Advances in Bioresearch
Adv. Biores., Vol 16 (6) November 2025: 129-146

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.6.129146

Advances in Bioresearch

ORIGINAL ARTICLE

Natural Antioxidant (*Curcuma Longa*) Supplementation on Irradiated Gonads of Male Albino Wistar Rats

Ekaete Vincent Ukpong[1], *Olaitan Ruth Asuquo[2,3], Emmanuel Esien-Umoh[1], Blessing Ibe[1], Itoro Efanga[1], Akpama Egong[1], Bassey Archibong[1], Paulinus Omini[1], Ndubuisi Chiaghanam[1], Nneoyi Egbe[1]

[1]Department of Radiography, Faculty of Allied Sciences, University of Calabar, Calabar, PMB 1115, Cross River State, Nigeria.

[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, PMB 1115, Cross River State, Nigeria.

[3]Department of Human Anatomy, Faculty of Medicine and Pharmaceutical Sciences, Kampala International University in Tanzania, Gongo la Mboto, P.O.Box 9790, Dar es Salaam, Tanzania

***Correspondence Author:** Olaitan Ruth Asuquo

Email address: ola_asuquo@yahoo.com

ABSTRACT

Natural antioxidants can reduce or prevent damage to cells caused by free radicals. Free radicals are generated when ionizing radiations interact with cells. Exposure of gonads to X-radiations could generate reactive oxygen species (ROS) and cause damage to the germ cells, which may be detrimental to the process of spermatogenesis and subsequently result in infertility in males. To assess the testicular antioxidants following exposure to radiation and ethanolic extract of Curcuma longa. A total number of 48 male albino Wistar rats aged 12 weeks were used for the experiment. These were divided into eight groups of 3 each, for 14 days and 28 days treatments respectively as follows: Group 1, control; group 2, single exposure to X-rays only; group 3, double exposure to X-rays only; group 4, ethanolic extract of Curcuma longa only; group 5, single exposure before Curcuma longa; group 6, Curcuma longa prior to single exposure to X-rays; group 7, double X-ray exposure before Curcuma longa; group 8, Curcuma longa prior to double exposure to X-rays. The X-ray dose for all exposures was 146μGy and dose of ethanolic extract of Curcuma longa for all treatments was 200mg/kg. The animals were sacrificed after 14 days and 28 days to study effects of x-rays and Curcuma longa supplementation on the cytoarchitecture of the testes, serum levels of Malondialdehyde, Super Oxide Dismutase, Catalase and Glutathione Peroxidase, body weight of the experimental animals. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 Statistical significance was at a p value of ≤ 0.05. Oxidative stress level was evident in the increased level of MDA (3.23 ± 0.32 IU/mg/protein), CAT (40.12 ± 1.05 IU/mg/protein), GPx (1.85 ± 0.10 IU/mg/protein) and decreased SOD (1.05 ± 0.02 IU/ mg/protein), levels in the blood serum. A decrease in MDA after 28 days pre-treatment was suggestive of decreased peroxidation and oxidative stress. There was loss of germ cells, nuclei fragmentation, poor nuclei outline, sparsely spaced seminiferous tubules, degenerative Leydig cells within the testicular architecture of experimental animals exposed to both single and double exposure groups exposed to X-radiation. Curcuma longa supplementation on gonads of irradiated Wistar rats has ameliorative effect. However, pre-irradiation treatment with Curcuma longa for 14 days- 28 days can boost endogenous antioxidants which helps in the reduction of the damaging effects of X-rays on gonad caused by free radicals.

***Keywords**: Natural antioxidants, testes, X-radiations, Supplementation, Curcuma longa.*

Received 26.09.2025 Revised 01.10.2025 Accepted 16.11.2025

How to cite this article:
Ekaete Vincent Ukpong, Olaitan Ruth Asuquo, Emmanuel Esien-Umoh, Blessing Ibe, Itoro Efanga, Akpama Egong, Bassey Archibong, Paulinus Omini, Ndubuisi Chiaghanam and Nneoyi Egbe. Natural Antioxidant (Curcuma Longa) Supplementation on Irradiated Gonads of Male Albino Wistar Rats. Adv. Biores. Vol 16 [6] November 2025. 09-20

INTRODUCTION

Increase in the use of X-radiations in diagnosis and treatment in CT scans and other imaging modalities utilizing X-rays in recent times is of major concern [1]. This is not unconnected with the carcinogenic effects and infertility in males that may result from high doses and frequent low doses used for these

investigations [2]. Radiation damage to cells is either by direct ionization of DNA or indirectly by formation of Reactive Oxygen Species (ROS) [3]. Studies have shown induction of oxidative stress at low doses of X-Rays on tissues exposed to it . The interaction of these low doses with the tissue results in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress under different pathophysiological conditions. Research has revealed widespread involvement of oxidative stress in several disease processes, including cancer [4]. Alterations caused by ionizing radiation can cause reproductive anomalies (morphological and functional gonadal dysfunction, e.g infertility and congenital mal-functions[21]. Furthermore, it has been shown that irradiation causes a marked change in the plasma total antioxidant capacity, and total body irradiation is known to cause a pronounced decrease in antioxidant capacity and an excessive increase in oxidative stress [5]. Antioxidant defense systems present in mammals help to minimize the formation of ROS by scavenging on them. However, these systems are not always continuously operative due to increase in the oxidative stress which may be a reason for such decrease and exhaustion of antioxidant defense system, as a result of increased endogenous production of the free radicals. Therefore, diet derived antioxidants become particularly important in diminishing cumulative oxidative damage, and several dietary antioxidants have been reported to decrease free radical attack on biomolecules. All these effects result from the formation of Reactive Oxygen series (ROS) when gonads are exposed to ionizing radiation. The formation of the ROS results in oxidative stress which subsequently leads to radiation damage. Cells have several enzymatic antioxidants that detoxify ROS and protect the cell from oxidative damage. It has been shown that following exposure to X-rays, natural antioxidants such as vitamins C and E exert a protective effect against chromosomal damage by reactive species generated by the irradiation [6]. Antioxidants prevent oxidative damage through one-electron reactions with free radicals which include superoxide radicals (O_2·−), hydroxyl radicals (OH·), singlet oxygen (O·), and hydrogen peroxide (H_2O_2) that adversely alter cellular lipids, protein, DNA, and polysaccharides. Both *in vitro* and *in vivo* studies on animal models have demonstrated the beneficial effect of antioxidants on damage induced by ionizing radiation. Studies in multiple antioxidant research indicated a reduction of chromosomal and oxidative damage in subjects undergoing radiotherapy. Many factors in the testes can induce oxidative stress, this is a pointer to the fact that the testis is vulnerable and highly dependent on oxygen to drive spermatogenesis and yet highly susceptible to the toxic effects of reactive oxygen metabolites [7]. The testicular tissues are often vulnerable to oxidative stress due to abundance of unsaturated fatty acids. Induction of oxidative stress in the testes activates induction of NF-kB mediated production of SOD and GPx activities. *Curcuma longa*, (Turmeric), a member of the *Curcuma* botanical group, is part of the ginger family of herbs, the *Zingiberaceae*. It contains a bioactive substance called curcumin, it could undergo several biochemical modifications, which helps it to fight cell damage [8]. Scientific evidence is mounting that curcuma offers innumerable health benefits which include, antioxidants, radioprotective, antibacterial, antifungal, antiviral, anti-inflammatory, anti-proliferative effects [9-10]. This study focuses on the direct assessment of the effects of *Curcuma longa (*turmeric) supplementation on testes of Wistar rats exposed to diagnostic range of X-rays.

MATERIAL AND METHODS

A total of forty-eight albino Wistar rats were divided into 8 of 3 rats each for 14 days and 28 days treatment respectively. The groups were weighed with Ashton Meyer's electronic weighing balance with Model number: 7765; Group 1 were fed with normal laboratory feeds and distilled water. Group 2 were given single exposure of 146µGy dose of X-rays and kept for 14 days and 28 days respectively before they were sacrificed. Group 3 were given double exposure of dose of X-rays and kept for 14days and 28days respectively before they were sacrificed. Group 4 animals were given 200mg/kg of ethanolic extract of *Curcuma longa* for 14 days and 28 days respectively. Group 5 were given 200mg/kg body weight dose of *Curcuma longa* for 14 days and 28 days respectively and then exposed to 146µGy dose of x-ray. Group 6 were given single exposure of 146µGy dose of X-rays then treated with a 200mg/kg body weight of *Curcuma longa* extract 24 hours after exposure for 14days and 28days respectively. Group 7 animals were treated with 200mg/kg body weight of ethanolic extract of *Curcuma longa* and given double exposure of 146µGy X-rays after 14days and 28days of the administration. Group 8 were given 200mg/kg body weight of ethanolic extract of *Curcuma longa* for 14days and 28days respectively and then given double exposure of 146µGy dose of X-rays.

Immobilization and exposure to X-radiation;

The animals were anesthetized with Chloroform then immobilized with surgical tape and fixed to the x-ray couch; their gonads were irradiated with 146µGy dose diagnostic range of x-rays with exposure factors of 40kVp, 4mAs and Film –focus distance (FFD) of 90cm. The field of irradiation was collimated to

the scrotum and 2cm above it, to cover the full extent of the testes. The rats were then sacrificed after 14 days and 28 days respectively.

