

Radioprotective effects of ethanolic extracts of *Gongronema latifolium* leaf against radiation induced oxidative stress in Wistar albino Rats

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ABSTRACT

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Background: The effects of low radiation exposures are naturally harmful to the humans and in this study we aimed to investigate the radioprotective effects of *Gongronema latifolium* extracts (GLE) against radiation induced oxidative stress.

Materials and Methods: Forty eight rats were divided into control and experimental groups. The control group had two sub-groups; (i) normal control (NC) was neither irradiated nor treated with GLE and (ii) irradiated control (IC) was irradiated (with 4 and 6 Gy) but no GLE treatment. The experimental group also had two sub-groups; (i) pre-treated (PRT) was treated with GLE before irradiation and (ii) post-treated (PST) was irradiated before treatment with GLE. Radioprotective effects were measured by noting changes in biochemical parameters of liver, lipid peroxidation, antioxidants enzymes and changes in body weight that occurred in the sub-groups. ANOVA with Tukey's test were used to determine significance differences in the sub-groups.

Results: There were significant changes in all biochemical enzyme markers between the NC and IC with values of % inhibition of lipid peroxidation 33 ± 1.5 and 71 ± 5.5 at 6 Gy dose of radiation exposure. The values of lipid peroxidation in PRT and PST differed significantly from the IC with values 35 ± 5.0 and 57 ± 1.0 at 6 Gy respectively; thus significant difference exists between PRT and PST. The IC sub-group recorded the highest radiation induced weight loss (56%). **Conclusion:** Preliminary result indicates that GLE ameliorated oxidative stress in pretreated sub-groups than in post-treated sub-groups, suggesting GLE as a potential prophylaxis.

INTRODUCTION

Humans are exposed to radiation from both natural and anthropogenic sources. Some of these exposures are intentional others are largely due to environmental factors ⁽¹⁾. Exposure to low level radiation is on the increase and common amid medical diagnostic procedures and advancement in technologies ⁽²⁾. The effects of these exposures are naturally deleterious to human body. Damage to cells can occur directly by ionization of critical components of the cell like deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins by radiation, and indirectly via generation of reactive oxygen species (ROS) ⁽³⁾.

In living cells, there exist efficient defense and repair mechanisms against ROS and they include enzymes such as glutathione peroxidase, superoxide dismutase and catalase. Increase in ROS production

depletes antioxidants defense and thus leads to chemical alterations of biomolecules which can disrupt structural and functional processes in the cell ⁽⁴⁾. ROS have the potential to initiate and promote oxidative damage in the form lipid of peroxidation ⁽⁵⁾. Radiation damage to tissues is still mostly attributed to overproduction of ROS in the biological system after radiation exposure ⁽⁶⁾. Increase in ROS possibly increases the level of lipid peroxidation in cells which results in an increase in oxidative stress. Hence protection of biological cells in the presence of ionizing radiation is of utmost importance in both planned and accidental exposures.

Radioprotectors are agents designed to mitigate radiation induced injury in normal cells. These compounds can be classified into two; prophylactic agents and mitigators or therapeutic agents. Prophylactic agents are administered before radiation exposure or radiotherapy to forestall the

development of acute or chronic effects of radiation. While mitigators are used to lessen toxicity even after radiation has been delivered (7). Attempts have been made to ameliorate these effects prophylactically by improving antioxidant level in the body or by mitigating the effects following exposures.

Several synthetic compounds have been investigated and tested for their radioprotective potency (8). Their mechanisms of actions are basically scavenging of free radicals and donation of hydrogen atoms. However, high toxicity and unfavorable routes of administration of these compounds limits their application and efficacy (9). Use of plants and its extract has been seen as a better alternative due to the high constituent of bioactive agents in addition to secondary metabolites with immunostimulants and antioxidants capacity. They are also essentially non-toxic relative to the synthetic compounds at their optimum protective dose (10). Studies in different strains of animals have shown that the administration of plant extracts and bioactive agents isolated from plant such as *Ocimum basilium* (2) *Alstonia scholaris* (11), genestein (12), *Tinospora cordifolia* (13), Curcumin (14), *Runus avium* (15), Cheonggukjang (16), Arbutin (17), naringin (18) and *Tanacetum parthenium* (19) protect different tissues against oxidative stress induced by radiation.

Gongronema latifolium belongs to family of *asclepiadaceae* plants. It is well known as utazi and arokeke in southeast and southwest Nigeria. *Gongronema latifolium* extract (GLE) was used in Nigerian ethnomedicine for the management of diabetes mellitus and high blood pressure (20). Studies have shown that GLE have strong activities against cancer cell lines such as A-549 (human lung carcinoma) and MCF-7 (human breast adenocarcinoma) (21). Essien and Effiong (22) reported the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of GLE and standard vitamin C to be 80.95% and 93.93% respectively. Their findings showed that there was no significant difference in DHHP scavenging activities of GLE and standard vitamin C. The phytochemical analyzes of GLE revealed antioxidant compounds such as alkaloids, phenols, tanins, flavonoids, saponins, alkaloids and Oligosaccharides (23, 24). Furthermore, vitamins such as Niacin, ascorbic acid, tocopherol, riboflavin and thiamine are also presence in GLE (25). The GLE contains bioactive agents that can change biological processes and overturn some disease conditions (26). Bioactive phytochemicals like polyphenols and flavonoids are essential antioxidants that eliminate ROS well known for causing many disease conditions (27).

