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FEED-BASED-DIET OF LUTEOLIN AND ASTRAGALIN AGAINST GAMMA-IRRADIATION-INDUCED GENOTOXICITY IN RATS

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Abstract

Human exposure to gamma irradiation have been scientifically linked to genetic instability. Exposure to gamma radiation exacerbate free radical to a level that is capable of causing genetic alterations. Natural constituents of plant origin like flavonoids and polyphenols have been validated to scavenge free radicals, repair and stabilizes unstable genetic component of DNA. This study validated the scientific mechanisms associated with gamma irradiation and genetic instability and investigated the protective and ameliorative effects of luteolin and astragalín-based diet of gamma-irradiated rats system. A total of thirty (30) albino rats (200 ± 5 g) were distributed into 5 groups of 6 each. All other groups except group i were exposed to 6 grey gamma irradiation. Such that group i and ii were fed with normal rat chow and water; groups iii were fed with luteolin-based diet, iv were fed with astragalín-based diet and v were fed with the combination of luteolin and astragalín (1:1) based diet. The rats were sacrificed, tissues and blood were collected for biochemical assays. Data were analyzed using SPSS and Duncan multiple range test. Irradiation significantly ($P < 0.05$) decreased p53 and Nrf-2 concentration and antioxidant enzymes activities of irradiated-rat, whereas it significantly ($p < 0.05$) increase the formation of micronuclei DNA of irradiated rats. Administration of Luteolin and astragalín-based diet have significantly ($p < 0.05$) increased p53 level, Nrf-2 concentration and antioxidant enzyme activities of administered rats, but the formation of micronuclei DNA is significantly ($p < 0.05$) reduced. It can therefore be concluded that irradiation is genotoxic and luteolin and astragalín are geno-protective.

Keywords: Genotoxicity, Luteolin, astragalín, leaves-based-diet, p53, Nrf-2, Nf-kB

Introduction

Gamma irradiation increased the generation of free radicals to a level that is capable of increasing micronuclei DNA formation, deplete antioxidant enzymes capacity, inactivate Nrf-2 and induced p53 damage.^[1] An increased micronuclei DNA formation is suggestive of chromosomal aberration and genomic instability.^[4] Genomic instability altered the process of cellular mitosis and meiosis and by extension cellular alterations. ^[5] An increase

exposure to gamma irradiation will exacerbate free radical generation and thus increasing cellular alterations. ^[6] Prolong damage to p53 protein caused lithium fraumeni syndrome, such that the cycle lose function of cellular regulations ^[7] Loss of cellular regulation is the underlying principle of genomic instability caused by aberration to chromosome structure. Several plant have been used in the treatment and management of irradiation-induced damages and alterations: *Adansonia*

digitata and *Corchorous olitorus* leaves have been used in the protection against irradiation-induced cellular damages in rats. [7] *Vernonia amygdalina* have been implicated in the scavenging of free radical in castor oil induced ulcerated rat. [8] *Adansonia digitata* and *Corchorous olitorus* leaves-based diet have offered protection against irradiation-induced atherosclerotic rats. [8] *Adansonia digitata* and *Corchorous olitorus* have ameliorated and protected the antioxidant capacity and lipid profile of rats exposed to gamma irradiation. [9] It is important to know that the pharmacological activities associated with *Adansonia digitata*, *Corchorous olitorus* and *Vernonia amygdalina* have been adduced to the bioactive constituents found in these plant, and this was further corroborated by the work of. [41] Contained in the leaves of *Adansonia digitata* and *Corchorous olitorus* are astragalin, luteolin, quercetin, catechin, epicatechin, tocopherol and saturated and unsaturated fatty acids as revealed by the high performance liquid chromatography (HPLC) analysis of *Adansonia digitata* and *Corchorous olitorus*. [41] Hence this work investigated the direct pharmacological potentials of luteolin and astragalin leaves-based diet on gamma irradiation-induced Genotoxicity in rats.

Materials and MethodS

Phyto-constituents used in the formulation of feed

Luteolin and astragalin used in the formulation of feed in this study were

product of Monobind International, Lake Forest, USA. Nuclear Related Factor-2 (Nrf-2) and p53 ELISA kits were products of Cayman Laboratory LTD., USA. Other chemicals were prepared in all-glass distilled water.