Sacrifice of rats

The rats were sacrificed using chloroform as an anesthesia. Inhalation of the anesthesia sedated the rats. The rats were then placed on the dissecting board in a supine position; a longitudinal midline incision was made; the testes were excised and fixed in Bouin's fluid for 24hours. The pituitary gland also was excised and placed in formalin, for further processing and studies

Fixation and paraffin tissue processing

Fixation was done in batches to cater for the histological and tinctorial investigations: The testes carefully excised and tissues were cut in 0.5cm and fixed Bouin's for 24 hours, after which it was transferred to 70% alcohol for dehydration. The tissues were then passed through 90% alcohol and chloroform at different durations. They were passed through equal concentration of Xylene and alcohol for clearing. The cleared tissues were dried, then transferred to 2 changes of molten wax for 20 minutes each in an oven at a temperature of 60 degrees Celsius. Sections of 5*u*m thick were gotten from a solid block of tissues. The blocks were chilled on a refrigerated plate for 10 minutes before sectioning, a microtome was used to slice the tissues into thin sections. These were then transferred to the hot water bath to melt the wax. The floating sections were then scooped up onto a slide and allowed to dry at 36 degrees Celsius, for 2 hours.

Staining Methods

Hemotoxylin & Eosin Staining method of Drury and Wallington [17]

Reagents: Hematoxylin, eosin, xylene, chloroform, alcohol.

Procedure: Hemotoxylin & Eosin staining methods of Drury and Wallington [17]
was carried out, on the anterior pituitary gland, testis and epididymis. The deparaffinized sections were hydrated by passing through decreasing concentration of alcohol baths and water (100%, 90%, 80%, 70%) for 15 minutes then stained in hematoxylin for 5 minutes and blue in running tap for 8 minutes. The slides were then stained in eosin for 60 seconds followed by rapid dehydration in varying grades of alcohol in ascending order. The dehydrated sections were then cleared with xylene and mounted with DPX (dibutylphthalate Polystyrene Xylene). The H&E stained slides were then viewed under microscope and photomicrographs were taken with x100 and x400 magnification for testes and x400 and x1000 magnification for anterior pituitary gland.

Estimation of Antioxidants in the testis

Blood samples were taken from Groups 1,2,3,4,5,6,7 and 8 into bottles and taken for antioxidant analysis including Super Oxide Dismutase (SOD), Melondialdehyde (MDA), Catalase (CAT), and Gluthathione peroxide (GPx), were carried out.

Determination of superoxide dismutase (SOD)

SOD activity was determined by using the method of Misra and Fridovich [4]. The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 to adrenochrome makes this reaction a basis for a simple assay of dismutase. 1 ml of sample was diluted in 9 ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample was added to 25 ml of 0.05 M carbonate buffer pH 10.2 to equilibrate in the spectrophotometer and the reaction was initiated by adding 0.3 ml of adrenaline. The change in absorbance was monitored at 430 nm for 5 minutes.

Determination of catalase (CAT)

Catalase activity was determined according to the method of Aebi [16]. Catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cells as a byproduct of various oxidase and superoxide reactions. Hydrogen peroxide is highly deleterious to the cells and its accumulation causes oxidation of cellular targets such as DNA, protein and lipid leading to mutagenesis and cell death. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell. 0.1 ml of the sample was pipetted into curette containing 1.9 ml of 50 MM phosphate buffer pH 7.0. The reaction was initiated by addition of 0.1 ml of freshly prepared 30% (w/v) hydrogen peroxide. The rate of decomposition of hydrogen peroxide was measured spectrophotometrically by monitoring the change in absorbance in thermogenesis 10s UV-US spectrophotometer at 240 nm.

Determination of Glutathione Peroxidase

Activity of Glutathione peroxidase was determined by use of assays measuring oxidation of NADPH in the presence of exogenous glutathione, cumene hydroperoxide, and glutathione reductase.

Determination of Malondialdehyde

Malondialdehyde (MDA) was measured according to procedure of Ohkawa et al., [18]. The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % aqueous solution of TBA. The mixture pH was adjusted to 3.5, and the volume was

finally made up to 4.0 ml with distilled water; 5.0 ml of the mixture of ***n***-butanol and pyridine (15:1, ***v/v***) was added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm.

Statistical Analysis

All statistical analyses were carried out using SPSS version 2.0 statistical software. The results were presented as the mean ± standard deviations and bar charts. Enzyme parameters and peroxidation levels of matched experimental and control pairs were analyzed with the Kolmogorov Smirnov test. One way ANOVA was used, when there was a significant difference among the groups. P values of less than 0.05 were considered statistically significant.

RESULTS

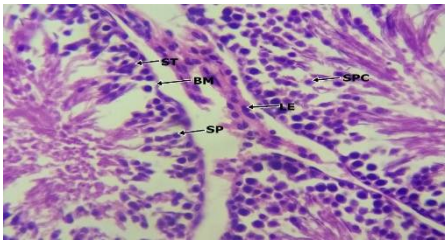


Plate 1: Photomicrograph of testis of Wistar rat (Control) for 14 days showing:

Closely packed seminiferous tubules (ST), intact basement membrane (BM) containing proliferating spermatogonia (SP), spermatocytes (SPC), insterstitium contains 3 to 5 Leydig cells (LE) per cluster. Sertoli cells population is 10 to 12 per tubule and majority contains spermatozoa. The cells are greater than 4 cell layers thick have deeply stained nuclei and coarse chromatin pattern, X400

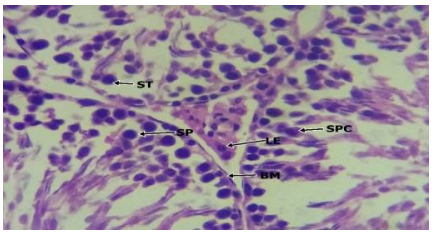


PLATE 2: Photomicrograph of testes of Wistar rats irradiated with single exposure of 146µGy dose of X-rays (14 days) showing:

prominent seminiferous tubules (ST) with an intact basement membrane consisting of proliferating spermatogonia (SP)of greater than four cell layers, interstium with Leydig cells, cells with uniform round to oval hyperchromatic nuclei comprising of the early series spermatogonia A and B (SP), spermatocytes (SPC) and late series spermatid (ST) and spermatozoa. The Sertoli cells are 9 to 10 per seminiferous tubules and the leydig cells (LE) are 3 to 4 per clusters. The lumina cavities of most of the tubules contain spermatogonia cells, some spermatids and few are empty, intact basement membrane. X400

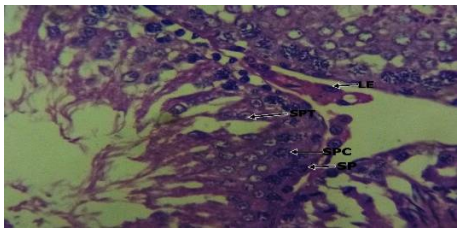


PLATE 3: Photomicrograph of testes of Wistar rats irradiated with double exposures of 146µGy dose of X-rays showing:

widely spaced seminiferous tubules (ST) containing atrophic and degenerated spermatogonia cells (SP). Interstitium (I) contains degenerative Leydig cell, empty luminal cavities and the spermatogonia cells (SPT) and spermatoytes (SPC) are mostly the early series having poor nuclei outline with nuclei fragmentation and karyolysis X 400

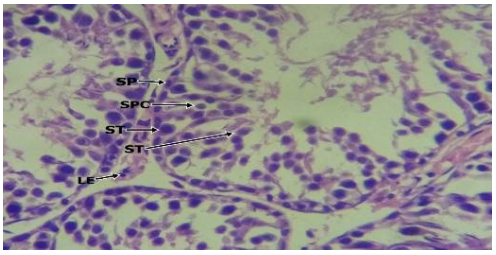


PLATE 4: Photomicrograph of testes of Wistar rats treated with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 14days, showing:

prominent seminiferous tubules (ST) with an intact basement membrane consisting of proliferating spermatogonia (SP) of greater than 4 cell layers. Interstitium (I) contains leydig cells, uniform round to oval hyperchromatic nuclei comprising of the early series spermatogonia A and B (SP), spermatocytes (SPC) and late series spermatid and spermatozoa. The Sertoli cells are 10 to 12 per seminiferous tubules (ST) and the Leydig cells (LE) are 3 to 6 per clusters. The lumina cavities of most of the tubules contain spermatogonia cells whiles few are empty.