However, till date, there is no report on the radioprotective potential of *Gongronema latifolium* leaf extract. Hence this study was designed to investigate the radioprotective effects of *Gongronema latifolium* leaf extract against whole-body irradiation

induced oxidative stress on wistar albino rats.

MATERIALS AND METHODS

Chemicals

Absolute ethanol, glacial acetic acid, sodium dodecyl sulphate (SDS), 1% thiobarturic acid (BDH, England), trichloroacetic acid, potassium dichromate (Sigma Aldrich, Germany) adrenalin (Merck Darmstadt, Germany) and other reagents used were of analytical grade.

Sources of animals

All the albino rats used in this study were purchased from the animal house, in the Department of Zoology, University of Nigeria Nsukka (UNN). Forty eight Wistar albino rats, comprising 31 males and 17 females, weighing about 130 – 150 grams and between ages of 16 to 20 weeks were used for this study. Prior to the studies the albino rats were given 14 days to acclimatize under standard environmental conditions with ambient temperature 22 ± 2 °C, air humidity of $50 \pm 10\%$, and light-darkness cycle 12/12 hrs at the animal house in University of Nigeria, Enugu campus (UNEC). The rats were housed in standard cages and fed with a standard Grower's mash rat pellets and water *ad libitum*, all through the experiment. This study was performed following guideline approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, UNN, with registration/approval reference number: FVM-UNN-IACUC-2021-0672.

Collection and preparation of *Gongronema latifolium* ethanolic leaf extracts

The fresh leaves of *gongronema latifolio* leaves were harvested from Rex Farm in Nsukka Local Government Area of Enugu State. The leaves were botanically identified by a Botanist in the department of Botany, UNN. Method of (28) was used for the preparation of GLE. The harvested fresh leaves of *gongronema latifolio* were washed severally with tap water to remove impurities. The leaves were dried in sun shade for 14 days to get rid of residual moisture and crushed into fine powder deploying electric mill. The samples were packed in air-tight plastic container. One kilogram (1 kg) of the pulverized sample was weighed and macerated in 2.5 L of 80% ethanol for 72 hrs. Then followed by periodic and thorough shaking at intervals and then stored at room temperature $26 - 28$ °C. The obtained sample was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and 16.2 grams of GLE was obtained. The extract was stored in an air-tight plastic container and kept in a refrigerator for the study. The GLE toxicity test and dose selection (250 mg/kg BW) for this research was based on previous report by Al-Hindi (29). They

reported no-observed-adverse-effect level (NOAEL) of GLE for both male and female rats as 250 mg/kg BW after extensive oral toxicity study on GLE at single dose of 250, 500 and 1000 mg/kg BW.

Experimental design

This experiment was divided into two batches based on the radiation dose given; batch one (4 Gy dose) and batch two (6 Gy). In each batch, the experiment was divided into two groups; the control and experimental group. The control group had two sub-groups; Normal control (NC) and Irradiated control (IC). On the other hand, experimental group also had two sub-groups; pre-treated (PRT) and post-treated (PST). Each sub-group contains six Wistar albino rats.

Batch one;

1. Control Group

- I. Normal control (NC): no treatment with GL extract, no radiation exposure
- II. Irradiated control (IC): no treatment with GL extract, but were exposed to 4 Gy dose

2. Experimental Group

- I. Pre-treated (PRT): were treated with 250mg of GLE/kg body weight (BW) for 7 consecutive days before being exposed to 4 Gy dose
- II. Post-treated (PST): were exposed to 4 Gy dose before being treated with 250mg of GLE/kg BW for 7 consecutive days.

In batch two, the same protocol and procedures were followed, except the radiation dose that was increased to 6 Gy.

Irradiation (IR)

Radiation doses of 4 and 6 Gy selected for this study was based on report by Debajit *et al.* They reported chronic changes in biochemical parameters such as hepatic enzymes, lipid peroxidation and antioxidant defense enzymes in Wistar albino rats that were exposed to the selected doses⁽³⁰⁾. The rats in IC, PRT and PST sub-groups were whole-body irradiated using 6 MV photon beam Linear accelerator (Elekta precise treatment System, UK, serial number 151315), at the Radiotherapy Unit, Radiation Medicine Department, University of Nigeria Teaching Hospital, Enugu. The animals were immobilized in special well ventilated plastic cage. In batch one, the sub-groups (IC, PRT and PST) were individually exposed to 4 Gy dose at dose rate of 245 MU/min, weighted 1:1 parallel opposed in posterior-anterior position. In batch two, each sub-group (IC, PRT and PST) was exposed to 6 Gy at dose rate of 245 MU/min, weighted 1:1 parallel opposed in posterior-anterior position. Six rats were irradiated at the same time at a field size of 22.5cm x 18cm at a source surface distance of 95.5cm. All IR was done at the same temperature (23.2 °C), pressure (984.5hPa), attenuation factor (0.9982), and treatment set-up

(source axis distance).

