

Original Research Article

Ameliorative effects of Astymin-SN® and vitamin A against X-ray radiation-induced oxidative stress of male Wistar albino rats

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Abstract

Purpose: To evaluate the ameliorative effects of Astymin-SN® and vitamin A against x-ray radiation-induced oxidative stress in selected organs of albino rats.

Methods: Forty rats were assigned to five groups of equal number of rats. Group 1 rats were unirradiated and untreated (control). Group 2 rats were irradiated and untreated. Group 3 rats received 10 ml/kg body weight (b.w) of Astymin-SN® subcutaneously 24-hours before irradiation and administration continued once daily for seven days. Groups 4 and 5 received 100 and 200 mg/kg b.w of vitamin A orally 24-hours before irradiation, and dosing continued once daily for seven days, respectively. All Irradiation groups were exposed to irradiation twice a day and every other day for seven days. A total radiation dose of 7,689.12 µGy was administered to each of the irradiated groups during the seven days. Organs (liver, kidney, and testis) were harvested for tissue homogenate preparations and malondialdehyde (MDA) and the activities of antioxidant enzymes – superoxide dismutase (SOD), glutathione (GPx) and catalase (CAT) were assessed.

Results: Marked increase in MDA and significant ($p < 0.05$) decline in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in the irradiated and untreated group 2 were observed in comparison with the (control) group 1 rats. Remarkably, the mean values of MDA, SOD, CAT, and GPx in the irradiated and treated groups (groups 3, 4 and 5) did not vary significantly from the unirradiated and untreated control.

Conclusion: The observed non-significant difference in the assessed parameters between the irradiated and treated groups is evidence of the possible ameliorative potential of Astymin-SN® and vitamin A against x-ray radiation-induced oxidative stress.

Keywords: X-Ray, Ionizing radiation, Radioprotection, Astymin-Sn, Vitamin A, Oxidative stress

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INTRODUCTION

Astymin-SN® represents a specialized amino acid infusion crafted in alignment with the guidelines set forth by the World Health

Organization (WHO) and the Food and Agriculture Organization (FAO), incorporating insulin-independent xylitol produced in India by Til Healthcare PVT. This formulation encompasses all essential and non-essential

amino acids along with xylitol. Studies have established that amino acids are crucial compounds that possess significant therapeutic potential across a myriad of diseases if utilized effectively [1]. Essential amino acids are deemed both vital and indispensable. Numerous studies have identified these amino acids for their remarkable antioxidative properties and anti-inflammatory effects [2].

Vitamin A, often referred to as retinoic acid, has been linked to a possible antioxidant function, indirectly influencing NF- κ B and NRF2 signaling pathways [3]. All-trans retinoic acid has been noted for its pivotal role in hindering the activation of hepatic stellate cells by inhibiting thioredoxin-interacting protein, thereby minimizing oxidative stress levels [4]. Additionally, retinoic acid, a derivative of vitamin A, has been shown to enhance the expression of antioxidant-related genes in mature buffalo oocytes in vitro [5]. The induction of superoxide dismutase and glutathione transferase activities by all-trans-retinoic acid has been documented, alongside a reduction in malondialdehyde (MDA) and reactive oxygen species in both healthy and varicocele-affected sperm, indicating that vitamin A boosts antioxidant enzyme function [6]. A concerning consequence of irradiation is the generation of reactive oxygen species (ROS), which consist of superoxide anion radicals ($\bullet\text{O}_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet\text{OH}$) [7]. ROS are extremely reactive, posing a threat to critical cellular components such as DNA, proteins, and membranes, ultimately leading to cell death [8]. Oxidative stress occurs under conditions of excessive free radical generation and/or insufficient antioxidant defense, resulting in chemical modifications of biomolecules that cause structural and functional impairments [9]. The damage inflicted by X-ray irradiation must be mitigated due to its various harms to cells and tissues. This study was conducted to assess the restorative effects of Astymin-SN (amino acids) and vitamin A against x-ray radiation-induced oxidative stress in selected organs of male Wistar albino rats.

EXPERIMENTAL

Animals

A cohort of forty (40) male Wistar albino rats, aged 12 - 14 weeks and weighing approximately 220-280g, was sourced from the Department of Veterinary Medicine, University of Nigeria, Nsukka, for this research. The rats were accommodated in the animal facility of the Department of Veterinary Surgery and Radiology in standard cages under optimal temperature

conditions of $25 \pm 2^\circ\text{C}$ and a light/dark cycle of 12 hours. They were fed twice daily with standard rat pellets and had unlimited access to clean drinking water.

