

Effects of Caffeine Administration on Body Weight and Memory in Healthy and Streptozotocine-Induced Diabetic Female Wistar Rats

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Abstract

Diabetes is a metabolic condition characterised by difficulty of the individual to regulate blood glucose within reference levels due to insufficient insulin or ineffective insulin sensitivity by the tissue. There have been reports and observations on the effect of type I diabetes on body weight and cognition especially during treatment. We therefore aim to determine the effects of caffeine on body weight and memory in Streptozotocine-Induced diabetic female Wistar rats.

Eight groups of female Wistar rats with mean weight of 180g were used for the study. Groups 1 and 2 were control and diabetic respectively, administered 20mg/kg of normal saline intraperitoneally. Groups 3, 4 and 5 were administered 15, 20 and 25 mg/kg of caffeine intraperitoneally (i/p) per day respectively for five weeks and maintained on caffeine for three weeks after induction of diabetes; groups 6, 7 and 8 were diabetic rats administered caffeine at 15, 20, and 25 mg/kg (i/p) respectively per day for three weeks. Diabetes was induced with 50 mg/kg STZ. Body weight and blood glucose level were monitored weekly. At the end of administration, Morris Water Maze was used to measure their spatial learning and memory. Animals were euthanized by cardiac puncture.

There was no significant ($p < 0.05$) difference in percentage weight change in healthy groups 3, 4, 5 when compared to control. There was significant ($p < 0.05$) decrease in percent weight change in groups 4, 5, 6 and 7 when compared to control.

There was significant ($p < 0.05$) decrease in duration to locate the hidden platform of Morris water maze in groups 3 and 5 when compared to control and diabetic group.

In conclusion, this study shows that caffeine has no effect on body weight in healthy rats but has a dose and time dependent effect on body weight as well as a preventive effect on cognitive decline in diabetic rats.

Keywords: Caffeine, Diabetes, Body weight, Learning, Memory.

Introduction

Caffeine is a highly consumed common source of 1,3,7 trimethylxanthine which is found in processed form analgesics-like anacin, weight control aids, allergy relief compounds, stimulant drug, and naturally in some seeds. The specific amount of caffeine needed to produce effects varies from person to person, depending on body size and degree of tolerance. The effects of which begins less than an hour after consumption, a moderate dose usually wears off in about five hours¹ and a bioavailability of ninety-nine percent², it is absorbed from the stomach, and rapidly through the walls of the intestine³ within forty-five minutes of ingestion, then distributed throughout all tissues of the body⁴, and is significantly altered by drugs or hormonal states.⁵ Caffeine is metabolized in the liver by the cytochrome P450 oxidase enzyme system, specifically by three dimethylxanthines CYP1A2 isozyme which constitute eighty-four percent of paraxanthine which increases lipolysis⁶. Caffeine acts via adenosine which functions normally as a synaptically released neurotransmitter in some part of the brain.⁷ It has been shown that caffeine reduce insulin sensitivity, tachycardia, tachypnoea, hyperthermia⁸, it affects weight loss through appetite suppression, when it acts as a stimulant; it increase calorie burning or energy expenditure by stimulating thermogenesis as one of its short term effects response by entering into the flight system activating the production of glycogen and using up the cyclic adenosine monophosphate to oxidise fat cells, making them readily available during thermogenesis^{9, 10} especially people living a sedentary lifestyle. It aids weight loss by increasing lipid metabolism in the liver¹¹⁻¹⁵ through indicators such as increase fat oxidation, lipolysis¹⁵ especially in obese persons¹⁶, inducing physical activity improving exercise performance especially for athletes.¹⁷ Chlorogenic acid is a natural ester of caffeic acid that causes weight loss by slowing down glucose absorption within the body after meal thereby reducing hunger or food craving, it is found in green coffee bean extract.¹⁸