Assessment of biochemical parameters

The blood samples of all sub-groups were collected through ocular puncture and were collected before and after IR. The blood samples were allowed to clot and then centrifuged to obtain serum which was used for measurement of the following biochemical parameters; alkaline phosphatase (ALP), alanine amino-transferase (ALT), aspartate amino-transferase (AST), malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD).

Determination of ALP was based on method of GSCC. Rec⁽³¹⁾. Concisely, the blank tubes (BT) and sample test tubes (STT) were assembled in duplicates, and then 0.05 ml of sample and 0.05 ml of distilled water was added into the STT and BT respectively, using a pipette (Pyrex, England). Three milliliters (3.0 ml) of substrate (phenolphthalein monophosphate) was added into each tube, mixed and the initial absorbance was captured at 405 nm using Spectrophotometer (E312, Jenway, UK). The stop watch was started and the absorbance of the sample as well as the blank was taken again thrice at interval of 1 min. The ALP activity was estimated using equation 1.

$$\text{Activity of ALP (in iU/L)} = \frac{\text{Absorbance of sample} \times 3300}{\text{Absorbance of Standard}} \quad (1)$$

Method of Reitman and Frankel⁽³²⁾ was utilized in estimating the ALT activity. Briefly, the BT and STT were assembled in duplicates and then 0.1 ml of serum was added into the STT. To these 0.5ml of buffer solution consisting of phosphate buffer, L-alanine and α -oxoglutarate were added. The blend were carefully mixed and then incubated for 30 min at 37 °C and pH of 7.4. Subsequently, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the two tubes while 0.1ml of sample was added to STT. The tubes were carefully mixed and incubated for 20 min at 25°C. Then 5 ml of NaOH solution was then added to each tube and mixed. The color intensity was taken against the blank after 5 min at wavelength 540 nm using Colorimeter LCD-52 (El, scientific co. India).

For the determination of AST, the method of Reitman and Frankel⁽³²⁾ was also deployed. However, AST was estimated by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The color intensity was taken against the blank at wavelength of 546 nm.

Lipid peroxidation was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) deploying method of Niehaus and Samuelsson⁽³³⁾. Briefly, 0.1 ml of liver homogenate (10 %w/v) was treated with 2 ml of thiobarbituric acid (0.37%), trichloroacetic acid (15 %) and 0.25 N Hydrogen chloride at ratio of 1:1:1. The mixture was kept in a boiling water bath (Water bath, Gallankamp England)

for 15 min, allowed to cooled and centrifuged (centrifuge 800D, Vickas Ltd, England) for 10 min. The obtained supernatant was analyzed by measuring its absorbance at 535 nm against reference blank. The lipid % inhibition was estimated using the equation 2.

$$\text{Lipids \% Inhibition} = \frac{A_0 - A_1}{A_0 \times 100} \quad (2)$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Reduced glutathione was determined using the modified method of Ellman ⁽³⁴⁾. Briefly, 0.1 ml of the sample and 0.9 ml of distilled water was mixed in a beaker, also added to the mixture was 0.02 ml of sodium sulphate, the blend was well shaken and kept in standing position at room temperature for 2 min. Then 0.02ml of lithium Sulphate (20%), 0.2ml of phosphor-18-tungstic acid and 0.2ml of 20% NaCO_3 were added to the beaker, the blend were shaken and kept in standing position for 4 min while monitoring peak color changes. Afterward, 2.5ml of 2% sodium sulphite was added and the absorbance was read at 680nm, the blank was set-up within 10 min. The absorbance was determined at wavelength of 412 nm. The % inhibition of GSH was estimated using equation 3,

$$\% \text{GSH of inhibition} = \frac{\{\text{Increase in absorbance of the sample} \times 100\}}{\text{Increase in absorbance of the blank}} \quad (3)$$

Catalase activity was examined adopting method of Sinha ⁽³⁵⁾. In brief, the sample was homogenized in 0.01 M phosphate buffer (at pH 7.0) and then centrifuged (at 5000 rpm). The reaction mixture includes 0.1 ml of supernatant, 0.4 ml of hydrogen peroxide (0.2 M) and 1 ml of 0.01 M phosphate buffer (pH 7.0). The reaction was put to a halt by adding 2 ml of dichromate-acetic acid (5% $\text{K}_2\text{Cr}_2\text{O}_7$ prepared in glacial acetic acid). The transition in the absorbance was estimated at 620 nm and noted. The % inhibition of CAT was calculated using the relation in equation 4,

$$\% \text{ Catalase inhibition} = \frac{100 - \{\text{Increase in absorbance of the sample} \times 100\}}{\text{Increase in absorbance of the blank}} \quad (4)$$

The SOD activities of the samples were determined using method of Arthur and Boyne ⁽³⁶⁾. A volume, 0.1 ml of serum and 0.9 ml of phosphate buffer (pH 7.8) were set-up in triplicates in 2.5ml buffer. Then 0.3ml of 0.01g adrenaline dissolved in 17 ml of DW was added and mixed in a cuvette. The SOD % inhibition was estimated by the changing rate of absorbance and was read at wavelength of 560 nm as described in equation 5,

$$\text{SOD \% inhibition} = \frac{\{\text{Increase in absorbance of the sample} \times 100\}}{\text{Increase in absorbance of the blank}} \quad (5)$$

Physical parameter

The mean BWs of all the rats in each sub-groups were determine before and after IR using a weighing balance (Vickas Ltd, England) to monitor changes.