Ethical approval

The care and handling of the animals during this study were conducted in accordance with the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, with the approval reference number: FVM-UNN-IACUC-2024-07/174.

Design

The forty (40) male Wistar albino rats were randomly divided into five groups, each comprising eight rats. Group 1 rats were neither irradiated nor treated, serving as the control group. Group 2 rats were subjected to irradiation without treatment (irradiation control). Groups 3, 4, and 5 were irradiated and received 10 ml/kg body weight of Astymin-SN®, and 100 and 200 mg/kg body weight of vitamin A, respectively.

Drug administration

Ten milliliters (10 mL) per kg body weight of Astymin-SN® was given subcutaneously to group 3 rats 24 hours prior to irradiation, and this treatment continued daily for seven consecutive days. In groups 4 and 5, rats were administered 100 and 200 mg/kg body weight of vitamin A orally 24 hours before irradiation, with dosing continuing daily for seven consecutive days, respectively.

Radiation source

A modern, high-frequency portable Veterinary X-ray machine (model: Hi-Ray Plus; manufacturer: PLH Medical Ltd, UK) equipped with a powerful HF inverter and a maximum tube voltage of 100 kV, featuring inherent aluminum tube filtration of 2.5 mm-Al, was utilized for whole-body irradiation of the experimental animals. For groups 3, 4, and 5, pre-irradiation treatment commenced 24 hours before the exposure. After a single irradiation event, post-irradiation treatment was initiated and continued daily for 7 days. The subsequent irradiations occurred every other day for a total of 7 days. The irradiated groups were exposed using exposure factors of 70 kV and 20 mAs. The radiation dosage (output) per exposure was calculated through the mathematical model for high-frequency x-ray machines as delineated by Ngankem *et al* [10].

$$\text{Output } (\mu\text{GY}) = 0.0095(\text{kV})^{2.18} / T^{0.8} \text{ mAs}$$

kV is the tube voltage; mAs is the product of tube current and exposure time and T is the inherent aluminum tube filtration, which is equivalent to 2.5 mm-Al.

Sample collection

All the rats from each group were sacrificed 24 hours after the last irradiation using chloroform. Liver, kidney and testis tissues were harvested, cleaned and washed twice with ice-cold normal saline, homogenized in chilled phosphate-buffered saline (PBS) and centrifuged at 3000 rpm for 15 minutes. The supernatants were obtained and stored at 20°C for biochemical analysis. Tissue homogenates of the organs were used to assess the levels of malondialdehyde (MDA) and the activities of the endogenous antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Assessment of oxidative stress biomarkers in tissues

SOD was determined according to Sun et al. based on inhibition of nitroblue tetrazolium reduction [11]. Catalase was measured by recording the decrease in absorbance due to decomposition of hydrogen peroxide to water [12]. The MDA concentration of tissue homogenates expressed as the thiobarbituric acid reactive substances (TBARS) was assessed spectrophotometrically as described by Placer et al [13]. GPx activity was assessed according to the method of Lawrance and Burk, recording the decrease of NADPH at 340 nm [14].

Statistical analysis

The obtained data were analyzed statistically with a statistical package for the social sciences (SPSS) version 22.0 (SPSS Inc., 111 USA, 2003). Data obtained were summarized as mean $\pm$ SD. The mean data of the parameters were compared between groups using one-way analysis of variance (ANOVA) followed by

Duncan multiple range test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Based on the exposure factors used and the tube filtration of the x-ray machine, radiation dose (output) administered to all the irradiated groups was 961.14 μ Gy per exposure, 1,922.28 μ Gy per day (961.14 x 2), and 7,689.12 μ Gy per week (4 days of irradiation per week (1,922.28 x 4); irradiation was every other day for seven days).

Findings of this study showed that, accumulated radiation dose of 7,689.11 μ GY during the seven days of diagnostic x-ray irradiation resulted in significant elevation ($p < 0.05$) in the mean levels of MDA of liver, kidney and testicular tissues as seen in the irradiated and untreated group 2 rats compared to the unirradiated and untreated control group 1 rats (Tables 1 to 3). In contrast, significant ($p < 0.05$) decline in the mean values of the endogenous antioxidants, SOD, CAT, and GPX was observed in the irradiated and untreated group 2 rats compared to the unirradiated and untreated control rats.