Diabetes, also known as diabetic mellitus is a group of metabolic disorder characterized by high blood glucose level, this is either due to the pancreas not producing enough insulin, or because cells of the body do not respond properly to the insulin that is

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produced.¹⁹⁻²⁰ Classification divides diabetes mellitus into three main types or causal cases²¹, type 1 diabetes mellitus is a polygenic immune-mediated or idiopathic disease¹⁷ found mostly in children, characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency.²⁰ Type 1 can be distinguished from type 2 diabetes via a C-reactive peptide assay, which measures endogenous insulin production.²² Type 2 diabetes mellitus, is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion¹⁹, this can be caused primarily due to lifestyle and genetics.²³ Other causes are excess: glucocorticoids, growth hormone; polycystic ovary disease; lipodystrophy; autoantibodies to the insulin receptor; mutations of insulin receptor, and of the peroxisome proliferation activator receptor, that cause genetic obesity and hemochromatosis.²⁴ Gestational Diabetes Mellitus occurs in about 2-10% of all pregnancies and may improve or disappear after delivery. Risks to the baby include; macrosomia (high birth weight), congenital cardiac anomalies, central nervous system anomalies, and skeletal muscle malformations²⁵.

Some of the main manifestations of diabetes are polyuria, polydipsia, polyphagia, thirst and weight loss. Symptoms may develop rapidly in type 1 diabetes while in type 2 diabetes they usually develop much more slowly which may be subtle or absent.²⁶ This can be diagnosed by fasting blood glucose level, plasma glucose, glycated haemoglobin, postprandial blood glucose.^{26, 27} The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis. Some health problems that may accelerate the deleterious effects of diabetes, includes smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise.²⁸ In type 1 diabetes mellitus patients, cognitive dysfunction is characterized by a diminished mental flexibility, speed sparing learning and memory²⁹, while in type 2 elderly diabetes mellitus adults, there are decline in memory, attention and other cognitive skills.³⁰

Aims of the Study

It has been observed that obesity is one of the risk factors in diabetes mellitus, and it is possible that caffeine and other constituents of coffee, such as chlorogenic acid and quinides, are involved in causing weight loss. The present study evaluates and analyses, the effect of chronic caffeine administration on risk reduction and management of diabetes

Materials and Methods

Experimental Animals

Female Wistar rats, aged 12-13 weeks,

weighing between the ranges of 150-200g were used for the study. These animals were purchased from the Department of Biochemistry, University of Ilorin-Ilorin, Nigeria. The animals were maintained, with free access to food (rat pellet) and water, with a 12-h light/12-h dark cycle (lights on at 07:00 h). Rats were used in accordance with the rules for the Care and Use of Laboratory Animals and the research was approved by the Ethics Committee of the University of Ilorin, Ilorin, Nigeria.

Study Design

Eight groups of 7 female Wistar rats each were used for the study. Groups 1 and 2 were control and diabetic untreated groups respectively that were administered normal saline.

Groups 3, 4 and 5 were healthy rats administered 15, 20 and 25 ml/kg caffeine intraperitoneally (i.p) per day respectively for five weeks; and were maintained on caffeine for three weeks after induction of diabetes; groups 6, 7 and 8 were diabetic rats placed on caffeine immediately after induction of DM at 15, 20 and 25 ml/kg (i.p) per day respectively for three weeks.

Induction of Diabetes

Type 1 diabetes was induced with freshly prepared 50 mg/kg STZ (in citrate buffer 0.1M, pH 4.5), which induced sustained hyperglycaemia after 3 days. Dextrose saline was given after induction of diabetes for rehydration. Food and water intake were closely monitored after induction. The animals were confirmed diabetic by the measurement of fasting blood glucose in the tail vein blood after 72 h of injection with a portable glucometer (on-call plus). The animals with hyperglycemia (blood glucose >8.1 mmol/L) and stable symptoms of diabetes mellitus such as polyuria and polydipsia were used for the study.

