JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 14, ISSUE 2: 677 – 688 (2024)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Effects of sub-acute oral administration of methanol ripe fruit extract of *Duranta erecta* on haematology, serum enzymes and oxidative stress markers of albino rats

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Abstract

Medicinal products should be evaluated for safety before use. Previous studies have demonstrated the anti-hyperglycemic and anti-dyslipidemic effects of methanol ripe fruit extract of *Duranta erecta* in albino rats. This study investigated the effects of sub-acute oral administration of methanol ripe fruit extract of *Duranta erecta* (MRFEDE) on the haematology, activity/levels of some serum enzymes and oxidative stress markers in albino rats. Twenty male albino rats were randomly assigned to four groups (1 – 4) of five rats each. Rats in group 1 served as the control and were given distilled water at the dose of 10 ml/kg body weight. Rats in groups 2 – 4 were given MRFEDE at the dose of 6.25, 12.5 and 25 mg/kg body weight, respectively. All treatments were given orally, once daily for 28 days. Blood samples for the evaluation of serum activity/levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase, malondialdehyde (MDA) and haematology were collected and assayed on days 0, 14 and 28. *In vitro* antioxidant potential of MRFEDE was also assayed using the diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. Administration of the extract caused significant ($p < 0.05$) dose-dependent decrease in serum ALT, AST and ALP activity. Rats in some of the treated groups (especially Group 3) also had significantly higher ($p < 0.05$) serum activity of SOD and catalase, and significantly lower ($p < 0.05$) serum levels of MDA. The extract did not have any significant effect on the haematological parameters assayed. The FRAP and DPPH assays showed that MRFEDE has good antioxidant potentials. Our findings showed that sub-acute administration of MRFEDE in rats did not produce any toxic effect, and was anti-oxidative in activity.

Keywords: Sub-acute toxicity; *Duranta erecta;* Antioxidant activity; Haematology; Serum biochemistry; Medicinal plant.

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Article History: Initial manuscript submission received – March 16, 2023; Final revised form received – July 01, 2024; Accepted for publication – July 06, 2024; Published – July 12, 2024.

Introduction

The development and mass production of synthetic drugs have revolutionized health care delivery all over the world. However, a reasonable percentage of the population in both developing and developed countries still rely on traditional practitioners and herbal remedies for their primary health care (Benzie and Wachtel-Galor, 2011). In Nigeria, just like other West African countries, herbal remedies have continued to gain acceptability, because of their low cost, affordability, availability, acceptability, and perceived low toxicity (Parmer, 2005). During the past two decades, public interest in natural therapies has increased sharply in both developing and developed countries, with increase in usage of phyto-pharmaceuticals. In 2007, about 38% of adults and 12% of children in the United States of America used some form of traditional medicine (Barnes *et al*., 2008).

Despite the widespread acceptability and use of herbal medicines globally and their reported benefits, they are not absolutely safe (Ezekwesili-Ofili and Okaka, 2019). The chances of adverse effects become more apparent due to indiscriminate or non-regulated use and lack of standardization. Also, prolonged use as is the case in the treatment of some chronic diseases like diabetes mellitus could predispose to toxicities. These have been a source of concern of many international fora on medicinal plants research (Angell and Kassier 1998).

In a study of liver and kidney functions in medicinal plant users in South-East Nigeria, it was found that liver problems were the most prominent indicator of toxicity as a result of chronic use of medicinal plants (WHO, 2002). According to Ezekwesili-Ofili and Okaka (2019), the level of liver toxicity in herbal medicine users increased proportionally with the duration of usage of the medicines. This was evidenced by the increases in the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), with the serum activity of ALP in the patients being always highest followed by AST and then ALT (Ezekwesili-Ofili and Okaka, 2019). As with synthetic drugs, the quality, efficacy, and safety of medicinal plants must therefore be evaluated and assured.

