Physiological Effects of Ethanolic Leaf Extract of *Duranta erecta* (L) on Albino Rat, *Rattus norvegicus*

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Gregory Ejikeme Odo^{1,*}, Juliana Ekenma Agwu¹, Nkechi Nweze², Clara Ikegbunam², Felicia Ekeh N¹, Vincent Ejere¹, Ngozi Ezenwaji¹, Reginald Njokuocha², Godwin Ngwu¹ and Fabian Okafor¹

¹Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu, Nigeria

²Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu, Nigeria

ABSTRACT

This study evaluated the physiological effects of ethanolic leaf extracts of Duranta erecta (L) in 48 albino wistar rats, Rattus norvegicus using biochemical and haematological indices of toxicity. The data were subjected to two-way analysis of variance (ANOVA) with Duncan's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. Group A served as control in which no leaf extract was administered while groups B, C and D were respectively administered with 200, 300 and 400 mg/kg body weight of the ethanolic leaf extract of D, erecta by gastric intubation for 28 days. The flavonoid contents were higher in relative high abundance (+++) more than other constituents such as the cardiac glycosides, steroids, saponins, and tannins are moderate in abundance (other constituents such as the cardiac glycosides, steroids, saponins, and tannins that were moderate in abundance (++). There was a dose duration dependent (p < 0.05) decrease in RBC in all the groups. There were also duration-dependent decrease in MCV values obtained in this research as against duration and dose dependent increases in MCH, and MCHC. Aspartate amino transferase, and alkaline phosphatase were significantly (p < 0.05) reduced in the groups treated with 200 and 400 mg/kg body weight of the extract. The reduced significant activity of these markers suggest that the ethanolic leaf extract of D. erecta offered some protections against diseases related to the muscles. The significant reduction of AST level has further eliminated any leakage of the enzyme in the liver due to hepatic injury in the treated rats. Previous biochemical studies have also confirmed the non toxicity of the plant extract of D. erecta to the kidney.

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Authors' Contribution
GEO, FNE and JEA conceived and
designed the project. NN, RN and CI
designed the project. VE and NE analysed the results.
GN and FO wrote the manuscript.

Key words Physiological effects, Rattus norvegicus, Duranta erecta, Haematology.

INTRODUCTION

Majority of Nigerians in the rural areas at some stages in their lives turn to traditional healthcare as a result of accessibility, availability, affordability and inherent trust on their efficacy (Inove, 2014). The acceptance of herbal remedies is gradually increasing worldwide (Inove, 2014). There is also growing concern about the haematological, histopathological and biochemical adverse effects of several hundreds of dietary herbs/vegetables which have remained either uninvestigated or poorly investigated and are increasingly used by patients with different diseases (Peyton et al., 2013). In contradiction to its usefulness, some herbal remedies used in treatment of some diseases (liver diseases inclusive) might as well induce some toxic effects on the patient (Chitturi and Farrell, 2014). The limitations of these herbal drugs revolve around

* Corresponding author: gregory.odo@unn.edu.ng 0030-9923/2020/0001-0001 \$ 9.00/0

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lack of documentation and quality control, dosage, and the common tendency to describe diseases and ailments vaguely (Okogun, 2013). The toxic effect caused by a drug is similar in man and some other animals, a premise for use of animal models in toxicological studies (Okogun, 2013).

Duranta erecta Linn. (Syn. Duranta plumier Jacq., repens v Linn. And Eng: Golden dewdrop) commonly known as pigeon berry and locally called "Katamehedi" belongs to the family Verbenaceae. The plant is reported to produce a wide range of steroids (Ahmed et al., 1998), flavonoids (Anis et al., 2001, 2002), glycosides (Takeda et al., 1995), steroidal glycosides (Hiradata et al., 1999) and terpenoids (Ahmed et al., 1998; Makboul and Abdul, 1981). Recently, Abou-Setta et al. (2007) reported six known compounds in addition to naringrnin, sucrose and raffinose isolated for the first time from this species.

