Journal of Pharmaceutical Research International

32(6): 9-18, 2020; Article no.JPRI.55990
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Toxicity of the Hydroethanolic Leaves Extract of *Duranta erecta* L. in Rat Models

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SD and CL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GK and BOE managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i630441
Editor(s):
(1) Dr. Mohamed Fathy, Assiut University, Egypt.
Reviewers:
(1) Victor B. Oti, Nasarawa State University, Nigeria.
(2) Mayur Porwal, IFTM University, India.
(3) Michel Archange Fokam Tagne, University of Ngaoundere, Cameroon.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55990

Original Research Article

Received 02 February 2020
Accepted 09 April 2020
Published 05 May 2020

ABSTRACT

Background and Objectives: *Duranta erecta* is used in folklore medicine for the treatment of myriad of diseases in Africa. The study was carried out to evaluate the safety of hydroethanolic leaves extract of *D. erecta* in experimental rats in order to ascertain its potential toxic effects. **Materials and Methods:** The acute toxicity study was performed by fixed dose method at 5000 mg/kg. In the subacute study performed on both male and female rats, group I (control) received 1 mL of freshly distilled water, groups II, III, IV were treated with 100, 250 and 500 mg/kg of freshly prepared extract respectively for 28 days. At the end of the study, haematological and biochemical

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parameters were determined. Internal organs (kidney, liver, lung, heart, spleen, stomach, testes and uterus) were weighed.
Results: 50% lethal dose (LD_{50}) of the extract was determined to be > 5 g/kg body weight. The subacute toxicity assessment resulted in overall body weight increase, a change in relative organ weight of the liver, lung, stomach, and changes in the haematological indices such as HCT%, LYM%, RDW- SD/fL, MCHC, MCV/fL, P-LCR% and biochemical parameters namely ALT, AST, LDH and creatinine of the tested group relative to the normal. The positive activity of the extract on liver enzymes and LDH is an indication of its good hepatoprotective potential.
Conclusion: The results affirmed that the extract is safe but could cause kidney problems when used for a prolonged period.

Keywords: Duranta erecta; acute toxicity; subacute toxicity; haematological indices; biochemical indicators.

1. INTRODUCTION

Plants based medicines have been integral part of the primary health care system in Africa and Asia and thus plays critical role in the well-being of herbal medicine users. The use of plant derived drugs is becoming popular in the developed countries with the intention of addressing the menace of development of resistance against synthetic drug and its associated adverse side effects [1]. Herbal medicines have been used for the treatment of various ailments by the general public as well as traditional medical practitioners worldwide. They are used as an alternative to orthodox medicine by folklore medical users. According to a report by Ezuruike and Prieto [2], 80% of the African population depend on traditional medicine to meet their healthcare needs. Herbal medicine has been generally considered safe because of preference for natural therapies and a greater interest in alternative medicine, making it difficult for its users acknowledge potential toxic effects associated them. There is a clamor for the use and inclusion of herbal products into the health care system, though most health care providers are hesitant in recommending herbal products to their patients due to safety issues [3]. Plants are generally rich in antioxidants and other bioactive compounds. In view of that, the use of the various parts of plants for disease treatment and control has focused on their beneficial properties to the neglect of possible toxicity.

Duranta erecta L. (family Verbenaceae) which is commonly known as 'golden dewdrop' is grown as a hedge or ornamental plants in various homes in Ghana. A number of bioactive compounds such as β-sitoserol, naringenin, acteoside, lamiide, coumarinolignoids, (E)-cinnamic acid, sucrose and raffinose have been identified and isolated from genus *Duranta* [4,5]. According to the results of our previous study, the leaves and fruits of *Duranta erecta* contain important phytochemicals such as flavonoids, phenols, saponins, sterols, tannins, alkaloids and triterpenoids [5]. The various parts of *Duranta erecta* are used in traditional folk medicine to treat malaria, intestinal worms and abscess and also known to possess anticancer and antibacterial properties [6]. Literature concerning the possible side effects of the plant is scanty. Thompson [7] reported that, the leaves and the fruits of *D. erecta* can be harmful to animals at higher doses. This raises concern about the potential toxic effect resulting from the unmonitored use of *Duranta erecta*. Adverse reactions associated with the use of herbal based products makes it imperative to scientifically scrutinize them to ensure their safe use. The aim of the study was to carry out a safety evaluation of *Duranta erecta* in laboratory rats to ascertain its potential toxic effects.