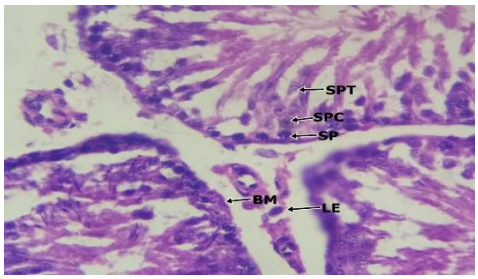
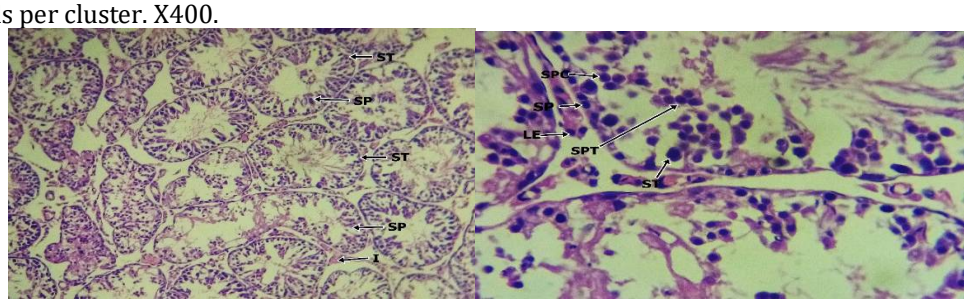


PLATE 5: Photomicrograph of testes of Wistar rats given single exposure of 146µGy dose of X-rays before treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 14days (Post – irradiation treatment), showing:

Widely spaced mildly atrophic seminiferous tubules (ST) containing moderately populated spermatogonia (SP) at various stage of maturation, interstitium contains Leydig cells. The cells (Spermatogonia (SP), Spermatocyte (SPC), Spermatids (SPT)are less than 4 layer thick in most of the tubules with empty lumina cavity. Few of the tubules contained mature spermatozoa. Leydig cells are less than 4 cells per cluster. X400.



(a) (b)

PLATE 6: Photomicrograph of testes of Wistar rats given single exposure of 146µGy dose of X-rays after treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 14days (Pre – irradiation treatment), showing:

a. widely spaced seminiferous tubule (ST) of various sizes and shape. Majority of the tubules are irregular in shape with distorted contours containing atrophic and degenerated spermatogonia cells. X 100

b. The fairly regular tubules contain proliferating spermatogonia cells (SP) of less than four layer thick, which includes mainly the early series spermatogonia and spermatocytes. The luminal cavities are mostly empty and few contain scanty spermatozoa. Finding is consistent with destruction of spermatogonia cells. X 400

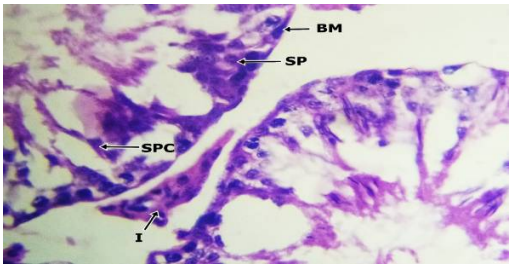


PLATE 7 Photomicrograph of testes of Wistar rats given double exposure of 146µGy dose of X-rays before treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 14days (Post – irradiation treatment), showing:

widely space seminiferous tubules (ST) consisting of sparsely populated spermatogonia cells (SP) and spermatocytes (SPC), Interstitium contains few Leydig cells, cell layer is less than 3 and majority of the tubules contain the early series comprising of spermatogonia A (SP) and spermatocytes (SPC). The cells have poor nuclei outline and fragmented nuclei, Leydig cells of the interstitium (I) have fragmented nuclei.

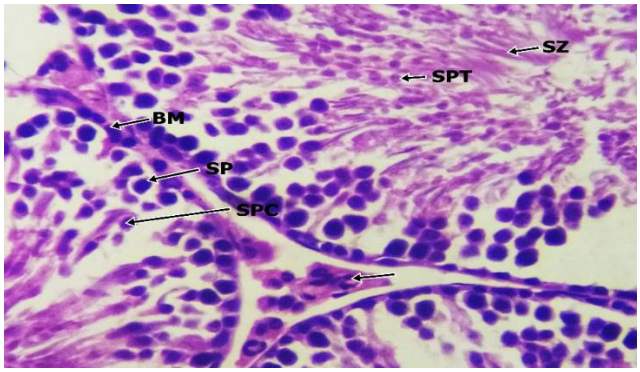


PLATE 8: Photomicrograph of testes of Wistar rats given double exposure of 146µGy dose of X-rays after treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 14days (Pre – irradiation treatment), showing:

prominent seminiferous tubules (ST) of various sizes containing proliferating spermatogonia (SP) at various stage of maturation. Interstitium (I) contains Leydig cells, spermatogonia (SP), spermatocytes (SPC), Spermatids (SPT) and Spermatozoa (SZ) are uniform having hyperchromatic oval to round deeply stained nuclei with coarse chromatin patterns. Cell are 3 to 5 cell layer thick. Sertoli cells are 9 to 11 per tubules and the Leydig cells are 3 to 6 per clusters. Luminal cavities are filled with spermatid (SPT) and spermatozoa (SZ). X 400.

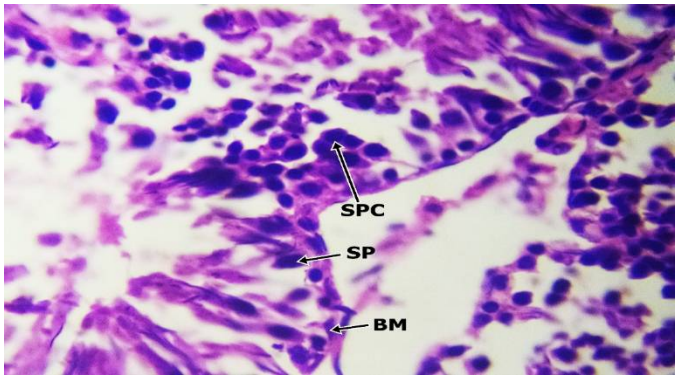


Plate 9 Photomicrograph of testis of Wistar rat (Control) for 28 days showing:

Closely packed seminiferous tubules (ST) containing proliferating spermatogonia SP), interstitium (I) contains Leydig cells, distinct cellular outline and oval to round hyperchromatic nuclei. An intact basement membrane (BM) with cells (SP, SPC) 3 to 5 cell layer thick and majority contains spermatozoa.

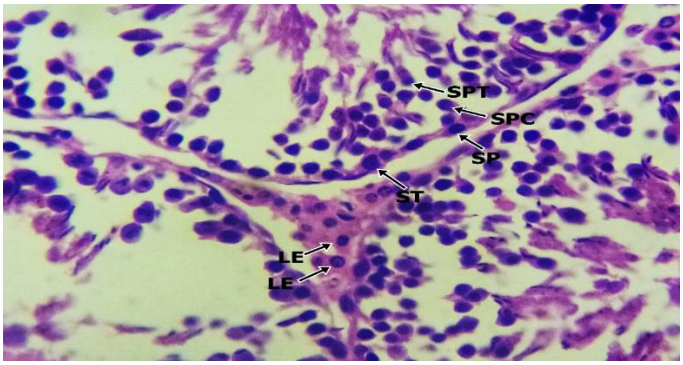


PLATE 10: Photomicrograph of testes of Wistar rats irradiated with single exposure of 146µGy dose of X-rays (28 days) showing:

intact basement membrane of prominent seminiferous tubules (ST), consisting of proliferating spermatogonia (SP)of greater than four cell layers, interstium with Leydig cells. cells is uniform round to oval hyperchromatic nuclei comprising of the early series spermatogonia A and B (SP), spermatocytes (SPC) and late series spermatid (ST) and spermatozoa. The Sertoli cells are 9 to 10 per seminiferous tubules and the Leydig cells (LE) are 3 to 4 per clusters. The lumina cavities of most of the tubules contain spermatogonia cells, some spermatids and few are empty, intact basement membrane. X400

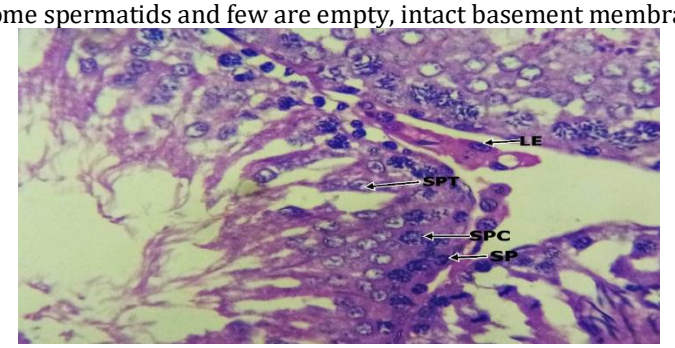


PLATE 11: Photomicrograph of testes of Wistar rats irradiated with double exposures of 146µGy dose of X-rays showing:

widely spaced seminiferous tubules (ST) containing atrophic and degenerated spermatogonia cells (SP). Interstitium (I) contains degenerative Leydig cell, empty luminal cavities and the spermatogonia cells (SPT) and spermatocytes (SPC) are mostly the early series having poor nuclei outline with nuclei fragmentation and karyolysis. X 400

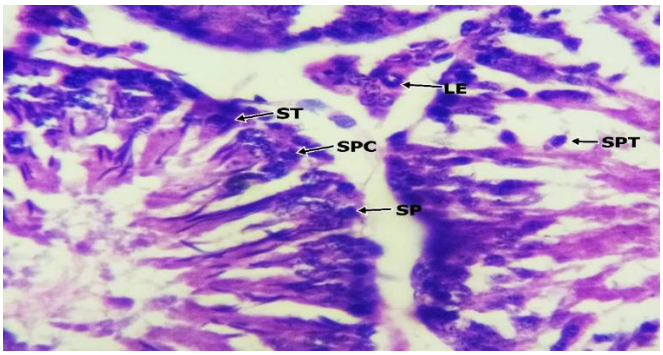


PLATE 12: Photomicrograph of testes of Wistar rats treated with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 28 days, showing:

widely spaced seminiferous tubules (ST) with empty lumina cavity. The cells are less than 3 to 4 cell layer thick (SP, SPC, SPT) with poor cytoplasmic and nuclei outline. The intervening interstitium are scanty and Leydig (LE) cell less than two per cluster.