2.2 Experimental Rats

A total of thirty (30), Wistar rats (200.00 ± 5.00 g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in clean cages and well-ventilated house conditions (Temperature: 28-30°C), (Photo-period; 12hr light, 12hr dark), (Humidity: 45-55%). They were fed with pelletized rats feed (Vital Feed, Grand Cereals, Jos, Nigeria) and clean tap water which were provided before and during administration of leaves-based diet.

Feed formulation

The following feed components from table 1 above were added together in the quantity arrived at in Table 2 below. The yellow corn was washed with clean water, dried at room temperature (29 °C) for 7 days. The dried yellow corn was grinded using grinding machine (Steelman K207, H.M and co Ltd, China).

Table 1: Feed Constituents and source

Feed Constituents	Sources
Corn starch	Was obtained from 'Oja-Tuntun' Market, Ilorin, Kwara State, Nigeria
Vitamins-mineral mix:	Product Ahirma,, India
D-methionine and L-Lysine	Product Ahirma,, India
Maize husk and Soybean oil	Were purchased from Olufadi, Ilorin
Luteolin	At 80 % inclusion
Astragalin	At 80 % inclusion
Luteolin + astragalin (1:1)	At 80 % inclusion

The Maize husk was grinded to shaft (50 % grinding) with grinding machine (Steelman K207, H.M and co Ltd, China). Soy bean was grinded with grinding machine (Steelman K207, H.M and co Ltd, China) to powdered form.

500 ml of Soy bean oil and 4 gram each of Vitamin and Mineral mix, D-methionine and L-lysin were added too. The luteolin and astragalin were adequately added to the mixture at 80 % inclusion.

Table 2: Formulation of luteolin and astragalin based-diet

Ingredients	Control diet	Luteolin-Diet	Astragalin-Diet	Luteolin+AstragalinDiet
Corn Starch	512	512	512	512
Soy Beans	250	250	250	250
Soy Oil	40	40	40	40
Maize Husk	40	40	40	40
Vit/Min Mix	50	50	50	50
Sucrose	100	100	100	100
D-Met	4	4	4	4
L-lysin	4	4	4	4
<i>Luteolin</i>	-	204	204	204
<i>Astragalin</i>	-	204	204	204
<i>Luteolin</i> + <i>Astragalin</i>	-	204	204	204

Compounded diets were administered to gamma irradiated rats for a period of 2 weeks before they were sacrificed.

(Oyegoke and Oladiji, 2018), which was briefly modified.

Exposure of Rats to Radiation and Induction of Genotoxicity in Rats

Experimental rats were exposed to

single dose of 6 grey whole-body gamma radiation by the method described by.^[5] Which was briefly modified by.^[4] The experimental animals

were kept in an improvised carton cage with holes made round the carton for ventilation (carton was used to restricts the movement and ensure uniform exposure of rats to radiation) instead of the usual radiation exposure coat that was used by.^[5]

Ethical approval

Ethical approval was obtained from the University of Ilorin Ethical Committee, University of Ilorin. With the ethical approval number obtained as follow UERC/ASN/2018/1409 on the 13th September, 2018. The study was conducted following the guidelines on the care and use of laboratory animals.

Animal Groupings and Feed-based Diets Administration

Table 3: Animal grouping

Group	Definition of Terms
Positive (NIR)	Rats were fed with normal rat chow and they were not exposed to irradiation
Negative (IR)	Rats were exposed to irradiation but not treated
IR-LBD	Rats were exposed to irradiation but were fed with luteolin-based diet
IR-ABD	Rats were exposed to irradiation but were fed with astragalin-based diet
IR-L+ABD	Rats were exposed to irradiation but were fed with combination of luteolin and astragalin-based diet

NIR-non-irradiated; IR-irradiated; IR-LBD- irradiated-luteolin-based-diet; IR-ABD- irradiated-astragalin-based-diet; IR-L+ABD- irradiated-luteolin+astragalin-based-diet.