2. MATERIALS AND METHODS

2.1 Plant Collection, Preparation and Extraction

Leaves of *Duranta erecta* were collected from the Kwame Nkrumah University of Science and Technology (KNUST) campus in October 2017 and authenticated by Dr George Sam at the Department of Herbal Medicine, Faculty of Pharmaceutical Sciences, KNUST. Herbarium specimen was deposited in the faculty's herbarium with voucher number KNUST/HM1/2017/L011. The leaves were washed under running tap water to remove dust and other extraneous substances. They were subsequently dried under shade for 2 weeks. The shade-dried materials were coarsely powdered using a grinder (Waring, USA). About 60 g of the pulverized samples were extracted with 50% ethanol for 48 hours. The mixture was separated by filtration and supernatant

evaporated under reduced pressure at 60°C using a rotary evaporator (Buchi R-205. Switzerland). The extract was dried using vacuum freeze dryer (Heto PowerDry LL3000, UK) to obtain hydroethanolic extract.

2.2 Animals

Thirty-two male and female Sprague-Dawley rats (93 – 133 g) and five albino mice (40 – 45 g) were used for the studies. The animals were obtained from the animal facility of the Department of Biochemistry and Biotechnology, KNUST and were housed in aluminium cages, suitably bedded with wood shaving. They were kept under standard laboratory conditions of temperature and humidity and 12-hour light and dark cycle. Animals were handled as stipulated in the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and the Guide for Care and Use of Laboratory Animals [8]. All animals were humanely handled during the experiment. The study protocols were reviewed and approved by a veterinarian on the research team.

2.3 Acute Toxicity Study

The acute toxicity study was carried out using five albino mice (40 - 45 g). Guideline 420 also referred to as the fixed dose method [9] and reported by Arthur et al. [10] was used. After an overnight fast, the rats were given 5000 mg/kg body weight (bw) of the freshly prepared hydroethanolic extract by oral gavage. The rats were observed for signs of toxicity focusing, specially, on the first 4 hours of drug administration. Visual observations were made for mortality and various changes in physical appearance such as paw-licking, stretching, respiratory distress, diarrhoea once daily for 7 days [9].

2.4 Subacute Toxicity Study

Thirty-two rats of both sexes were used for the subacute study. For each sex, animals were assigned to four groups of four animals per group. The 'resource equation' method was used in determining the sample size (animals) for the study. This method applies the law of diminishing return [11-13]. Using all variables, a minimum of 3 animals per group was considered significant. The normal group received 1 mL of freshly distilled water daily while the test groups were treated with 100, 250 and 500 mg/kg bw. of the freshly prepared aqueous solution of the extract once daily for 28 days respectively. All animals were fasted 12 hours prior to first oral administration of the extract and had free access to food and water throughout the duration of the experiment. The animals were observed daily for general signs of toxicity and mortality.

2.5 Body Weight Changes

Rats in all groups were weighed on the first day (D0) and every 4 days afterwards to day 28. The percent change in body weight was calculated using the formula:

$$\text{Percentage change in body weight} = \left(\frac{\text{Wn} - \text{Wo}}{\text{Wo}}\right) \text{X } 100$$

Where Wn = weight of animals on respective days and Wo = weight on the first day (DO).

2.6 Blood Collection and Subacute Assessment

At the end of the experiment period of 28 days, animals were fasted and euthanized by cervical dislocation. Blood samples were collected into EDTA tubes via incision in the cervical region with the aid of a sterile blade for haematological analyses using Sysmex Haematology Systems (USA). The haematological profile white blood cell count (WBC) red blood cell count (RBC), haemoglobin (HGB) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocyte (LYM), platelet (PLT), haematocrit (HCT), red cell distribution width (RDW), mean platelet volume (MPV), neutrophil (NEUT), plateletcrit (PCT), platelet large cell volume (P-LCR) and plate volume distribution width (PDW) were analysed.