It is widely cultivated as an ornamental plant in tropical and subtropical gardens throughout the world with some common names as; golden dewdrop, pigeon berry sky flower, Brazilian sky flower, yellow bush *etc. Duranta erecta* is native to southern USA (Texas and Southern

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Florida), Mexico, Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) the Caribbean and South America (Surinam, Venezuela, Brazil, Bolivia, Colombia, Ecuador, Peru, Argentina and Paraguay). It is widely naturalized in the coastal districts of eastern Australia, south-eastern, central and northern Queensland and in the coastal districts of northern New South Wales). Duranta erecta which is generally called 'yellow bush' is wide spread in Nigeria. It is a sprawling, sometimes Vine like tender evergreen shrub or small tree that can be up to 5.5m (18 ft) tall and can spread to an equal width (Nebedum, 2013). Mature specimens possess auxiliary thorns, which are often absent on younger specimens. The leaves are light green, elliptic toovate, opposite, and grow up to 7.5cm long and 3.5cm broad, with a 1.5cm petiole. It usually forms a multi stemmed clump with branches that drop and trail. The individual flowers are tubular with five petals, light blue to violet or purple and flare out at the mouth about 0.5 in (1.3cm) across (Nebedum, 2013).

The fruit is a spherical yellow drupe about 0.5 in (1.3cm) in diameter borne in showy hanging branches. The leaves and berries of *D. erecta* are toxic, and are confirmed to have killed children, dogs and cats (Nebedum, 2013). Reports have shown that *D. erecta* leaf extracts have powerful biological effects which include therapeutic effects, hypoglycemic effects, anticancer, antimicrobial, hepatoprotective and antispasmodic activities. The plant is not browsed by cattle, however birds feed on the fruits without difficulty (Whistler, 2013). The fruits are used in the treatment of malaria and intestinal worms (Whistler, 2013). The leaves are used in the treatment of abscess (Nebedum, 2013).

With regards to the perception of rural dwellers generally, herbal remedies are considered safe and more effective, therefore consumers view them as natural alternatives to modern medicine (Chitturi and Farrell, 2014). Presently, Nigerians are not (grossly or holistically) conversant with the physiological effects of *Duranta erecta* leaf extracts, hence the current research work to the physiological effects of *D. erecta* leaf extracts on albino rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Collection of plant materials

The *D. erecta* leaves were collected from International Centre for Ethno-medicine and Drug Preparation in Nsukka, Enugu State, Nigeria. The leaves were identified and authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. A specimen of the leaves was deposited

in the herbarium, of Department of Plant Science and Biotechnology with the voucher and protocol numbers of UNN-EGACC, 0330/2013, respectively.

Extraction/Preparation of leaf extract

The leaves of *D. erecta* were sorted out to eliminate all extraneous materials and air dried in the laboratory for two weeks after which they were blended into fine powder. Four hundred and thirty five grams (435g) of the powder were extracted with 95% ethanol for 18h at 85°C and concentrated in a rotary evaporator (Buchi 461, Switzerland) at 50°C to get 78 g (17.9% yield) of the crude extract (Adebayo et al., 2006). They were latter grounded into smooth powder and stored in an airtight container. Four hundred and thirty-five grams (435g) of the powdered leaves were brewed in 1000 ml of distilled water and allowed to stand for 12 h and stirred at intervals. Then the decoction was filtered with Whatman filter paper No. 2. The filtrate was dried by heating in a water bath at a regulated temperature of 40°C to give a solid pastelike extract which was finally stored in a clean bottle to be administered to the rats. The stock solution was prepared daily before administration. The aqueous extraction was carried out according to the protocols described by Ekenyong *et al.* (2012).

Phytochemical analysis

The extracts of *D. erecta* leaves were subjected to qualitative analysis for the various phytochemical tests such as alkaloids, phytosterols, flavonoids (using ammonia and aluminium chloride test); resins (using precipitation and colour test; saponins (using frothing, emulsion and haemolysis tests); tannins (using ferric chloride and lead sub acetate tests), steroids and terpenoids (Ghasemi *et al.*, 2014).

Determination of lethal dosage (LD_{50})

This test was carried out to determine the LD₅₀ value of the experimental animals. This test was carried out according to the procedures described by Lorke (1983).

Collection of animals

Twenty seven (27) rats weighing 120-196 g were procured from the animal house of Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were randomly divided into 3 groups (with the sum total) of 9 (3 sub-group containing 3 rats each) rats in each group. The first group was treated with 1000 mg/kg while group 2 and 3 were administered with 2000 mg/kg and 3000 mg/kg, respectively for the lethal dose test.

