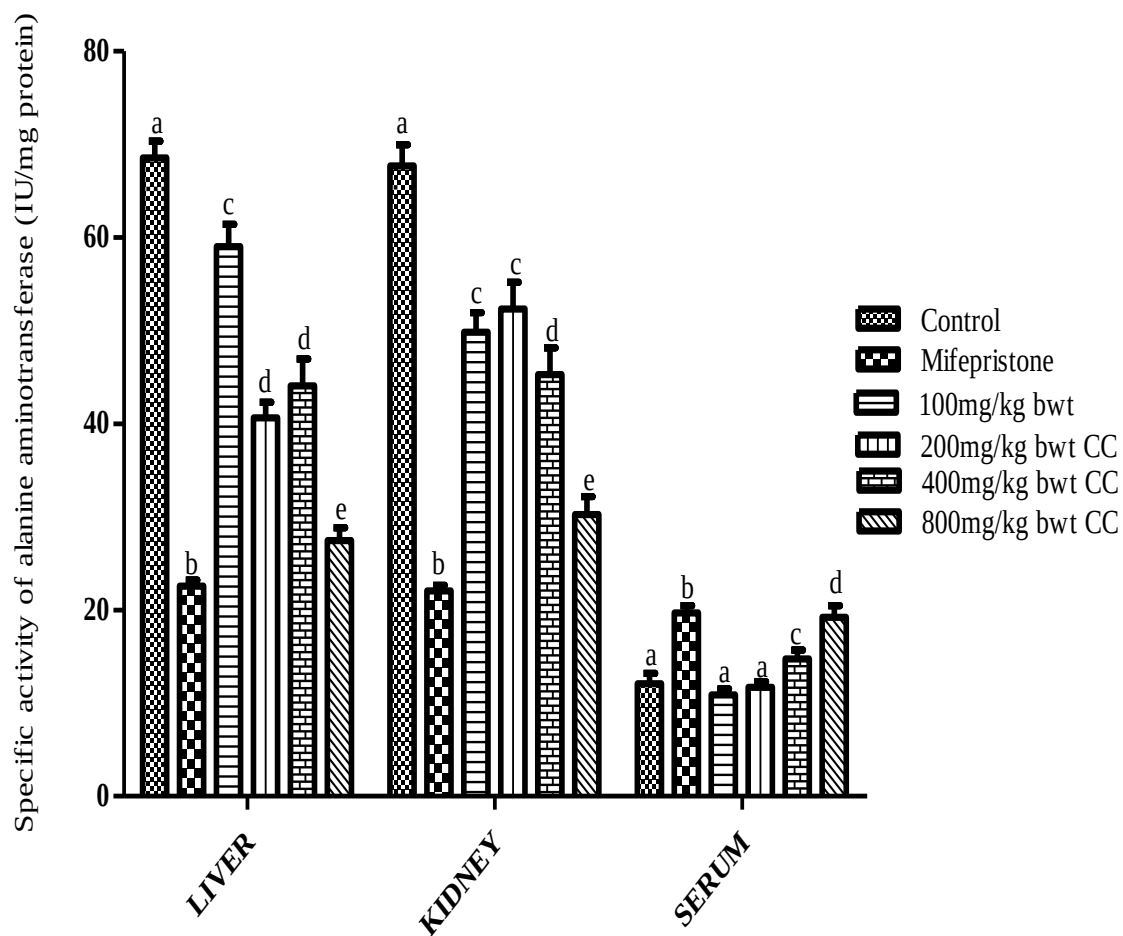


Figure 4.8: Specific Activity of Alanine Aminotransferase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pod for Nine Days
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.4.3 Alkaline phosphate activity

Administration of 100, 200, 400 and 800 mg/kg body weight ethanolic extract of *Citrullus colocynthis* pod and mifepristone significantly ($p < 0.05$) increased the activity of alkaline phosphatase in the serum of all pregnant female rats when compared with control but caused significant ($p < 0.05$) reduction in the activity of the enzyme in the liver and kidney tissues respectively (Figure 4.9).

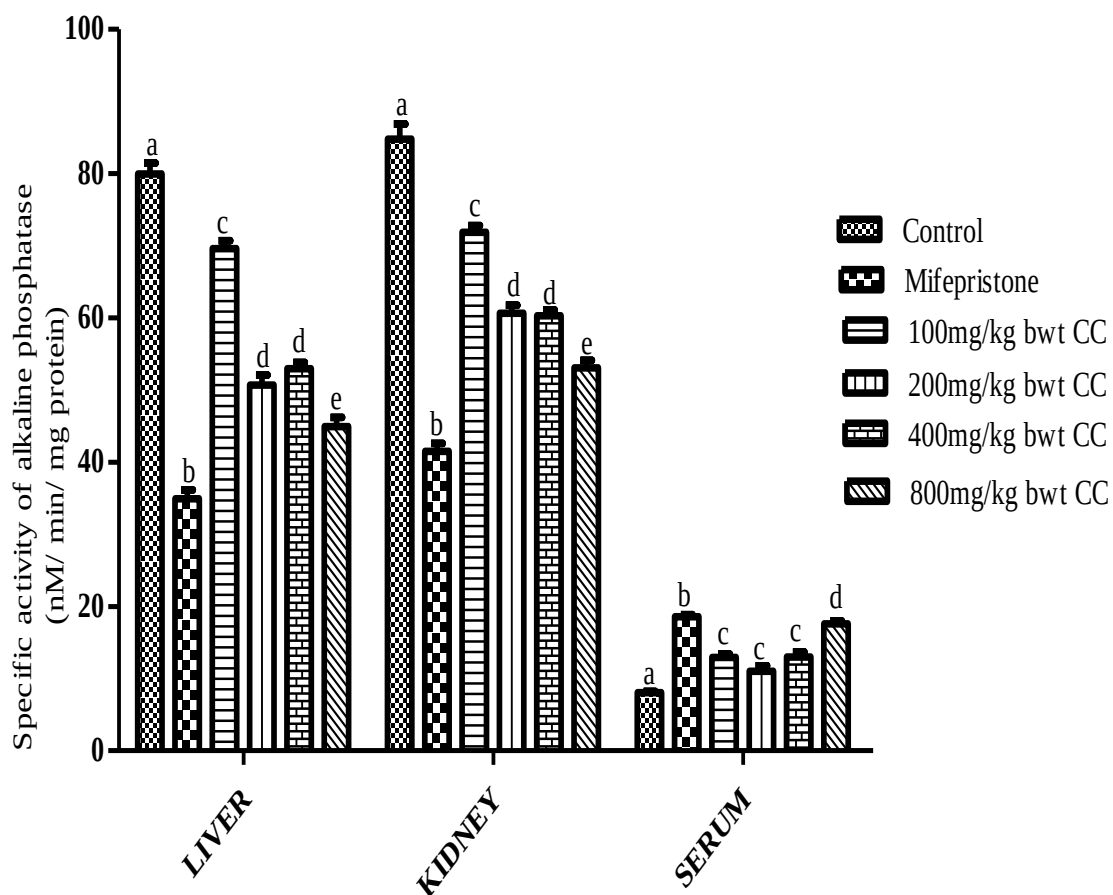


Figure 4.9: Specific Activity of Alkaline Phosphatase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Nine Days

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4. 5 Biochemical Assessment

4.5.1 Organ- Body Weight Ratios

Administration of *Citrullus colocynthis* pod ethanolic extract at 100, 200 and 400mg/kg body weight did not significantly ($p>0.05$) altered organ-body weight ratio of the uterus and ovary whereas a significant reduction ($p<0.05$) at high dose of 800mg/kg body weight and mifepristone was observed when compared with control (Table 4.5). Also, a significant ($p<0.05$) increase in organ-body weight ratio of the liver of rats administered with the different doses and mifepristone except the group treated with 200mg/kg body weight when compared with the control (Table 4.5).

Table 4.5: Organ-Body Weight Ratio of Wistar Rats Treated with Ethanolic Extract of *Citrullus colocynthis* Pods

Groups	Uterus	Ovary	Kidney	Liver
Control	0.14±0.01 ^a	0.18±0.01 ^a	0.51±0.03 ^a	2.50±0.13 ^a
Mifepristone	0.10±0.01 ^b	0.14±0.03 ^b	0.45±0.01 ^b	2.75±0.13 ^b
100mg/kg bwt	0.14±0.01 ^a	0.17±0.01 ^a	0.45±0.02 ^b	2.70±0.07 ^b
200mg/kg bwt	0.14±0.01 ^a	0.18±0.01 ^a	0.39±0.01 ^c	2.44±0.06 ^a
400mg/kg bwt	0.13±0.01 ^a	0.18±0.02 ^a	0.52±0.01 ^a	2.68±0.08 ^b
800mg/kg bwt	0.11±0.00 ^b	0.16±0.01 ^b	0.46±0.01 ^b	2.94±0.11 ^b

Values are mean ± SEM of five determinations. Columns with different superscripts are significantly different (P<0.05)

4.5.2 Effect of Twenty-one Days Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Marker Enzymes in Serum and Tissues of Pregnant Wistar Rats

4.5.2.1 Aspartate Aminotransferase Activity

Administration of *Citrullus colocynthis* pod ethanolic extract for twenty-one days at different doses and mifepristone to pregnant rats caused significant ($p < 0.05$) increase in serum aspartate amino transferase activity of all pregnant female rats treated when compared with control except for group administered with 100mg/kg body weight (Figure 4.10). In contrast, a sharp reduction was observed in aspartate aminotransferase activity of both the liver and kidney tissues of pregnant female rats administered with ethanolic extract of *Citrullus colocynthis* pods and mifepristone when compared with control (Figure 4.10).

4.5.2.2 Alanine Aminotransferase Activity

Ethanolic extract of *Citrullus colocynthis* pod administration to rats at different doses and mifepristone for 21 days increased significantly ($p < 0.05$) the activity of alanine amino transferase in the serum of the pregnant female rats when compared with control whereas significant reduction was observed in alanine aminotransferase specific activity of the liver and kidney tissues of pregnant female rats (Figure 4.11).

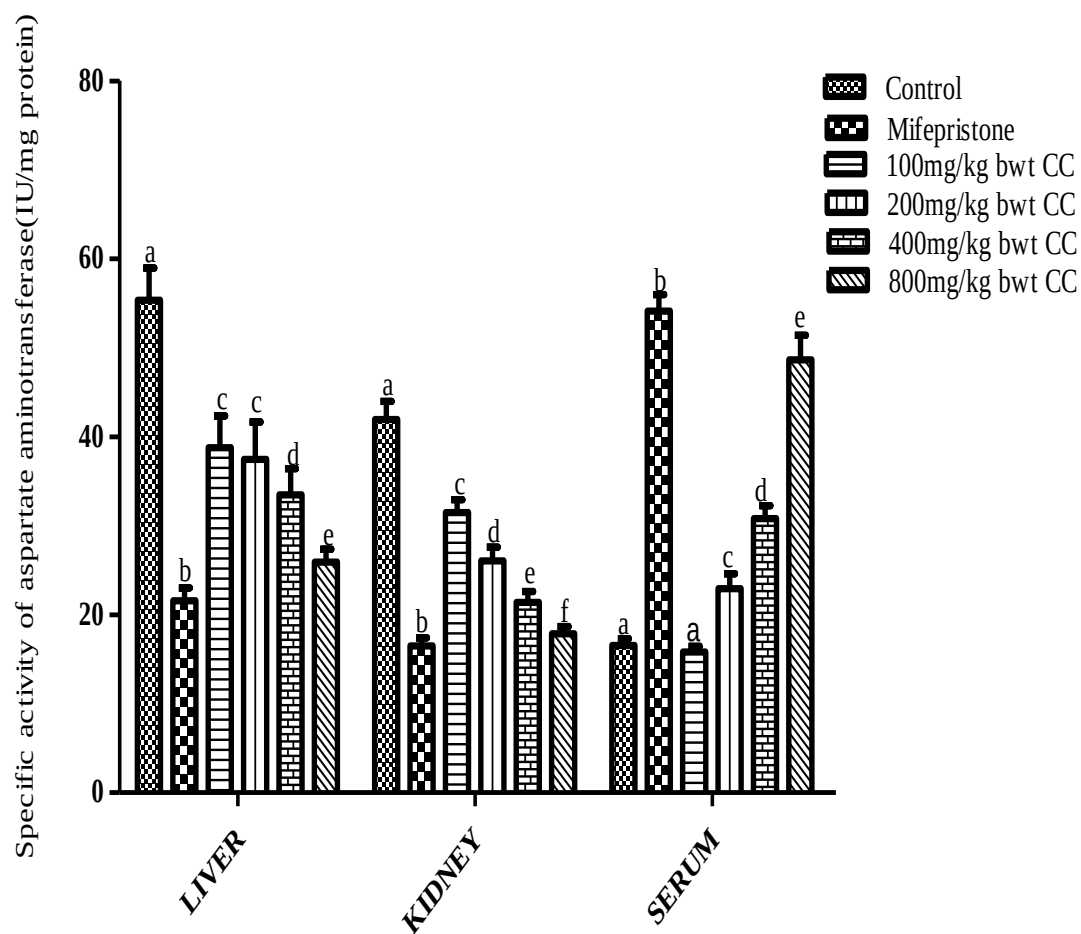


Figure 4.10: Specific Activity of Aspartate Aminotransferase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pod for Twenty One Days
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

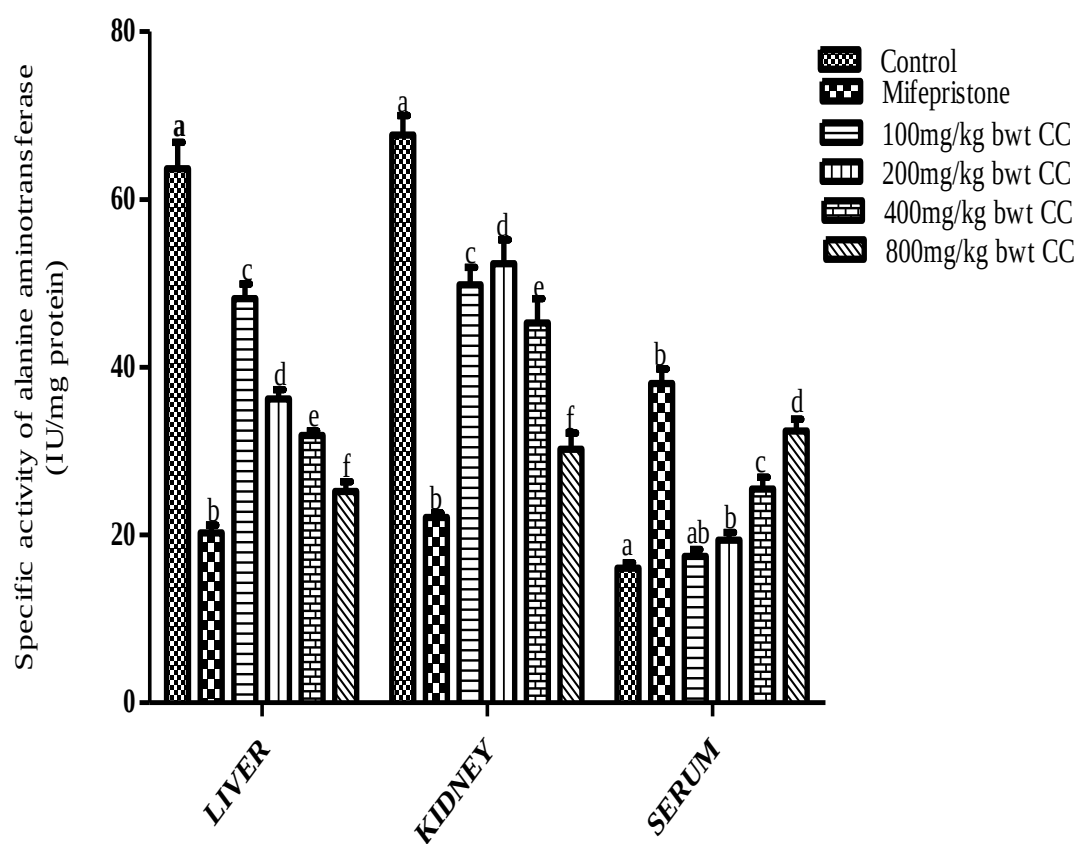


Figure 4.11: Specific Activity of Alanine Aminotransferase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.2.3 Alkaline Phosphatase Activity

The ethanolic extract of *Citrullus colocynthis* pod administered at various doses and mifepristone for 21 days significantly increased ($p < 0.05$) the activity of serum alkaline phosphatase of the pregnant female rats when compared with control. The highest concentration of ALP was observed in 800mg/kg body weight of extract treated group serum. It was also observed that there was a reduction in the enzyme activity in the liver as well as the kidney of the rats administered with the required doses when compared with the control (Figure 4.12).

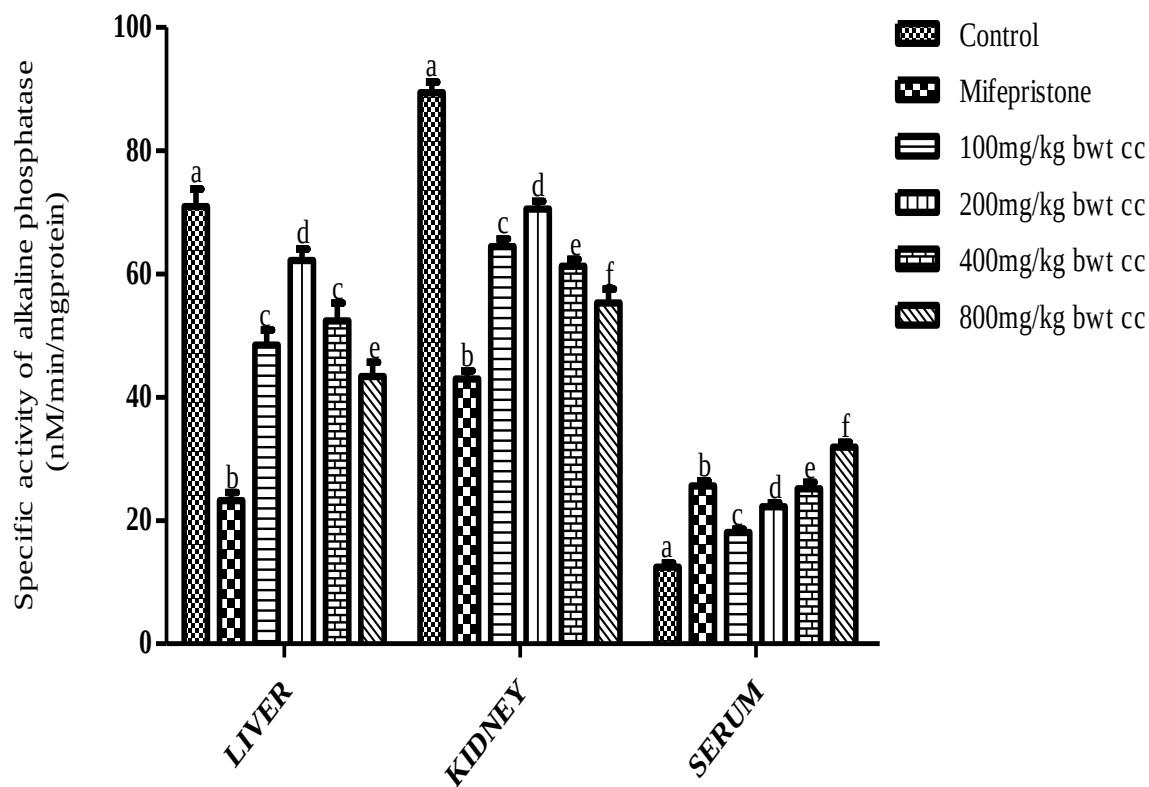


Figure 4.12: Specific Activity of Alkaline Phosphatase in Pregnant Rats Serum and Tissues Following the Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.3 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Liver Functions Indices of Pregnant Female Wistar Rats

The extract caused a decreased in protein concentration at doses 200, 400 and 800mg/kg body weight and mifepristone while there was no significant ($p>0.05$) difference observed in the group administered with 100mg/kg body weight when compared with the control (Figure 4.13). The extract reduced the albumin concentration in the serum of all the treated rats except for the group which received 800mg/kg body weight that showed no significance ($p<0.05$) when compared with control (Figure 4.14). It caused a decrease in the extract serum direct bilirubin at doses 100 and 400mg/kg body weight and mifepristone treated group but an increase was observed for those group treated with 200 and 800mg/kg body weight when compared with the control (Figure 4.15). The extract and the mifepristone treated rats recorded a significant ($p<0.05$) decrease in total bilirubin in the serum when compared with control (Figure 4.16).

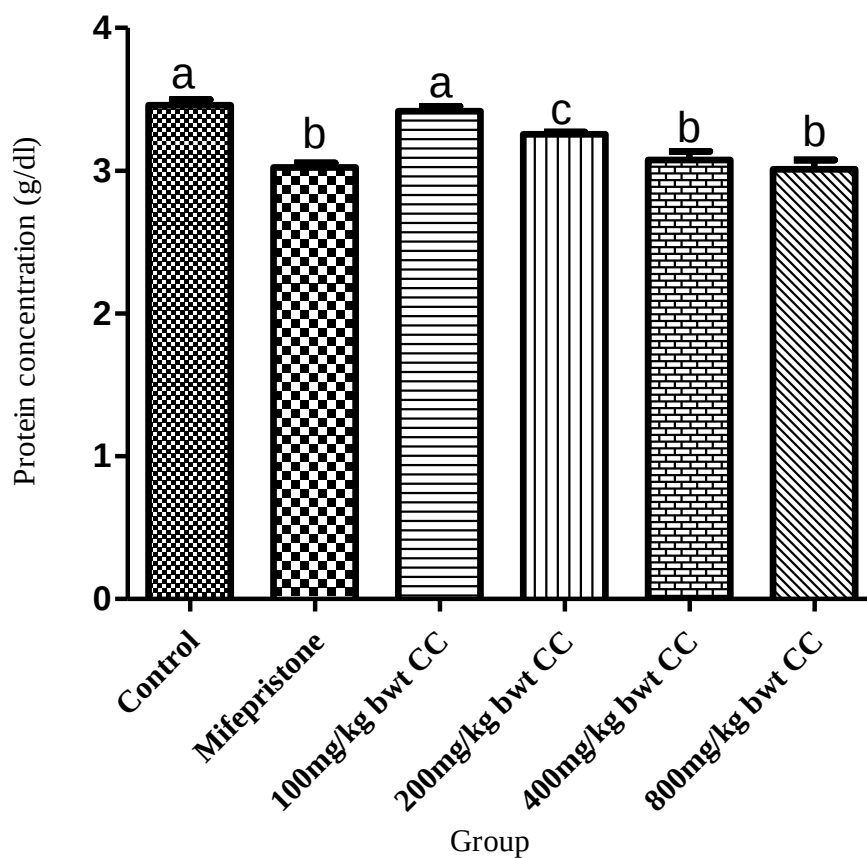


Figure 4.13: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Protein Level in Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinations. Bars with different letters are significantly different at ($p < 0.05$) from the control.