2.7 Organ Assessments

The euthanized animals were dissected and their internal organs namely liver, kidney, heart, stomach, spleen, testes or uterus excised, washed with normal buffered saline, and blotted with clean tissue paper. These were observed macroscopically and weighed to obtain absolute organ weight (AOW). The relative organ weights (ROW) were calculated for each of the rat using the formula;

$$\text{ROW} = \frac{Absolute\ Organ\ Weight}{Body\ Weight\ at\ Sacrifice} \text{ X } 100$$

2.8 Biochemical Assays

Five millilitres of blood was dispensed into gel activated tubes, allowed to clot and centrifuged at 3000 rpm for 5 minutes to obtain sera for the various biochemical assays using the using the Selectra E (Vital Scientific, Japan) and reagents from ELITECH (France). Parameters determined included alanine aminotransferase (ALT), aspartate amino transferase (AST), total, direct and indirect bilirubin, creatinine, urea, sodium, potassium, chloride, total cholesterol (TChol), high density lipoproteins (HDL), total triglycerides (Trigs), glucose, and lactase dehydrogenase. Low density lipoprotein (LDL) concentration was calculated using the Friedewald's equation [14].

2.9 Statistical Analysis

A GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. Experimental values were expressed as mean ± standard error of mean (SEM) and assessed by one-way analysis of variance followed by Tukey's multiple comparison test to evaluate the significance between the various group. A P value of ≤ 0.05 was considered as significant.

3. RESULTS

3.1 Observation for Sings of Toxicity in Animals in Acute Toxicity Study

In the acute toxicity study, no clear signs of toxicity were observed in the rats at 5000 mg/kg p.o. The extract produced no unwanted effect on the behavioural responses of the treated rats. There were no signs of changes in the behaviour patterns, skin, eyes, salivation, and diarrhoea of the rats. The nature of stool, urine and eye colour remain the same even after drug administration and throughout the period of observation. Also, there was no stretching, paw liking or diarrhoea. Since no adverse effect or lethality of the extract was recorded at the dosage of 5000 mg/kg, the LD_{50} was estimated to be greater than 5000 mg/kg (n=5).

3.2 Subacute Toxicity Study

3.2.1 Effect of treatment on body weight

The effect of crude extract on the body weight of animals is shown in Fig. 1. The body weights of the animals were not affected by administration of the extract. There was no significant change in the body weight of treated animals when compared to the normal group. Animals gained but insignificant weight and appeared active and normal. There was, however, a marginal drop in the body weight of treated animals from day 24 to 28. Exception was that female rats treated with 500 mg/kg gained weight. This showed that the extract was tolerated at all doses and did not interfere in their nutritional status.

3.2.2 Effect on relative organ weight of animals

The relative organ weight of male and female animals yielded varied results (Figs. 2 and 3). No significant change in relative weights of testes, lung, stomach, heart, kidney and spleen of male rats was observed (Fig. 2). There was, however, significant reduction (P<0.001) in the relative organ weight of the liver of treated male rats when compared to that of the normal.

For females, significant increase was observed in relative organ weight of lung (P<0.05) and stomach (P<0.01) for animals that received extracts at 500 mg/kg (Fig. 3). Contrary, reduction in relative weight of the liver (P<0.001) was observed.

3.2.3 Effect of treatment on haematological indices of animals

The extract had no significant effects on most of the haematological indices. However, there was a significant increase in LYM% (P<0.01) and MCHC (P<0.001). On the other hand, significant reduction was observed in the HCT% (P<0.05), RDW-SD/FI (P<0.001), and P-LCR% (P<0.001) (Table 1) of treated male rats. These changes were unrelated to the dose administered.

Treated female rats showed significant but dose-independent increase in LYM% (P<0.001) and reduction in MCV/FI (P<0.001) and RDW-SD (P<0.001). There was, however, significant dose-dependent reduction P-LCR% (P<0.001) compared with normal (Table 2).

3.2.4 Effect of treatment on biochemical indicators

The effect of extract treatment on biochemical indicators is summarised in Tables 3 and 4. There was a decrease in the ALT of treated male rats but this was significant (P<0.01) only at the lower doses (100 mg/kg and 250 mg/kg). Also, dose-unrelated increases were recorded for AST

with the lower dose causing significant increase (P<0.05). AST/ALT level increase with inversely with concentration with significant increases at 100 mg/kg (P<0.001) and 250 mg/ (P<0.01). The extract caused general increase, independent of dose, in the serum creatinine level of treated male rats with the dose at 250 recording a significant increase (P<0.01). Other parameters, on the other hand, did shows marked changes (Table 3).