Glycaemia Assessment

Prior to glucose level estimation, the rats underwent 12h of overnight fasting. After fasting, to determine baseline glucose levels, blood samples (0.1 µl) were collected from the distal end of the tail and analysed immediately using an On-Call® Plus glucometer with the corresponding On-Call® Plus test strips (Roche, Mannheim, Germany).

Caffeine Dose and Administration

Caffeine (Sigma-Aldrich, St. Louis, MO, USA) was administered intraperitoneally. Doses of 15, 20 and 25 ml/kg (BW)/day were given to specified groups.

Memory Test

Morris water maze described by Richard Morris was used for this study. The maze is widely used in behavioural neuroscience to study spatial learning and memory in rats; assess damage to cortical regions

of the brain. The water maze consisted of a round tank 170 cm in diameter and 70cm deep, filled with water, and water temperature was maintained at 25°C. A platform of 10cm in diameter was submersed 2 cm above the water surface, placed at the center of one of the four imaginary quadrants of the tank and maintained in the same position during the training session. The experimental procedures were videotaped, and the scores for latency to escape to the platform were computed. This period consisted of two trials (training and memory test), during which the experimental rats after been administered caffeine depending on groups dosage were submerged in the tank, while facing the wall of the tank, and allowed to swim freely in water to locate a hidden platform. The first trial served as the training-learning period, while the second served as memory test (short term). If the rat did not locate the platform in sixty second during learning, it was gently guided to the escape platform. The rats were allowed to remain on the platform for ten seconds after locating the platform before being removed from the tank to rest for thirty minutes. The experimental rats were then placed again at the same starting point, maintaining same quadrant in the tank, and memory test were conducted, observed, and results computed for short term memory.

Statistical Analyses

Statistical analyses were performed using the SPSS® statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Data were presented as mean $\pm$ SEM. Differences between continuous data were analysed using the paired Student's *t*-test and analysis of variance using a Duncan *post hoc* comparison, as appropriate.

Results

Body weight in healthy and diabetic rats

There was a significant ($p < 0.05$) increase in body weight of animals at weeks five and six when compared to week 1 during caffeine administration, and no significant difference in percentage weight change in healthy rats when compared to control as highlighted in both table 1 and 2 respectively. A significant decrease in body weight at the 6th week was observed when compared to week 1 in diabetic rats, so also a significant decrease in percentage weight change when compared to control in diabetic rats as indicated in table 2 and 3.

Blood glucose level in healthy and diabetic rats

Healthy rats administered caffeine 15mg/kg, 20mg/kg and 25mg/kg respectively before induction of diabetes showed no significant difference in blood glucose level (4.60 ± 0.16 , 4.17 ± 0.20 , 4.41 ± 0.07) when compared with control (4.14 ± 0.23) in figure 1. While, a significant reduction was noticed in rats post-treated with caffeine (11.80 ± 1.54) when compared with diabetes untreated (18.77 ± 2.69), there was also a significant increase in blood glucose in post-treated animals with caffeine (32.22 ± 1.24 and 30.66 ± 1.39) when compared with diabetes untreated and control groups respectively as indicated in Figure 2.

Memory test in healthy and diabetic rats

Healthy rats administered caffeine before induction of diabetes, showed no significant difference during learning, but a significant decrease was noticed in group 3 (51.71 ± 6.58) when compared with control (44.71 ± 8.18) during memory test as shown in Figure 3. In diabetic rats maintained and/or administered

Table 1: Effect of caffeine on body weight in healthy female Wistar rats

Weeks	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Groups						
C (g)	187.00 ± 6.48	189.86 ± 5.99	199.14 ± 5.95	195.57 $\pm$ 7.03	214.86* $\pm$ 6.71	222.00 * ± 6.71
CL (g)	187.29 ± 8.83	189.71 ± 8.70	194.86 ± 7.99	203.71 ± 8.78	213.43* ± 9.85	229.29* ± 9.87
CM (g)	188.00 ± 7.88	194.86 ± 9.26	205.14 ± 10.14	216.29 ± 10.49	224.71* ± 9.96	239.86* ± 11.91
CH (g)	204.43 ± 6.00	205.43 ± 4.91	205.14 ± 3.75	211.71 ± 3.89	214.43 ± 3.70	227.43* ± 4.26

