Mitigating effects of methanolic leaf extract of *Duranta erecta* against X-ray induced oxidative stress in Wistar albino rats

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Carcinogenic effects and other harmful effects associated with low dose-rate and low dose radiation have recently been reported. Therefore, it is important to identify effective and safe radioprotectors or radiation mitigators that can counter the effects of ionizing radiation damage. Therefore, the aim of the study was to evaluate the mitigating potential of methanolic leaves extract of *Duranta erecta* (DE) against acute whole-body X-ray irradiation-induced oxidative stress in Wistar albino rats. Rats in groups B, C and D, were exposed to double X-rays radiation at a source to skin distance (SSD) of 70 cm to deliver a total radiation dose of 18.4mGy at the rate of 9.2mGy/exposure using 100kVp and 30mAs exposure factors and quality factor of the X-ray machine (P) of 15. Rats in group A (control) were neither irradiated nor administered with DE extract. In contrast, group B rats were irradiated but not treated. DE extract at doses of 500 and 1000 mg/kg body weight was administered to groups C and D rats for 15 consecutive days. On post-irradiation days 1, 7, 14 and 21, the antioxidant status of the rats was evaluated and compared with control. Parameters assessed were catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA). Mean serum levels of GSH, SOD, CAT and MDA in the irradiated and treated groups did not vary significantly (P > 0.05) from the un-irradiated control group. In conclusion, methanolic extract of DE mitigated against radiation-induced decline in serum GSH, SOD, CAT, and radiation-induced MDA elevation. Results obtained indicated that methanolic extract of DE can be used potentially as a natural radiation mitigating agent.

**Keywords:** Free radicals, Ionizing radiation, Oxidative stress, Plant extract, Radiobiology

**Introduction**

X-rays are extensively used for both diagnostic and therapeutic purposes (Semiha *et al*., 2009). Exposure to ionizing radiation produces oxygen-derived free radicals termed reactive oxygen species in the tissue, which include hydroxyl radical (OH−) and superoxide radical anion (O2−), as well as other oxidants such as hydrogen peroxide (H2O2) (Adler *et al*., 1999). These reactive free radicals are considered one of the most important causes of radiation-induced carcinogenesis (Onoda & Inano, 1998). They react with body tissues and generate lipid peroxidation, DNA lesions and enzyme inactivation, which are mediators of radiation damage (Semiha *et al*., 2009). Detrimental biological effects of ionizing radiation are partly mediated by...
free radicals generated through the decomposition of cellular water (Wambi et al., 2008). However, organisms have protective systems against free radical reactions, for example, endogenous antioxidants and antioxidative enzymes (Guney et al., 2005). High dose radiations are known to induce deleterious consequences, including cancers. Recently, a concern on the carcinogenic effects of low dose radiation especially in occupational exposure and medical exposure has been increasing (Takai et al., 2021). Total body exposure to ionizing X-ray radiation has been found to exert many alterations in biological systems resulting in oxidative stress imbalance (Azab et al., 2004; Cherdyntseva et al., 2005). With respect to radiation damage to both humans and animals, it is important to protect or mitigate against the adverse effects induced by ionizing radiation (Verma et al., 2011). Radioprotectors are agents administered before exposure to prevent radiation-induced cellular and molecular damage (Stone et al., 2004). Radiation mitigators are drugs administered shortly after radiation exposure that accelerates recovery or repair of injury caused by radiation (Singh et al., 2013). Many synthetic antioxidant agents have been developed to alleviate radiation-induced oxidative stress but have major drawbacks such as high cost, lack of availability, and potential for health risk (Patro et al., 2016). Hence, the search for alternative sources, including bioactive principles of plant origin (Kitts et al., 2000). In an attempt to find potent natural antioxidants, some herbal medicines have recently gained recognition as biological response modifiers (Jagetia & Baliga, 2002). In particular, the use of herbs for their potential as possible modifiers of the radiation response is receiving considerable attention (Ben-Hur & Fulder, 1981; Zhang et al., 1987). Duranta erecta (DE) is a species of flowering shrub in the verbena family of Verbenaceae and it is a native plant of Asia, Africa, and South and Central America. The Common names include golden dewdrop, pigeon berry, and skyflower (Pereira et al., 2006; Aymard & Grande, 2012). Duranta erecta stem and leaves are used in traditional folk medicine for the treatment of malaria, intestinal worms and abscess (Aymard & Grande, 2012) Antitumor, antibacterial and antifungal activities and as well as the insecticidal potential of Duranta erecta have been reported (Bhar et al., 2016; Agwu et al., 2018). However, to the best of our knowledge, the mitigating potential of DE extract against radiation-induced oxidative stress has not been scientifically evaluated. This study aimed to evaluate the mitigating potential of methanolic leaf extract of Duranta erecta against whole-body X-ray induced oxidative stress in Wistar albino rats.