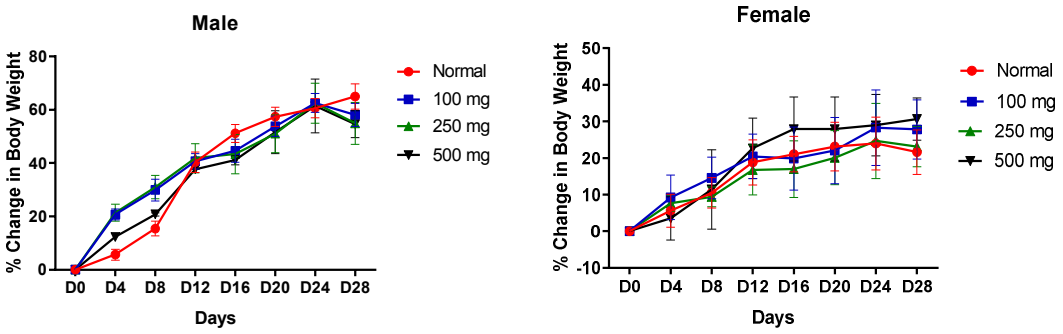

Fig. 1. Change in body weight of animals

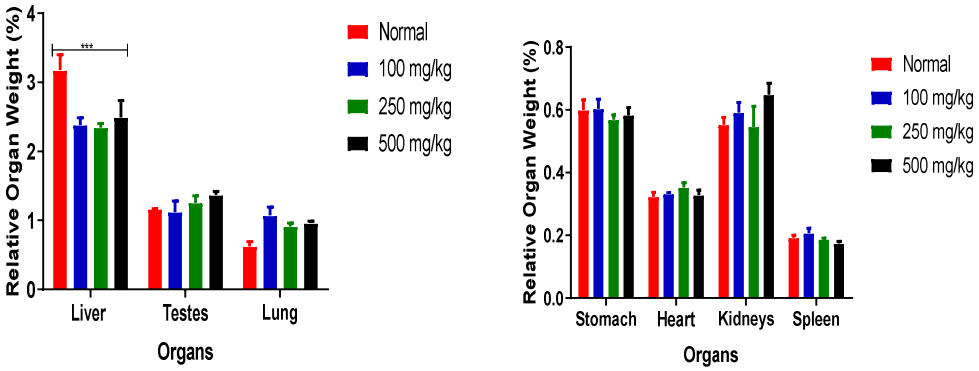

Fig. 2. The effect of the extract on ROW of male rats. Significant difference: ***p<0.001 against normal

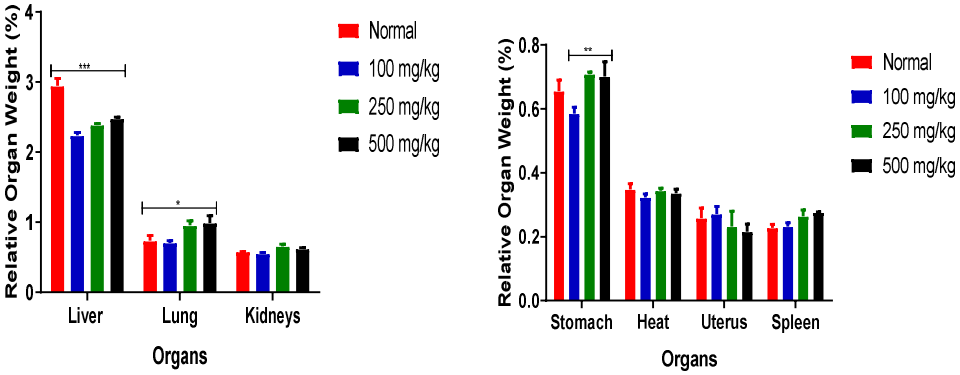

Fig. 3. The effect of the extract on ROW of female rats. *P<0.05; **P<0.01; ***P<0.001 against normal