Values are mean $\pm$ SEM, n = 7, * significantly $P < 0.05$ different from week 1. C = control, CL = caffeine administration 15mg/kg maintained before induction of diabetes; CM = caffeine administration 20mg/kg maintained before induction of diabetes; CH = caffeine administration 25mg/kg maintained before induction of diabetes.

Table 2: Effect of caffeine on body weight change in healthy and diabetic female Wistar rats

Groups	Weight Change (%)
C (g)	18.72 ± 6.48
CL (g)	22.43 ± 3.25
CM (g)	27.13 ± 3.71
CH (g)	11.25 ± 2.98
D (g)	6.34 ± 8.03*
PL (g)	15.24 ± 6.18*
PM (g)	15.10 ± 4.03*
PH (g)	16.44 ± 6.49*
DL (g)	13.07 ± 4.03*
DM (g)	10.25 ± 6.48*
DH (g)	8.45 ± 3.37*

Values are mean ± SEM, n = 7, *significantly different (P<0.05) when compared to control.

C = control, CL = caffeine administration 15mg/kg maintained before induction of diabetes; CM = caffeine administration 20mg/kg maintained before induction of diabetes; CH = caffeine administration 25mg/kg maintained before induction of diabetes. D = diabetic untreated; PL = caffeine administration 15mg/kg maintained after induction of diabetes; PM = caffeine administration 20mg/kg maintained after induction of diabetes; PH = caffeine administration 25mg/kg maintained after induction of diabetes; DL = caffeine administration 15mg/kg after induction of diabetes; DM = caffeine administration 20mg/kg after induction of diabetes; DH = caffeine administration 25mg/kg after induction of diabetes.

Table 3: Effect of caffeine on body weight in diabetic female Wistar rats

Weeks	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Groups						
C (g)	187.00 ±6.48	189.86 ±5.99	199.14 ±5.95	195.57 ±7.03	214.86* ±6.71	222.00* ±6.71
D (g)	184.71 ±11.42	157.29 ±9.06	183.00 ±11.80	183.86 ±11.87	171.71 ±11.81	173.00 ±18.45
PL (g)	212.83 ±11.50	163.20 ±14.32	188.00 ±18.04	188.80 ±23.20	176.60 ±20.05	180.40 ±21.76
PM (g)	216.50 ±12.54	172.50* ±10.04	191.67 ±6.44	191.80 ±7.17	172.20* ± 8.10	183.80* ±7.64
PH (g)	215.66 ±4.08	183.60* ±9.52	189.60 ±9.54	189.60 ±9.37	177.00 ±10.64	180.20* ±16.77
DL (g)	200.00 ±1.95	179.00* ±1.99	184.57 ±6.41	198.43 ±6.41	181.71* ±6.23	173.86* ±7.44
DM (g)	196.57 ±6.66	180.00 ±7.20	184.43 ±7.36	181.86 ±7.41	173.86* ±7.44	176.43 ±6.63
DH (g)	192.71 ±11.50	171.29 ±12.00	187.14 ±9.13	199.71 ±9.17	176.57 ±9.22	176.43 ±6.63

Values are mean±SEM, n = 7, * significantly P<0.05 different from week 1. C = control, D = diabetic untreated; PL = caffeine administration 15mg/kg maintained after induction of diabetes; PM = caffeine administration 20mg/kg maintained after induction of diabetes; PH = caffeine administration 25mg/kg maintained after induction of diabetes; DL = caffeine administration 15mg/kg after induction of diabetes; DM = caffeine administration 20mg/kg after induction of diabetes; DH = caffeine administration 25mg/kg after induction of diabetes.

caffeine before induction of diabetes, a significant increase in groups 2, 3, 4 and 5 (29.86±7.25, 26.80±11.00, 24.40±8.32, and 18.40±7.49) was observed when compared to control (44.21±08.00) during learning, a significant increase in groups 3 and 5 was observed (17.40±4.18 and 5.40±0.40) during memory test when compared to group 1 and 2 (44.71±8.18 and 21.86±5.73) as illustrated in Figure 4.