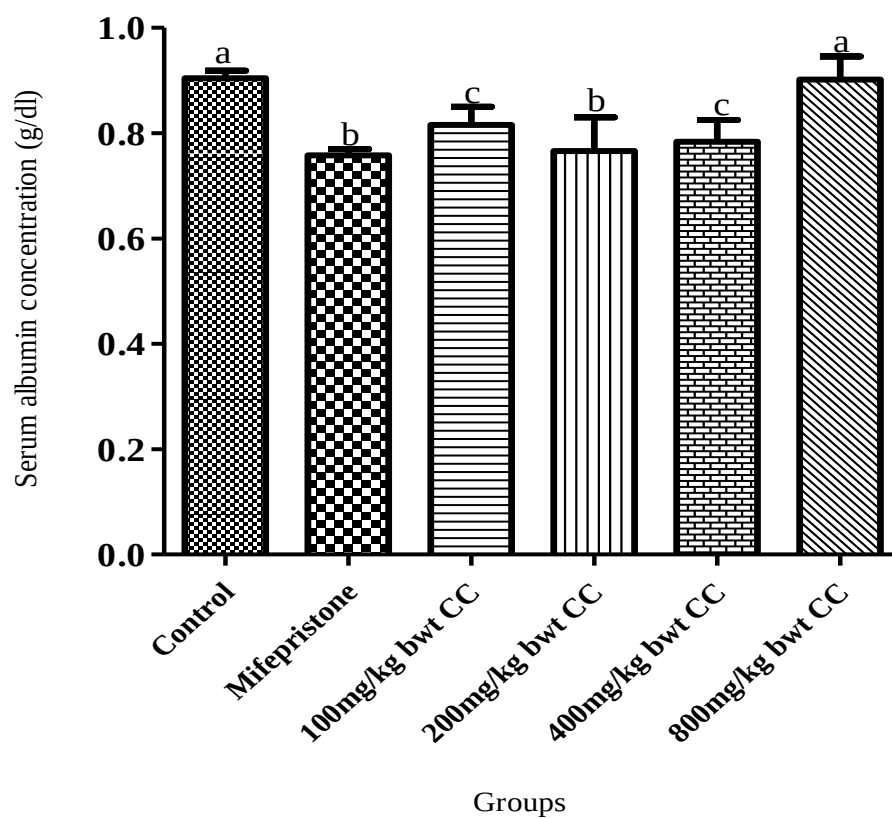


Figure 4.14: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Albumin Level in Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

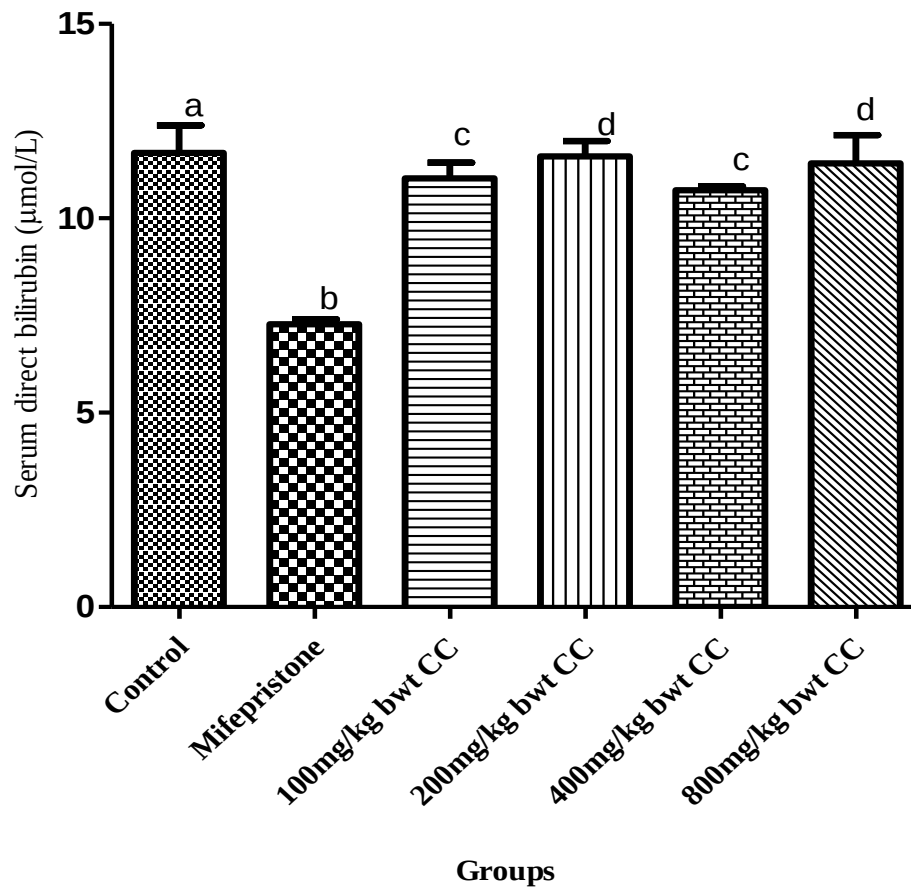


Figure 4.15: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Direct Bilirubin Level in Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

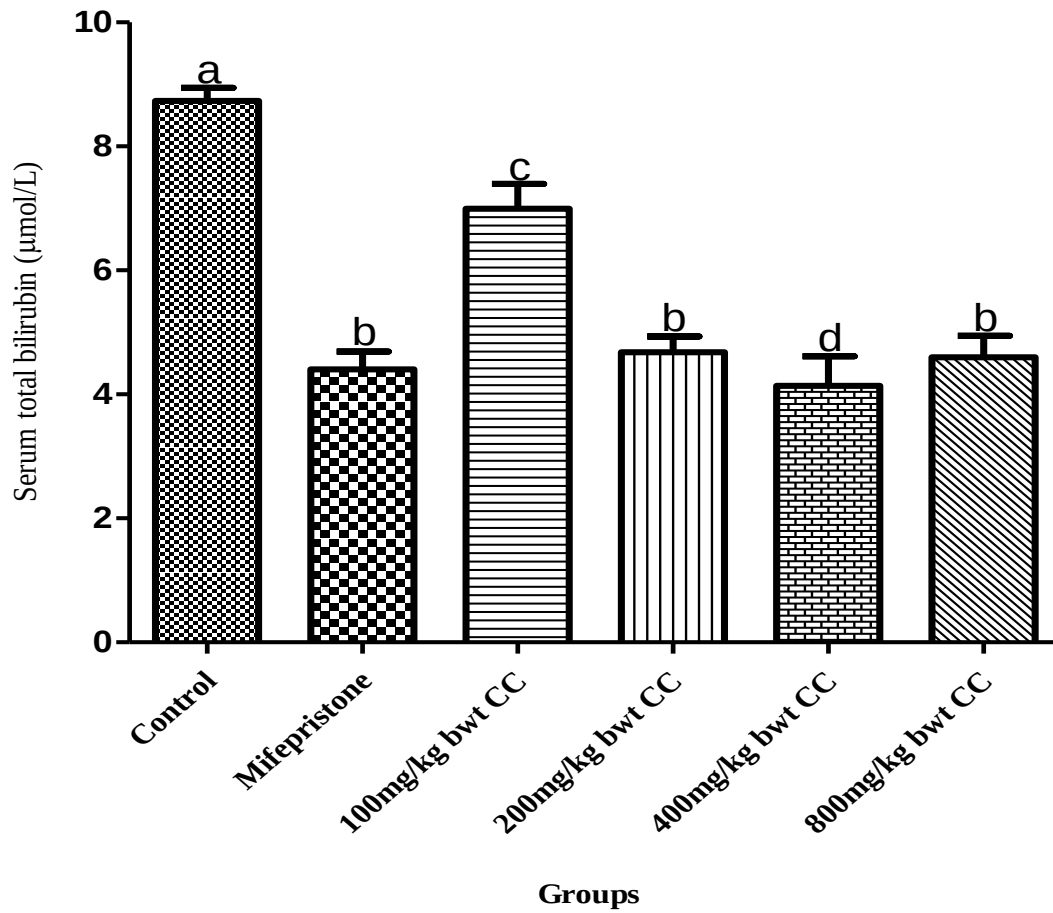


Figure 4.16: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Total Bilirubin Level in Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinants. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

4.5.4 Effect of Ethanolic Extract of *Citrullus colocynthis* on Glucose Concentration

Citrullus colocynthis pods extract and mifepristone caused a drastic reduction in the concentration of glucose across the treated groups especially at dose 400, 800mg/kg body weight and mifepristone when compared with the control (Figure 4.17).

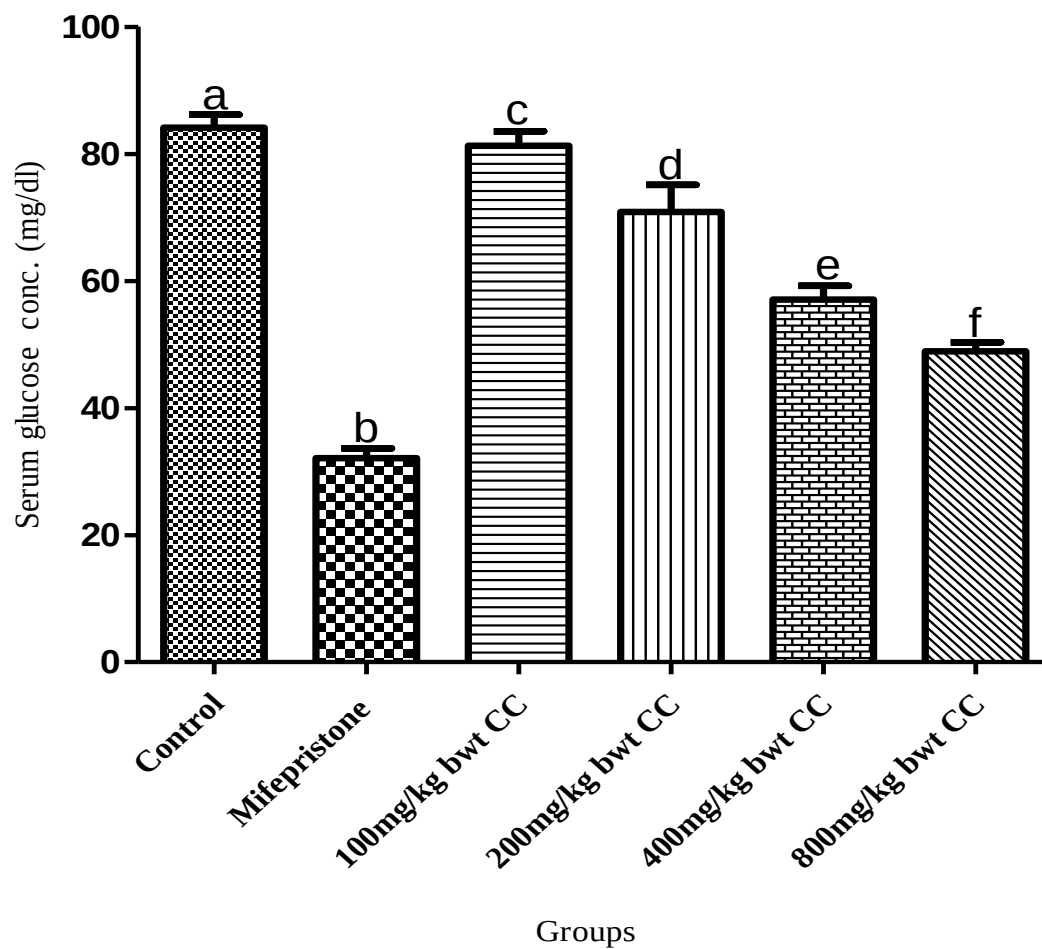


Figure 4.17: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Glucose Level in Pregnant Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC:*Citrullus colocynthis*

4.5.5 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Kidney Functions Indices of Pregnant Female Wistar Rats

It was also observed that ethanolic extract of *Citrullus colocynthis* pod drastically reduced the uric acid concentration in a dose dependent manner when compared with the control in all the groups treated and mifepristone (Figure 4.18).

The extract at doses 400 and 800mg/kg body weight and mifepristone produced significant decrease ($p<0.05$) in concentration of serum creatinine whereas, the group administered with 200 mg/kg body weight showed no significant difference while an increase was observed for 100mg/kg group when compared with control (Figure 4.19). A significant decrease ($p<0.05$) was observed in concentration of serum urea in the groups administered the extract and mifepristone when compared with the control (Figure 4.20). Also, the serum potassium and sodium electrolyte concentration for all groups administered with the extract produced no significant difference ($p>0.05$) when compared with the control except for 200mg/kg body weight group of sodium electrolyte concentration (Figure 4.21 and 4.22). *Citrullus colocynthis* pod administered at various concentrations of 100, 200, 400 and 800mg/kg body weight including the mifepristone group revealed alterations in the concentration of phosphorus in the serum when compared with the control (Figure 4.23). A significant elevation ($p<0.05$) was observed in chloride ion concentration in the treated groups and mifepristone when compared with the control (Figure 4.24). There was no significance ($p<0.05$) difference in calcium ion concentration in all the treatment groups and mifepristone except for 200mg/kg body weight group that shows an increase in concentration when compared with the control (Figure 4.25).

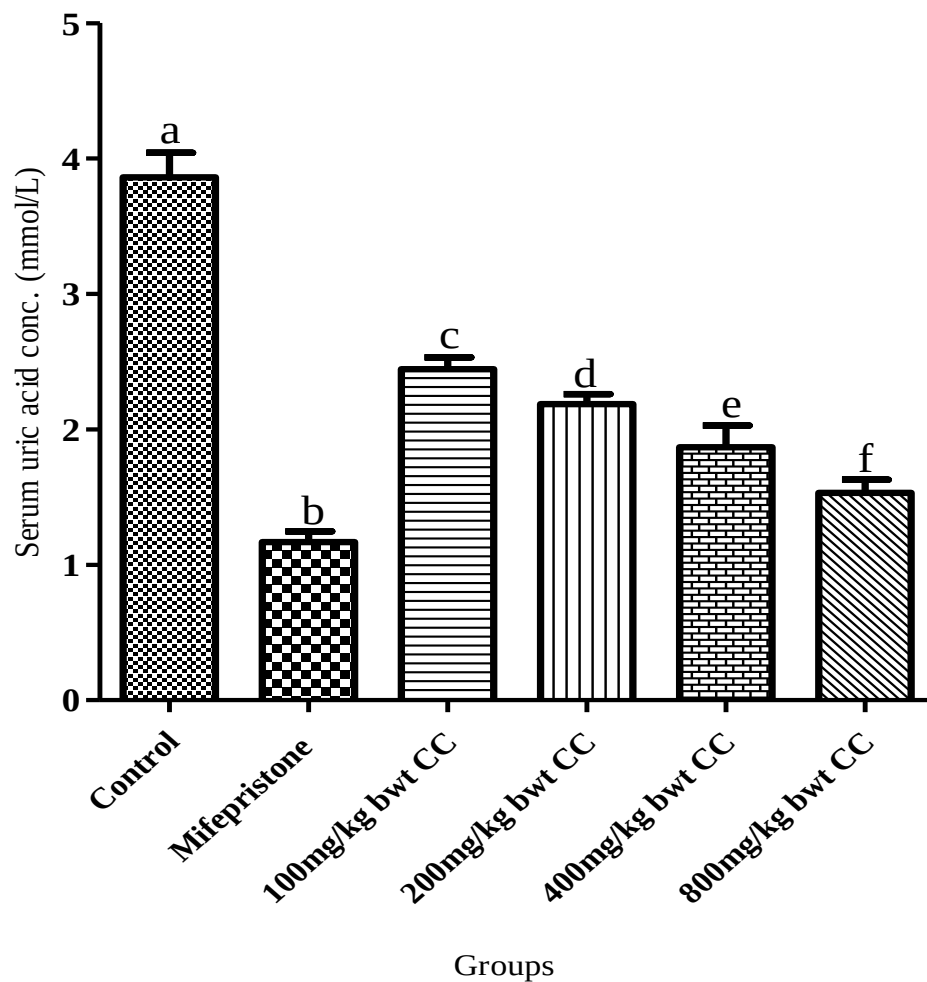


Figure 4.18: Effect of Ethanolic Extract of *Citrullus colocynthis* on Serum Uric Acid Level in Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

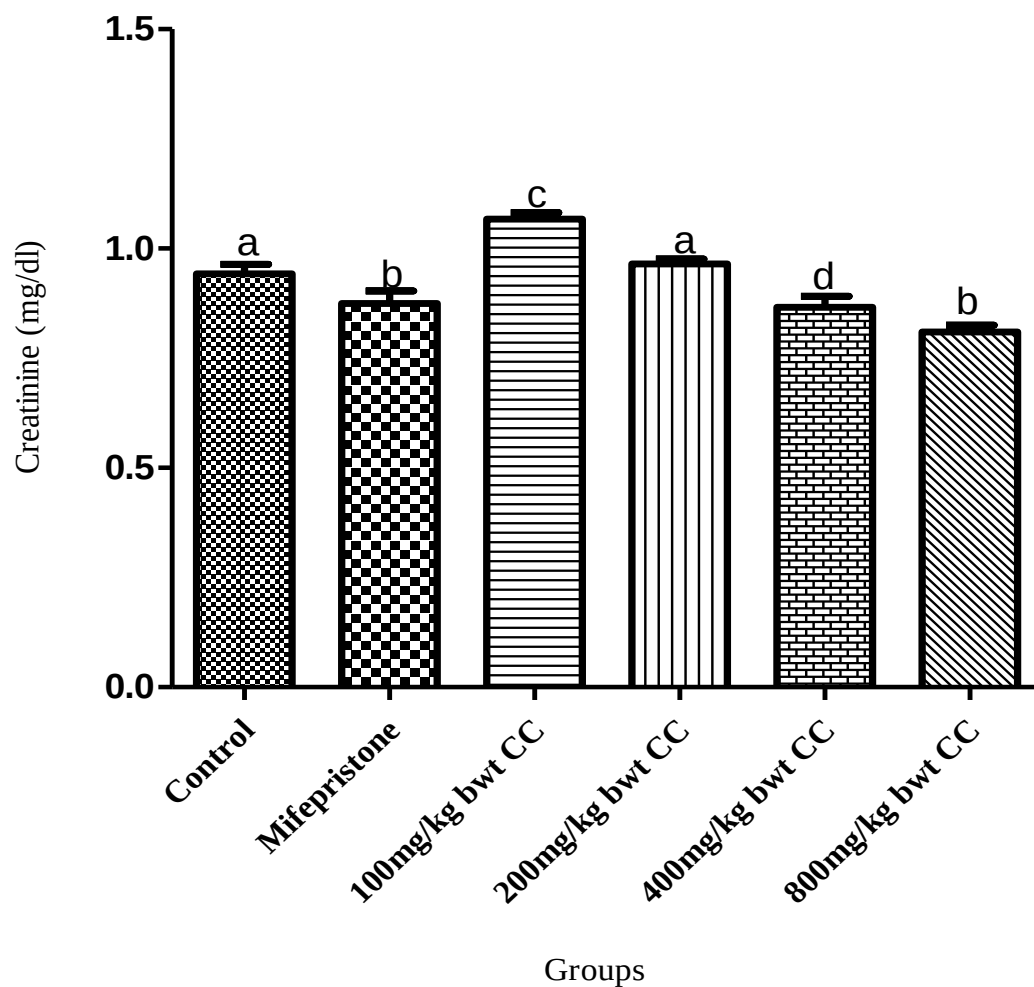


Figure 4.19: Effect of Ethanolic Extract of *Citrullus colocynthis* on Creatinine Level in Serum of Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

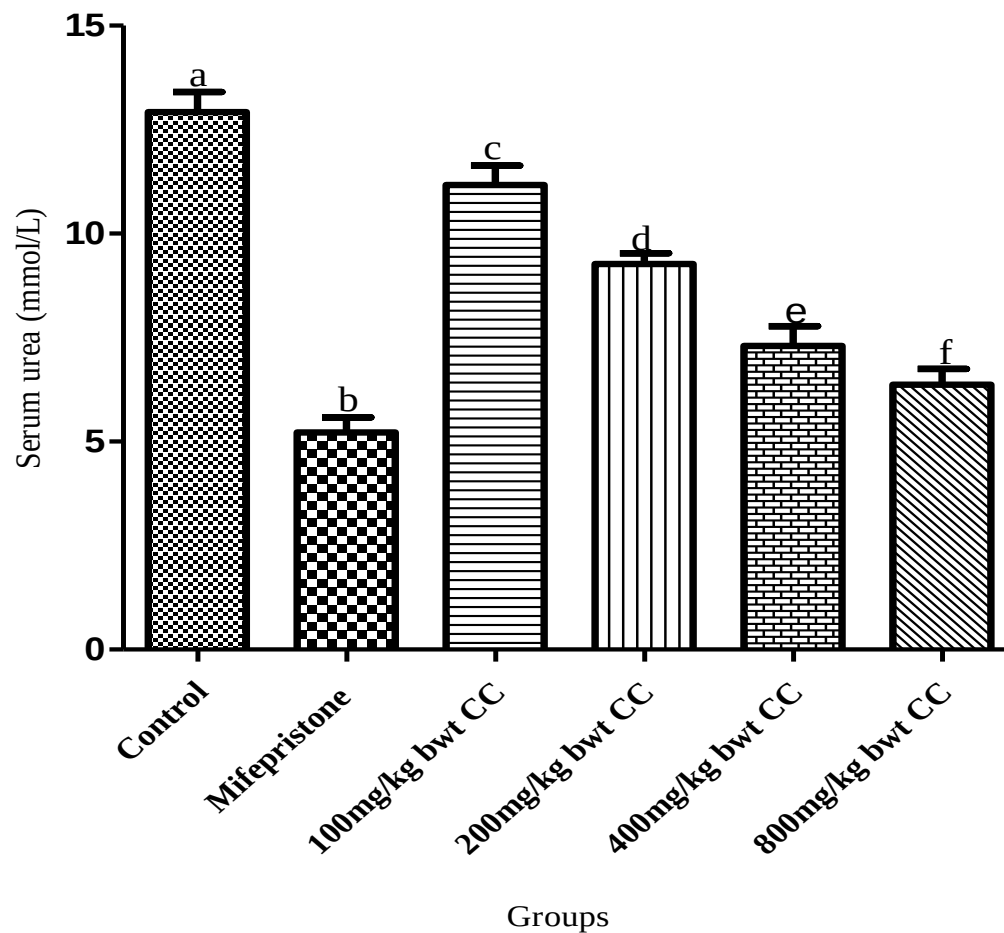


Figure 4.20: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Urea Level in Serum of Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

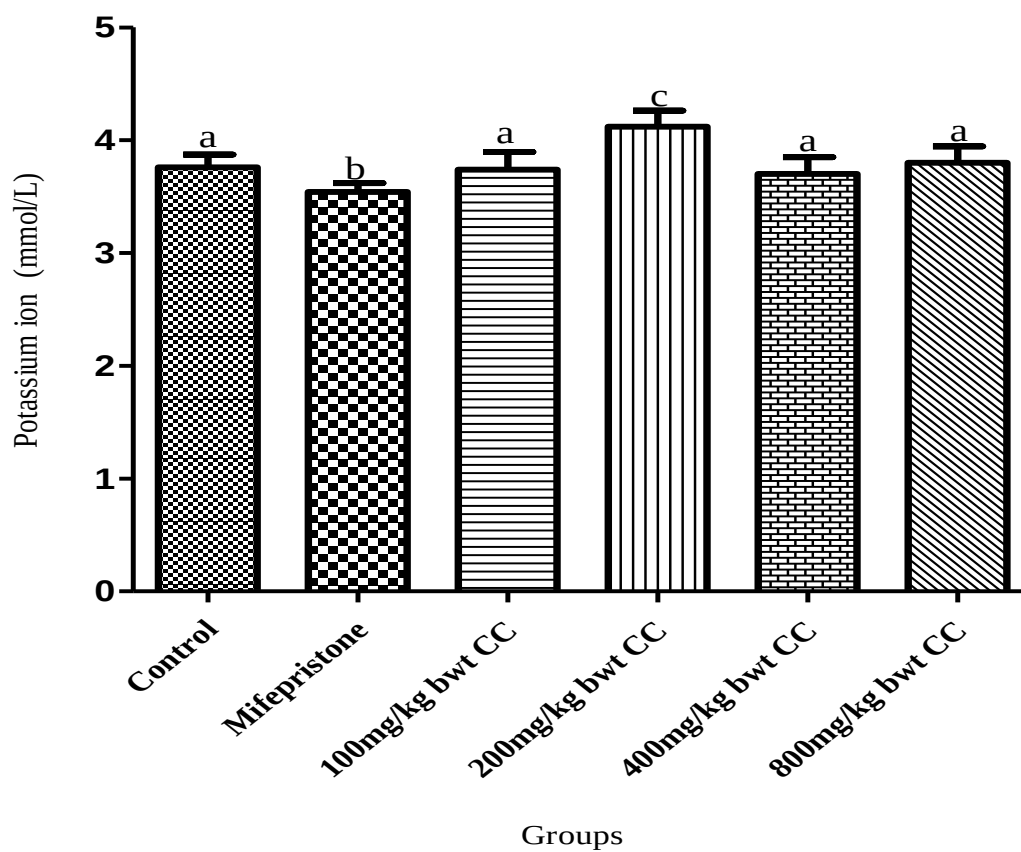


Figure 4.21: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Plasma Potassium ion Level in Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

