EVALUATION OF RADIOPROTECTIVE POTENCY OF LYCOPERSI C ON ESCU LENT U M (LE) ON RADIATION-INDUCED CYTOLYSIS IN ALBINO RATS

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Introduction

Radiation is the energy that travels through space or matter. There are two basic types of radiation: ionizing and non-ionizing radiation. Non-ionizing radiation is not energetic enough to ionize atoms and interact with materials in ways that create different hazards like ionizing radiation. Examples of non-ionizing radiation include microwaves, visible light, radio waves etc (Ng 2003). Ionizing radiation consists of both particles and electromagnetic (EM) radiation. The eventual discovery of X-rays in 1895 and radioactivity in 1896 generated the biological effects that were observed afterward. The first report of skin cancer associated with X-rays was reported in 1902; experimental confirmation was made eight years later (Bushberg et al. 2012). According to the National Commission on Radiation Protection (NCRP 2009) report No 160, sources of ionizing radiation are categorized into two sources: (1)Natural sources (2) anthropogenic sources. Naturally occurring sources of radiation in- clude (i) cosmic rays, (ii) cosmogenic radionuclides, and (iii) primordial radionuclides and their radioactive decaying products. Cosmic radiation includes both the primary extraterres- trial radiation that strikes the Earth's atmosphere and the secondary radiations produced by the interaction of primary cosmic rays with the atmosphere. Primary cosmic rays predom- inantly consist of extremely penetrating high-energy (mean energy ~10 GeV) particulate radiation, approximately 80% of which is high-energy protons. Cosmogenic radionuclides are some of the secondary cosmic ray particles which collide with stable atmospheric nuclei. Although many cosmogenic radionuclides are produced, they contribute very little (~0.01 mSv per year or less than 1%) to natural background radiation. Primordial radionuclides are the radioactive materials that have been present on the Earth since its formation. Primordial

radionuclides with physical half-lives comparable to the age of the Earth (~4.5 billion years) and their radioactive decaying products are the largest sources of terrestrial radiation expo- sure (Bushberg et al. 2012). Anthropogenic sources are categorized into two: (i) Artificial source and (ii) Enhanced natural source. On a general note, it is obvious from the evidences above that harm caused to living cells in animal can result in devastating health defect of the animal or human; and ionizing radiation possesses the characteristic of generating harmful activity of free radicals, which are agents of mass destruction in the body depending on either these are stochastic or deterministic. Several attempts have been made to protect personnel working in radiation medicine departments, radiopharmaceutical centers, aviation, nuclear power operations, uranium miners and other sources of ionizing radiation by the provision of the following: personnel dosimeter, shielding devices, radiation detection equipment and other safety procedures, policies among others, with the intent of ensuring safety for patients, occupational personnel and the society at large. Ionizing radiation can produce reactive oxy- gen species such as superoxide anion radical O^{-}, hydrogen peroxide (H_2O_2), hydroxyl radical

OH^{*} and nitrogen dioxide NO_2 through the decomposition of cellular water (Takenshita et al. 2004). A number of dietary antioxidants have been reported to decrease free radical attack on biomolecules (Halliwell and Gutteridge 2004). The choice of Nsukka LE is as a result of the fact that Nsukka is one of the towns in the tropics, where the geochemistry com- position of the soil is polluted with heavy metals naturally, geologically, and anthropogenical activities increase the concentration of these elements to amounts that are harmful to both plants and animals (Chibuike and Obiora 2014 and Song et al. 2001). Numerous chemical compounds have been synthesized and tested for their radio-protective efficacy (Sweeney 1979). The major demerit of some of these compounds has been their high toxic nature at the best protective dose (Sweeney 1979), which forestall their effectiveness in man. LE is known to be an edible fruit, with negligible toxicity; it is very cheap in its season and read- ily available. This informed our research intent toward evaluating radioprotective potency of LE on radiation-induced cytolysis in albino rats. By determining antioxidant enzymes (Catalase (CAT), Superoxide Dismutase (SOD) and Gluthathione Peroxidase (GPx)) activ- ity, liver function enzymes (Alkaline

Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)) activity, serum nutrients (Total Protein (TP) and Albumin (ALB)) and non-enzymatic oxidative stress indices (Vitamin C (VIT C), Vitamin E (VIT E), Glutathione (GSH) and Malondialdehyde (MDA)) concentrations they will serve as indicators that will enable us understand the rate of damage to different cell organs in the experimental animals and radio-protective measures of LE extract.