Collection of Biological Fluids

haematological and biochemical serum analysis.^[15]

Blood Collection

Serum

Feed administration was discontinued 24 hours to blood collection from the irradiated rats administered with *luteolin* and astragalin-based diets. The animals were anaesthetized using diethyl ether soaked in cotton wool. The soaked cotton wool was housed in a closed glass container. The rats were placed in the glass container to lose consciousness, the jugular veins were slightly displaced for incision. Blood samples were collected into heparinized and non-heparinized sample bottles for

The blood collected into the non-heparinized sample bottle were left undisturbed for 30 minutes at room temperature. The bloods were centrifuged at 4500 g for 10 minutes using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England). The sera were pipetted using Pasteur pipette into clean, dry sample bottles and refrigerated for further analysis.^[21]

Preparation of Tissue Homogenate

After sacrifice, the rats were dissected to isolate the liver. The isolated liver was gently cleansed with 1.14 % KCl washing solution to remove blood stains and dirty. The liver was weighed and immediately stored in ice cold 0.25M sucrose solution. 1g of the liver was cut with a clean scapel, homogenized in ice-cold 0.25M sucrose solution (1:5^{w/v}). The homogenates were cold centrifuge at 12000g for 15 minutes, the supernatant and sediments were separated into different sample bottles and stored in the freezer (-5°C) until required for further analysis.

**Determination of Genotoxicity in irradiated Rats and the effects of Luteolin and astragalin diet
Micronuclei assay and mean number of micro nucleated polychromatic Erythrocytes**

Determination of the number micro nuclei DNA formed and the extent of chromosomal aberration was assayed for by the method adopted by^[12]and modified by^[10];the rats were sacrificed by cervical dislocation. The femurs were removed and bone marrow was flushed with Fetal Bovine Serum (FBS) (PAA Laboratories GmbH, PAA-Strasse 1, Pasching, Austria). The flushed cells were centrifuged at 2000 rpm for 5 minutes. The supernatants were discarded and the sediment were slides and stained with May-Grunwald and Giemsa stains. At least 2000 erythrocytes per rats were scored at ×1000 for micro nuclei in polychromatic erythrocytes (MNPCE) using automated electron microscope

(KM80 R10B, Aust-Elect, Gabson, UK).

Preparation of Nuclear Extract, from which p53 and Nrf-2 are assayed for.

Irradiated rats that were fed with *A. digitata* and *Corchorus olitorius* leaves based-diet, administered with n-hexane, ethylacetate and n-butanol fractions and Subfractions were sacrificed by cervical dislocation and the liver were isolated. The isolated liver was homogenized (phosphate buffer saline media) and cold-centrifuged at 12000 g for 15 minutes. The supernatants were kept for other biochemical analysis. The sediment was treated with DNase inhibitor to prevent degradation of DNA of interest, they sediments were also treated with an alcohol (calcitol) to kill and coagulate impurities, the treated sediment was further ultra-centrifuge at 4500 g for 10 minutes. The sediment was discarded and supernatant was retained. The retained supernatant was treated with NP40 and Tris-chloride, to aid the separation of the cytoplasmic fraction from nuclear fraction. The mixture (supernatant and NP40 and Tris-chloride) was ultra-cold-centrifuge at 8,500 g for 10 minutes. The sediment was discarded and the supernatant (Nuclear extract) was obtained and was further used for p53 and Nrf-2 ELISA analysis.

Determination of p53 protein assay

Determination of p53 concentration was assayed for by adopting the method described by.^[18]

Determination of Nrf-2 protein assay

Determination of p53 concentration was assayed for by adopting the method described by.^[18]

Determination of Antioxidant Enzymes Assay

Superoxide dismutase activities

The Superoxide dismutase activities was determined by the method adopted by.^[21]

Catalase activities

The Catalase activities was determined according to the method of [20].