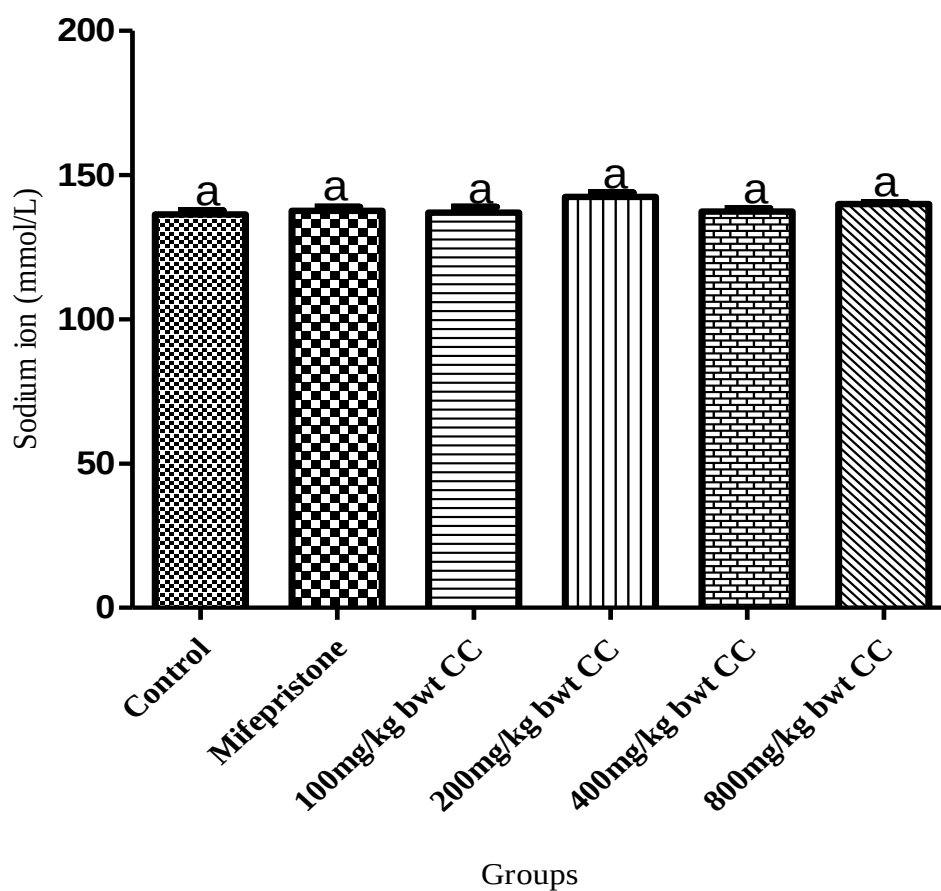


Figure 4.22: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Plasma Sodium ion Level in Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

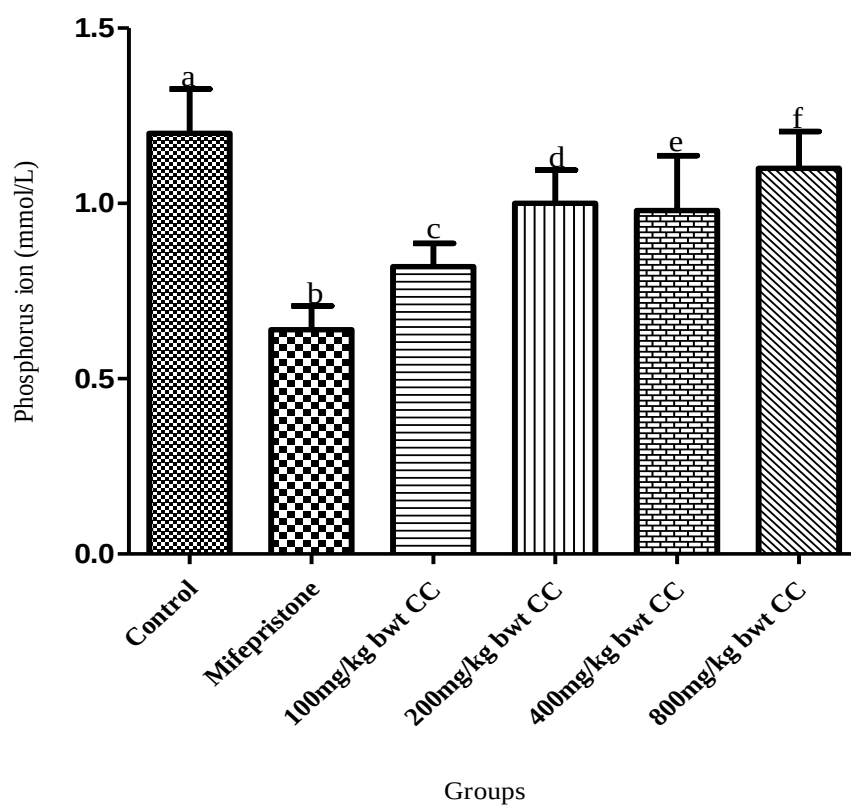


Figure 4.23: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Plasma Phosphorus ion Level in Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

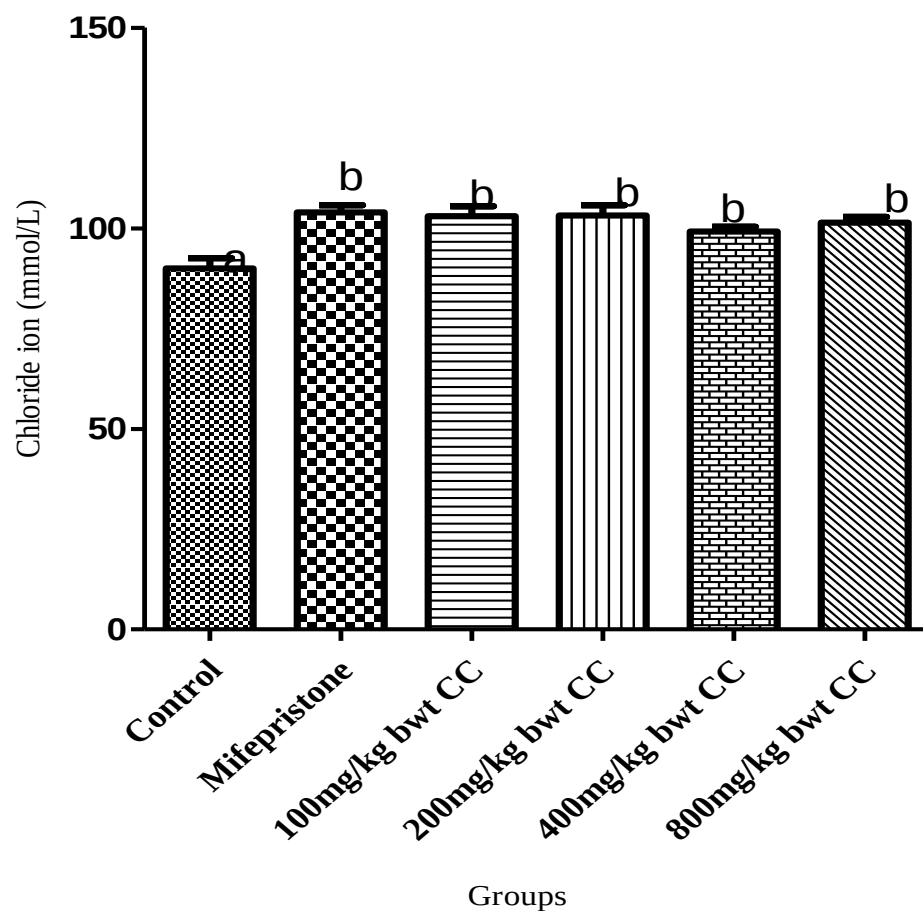


Figure 4.24: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Plasma Chloride ion Level in Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

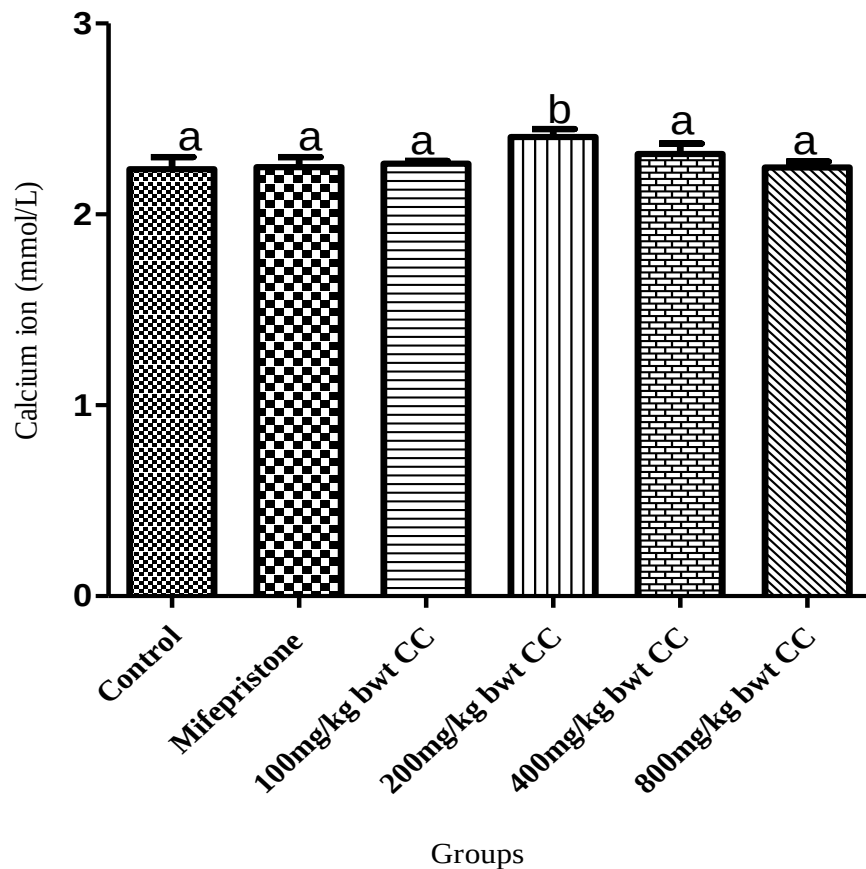


Figure 4.25: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Plasma Calcium ion Level in Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control

CC: *Citrullus colocynthis*.

4.5.6 Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* on Lipid Peroxidation

The administration of *Citrullus colocynthis* pods of ethanolic extract at various concentrations increased significantly ($p < 0.05$) malondialdehyde activity level in the serum of all the treated rats and the mifepristone group when compared with control (Figure 4.26)

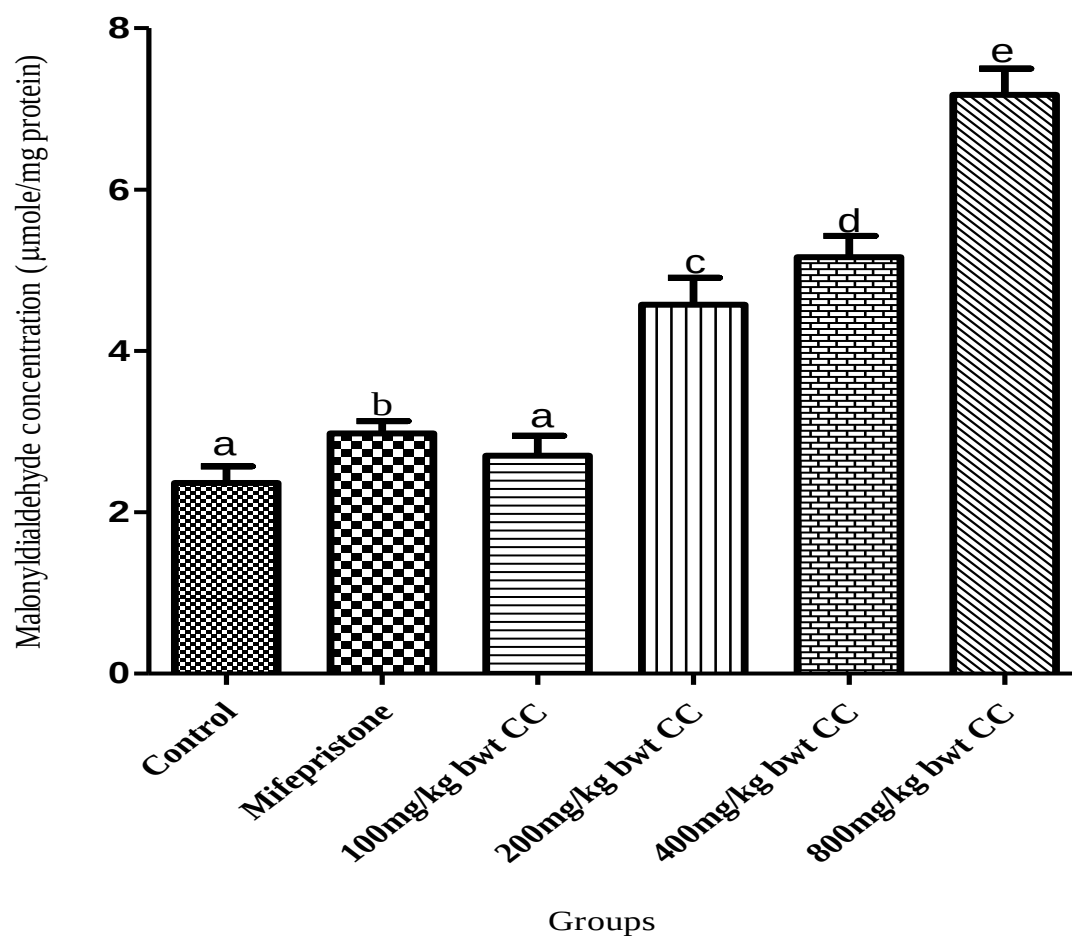


Figure 4.26: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Malonyldialdehyde Activity in Serum of Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.7 Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* on some Antioxidant Enzymes

A significant ($p < 0.05$) decreased was obtained for the superoxide dismutase activity concentration for all treatment groups and mifepristone treated as compared with the control (Figure 4.27). The catalase activity on the other hand was reduced significantly ($p < 0.05$) in all the groups administered with the extract and mifepristone except for 100mg/kg body weight group that showed no significant ($p > 0.05$) alteration in its activity from the control (Figure 4.28).

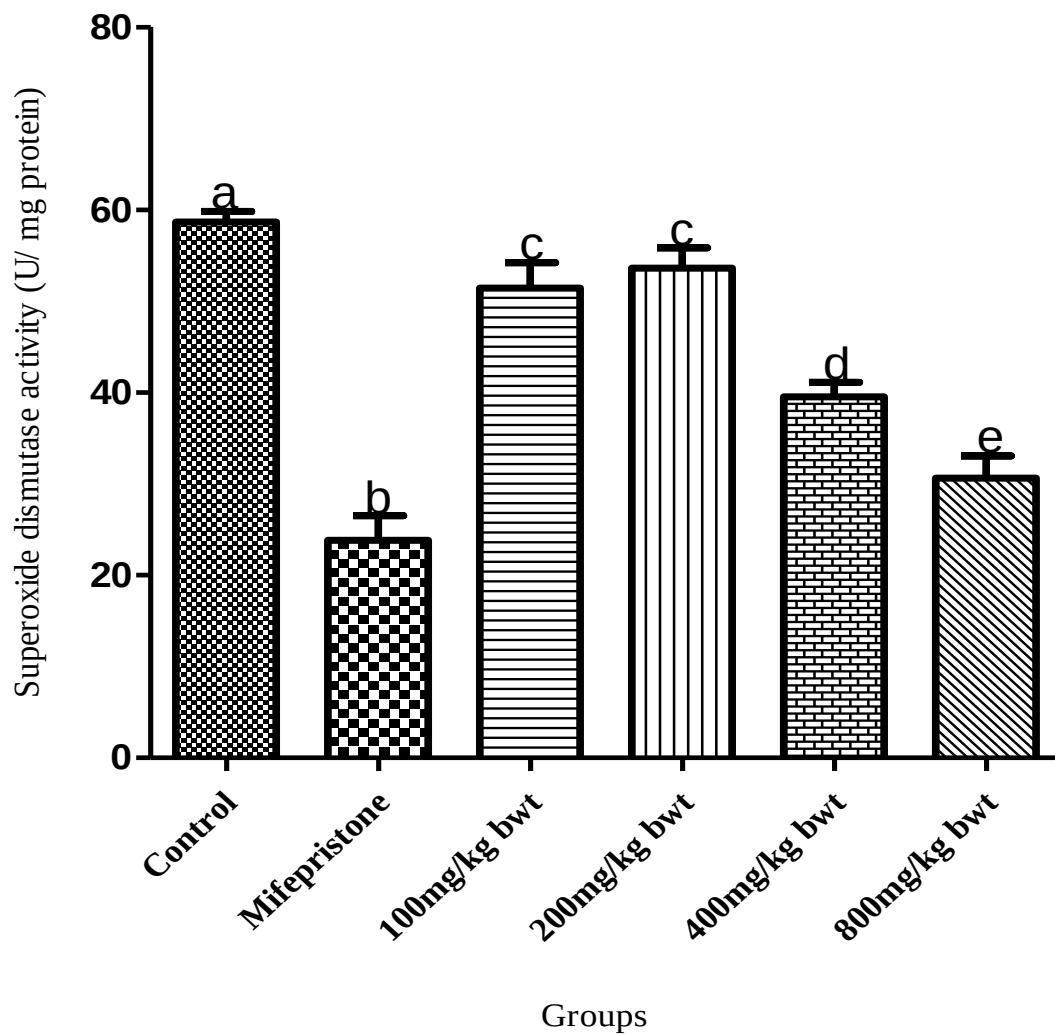


Figure 4.27: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Superoxide Dismutase Activity in Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

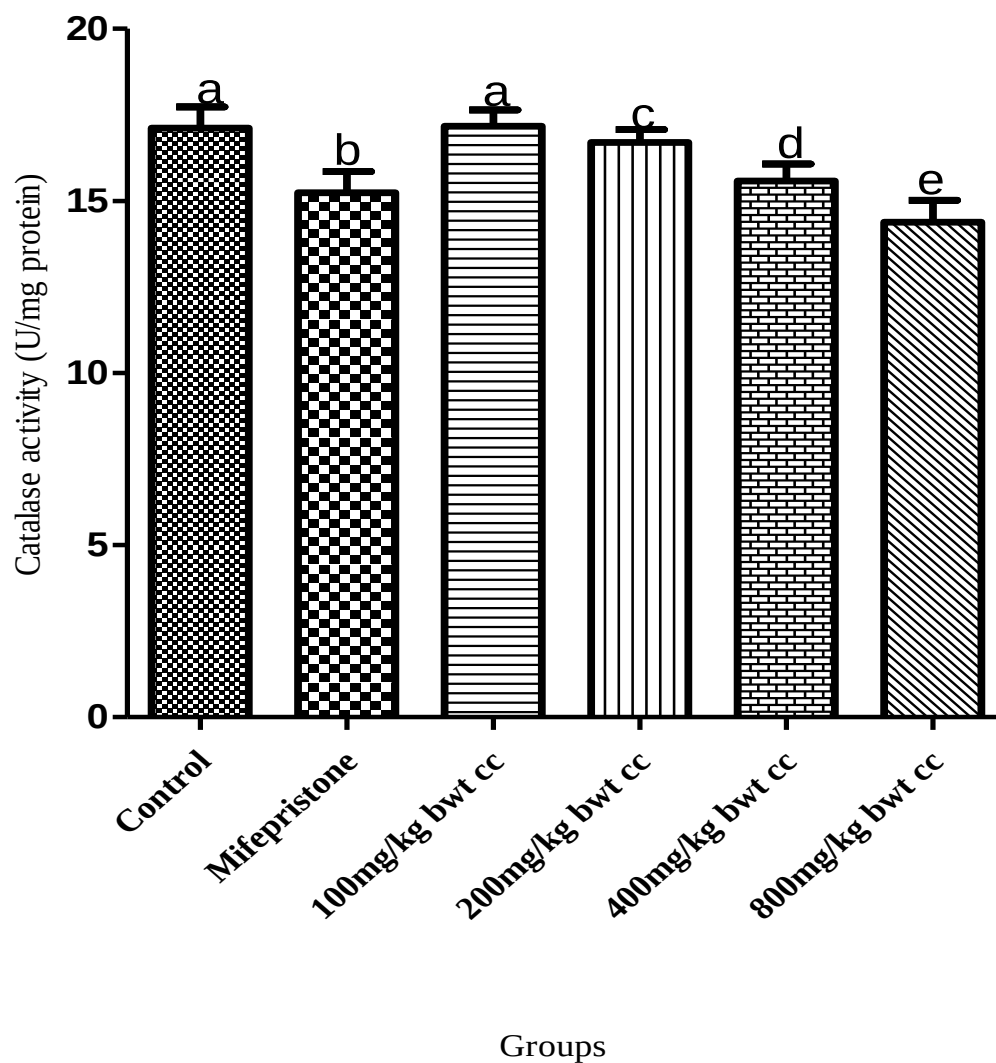


Figure 4.28: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Catalase Activity in Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

4.5.8 Effect of Twenty-one Days Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Pregnant Female Wistar Rats Hormonal Profile

It was observed that a prolonged administration of *Citrullus colocynthis* pod extract at various doses 100, 200, 400, 800mg/kg body weight as well as mifepristone to pregnant rats caused a significant decrease ($p < 0.05$) on progesterone, oestrogen, luteinizing and follicle stimulating hormone, and prolactin concentration levels in a dose dependent manner respectively (Figure 4.29 - 4.33).