Methodology

Design

An experimental cross-sectional approach using healthy adult male white albino rats was adopted for the study. Eighteen white albino rats were divided into 6 groups of 3 rats each. The animals were grouped in the order below: 1, Normal control (NC); 2 and 3 Administered extract before irradiation, that is pre-treatment (PRT); 4 and 5 Administered extract after irradiation, that is post-treatment (PST); 6 Irradiated without treatment, that is negative control(NTC)

Target Population

The animal model was chosen because the body chemistry accurately reflects that of the human and also imitate human disease to some extent. White adult albino rats have the set of organs-heart, kidney, lungs, liver etc. which work in some ways as they do in human (Giridharan et al. 2000); and is therefore, a fairly accurate representation.

Fruit Sample Identification and Collection

Fresh ripe samples of tomato fruit LE was bought from a farmer at Nsukka, who harvested it from his farm; and Dr.Mrs N. O. Nweze, a plant taxonomist, head of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (UNN), graciously confirmed the botanical identity of the said fruit.

Figure 1.1: Lycopersicon Esculentum

Extraction of LE

Apparently healthy fruits of LE were purchased from a farmer in Nsukka and extracted using Ethyl Acetate reagent, which the extract was further analyzed qualitatively and quantitatively confirming it to contain flovanoid, tannin and phenol.

Administration of Extract

The extract was administrated to the rats at on a once daily basis in this order: Group 1 normal control; groups 2 and 3 (20mg/kg body weight) and (40mg/kg body weight) before irradiation respectively; groups 4 and 5 (20mg/kg body weight) and (40mg/kg body weight) after irradiation respectively. This was done for seven consecutive days via oral intubation. This is because natural compounds showed their radio-protective effects after 7 days of oral administration (Kumar et al. 2005). The pre-irradiation groups were administered for 7 days. Also, the post-irradiation groups were administered for 7 days. Group 6 rats were not treated with extract but were exposed to irradiation (negative control).

Figure 1.2: White male adult albino rats after acclimatization period

Irradiation of the Animals

Five groups (2, 3, 4, 5 and 6) of the experimental animals were irradiated except group1 which is normal control. Three radiation doses (2Gy, 3Gy, and 4Gy) were administered sequentially to three sets of five rats each. The 6MV photon beam used to irradiate the albino rats was out putted from Elekta precise linear Accelerator, installed at the Radiotherapy unit of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. Animals were placed in ventilated perspex containers and subjected to whole-body irradiation.