Discussion

Findings from the present study showed that, caffeine has no significant weight change in healthy rats when compared to control. Katherine et al.,⁹ and Maura,³¹ both shared a contrary view by stating the fact that caffeine consumption leads to weight loss due to the stimulatory effect on fluid and not on stored fat in tissues, thereby increasing body metabolism via calorie burning or thermogenic response in the human body.

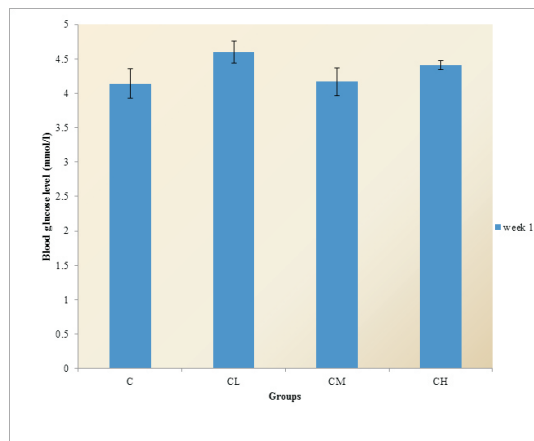


Figure 1: Blood glucose level in healthy rats administered caffeine in week 1

Values are mean $\pm$ SEM, $n = 7$, $P < 0.05$ shows no significant difference with control group. C = control, CL = caffeine administration 15mg/kg before induction of diabetes; CM = caffeine administration 20mg/kg before induction of diabetes; CH = caffeine administration 25mg/kg before induction of diabetes

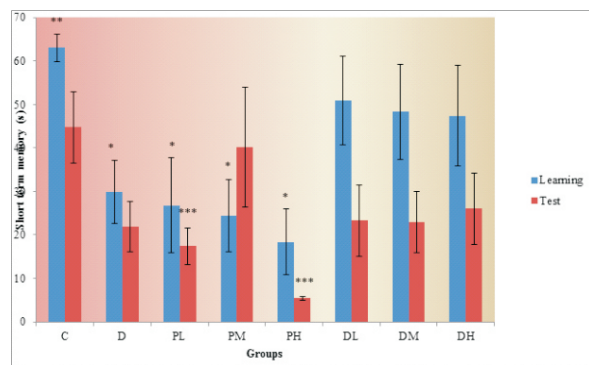


Figure 4: Effect of caffeine on memory in diabetic rats

Values are mean $\pm$ SEM, $n = 7$, * significantly $P < 0.05$ different from control, ** significantly $P < 0.05$ different from diabetic group, *** significantly $P < 0.05$ different from control and diabetic group.

C = control, D = diabetic untreated; PL = caffeine administration 15mg/kg maintained after induction of diabetes; PM = caffeine administration 20mg/kg maintained after induction of diabetes; PH = caffeine administration 25mg/kg maintained after induction of diabetes; DL = caffeine administration 15mg/kg after induction of diabetes; DM = caffeine administration 20mg/kg after induction of diabetes; DH = caffeine administration 25mg/kg after induction of diabetes.