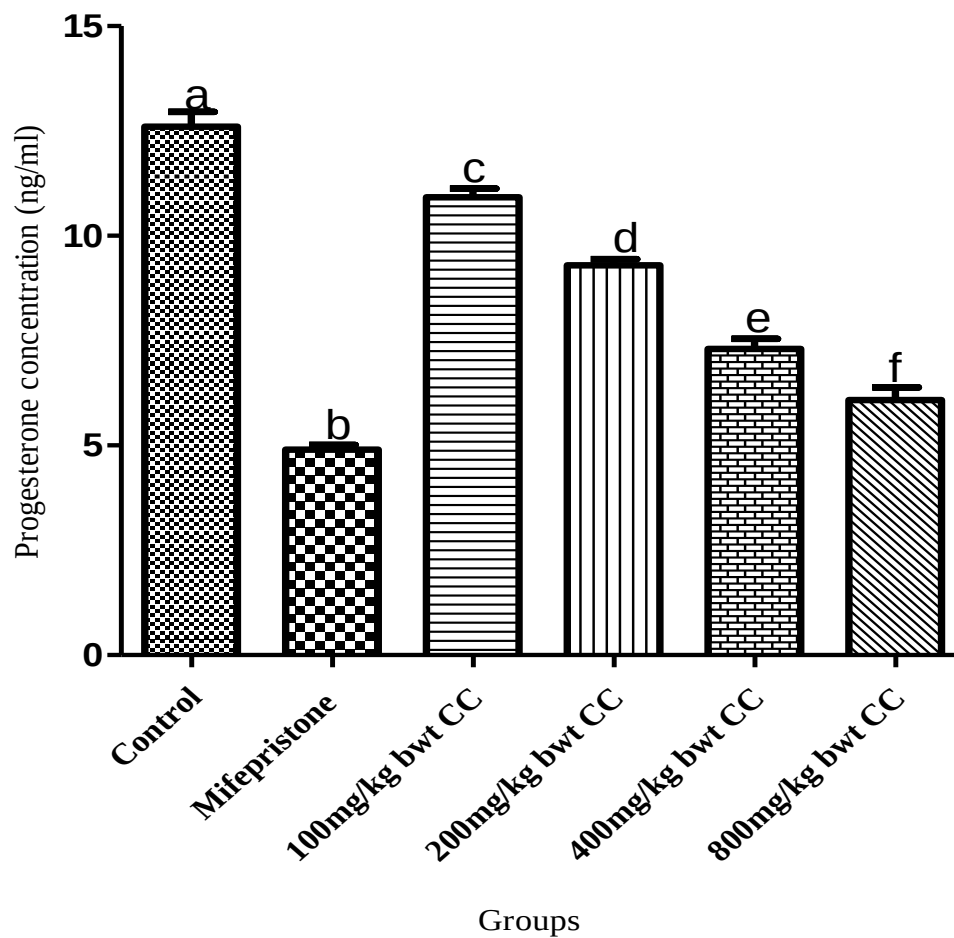


Figure 4.29: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days on the Progesterone Level of Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

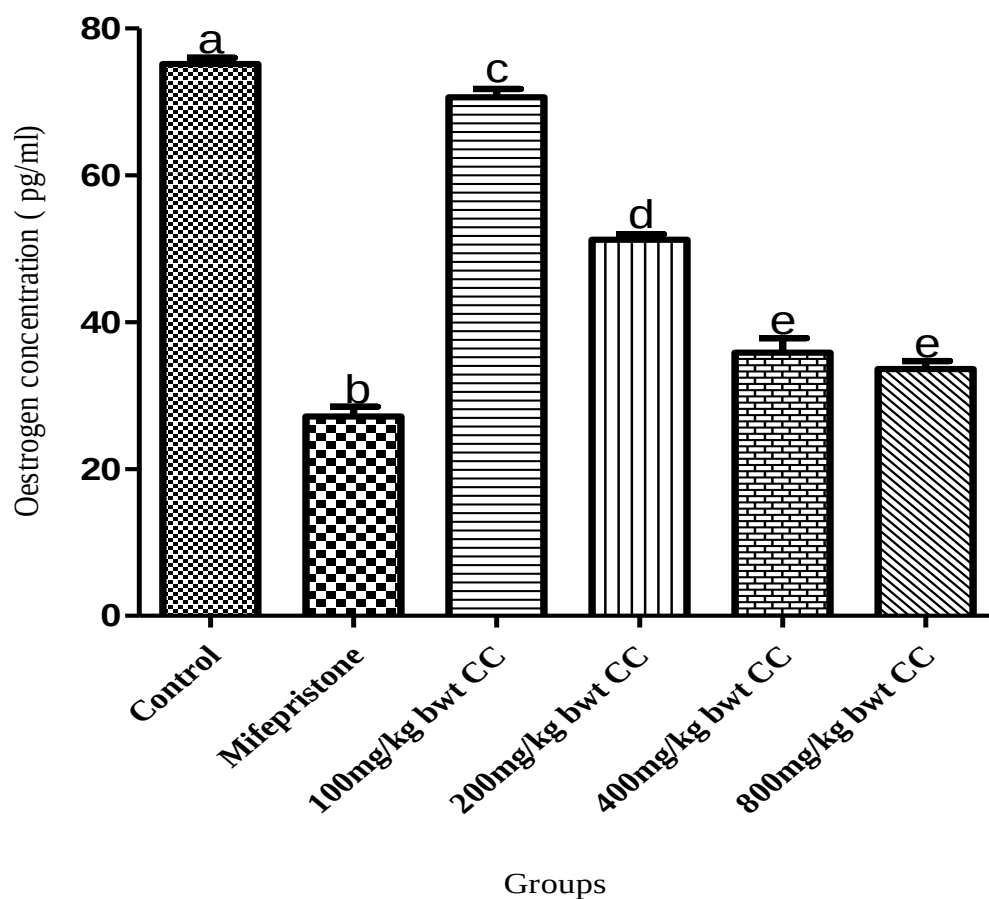


Figure 4.30: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days on the Oestrogen Level of Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

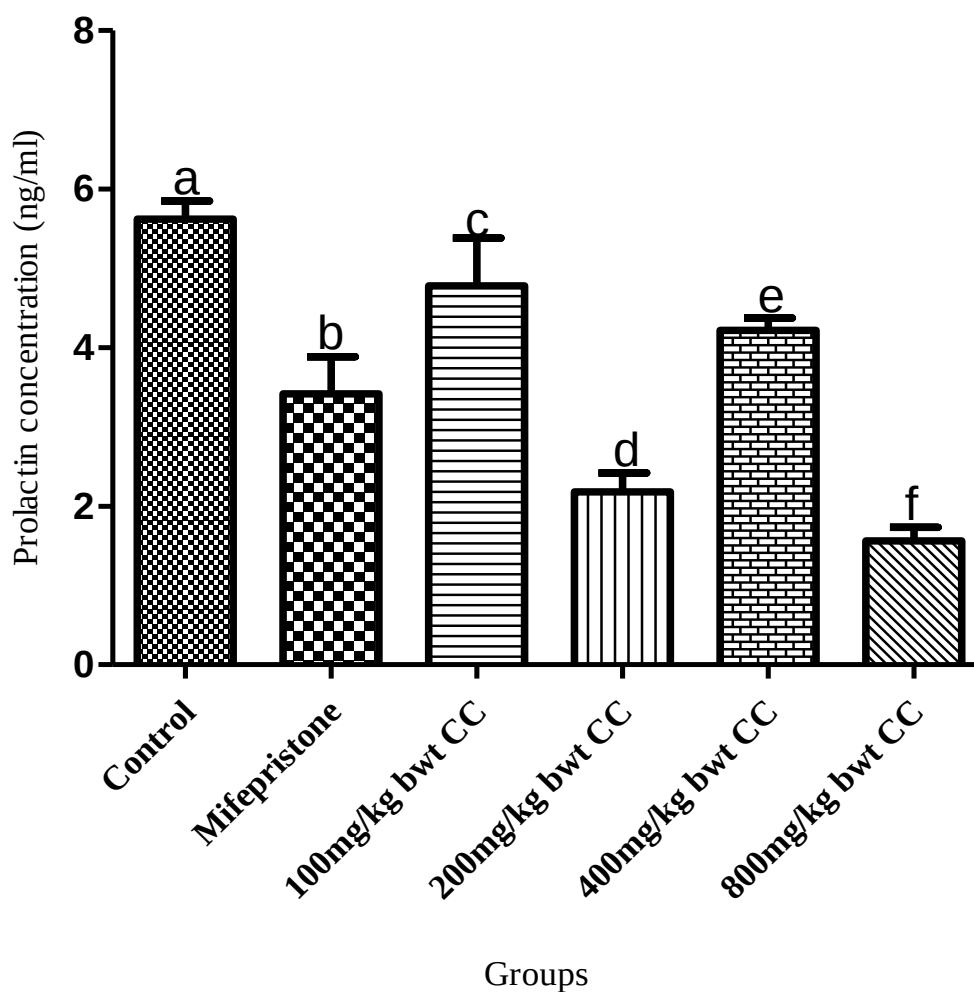


Figure 4.31: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days on the Oestrogen Level of Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

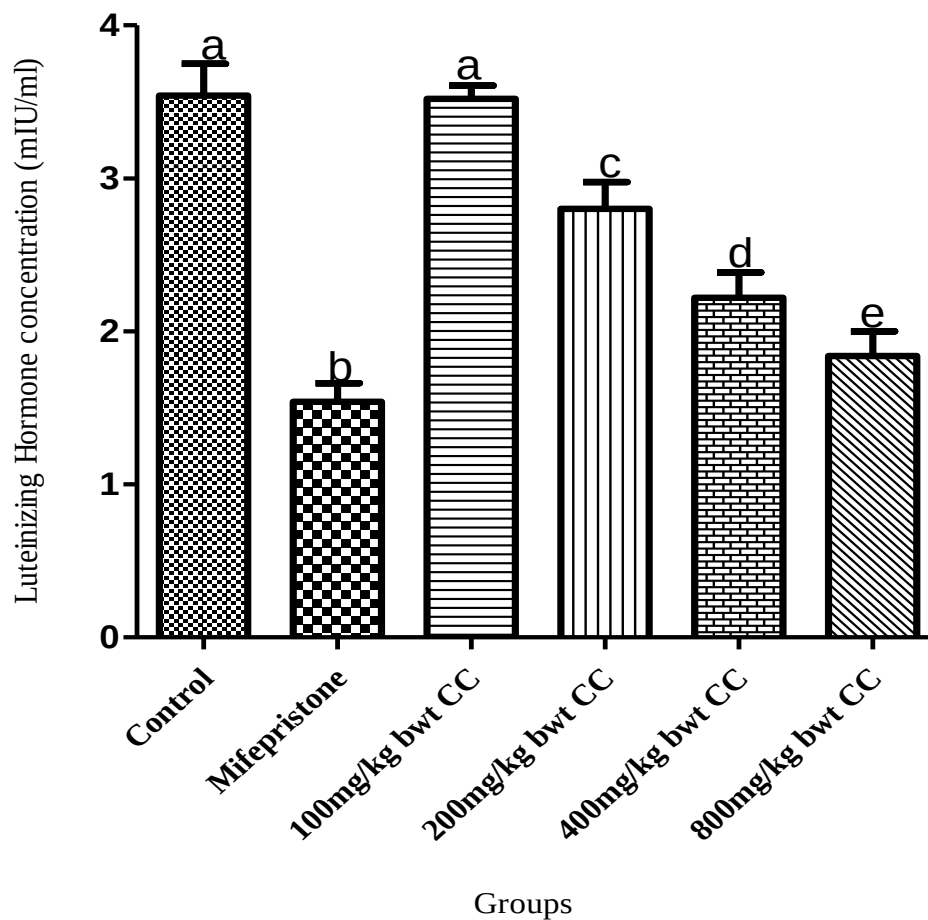


Figure 4.32: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days on the Luteinizing Hormone Level of Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

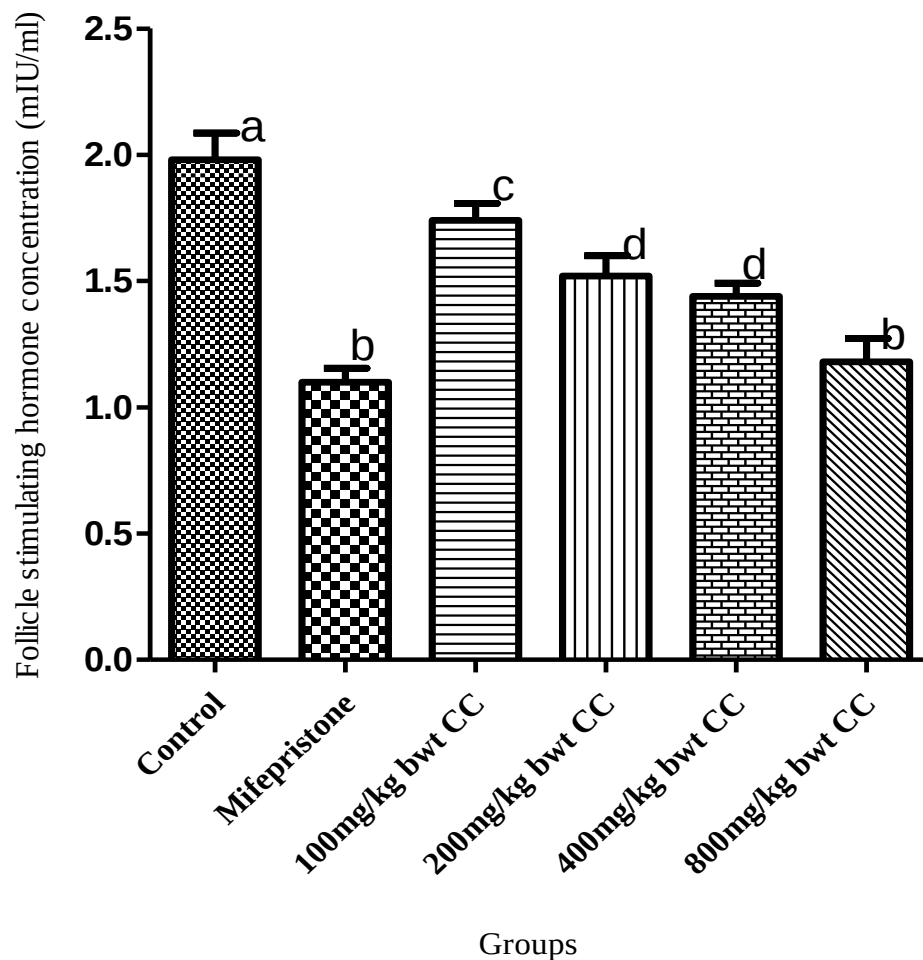


Figure 4.33: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods for Twenty One Days on the Follicle Stimulating Hormone Level of Pregnant Wistar Rats
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.9 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Lipid Profile of Pregnant Female Wistar Rats.

Ethanolic extract of *Citrullus colocynthis* pods administered showed significant ($p < 0.05$) reduction in triacylglycerol of pregnant rats at dose 200 and 400mg/kg body weight while there was no significant difference ($p > 0.05$) in the other treated groups with mifepristone when compared with the control (Figure 4.34). Consequently, significant ($P < 0.05$) increase observed in concentration of total cholesterol as well as low density lipoprotein cholesterol of all the treated groups alongside mifepristone group when compared with the control, whereas the reverse is the case of high density lipoprotein cholesterol (Figure 4.34- 4.37). The atherogenic index significantly ($p < 0.05$) increased with increase in dose (Figure 4.38).

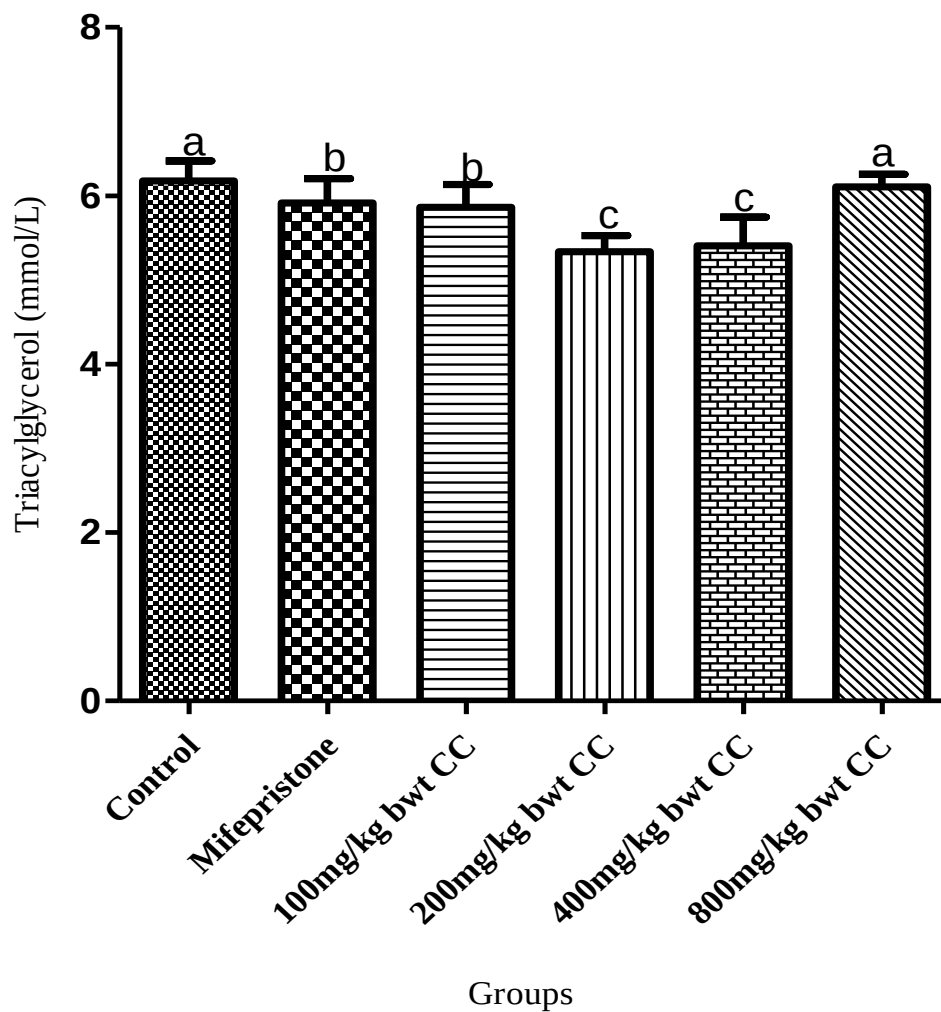


Figure 4.34: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Triacylglycerol Level of Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

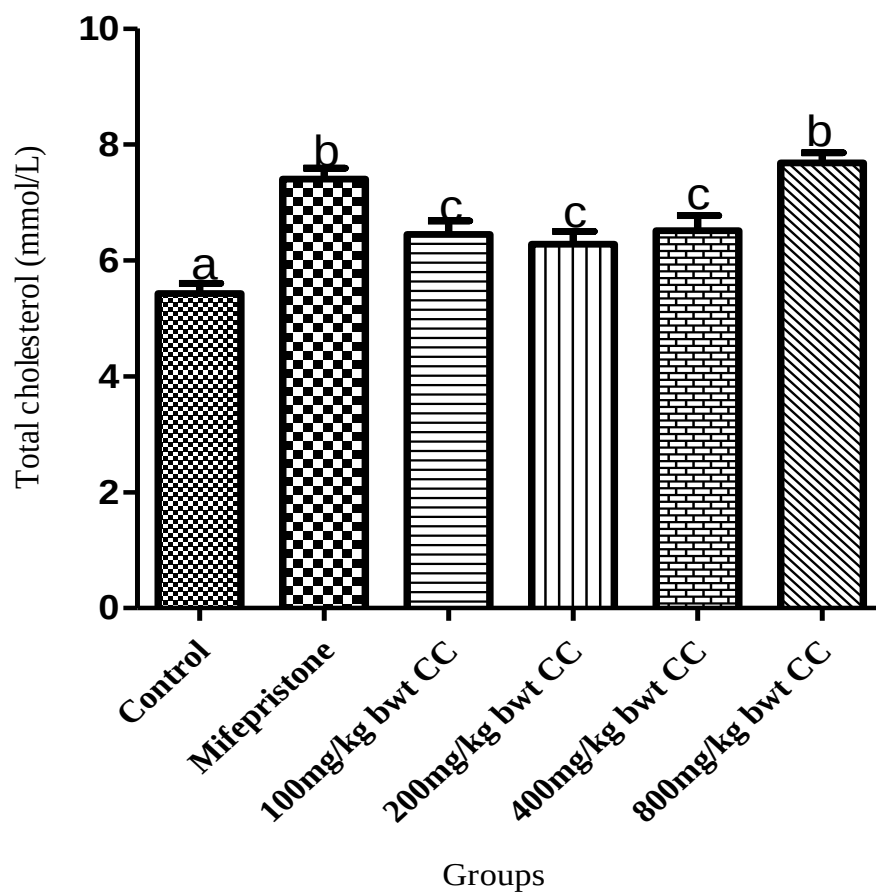


Figure 4.35: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Total Cholesterol of Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

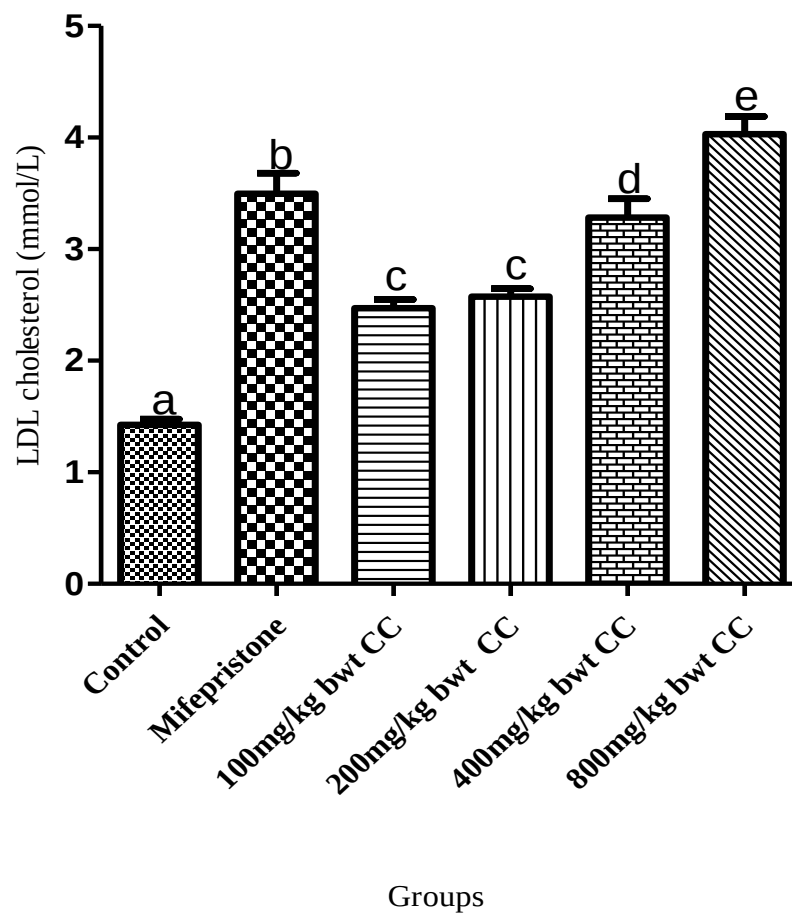


Figure 4.36: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum Low Density Lipoprotein Cholesterol Level of Pregnant Wistar Rats.

Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

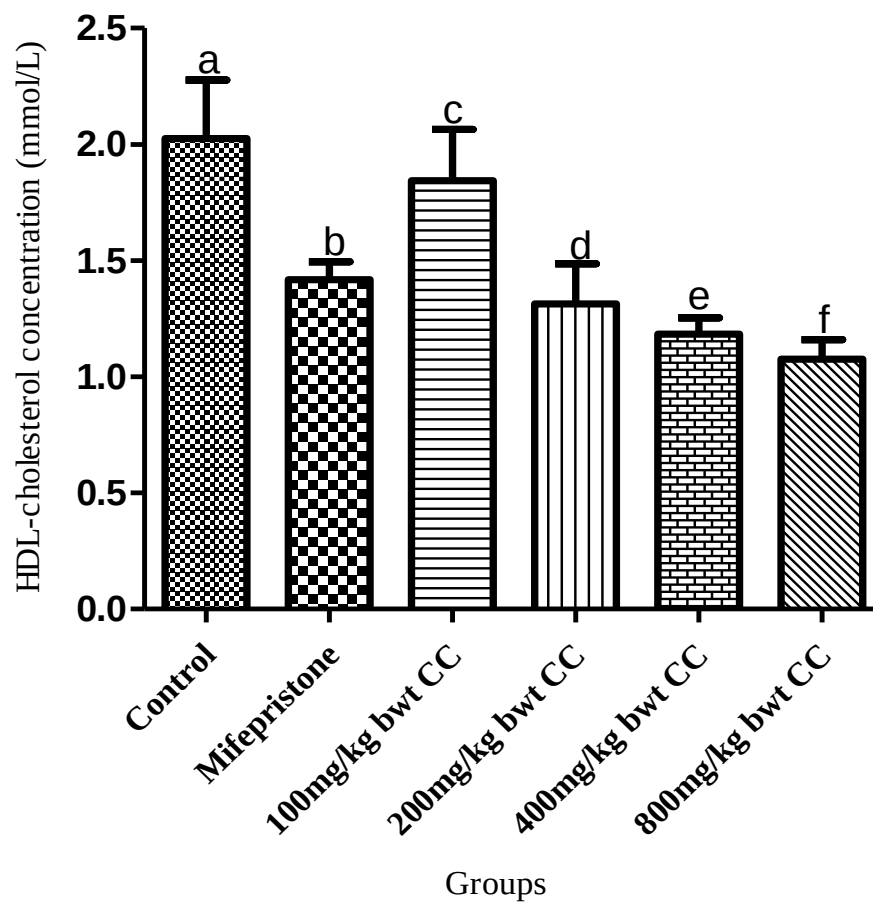


Figure 4.37: Effect of Administration of Ethanolic Extract of *Citrullus colocynthis* Pods on Serum High Density Lipoprotein Cholesterol of Pregnant Wistar Rats. Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