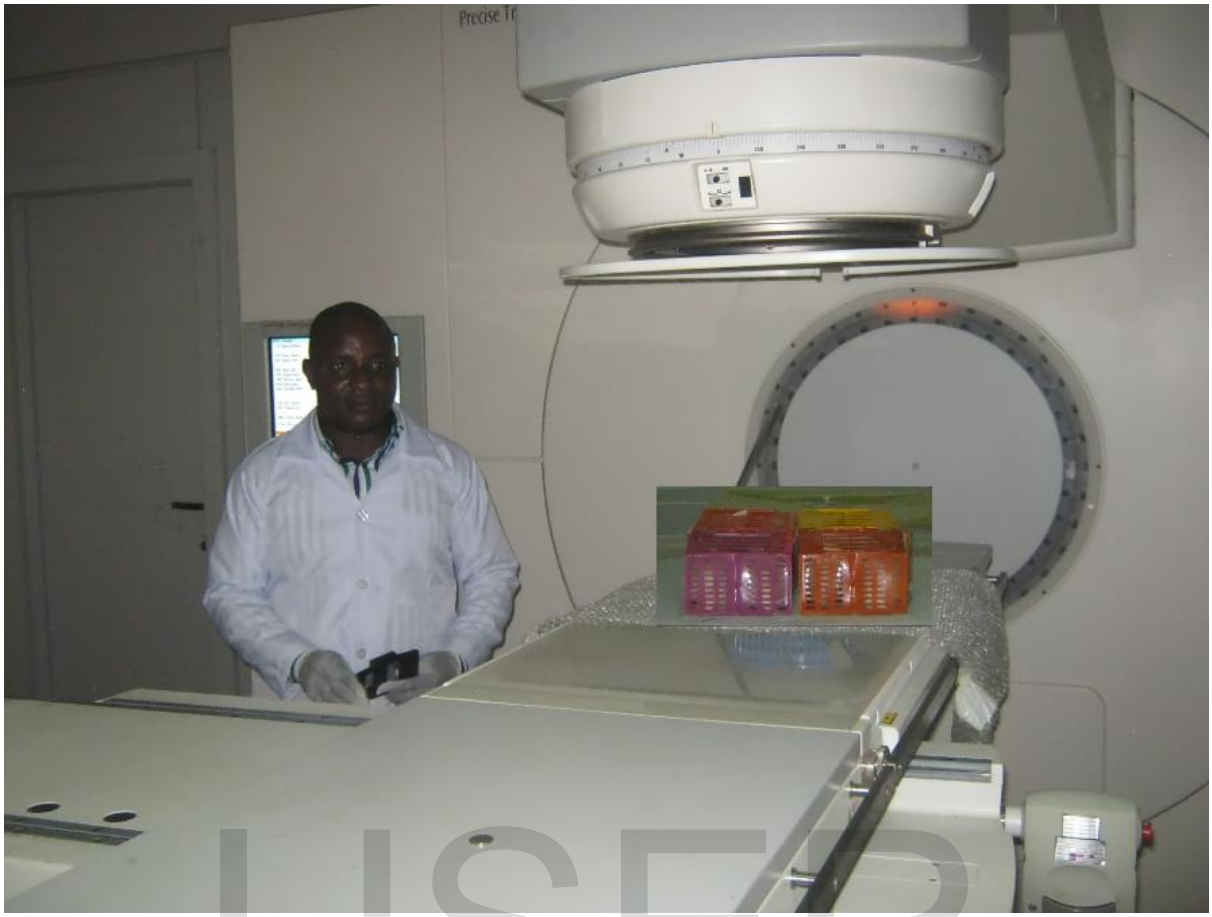

Figure 1.3: Aligning rat container with the collimator field size of the Linear Accelerator with the help of the laser light