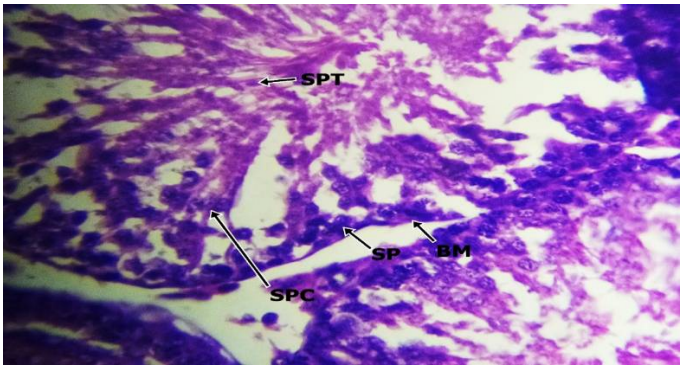


PLATE 13: Photomicrograph of testes of Wistar rats given single exposure of 146µGy dose of X-rays before treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 28days (Post –irradiation treatment), showing:

closely packed seminiferous tubules separated by sparse interstitum (I). The tubules contain proliferating spermatogonia (SP) at various level of maturation, enlarged cells with hyperchromatic with coarse chromatin. The cells are 3 to 5 layer thick consisting of both the early and late series and majority of the tubules contained mature spermatozoa within their lumina cavity. The Leydig cells numbered 3 to 4 per cluster. X 400

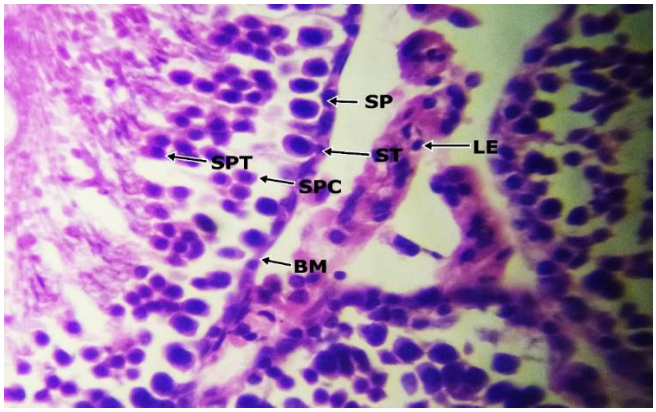


PLATE 14: Photomicrograph of testes of Wistar rats given single exposure of 146µGy dose of X-rays after treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 28 days (Pre – irradiation treatment), showing:

closely packed seminiferous tubules (ST) of various sizes separated by moderate interstitium (I). The tubules contain proliferating spermatogonia at various level of maturation (SP), uniform, hyperchromatic cells with coarse chromatin. The cells 3 to 5 layer thick consisting of both the early and late series and majority of the tubules contained mature spermatozoa within the lumen. The Leydig cells numbered 3 to 4 per cluster.

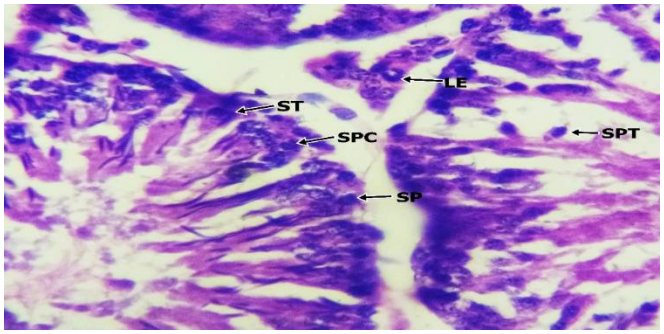


PLATE 15: Photomicrograph of testes of Wistar rats given double exposure of 146µGy dose of X-rays before treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 28 days (Post –irradiation treatment), showing:

seminiferous tubules (ST) containing deeply stained spermatogonia (SP) cells with poor nuclei outline and coarse chromatin pattern. The cells are less than four layers thick and lumina cavity contain scanty spermatid (SPT) and spermatozoa. There is hyperplasia of the Leydig cells (LE). The cells are enlarged with prominent nucleoli. X100

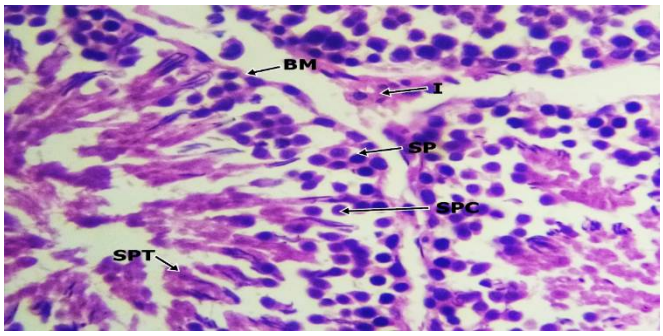


PLATE 16: Photomicrograph of testes of Wistar rats given double exposure of 146µGy dose of X-rays after treatment with 200mg/kg b.w.t of ethanolic extract of *Curcuma longa* for 28 days (Pre – irradiation treatment), showing:

closely packed seminiferous tubules of various sizes separated by sparse interstitum (I). The tubules contain proliferating spermatogonia (SP) at various level of maturation, uniform, hyperchromatic cells with coarse chromatin. The cells (spermatogonia (SP), spermatocyte (SPC), spermatids (SPT) are 3 to 5 layer thick consisting of both the early and late series and majority of the tubules contained mature spermatozoa within the lumen, intact basement membrane (BM). The Leydig cells numbered 3 to 4 per cluster

ANTIOXIDANTS RESULTS

Superoxide Dismutase (SOD)

Observations of experimental animals in the 14 days treatment group showed a significant decrease in SOD in animals exposed to X-rays only and the pre-irradiation treatment groups. There was no significant difference in the post-irradiation treatment group and in animals treated with *Curcuma longa* only (Fig. 1). At $P < 0.05$.

Curcuma longa supplementation for 28days showed no significant difference in all groups compared to control. However, there was a significant increase in SOD concentration in single exposure post-irradiation treatment group compared to the animals given single exposure only. A significant decrease was observed in double exposure post – irradiation treatment animals when compared to single exposure post irradiation animals (Fig. 2). $P<0.05$

Comparison of the 14 days and 28 days treatment groups showed a marked decrease in SOD concentration by the 28th day when compared to 14 days treatment groups (Fig. 3). $P<0.05$

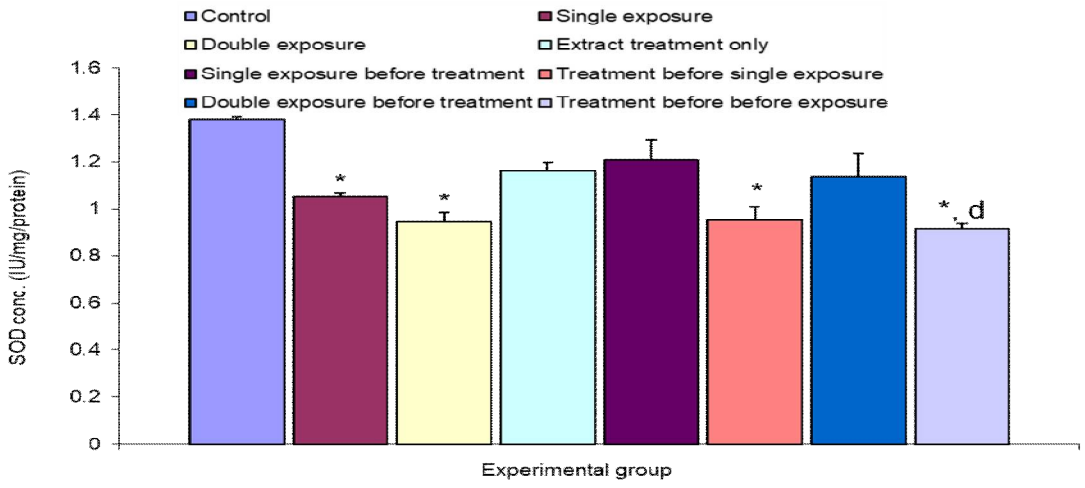


FIG. 1: Curcuma longa supplementation for 14 days on superoxide dimutase (SOD) concentration in Wistar rats exposed to X-radiation.