Experimental design

The experimental design was the modified versions of that reported earlier (Moura et al., 2005; Igboasoiyi et al., 2007). Four groups A, B, C and D were used for the subchronic experiment. Groups B, C and D were respectively administered 200, 300 and 400 mg/kg body weight of ethanolic leave extract of D. erecta for 28 days while Group A served as control in which no extract was administered except distilled water. They were subsequently dissected from the abdominal region of the rat; blood was collected from the pulmonary vein using the vacutainer system into EDTA anti-coagulated and non anti-coagulated tubes. The non-coagulated blood was allowed to clot at room temperature; the resultant clear part were centrifuged at 1500 x g for 10 min to obtain the serum; biochemical analyses were run immediately using the automated multiitem analyzer (TMS-1024, Tokyo Boeki Medical System Ltd., Japan). Similarly, the anti-coagulated blood was immediately used for haematological studies.

Biochemical and haematological assays

Commercial test kits (Biosino Bio-Technology and Science Inc. Beijing, China) were used for all biochemical parameters measured. Standard methods were used to estimate, aspartate aminotransferase (AST) (Bergmeyer et al., 1986a), alanine aminotransferase (ALT) (Bergmeyer et al., 1986b), alkaline phosphate (ALP). These parameters were determined using the automated biochemical multiitem analyzer (TMS-1024; Tokyo Boeki Medical System Ltd., Japan). The whole blood was used to assay white blood cell count (WBC), red blood cell count (RBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCHC), platelet count, percentage concentration lymphocyte (%LYM) and percentage (%MON). The parameters were determined by automated haemotology system analyzer (ADVIA 60 Open Tube; Bayer Corporation, Tarrytown, Newyork, USA)

Statistical analysis

Data obtained were expressed as mean±SD and were statistically analyzed using the statistical package SPSS 17.0 computer program. The data were subjected to two-way analysis of variance (ANOVA) with Duncan's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. The values of P<0.05 and P<0.0 were considered significant.

RESULTS

Qualitative phytochemical composition of the crude extract of D. erecta

The flavonoid contents are in relative high abundance

(+++) while other constituents such as the cardiac glycosides, steroids, saponins, and tannins are moderate in abundance (++). The alkaloid is low in abundance (+) as shown in Table I.

Table I.- Qualitative phytochemical screening of *D. Erecta*.

S. No.	Constituents	(mg/100g)
1	Alkaloids	+
2	Flavonoids	+++
3	Tannins	++
4	Saponins	++
5	Steroids	++
6	Glycosides	++

^{+,} present in small quantity; ++, moderately present; +++, abundantly present.

Acute toxicity studies of D. erecta on albino rats

In the first phase of the acute toxicity studies, there were no remarkable signs of toxicity observed at 1000 mg/kg dosage, while at 2000 mg/kg dosage, there were however salivation, rubbing of nose and mouth on the floor of the cage and restlessness. However, at 3000 mg/kg dosage, all the animals died 2 hours after the extract administration. Therefore, the median lethal (LD $_{50}$) dose was determined to be 2430 mg/kg dosage by plotting the graph of probit kill values against log of concentration and finding the values of 50% probit kill as against the antilog value on log concentration value (Fig. 1).

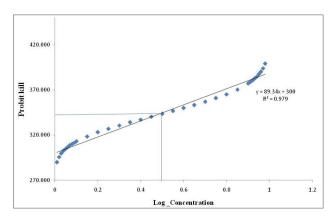


Fig. 1. Median lethal (LC $_{50}$) dose concentration of albino rats exposed to different concentration of *D. erecta*.

Biochemical parameters

Table II shows the values for the biochemical parameters analyzed during the experiment the Aspartate transaminase (AST) showed a significant effect (p=0.00). The groups treated with the extract have lowered AST when

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compared to the control. Group 1 (200 mg/kg) lowered the AST value more than the other groups with higher doses when compared with the control. Similarly, Group 300 mg/kg lowered the ALP value more than the other groups with

higher doses when compared to the control. The Alkaline phosphatase (ALP) was not statistically significant (p= 0.140) and all the animals used were healthy showing no evidence of negative behaviour or mortality at this stage.

Table II.- Effects of different treatment of *D. erecta* on the biochemical parameters of albino rats.