Duranta erecta commonly called golden dewdrop or yellow bush is a perennial shrub that belongs to the family *Verbenaceae.* Its leaves are light green to yellow; the flowers are light blue or lavender, and its fruits are small, orange and globose, hanging in clusters (Huxley, 1998). In ethnomedicine, the fruits are used for treatment of malaria, abscesses and parasitism (Udobi *et al.,* 2018), while extracts of the whole plant is used for the treatment of asthma, bronchitis, fever, itches, infertility, pneumonia amongst other uses (Rahmatullah *et al*., 2011; Savithramma *et al.,* 2015). Various reported studies that used the extracts of *D. erecta* whole plant, fruits or plant parts have shown its anti-bacterial (Ogbuagu *et al*., 2015), anti-fungal (Sharma *et al.,* 2012), anti-urolithiasis (Agawane *et al*., 2019), gastroprotective (Aziz *et al.*, 2013), anti-parasitic (Ijaz *et al*., 2010), anti-cancer (Wagh and Butle, 2019), anti-viral (Abou-Setta *et al.*, 2007), and anti-hyperglycemic (anti-diabetic) (Eke and Okpara, 2021) activities.

There is an increased and widespread use of *D. erecta* fruit due to its potency in the management of various disease conditions but there is also a concurrent paucity of reports in available literature on the possible long-term toxicity of this plant. The anti-diabetic and anti-dyslipidemic effects of the ripe fruit extract of *D. erecta* was reported recently (Eke and Okpara, 2021). Diabetes mellitus is a chronic disease requiring long-term treatment. Thus, the long-term safety of any agent recommended for the treatment of diabetes should be established. The present study investigated the effects of sub-acute oral

administration of methanol ripe fruit extract of *D. erecta* on haematology and activities/levels of some serum enzymes and oxidative stress markers in albino rats, and also its *in vitro* anti-oxidant potentials.

Materials and Methods

Plant materials: Fresh ripe fruits of *Duranta erecta* were collected from the botanical garden of University of Nigeria, Nsukka (UNN) and identified by a botanist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Chemical, Reagents and Drugs: Methanol (Sigma Aldrich®, USA), aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase test kits (Dialab, Wiener Neudorf, Austria), catalase, superoxide dismutase, malondialdehyde test kits (Sigma St. Louis, MO, USA), Drabkin's reagents, red blood cell and white blood cell diluting fluids.

Animals: Twenty male albino rats weighing 120 – 150 g were procured for the study from the Department of Veterinary Medicine, University of Nigeria, Nsukka Laboratory Animal House. They were housed in clean stainless wire mesh cages and fed with rat chow and clean drinking water *ad libitum*. Adequate ventilation was provided and twelve-hour light and dark cycles were maintained throughout the experiment. They were acclimatized for two weeks before the commencement of the experiment. Animal care and welfare was as prescribed by the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Preparation of the plant extract: The fresh ripe fruits of *Duranta erecta* were collected, washed, dried under shade, pulverized and stored in an air tight container pending its use. Cold maceration method of extraction was employed as described by Sukhdev *et al.* (2008). Crude methanol extract was made by mixing 300 g of the dry pulverized fruit with 1.5 litre of 70% methanol and shaking the mixture regularly for 72 hours. The mixture was then filtered using Whatman No. 1 filter paper into pre-weighed beakers. The filtrate was concentrated and dried in a hot air oven at 37^{o}C. The extract, named methanol ripe fruit extract of *Duranta erecta* (MRFEDE), was recovered, weighed and stored at 4^{o}C.

Experimental design: The twenty male albino rats procured for the study were randomly assigned to four groups (1 – 4) of five rats each, and were treated as follows: Group 1 – Distilled water (10 ml/kg), Group 2 – 6.25 mg/kg of the extract; Group 3 – 12.5 mg/kg of the extract, Group 4 – 25 mg/kg of the extract. The doses chosen were based on the findings of Eke and Okpara (2021) which showed that 25 mg/kg of the extract had a more potent anti-hyperglycemic effect compared to other doses. The treatments were administered daily through a stomach tube for 28 days.