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
d = p<0.05 vs Single exposure before treatment

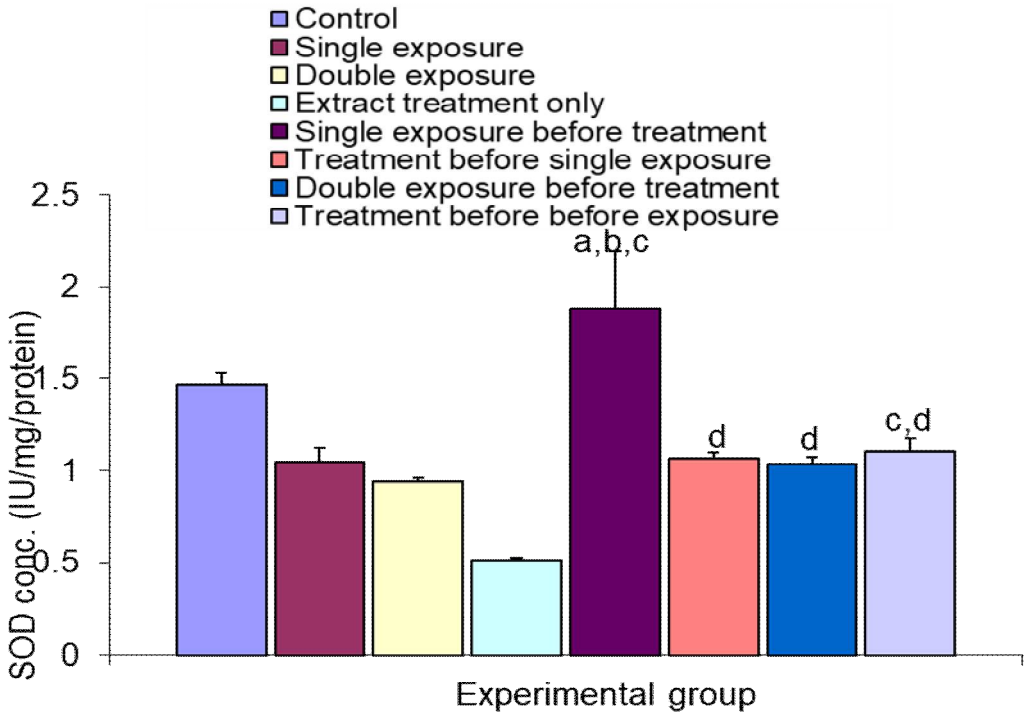


FIG. 2: *Curcuma longa* supplementation for 28 days on superoxide dismutase (SOD) concentration in Wistar rats exposed to X-radiation.

Values are expressed as mean ±SEM, n = 3
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
c = p<0.05 vs extract only
d = p<0.05 vs single exposure before treatment

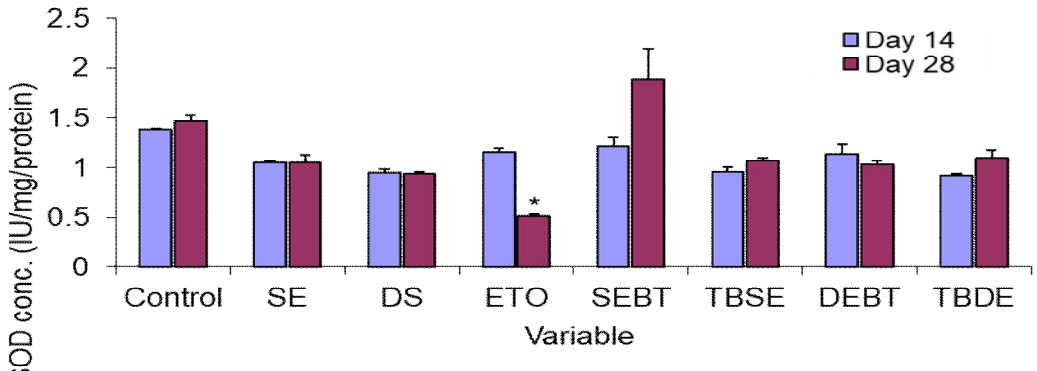


FIG. 3: Comparison of superoxide dimutase (SOD) concentration between 14 and 28 days of treatment in the different experimental groups.

Values are presented as mean +SEM, n = 3.
* = signiicantly different from day 14 at p<0.05

SE - Single exposure
DS - Double exposure
ETO - extract from Curcuma longa only
SEBT - Single exposure before treatment
TBSE - Treatment before single exposure
DEBT - Double exposure before treatment
TBDE - Treatment before double exposure

MELONDIALDEHYDE RESULTS

There was significant increase in MDA observed in all experimental animals in the 14 days treatment group. However, double exposure post-irradiation treatment animals showed no significant difference compared to control. A marked increase in MDA was observed in post treatment single exposure group compared with the pre-irradiation single exposure group. The double exposure post-irradiation treatment group showed a marked increase in MDA compared with the pre-irradiation treatment animals (Fig. 4). P<0.05

Observations of the 28 days treatment groups showed a significant increase animals exposed to X-rays only compared to control. A significant decrease in MDA was observed in all animals supplemented with *Curcuma longa* compared to groups exposed to X-rays only. A marked decrease was observed in pre-irradiation treatment double exposure group by the 28th day (Fig. 5). P<0.05

Comparison of MDA concentration between 14 and 28days of treatment showed significant decrease in animals treated with *Curcuma longa* only, single exposure pre-irradiation and post-irradiation treatment and double exposure pre-irradiation treatment groups by 28th day when compared to the 14th days treatment groups (Fig. 6). P<0.05

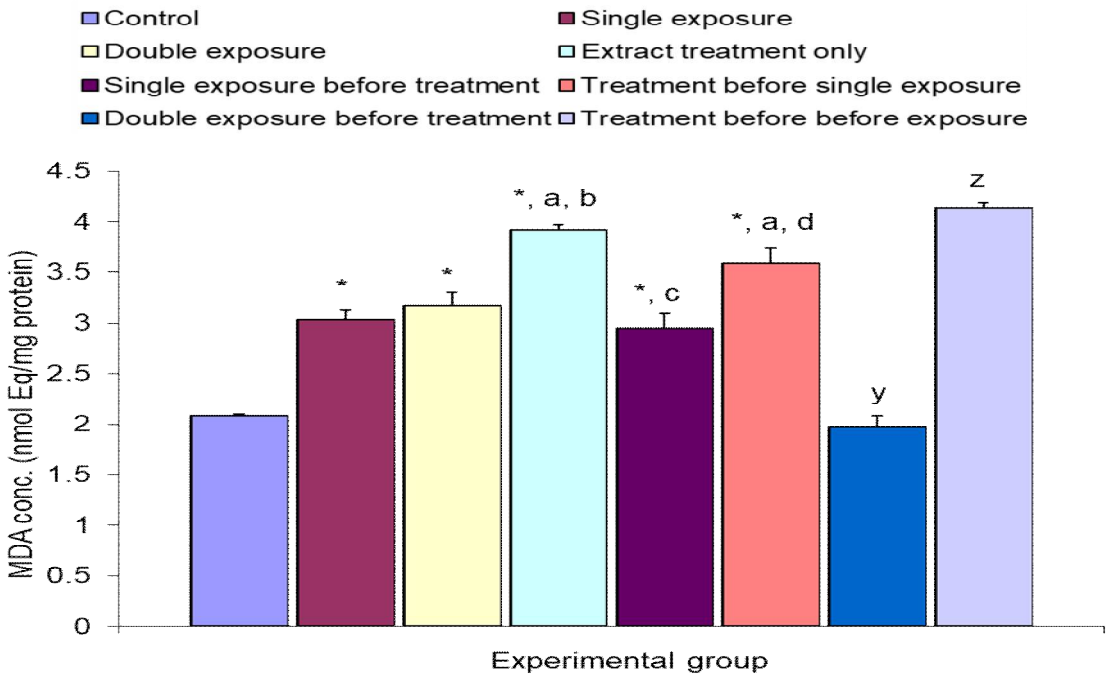


FIG. 4: *Curcuma longa* supplementation for 14 days on malondialdehye (MDA) concentration in Wistar rats exposed to X-radiation

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
c = p<0.05 vs extract only
d = p<0.05 vs Single exposure before treatment
y = p<0.05 vs all group except ith control
z = p<0.05 vs all group except with extract only

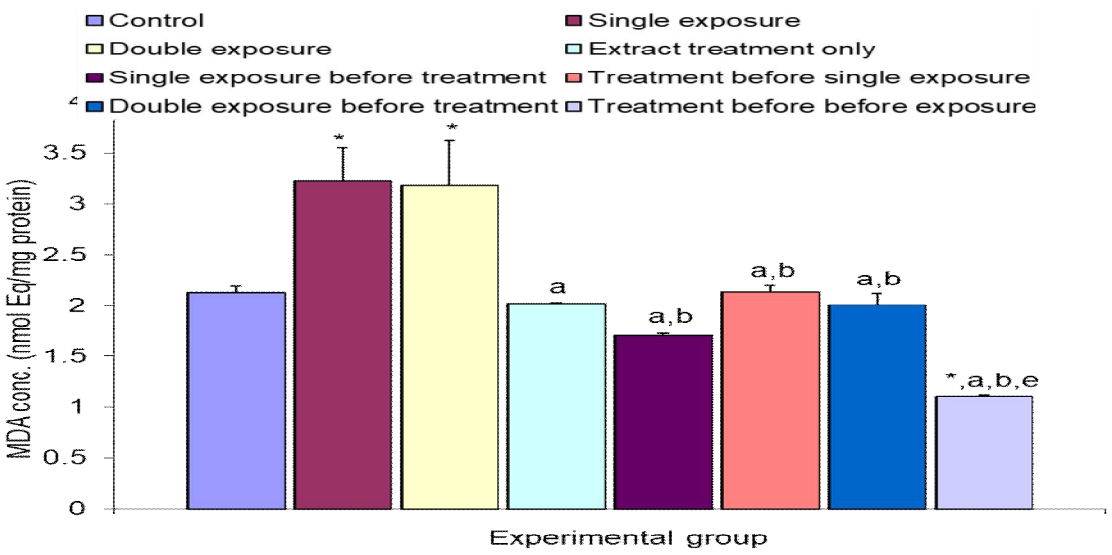


FIG. 5: *Curcuma longa* supplementation for 28 days on malondialdehye (MDA) concentration in Wistar rats exposed to X-radiation