Shimoda et al.,³² share the same view, based on the fact that weight loss was effective through chemical and neurological processes on a short-term caffeine consumption basis, similarly Liu et al.,³³ shared Shimoda's idea though, on the long term basis. In a review by Bethany,¹⁰ caffeine neither causes weight loss nor reduce weight gain from its long or short term consumption even in the absence of physical activities after working on the effect of chronic caffeine consumption, reasons given was supported by Pricilia and Maria,³⁴ Altamari et al.,³⁵ and Mendes and Brito,³⁶ that 100% of caffeine ingested orally was absorbed by the gastrointestinal tract. Pettenuzzo et al.³⁷ experimented on both sexes in rats and concluded that chronic caffeine consumed orally does not have any binding effect irrespective of sexes on body weight gain, though the responses were stronger in females

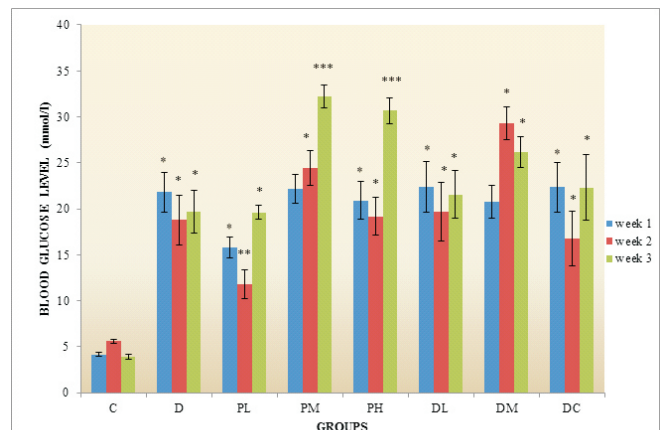


Figure 2: Weekly blood glucose level in diabetic rats administered caffeine

Values are mean $\pm$ SEM, $n = 7$, * $P < 0.05$ significantly different from control; ** $P < 0.05$ significantly different from diabetic group; *** $P < 0.05$ significantly different (P<0.05) from control and diabetic group.

C = control, D = diabetic untreated; PL = caffeine administration 15mg/kg maintained after induction of diabetes; PM = caffeine administration 20mg/kg maintained after induction of diabetes; PH = caffeine administration 25mg/kg maintained after induction of diabetes; DL = caffeine administration 15mg/kg after induction of diabetes; DM = caffeine administration 20mg/kg after induction of diabetes; DH = caffeine administration 25mg/kg after induction of diabetes.

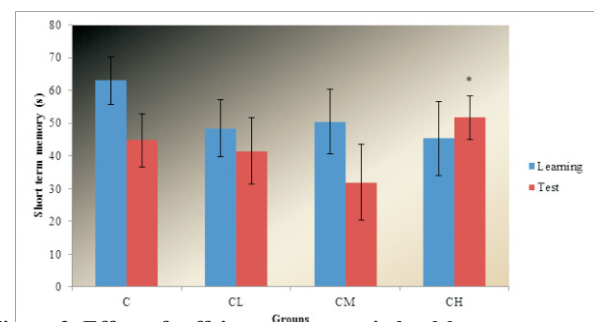


Figure 3: Effect of caffeine on memory in healthy rats

Values are mean $\pm$ SEM, $n = 7$, * significantly $P < 0.05$ different from control. C = control; CL = caffeine administration 15mg/kg before induction of diabetes; CM = caffeine administration 20mg/kg before induction of diabetes; CH = caffeine administration 25mg/kg before induction of diabetes

compared to the male Wistar rats.

Diabetic post-treated groups administered 15ml/kg and 25ml/kg caffeine showed significant ($p < 0.05$) reduction in body weight during week 5 and 6 when compared with week 1. It has been reported that the relationship between caffeine and diabetes mellitus is controversial, caffeine from review have both positive and negative effects on diabetes mellitus,³⁸ Richard et al. reported in their literature that caffeine consumption has been associated to lower risk of diabetes mellitus type 2, and a loss of weight in type 1 diabetes mellitus patient.