Materials and Methods

Plant material and extraction

Duranta erecta leaves were collected from the vicinity of University of Nigeria, Nsukka. The plant was sent to botanist of University of Nigeria, Nsukka for specific identification. An analytical grade methanol solvent which was purchased from Lavans Nigeria Limited was used for the extraction.

Preparation of methanolic extract

Duranta erecta leaves were washed thoroughly and oven-dried at 40°C for 5 days until the leaves became fully dried. The dried leaves were then ground to fine powder using stainless steel blender. The powder form of oven-dried DE was subjected to solid-liquid extraction by using 100% methanol solvent. Each 1g of the dried DE leaf sample was extracted by using 10 mL of solvent (methanol) with a solid to solvent ratio of 1:10 (w/v). The mixture of powdered DE and methanol was continuously swirled at 150 rpm in a shaker incubator for 2 hours at 37°C before being filtered using Whatman Filter Paper number 1. The residue was then re-extracted twice following the same procedure. The collected filtrates were subjected to a rotatory evaporator to remove the entire methanol. Finally, the crude extract was formed and was later concentrated to dryness in a hot-air oven at 40°C. The extract was then stored in a refrigerator at 4°C throughout the duration of the study.

Acute toxicity study

This was performed to determine the safe dose(s) to be used on the rats. Twenty (20) apparently healthy rats weighing 140 – 150g and assigned into four groups of 5 rats each were used for the toxicity test. The rats were housed 5 per plastic cage, and the photoperiod (light on from 06:00 to 18:00h), air changes and room temperature (24 ± 1°C) were controlled. All animals had free access to tap water and food, except for a short fasting period of 24 hours before oral administration of the DE extract. The DE extract was dissolved/suspended in distilled water and administered orally at doses of 400, 800, 1200 and 1600 mg/kg body weight. The general behaviour of rats was observed continuously for 1 hour after the treatment and then intermittently for 4 hours, and thereafter over a period of 24 hours (Twaij et al., 1983; Halliwell & Chirico, 1993). The rats were further
observed for up to 14 days following treatment for any signs of toxicity and death, and the latency of death. The methanol extract of leaves of *Duranta erecta* was found to be nontoxic up to the maximum dose of 1600 mg/kg body weight. Doses selected for in vivo antioxidant study were 500 and 1000 mg/kg body weight.

**Phytochemical Screening**
The crude extract of leaves of DE was examined for the presence of alkaloids, flavonoids, tannins, glycosides and saponins using the conventional method of Evans (1996).

**Animals care, handling and grouping**
Handling of the rats was carried out according to the legal requirement of the relevant local or national authority, and the study was approved by the Ethical Committee of the University of Nigeria, Nsukka. A total of forty (40) male Wistar albino rats, 7 to 9 weeks old and weighing 130-150g from an inbred colony, were used for the study. The rats were maintained under controlled conditions of temperature and light. They were provided standard rat feed (procured from Animal Care Consult Nig. Limited) and water ad libitum. Tetracycline water once a fortnight was given as a preventive measure against infections.
The 40 rats were assigned into four groups (A, B, C and D) of 10 rats each.