Statistical analysis

Statistical analysis was performed using statistical package for the social sciences (SPSS) version 20, Chicago, IL, USA. The experiments were carried out in triplicate and data were presented as mean $\pm$ standard deviation (SD). Mean difference were considered statistically significant at $p \leq 0.05$. One way analysis of variance (ANOVA) in conjunction with Tukey's honest significance different (HSD) test was used to determine variation in both biochemical and physical parameters.

RESULTS

Biochemical parameters

Hepatic enzymes

Table 1 shows the hepatic enzyme activities in all the sub-groups, before and after whole-body exposure to 4 and 6 Gy doses. From the analyses there was no statistical significant difference ($p > 0.05$) in the hepatic enzymes (ALP, ALT and AST) in all the sub-groups before IR. However, serum activities of the hepatic enzymes (ALP, ALT and AST) in IC sub-groups increased significantly ($p < 0.05$) in comparison to NC sub-group on day 8 after IR. The serum activities of ALP, ALT and AST in IC sub-groups that were exposed to 4 Gy increased by 89, 60 and 91% respectively. In the same way, the serum activities of ALP, ALT and AST in IC sub-groups that were exposed to 6 Gy dose elevated by 105, 89 and 91% respectively. Remarkably, the serum activities of ALP, ALT and AST in PRT sub-groups that were exposed to 4 and 6 Gy doses indicated no significant differences ($p > 0.05$) in the observed mean values compared to the NC sub-groups. The PST sub-groups exposed to 4 and 6 Gy doses showed no significant difference ($p > 0.05$) in the serum activities of ALP and ALT relative to NC group, except for serum activity of AST at 6 Gy dose.

Oxidative stress

Figure 1 demonstrates MDA % inhibition in all the sub-groups, before and after whole-body exposure to 4 and 6 Gy doses. The results showed that there was no significant difference ($p > 0.05$) in MDA % inhibition in all the sub-groups before whole-body exposure to radiation. In comparison to NC sub-groups, the MDA % inhibition in IC sub-groups that were exposed to 4 and 6 Gy doses significantly increased ($p < 0.05$) by 130 and 91% respectively. Interestingly, there was no significant difference ($p > 0.05$) in the MDA % inhibition among NC, PRT and PST that was exposed to 4 Gy. For sub-groups that

were exposed to 6 Gy, the same trend was observed between NC and PRT sub-groups, however, the PST sub-groups showed slight significant increase ($p < 0.05$) compared to NC sub-group.

Table 1. The alkaline phosphatase (ALP), alanine amino-transferase (ALT), aspartate amino-transferase (AST) activities of all the sub-groups exposed to 4 and 6 Gy doses.

	4Gy dose, ALP (iU/L) Mean ± SD		6Gy dose, ALP (iU/L) Mean ± SD	
Sub-groups	Before IR (n=6)	Day 8 after IR (n=6)	Before IR (n=6)	Day 8 after IR (n=6)
NC	38 ± 2.0	39 ± 1.0	38 ± 2.0	39 ± 1.0
IC	35 ± 2.5	74 ± 3.4*†	40 ± 1.5	80 ± 2.0*
PRT	41 ± 1.0	37 ± 4.0*	31 ± 3.0	43 ± 3.0*
PST	36 ± 2.5	43 ± 7.0*	32 ± 1.5	52 ± 3.0*
	ALT (iU/L)		ALT (iU/L)	
NC	37 ± 2.5	37 ± 1.0	37 ± 2.5	37 ± 1.0
IC	37 ± 1.5	60 ± 2.0*†	37 ± 3.5	70 ± 7.5*†
PRT	38 ± 2.1	32 ± 2.5*	29 ± 1.0	41 ± 9.4*
PST	38 ± 1.2	39 ± 4.5*	31 ± 1.0	46 ± 2.5*
	AST (iU/L)		AST (iU/L)	
NC	38 ± 2.0	33 ± 1.0	38 ± 2.5	33 ± 1.0
EC	39 ± 6.1	63 ± 4.0*†	37 ± 8.1	63 ± 4.0*†
PRT	39 ± 2.5	32 ± 3.7*	32 ± 3.8	35 ± 1.1*
PST	38 ± 2.0	44 ± 3.0*	30 ± 3.6	44 ± 3.0*

*Significantly different ($p < 0.05$) from NC, † Not significantly ($p > 0.05$) different from NC. ‡ Significantly different ($p < 0.05$) from PRT and PST.