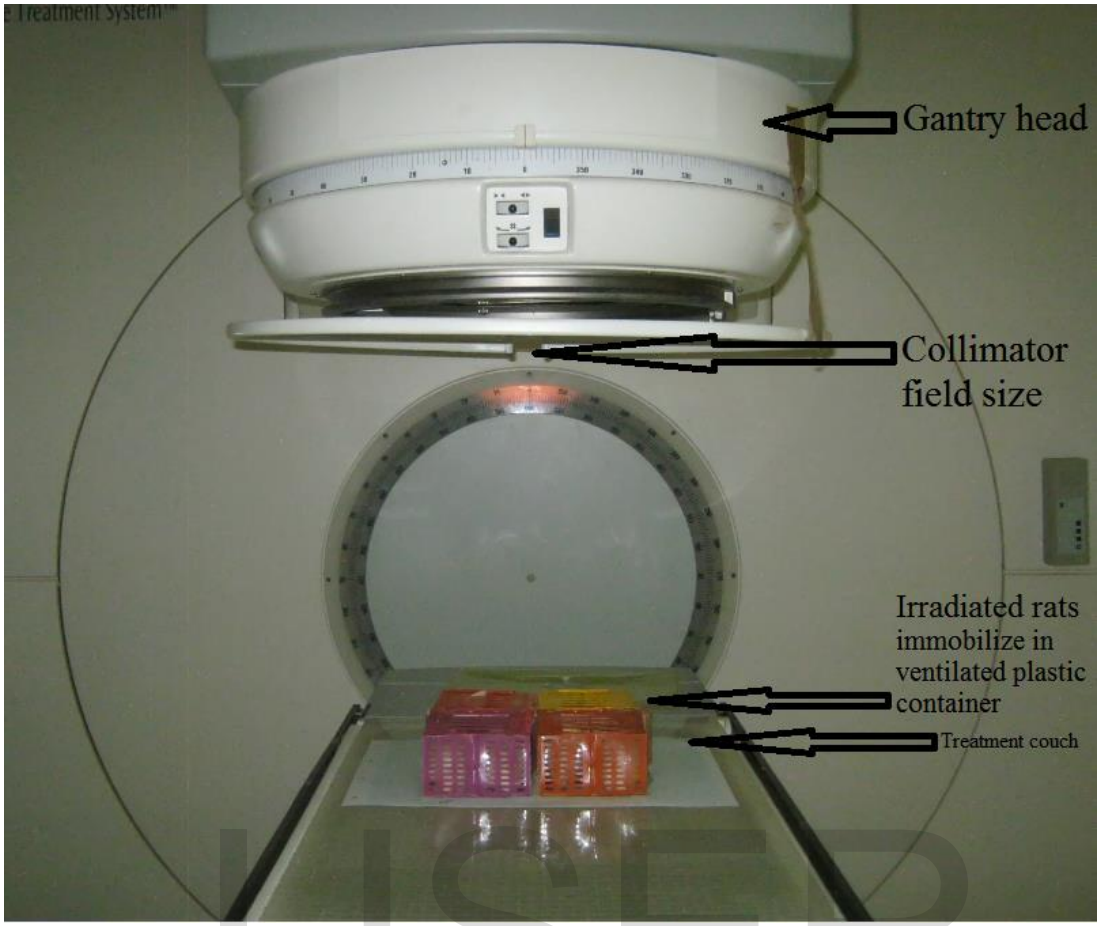

Figure 1.4: Ventral (anterior) Irradiation

Figure 1.5: Dorsal (posterior) Irradiaton

Blood Samples Collection

After a whole-body irradiation of the animals, blood samples were collected from all the animals in groups (1, 2, and 3) by ocular puncture and this was done after an hour interval, and the blood were obtained to the laboratory for screening test. Similarly, after 7 days of

post-administration of the extract on groups 4, 5 and 6 experimental animal, blood sam- ples were also obtained via ocular puncture and were taken to the laboratory for screening test. The screening test was replicated, considering Antioxidant enzymes, Liver function enzymes, Serum nutrients and Non-enzymatic oxidative stress indices, after which the data were analyzed using one way analysis of variance.

Results

The phytochemical constituents of LE that were determined qualitatively and quantitatively using ethyl acetate (reagent), revealed the presence of flavonoids, tannins and phenols as contents of the extract. The results are sequentially shown on

tables and in figures below.

Table 1 Quantitative phytochemical constituents of LE extract

Antioxidant Phytochemical	Mean±Std mg/g	%
Phenols	1.437±0.216	14.37%
Flavonoid	7.041±0.297	70.41%
Tannin	0.565±0.212	5.65%

Table 2 The summarized mean of antioxidant enzymes potency of the extract of LE in radiation-induced oxidative stress in albino rats cytolysis

Groups	CAT (µ/l)	SOD (µ/l)	GPx (µ/l)
NC	1.76	10.92	17.52
PRT	1.77	11.30	14.79
PST	2.75	11.15	24.49
NTC	2.45	11.31	21.34

CAT mean levels obtained in rats exposed to 2, 3 and 4 Gys increased ($p < 0.05$) significantly
in NTC (2.45µ/l) when compared to PRT (1.77µ/l) and NC (1.76µ/l); PST (2.75µ/l) showed a non-significant ($p > 0.05$) increase when compared to NTC (2.45µ/l). GPx is of the same trend with CAT in data analysis; but in the case of SOD, the mean levels obtained showed a non-significant ($p > 0.05$) decrease in NTC (11.31µ/l) when compared to PRT (11.30µ/l) and PST (11.15µ/l) respectively, though they do not increase ($p > 0.05$) significantly when compared to NC (10.92µ/l).

Table 3 The summarized mean of liver function enzymes potency of the extract of LE in radiation-induced oxidative stress in albino rats cytolysis

Groups	ALP (iu/l)	ALT (iu/l)	AST (iu/l)
NC	46.33	45.00	39.33
PRT	46.67	26.17	46.84
PST	34.67	38.00	59.84
NTC	36.67	40.00	44.67

AST mean levels obtained in rats exposed to 2, 3 and 4 Gys increased ($p < 0.05$) significantly
in NTC (44.67iμ/l) when compared to NC (39.33iμ/l) and PRT (46.84iμ/l); and showed a significant ($p < 0.05$) decrease when compared to PST (59.84iμ/l). Though in liver function enzymes, ALP and ALT were not in the same increased levels with AST. They rather decrease ($p < 0.05$) significantly at PST stage.