**Irradiation**
A portable X-ray machine (model; Hi portal 100 Hf) at the Department of Veterinary Surgery and Radiology, University of Nigeria, Nsukka was used. The rats in groups B, C and D were restrained in well-ventilated perspex boxes. The rats were exposed to acute double exposure to X-rays radiation at a source to skin distance (SSD) of 70 cm to deliver a total radiation dose of 18.4 mGy at the rate of 9.2 mGy/exposure using 100 kVp and 30 mAs exposure factors and quality factor of the X-ray machine (P) of 15.

Radiation dose from the diagnostic X-ray machine was indirectly measured by using exposure parameters such as the source to skin distance (SSD), the product of tube current and exposure time (m.A.s), the kilovoltage peak (kVp) and the quality factor of the X-ray machine (P) (Ayad et al., 1994; Ayad et al., 2001; Simol et al., 2021).

The radiation dose (mR) was determined using equation as described by Ayad et al. (1994) and Ayad et al. (2001): Radiation dose (mR) per exposure = P(kVp)²m.A.s/ R², where P, kVp, mAs and R² stand for: the quality factor of the X-ray machine (P), the kilovoltage peak (kVp), the product of tube current and exposure time (m.A.s) and source to skin distance respectively.

**Administration of crude leaf extract of Duranta erecta (DE)**
500 and 1000 mg/kg body weight of leave extract of DE was administered orally to groups C and D rats, respectively, immediately after irradiation (post-irradiation) and continued daily for a period of 15 consecutive days. Group A rats were not irradiated and not treated (control), while group B rats were only irradiated and but not treated.

**Blood/serum collection**
On 1, 7, 14 and 21 days post-irradiation, two millilitres (2 mL) of blood for serum preparations were collected from each of the rats in the four groups (A, B, C and D) from the retro-orbital plexus using a clean capillary tube into sample bottles. The collected blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes, before serum was harvested (Yesufu et al., 2010).

**Determination of malondialdehyde (MDA) production in serum**
The malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper & Hadley (1990). 50 µL of serum was deproteinized by adding 1mL of 14% trichloroacetic acid and 1mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56x105 mol/L/cm) using the formula, A=ΣCL, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in µmol/L.

**Determination of catalase activity in serum**
Catalase activity was determined according to the method described previously (Goth, 1991). This involves the combination of optimized enzymatic conditions and the spectrophotometric assay of hydrogen peroxide-based on the formation of its stable complex with ammonium molybdate. A volume of 0.2 mL of serum was incubated in a New Brunswick Scientific incubator in 1.0 mL substrate (65 µmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 60 sec. Serum catalase activity is linear up to 100 kU/L.
One-unit catalase decomposes one µmol of hydrogen peroxide/1 min under these conditions. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate (NH₄)₆ Mo₇O₂₄ ·4 H₂O), and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm using a spectrophotometer (Jenway 6305; Jenway, Essex, UK) against reagent blanks. Serum catalase activity was expressed in kU/l.

**Determination of superoxide dismutase (SOD) activity**

Superoxide dismutase activity was assayed according to the method of Fridovich (1990). In this method, the xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of the enzyme, providing 50% inhibition of NBT reduction. Results were expressed as U/ml.

**Measurement of reduced glutathione (GSH)**

Serum reduced glutathione was measured by the method of Moron et al. (1979). About 0.1 mL of serum was deproteinized by 3 mL of 5% TCA. After mixing, tubes were kept for 5 min at room temperature and then centrifuged. To 1 mL of supernatant 4 mL of 0.3M Na₂HPO₄ (pH: 8.0) and 0.5 mL of 0.6 mM DTNB was added. The contents were mixed by vortexing and the absorbance of yellow color produced was recorded within 10 min at 412 nm. The concentration of GSH from serum was calculated by the use of a standard curve of GSH. The values were expressed as µmol/L.