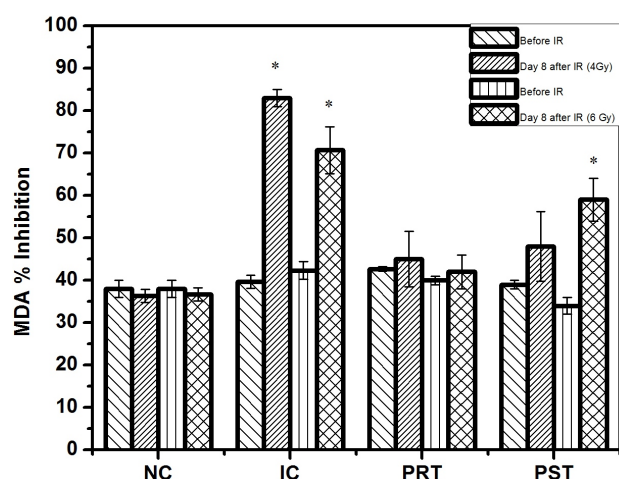


Figure 1. Malondialdehyde (MDA) % inhibition in Normal control (NC), Irradiated control (IC), Pre-treated (PRT) and Post-treated (PST) sub-groups. Values are expressed as mean ± Standard deviation (SD) and * indicates $p \leq 0.05$ (level of significance).

Antioxidant enzymes

Table 2 indicates GSH, CAT and SOD % inhibition of all the sub-groups, before and after exposure to 4 and 6 Gy doses. The analyses revealed that there was no significant difference in the mean values of the antioxidant enzymes (GSH, CAT and SOD) in all the groups before exposure to 4 and 6 Gy doses.

The GSH % inhibition in IC sub-group that was exposed to 4 Gy decreased significantly ($p < 0.05$) compared to NC and PRT sub-groups on day 8 after IR. The same trend was also noted in GSH % inhibition in the IC sub-group that was exposed to 6 Gy. The % decrease in GSH activities of IC sub-groups

that were exposed to 4 and 6 Gy in comparison to NC sub-group were 57% and 48% respectively. The PRT and PST sub-groups that were exposed to 4 and 6 Gy dose also decreased significantly relative to NC sub-group but with improved recovery when compared to IC sub-groups.

The CAT % inhibition in IC sub-groups exposed to 4 and 6 Gy decreased significantly ($p < 0.05$) compared to NC, PRT and PST sub-groups on day 8 after IR. The decrease in CAT activities for IC sub-groups exposed to 4 and 6 Gy relative to NC were 61% and 51% respectively. Rats in PRT and PST sub-groups indicated significant decrease in CAT activities compared to NC sub-groups but with improved recovery relative to IC sub-groups.

The SOD % inhibition in IC sub-groups irradiated at doses of 4 and 6 Gy decreased significantly ($p < 0.05$) in comparison to NC, PRT and PST sub-groups. The decrease in the mean value of SOD activities in IC sub-groups exposed to 4 and 6 Gy relative to NC were 63% and 56% respectively. Relative to NC sub-groups, rats in PRT and PST sub-groups also exhibited significant decrease in SOD mean activities but with better recovery compared to the IC sub-groups.

Table 2. The glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) percentage inhibition % of all the sub-groups exposed to 4 and 6 Gy doses.

	GSH , 4Gy, MEAN ± SD, n=6 % Inhibition		GSH , 6Gy, MEAN ± SD, n=6 % Inhibition	
Group	Before IR (n=6)	Day 8 after IR (n=6)	Before IR (n=6)	Day 8 after IR (n=6)
NC	69 ± 2.5	69 ± 3.0	69 ± 2.5	69 ± 1.0
IC	68 ± 2.0	30 ± 2.8*†	65 ± 1.0	36 ± 2.5*
PRT	62 ± 1.0	60 ± 1.5*	68 ± 1.0	60 ± 7.0*
PST	65 ± 2.0	45 ± 4.5*	74 ± 2.5	42 ± 4.0*
	CAT		CAT	
NC	81 ± 1.5	79 ± 2.6	80 ± 1.5	79 ± 2.6
IC	76 ± 5.0	31 ± 5.0*	77 ± 4.5	38 ± 1.5*
PRT	77 ± 2.5	50 ± 8.5*	77 ± 4.6	60 ± 8.5*
PST	78 ± 3.7	44 ± 6.0*	76 ± 10.9	41 ± 8.0*
	SOD		SOD	
NC	78 ± 2.0	79 ± 1.0	78 ± 2.0	79 ± 1.0
EC	74 ± 4.7	29 ± 4.0*†	72 ± 2.6	34 ± 2.0*†
PRT	66 ± 1.5	51 ± 3.5*	71 ± 6.7	64 ± 2.5*
PST	76 ± 3.5	41 ± 6.5*	77 ± 2.0	50 ± 7.3*

*Significantly different ($p < 0.05$) from NC, † Not significantly ($p > 0.05$) different from NC. ‡ Significantly different ($p < 0.05$) from PRT and PST.

Body weight

Figure 2 indicates the mean BW of the rats in each sub-group, before and after exposure to 4 and 6 Gy doses. The mean BW of the rats before IR across the sub-groups ranged from 109-115 grams. Rats in NC sub-groups maintained fairly constant weight all through the period of experiment and no significant differences were noted in their mean BW. However, significant decrease in BW was noted in the sub-groups (IC, PRT and PST) that were exposed to 4 and 6 Gy doses. The observed weight loss noted in IC, PRT and PST sub-groups that were exposed to 4

Gy dose were 40, 20 and 25% respectively, while IC, PRT and PST sub-groups that were exposed to 6 Gy dose were 56, 39 and 48% respectively. From the analyses the weight loss in IC sub-group was significantly higher compared to PRT and PST groups. In addition to weight loss, other physical changes noted on day 8 after whole-body irradiation were lethargy, diarrhea, ruffled hair and decline in water and food consumption.