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
e = p<0.05 vs treatment before single exposure

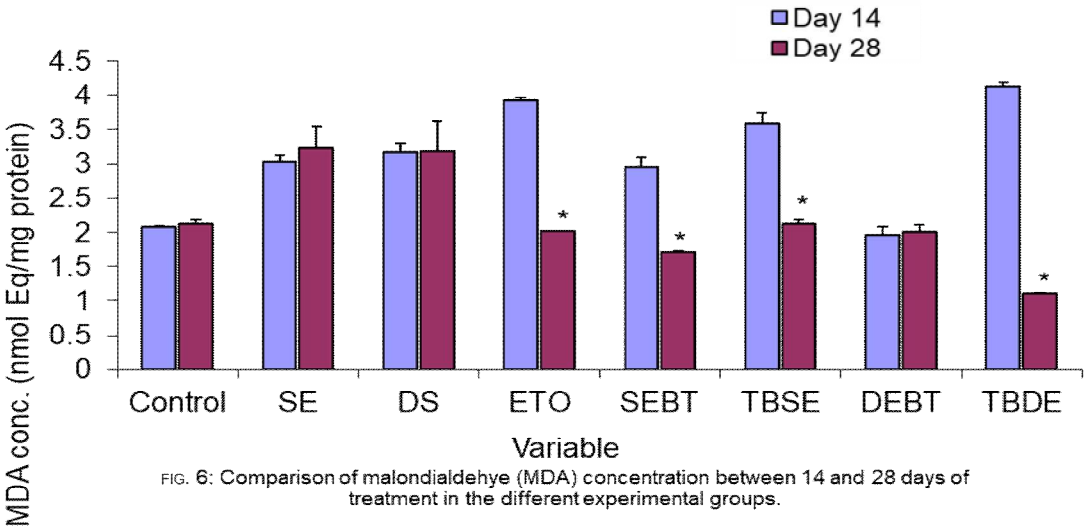


FIG. 6: Comparison of malondialdehye (MDA) concentration between 14 and 28 days of treatment in the different experimental groups.

Values are presented as mean +SEM, n = 3.
* = signiicantly different from day 14 at p<0.05

SE - Single exposure
DS - Double exposure
ETO - extract from Curcuma longa only
SEBT - Single exposure before treatment
TBSE - Treatment before single exposure
DEBT - Double exposure before treatme
TBDE - Treatment before double exposure

Glutathione peroxidase

Experimental animals treated for 14 days showed no significant difference in Glutathione peroxidase (GPx) concentration in all groups when compared to control. Double exposure post-irradiation treatment experimental animals showed a significant increase when compared to all groups (Fig. 28). Observations showed a significant increase in the double exposure pre –irradiation treatment group when compared to animals treated with *Curcuma longa* only (Fig. 7). $P < 0.05$

Curcuma longa supplementation for 28 days on GPx concentration on animals exposed to x-rays showed significant increase in double exposure pre- irradiation and post-irradiation treatment animals compared to control, X-rays exposure only and single exposure pre-irradiation treatment groups. A marked increase was observed in double exposure pre-irradiation experimental animals compared to the single exposure pre-irradiation experimental animals (Fig. 8). $P < 0.05$

GPx concentration increased significantly in animals supplemented with *Curcuma longa* only and in single exposure before treatment group by 28th day compared to those of the 14 days treatment. A marked decrease was noticed in the double exposure before treatment group on the 28th day of treatment compared to result seen on the 14th days treated animals (Fig. 9). $P < 0.05$

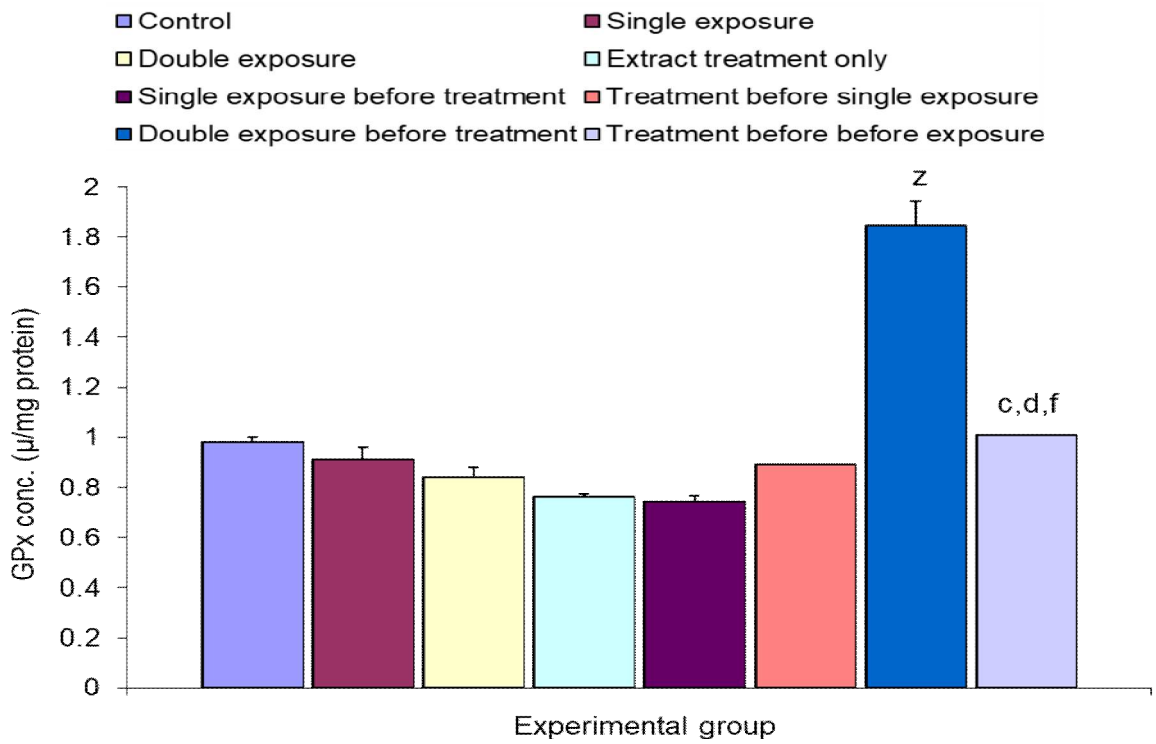


FIG. 7: *Curcuma longa* supplementation for 14 days on glutathion peroxidase (GPx) concentration in Wistar rats exposed to X-radiation.

Values are expressed as mean ±SEM, n = 3
c = p<0.05 vs extract only
d = p<0.05 vs Single exposure before treatment
f = p<0.05 vs double exposure before treatment
z = p<0.05 vs all group

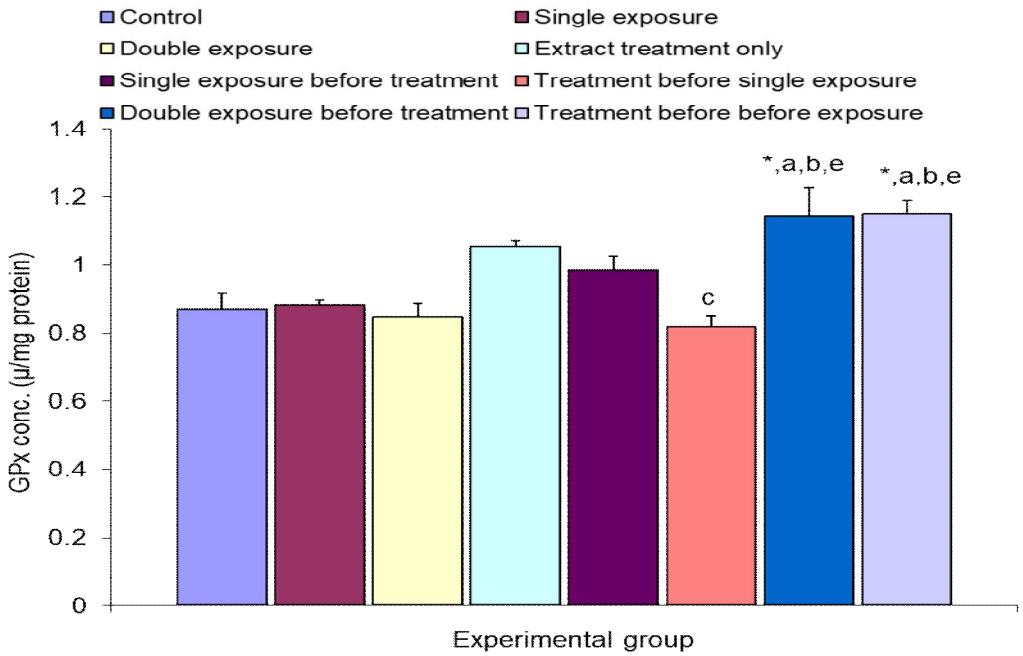


FIG. 8: *Curcuma longa* supplementation for 28 days on glutathion peroxidase (GPx) concentration in Wistar rats exposed to X-radiation.