Parameter	Concentration	Duration (Weeks)			
(s)	(mg/kg)	1	2	3	4
ALT	Control	45.00 ± 0.58^{a1}	80.00 ± 2.89^{c2}	86.33 ± 0.33^{c3}	96.00 ± 0.58^{c4}
	200	49.67 ± 0.33^{b1}	67.67 ± 1.45^{b2}	72.00 ± 0.00^{a3}	80.33 ± 0.33^{a4}
	300	49.67 ± 0.67^{b1}	70.00 ± 0.58^{b2}	87.00 ± 0.00^{c3}	98.00 ± 0.58^{d4}
	400	56.00 ± 0.58^{c1}	60.00 ± 0.00^{a2}	77.67 ± 0.33^{b3}	87.67 ± 0.33^{b4}
ALP	Control	67.67 ± 0.88^{c2}	64.00 ± 2.31^{b12}	62.00 ± 0.58^{c1}	61.33 ± 0.33^{c1}
	200	74.33 ± 0.33^{d4}	50.33 ± 0.33^{a3}	48.00 ± 0.00^{62}	46.33 ± 0.88^{b1}
	300	65.67 ± 0.33^{64}	48.00 ± 0.58^{a3}	44.00 ± 0.58^{a2}	40.67 ± 0.33^{a1}
	400	60.00 ± 0.58^{a4}	50.67 ± 0.67^{a3}	45.00 ± 0.58^{a2}	42.33 ± 0.33^{a1}
AST	Control	82.00 ± 1.15^{ab1}	94.00 ± 1.15^{c2}	96.33 ± 0.33^{a23}	98.67 ± 0.33^{b3}
	200	80.00 ± 2.31^{a1}	90.33 ± 0.33^{b12}	86.00 ± 10.00^{a12}	98.00 ± 0.57^{b2}
	300	91.00 ± 0.58^{c2}	87.67 ± 0.33^{ab1}	96.33 ± 0.33^{a3}	98.33 ± 0.33^{64}
	400	86.00 ± 1.15^{b1}	86.00 ± 1.15^{a1}	87.67 ± 0.33^{a1}	90.67 ± 0.33^{a2}

Table III.- Effects of exposure to various sub-lethal levels of *D. erecta* on haematological parameters in albino rats.