Blood samples were collected on days 0, 14 and 28 into EDTA sample bottles for haematology and also into plain sample bottles for serum biochemistry. The haematological parameters and serum chemistry parameters were evaluated using standard procedures and according to the kit manufacturer's instructions. Heamatological parameters were evaluated according to the method of Cheesbrough, (2009). Packed cell volume (PCV) was determined with a microhaematocrit centrifuge and reader (Hawksley, England). Haemoglobin (Hb) concentration was measured following the cyanomethaemoglobin method using a spectrophotometer (CHEM-5V3; Erba, Mannheim, Germany) read at 546 nm against the reagent blank and a standard solution of cyanmethaemoglobin. The white blood cell (WBC) counts were determined using the improved Neubauer haemocytometric method.

The serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined following the specified instructions provided by the manufacturers (Dialab, Wiener Neudorf, Austria). Absorbance for each assay was measured using a spectrophotometer (CHEM-5V3; Erba, Mannheim, Germany) at the specified wavelengths. The absorbance of each sample was read against the reagent blank, and the absolute concentration values were determined using the standards and calibrators for the respective assay kits.

Serum superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972). This involved the ability of SOD to inhibit the autoxidation of epinephrine to adrenochrome at pH 10.2. Serum catalase activity was determined according to the method of Goth (1991) which is based on the combination of optimized enzymatic conditions and the spectrophotometric assay of hydrogen peroxide, and the formation of its stable complex with ammonium molybdate. Serum concentration of malondialdehyde (MDA), an end product of lipid peroxidation, was determined according to the method of Stocks and Dormandy (1971); this was based on the formation of thiobarbituric acid reactive substances (TBARS). Thiobarbituric acid reacts with MDA to produce a stable chromogen that is quantified spectrophotometrically. The color intensity of the chromogen was measured at 532 nm, and was directly proportional to MDA content.

The free radical scavenging activity of the extract was evaluated *in vitro* using the DPPH assay procedure as described by Rao *et al.* (2009) which utilized ascorbic acid as standard. The DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution was prepared by dissolving 25 mg DPPH in 100 mL methanol and stored at 20 °C till further use. The plant extract and the standard were prepared at various concentrations: 25, 50, 100, 200 and 400 µg/mL in methanol. The prepared DPPH solution (150 µL) was added to 50 µL of various concentrations of plant extract and standard. The reaction mixture was mixed well and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm on a microplate reader. The blank was prepared using methanol. The assay was done in triplicates for each concentration of the extract and ascorbic acid. The free radical scavenging activity was calculated using the standard formula.

The ferric reducing antioxidant power (FRAP) assay was carried out as described by Benzie and Strain (1999). The FRAP reagent was prepared by mixing 25 mL of 300 mmol/L acetate buffer, 2.5 mL of 10 mmol/L of 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) solution, and 2.5 mL of 20 mmol/L $FeCl_3$ solution in a 10:1:1 ratio. The sample (10 µL) was mixed with 200 µL of FRAP reagent; the contents were mixed vigorously. Ferric tripyridyltriazine (FeIII-TPTZ) complex was reduced to ferrous tripyridyltriazine (Fe II-TPTZ) form in the presence of antioxidants and developed an intense blue color, with maximum absorption at 593 nm. Absorbance readings are taken after 0.5 seconds and every 15 second until 4 minutes. The change of absorbance was calculated and related to the change in absorbance of an Fe (II) standard solution. The procedure was repeated for the various concentrations of the extract and ascorbic acid: 25, 50, 100, 200 and 400 µg/mL. The assay was done in triplicates for each concentration of the extract and ascorbic acid. The change in absorbance was linearly proportional to the concentration of the antioxidant. One FRAP unit was arbitrarily defined as the reduction of 1 mol of Fe (III) to Fe (II) and expressed as µmol of Fe^{2+} equivalents/L of the extract.