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
c = p<0.05 vs extract only
e = p<0.05 vs treatment before single exposure

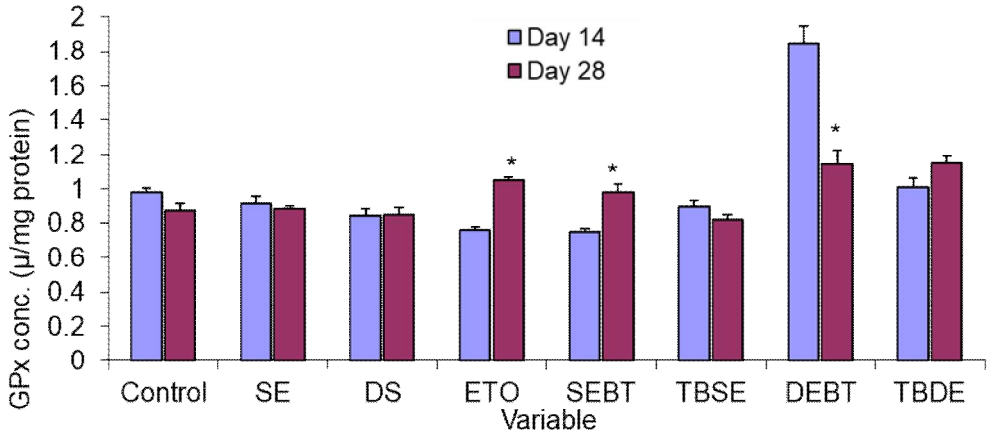


FIG. 9: Comparison of glutathion peroxidase (GPx) concentration between 14 and 28 days of treatment in the different experimental groups.

Values are presented as mean +SEM, n = 3.
* = signiicantly different from day 14 at p<0.05

SE - Single exposure ; DS - Double exposure
ETO - extract from Curcuma longa only
SEBT - Single exposure before treatment
TBSE - Treatment before single exposure' DEBT - Double exposure before treatment
TBDE - Treatment before double exposure

CATALASE

Catalase (CAT) concentration in 14 days treatment experimental animals showed no significant difference in animals exposed to X-rays only. A marked increase in animals treated with *Curcuma longa* only, single exposure animals treated with *Curcuma longa was* observed when compared with control. A marked decrease was observed in double exposure animals treated with *Curcuma longa* for 14days compared to single exposure groups (Fig. 10). $P < 0.05$

No significant difference was observed in animals exposed to X-rays only in 28 days treatment group compared to control. A significant increase in CAT concentration was noticed in single exposure post-irradiation treatment (SEBT) and double exposure pre-irradiation treatment (TBDE) group when compared to control. Similar result was observed in single exposure animals treated with *Curcuma longa* and the double exposure pre-irradiation treatment group compared to animals exposed to X–rays. Comparing CAT concentration between the animals supplemented with *Curcuma longa* and those exposed to X-rays and *Curcuma longa* treatment, a significant increase was observed (Fig. 11). $P < 0.05$

A marked decrease in CAT concentration was observed in animals supplemented with *Curcuma longa* only by 28th day compared to those treated for 14days. A significant increase in single exposure animals and double exposure groups supplemented with *Curcuma longa* was observed in the 28th day compared to experimental animals treated for 14 days (Fig. 12). $P < 0.05$

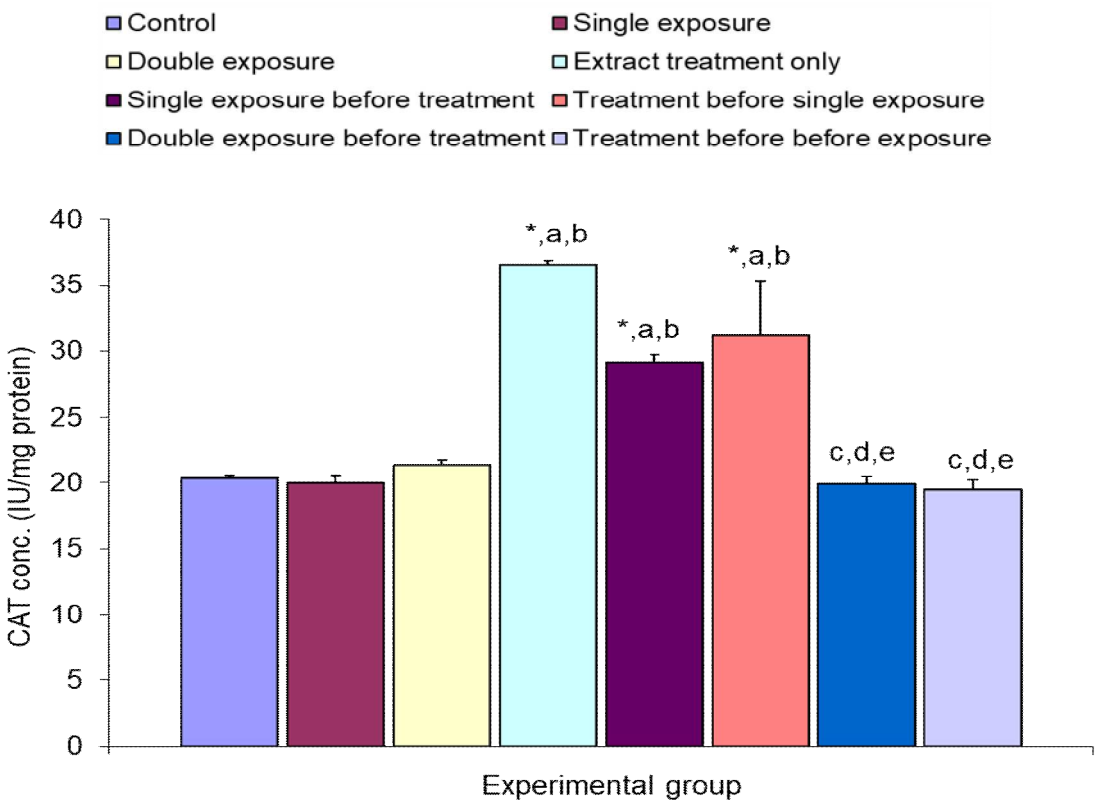


FIG. 13: *Curcuma longa* supplementation for 14 days on catalase (CAT) concentration in Wistar rats exposed to X-radiation.

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
c = p<0.05 vs extract only
d = p<0.05 vs Single exposure before treatment
e = p<0.05 vs treatment before single exposure

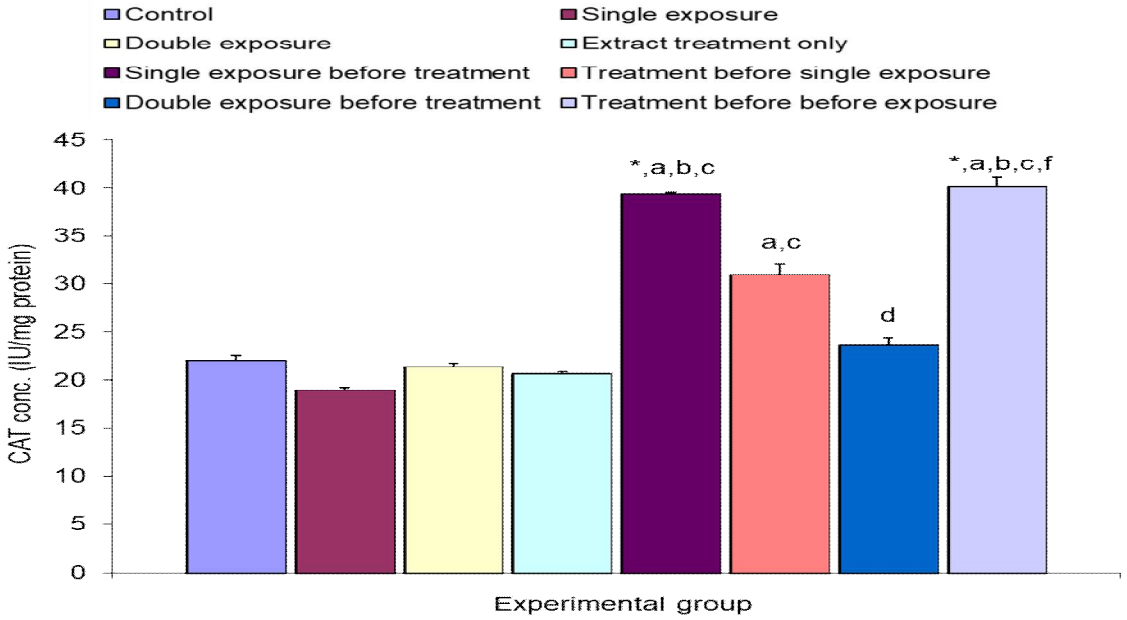


FIG. 14: *Curcuma longa* supplementation for 28 days. on catalase (CAT) concentration in Wistar rats exposed to X-radiation