Remarkably, the mean values of MDA, SOD, CAT, and GPX in the groups 3, 4, and 5 rats that were irradiated and treated with 10 ml/kg body weight of Astymin-SN[®], 100 and 200 mg/kg body weight of vitamin A, respectively, did not vary significantly from the unirradiated and untreated group 2 rats.

DISCUSSION

Radiation exposure induces injuries due to damage at the cellular and systemic levels. Radiation produces reactive oxygen species, which lead to lipid peroxidation, protein oxidation, and DNA damage [15]. Cellular and tissue resistance against ionizing radiation depends on many endogenous parameters, including antioxidant systems and their capacity for adaptive response [16].

Table 1: Post-irradiation oxidative stress markers of the liver tissue homogenates of male Wistar albino rats

Group	MDA (mg/g)	SOD (μ /mg)	CAT (U/g)	GPX (U/g)
1 (0.0 mGy) control	5.59 $\pm$ 0.32 ^a	9.19 $\pm$ 0.34 ^a	0.84 $\pm$ 0.06 ^a	12.38 $\pm$ 0.49 ^a
2 (7,689.11 μ Gy)	8.94 $\pm$ 0.37 ^b	6.23 $\pm$ 0.11 ^b	0.44 $\pm$ 0.03 ^b	7.97 $\pm$ 0.90 ^b
3 (7,689.11 μ Gy+10 mL Astymin-SN [®])	6.18 $\pm$ 0.49 ^a	8.22 $\pm$ 0.64 ^a	0.83 $\pm$ 0.07 ^a	12.51 $\pm$ 0.78 ^a
4 (7,689.11 μ Gy+100 mg/kg V.A)	5.95 $\pm$ 0.13 ^a	8.96 $\pm$ 0.38 ^a	0.81 $\pm$ 0.05 ^a	12.27 $\pm$ 0.60 ^a
5 (7,689.11 μ Gy+200 mg/kg VA)	6.04 $\pm$ 0.23 ^a	9.10 $\pm$ 0.24 ^a	0.84 $\pm$ 0.04 ^a	12.40 $\pm$ 0.67 ^a

The different superscripts ^a and ^b across the groups denote statistical significance ($P < 0.05$). Values are Mean $\pm$ SD; MDA – malondialdehyde; SOD - superoxide dismutase; GPx – glutathione peroxidase (GPx), and CAT - catalase

Table 2: Post-irradiation oxidative stress markers of the kidney tissue homogenates of male Wistar albino rats

Group	MDA (mg/g)	SOD (μ/mg)	CAT (U/g)	GPX (U/g)
1 (0.0 μGy control)	2.91±0.45 ^a	0.59±0.01	5.88±0.29 ^a	8.56±0.37 ^a
2 (7,689.11 μGy)	5.24±0.46 ^b	0.21±0.05	4.06±0.45 ^b	2.76±0.64 ^b
3 (7,689.11 μGy + 10 mL Astymin-SN®)	3.01±0.61 ^a	0.52±0.07	5.83±0.22 ^a	7.77±0.96 ^c
4 (7,689.11 μGy + 100 mg/kg V.A)	2.93±0.41 ^b	0.49±0.08	6.18±0.28 ^a	8.69±0.14 ^a
5 (7,689.11 μGy + 200 mg/kg V.A)	2.99±0.60 ^a	0.55±0.04	6.12±0.28	8.54±0.44 ^a

The different superscripts ^a and ^b across the groups denote statistical significance ($P < 0.05$). Values are Mean $\pm$ SD; MDA – malondialdehyde; SOD - superoxide dismutase; GPx – glutathione peroxidase (GPx), and CAT - catalase

Table 3: Post-irradiation oxidative stress markers of testicular tissue homogenates of male Wistar albino rats

Group	MDA (mg/g)	SOD (μ/mg)	CAT (U/g)	GPX (U/g)
1 (0.0 μGy control)	3.58±0.46 ^a	0.72±0.08 ^a	7.32±0.47 ^a	5.79±0.14 ^a
2 (7,689.11 μGy)	6.55±0.73 ^b	0.41±0.06 ^b	5.84±0.23 ^b	1.76±0.55 ^b
3 (7,689.11 μGy + 10 ml Astymin-SN®)	4.08±0.62 ^a	0.60±0.08 ^c	7.48±0.71 ^a	4.68±0.72 ^c
4 (7,689.11 μGy + 100 mg/kg V.A)	3.58±0.44 ^a	0.76±0.05 ^{ac}	7.06±0.53 ^a	5.18±0.86 ^{ac}
5 (7,689.11 μGy + 200 mg/kg V.A)	4.18±0.38	0.72±0.03 ^{ac}	7.68±0.37 ^a	5.18±0.86 ^{ac}