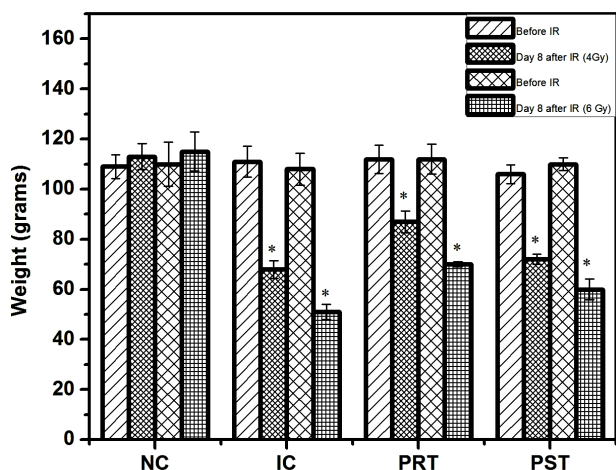


Figure 2. The body weight of rats in Normal control (NC), Irradiated control (IC), Pre-treated (PRT) and Post-treated (PST) sub-groups. Values are expressed as mean $\pm$ Standard deviation and * indicates $p \leq 0.05$ (level of significance).

DISCUSSIONS

Both planned and unplanned exposure of human cells to ionizing radiation induces free radicals causing oxidative stress that could alter biological processes and cause some disease conditions including cancer. Amelioration of oxidative stress is crucial in mitigating diseases that may arise from exposure to radiation. This experimental study tried to investigate the radioprotective efficacy of GLE against radiation induced oxidative stress by measuring changes in hepatic function enzymes, lipid peroxidation and antioxidant defense enzymes.

From the results, the observed elevation in hepatic enzymes (ALT, AST and ALP) activities are in agreement with the finding made by (2, 37). The conditions of these enzymes are keen indicators of hepatocellular necrosis (38). Increase in ALP level is attributed to pathological modification in biliary flow and damage to liver cell membrane (39), while increase in AST and ALT activity levels could be associated to the severe impairment in hepatic cells as a result of radiation interaction with the membrane of cells and considerable breakdown in the liver parenchyma (40). ALP plays crucial role in supporting cellular membrane permeability. The administration of GLE resulted in a reduction of mean values of hepatic enzymes which suggests that GLE have hepatoprotective potentials.

Malondialdehyde (MDA) remains one of the major indexes for lipid peroxidation as well as radiation induced toxicity. The significant increase noted in MDA % inhibition is consistent with the previous observation by (18, 41, 42). This is attributed to membrane lipid peroxidation caused by free radicals and depletion in antioxidant enzymes (43). The recorded improvement in MDA % inhibition mean value in PRT and PST sub-groups suggest that GLE have the potentials to mitigate oxidative stress especially when it's in the biological system prior to radiation exposure.

As indicated in table 2, the observed decrease in GSH, CAT and SOD % inhibition was in concordance with previous report by (2, 20, 44). The decline in GSH activities may be attributed to direct utilization as antioxidant in mopping up free-radicals generated by radiation. The observed decrease in the % inhibition of SOD and CAT might also be associated to the presence of excess ROS. Decline in activity of SOD is an indicator of hepatocellular injury and remains most sensitive biomarker in liver damage (45). These ROS interacts with enzyme molecules causing their denaturation and partial inactivation (46). The observed improvement in the endogenous enzymes in PRT and PST sub-groups when compared to IC subgroup suggests that GLE alleviated the radiation induced stress. It is noteworthy to suggest that oxidative stress caused by radiation were mitigated due to the antioxidant activities of phytochemicals like flavonoids and polyphenols present in GLE. Hence we attribute GLE mechanism of action to scavenging of free radicals, donation of hydrogen and regulation of endogenous enzymes.

As shown in figure 2, the observed decrease in body weight after whole-body exposure to radiation is in an agreement with the observation made by (47). Decrease in the body weight of rats exposed to radiation could be associated to acute radiation syndrome (ARS) or radiation sickness. The main cause of this syndrome is attributed to reduction of immature parenchymal stem cells in specific tissues. It could also be as a result of harmful and irreversible alterations in gastro-intestinal tract (GIT) and bone marrow that usually cause dehydration, infection, and electrolyte imbalance (48). The body weight of rats in PRT and PST groups made significant recovery which suggests that GLE ameliorated radiation induced weight loss in wistar albino rats.

CONCLUSION

We have evaluated the radioprotective effects of *Gongronema latifolium* extract against whole body induced oxidative stress in Wistar Albino Rats. Preliminary result shows that GLE ameliorated oxidative stress more in pretreated sub-groups than in post-treated sub-groups, suggesting GLE as a

potential prophylaxis.

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Ethical consideration: All the procedures on animals were reviewed by a local animal care committee to ensure that all the procedures are convenient and humane.