Statistical Analysis: Data obtained from the study were analyzed with one way analysis of variance (ANOVA) using SPSS version 26 (IBM

Corp, Armonk, NY, USA), and variant means separated with Duncan's multiple range post-hoc. Probability (p) values less than 0.05 were considered statistically significant. Results generated were presented as mean ± standard error of mean (SEM).

Results

There were no significant variations ($p > 0.05$) in the mean Hb concentrations among the rat groups on all the days of assay (Day 0, 14 and 28) (Table 1). On days 0 and 28, there were no significant ($p > 0.05$) variations among the rat groups in their mean PCV, but on day 14, the mean PCV of the Group 2 rats was significantly lower ($p < 0.05$) than that of the Group 1 Control rats (Table 2). The TWBC counts of the rat groups did not significantly ($p > 0.05$) vary among the rat groups on days 0, 14 and 28 (Table 3).

Table 1. Mean haemoglobin concentration (g/dl ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control.

Groups and their Treatments	Day 0	Day 14	Day 28
Group 1 (10 ml/kg Distilled Water)	18.76 ± 0.71	11.06 ± 0.95	16.28 ± 0.76
Group 2 (Extract 6.25 mg/kg)	19.16 ± 0.56	11.09 ± 0.85	16.60 ± 0.99
Group 3 (Extract 12.5 mg/kg)	18.06 ± 0.36	10.95 ± 0.87	16.81 ± 0.67
Group 4 (Extract 25 mg/kg)	18.91 ± 0.74	12.34 ± 0.75	18.60 ± 0.77

Table 2. Mean packed cell volume (% ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control.

Groups and their Treatments	Day 0	Day 14	Day 28
Group 1 (10 ml/kg Distilled Water)	35.00 ± 0.82	35.75 ± 1.11	31.75 ± 1.44
Group 2 (Extract 6.25 mg/kg)	34.00 ± 0.41	29.75 ± 1.03 [a]	32.25 ± 0.48
Group 3 (Extract 12.5 mg/kg)	34.00 ± 1.00	33.00 ± 1.47	32.25 ± 0.49
Group 4 (Extract 25 mg/kg)	33.00 ± 1.08	32.50 ± 1.19	31.25 ± 0.85

'a' indicates significant ($p < 0.05$) difference when compared with the Group 1 (Control) in the column

Table 3. Mean total white blood cell counts ($10^3/\mu L$ ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control.

Groups and their Treatments	Day 0	Day 14	Day 28
Group 1 (10 ml/kg Distilled Water)	6.06 ± 3.75	5.98 ± 2.50	5.48 ± 3.23
Group 2 (Extract 6.25 mg/kg)	6.04 ± 2.36	5.55 ± 2.89	6.46 ± 2.39
Group 3 (Extract 12.5 mg/kg)	6.08 ± 2.50	6.03 ± 2.50	5.53 ± 2.29
Group 4 (Extract 25 mg/kg)	6.04 ± 2.39	5.56 ± 3.75	5.61 ± 4.27

There were no significant variations ($p > 0.05$) in the serum AST activity of all the rat groups on day 0, but on day 14, the serum AST activity of the Groups 2, 3 and 4 rats were significantly lower ($p < 0.05$) than that of the Group 1 rats (Figure 1). Further on day 14, the serum AST activity of the Groups 3 and 4 rats were also significantly ($p < 0.05$) lower than that of the Group 2 rats (Figure 1). On day 28, however, the serum AST activity of the Group 2 rats was significantly higher ($p < 0.05$) than those of all other groups, and that of Groups 3 and 4 were further significantly lower ($p < 0.05$) than that of the Group 1 (Figure 1).