**Data analysis**

The parameter values were all expressed as the mean ± standard deviation. Significant differences among the groups were determined by one-way ANOVA using SPSS 12.0 software package programme. The results were considered significant at p < 0.05.

**Results**

The methanol extract of *Duranta erecta* leave gave a dark brown, semi-solid substance that was readily soluble in de-ionized water. Acute toxicity investigation revealed no death or any signs of toxicity (Table 1) after oral administration of single dose of the methanolic extract at any dose level up to the maximum dose tested (1600 mg/kg b.d wt), which was the no-observed-adverse-effect level (NOAEL).

Phytochemical screening of methanolic extract of *Duranta erecta* revealed the presence of alkaloid, flavonoid, glycosides, saponins, steroids and tannins. The results of the *in vivo* antioxidative effects of *Duranta erecta* showed that activities of superoxide dismutase (Table 2), catalase (Table 3) and glutathion...
(Table 4) did not differ significantly in the irradiated and treated groups compared to the control at post irradiation days. However, a significant decrease ($P < 0.05$) in the means of serum SOD, CAT and GSH in the irradiated and untreated group (group B) compared to the treated and the control groups was observed. *Duranta erecta* extract also showed a dose dependent increase in the serum levels of SOD and GSH activities. The means of serum SOD and GSH of the group D rats treated with 1000 mg/kg body weight of DE extract were slightly higher compared with group C rats treated with 500 mg/kg and the control group, though the difference was not significant ($P > 0.05$). Similarly, at post irradiation days 1, 7, 14 and 21, the mean serum MDA (Table 5) in the irradiated and treated groups (C and D) did not differ significantly ($P > 0.05$) from the control (group A). A significant increase ($P < 0.05$) in the mean serum level of MDA of group B rats that were irradiated and not treated compared to the control group and the irradiated and treated group was observed.

**Table 3**: The mean ± standard deviation of CAT activity (Ku/l) at post irradiation days

<table>
<thead>
<tr>
<th>Group</th>
<th>Post irradiation days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 (24hurs)</td>
</tr>
<tr>
<td>Group A</td>
<td>54.46 ± 5.18a</td>
</tr>
<tr>
<td>Group B</td>
<td>46.14 ± 3.36b</td>
</tr>
<tr>
<td>Group C</td>
<td>52.88 ± 3.52a</td>
</tr>
<tr>
<td>Group D</td>
<td>53.02 ± 2.72a</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts are significantly different ($p < 0.05$). Group A (control) = no irradiation and no treatment. Group B = irradiation only. Group C = irradiated & treated with 500mg/kg of *Duranta erecta* extract. Group D = irradiated & treated with 1000mg/kg of *Duranta erecta* extract

**Table 4**: Mean ± standard deviation of GSH level ($\mu$mol/mL) in irradiated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Days post irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 (24hrs)</td>
</tr>
<tr>
<td>Group A</td>
<td>77.41 ± 6.22a</td>
</tr>
<tr>
<td>Group B</td>
<td>60.28 ± 2.14b</td>
</tr>
<tr>
<td>Group C</td>
<td>78.04 ± 8.24a</td>
</tr>
<tr>
<td>Group D</td>
<td>80.11 ± 3.96a</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts are significantly different ($p < 0.05$). Group A (control) = no irradiation and no treatment. Group B = irradiation only. Group C = irradiated and treated with 500mg/kg of *Duranta erecta* extract. Group D = irradiated and treated with 1000mg/kg of *Duranta erecta* extract