Values are expressed as mean ±SEM, n = 3
* = p<0.05 vs control
a = p<0.05 vs single exposure
b = p<0.05 vs double exposure
c = p<0.05 vs extract only
d = p<0.05 vs single exposure before treatment
f = p<0.05 vs double exposure before treatment

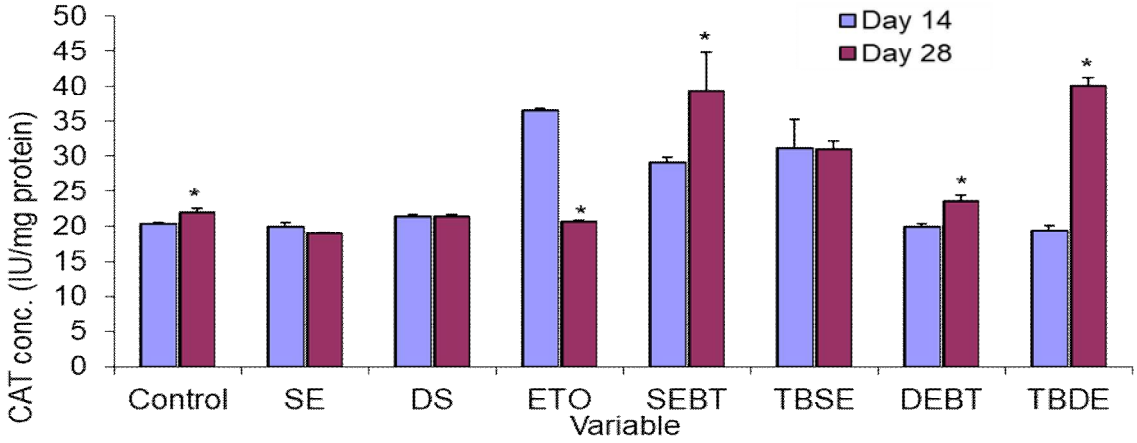


FIG. 15 : Comparison of catalase (CAT) concentration between 14 and 28 days of treatment in the different experimental groups.

Values are presented as mean +SEM, n = 3.
* = signiicantly different from day 14 at p<0.05

SE - Single exposure ; DS - Double exposure
ETO - extract from Curcuma longa only; SEBT - Single exposure before treatment
TBSE - Treatment before single exposure; DEBT - Double exposure before treatment
TBDE - Treatment before double exposure

DISCUSSION

Sperm cells are more likely to be influenced by oxidative stress than other cells because of the limited amount of cytoplasm in a mature sperm, the concentration of antioxidants that suppresses reactive oxygen species in the sperm and the presence of high levels of unsaturated fatty acids in the sperm

structure (Saleh & Agarwal, 2002). Antioxidant enzymes in the sperm fail to protect plasma membrane surrounding acrosome and tail. Therefore, sperm viability is greatly dependent on the availability of the antioxidants, which mostly is related to the antioxidant systems in the plasma of the semen. ROS generated in the semen is constantly deactivated by seminal antioxidants under normal conditions (REF). Study by Showel, et al., [8] stated that sperms can fight oxidative stress conditions mainly due to the antioxidant properties of semen. Imbalance between ROS production in the testes and antioxidants systems to activate the production of antioxidants is the major reason for the establishment of oxidative stress when testes are exposed to x-rays. Super oxide dismutase (SOD), catalases (CAT) and Glutathione peroxidase are antioxidant enzymes that protect the testicular cells from damage produced by ROS such as super oxide ions, hydroxyl radicals and hydrogen peroxide. SOD protects cells from oxygen radicals by scavenging on the superoxide, converting it to hydrogen peroxide which in turn is broken down into water [4]. Therefore, super dismutase is necessary in the dismutation of Hydrogen peroxide. A decrease SOD, and a significant increase in MDA, GPx, CAT in experimental animals observed in this study is indicative of oxidative stress in the testicular environment activated by ionizing radiation. Oxidative stress is a major factor in the aetilogy of male infertility and carcinogenesis. ROS attacks and induce lipid peroxidation and DNA fragmentation disrupting both the motility of these cells (Aitken, 2008). At the level of the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells as well as the ability for germinal epithelium to differentiate to spermatozoa. The vulnerability of testis to oxidative stress is due to abundance of highly unsaturated fatty acids and the presence of potential reactive oxygen species. SOD was observed to decrease significantly from values in the control groups (1.38$\pm$0.01 IU/mg/protein and 1.47IU/mg/protein) in animals exposed to X-rays in both the experimental groups of the 14th days (1.05 $\pm$ 0.02 IU/ mg/protein) and 28 days (1.05 $\pm$ 0.08 IU/mg/protein) duration of the study. Observations showed that the concentration of SOD increased by the 28th day of the experiment in animals exposed to X-rays and treated with *Curcuma longa* (1.88 $\pm$ 0.31UI/mg/protein). Similar result was seen in a study by Yarru et al., 2009, he stated that decreased expression of SOD, was alleviated by inclusion of supplementation in the diet. Therefore, dietary antioxidants are necessary to boost the endogenous antioxidant as also seen in this study. Exposure of testicular tissues to X-rays causes ionization as X-rays penetrates them. The resultant effect is the production of ROS which subsequently causes damage to the testes and increase in the testicular antioxidant in animals supplemented. Increased MDA is indicative of peroxidation of the seminiferous tubule membrane. MDA is the most frequently used biomarker for oxidative stress; this is a by -product of PUFA (polyunsaturated fatty acid) peroxidation of cell membrane. Increase in MDA was observed in animals exposed to both single dose (3.23 $\pm$ 0.32 IU/mg/protein) and double dose (3.19 $\pm$ 0.44 UI/mg/protein) of x-radiation, when compared to control group (2.12 $\pm$ 0.06 IU/mg/protein) indicating peroxidation in these animals. A reduction of MDA was observed in animals treated with *Curcuma longa* for 28days (1.70 $\pm$ 0.02 IU/mg/protein) compared to those given similar treatment for 14 days (2.95$\pm$0.16 UI/mg/protein) (Fig.5 and 6). Fan, 2003, noted similar results; an increase in MDA on irradiated animals which indicated the activation of oxidative stress. Increase in MDA was observed in animals treated for 14 days but there was a significant decrease with the 28 days treatment group. A similar result was obtained in an experiment by Shakeri, et al., (2017), they observed an increase in MDA when treated with a lower dose of *Curcuma longa*, an increase in the dosage on the experimental animals showed a significant decrease. In this study, a significant increase (3.19 $\pm$ 0.13 IU/mg/protein and 3.92 $\pm$ 0.05 IU/mg/protein) was observed in animals exposed to single and double doses of X-radiation only in 14 days post irradiation group. This persisted through the duration of the experiment as it was observed in the single (3.23 $\pm$ 0.32 IU/mg/protein) and double exposure groups (3.19 $\pm$ 0.44 IU/mg/protein), in the 28th post irradiation group (Figure 4 and 5). Catalase (CAT) in the animals treated with *Curcuma longa* before exposure to double dose of x-rays was observed to increase (40.12$\pm$1.05 IU/mg/protein) by the 28th day post-irradiation. This indicated a boost in the endogenous antioxidant with increased duration of administration of the *Curcuma longa* supplement. Also observed was a marked decrease in GPx concentration noticed in the double exposure before treatment group, on the 28th day of treatment compared to result seen on the 14th days treated animals showed that the supplementation with *Curcuma longa* reduced the oxidative stress from the level notice on the 14th day post-irradiation duration (1.85 $\pm$ 0.10 IU/mg/protein). This was observed by the marked decrease in GPx by the 28th day post- irradiation (1.14 $\pm$ 0.03 IU/mg/protein). Observation of the damage to the germ cells and the loss of Leydig cells shows the oxidative stress caused by the formation of Reactive Oxygen Species (ROS) in animals exposed to X-radiations (Plate 7, 11, 12). However, the scavenging properties of CAT and GPx ameliorated the effect of peroxidation. This is observed in the recovery of the germ cells to control observed on the 28th day of treatment (Plate 14, 16). The presence of the phenolic groups gives Curcumin the ability to react

with RNS and ROS. This might be the reason of its protective properties on the epithelial cells. Curcumin, the most active ingredient in *Curcuma longa rhizome* is observed to induce the expression of the cytoprotective proteins such as superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), glutathione-*S*-transferase (GST) [15]. These protective effects, according to studies is seen to increase SOD, GP_x, GR, CAT, and reduce the production of melondialdehyde (MDA) which results in reduction of oxidative stress. According to Soetikno et al, [10], Curcumin attenuates malondialdehyde (MDA) levels (a lipid peroxidation marker).

CONCLUSION

The use of *Curcuma longa* supplementation and development of antioxidant therapy can break the oxidative stress chain reaction, caused by exposure to x-radiation and play a very significant role in increasing the capacity of the gonad to fight free radical-induced oxidative stress, and therefore improve the process of spermatogenesis.

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