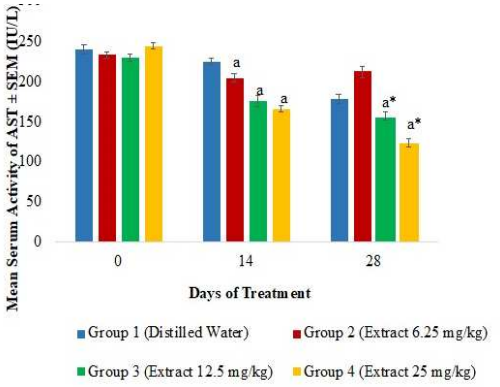

Figure 1. Mean serum aspartate aminotransferase (AST) activity (IU/L ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group mean significantly ($p < 0.05$) lower when compared with Group 1 (Control); * Indicates significant ($p < 0.05$) difference when the given graded doses of the extract were compared.

The mean serum ALT activity of all the rat groups did not significantly vary ($p > 0.05$) at baseline (day 0), but on day 14, the serum ALT activity of the Groups 2, 3 and 4 rats were significantly lower ($p < 0.05$) than that of the Control (Group 1) rats (Figure 2). The mean serum ALT activity of all the extract treated groups (Groups 2, 3 and 4) were also significantly lower ($p < 0.05$) than that of the Group 1 rats on day 28, with the mean serum ALT activity of the Group 4 rats being further significantly lower ($p < 0.05$) than that of the other two extract treated groups (Groups 2 and 3) (Figure 2).

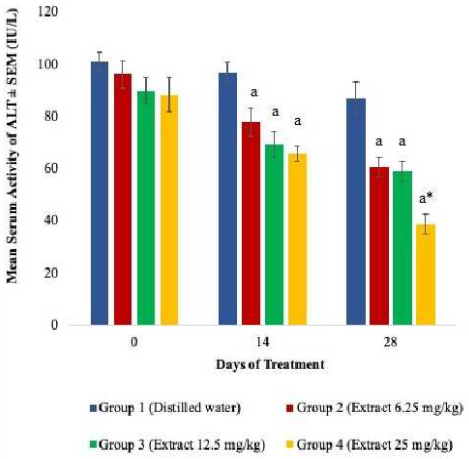

Figure 2. Mean serum alanine aminotransferase (ALT) activity (IU/L ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group means significantly ($p < 0.05$) lower when compared with Group 1 (Control); * Indicates significant ($p < 0.05$) difference when the graded doses of the extract were compared.

There were no significant variations among all the rat groups in their mean serum ALP activity on day 0, but on day 14, the serum ALP activity of the Groups 2, 3 and 4 rats were significantly lower ($p < 0.05$) than that of the Group 1 rats (Figure 3). Further on day 14, the mean serum ALP of Groups 3 and 4 rats were significantly lower ($p < 0.05$) than that of the Group 2 rats (Figure 3). On day 28, the mean serum ALP activity of all the extract treated groups (Groups 2, 3 and 4) were also significantly lower ($p < 0.05$) than that of the Group 1 rats, with the mean serum ALP activity of the Group 4 rats being further significantly lower ($p < 0.05$) than that of the other two extract-treated groups (Groups 2 and 3) (Figure 3).

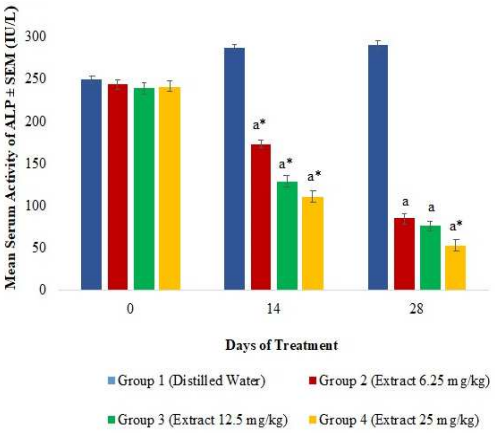

Figure 3. Mean serum alkaline phosphatase (ALP) activity (IU/L ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group means that are significantly ($p < 0.05$) lower when compared with Group 1 (Control); * Indicates significant ($p < 0.05$) difference when the graded doses of the extract were compared.