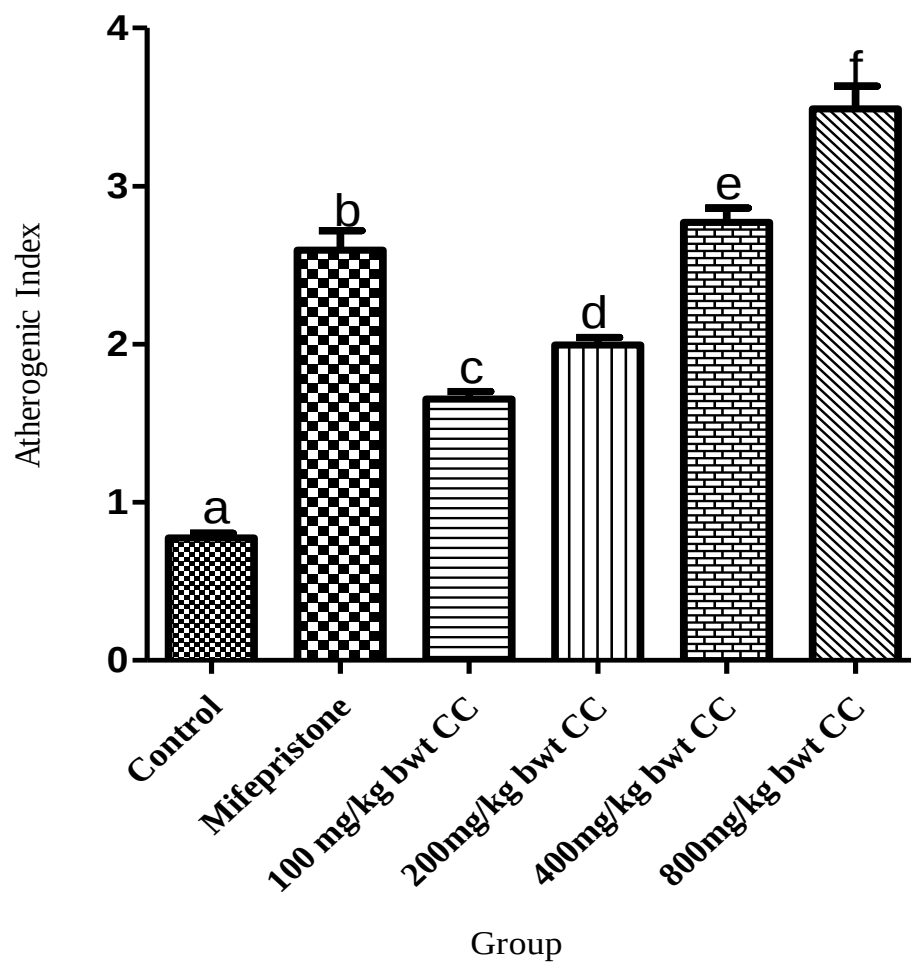


Figure 4.38: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Atherogenic Index of Pregnant Wistar Rats.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.10 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Chromosomal Aberration of Pregnant Female Wistar Rats Bone Marrow

Figure 4.39 and 4.40 reveals a significant ($p < 0.05$) increase in metaphase chromosomal aberration (MCA), percentage chromosomal aberration count (CA %) of all the treated groups and mifepristone treated in comparison with the control while the reverse was seen in total number of metaphase analysed (TNMA) as well as the mitotic index (MI) when compared with the control (Figure 4.41 and 4.42)

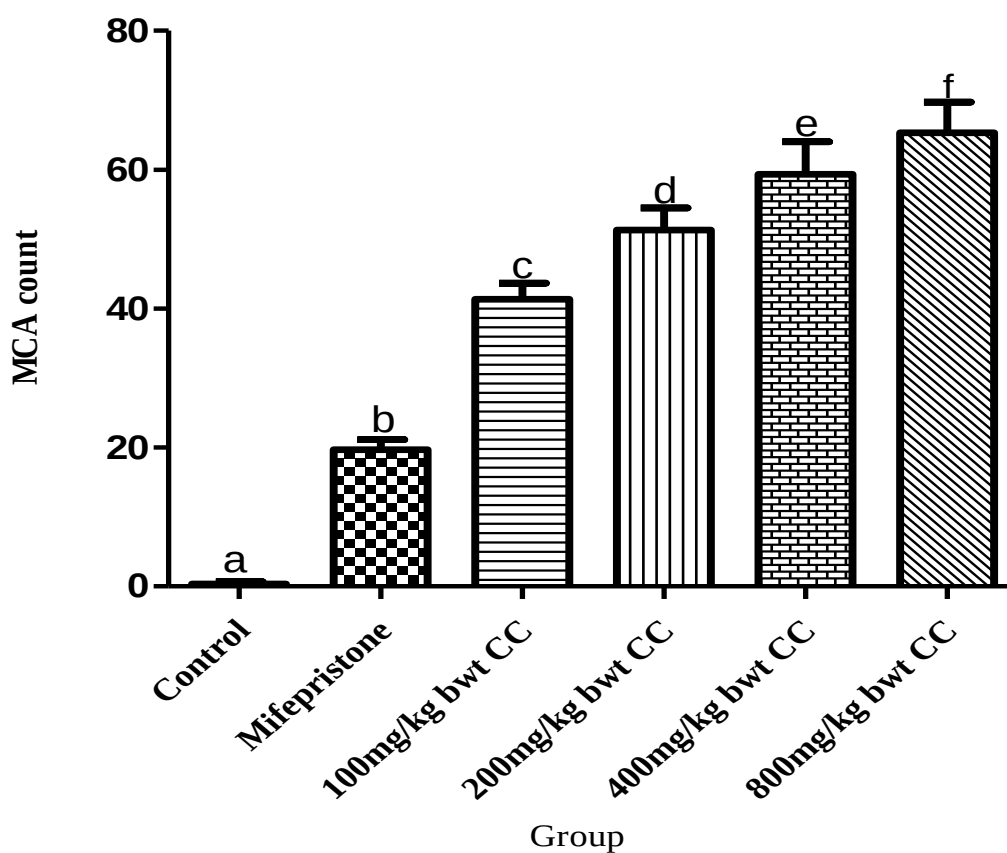


Figure 4.39: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on MCA count of Pregnant Wistar Rats Bone Marrow.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

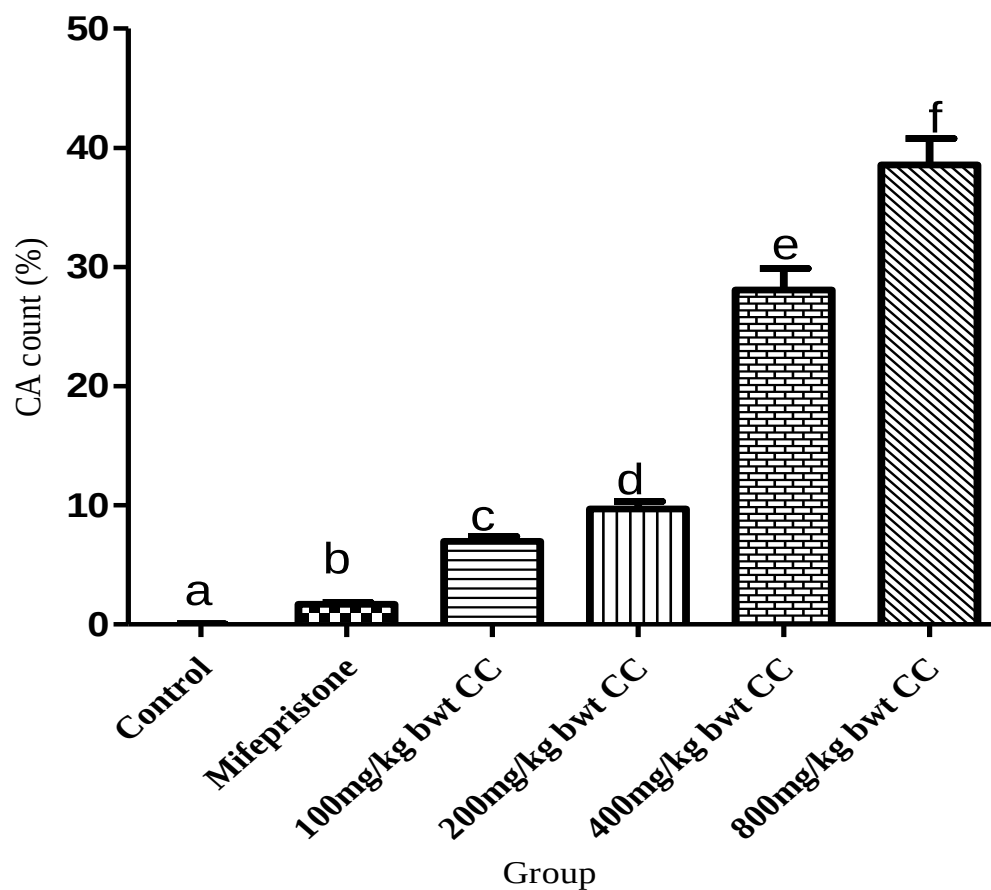


Figure 4.40: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Chromosomal Aberration Count of Pregnant Wistar Rats Bone Marrow.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

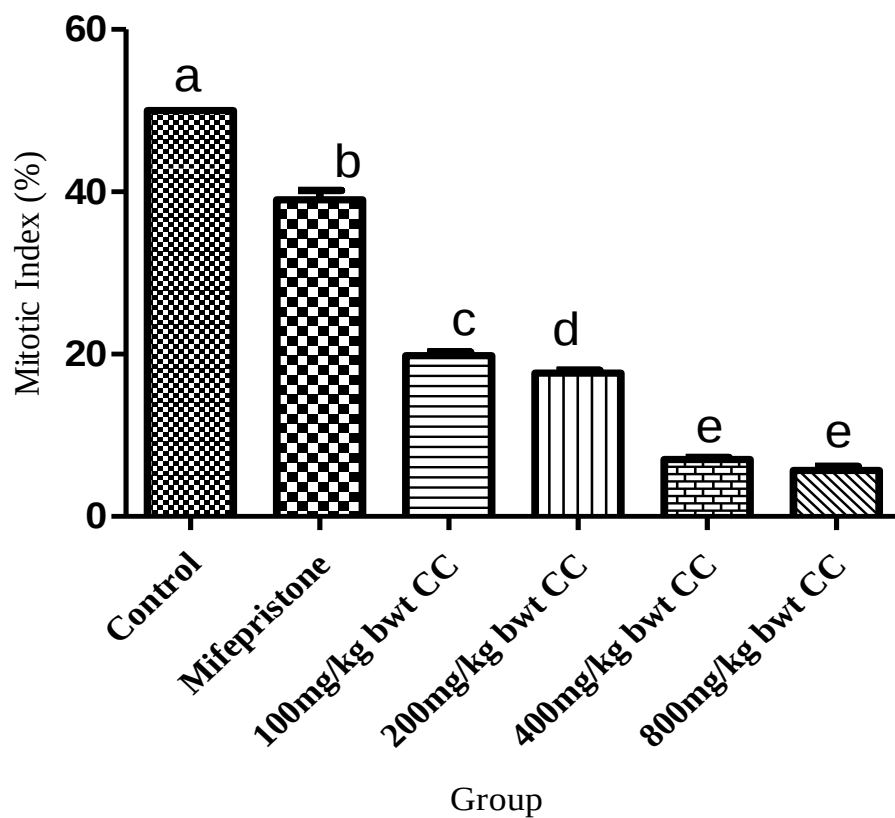


Figure 4.41: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Mitotic Index Level of Pregnant Wistar Rats Bone Marrow. Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

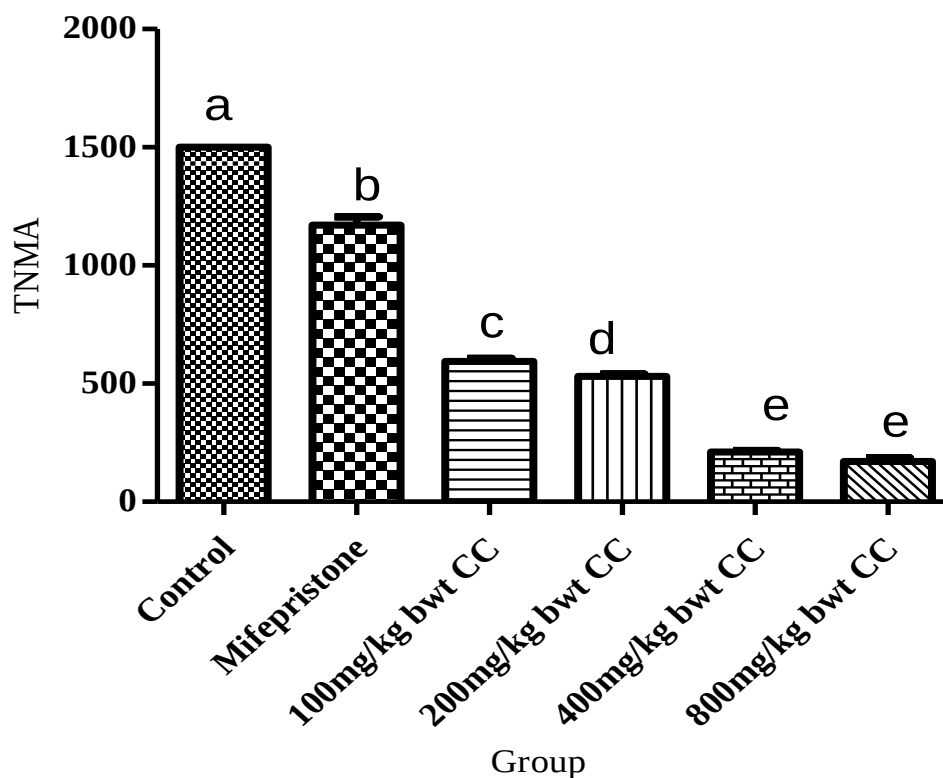


Figure 4.42: Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on TNMA of Pregnant Wistar Rats Bone Marrow.
 Bars are mean $\pm$ SEM of five determinations. Different letters on the bars shows they are significantly different at ($p < 0.05$) from the control.

CC: *Citrullus colocynthis*

4.5.11 Effect of Ethanolic Extract of *Citrullus colocynthis* Pods on Pregnant Female Wistar Rats Haematological Parameters

The ethanolic extract *Citrullus colocynthis* administered to pregnant rats as well as mifepristone for twenty-one days showed that there was significant decrease ($p < 0.05$) in Platelet, Total WBC, MCV, and MCH levels (Table 4.6) while there were no ($p > 0.05$) significant changes in the levels of Hb Count, RBC concentration and PCV (%) compared with control.

Table 4.6: Haematological Parameters on Pregnant Female Wistar Rats Treated Orally with *Citrullus colocynthis* Pods Extract

Parameters	Control	Mifepristone	100mg/kg bwt CC	200mg/kg bwt CC	400mg/kg bwt CC	800mg/kg bwt CC
PCV (%)	35.80 ± 0.86 ^a	36.40 ± 1.91 ^a	34.20 ± 2.40 ^a	35.40 ± 2.62	36.00 ± 1.45 ^a	37.20 ± 2.62 ^a
Hb Count (g/dl)	11.36 ± 0.43 ^a	11.04 ± 0.64 ^a	10.74 ± 0.91 ^a	10.92 ± 0.78 ^a	10.54 ± 0.52 ^a	11.36 ± 0.86 ^a
RBC Conc. (x 10¹²/L)	5.84 ± 0.16 ^a	6.66 ± 0.36 ^a	7.76 ± 0.78 ^a	7.42 ± 0.78 ^a	6.72 ± 0.39 ^a	5.94 ± 1.15 ^a
MCHC (gm%)	31.72 ± 0.55 ^a	30.30 ± 0.46 ^{a,b}	31.26 ± 0.64 ^a	30.88 ± 0.26 ^a	29.26 ± 0.48 ^b	30.52 ± 0.40 ^{a,b}
MCH (pg)	19.50 ± 0.94 ^a	16.60 ± 0.67 ^b	14.26 ± 1.74 ^c	15.78 ± 2.58 ^{b,c}	15.84 ± 0.92 ^{b,c}	21.96 ± 4.23 ^a
MCV (FL)	61.60 ± 2.32 ^a	54.80 ± 2.27 ^b	45.40 ± 5.16 ^c	51.00 ± 3.54 ^b	54.00 ± 3.27 ^b	57.20 ± 4.59 ^{a,b}
Platelet Count (x 10⁹/L)	388.76 ± 3.58 ^a	370.68 ± 1.33 ^b	347.96 ± 6.34 ^c	342.82 ± 1.80 ^c	329.32 ± 5.20 ^d	329.56 ± 1.45 ^d
Total WBC (x 10⁹/L)	25.82 ± 3.53 ^a	16.26 ± 0.35 ^b	15.40 ± 1.00 ^b	13.48 ± 1.41 ^b	15.84 ± 1.54 ^b	16.66 ± 1.03 ^b

Values are mean (n=5) ± SEM

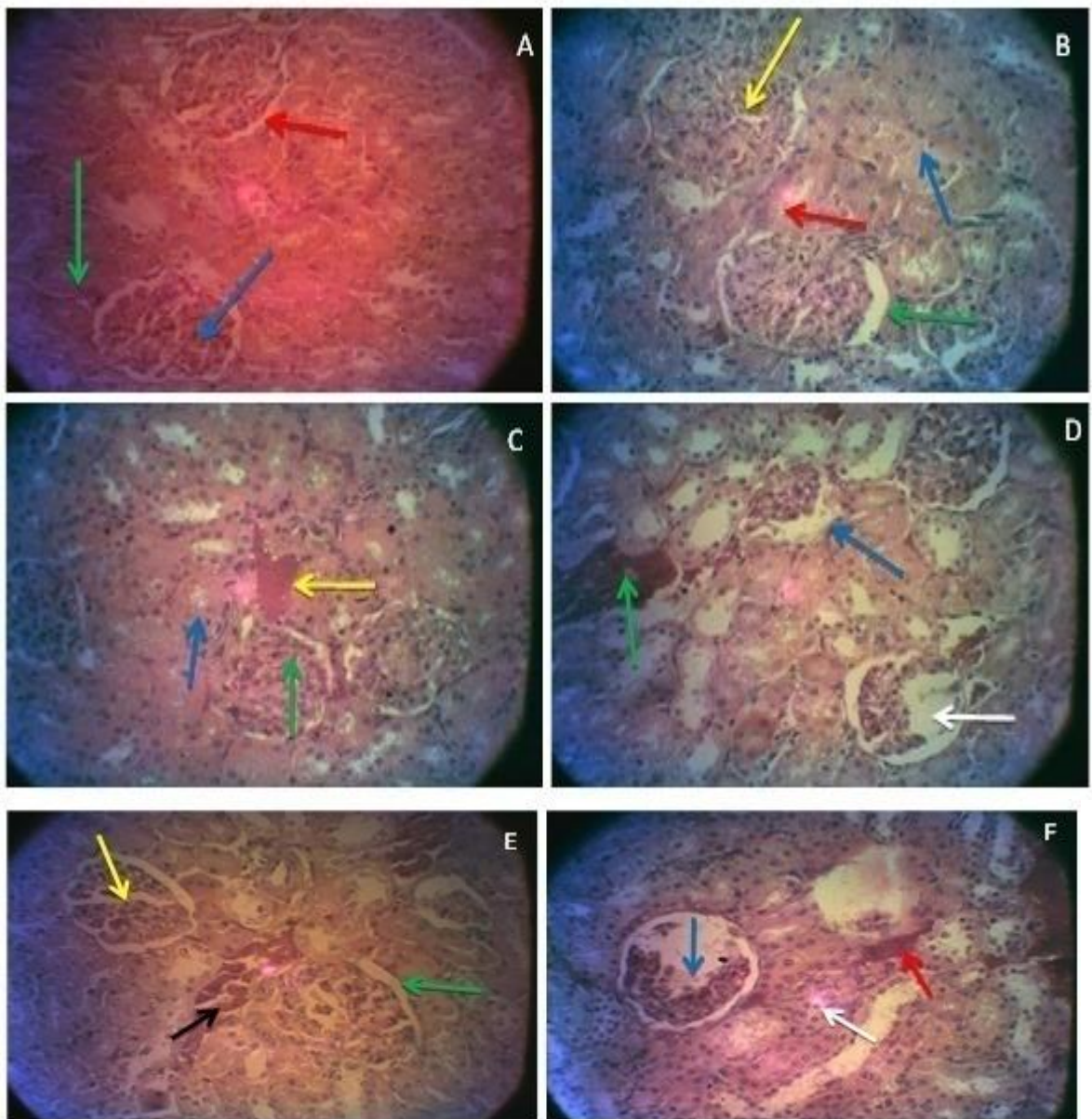


Figure 4.43: Cross Section of the Kidney

- A:** Photomicrograph of rat kidney administered with 0.5ml olive oil. The (blue arrow) indicate normal renal histo architecture with intact glomerulus, (red arrow) shows the bowman's capsule and renal tubules (green arrow). (x 400; H&E)
- B:** Photomicrograph of rat kidney administered with 1.84mg/kg bwt of Mifepristone. The (red arrow) specify mild hemorrhage in the renal tubules, intact glomerulus (yellow arrow), capsular space (green arrow) and renal tubules (blue arrow) are also intact. (x400; H&E)

- C:** Photomicrograph of rat kidney administered with 100mg/kg bwt *Citrullus colocynthis* pods. There is hemorrhage in the renal tubules (red arrow). The glomerulus (yellow arrow), capsular space (green arrow) and renal tubules (blue arrow) are all intact. (x400; H&E)
- D:** Photomicrograph of rat kidney administered with 200mg/kg bwt *Citrullus colocynthis*. Hemorrhage was observed in the renal tubules (green arrow) and degeneration of the glomerulus (white arrow), the capsular space are dilated (blue arrow). (x400; H&E)
- E:** Photomicrograph of rat kidney administered with 400mg/kg bwt *Citrullus colocynthis*. There is vacuolization in the glomerulus (yellow arrow) surround by Bowman's capsule (green arrow). Hemorrhage is seen in the renal parenchymal (black arrow). (x400; H&E)
- F:** Photomicrograph of rat kidney administered 800mg /kg bwt *Citrullus colocynthis*. It shows degeneration of glomerulus (blue arrow) surround by Bowman's capsule (white arrow). Hemorrhage is clearly seen in the renal parenchymal (red arrow). (x400; H&E)

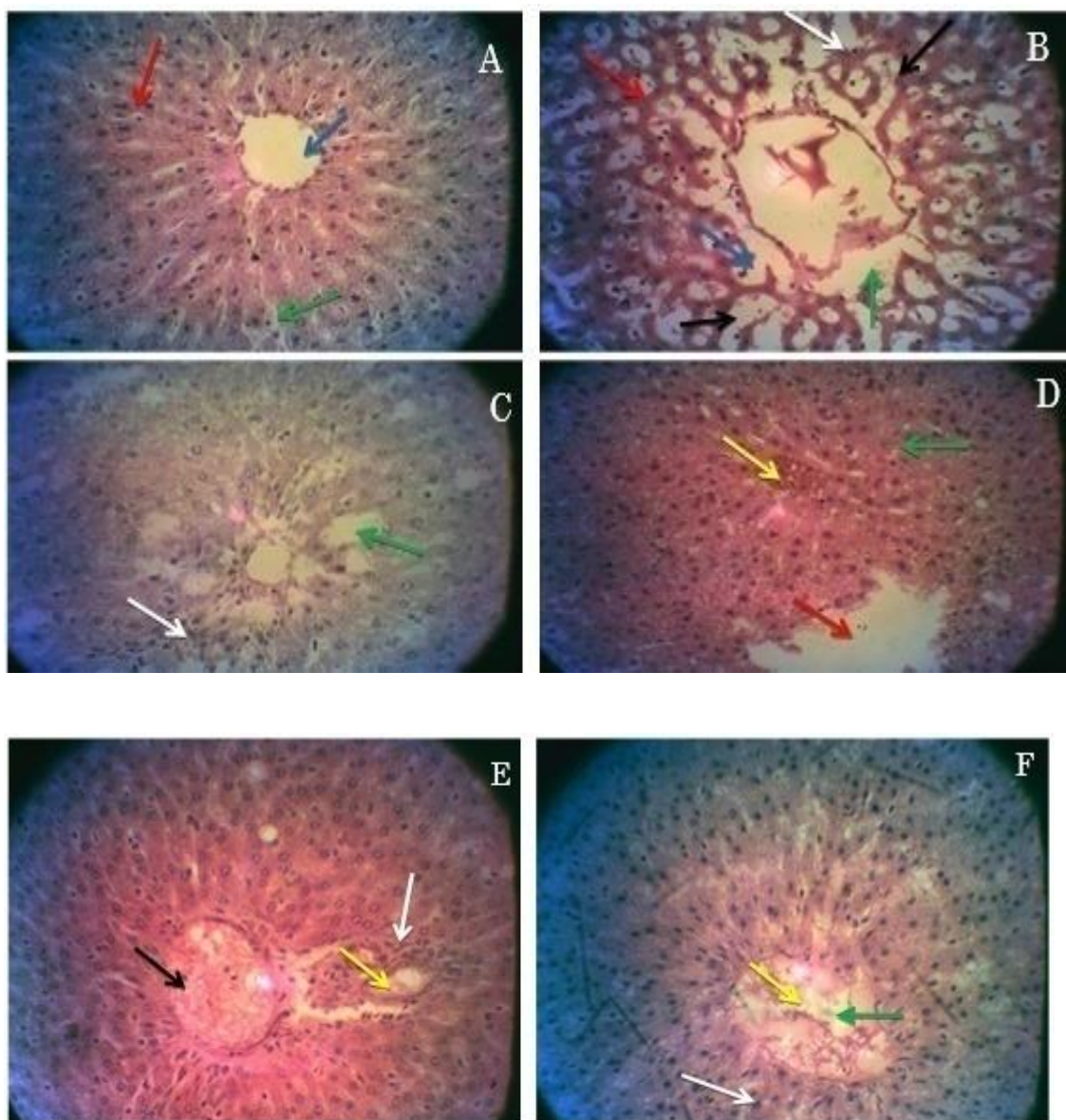


Figure 4.44: Cross Section of the Liver

- A:** Photomicrograph of rat liver administered with 0.5ml of olive oil. It shows normal hepatic histo- architecture with no congestion in the central vein (blue arrow) and hepatocyte (red arrow). The hepatic cells are arranged in a cord from the central vein and are separated by sinusoid (green arrow). (x400; H&E)
- B:** Photomicrograph of rat liver administered with 1.84mg/kg bwt mifepristone. It indicates a fairly distorted hepatic parenchyma (green arrow) characterized by

widened sinusoid (black arrow), visible hepatocytes with no orderly arrangement (blue arrow) and vacuolization around the hepatocytes (red arrow) (x400; H&E)