Parameter (s)	Concentration		-		
	(mg/kg)	1	2	3	4
PCV	Control	38.00 ± 0.58^{a3}	30.00 ± 1.15^{a2}	28.33 ± 0.33^{a12}	26.00 ± 1.15^{a1}
	200	40.00 ± 0.58^{ab3}	35.00 ± 1.15^{c2}	32.67 ± 0.88^{b12}	31.33 ± 0.33 ^{b3}
	300	41.33 ± 0.88 ^{b3}	34.00 ± 0.58^{bc2}	32.33 ± 0.33^{b2}	29.00 ± 1.15^{b1}
	400	41.33 ± 0.88 ^{b3}	31.33 ± 0.88^{ab2}	29.33 ± 0.33^{a2}	24.33 ± 0.33^{a1}
WBC	Control	10800.00 ± 0.58^{b4}	4600.33 ± 3.18^{a1}	6260.27 ± 0.03^{a2}	7700.83 ± 0.33^{a3}
	200	11500.00 ± 0.58^{d4}	5200.33 ± 29.16^{b1}	6577.50 ± 0.35^{b2}	7953.10 ± 3.51 ^{b3}
	300	10600.00 ± 0.58^{a4}	5901.67 ± 1.67^{c1}	7058.33 ± 1.67^{c2}	8250.07 ± 0.03^{c3}
	400	10900.00 ± 0.58^{c4}	$6600.00 \pm 0.00^{\rm d1}$	7836.33 ± 0.33^{d2}	8850.63 ± 0.24^{d3}
RBC	Control	10.41 ± 0.01^{a3}	8.88 ± 0.01^{a2}	7.68 ± 0.57^{a1}	6.99 ± 0.00^{a1}
	200	10.69 ± 0.68^{a2}	9.42 ± 0.00^{b1}	9.37 ± 0.26^{b1}	8.20 ± 0.15^{b1}
	300	10.66 ± 0.50^{a2}	9.58 ± 0.01^{c1}	9.58 ± 0.00^{b1}	9.52 ± 0.04^{d1}
	400	10.53 ± 0.85^{a2}	9.66 ± 0.01^{d12}	9.03 ± 0.03^{b1}	8.89 ± 0.00^{c1}
Hb	Control	9.27 ± 0.09^{a1}	10.55 ± 0.00^{a2}	11.43 ± 0.03^{a3}	12.47 ± 0.03^{a4}
	200	10.20 ± 0.64^{ab1}	11.43 ± 0.26^{b2}	12.63 ± 0.03^{b3}	13.80 ± 0.00^{b4}
	300	$10.80 \pm 0.29^{\mathrm{b1}}$	11.10 ± 0.06 ^{b1}	11.27 ± 0.15^{a1}	11.83 ± 0.52^{a1}
	400	9.70 ± 0.29^{ab1}	12.47 ± 0.03^{d2}	15.73 ± 0.03^{c3}	17.53 ± 0.55^{c4}
МСН	Control	2.42 ± 0.02^{a1}	3.54 ± 0.14^{ab2}	4.06 ± 0.03^{b3}	4.82 ± 0.23^{b4}
	200	2.59 ± 0.18^{a1}	2.94 ± 0.34^{a1}	3.97 ± 0.08^{b2}	4.44 ± 0.06^{ab2}
	300	2.59 ± 0.04^{a1}	3.28 ± 0.06^{a2}	3.49 ± 0.03^{a3}	4.08 ± 0.02^{a4}
	400	2.40 ± 0.11^{a1}	4.17 ± 0.09^{b2}	5.37 ± 0.04^{c3}	7.24 ± 0.24^{c4}
MCHC	Control	24.17 ± 0.15^{a1}	35.39 ± 1.45^{a2}	40.36 ± 0.54^{b3}	48.17 ± 2.25^{b4}
	200	25.77 ± 1.79^{a1}	32.71 ± 0.33^{a2}	38.77 ± 1.16^{b3}	44.38 ± 0.62^{ab4}
	300	25.99 ± 0.44^{a1}	32.66 ± 0.50^{a2}	34.90 ± 0.27^{a3}	40.81 ± 0.21^{a4}
	400	24.03 ± 1.05^{a1}	40.11 ± 1.16^{b2}	53.76 ± 0.38^{c3}	72.10 ± 2.51^{c4}
MCV	Control	35.83 ± 0.33^{a1}	32.77 ± 0.67^{a1}	37.29 ± 2.52^{a1}	37.23 ± 1.65 ^{b1}
	200	37.90 ± 1.74^{a1}	37.15 ± 1.23^{b1}	35.96 ± 0.88^{a1}	$38.43 \pm 0.30^{\rm b1}$
	300	38.88 ± 1.34^{a3}	35.17 ± 1.15^{ab2}	33.75 ± 0.35^{a12}	30.40 ± 1.40^{a1}
	400	39.16 ± 3.44^{a2}	32.28 ± 1.12^{a1}	32.58 ± 0.37^{a1}	27.40 ± 0.35^{a1}

Mean values with different alphabets as superscripts in a column differ significantly (p < 0.05). Mean values with different numbers as superscripts in a row differ significantly (p < 0.05). Hb, Haemoglobin; LYM, percentage lymphocyte; MCHC, mean corpuscular haemoglobin concentration; MCV, mean cell volume; PCV, platelet count volume; RBC, red blood cell count and WBC, white blood cell count.

Haematological and morphological parameters

The effects of different treatments of the *D. erecta* on the haematological indices of albino rats are shown in Table III and Figure 2. The PCV values of B, C and D treatment groups were significantly (p < 0.05) high when compared to the control values. Generally, significant (p < 0.05) duration – dependent decrease in PCV was observed in all the experimental groups. The groups administered with different does of *D. erecta* recorded significantly (p < 0.05) higher values of WBC than the control values. Increased dose of the *D. erecta* produced a significant (p < 0.05) dose-dependent increase in WBC. The RBC values of the treatment groups were higher than the control values significantly (p < 0.05). There was a dose dependent increase in RBC, however, a duration dependent (p < 0.05) decrease in RBC was recorded in all the groups (Table III).

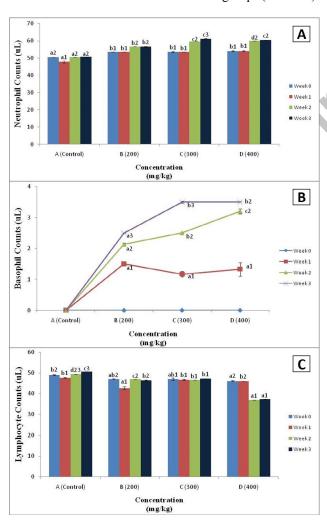


Fig. 2. Effects of exposure to various sub-lethal levels of *D. erecta* on neutrophil (A), basophil (B) and lymphocyte (C) count of albino rats.