Glutathione-S-transferase (GST) activities

The Glutathione-S-transferase (GST) activity was determined by according to.^[21]

Results

The effect of Luteolin and Astragal-based diet on the Chromosomal aberration status of irradiated rats, taking consideration of the mean number of micro nuclei DNA formed

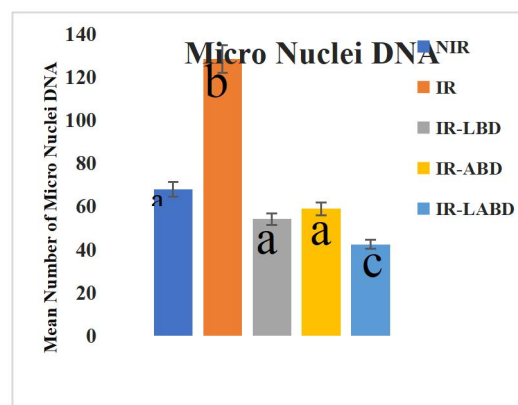


Figure 1: The effect of Luteolin and Astragal-based diet on the mean number of micro nuclei DNA of irradiated rats. NIR- non-irradiated; IR- irradiated; IR-LBD: irradiated-luteolin-based diet; IR-ABD- irradiated-astragal-based-diet; IR-LABD- irradiated-luteolin-astragal-based-diet.

The effect of Luteolin and Astragal-based diet on the p53 status of irradiated rats

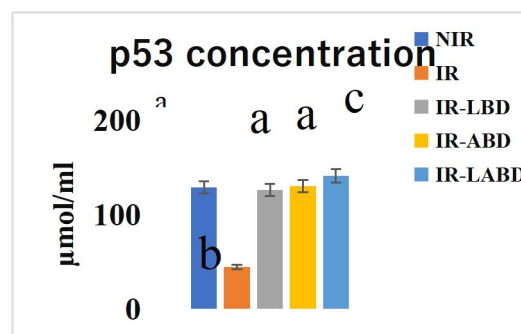


Figure 2: The effect of Luteolin and Astragal-based diet on the p53 status of irradiated rats. NIR- non-irradiated; IR- irradiated; IR-LBD: irradiated-luteolin-based diet; IR-ABD- irradiated-astragal-based-diet; IR-LABD- irradiated-luteolin-astragal-based-diet.

The effect of Luteolin and Astragalin-based diet on the Nrf-2 status of irradiated rats

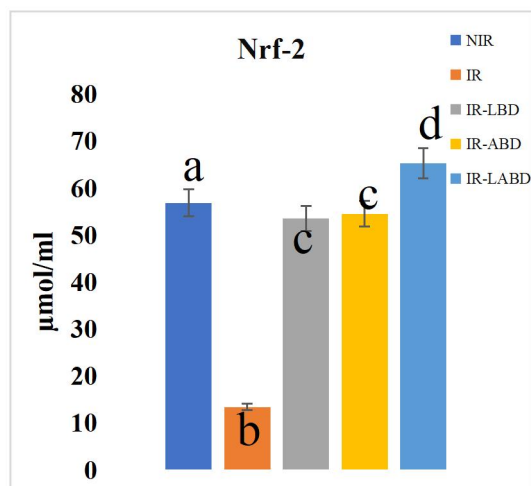


Figure 3: The effect of Luteolin and

Astragalin-based diet on the Nrf-2 status of irradiated rats. NIR- non-irradiated; IR-irradiated; IR-LBD: irradiated-luteolin-based diet; IR-ABD- irradiated-astragalin-based-diet; IR-LABD- irradiated-luteolin-astragalin-based-diet.

Discussion

Exposure to gamma Irradiation exacerbate free radical generation from subtoxic to very toxic concentration. This concentration is capable of mediating the synthesis of micronuclei DNA and eliciting chromosomal aberration that eventually lead to Genotoxicity, cellular alterations and eventual damage to cellular system and then death.^{[6][42]}

Table 3The effect of Luteolin and Astragalin-based diet on the Nrf-2 status of irradiated rats. NIR- non-irradiated; IR-irradiated; IR-LBD: irradiated-luteolin-based diet; IR-ABD- irradiated-astragalin-based-diet; IR-LABD- irradiated-luteolin-astragalin-based-diet.