- C:** Photomicrograph of rat liver administered with 100mg/kg bwt *Citrullus colocynthis*. There is presence of inflammatory cell (white arrow) around the central vein. There are areas of mild degeneration of hepatic parenchyma (green arrow). (x400; H&E)
- D:** Photomicrograph of rat liver administered with 200mg/kg bwt *Citrullus colocynthis* shows congestion in the central vein (yellow arrow) and degeneration of hepatic parenchyma (red arrow). Hepatocytes are visible with necrosis (green arrow). (x400; H&E)
- E:** Photomicrograph of rat liver administered with 400mg/kg bwt *Citrullus colocynthis* shows congestion in the central vein (yellow arrow) and portal vein (black arrow). There is also mild inflammation around the portal vein (white arrow). (x400; H&E)
- F:** Photomicrograph of rat liver administered with 800mg/kg bwt *Citrullus colocynthis* indicate visible central vein with slight congestion (yellow arrow) and hepatocyte are arranged in a cord from the central vein and are separated by sinusoid (white arrow). There is also sinusoidal hemorrhage (green arrow). (x400; H&E)

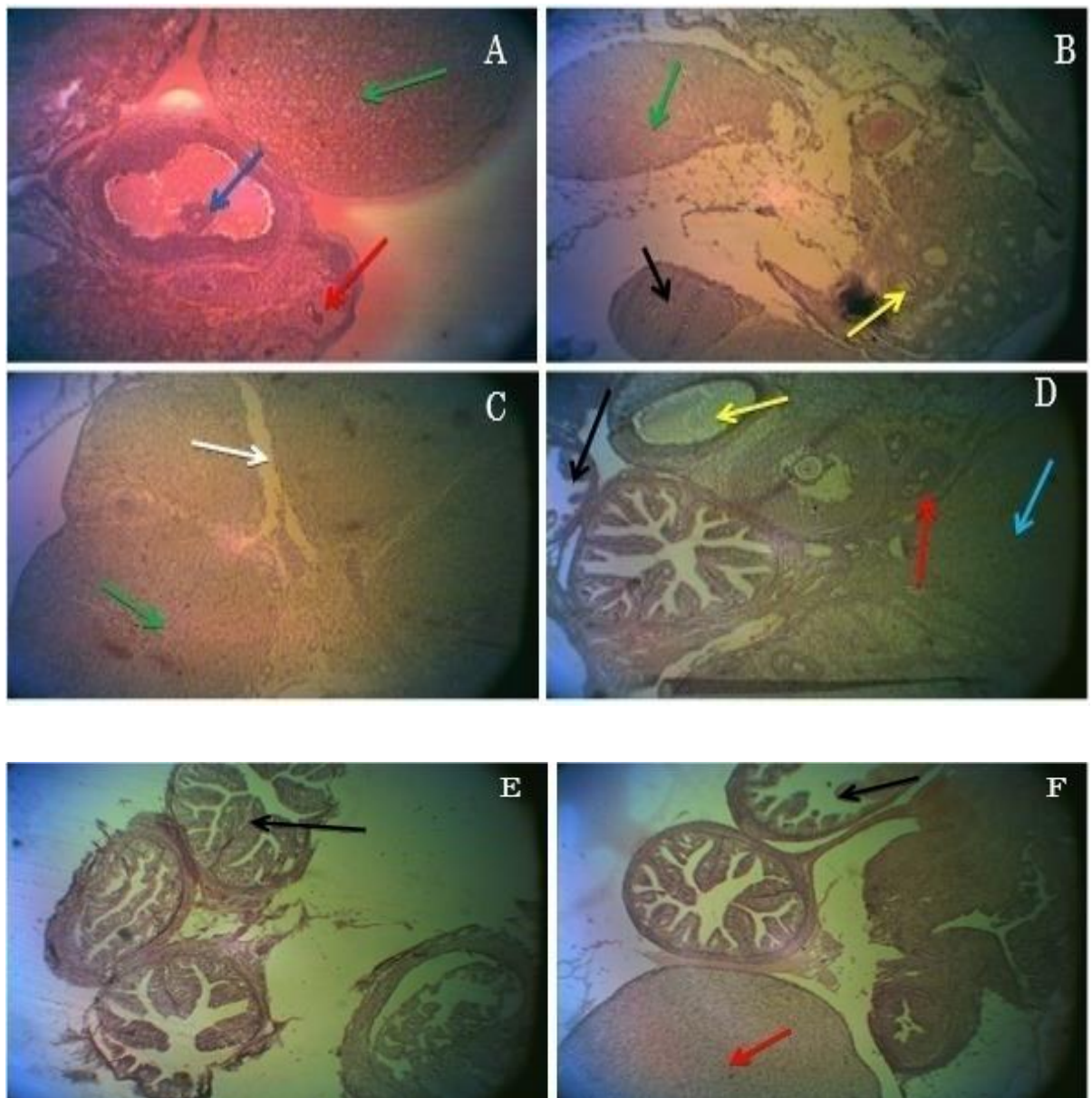


Figure 4.45: Cross Section of the Ovary

- A:** Photomicrograph of rat ovary administered with 0.5ml of olive oil. It specifies the presence of mature follicle with primary oocyte (blue arrow), regressive corpus luteum (green arrow) and Primary oocytes and primary follicles (red arrow). (x400; H&E)
- B:** Photomicrograph of rat ovary administered with 1.84mg/kg bwt of mifepristone. It indicates the presence of Primordial follicles (yellow arrow),

regressive corpus luteum (black arrow) and corpus luteum (green arrow).
(x400; H&E)

- C:** Photomicrograph of rat ovary administered with 100mg/kg bwt *Citrullus colocynthis* showing damage to the medulla (white arrow), regressive corpus (green arrow). (x400; H&E)
- D:** Photomicrograph of rat ovary administered with 200mg/kg bwt *Citrullus colocynthis* shows presence of mature follicle with primary oocyte(yellow arrow), regressive corpus luteum (blue arrow), corpus luteum (black arrow) and Primary oocytes and primary follicles (red arrow). (x400; H&E)
- E:** Photomicrograph of rat ovary administered with 400mg/kg bwt *Citrullus colocynthis*. It indicates degenerating corpus luteum (black arrow). (x400; H&E)
- F:** Photomicrograph of rat ovary administered with 800mg/kg bwt *Citrullus colocynthis* shows presence regressive corpus luteum (red arrow) and degenerating corpus luteum (green arrow) (x400; H&E)

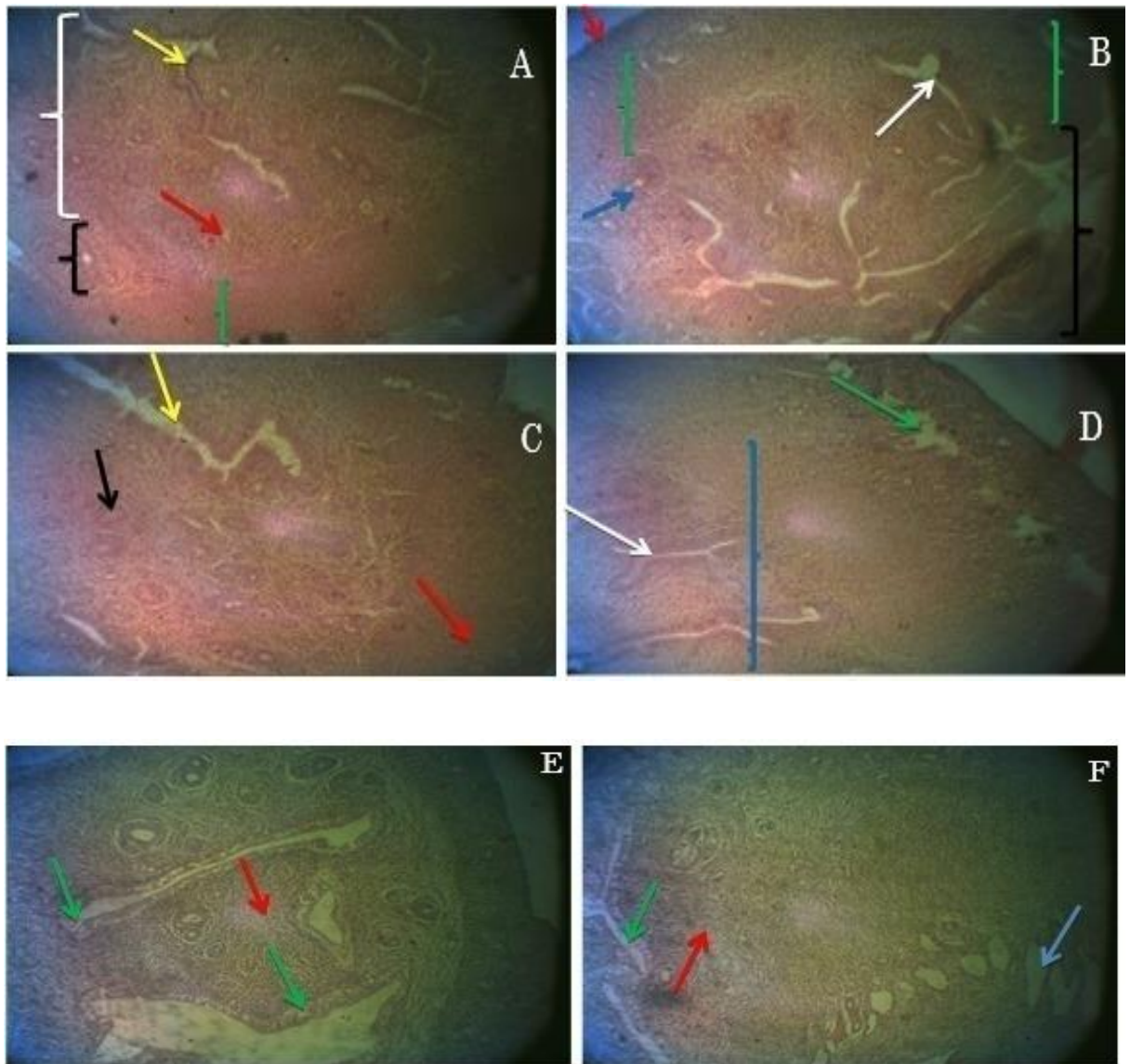


Figure 4.46: Cross Section of the Uterus

- A:** Photomicrograph of rat uterus administered with 0.5ml of olive oil. It shows intact functionalis layer (white brace), basalis layer (black brace), and myometrium (green brace) with the presence of large and coiled uterine gland (yellow arrow), coiled arteries (red arrow). (x400; H&E)
- B:** Photomicrograph of rat uterus administered with 1.84mg/kg bwt mifepristone. Three layer of the uterus the endometrium (black brace), and myometrium (green brace), serosa (blue arrow) with the presence of large and coiled

uterine gland (white arrow), coiled arteries (blue arrow) was observed (x400; H&E)

- C:** Photomicrograph of rat uterus administered with 100mg/kg bwt *Citrullus colocynthis* shows numerous blood vessel in the myometrium (red arrow) and endometrium layer is intact (black brace) with the presence of large and coiled uterine gland (yellow arrow). (x400; H&E)
- D:** Photomicrograph of rat uterus administered with 200mg/kg bwt *Citrullus colocynthis* shows mild vacuolization of the myometrium (green arrow). The endometrium layer is intact (blue brace) with the presence of large and coiled uterine gland (white arrow) (x400; H&E)
- E:** Photomicrograph of rat uterus administered with 400mg/kg bwt *Citrullus colocynthis* shows mild degeneration of the simple columnar epithelium lining the uterine gland (green arrow). The supporting connective tissue are loose and less dense (red arrow). (x400; H&E)
- F:** Photomicrograph of rat uterus administered with 800mg/kg bwt *Citrullus colocynthis* shows vacuolization in the myometrium (blue arrow) and mild degeneration of the simple columnar epithelium lining the uterine gland (green arrow). The supporting connective tissue are lose and less dense (red arrow). (x400; H&E)

CHAPTER FIVE

Discussion

5.1 Abortifacient Effect of Ethanolic Extract of *Citrullus colocynthis* Pods

Substances that can interrupt pregnancy could be of significant importance in human fertility control (Gooasekera *et al.*, 1995). There is a wide ranged data available over the years for the use of medicinal plants as an abortifacient from the ethnobotanical survey, but there is need to explore for harmless, efficient, convenient and cheaper alternatives for abortion which must be scientifically proven. This ideology has motivated our interest to search for a plant that will have abortifacient activity and be scientifically justified. Though, research have carried out to scientifically validate the use of some plants such as *Anthocephalus cadamba* (Shaikh *et al.*, 2015), *Musa rosacea* (Srikanth *et al.*, 2013), *Senna alata* (Yakubu *et al.*, 2010) and *Bambusa vulgaris* (Yakubu *et al.*, 2009) as an abortifacient. *Citrullus colocynthis* pods is also one of the medicinal herbs claimed to have abortifacient activity in folklore medicine but the mechanism of it action not validated.

5.1.1 Secondary Metabolites

The screening of ethanolic extract of *Citrullus colocynthis* has revealed that the presence of many phytoconstituents like alkaloids, saponins, phenolics, steroids, glycosides, flavonoids, tannins, phlobatannins which either work alone or combine to induce abortion in rats by disruption of pregnancy or altering the development of the fetus. Alkaloids, phenolics, saponins, steroids, glycosides have been reported to either act alone or in combination to bring about the pregnancy terminating effects in rats (Yakubu *et al.*, 2010; Srikanth *et al.*, 2013). Also, sex hormones such as sterols present in plants had been suggested to be liable to the antifertility effects observed in the administration of leaves of *Indigofera trifoliata* to pregnant animals (Sani and

Sule, 2007). *Graptophyllum pictum* was also reported to suppress normal contraction of the uterine and elevated anti-implantation activity due to the presence of an alkaloids-like constituent in the extract (Stella *et al.*, 2009). Thus, these secondary metabolites like alkaloids, flavonoids, steroids, phenols, saponins present in *Citrullus colocynthis* ethanolic extract could contribute to its abortifacient and anti-implantation activity. Furthermore, the presence of some other metabolites like the gallic acid, catechin, curbitacin B and quercetin with myristin suggest that the ethanolic extract of *Citrullus colocynthis* contain some active polyphenol of medicinal importance.

These phytochemicals can also serve as an active component in synthetic drug production.

5.1.2 Abortifacient parameters

The reduction observed in body weight gain by the pregnant rats administered with 800 mg/kg bwt and mifepristone may be used to assess the integrity of the maternal homeostasis while the increase observed in weight of rats in other groups administered with extract showed that growth of the rats was not tampered with by the extract. As stated earlier that there were no clinical signs of toxicity recorded except for the mifepristone treated group that showed signs of tiredness which further support the side effects of the drug.

It is therefore, suggested that the extract might be more tolerable than mifepristone by the animals. Reduction in the number of life fetus, the weight of fetus and survival ratio of the fetus might be linked with the abortifacient potential of the extract which is dose-dependent. Increase shown in the number of the dead fetus with an increased dose of the extract can be attributed to the observed increase during the post-implantation period in the animals which may be attributed to the

abortifacient activity. Shaikh *et al.*, 2015, also reported that *Anthocephalus cadamba* stem barks have abortifacient potential in mice.

Pre-implantation loss and implantation index estimate numbers of implanted blastocytes found in the uterus while the connection between the number of implanted blastocytes and those that have not developed is established by blastocytes index and post-implantation loss (Almeida and Lemonica, 2000). Decreased in the number of the implantation sites due to the increase in doses of the extract have suggested the anti-implantation consequence of extract and the synthetic drug mifepristone which consequently, led to the reduction observed in the implantation index. Also, it was established during this study, that implantation index has a connection with the number of corpora lutea and the blastocytes implantation that occurs in the endometrium (Tafessel *et al.*, 2006). Reduction in implantation index with the contrasting increase in the pre- and post-implantation losses which are dose-dependent imply the abortifacient or foetus resorptive properties of *Citrullus colocynthis* pods, which are a signal of failure or malfunction in the development of the embryo this is in support of the report of Dabhadkar and Zade (2013), on abortifacient value of *Indigofera trifoliata* leaves on albino rats. The occurrence of foetus destruction implies that disruption of pregnancy takes place after implantation of a foetus (Elbetieha *et al.*, 2000). This may be due to modification in the uterine environment. The decrease was seen in corpora lutea with respect to increase in dose, as well as mifepristone which elucidates that reproductive performance of the mother was altered leading to the anti-pregnancy potentials of the extract and mifepristone. These results have shown that ethanolic extract of *Citrullus colocynthis* pods may have pregnancy terminating potentials.

5.1.3 Reproductive hormone

During pregnancy a number of physiological, biochemical, morphological, and histological changes takes place in the uterus and ovaries. These require the regulation of follicular growth and ovulation by endocrine hormones like follicle stimulating hormone, luteinizing hormone, and prolactin as well as ovarian hormones such as progesterone and oestrogen. Ovarian hormones are mostly produced by various cells of the ovary such as granulosa cells of the mature follicles and the corpus luteum (Katalinic *et al.*, 2006).

Hence, the female reproductive hormones progesterone, oestrogen, FSH, LH, and prolactin were quantitatively analyzed in the current study to diagnose pregnancy terminating potentials of ethanolic extract of *Citrullus colocynthis* pods.

5.1.3.1 Follicle Stimulating Hormone

FSH serves as the prime hormone of reproduction mammals. FSH is mainly responsible for the development of female reproductive gonads, maturation at the time of puberty and for the genesis of mature Graafian follicles during the fertile period (Kumar *et al.*, 1997). However, reduction observed in the concentration of FSH is a sign of impairment caused on the reproductive cycle of the animals as well as failing pregnancy.

Also, FSH act often on the granulosa receptor cell directly thereby stimulating the growth and maturation of ovarian follicles. Hence, the reduction then suggests that there is a gonadal dysfunction which may lead to impair folliculogenesis and delay in follicle maturation. Therefore, reduction in the FSH levels through the administration of the extract and mifepristone may be ascribed to obstruction of folliculogenesis and disruption in the maturation of the ovarian follicles during the preovulatory phase confirms the report of Andersen and Ezcurra (2001). This may be due to the effect of

the extract or drug on the anterior pituitary or the hypothalamus because gonadotropic releasing hormone released through the hypothalamus helps to regulate FSH secretion. Therefore, reduction in the hormone may be attributed to the abortifacient activity of the extract on pregnant Wistar rats.

5.1.3.2 Luteinizing Hormone

LH is the required hormone needed for continuous development and functioning of the corpora lutea including ovulation. Consequently, observed reduction of LH concentrations in this study can be linked with hindrance to ovarian follicle luteinization which could account for the reduction observed in the serum progesterone. The decrease could suggest that the endometrium was impaired and can possibly facilitate abortion.

LH stimulates the release of sex hormones from the gonads. Normally, luteinizing hormone in animals during preovulatory period suddenly surges and stimulates ovulation of mature follicles in the ovary to rupture. Therefore, the sudden decrease in serum LH concentration may suggest an inhibitory effect of the extract or mifepristone on the secretion of LH which may interfere with ovulation and the functional capacity LH hormone (Ogilvie and Rivier, 1997). This can leads to impairment of estrous cycle and hinders conception, implantation and normal reproduction in the pregnant rats resulting in abortion.

5.1.3.3 Progesterone

Progesterone helps in the regulation of menstrual cycle and preparing the uterus for conception and pregnancy. The hormone promotes the growth of lactation glands in the breast during pregnancy. It maintains the uterine environment for implantation,

development, and maturation of fertilized egg and prevents further egg release until the pregnancy has been terminated (Goodger and Rogers, 1993).

The reduced level of progesterone hormone caused by the extract may be due to inactivation of ovarian follicles luteinization thereby leading to decreased secretion of oestrogen. This might be accountable for the reduction seen in serum progesterone level in this study. Furthermore, the decrease in progesterone concentration by the extract and mifepristone implies that the uterine surroundings supporting fetal development may be impaired.

5.1.3.4 Oestrogen

Oestrogen stimulates proliferation of uterine lining and increases its thickness in the cycle pre-ovulatory phase. Growth and development of reproductive organs are mostly achieved by estradiol. Also, during follicular development, FSH in conjunction with oestrogen stimulates granulosa cell proliferation. Therefore, a reduction in serum levels of oestrogen observed may be linked with decreased synthesis which might imply that the extract or mifepristone can block ovulation by directly acting on the pituitary gland and influence minor modification of LH and FSH. These findings, compared favourably with some studies carried out on animals using several plants which have showed that reduction occurs in the concentrations of hormones such as FSH, LH, progesterone and oestrogen. Some of these plants include *Cola nitida*, *Afrormosia axiflora* and *Pterocarpus erinaceus* (Benie *et al.*, 2003), *Garcinia kola* seed (Braide *et al.*, 2003) had been reported to cause a reduction in serum or plasma levels of these hormones. Also, Kumar *et al* (1997) reported that a reduction in FSH hinders follicle maturation during the preovulatory phase which in turn affects conception. It is evident from these reports that a reduction in FSH and LH impaired ovarian steroidogenesis and function.

Consequently, such decrease in oestrogen levels can obstruct ovulation, reproductive tract readiness for zygote implantation, and ability to maintain the pregnancy state (Benie *et al.*, 1990). The extract could have inhibited embryo implantation, placenta development and alters oestrogen metabolism which might have result in abortion.

5.1.3.5 Prolactin

Prolactin plays important roles in the implantation, decidualization and subsequent placentation of the endometrium. It has also been reported that among the numerous genes that are upregulated during decidualization, is one of the most abundant secretory products in the amniotic fluid (Bao *et al.*, 2007; Gallersen *et al.*, 2007). Therefore, decrease in serum levels of reproductive hormones strongly supported the abortifacient activity. Furthermore, lack of endometrial prolactin during implantation window seems to be implicated in reproductive failure (Garzia *et al.*, 2004). In a few mammals, prolactin is imperative for the maintenance of the secretory function of the corpus luteum in most rodents. In addition, the decrease observed could be attributed to the reduction in the concentration of other reproductive hormones which suggest that the extract tends to block the rate-limiting enzymes involved in the synthesis of the hormones.

5.2 Biochemical Assessment

5.2.1 Organ- Body Weight Ratio

The alteration of organs in relation to their body mass is always determined to ascertain the value for the organ-body weight ratios generally (Adebayo *et al.*, 2013). The modification in organ body weight ratio may be signal of cell constraint or inflammations because the cells are the unit components of the organs. The constriction in the organ may occur as a result of loss of fluid due to permeability

effect whereas inflammation may be a consequent of increase in organ-body weight ratio (Moore and Dalley, 1999). The significant reduction in the uterus and ovary organ-body weight ratios at 800mg/kg body weight in this study might be a signal or pointer to the abortifacient effect of the extract on the pregnant Wistar rats. This is in support of the findings of Qazan *et al.* (2007) that administration of *Citrullus colocynthis* caused a sharp decrease in the relative ovarian and embryo weight of pregnant pregnant rats. Also, the significant increase observed in the liver body weight ratio suggests that the *Citrullus colocynthis* extract induced an inflammation on the organ at these doses.