Table 1. Haematological profile of male rats in the subacute toxicity study

	Normal	100 mg/kg	250 mg/kg	500 mg/kg
WBCx10^3/µL	6.10±0.50	5.68±1.68	6.00±0.72	6.50±0,20
RBCx10^6/µL	8.09±0.47	8.04±0.22	8.40±0.17	8.10±0.31
HGB (g/dL)	14.10±0.67	14.10±0.18	14.60±0.32	13.75±0.05
HCT (%)	58.27±3.45	49.58±1.00*	51.97±1.23*	48.35±1.25*
MCV (fL)	72.03±0.74	61.73±0.46	61.90±0.51	59.75±0.75
LYM (%)	61.20±3.92	69.60±2.12**	79.30±2.84**	77.70±8.60**
RDW-SD (fL)	43.80±1.28	34.23±0.22***	34.17±0.48***	35.75±0.15***
MCHC (g/dL)	24.23±0.35	28.48±0.23***	28.10±0.06***	28.45±0.65***
MCH (pg)	17.43±0.20	17.55±0.25	17.37±0.15	17.00±0.60
RDW-CV (%)	15.17±0.29	13.98±0.17	13.93±0.54	15.45±0.45
P-LCR (%)	13.57±1.01	6.13±0.78***	6.83±1.45***	3.95±0.35***
PDW (fL)	10.23±0.23	8.20±0.25	8.57±0.38	7.60±0.20
MPV (fL)	8.40±0.12	7.05±0.18	7.20±0.26	6.60±0.10
LYM × 10^3/µl	3.73±0,43	3.95±1.16	4.80±0.75	5.10±0.70
PLT (10^3/µl)	1058.67±80.76	1439.25±151.77	1060.67±54.81	1256.50±25.50
PCT (%)	0.89±0.06	1.02±0.12	0.76±0.03	0.83±0.00

*Mean±SEM; *P<0.05, **P<0.01, ***P<0.001 compared to normal*

Table 2. Haematological profile of female rats in the subacute toxicity study

Index	Normal	100 mg/kg	250 mg/kg	500 mg/kg
WBCx10^3/µL	7.30±0.31	5.95±0.50	5.75±0.44	6.30±0.26
RBCx10^6/µL	7.20±0.07	8.23±0.50	8.16±0.22	8.05±0.03
HGB (g/dL)	13.43±0.09	13.48±0.88	14.08±0.28	14.10±0.15
HCT (%)	52.07±0.88	50.50±2.72	48.63±0.82	48.73±0.34
MCV (fL)	72.30±0.60	61.45±0.80***	59.68±0.82***	60.53±0.64***
LYM (%)	63.47±0.48	72.08±2.09***	69.30±3.41***	79.03±0.41***
MCHC (g/dL)	25.80±0.32	27.00±2.51	28.93±0.27	28.93±0.26
RDW-SD (fL)	39.83±0.75	32.35±0.82***	31.10±0.93***	34.40±2.910***
MCH (pg)	18.63±0.12	16.63±1.61	17.28±0.11	17.53±0.20
P-LCR (%)	11.90±0.56	7.50±2.01***	6.33±0.75***	5.23±0.72***
RDW-CV (%)	13.40±0.25	13.20±0.26	12.73±0.33	14.33±1.59
PDW (fL)	9.83±0.19	8.75±0.67	8.35±0.34	8.10±0.35
MPV (fL)	8.13±0.09	7.18±0.37	7.00±0.16	6.90±0.21
LYM (× 10^3/µL)	4.67±0.18	4.30±0.46	6.90±1.70	4.47±0.15
PLT 10^3/µl	1323.00±156.52	1078.25±218.18	1094.50±115.47	988.33±139.70
PCT %	1.08±0.13	0.76±0.13	0.76±0.07	0.68±0.09

*Mean±SEM; *P<0.05, **P<0.01, ***P<0.001 compared to normal*

In the treated female rats, all parameters but AST, creatinine and LDH showed no marked changes at the doses used. There was increase in the AST and creatinine levels of treated female rats while LDH level decrease. The increase in AST was dose-dependent and significant (P<0.05) at 500 mg/kg. Contrary, the increase in creatinine was not related to the dose of extract and was significant (P<0.0001) at the lowest dose tested. LDH, on the other hand, showed a dose-dependent reduction and this was significant (P<0.05) at 500 mg/kg (Table 4).

4. DISCUSSION

Herbal medicines, and for that matter medicinal plants, are considered to be generally 'safe'. This assertion is based on the premise that they are of natural origin [15]. As a result, their toxicity is often taken for granted even when they are used over a long period of time. There are however, instances when medicinal plants have been shown to demonstrate one toxicity or the other [3]. The current study assessed the acute and subacute toxicity of hydroethanolic leaf extract of *Duranta erecta*.

The acute toxicity study showed no evident toxicity (the primary endpoint for the OECD guideline 420 [9] or mortality (LD_{50} >5000 mg/kg). This is an indication that the extract has a high degree of safety [16].