The mean levels of serum SOD did not significantly vary ($p > 0.05$) among all the rat groups on day 0, but on day 14, the mean serum SOD activity of rats in Groups 3 and 4 were significantly higher ($p < 0.05$) than those of the Control (Group 1) rats (Figure 4). Among the two (Group 3 and 4), still on day 14, the mean serum SOD levels of the Group 3 rats were significantly ($p < 0.05$) higher than that of the Group 4 rats (Figure 4). On day 28 however, the mean SOD levels of all the extract treated groups (Groups 2, 3 and 4) were significantly higher than that of the control (Group 1), and in addition the mean SOD levels of the Group 3 rats was still highest among all and was significantly higher than those of the two other extract-treated groups (Group 2 and 4) (Figure 4). The mean serum catalase activity of all the rat groups did not significantly vary ($p > 0.05$) on days 0 and 14, but on day 28, the serum catalase activity of the Group 3 rats was significantly higher ($p < 0.05$) than those of all other groups (Figure 5). On days 0 and 14 of the experiment, the mean MDA levels of all the rat groups did not significantly vary ($p > 0.05$), but on day 28, the serum MDA levels of the Group 3 and 4 rats were significantly lower ($p < 0.05$) than those of rats in Groups 1 and 2 (Figure 6).

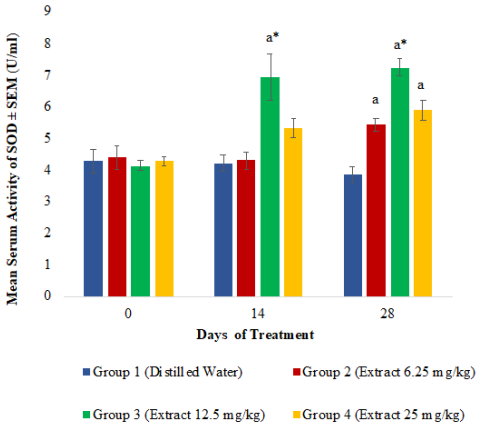

Figure 4. Mean serum superoxide dismutase (SOD) activity (U/ml ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group means that are significantly ($p < 0.05$) higher when compared with Group 1 (control); * Indicates significant ($p < 0.05$) difference when the graded doses of the extract were compared.

In the DPPH assay, at 25 µg/mL, *Duranta erecta* fruit extract exhibited an *in vitro* free radical scavenging activity that was about half that of ascorbic acid (Figure 7). However, at other concentrations (50 – 400 µg/ml), *D. erecta* extract and ascorbic acid had comparable mean percentage inhibition of DPPH radical, which was not significantly ($p < 0.05$) different from each other (Figure 7) Results of the FRAP assay showed that the *in vitro* ferric reducing antioxidant power of the MRFEDE at the various concentrations assayed were significantly lower ($p < 0.05$) than that of ascorbic acid at the same concentrations (Figure 8). Each increase in the concentration of the extract produced a concomitant increase in its *in vitro* ferric reducing antioxidant power (Figure 8).

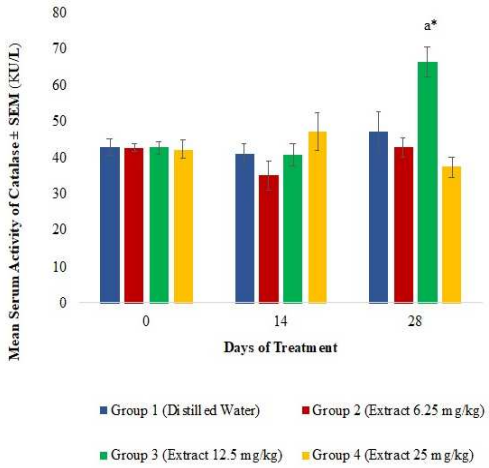

Figure 5. Mean serum catalase activity (KU/L ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group mean that is significantly ($p < 0.05$) higher when compared with Group 1 (control); * Indicates significant ($p < 0.05$) difference when the graded doses of the extract were compared.