5.2.2 Haematological Parameters

Evaluation of haematological parameters is a useful blood marker which describes haematological functions of some chemical components or plant extracts in animals. Hence, the mild increase in RBC caused by 100 and 200mg/kg body weight of *Citrullus colocynthis* extract on the experimental animals indicated that there is no adverse effect on the blood oxygen-carrying capacity, quantity of oxygen delivered and thrombopoietin (McLellan *et al.*, 2003). Platelets play a fundamental role in the prevention of blood loss as well as alteration in the secretory capacity of these haemopoietic components could cause a detrimental effect. Consequently, the observed decrease in platelets counts from this study suggests that *Citrullus colocynthis* contains some active secondary metabolites which may be a potential cause of disruption or inhibition of the platelet activation pathway leading to hampering of the blood clotting factor thereby causing haemorrhage (Vita, 2005). Thus, might lead to the toxic effect of mifepristone on the pregnant rats thereby causing abortion while *Citrullus colocynthis* pods extract did not alters the haemoglobin concentration.

Iron is a major constituent of various enzymes present in the cells and also form part of the heme group present in haemoglobin (a porphyrin ring containing iron). Almost all the stored body's iron is in the red blood cells where iron is central for haemoglobin synthesis. The deficiency in iron levels observed might be due to inadequate absorption of iron, unnecessary loss of blood with external haemorrhage, or obstruction in iron metabolism (Kee, 2001; Chernecky and Barbara, 2001).

5.2.3 Serum Lipid Profile

Alterations in the concentration of important lipids like high-density lipoprotein cholesterol (HDL-C), cholesterol, low-density lipoprotein cholesterol (LDL-C) as well as triacylglycerols (TAG) can provide useful information (Abolaji *et al.*, 2007) which relates to tendency of the body to be expose to atherosclerosis or coronary heart diseases. Elevations of the lipids like total cholesterol, triacylglycerol, and LDL-C except the HDL-C are indicators of an increased risk of exposure to atherosclerosis. High levels of triglycerides and LDL are associated with coronary artery diseases (Yakubu *et al.*, 2008). The alteration in serum lipids by *Citrullus colocynthis* in this study indicates a dose and parameter dependent effect of the plant extract.

For instance, the non-significant effect of *Citrullus colocynthis* pod in this study at dose 100 and 800mg/kg on the serum triacylglycerols suggest that the extract might not hamper with triacylglycerols metabolism while the reduction in the concentration of triacylglycerol shows that the extract might alter the synthesis of triacylglycerols. Furthermore, the significant increase on total cholesterol in this study by the ethanolic extract of *Citrullus colocynthis* at all doses might be due to increase in acetyl CoA generation arising probably from increased β -oxidation of fatty acid as acetyl CoA is a key precursor or substrate in the biosynthesis of cholesterol.

HDL- cholesterol have been reported to contain anti-atherogenic properties, this is because there is no correlation existing between HDL-C and threat of cardiovascular disease, while LDL-C builds up cholesterol gradually in the walls of arteries that feed vital tissues like the heart and brain. The accumulation of cholesterol forms plaque that clots the arteries thereby leading to atherosclerosis and hence high blood pressure (Jackson, 1996). The greatest single pointer of the likelihood to develop atherosclerotic heart disease is the ratios of plasma LDL-cholesterol to plasma HDL-cholesterol not total plasma cholesterol (Arise *et al.*, 2012). Therefore, the significant decreases in HDL-C, accompanied by an increase in LDL-C suggest that the animals may be predisposed to cardiovascular risk. This is also evident from the dose-dependent increase in the computed atherogenic index.

5.2.4 Tissues Marker Enzymes

Measurement of the activities of ‘marker’ enzymes presents in most tissues as well as some fluids of the body can be used to assess the degree of toxicity of a plant extract on organ or tissues (Yakubu *et al.*, 2003).

Most of the enzyme tests used in diagnosis or as ‘markers’ for cellular damage depend on the very high concentration of such enzyme within the cell relative to that in the plasma or serum. Consequently, cellular damage arising from drug toxicity or diseases often result in a measurable increase in enzyme activity, in the blood as the enzyme is released from the damaged cell. Their presence in an elevated form in the serum might reveal informations on tissue or organ injury and dysfunctions (Withawaskul *et al.*, 2003).

Alanine aminotransferase (ALT) is a marker enzyme located mostly in the liver, pancreas, kidney, lungs, brain, cardiac and skeletal muscles, but aspartate

aminotransferase (AST) is primarily found in the liver (Mukinda and Syce, 2007).

Alkaline phosphatase is mostly used to ascertain membrane integrity.

5.2.4.1 Alkaline Phosphatase (ALP)

ALP is an ubiquitous enzyme localized within the plasma membrane, where it helps in the hydrolysis of phosphate esters. It is the most abundant 'marker' enzyme present in the plasma membrane and endoplasmic reticulum (Akanji *et al.*, 1993). Furthermore, some reports have linked the connection of ALPs in cellular processes like cell growth, regulation of protein phosphorylation, cell death and apoptosis with cellular migration during embryonic development.

Serum ALP is an indicator of alteration in tissues or organ since the level of the enzyme is always raised when there is tissue damage arising from permeability changes. Thus, the elevation in ALP observed in the serum due to the administration of *Citrullus colocynthis* indicates that there can be bony metastasis as ALP is an osteoblast activity by-product or a disease which affects hyperparathyroidism (blood calcium level), damaged liver cells (such as infiltrative liver diseases, abscesses, amyloidosis or granulomatous) and cause vitamin D deficiency (Rosalki and McIntyre, 1999). The increase in the serum can also be associated with intrahepatic cholest infiltrative liver disease or obstruction of bile duct.

The significant decrease in liver and kidney ALP activity by ethanolic extract of *Citrullus colocynthis* pod and consequent elevation in serum ALP activity recorded in this study indicates that integrity of the plasma membrane of the hepatocytes and the kidney cells had been compromised. This will consequently affect the normal transport of ions and other substances across the cell membrane which can lead to starvation and eventual cell death (Akanji *et al.*, 1993). Consequently, the reduction observed in ALP activity might be contributed to by the extract. While the increase

observed in the serum ALP activity might be an indication of likely damage to the liver and the kidney. Kaneko *et al* (1997) supported this finding by establishing the fact that elevated level of ALP is a pointer to cholestatic liver disease.

5.2.4.2 Aminotransferases

Aminotransferases are considered to be useful marker enzymes of liver cytolysis and can be used to assess membrane damage in the liver and heart (Shahjahan *et al.*, 2004). They are involved in transferring an amino group from alpha-amino to alpha-keto acids in the biochemical regulation of intracellular amino acids pool.

5.2.4.2.1 Aspartate Aminotransferase

AST is a very important enzyme in assessing liver cytolysis. The significant increase in the serum AST activity and corresponding decrease in the liver AST activity caused by the administration of *Citrullus colocynthis* pods extract could be due to De novo synthesis of the enzyme molecule or destruction caused on the tissue leading to an activity higher or than the control in the serum. This suggest that the functions of the liver in metabolism of carbohydrate, fats, protein, ile secretion, vitamins storage with detoxification might have been impaired (Ahsan *et al.*, 2009). Furthermore, the decrease in kidney AST activity and the corresponding increase in serum AST activity by the *Citrullus colocynthis* extract can be attributed to damage of the plasma membrane of the kidney. This will have corresponding effects on amino acid metabolism. Thus an elevation in the level of this marker enzymes in the serum suggest that the extract is toxic at high dose with mild toxicity at low dose of 100 and 200mg/kg body weight thereby leading to the induction of hepatocellular or nephrotoxic damage on the tissues and is in line with earlier reports (Adedapo *et al.*, 2004).

5.2.4.2.2 Alanine Aminotransferase

The observed significant increase in ALT activity of the serum after the administration of extract at doses 400 and 800mg/kg body weight and mifepristone in this study indicate that there is a membrane damage done to the liver and the kidney that led to the increase observed. Furthermore, the corresponding decrease in the ALT activity suggests that damage to the plasma membrane of the organs. The dose specific alterations of the marker enzymes supported toxicity of the *Citrullus colocynthis* extract. Meanwhile, increase in serum ALT activity is widely used as a surrogate marker for tissue damage (Miyazaki *et al.*, 2009). ALT has been used as a marker for liver injury in people and in preclinical toxicity studies (Yang *et al.*, 2009).

5.2.5 Liver Function Indices

These indices are used commonly to assess liver injury rather than hepatic functions. It reflects problems arising in the liver. An excellent criteria used in assessing functional capacity of the liver is by evaluating serum proteins such as albumin and globulin (Yakubu *et al.*, 2003). The significant decrease in albumin by the extract at dose 100, 200 and 400mg/kg body weight may be adduced to decrease in the rate of synthesis of albumin in liver without a proportionate decrease in the rate of its catabolism. This decrease may negatively affect the movement of a variety of ligands to their point of utilization or excretion in the organs or tissues. It may also lead to inability of albumin to neutralize some free radicals, exogeneous drugs, bile pigments and cholesterol in the liver (Aquirre *et al.*, 2014). It was also reported by Ruben (2017) that alterations in the level of albumin can alter the functioning of the platelet. The significant decrease in total bilirubin could indicate a diminished synthetic capacity of the liver, leading to hepatocellular damage (Woodman, 1996). The reduction may also suggest a reduced rate of transportation of nutrients, defense,

coagulation processes, buffering capacity of the blood and homeostasis (Ganong, 2001).

Therefore, the reduction in serum total bilirubin by the *Citrullus colocynthis* pods extract at all doses in this study is a signal of impairment on the tissues functional capacity like liver damage as supported by the report of Mouldgil and Narang (1989) which in turn might affect the normal functioning of the hepatocytes.

5.2.6 Kidney Function Indices

Kidney function indices mainly creatinine, urea, uric acid and serum electrolytes are mostly used to estimate the functional capability of animal nephron at glomerular and tubular levels (Ghasi *et al.*, 2000, Yakubu *et al.*, 2003). There was a selective and dose-dependent effect of *Citrullus colocynthis* pods extract on kidney function indices considered. Urea, creatinine and uric acids are significant byproducts of protein, muscle and purine degradation respectively. The decrease in creatinine concentration by the *Citrullus colocynthis* extract pods at dose 400 and 800mg/kg body weight as well as mifepristone could be an alteration in the secretory functions of the kidney. While the elevated levels of creatinine at doses 100 and 200mg/kg body weight suggest glomerular dysfunction (Chawla, 1999). Furthermore, decrease in urea and uric acid in this study implies toxic effect of the *Citrullus colocynthis* extract. It might result from the interference of the components of *Citrullus colocynthis* extract with the route of getting rid of the ammonia produced from amino acid metabolism in the liver (Whealton *et al.*, 1994).

The *Citrullus colocynthis* pods extract might have alters creatinine metabolism, thereby leading to elevated synthesis in the tissue and does not compromise tubular excretion functional capacity (Zilva *et al.*, 1991). Therefore, renal disease resulting

from reduced glomerular filtration rate can lead to retention of creatinine in the serum. It could also be a signal of some adverse effect occurring in the muscle or alteration in creatinine synthesis. The reduction in the levels of urea and uric acids is an indication of some abnormalities which takes place during the excretion of urea and uric acids; this could be caused by some non-renal factors present in *Citrullus colocynthis* extract in this study. In addition, reduced level of urea and uric acids in rat serum by *Citrullus colocynthis* pods ethanolic extract at all doses administered and mifepristone further corroborates the impaired functional capacity of the nephron.

The non-significant result produces by *Citrullus colocynthis* pod ethanolic extract as well as mifepristone on sodium and calcium ions with reduction in phosphate ions levels further supports the selective function of the extract of *Citrullus colocynthis* on some biochemical parameters used to evaluate toxicity in this study. Reduction in the levels of phosphate ions, as well as decrease calcium ions levels caused by administering the extract and mifepristone, might be due to tubular dysfunction (Chawla, 1999).

Furthermore, the non-significant effects of the extract on the serum sodium ion concentrations on pregnant rats in this study suggest that the secretory ability and normal organ functioning in relation to the electrolyte are unaffected. The finding is in line to the established statement by Ashafa *et al.* (2009) and Pendota *et al.* (2010). All alterations in liver and kidney function induced by the *Citrullus colocynthis* pods extract and mifepristone are pointers to the undesirable effect on the secretory, synthetic, reabsorptive and excretory functions of these organs.

5.2.7 Chromosomal Aberration

A number of researches have been carried out on the genotoxicity effect of some chemicals and biological toxic compounds. Thus, administration of *Citrullus colocynthis* pods ethanolic extract at various doses resulted in an increase in the percentage chromosomal aberration count and decrease in the mitotic index. It is suggestive that the extract has the ability to induce DNA damage that can arrest the cells by decreasing the mitotic index thereby, leading to cell death. This might have been caused by toxic effect of some plant metabolites present in the extract and oxidative stress induced by the extract on the pregnant Wistar rats. This is in support of the findings of Monica *et al.* (2013) on crude extract of orchid *Oncidium flexuosum* Sims inducing DNA damage and cell death.

5.2.8 Histology of some Tissues after Extract Administration

Renal, hepatic, ovarian and uterine integrities were maintained among the control animals as shown in Figure 4.43A, 4.44A, 4.45A and 4.46A respectively. The glomerulocytes were closely apposed, moderate peri-capsular spaces, distal and proximal convoluted tubules showed intact cell lining. A well distinct hepatocytes plate's arrangements and arrays of moderate sinusoidal spaces toward the centrally placed veins depicting normal histological appearances of the liver as well as the presence of various follicular stages with mature follicles that characterized the histological features of the ovary. Well intact uterine lining showing the endometrial glands and characteristically tall endothelium were maintained in the control animals. However, these features observed in the control animals showed mild to severe alterations in the renal, hepatic, ovarian and uterine architecture among the treated animals. Mild degeneration of glomerulocytes, derangement in the alignment of the hepatocytes leading to abnormal spaces widening of the capsular spaces and the

hepatic sinusoids demonstrated the action of the administration of the mifepristone among the treated animals Figure 4.43B, 4.44B, 4.45B and 4.46B respectively.

Moreover, increased loss of glomerulocytes, hepatocytes, complete arrest of follicular development and presence of atretic follicles figure 4.43D - F, 4.44C – F, and 4.45C - F, 4.46C - F and loss of endometrial lining associated with hemosiderin deposition particularly in the renal parenchymal evidently demonstrated the necrotic actions of the extract administered.

The renal architecture of the treated animals were identified by mass degeneration of the glomerulocytes evidence in the increased capsular spaces figure 4.43D, 4.43E, and 4.43F and the endothelia lining of the distal and proximal convoluted tubules with marked hemorrhagic deposits, an indication of ruptured blood vessels; the administration of the extract tends to complicate blood supply to this organ, which could explain enormous loss of glomerulocytes and endothelia lining of the renal tubules. This could as well be associated with hepatocytes loss due to nuclei loss which was preceded by pyknotic, chromatolysis and karyolysis of the hepatic nuclei with increased sinusoidal spaces compromising the integrity of the liver and kidney functions as observed in figure 4.43D – F and figure 4.44B-D. It is worth to note here, that the extract clearly demonstrated histologically hepatotoxic and damaging actions on the renal integrity.

More importantly, abortifacient actions of the *Citrullus colocynthis* pods were revealed by arrest of follicular development and presence of atretic or atrophy follicles. This however, produced a mirage effects on the endometrial lining, loss of endometrial glands creating vacuolation in the uterine parenchyma as shown in figure 4.44C - F and 4.45D - F. These changes might have resulted from the interference with the level of hormonal balance or the necrotic action of the administered extract on the developing follicles and the endometrial lining.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

1. The results showed that ethanolic extract of *Citrullus colocynthis* pods extract contained seven (7) secondary metabolites and eleven (11) minerals. Alkaloids (5.2mg/ml) and calcium (27.17mg/l) were the most abundant.
2. The study also revealed that ethanolic extract of *Citrullus colocynthis* pods induced abortion in the pregnant female wistar rats via oral administration by increasing the number of dead foetus, resorption index, pre- and post- implantation loss with decrease in life fetuses, survival ratio, corpora lutea and foetal toxicity, this support the folkloric use of the plant concoction as an abortifacient.
3. The mechanism of action of *Citrullus colocynthis* pods extract in inducing abortion can be attributed to the reduction observed in the concentrations of the female reproductive hormones such as progesterone, oestrogen, luteinizing hormone, follicle stimulating hormone and prolactin assayed for in the sream of the pregnant female wistar rats.
4. It is evident from the result that the extract might predispose pregnant female wistar rats to risk of cardiovascular diseases due to the elevation observed in the concentration of total cholesterol, low density lipoprotein, and atherogenic index with decrease in high density lipoprotein.
5. The extract could also distrust the integrity of the plasma membrane and alter amino acid metabolism through the observed increase in malondialdehyde and a corresponding decrease in catalase and superoxide dismutase.

6. The study showed that the extract increased the activity of serum alanine and aspartate aminotransferases and alkaline phosphatase with corresponding decrease in the serum.
7. Ethanolic extract of *Citrullus colocynthis* pods elevated the rate of metaphase chromosomal aberration and decrease in mitotic index which are resulted in damaged DNA of the pregnant female wistar rats. This hindered the cells from dividing, thereby resulting in abortifacient or teratogenic effect of the extract on the pregnant wistar rats.
8. Histological results of the tissues studied showed that *Citrullus colocynthis* extract may be injurious to the tissues in a dose dependent manner.

6.2 Conclusion

It was concluded for this study that ethanolic extract of *Citrullus colocynthis* pods caused abortion of foetus in pregnant Wistar rats with the most effective dose being 400 mg/kg body weight. It can thus be used as an abortifacient agent. However biochemical and histological results confirmed that the extract could be toxic to the tissues studied.

6.3 Recommendation

It is recommended that further research should be done to ascertain the active constituent present in the plant pod that is responsible for the abortifacient action of the plant. Hence, the plant can further be explored for the development of abortifacient drugs.

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APPENDIX

0.25M Sucrose solution

0.25M Sucrose Solution 85.75g sucrose was dissolve in small quantity of distilled water and make up to 1L with distilled water.

1M Sodium Chloride

58.5g of Sodium chloride was dissolved in a little quantity of distilled water and made up to 1L with distilled water

Mayer's Reagent

1.4g of mercuric was dissolved in 60ml of distilled water and 4.5g of Potassium Iodide in 20ml distilled water. The two solutions were mixed and made up to 100ml

Wagner's Reagent

2g of Iodine and 3g of Potassium Iodide were dissolved in 20ml and made up to 100ml

Reagent for Protein Determination

Bovine Serum Albumin (1% BSA)

Bovine Serum Albumin (1%) was prepared by dissolving 1g of BSA powder in a little quantity of distilled water with continuous stirring and the solution made up to 100mls.

Biuret Reagent

Solution A

1.5g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 6.0g of sodium potassium tartarate ($\text{NaK} \cdot \text{C}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) were dissolved in a little distilled water and then made up to 500ml. solution

Solution B

30g of Sodium Hydroxide (NaOH) pellet was dissolved in a little distilled water and made up to 300ml and added slowly to solution A with constant stirring in a 1L standard flask and made up to 1L with distilled water. The solution was stored in polythene bottle

Carbonate Buffer (0.1M, pH 10.1)

A. 2.10g of Sodium hydrogen carbonate was dissolved in a little distilled water and made up to 250ml.

B. 3.10g of hydrated sodium carbonate ($\text{NaCO}_3 \cdot \text{H}_2\text{O}$) salt was dissolved in a little distilled water and made up to 250ml.

The two solutions A and B was then mixed together with constant stirring, the Ph meter was then use to check for the solution pH until 10.1 was obtained.

Magnesium Sulphate (0.1M)

2.464g of hydrated magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) salt was dissolved in distilled water to make 100ml solution

Para-nitro Phenyl phosphate (10mM)

36.7mg para –nitro phenyl orthophosphate was dissolved in distilled water and made up to 100ml solution

Sodium Hydroxide (1N)

40g of sodium hydroxide pellet was dissolved in distilled water and made up to 1L.

5% K₂Cr₂O₇ (Dichromate Solution)

5g of K₂Cr₂O₇ (Hopkins & Williams, England) salt was weighed and dissolved in 80ml of distilled water and later made up to 100ml.

0.2M H₂O₂ (Hydrogen peroxide)

11.50ml of 30% (w/w) H₂O₂ stock was diluted with distilled water in a volumetric flask and the solution made up to 500ml.

Dichromate/acetic acid

This was prepared by mixing 5% solution of K₂Cr₂O₇ with glacial acetic acid (1: 3).

Phosphate buffer (0.01M, pH 7.0)

3.58g of Na₂HPO₄ .12H₂O and 1.19g NaH₂PO₄ .2H₂O salts were dissolved in 900ml of distilled water and the pH was adjusted to 7.0 after which it was made up to 1L.

0.05M Carbonate buffer (pH 10.2)

3.58g of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ and 1.05g of NaHCO_3 hydrated salts were weighed and dissolved in 200ml of distilled water. The pH was checked and adjusted to 10.2, thereafter it was then made up to 250ml.

30% Trichloroacetic acid (TCA)

It was prepared by dissolving 9g of TCA (CCl_3COOH) in a little quantity of distilled water and made up to 30ml with distilled water..

0.75% Thiobarbituric acid (TBA)

0.23g of TBA was weighed and dissolved in 0.1M HCl and made up to 30ml with the same solution.

0.15M Tris-KCl buffer (pH 7.4)

It was prepared by weighing 1.12g of KCl and 2.36g of Tris base and dissolved it separately in distilled water and made up to 100ml and pH was adjusted to 7.4.