Table 4 The summarized mean of serum nutrients potency of the extract of LE in radiation-induced oxidative stress in albino rats cytolysis.

Groups	TP (g/dl)	ALB (g/dl)
NC	5.37	3.00
PRT	6.02	2.94
PST	4.22	2.54
NTC	3.57	2.13

TP concentration levels for rats exposed to 2, 3 and 4 Gys showed a significant ($p < 0.05$)
decrease in NTC (3.57 g/dl)when compared to NC (5.37 g/dl)and PRT (6.02 g/dl), but showed a non-significant ($p > 0.05$) increase in PST (4.22 g/dl). Albumin has the same mean levels obtained in TP.

Table 5 The summarized mean of non-enzymatic oxidative stress indices of the extract of LE in radiation-induced oxidative stress in albino rats cytolysis

Groups	VIT C (mg/dl)	VIT E (mg/dl)	GSH (mg/dl)	MDA (mg/dl)
NC	1.43	0.67	0.20	1.42
PRT	1.39	0.95	0.29	2.76
PST	1.67	1.08	0.35	5.41
NTC	2.25	1.20	0.33	6.35

MDA activity levels for rats exposed to 2, 3 and 4 Gys showed a significant ($p < 0.05$) increase NTC (6.35) when compared to NC (1.42 mg/dl) and PRT (2.76 mg/dl); and showed a non- significant ($p > 0.05$) decrease when compared to PST (5.41 mg/dl).

Vitamin C and E and GSH showed a non-significant ($p > 0.05$) increase when their NC was compared to their PRT, PST and NTC. In all the parameters considered in this study, the extract proved a noticeable potency in the PRT phase, while PST phase showed a weak recovering mechanism.

Discussion

Phytochemical Constituents of LE

There is a large number of tomato cultivars with a wide range of morphological chemicals, nutritional and sensorial characteristics. Many factors are known to affect the nutrient con- tent of tomato cultivated. The phytochemical constituents of LE considered in this study are basically those biochemical contents in the fruit that could help function as antioxidant. At the same time, the phytochemical constituents obtained from LE in this study are com- pared with other LE cultivated in other parts of the world, to see if there are geographical variation with the ones planted in Nsukka. The phytochemical determination of LE extract revealed the presence of flavonoids and phenols, which were the highest bioactive phyto- chemical present, and tannins being the lowest. Other research works pointed to the fact that they are most effective when used for short term administration (Fonceka et al. 2012).

Stewart et al. (2000) reported from a research conducted by them in Scotland, UK, that flavonoids contents were the highest of the different varieties of tomato that were analyzed; and this is in agreement with the phytochemical findings in the current study. Khalaf et al. (2014) noticed that ethyl ether and ethyl acetate are very efficient in the recovery of flavonoid aglycons, lower molecular-weight phenols, and tannins. Khalaf et al. (2014) had similar observation in a study conducted in Egypt. They equally viewed that flavonoids, which are the major components of the total phenolic content of tomato LE pomace, be quantified in different solvent extract. In a research carried out in New York City, USA, there were, also, results confirming a previous report that flavonoids represent the main group of phenolic compounds in white onion (Yang et al. 2004).

Nishium et al. (2011) and Assunta et al. (2014) also discovered the presence of flavonoids, phenoils and tannins in phytochemical constituents of LE. They found flavonoids to be the highest and tannins to be the lowest. Although the two research

works were carried out in Kobe, Japan and Naples, Italy respectively, what they noticed in their studies are in line with the present study in the range of bioactive content of LE extract.

Chang et al. (2006) studied the effects of hot-air-drying treatment on tomato in North- ern California, USA. They found that this process could enhance the nutritional value of tomatoes by increasing parts of the total flavonoids, total phenolics, and lycopene contents.

The result of the present study identifies that antioxidant phytochemical constituent's test is actually in agreement with several researchers' works: (Assunta et al. 2014, Nishium et al. 2011, Khalaf et al. 2014, Stewart et al. 2000, Heim et al. 2000 and Kang et al.
2007). There were no variation in the quantity of bioactive agents, showing that the LE extract possesses some bioactive properties which could serve as antioxidants.