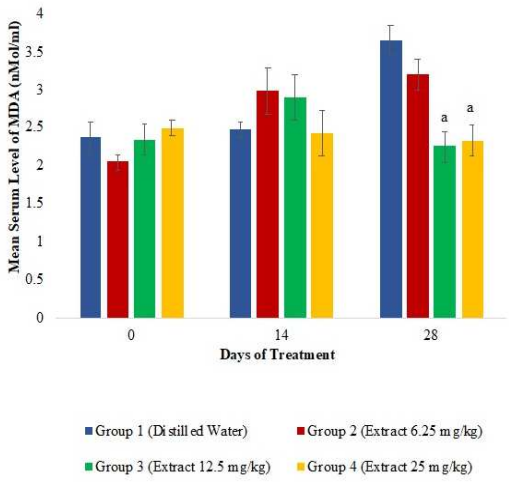

Figure 6. Mean serum malondialdehyde (MDA) levels (nMol/ml/ ± SEM) of rat groups treated for 28 days with methanol ripe fruit extract of *Duranta erecta (MRFEDE),* compared with untreated control. 'a' indicates group means that are significantly ($p < 0.05$) lower when compared with Group 1 (control).

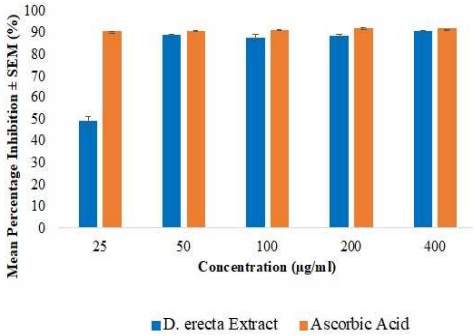

Figure 7: Comparison of the *in vitro* free radical scavenging activities of various concentrations of the methanol ripe fruit extract of *Duranta erecta* and with an ascorbic acid standard, based on the DPPH Assay.

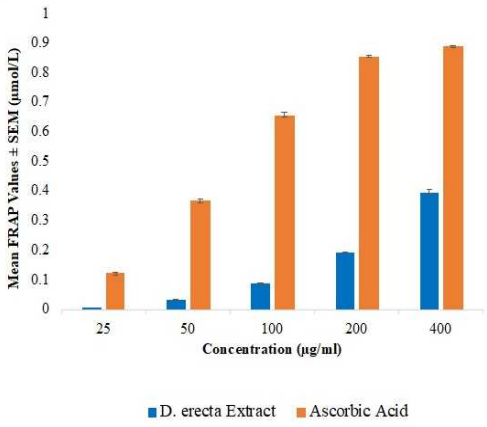

Figure 8: Comparison of the *in vitro* ferric reducing antioxidant power of various concentrations of the methanol ripe fruit extract of *Duranta erecta* with ascorbic acid standard based on the ferric reducing antioxidant power (FRAP) assay.

Discussion

Treatment with the extract did not significantly alter the haematological profile (Hb, PCV and TWBC) of treated rats. This might be an indication that the extract has no significant effect on haematopoietic organs and blood cells at the administered doses and duration of administration. It could also imply

that the extract did not suppress the immune system since it did not significantly alter the TWBC all through the period of the study. These haematological findings underscore the safety of the extract at the tested doses and duration of administration. However, treatment with the extract led to significantly lower serum activity of AST, ALT and ALP in the treated rats compared to the control. This decrease was dose-dependent, since the rat group treated with 25 mg/kg (high dose) of the extract had the lowest serum enzyme activity. It is thought that this decrease shows the ability of the extract to stabilize the cell membranes and thus protect the liver and other tissues from which these enzymes are elaborated. This protective activity may be attributed to the earlier reported presence of high levels of flavonoids in the ripe fruits of *D. erecta* (Eke and Okpara, 2021). These findings also suggest that the extract at the administered doses and duration of administration was not toxic to the organs and tissues of the body that elaborate these enzymes.