The lymphocyte counts of the control (A) group was significantly (p < 0.05) higher than the values recorded in other extract administered groups. A significant (p < 0.05) dose-dependent decrease in lymphocyte values were observed during period of the research (Fig. 2C). There were also duration-dependent decrease in MCV values obtained in this research as against duration and dose dependent increases in MCH, and MCHC. The neutrophil level of all the treatment groups were significantly (p < 0.05) higher than the control values. Significant durationdependent increase in neutrophil level was recorded in group A, C and D (Fig. 2A). Groups C and D recorded a significantly (p < 0.05) higher basophil values than the group B. A significant duration dependent basophil values were observed in the results (Fig. 2B). In overall, the lymphocyte counts of the control (A) group was significantly (p < 0.05) higher than the values recorded in other extract administered groups. A significant (p < 0.05) dose-dependent decrease in lymphocyte values were observed in this study (Fig. 2C).

DISCUSSION

The safety of drugs and plant products for human use can be determined using toxicological evaluation which is usually carried out in various experimental animals to predict toxicity and to be used as guidelines for selecting a safe dose in humans. Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for studies (Olson et al., 2000). From the observed values of WBS, it is clear that an increase in the number of WBS is a normal reaction of rats to foreign substances which alter their normal physiological processes. The leucocytosis observed in the present study indicates a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections. Leucocytosis which could be directly proportional to the severity of the causative stress condition may be attributed to an increase in leucocyte mobilization (Celik and Suzex, 2008).

The elevated level human toxicity when the data are translated from animal MPV and PDW in the extract may be due to high rate of erythropoiesis occurring in the bone marrow of the rats (Rhiouani et al., 2008). The rise in ALT in the treated groups is an indication of hepatocellular injury. ALT is a cytoplasimic enzyme found in very high concentration in the liver (Aliyu et al., 2007), and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function. The toxicity exerted by ethanolic leaf extract of *Duranta erecta* was confirmed from histological

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sectioning which indicated various degrees of spotty hepatocellular necrosis in all the treated groups.

The observed damage may be due to the fact that the liver being the first target of acute toxicity and the first organ exposed to everything that is absorbed in the small intestine, may metabolize foreign substances to highly reactive metabolites which may be hepatotoxic. In addition, because of the short duration of treatment, the alterations might be incipient and reversible, and not pronounced enough to change significantly serum ALT levels (Grance et al., 2008). ALP, is a marker of obstructive jaundice and intraheptic cholestatis (Davern and Scharschmidt, 2002) was significantly reduced; this further supported that the observed liver damage was not connected to biliary obstruction of the liver.

The result obtained was partly in agreement with the work of Moura et al. (2005) involving rats treated with the hydro-alchoholic extract of *Duranta erecta* leaves. ALT activity was high but not significant in one of the treated groups; they however, suggested that histological study be accessed in order to establish heptatotoxicity of the plant. AST, is one of the markers of skeletal and myocardial muscles (Rosalki et al., 2004; Laterza et al., 2008; Rasekh et al., 2008). When myocardial cells, containing one of the markers (AST) is damaged or destroyed due to deficient oxygen supply or glucose, the cell membrane becomes permeable or may rapture, which results in the leakage of enzymes (Prince et al., 2008).

The reduced significant activity of these markers suggest that the ethanolic leaf extract of *Duranta erecta* offered some protections against diseases related to the muscles. The significant reduction of AST level has further eliminated any leakage of the enzyme in the liver due to hepatic injury activities that were significantly decreased in the treated rats. Previous biochemical studies have also confirmed the non-toxicity of the plant extract of the plant extract of *D. erecta* to the kidney (Moura *et al.*, 2005; Igboasoyi *et al.*, 2007).

CONCLUSION

The reduced significant activity of the markers suggest that the ethanolic leaf extract of *Duranta erecta* offered some protections against diseases related to the muscles. The significant reduction of AST level has further eliminated any leakage of the enzyme in the liver due to hepatic injury activities that were significantly decreased in the treated rats. Previous biochemical studies have also confirmed the non-toxicity of the plant extract of the plant extract of *D. erecta* to the kidney.

Statement of conflict of interest

Authors have declared no conflict of interest.

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