Antioxidant Enzymes

Radiation damage in cell is known to involve the production of free radicals such as super- oxide radical (O_2^*), hydroxyl radical (OH^*), lipid radical and lipid peroxide radical (H_2O_2), which produce lipid peroxide in biomembrane, that will develop various episodes of bio- hazards, beside direct damage to DNA.

The naturally found antioxidant enzymes in the body chemistry of every living organism are regularly in constant fight with any toxins and free radicals generated to cause harm in the organism's body system; and at the same time, function to scavenge their unwanted activities. But for the purpose of this study, the extract from LE was used to enrich natural antioxidant enzymes in the body of the white albino rats, so that, it will be able to scavenge radiation-induced oxidative stress generated.

Many antioxidant compounds naturally occurring from plant sources have been identified as free radical or reactive oxygen species scavengers (Duh 1998). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Lai et al 2001).

It is likely that the antioxidants have the ability to intercalate into the plasma

mem- brane, change its fluidity, and inhibit lipid peroxidation by chelating transition metals and scavenging ROS (Kang et al. 2007). The antioxidant effect is mainly due to phenolic com- pounds which are able to donate a hydrogen atom to the free radicals; thus, stopping the propagation chain reaction during lipid peroxidation process (Sanchez-Mareno et al. 1998 and Yanishlieva and Marinova 1998).

Naturally, existing scavenger systems, that is, CAT, SOD and GPx systems, work to quench these oxidized substances (Nada 2008). The increase in lipid peroxidation levels in X-ray-irradiated rats might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids portion of cellular membranes (Prasad et al. 2005).

From the data analysis, a clear level of the extract in the PRT groups was observed, while the PST groups were relatively different. The X-ray irradiation exposure resulted in ROS activity was evidenced in the PST groups in the current study, which is similar to what was observed by Spitz et al. (2004). The treatment with different dosage of antioxidant resulted in an increase in GSH level, CAT and GPx activity. The increase in the activities of SOD, CAT and GPx level in the presence of GSH might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules, causing their denaturation and partial inactivation. Under normal conditions, the inherent defense system, including GSH and the antioxidant enzymes, protect against oxidative damage. Post-administration are absolutely opposite of what we have in the pre-administration, showing that preventive intake of this extract is desirable.

Liver Function Enzymes

Indirect interaction occurs when radiation energy is deposited in the cell and the radiation interacts with cellular water rather than with macromolecules within the cell. The reaction that occurs is hydrolysis of water molecules, resulting in a hydrogen molecule and hydroxyl free radical molecule (Dowd and Tilson 1999). The identified liver function enzymes are naturally found in the body system of animals, functioning in a protective manner to ensure that there is no distortion in the liver functional tissue/cell. A slighted distortion in the hepatocytes will be discovered in the activity of the liver enzymes.

The data indicated PRT and PST when compared to NC and NTC in ALP (a significant [$p < 0.05$] decrease at the PST phase), showing that the extract proved its

potency at the PRT phase, ALT (a significant [$p < 0.05$] decrease in the PRT and PST) and AST showed that the extract was more efficacious at PRT phase than at PST phase. This is in agreement with the findings of Srinivasan et al. (2009). Though we had a tremendous decrease in ALP, (as was observed in PST groups, indicating a weak recovery), potency in the extract was seen in the pre-administration. The increase in the activity of AST as was observed in the present study is in accordance with the findings of Roushdy et al. (1984), Kafafy et al. (2006), Ramadan et al. (2001) and Nada (2008). They explained that changes in the enzymatic activities after irradiation may be due either to the release of enzymes from radiosensitive tissues or to the changes in its synthesis, and that may be related to the extensive breakdown of liver parenchyma and renal tubules. What was observed in the liver function enzymes point to the fact that there will be a degree of hepatocellular dysfunction caused by the radiation at the post-administration.

Serum Nutrients

Radioactive nuclide or ionizing radiation has the capacity to induce oxidative stress through the generation of ROS in an imbalance in pro-oxidant, and antioxidant status in the cells.

Serum proteins are synthesized and secreted by several cell types depending on the nature of the individual serum protein. An important function of serum protein is the maintenance of the normal distribution of body water by controlling the osmotic balance between the circulating blood and the membrane of tissues, and the transport of the lipids, hormones and inorganic materials (Harper et al. 1977). The results obtained in this work showed that there is a significant ($p < 0.05$) decrease in serum TP and ALB. Saada et al., (1999) and Haggag et al. (2008) suggested that the decrease in serum protein in irradiated rats might be the result of the damage of vital biological processes or due to changes in the permeability of liver, kidney and other tissues resulting in the leakage of protein, especially albumin via the kidney.