Groups	SOD	CAT	GST	GPX	MDA
NIR	34.12±0.06 ^a	24.12±0.16 ^a	87.12±0.65 ^a	41.56±0.23 ^a	19.45±0.10 ^a
IR	9.56±0.07 ^b	12.12±0.15 ^b	22.23±0.55 ^b	8.65±0.24 ^b	89.45±0.13 ^b
IR-LBD	38.12±0.02 ^c	21.11±0.13 ^c	74.43±0.45 ^c	39.78±0.29 ^c	21.22±0.14 ^c
IR-ABD	37.34±0.02 ^c	22.00 ±0.16 ^c	74.54±0.65 ^c	38.76±0.21 ^c	20.45±0.17 ^c
IR-LABD	40.12±0.05 ^d	27.78±0.17 ^d	80.12±0.48 ^d	45.23±0.26 ^d	15.34±0.89 ^d

As observed in the present study, exposure of rats to 6 grey gamma irradiation, might be adduced to the increase concentration of micro nuclei DNA formation in the irradiated rats, when compared with the non-irradiated control.

^[42]Have established the direct linkage between gamma irradiation and depletion of antioxidant enzyme activities of gamma irradiated rats. This depletion have been associated with increased free radical generation by gamma irradiation ^[41]. In the present study, the exposure of rats to 6 grey gamma irradiation maybe suggestive of the decreased in the antioxidant enzyme activities in the rat exposed to 6 grey gamma irradiation, when compared with the non-irradiated control.

Rat exposure to irradiation have induced p53 damage, leading to lithium fraumeni syndrome. This syndrome predisposes irradiated rats to carcinogenesis.^[42]In the present study, the reduction in the p53 concentration observed in rats exposed to gamma irradiation, maybe associated with p53 damage caused by irradiation. This maybe suggesting that, a diseased condition called lithium fraumeni syndrome might have been inflicted in the group exposed to irradiation. Also, Irradiation-induced free radical generation mediated the inactivation of Nrf-2 in irradiated rats, thus preventing the movement of Nrf-2 from the cytosols to the nucleus. This excessive inactivation is the underlying cause of antioxidant system depletion and increase Nf-kB activation. ^[40] In the

present study, there is observable reduction in the Nrf-2 concentration in the rat exposed to irradiation, this reduction maybe due to gamma irradiation-induced inactivation.

^[41]Have established the protective effective of *Adansonia digitata* and *Corchorous olitorous*leaves-based dietin gamma-irradiated-rat. Also ^[41] have established the amelioration of radiation-induced cellular alterations in rat administered with solvent fractions of methanol extract of *Adansonia digitata* and *Corchorous olitorous*Leaves in rats.

^[40]Have also established that, thebioactive constituents of *Adansonia digitata* and *Corchorous olitorous* against irradiation-induced cellular damages in rats.Also ^[43] have also established that, the bioactive constituents in*Adansonia digitata* and *Corchorous olitorous* that maybe responsible for all the above pharmacological properties mentioned aboveare quercetin, catechin, luteolin, astragalin, epicatechin, tocopherol and unsaturated fatty acids in varying concentrations respectively. These constituents maybe responsible for the pharmacological activity against gamma irradiation-induced cellular alterations. In the present study, the presence of luteolin and astragalin in the diet administered to irradiated rats were responsible for the reversal in the concentration of micronuclei DNA formed in diet-administered rats when compared with the negative control rats. This implied that luteolin and astragalin based-diet administered restored genetic

stability and proper chromosomal architecture.

Luteolin and astragalin are polyphenol and they scavenge free radicals and as well stabilized reactive intermediate [31]. The free radicals scavenging properties of luteolin and astragalin will prevent antioxidant enzyme depletion. Thus preventing cellular alterations that might be induced from free radical generation. As observed in the present study, there is increased in the antioxidant enzyme capacity in the luteolin and astragalin based-diet administered rats, when compared with the negative control group. This implied that, the luteolin and astragalin successfully restored and further increased the antioxidant enzyme capacity of irradiated rats.

Recommendation

As observed in the present study, that luteolin and astragalin based-diet is safe have successfully reversed cellular alterations in rat exposed to gamma irradiation, I therefore recommended luteolin and astragalin to be further worked on as a component of human diet against any cellular alterations.

Conclusion

It can therefore be concluded that Luteolin and astragalin are in part responsible for the pharmacological properties of *Adansonia digitata* and *Corchorus olitorius* leaves. The duo constituents are potential nutraceuticals.

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