Findings from the present study showed that caffeine has no significant effect on blood glucose level in healthy rats corresponding with reports by Urzua et al.,⁴⁵ who reported same on their non-diabetes male Wistar rats. Also, reports given by, Cheraskin et al.,⁴³

stated that caffeine does not have an effect on blood glucose in non-diabetic rats when compared to control in their experiment supporting the claim of this study. This study also revealed a significant ($p < 0.05$) reduction in blood glucose in diabetic post-treated groups administered 15ml/kg caffeine during the first two weeks and, 25ml/kg caffeine in the second week respectively. There has been controversial views on caffeine effect of diabetes. Maria,³⁹ demonstrated that 25mg/kg per day caffeine is enough to impair insulin action, strictly not affecting blood glucose level in young and healthy adults, but in diabetes mellitus type 2, a detectable rise in blood glucose is seen particularly after meals. In another literature by van Dam and Hu,⁴⁰ showed that habitual coffee consumption is associated with a substantially lower risk of type 2 diabetes mellitus, which might be due to caffeine present in coffee and other contents such as magnesium. This theory was clearly explained by Ceriello and Motz,⁴¹ that coffee contains antioxidant effect on the body which would reduce the kind of oxidative stress associated with type 2 diabetes mellitus, de Valk,⁴² stressed on coffee containing magnesium which improves insulin functions. In an experiment conducted by Siham *et al.*,⁴⁴ in which streptozotocine induced diabetic male Wistar rats were given (10, 50, and 100) mg/kg doses of caffeine per day, their result showed that caffeine at 100mg/kg/day serve as a protective role in the biochemical and microscopic change in pancreatic β -cells in diabetes induced rats. Julia *et al.*⁴⁶ in their literature, stated that oral intake of coffee Arabica at doses of 63mg/kg and 93mg/kg by Holtzman (breed type) male rats did reduce blood glucose level when compared to diabetes untreated.

Findings from the present study also reflected no effect on learning but an improvement was seen in pre-treated group administered 25ml/kg caffeine in memory test when compared to control contrary to the report that caffeine sharpens our memory, increase our awareness as well as boost our long-term memory according to Gatlin.⁴⁷ Renata *et al.*,⁴⁹ in their report on the effect of chronic coffee and caffeine ingestion effect on cognitive function and antioxidant system of rats' brain shows that 20mg/kg and 40mg/kg of caffeine, before been subjected to behavioural tasks open-field maze and novel preference tasks concluded that intake of coffee improved long term memory. van Gelder *et al.*,⁵⁰ inclined on a protective influence of caffeine due to its endogenous antioxidant system, in their report, 375 ml/day approximate body weight of 60-70kg of coffee was given to elderly experiencing cognitive decline when they noticed an improve in capability and memorization in animals given coffee. Angelucci⁵¹, indicated that adult male Wistar rats after receiving caffeine (0.3-30 mg/kg) administered 30 min before training, caused a small increase in memory retrieval did not alter the performance of the animals either in the

training or in the test session during Morris water maze but post-training administration of caffeine improves memory retention but not memory acquisition.

In diabetes rats, findings showed a significant decrease in learning of control group when compared to diabetes untreated, and a significant increase during test was noticed in post-treated groups' administered 15mg/kg and 25mg/kg caffeine when compared to diabetes untreated and control. Augustina²⁹ placed emphasis on cognitive dysfunction sparing learning and memory in type 1 diabetes, while Anahad,³⁰ postulated a decline in memory attention and other cognitive skills in type 2 elderly diabetic. Elizabeth,⁴⁸ worked on midlife adults with type 1 diabetes and reported a reduction in motor speed, psychomotor efficiency due to reduction in white matter volume in some but not all, and a reduction in grey matter density in brain region responsible for language processing and memory.

Conclusion

This study was able to show that caffeine has no effect on body weight and blood glucose in normal rats, but may lead to either an increase or decrease body weight and blood glucose level in diabetic state depending on doses and periods of exposure. The effect of caffeine on learning and memory is also dependent on dosage and diabetic status of the rats.

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