Superoxide dismutase is an important antioxidant defense in nearly all living cells exposed to oxygen (Tomusiak-Plebanek *et al.,* 2018). Catalase works hand in hand with SOD to protect the cell from oxidative damage by reactive oxygen species (ROS) (Chelikani *et al*., 2004). Results of the study showed that administration of the extract led to significant increase in SOD and catalase activities in extract treated rats. However, the rats which received the mid-dose (12.5 mg/kg) of the extract had the highest SOD and catalase activities compared to those of the other extract-treated rats. The fact that the mid dose of the extract (12.5 mg/kg) produced the highest SOD and catalase activities will suggest extract accumulation in the body of the rats over time, the ratio of the saturated receptor sites to the total receptor sites increased, leading to an increase in the effect produced by the same dose. This further buttresses the theory of receptor occupancy which states that drug effect is proportional to the number of receptors occupied by drug (Christopoulos and El-Fakahany, 1999). Maximal effect occurs when all receptors are occupied.

The higher activity of SOD and catalase recorded in the extract-treated rats may be due to the presence of phytochemicals especially phenolics and flavonoids which act as indirect antioxidants. These phytochemicals have been reported to act through the augmentation of cellular antioxidant capacity by enhancing specific genes encoding antioxidant proteins (Sahin *et al*., 2010; Jung and Kwak, 2010). Their physiological effects therefore last longer than those of direct antioxidants. Since the highest activities of catalase and SOD were recorded in the rats treated with 12.5 mg/kg of the MRFEDE, it follows that this indirect effect of the extract in increasing cellular antioxidant capacity was not dose-dependent. This is thought to be as a result of the fact that plant extracts are essentially a mixture of many compounds with complex interactions between one another and so it is not unusual to find compounds that promote a particular effect and other compounds that antagonize that effect in the same extract. The overall effect of the extract is ultimately determined by the compounds that produce a greater effect at the dose at which the extract is administered (Caesar and Cech, 2019). Since 12.5 mg/kg produced a more potent antioxidant activity, it may mean that at this dose, all the various components of the extract work synergistically to promote an antioxidant effect.

Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids (Davey *et al*., 2005). Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage induced by free radicals (Negre-Salvayre *et al.*, 2010). Therefore, higher MDA levels indicate a higher degree of lipid peroxidation. In this study, rats that received

the mid and high doses of the extract had significantly lower MDA levels on day 28. This may be acting in concert with the activities of catalase and SOD as observed in the same groups of rats. Hence, a decrease in oxidative damage and lipid peroxidation in these rat groups led to low MDA levels.

The DPPH assay on the extract showed high free radical scavenging activity comparable to ascorbic acid. However, the FRAP protocol showed a weak activity compared to ascorbic acid which is a standard antioxidant. The reason for the disparity between the antioxidant activity of MRFEDE using the DPPH and FRAP protocols may be because the extract is rich in flavonoids which are protic compounds that exert their antioxidant activity by forming complexes with free radicals and reactive metals through electron transfer (Kasprzak *et al*., 2015). The extract therefore may have showed good radical scavenging activity by readily forming complexes with the DPPH free radical but was unable to readily reduce TPTZ-Fe^{3+} complex to TPTZ-Fe^{2+} using the FRAP protocol, hence, the weak ferric reducing antioxidant power observed in the FRAP protocol.

Considering both the *in vitro* antioxidant and *in vivo* antioxidant activities of the extract, it is evident that MRFEDE possesses antioxidant activity, with strong *in vitro* and *in vivo* antioxidative effects.

Conclusion: This study demonstrated that the methanol ripe fruit extract of *Duranta erecta* is not sub-acutely toxic at the tested doses and duration of administration. The study also showed that the extract has both *in vitro* and *in vivo* direct and indirect antioxidant activity.

Conflict of interest

The authors declare no conflict of interests.

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