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REFERENCES

- Jerrold T, Bushberg JT, Anthony JS, Leidholdt EM, Boone JM (2012) The essential physics of medical imaging. 3rd Edition. Philadelphia, PA 19103 USA: Lippincott Williams & Wilkins, a Wolters Kluwer.
- Farag MFS (2013) Utilization of basil extract as a radioprotector in male rats. *Arab Journal of Nuclear Science and Applications*, **46**(1): 274-281.
- Mun GI, Kim S, Choi E, Kim CS, Lee YS (2018) Pharmacology of natural radioprotectors. *Arch Pharm Res*, **41**: 1033-1050.
- Robbins ME, Zhao W, Davis CS, Toyokuni S, Bonsib SM (2002) Radiation-induced Kidney Injury: A Role for Chronic Oxidative Stress? *Micron*, **33**(2): 133-141.
- Ozkan A and Fiskin K (2004) Free radicals, carcinogenesis and antioxidant enzymes. *Turkish Journal of Hematology and Oncology*, **14**: 52-60.
- Abd El-Rahman NA and Sherif NH (2015) Caffeine and Aspirin Protecting Albino Rats Against Biochemical and Histological Disorders Induced by Whole Body Gamma Irradiation. *Arab Journal of Nuclear Science and Applications*, **48**(1): 99-111.
- Citrin D, Cotrim, AP, Hyodo F, Baum BJ, Krishna MC, Mitchell JB (2010) Radioprotectors and Mitigators of Radiation-Induced Normal Tissue Injury. *The Oncologist*, **15**(4): 360-371.
- Sweeny TR (1979) A survey of compounds from the antiradiation drug development program of the U.S. Army Medical Research and development command. Washington, D.C.
- Qian L, Cao F, Cui J, Wang Y, Huang, Y, Chuai Y, et al. (2010) The potential cardioprotective effects of hydrogen in irradiated mice. *Journal of Radiation Research*, **51**(6): 741-747.
- Bhatia AL, Kamal R, Verma G, Sharma KV, Jain M (2008) Study on Acetogenin against Radiation-induced Hepatic Biochemical Alterations in Mice. *Asian J Exp Sci*, **22**(3): 433-438.
- Gupta U, Chaudhary R, Goyal PK (2010) Post-treatment effects of *Alstonia scholaris* extract against radiation-induced biochemical alterations in Swiss albino mice. *Int J Radiat Res*, **8**(3): 169-177.
- Gaur A (2010) Ameliorating effects of genestein: Study on mice liver glutathione and lipid peroxidation after irradiation. *Int J Radiat Res*, **7**(4): 187-199.
- Arya S and Sharma J (2010) Modulation of radiation induced changes in nucleic acid content of liver of Swiss albino mouse by *Tinospora cordifolia* (Miers). *Int J Radiat Res*, **8**(3): 179-185.
- Nair CKK and Menon A (2013) Consumption of antioxidant dietary agents, Curcumin and vitamin C, protects cellular DNA from gamma-radiation. *Int J Radiat Res*, **11**(1): 11-16.
- Sharma KV and Sisodia R (2014) Radioprotective efficacy of *prunus avium* fruit in mice brain with reference to in-vitro and in-vivo studies. *Int J Radiat Res*, **12**(1): 21-31.
- Kim JH, Li L, Park DH, Lee IW, Ham N, Kim HS, et al. (2019) Inhibitory effects of Cheonggukjang extracts on radiation-induced micronucleus formation and inflammasome activation. *Int J Radiat Res*, **17**(1): 57-65.
- Nadi S, Banaei A, Mozdarani H, Monfared AS, Ataei GR and Abedi-Firouzjah R (2020) Evaluating the radioprotective effect of arbutin on mice exposed to megavoltage X-rays based on hematological parameters and lymphocytes micronucleus assay. *Int J Radiat Res*, **18**(2): 275-282.
- Ali MM, Ahmed OM, Nada A Sh, Abdel-Reheim ES, Amin NE (2020) Possible protective effect of naringin (a citrus bioflavonoid) against kidney injury induced by γ -irradiation and /or iron overload in male rats. *Int J Radiat Res*, **18**(4): 673-684.
- Pooja Sh, Shetty P, Kumari NS, Shetty KJ (2021) Radioprotective and antioxidant potential of *Tanacetum parthenium* extract and synthetic parthenolide in Swiss albino mice exposed to electron beam irradiation. *Int J Radiat Res*, **19**(1): 145-154.
- Ugochukwu NH and Babady NE (2002) Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sci*, **73**(15): 1925-1938.
- Iweala EEJ, Liu F, Cheng R, Li Y, Omonhinmin CA, Zhang Y (2015) Anti-Cancer and Free Radical Scavenging Activity of Some Nigerian Food Plants *in-vitro*. *Int J Cancer Res*, **11**: 41-51.
- Essien GE and Effiong GS (2017) Evaluation of antioxidant potentials in methanol extract of *gongronema latifolium* and *lasianthera africana* leaf. *European Journal of Pharmaceutical and medical research*, **4**(9): 841-846.
- Atangwho IJ, Ani IF, Agiang MA, Effiong GS, Ebong PE (2010) Comparative effect of chlorpropamide and combined leaf extracts of *Azadirachta indica* and *Vernonia amygdalina* on blood glucose and biochemical parameters of alloxanized rats. *J Med Med Sci*, **1**(6): 248-253.
- Morebise O, Fafunso MA, Makinde JM, Olayide OA, Awe EO (2002) Anti-inflammatory Property of *Gongronema latifolium*. *Phytother Res*, **16**(1): 575-577.
- Alobi NO, Ikpeeme EM, Okoi AI, Etim KD, Eja ME (2012) Phytochemical and nutritional profile of *lasianthera africana*, *Heinsia crinata* and *Gongronema latifolium*. *New York Sci J*, **5**(3): 45-48.
- Balogun ME, Besong EE, Obimma JN, Mbamalu OS, Djobbissie SFA (2016) *Gongronema latifolium*: A Phytochemical, Nutritional and Pharmacological Review. *J Phys Pharm Adv*, **6**(1): 811-824.
- Dailey A and Vuong QV (2015) Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. *Cogent Food Agric*, **7**: 1-10.
- Iweala EEJ, Uhegbu FO, Adesanoye OA (2013) Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan induced diabetic rats. *J Pharmacogn Phyther*, **5**(5): 91-7.
- Al-Hindi B, Yusoff, NA, Ahmad M. Atangwho IJ, Asmai MZ, Al-Mansoub MA, et al. (2019) Safety assessment of the ethanolic extract of *Gongronema latifolium* Benth. leaves: a 90-day oral toxicity study in Sprague Dawley rats. *BMC Complement Altern Med*, **19**: 152.
- Debajit C, Reshma K, Sudha K, Chiranth, Rahul Charu Y, et al. (2018) Study of chronic effects of varying dosage of X-rays on hepatotoxicity in wistar albino rats. *Asian J Pharm Clin Res*, **11**(2): 293-296.
- Rec GSCC (1972) Optimised standard colorimetric methods. Serum alkaline phosphatase (DGKC). *J Clin ChemClin Biochem*, **10**: 182-182.
- Reitman S and Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*, **28**(1): 56-63.
- Niehaus WG and Samuelsson BS (1968) Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Resources Eur J Biochem*, **6**(1): 126-130.
- Ellman GL (1959) Tissue sulfhydryl groups. *Arch of Biochem and Biophys*, **82**(1): 70-77.
- Sinha KA (1972) Colorimetric assay of catalase. *Anal Biochem*, **47**: 389-394.