**Table 5**: Mean ± standard deviation of MDA level ($\mu$mol/L) in rats at post irradiation days

<table>
<thead>
<tr>
<th>Group</th>
<th>Days post irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 (24hrs)</td>
</tr>
<tr>
<td>Group A</td>
<td>16.27 ± 2.02a</td>
</tr>
<tr>
<td>Group B</td>
<td>25.35 ± 6.20b</td>
</tr>
<tr>
<td>Group C</td>
<td>15.11 ± 4.6a</td>
</tr>
<tr>
<td>Group D</td>
<td>15.86 ± 6.22a</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts are significantly different ($p < 0.05$). Group A (control) = no irradiation and no treatment. Group B = irradiation only. Group C = irradiated and treated with 500mg/kg of *Duranta erecta* extract. Group D = irradiated and treated with 1000mg/kg of *Duranta erecta* extract

**Discussion**

Reactive oxygen species (ROS) or free radicals generated by radiation are well known to induce inflammation and tumorigenesis in target tissues (Ibuki & Goto, 1997; Agarwal & Prabakaran, 2005). Ionizing radiation causes a pro-oxidant state as a result of the intracellular generation of reactive
oxygen species (Agarwal & Prabakaran, 2005). From our findings, X-rays radiation induced a significant increase in serum MDA in group B rats that were irradiated and not treated compared to the control and the treated groups. A significant increase in MDA level was observed only at days 1 and 7 post irradiation indicating radiation induced lipid peroxidation in the rats (Halliwell & Chirico, 1993). Impressively, methanolic leaf extract of Duranta erecta mitigated against radiation induced lipid peroxidation as evidenced in the non-significant variation between the treated groups and the control. Furthermore, at post irradiation days 1 and 7, a significant decline (P < 0.05) in serum GSH, SOD and CAT was only observed in group B rats that were irradiated and not treated compared to the irradiated and treated groups (group C and D) and the control group. Remarkably, methanolic leaf extract of Duranta erecta protected against radiation induced decline in serum GSH, SOD and CAT in the irradiated and treated groups of rats. The findings of this study were similar with the previous works of Uma & Ganasoundari (1995) and Ganasoundari et al. (1997), who reported that Osimum sanctum protects against radiation-induced lipid peroxidation and reduction in glutathione concentration in rats. Results of this study were also related to the previous works of Krishna & Kumar (2005), who reported that daily oral administration of 800mg/kg body weight of Raigira (Amaranthus paniculatus) leaf extract for 15 consecutive days before whole body exposure to γ-radiation was able to arrest radiation induced lipid peroxidation and the decline in reduced glutathione in the liver and blood of mice. Similarly, extract of ginger has also been reported to increase glutathione, reduce lipid peroxidation in vivo and scavenging of various free radicals in vitro (Jagetia et al., 2003; Jagetia et al., 2004). Findings of the current research are further related with the results of Sunila & Kuttan (2005), who reported that ethanolic extract of Piper longum (pippali) fruits protected mice against the radiation-induced decline in GSH. Our findings are also related to the work of Kumar & Kuttan (2004) which reported that Phyllanthus amarus extract protects against radiation-induced decline in superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase, and glutathione reductase. The observed mitigating effects of methanolic leaf extract of Duranta erecta in this study may be mediated through several mechanisms, since it consisted of complex mixtures of many chemicals as seen in the phytochemical screening. Some of the phytochemical constituents of Duranta erecta extract may be responsible for the antioxidative activity as demonstrated in our study. Flavonoid’s ability for scavenging of radiation induced free radicals and elevation of cellular antioxidants in the systems of the irradiated rats may be a leading mechanism for the mitigating effects observed in this study (Jagetia, 2007). The polyphenols (alkaloids) present in the Duranta erecta leaf extract may have upregulated mRNAs of antioxidant enzymes such as catalase, glutathione transferase, glutathione peroxidase, superoxide dismutase and thus may counteract the oxidative stress-induced by ionizing radiations (Jagetia, 2007).

In conclusion, methanolic leaf extract of Duranta erecta mitigated against X-rays radiation induced oxidative stress in Wistar albino rats. Results obtained indicated that methanolic leaf extract of DE can be used potentially as a ready accessible and valuable bioactive source of natural radiation mitigator.

Conflict of Interest
The authors declare that there is no conflict of interest.

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