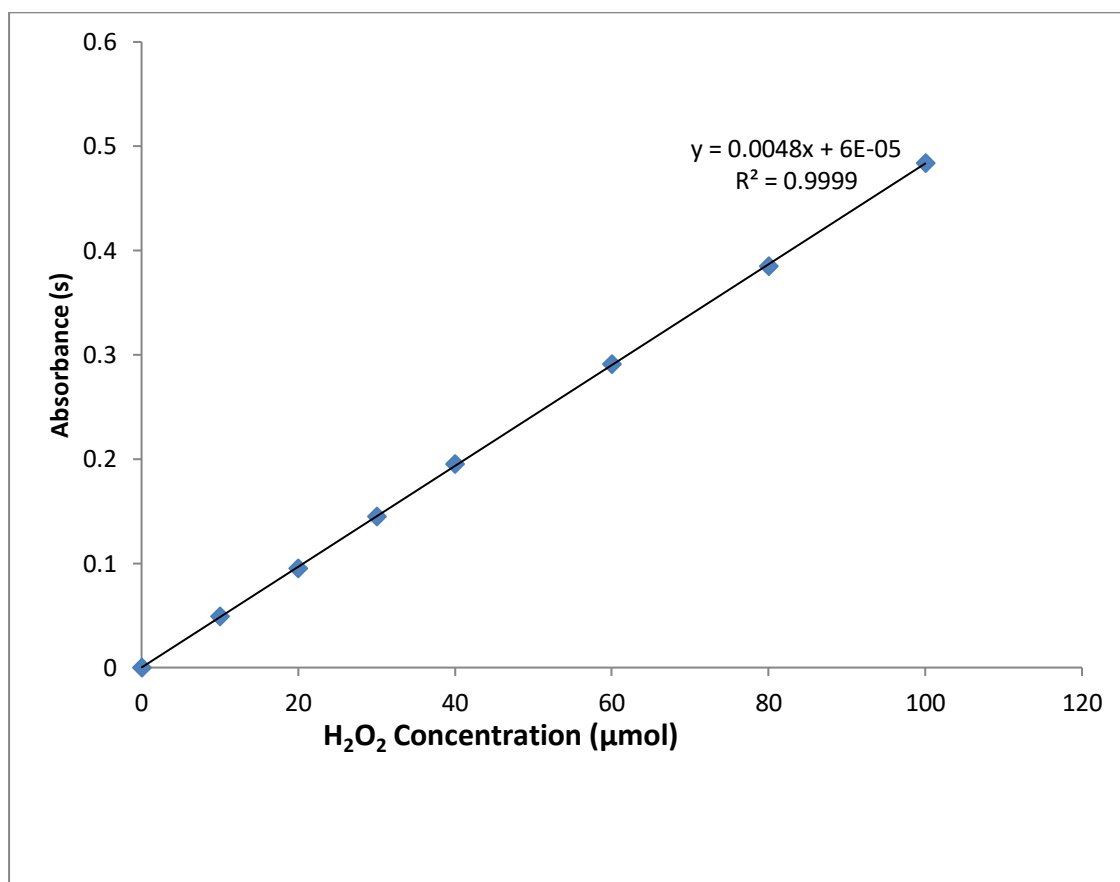


Figure 4.47: Standard Curve for H₂O₂

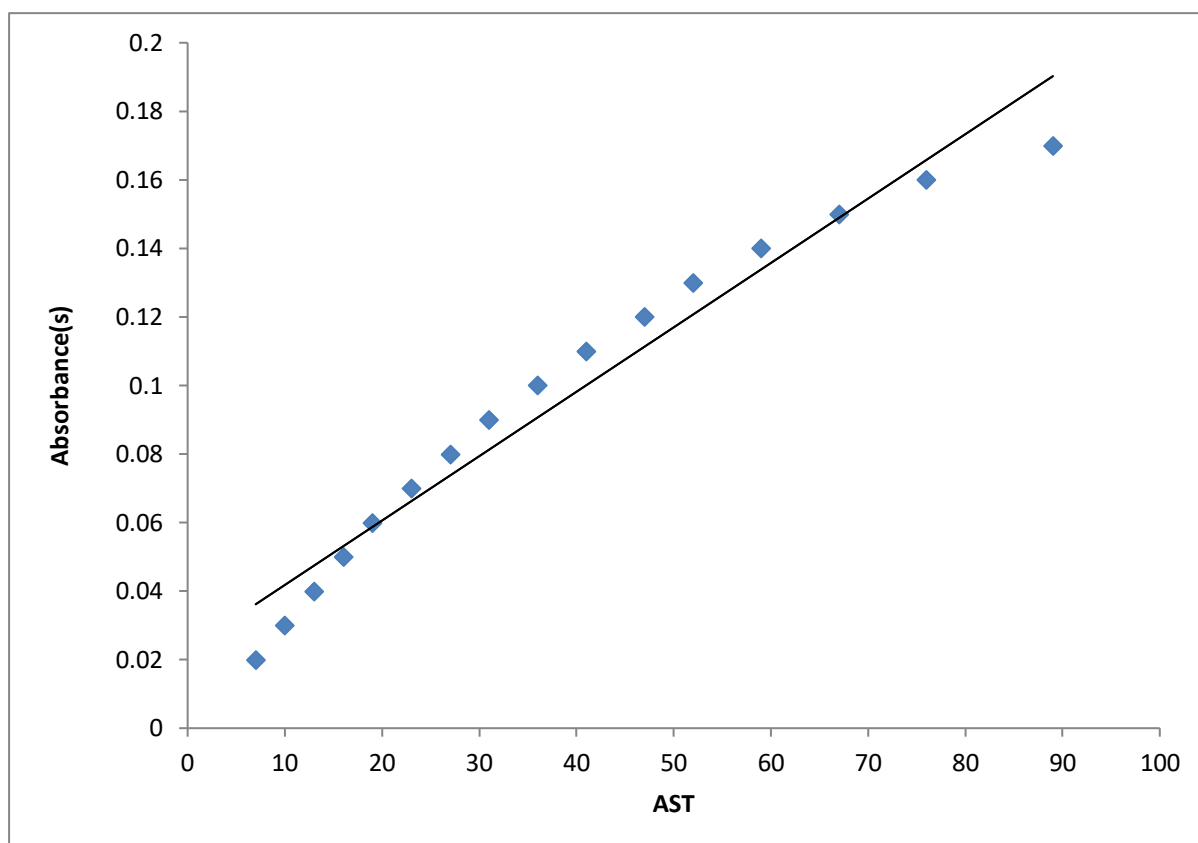


Figure 4.48: Standard Curve for Aspartate Aminotransferase

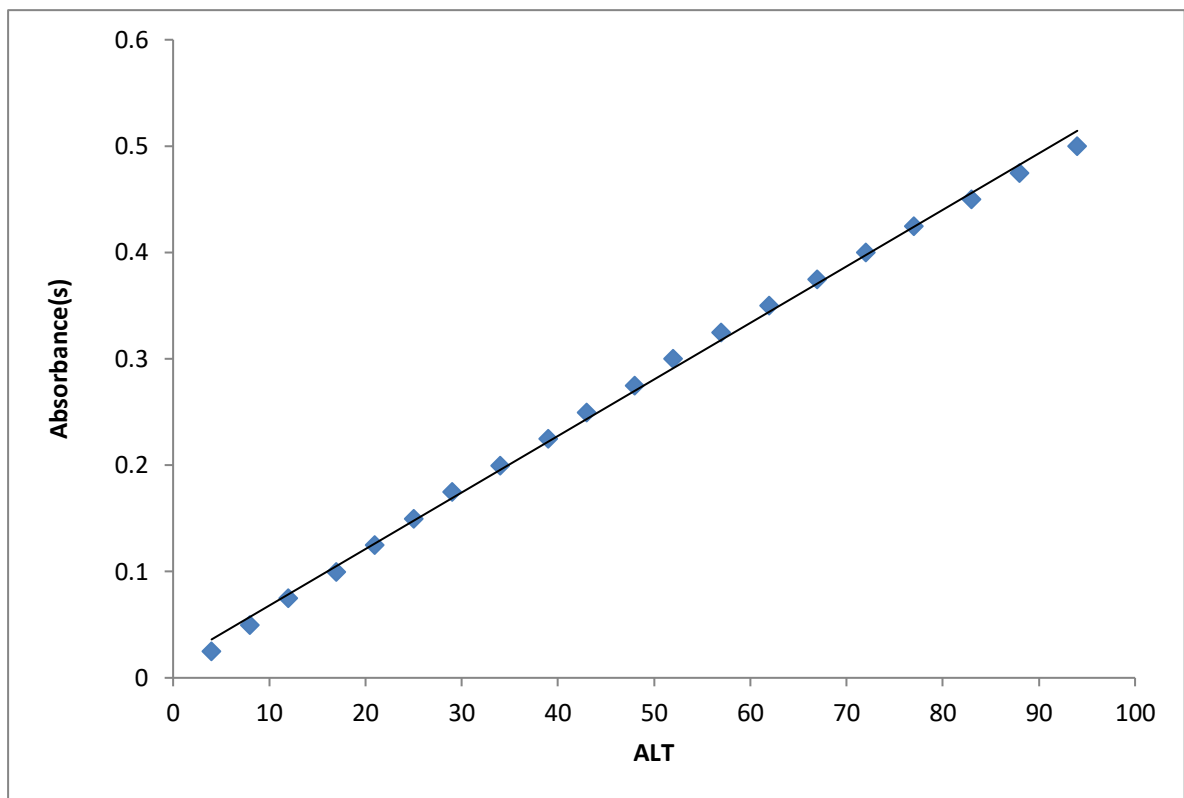


Figure 4.49: Standard Curve for Alanine Aminotransferase

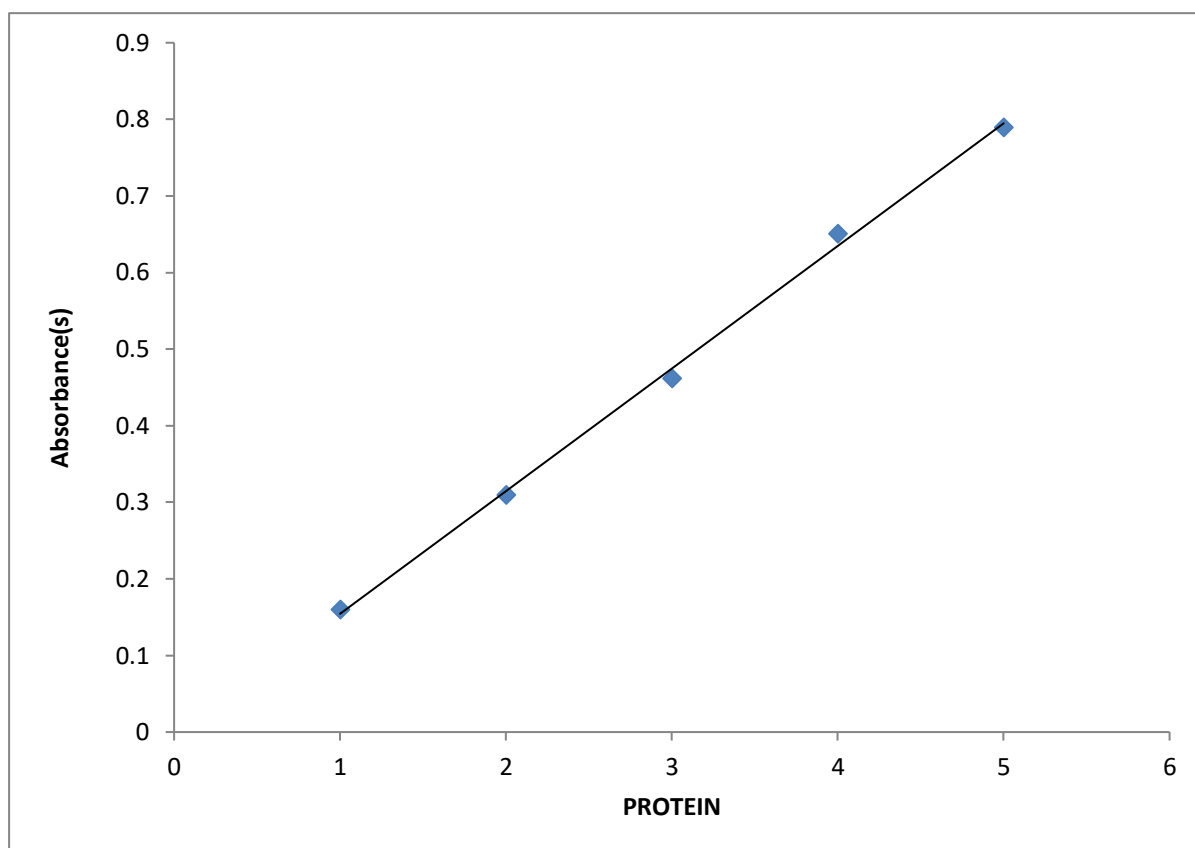


Figure 4.50: Standard Curve for Protein

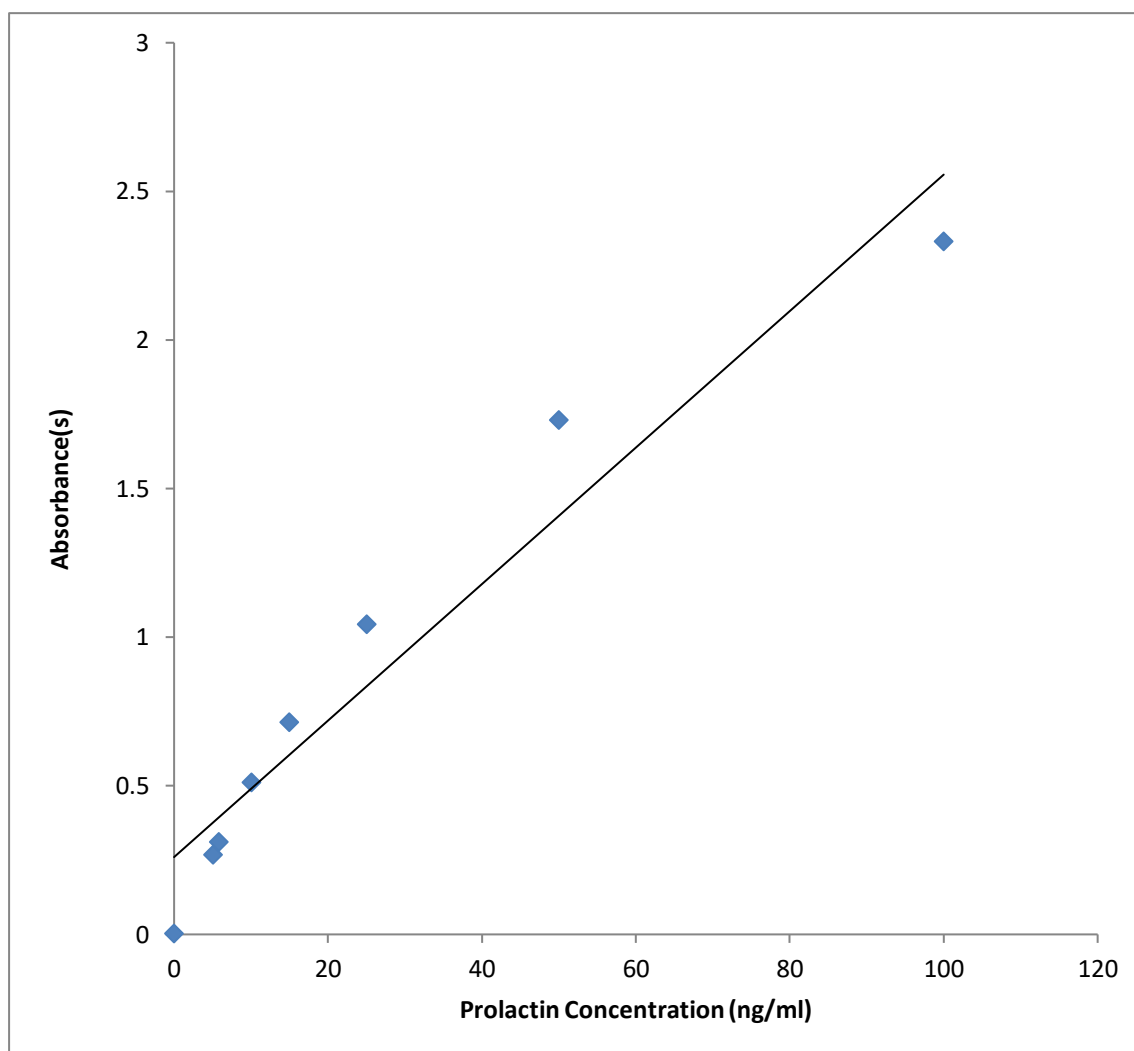


Figure 4.51: Standard Curve for Prolactin

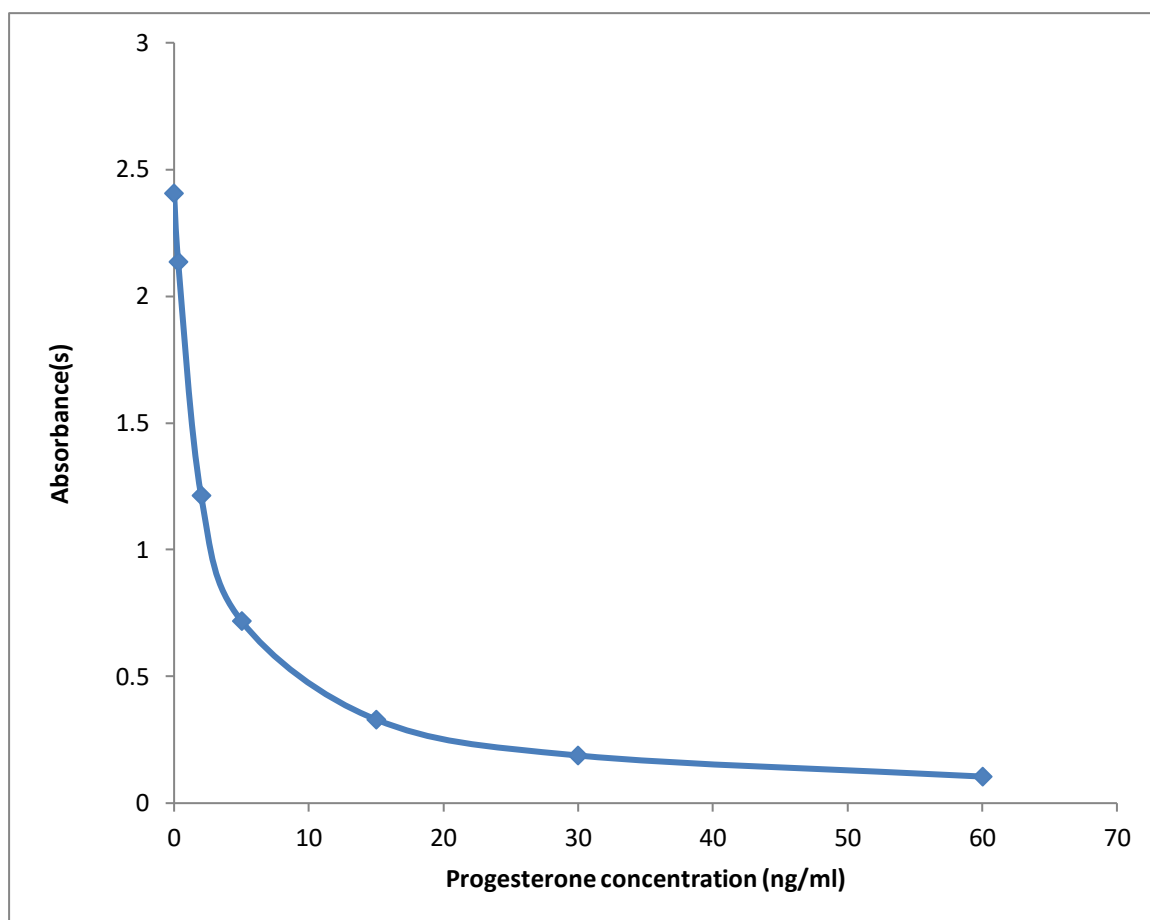


Figure 4.52: Standard Curve for Progesterone

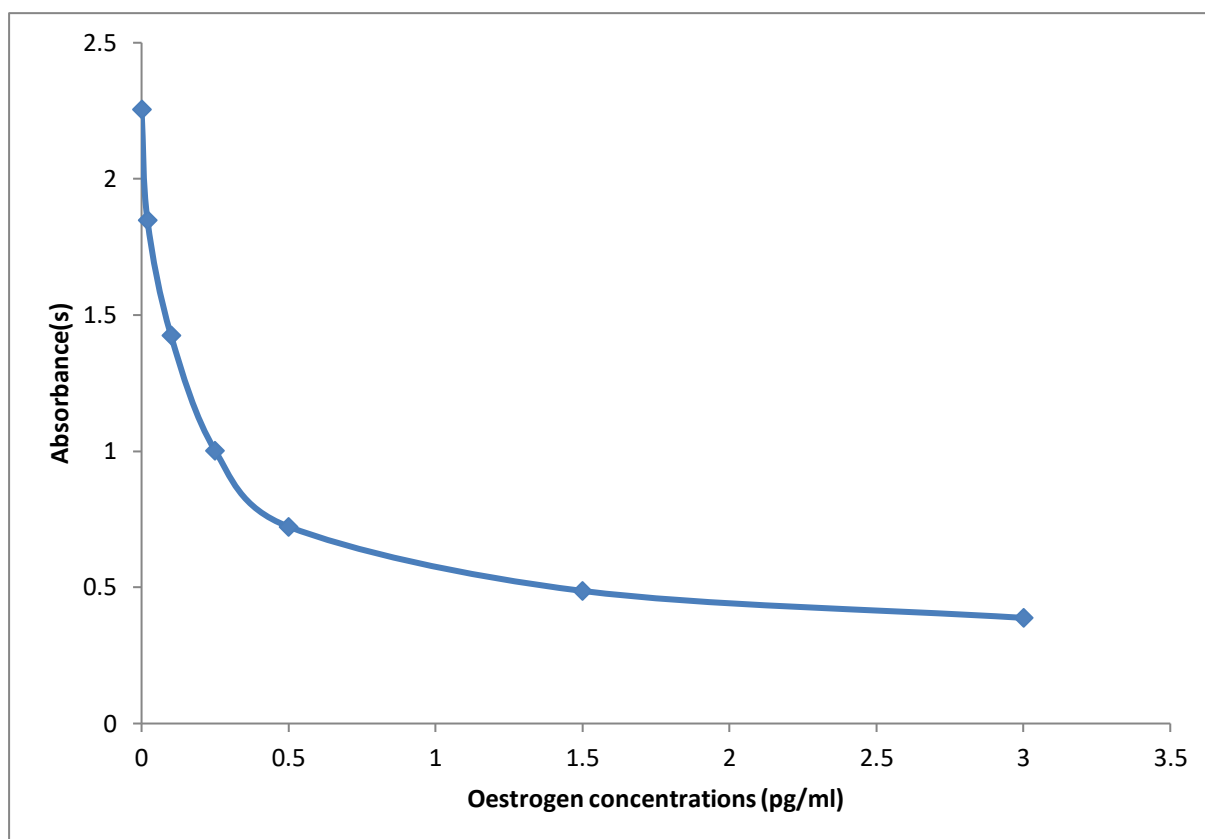


Figure 4.53: Standard Curve for Oestrogen

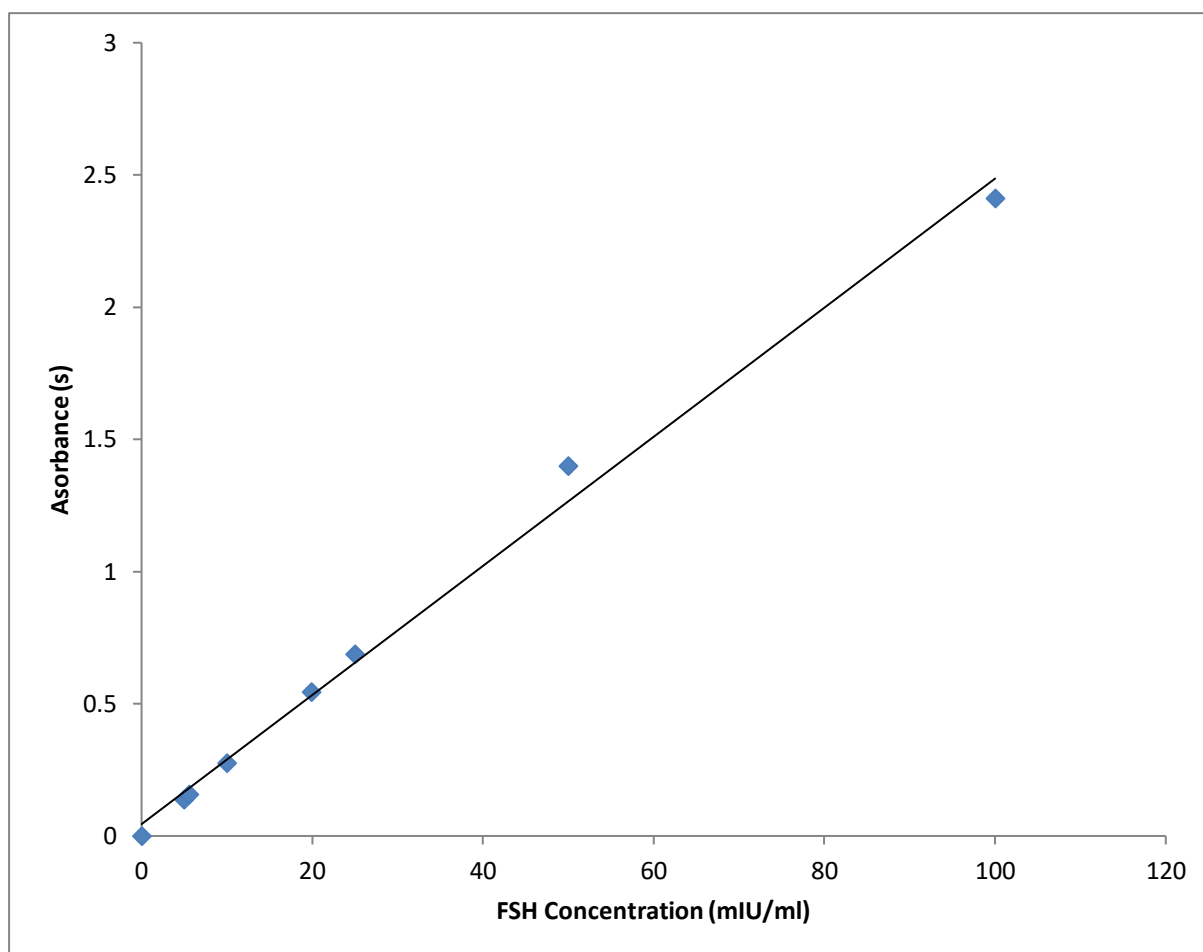


Figure 4.54: Standard Curve for Follicle Stimulating Hormone