The measurement of TP and ALB in the blood helped in the understanding of nutritional status of the white albino rats used in this study. It was observed that the PRT groups proved that LE extract had a noticeable potency, while PST groups had a weak recovering mechanism owing to the fact that so much harm had been caused to the cells before the extract was administered. In the case of TP and ALB, they equally decrease significantly in sera of irradiated rats with doses of 2, 3 and 4 Gy, which is in line

with the observation of Ali et al. (2007).

Non-Enzymatic Oxidative Stress Indices

Ionizing radiation is known to induce oxidative stress through the generation of reactive oxygen species (ROS) in an imbalance in pro-oxidant and antioxidant status in the cells (Bhosle et al. 2005).

The role of these few biomolecules are necessary to maintain the immmunological system cells redox balance and preserve their function until they are innervated by sympathetic nerves when there is an invasion in the system. A desirable position of the extract in the PRT phase to the induced X-ray irradiation on the white adult albino rats was observed. The increasing nature of the PST groups indicated that so much injury had been inflicted on the cells of the rats. However, the harmful effect of a high dose of ionizing radiation is well established.

The data obtained from rats treated with LE extract before and after whole-body X-ray irradiation revealed significant modulation in the biochemical tested parameters; and profound improvement in the activity of antioxidant status and GSH agrees with the work of Mansour (2013). He explained that the treatment of irradiated rats with the extract also appeared to be effective in minimizing the radiation-induced increase in lipid peroxidation as well as the changes in the liver.

Glutathione, as a well-known antioxidant, provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage (Sener et al. 2006 and Reiter et al. 2001).

The efficacious nature of the extract that swept through the pre-treatment groups in the present study and invaded group 4 (post-treatment) in vitamin C was not unconnected with the report of Giovanelli and Paradise (2002), which says that VIT C and E among others are contained in tomato; then, leaving the other groups in an uncontrollable increase, showing that the potency of the extract was weakened in the post-administration.

The increase in MDA level on treatment with different doses of antioxidants might be related to the antioxidative properties of the antioxidant, which protect the outer membrane of mammalian cells (Block and Mead 2003).

Ali et al. (2007) noticed an increase of MDA in few hours after radiation exposure. The cell's natural enzymatic and antioxidant mechanisms may be the main cause of irradiation-induced peroxidation.

Maha (2010) observed that the essential oil of feoniculum vilgare mill was effective in minimizing damage caused by gamma-irradiation which is inline with the present work at the pre-administration phase. The study by Dowd and Tilson (1999) indicated that free radicals are generated by the activity of the serum. This agrees with the result of this work at the post-administration phase. Waer and Shalaby (2012) recognized that lycopene extract of LE proved efficacious at the pre-administration phase. And this is in agreement with the result of the present study.

Conclusion

The present study was undertaken to determine the role of graded doses of antioxidants to overcome the hazards of ionizing radiation. It is confirmed by the antioxidant phytochem- ical constituents that the extract of LE cultivated in Nsukka has radioprotective potency which minimizes radiation-induced cytolysis in white albino rats used for the experiment. Therefore, the constituent has no geographical variation that is noticeable. The parameters studied in the current work were antioxidant enzymes (CAT, SOD and GPx), liver func- tion enzymes (ALP, ALT and AST), serum nutrients (TP and ALB) and non-enzymatic oxidative stress indices (VIT C and E, GSH and MDA). Rats treated with graded doses of ethyl acetate extract before and after whole body X-ray irradiation showed significant modulation in antioxidant enzymes and liver function enzymes activity, serum nutrients and non-enzymatic oxidative stress indices concentration . The treatment was also effective in minimizing the radiation-induced increase in lipid peroxidation in some tissue organs when compared with irradiated control rats. Therefore, the intake of LE supplement may be desirable for any patient that will be undergoing either any diagnostic modalities or radio- therapeutic treatment. It could be concluded that graded doses of the extract of LE exert a beneficial protective potential against many radiation-induced biochemical changed and disturbed oxidative stress markers.

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