The different superscripts ^a and ^b across the groups denote statistical significance ($P < 0.05$). Values are Mean $\pm$ SD; MDA – malondialdehyde; SOD - superoxide dismutase; GPx – glutathione peroxidase (GPx), and CAT - catalase

Some studies have revealed that irradiation induces oxidative damage by causing an increase in MDA levels and a decrease in the levels of the antioxidants such as SOD, GSH, and GPX, depending on time and dosage [17]. In this study, MDA levels in the liver, kidney, and testis were significantly elevated, whereas a significant decline in the mean values of SOD, CAT, and GPX activities was observed in the irradiated and untreated rats. The reduction in the antioxidant enzymes is associated with the depletion of enzymes during oxidative stress that occurred as a result of irradiation [18]. As evident in the current study, whole body x-ray irradiation probably generates reactive oxygen species that induce lipid peroxidation, leading to elevation in the mean levels of MDA. The observed high MDA levels in the liver, kidney and testicular tissue homogenates in the irradiated untreated group 2 rats may be indicative of oxidant damage to the mitochondria of the cells and cellular membranes, which may in turn lead to radiation-induced apoptosis [19]. Similarly, Liren et al observed increased MDA levels in cardiac tissue of mice exposed to gamma rays [20]. Findings of our study corroborate with the results of Osman and Abd El, who assessed a variety of tissues such as brain, liver, spleen, kidney, and testis after exposing rats to whole body gamma radiation of 4Gy and 6Gy and found increased lipid peroxidation in all the tissues [21].

However, the observed non-significant difference in the mean values of MDA in the irradiated and treated groups 3, 4, and 5 rats in comparison with the unirradiated untreated control group 1

indicates possible radioprotective effects of Astymin-SN® and vitamin A. This could imply that the two test drugs protected against radiation-induced lipid peroxidation biochemical event.

A GPX has a versatile role as an antioxidant and executes its radio protective function through free radical scavenging, restoration of the damaged molecules by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state [22]. Superoxide dismutase is the most important enzyme that protects from radiation [16]. In the present study, the mean levels of SOD, CAT, and GPx in the tissue homogenates of the liver, kidney and testis of the irradiated untreated rats declined significantly compared to the unirradiated untreated control. The observed significant fall in the mean values of the endogenous antioxidant enzymes in the current study could be attributed to their utilization to mitigate oxidant damage induced by X-ray radiation. The marked reduction in the enzymes could be associated with excess reactive oxygen species production, which caused their denaturation and partial inactivation [23]. Similarly, Heba et al. observed that gamma radiation exposure resulted in a decrease in SOD and GSH content and an increase in MDA content in the body tissues of irradiated rats compared to control [24]. In order to protect the body against radiation-induced oxidative stress, exogenous antioxidants may be used [25]. Protective effects of Astymin-SN® and vitamin A against X-ray radiation-induced oxidative stress were evaluated in the study. Remarkably, in the current study, the mean levels of MDA, SOD,

CAT, and GPx in the tissue homogenates of the liver, kidney and testis of rats irradiated and treated with Astymin-SN® and vitamin A were similar to the mean values of the unirradiated and untreated control group. This observed non-significant variation in the assessed parameters in the irradiated and treated rats compared to the unirradiated and untreated control group denotes possible radioprotective potential of the vitamin A and Astymin-SN against x-ray radiation-induced oxidative stress. Therefore, findings of the current study support growing evidence that vitamin A and Astymin-SN® could play a role in protecting the body against oxidative stress damage.

CONCLUSION

A portable high-frequency diagnostic X-ray machine has been observed to induce a significant increase in oxidative stress, as indicated by increased lipid peroxidation (MDA) and decreased endogenous antioxidant enzymes' activities, as evident in the irradiated and untreated rats. The observed non-significant difference in the assessed parameters in the treated groups, though exposed to radiation, is proof of possible ameliorative potential of Astymin-SN® and vitamin A against x-ray radiation-induced oxidative stress.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Ogbanya Kenneth: conceptualization and design of the study, Nnaji Theophilus, Mgbe Emeka and Chukwu Jude: collection of data and data analysis, Ogbanya Kenneth: drafting of the manuscript. All authors revised and approved the manuscript.

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