36. Arthur JR and Boyne R (1985) Superoxide dismutase and glutathione peroxidase activities in neutrophil from selenium deficient and copper deficient cattle. *Life Sci*, **36**: 1569-1575.
37. Abdou MI and Abbas OA (2009) Evaluation of diphenyl dimethyl bicarboxylate (DDB) as a probable hepato-protector in rats against whole body gamma irradiation. *Bioscience Research*, **6**(1): 1-11.
38. Wroblewski F (1959) The clinical significance of transaminase activities of serum. *Am J Med*, **27**: 911-923.
39. Dunsford HA, Karnasuta C, Hunt JM, Sell S (1989) Production of monoclonal antibodies to preneo-plastic liver cell populations induced by chemical carcinogens in rats and to transplant Morris hepatomas. *Cancer Research*, **49**(17): 4887-4893.
40. Kafafy Y (2000) Protective effect of cytochrome and vitamin E on gamma irradiation injury in rats. *Egypt J Rad Sci Applic*, **13**(2): 17-25.
41. Hafez EN, Ebrahim RM, Barakat AM (2021) Evaluation of the oxidative stress in liver of mice vaccinated with gamma radiation-attenuated *Toxoplasma gondii*. *Int J Radiat Res*, **19**(2): 339-347.
42. Makhoulouf R and Makhoulouf I (2012) Evaluation of the effect of Spirulina against Gamma irradiation induced oxidative stress and tissue injury in rats. *Int J Applied Science and Engineering Research*, **1**(1): 152-164.
43. Khan MR, Mariam A, Shabbir M, Saeed N, Bokhari J (2012) Antioxidant and hepatoprotective effects of *Oxalis corniculata* against carbon tetrachloride (CCl₄) induced injuries in rats. *Afr J Pharm Pharmacol*, **6**(30): 2255-2267.
44. Park SH, Pradeep K, Choi MH, Ko KC, Park HJ (2010) Hesperidin and Curdlan treatment ameliorates γ -radiation induced cellular damage and oxidative stress in the liver of Sprague-Dawley rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **1**(3): 165-177.
45. Tamilarasi R, Sivanesan D, Kanimozhi P (2012) Hepatoprotective and antioxidant efficacy of *Anethum graveolens* linn in carbon tetrachloride-induced hepatotoxicity in albino rats. *J Chem Pharm Res*, **4**(4): 1885-1888.
46. Kregel KC and Zhang HJ (2007) An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am J Phys*, **292**(1):18-36.
47. Mihandoost E, Shirazi A, Mahdavi SR, Aliasgharzadeh A (2014) Consequences of Lethal-Whole-Body Gamma Radiation and Possible Ameliorative Role of Melatonin. *The Scientific World Journal*, **2014**, 9. <https://doi.org/10.1155/2014/621570>
48. CDC (2020) Acute Radiation Syndrome: A Fact Sheet for Clinicians. Retrieved from CDC.