Table 3. Biochemical parameters of male rats in the subacute toxicity study

Male	Normal	100 mg/kg	250 mg/kg	500 mg/kg
ALT U/L	63.87±5.27	34.90±1.84**	34.30±5.02**	51.27±1.87
AST U/L	148.00±2.55	169.55±7.17*	143.77±5.26	158.27±0.55
AST/ALT	2.37±0.19	4.88±0.15***	4.33±0.52**	3.07±0.09
TBil mmol/L	1.63±0.55	2.03±0.62	1.73±0.55	1.66±0.16
DBil mmol/L	0.95±0.05	0.90±0.18	1.22±0.34	0.95±0.24
IBil mmol/L	0.67±0.52	1.13±0.59	0.50±0.23	0.70±0.15
Creat µmol/L	28.50±6.22	41.85±7.68	46.63±7.65*	43.33±4.98
Urea mmol/l	11.04±0.85	8.94±0.31	8.63±0.22	10.10±1.47
K mmol/L	7.30±0.45	7.43±0.48	7.20±0.20	7.40±0.12
Na mmol/L	143.00±0.44	143.93±1.21	143.17±1.22	141.43±0.67
Cl mmol/L	105.57±0.53	105.13±0.37	105.80±1.05	104.57±0.50
TChol mmol/L	1.87±0.07	2.33±0.15	1.96±0.29	2.03±0.16
Trigs mmol/L	1.19±0.15	0.92±0.22	0.92±0.46	0.49±0.00
VLDL mmol/L	0.53±0.09	0.43±0.09	0.43±0.19	0.21±0.01
HDL mmol/L	0.58±0.02	0.73±0.18	0.73±0.08	0.79±0.03
LDL mmol/L	0.74±0.03	1.18±0.24	0.81±0.24	1.00±0.13
FBG mmol/L	1.38±0.40	2.01±0.70	1.46±0.34	1.56±0.31
LDH U/L	4617.77±101.24	3844.60±244.26	4000.40±524.02	3557.20±28.56

*Mean±SEM; *P<0.05, **P<0.01, ***P<0.001 compared to normal*

Table 4. Biochemical parameters of male rats in the subacute toxicity study

Female	Normal	100 mg/kg	250 mg/kg	500 mg/kg
ALT U/L	56.70±4.22	53.35±4.55	37.78±6.96	51.53±7.94
AST U/L	149.77±8.24	152.63±11.46	187.60±30.60	221.60±10.95*
AST/ALT	2.67±0.19	2.83±0.57	3.10±0.92	3.57±0.83
TBil mmol/l	1.36±0.27	3.36±1.02	2.81±0.99	2.63±1.00
DBil mmol/l	0.93±0.11	1.76±0.60	1.33±0.45	1.24±0.61
IBil mmol/l	0.43±0.19	1.58±0.56	1.48±0.58	1.40±0.55
Creat µmol/L	28.50±0.67	54.70 ±3.00****	35.38±3.10	29.60±2.75
Urea mmol/l	10.61±0.46	10.71±1.77	11.05±0.92	12.06±1.78
K mmol/L	7.07±0.29	8.30±1.26	8.18±0.80	7.33±0.29
Na mmol/l	142.73±0.43	143.73±1.14	141.30±1.10	142.17±1.02
Cl mmol/l	106.33±0.80	106.58±1.63	106.73±1.48	107.17±1.85
TChol mmol/l	2.15±0.03	1.96±0.22	1.99±0.31	1.97±0.39
Trigs mmol/l	1.27±0.22	0.55±0.15	0.89±0.19	0.37±0.10
VLDL mmol/l	0.57±0.09	0.25±0.06	0.40±0.09	0.17±0.03
HDL mmol/l	0.87±0.11	0.63±0.23	0.61±0.19	0.45±0.17
LDL mmol/l	0.70±0.13	1.08±0.37	0.98±0.17	1.35±0.55
FBG mmol/l	1.20±0.10	1.04±0.15	1.17±0.34	1.34±0.17
LDH U/L	4052.10±101.65	3524.93±448.53	2929.00±342.21	2252.87±446.06*

*Mean±SEM; *P<0.05, **P<0.01, ***P<0.001. ****p<0001 compared with normal*

Differences in organ weight between treated and control groups of animals have been used as indicators to evaluate the toxic effect of test substance during the subacute toxicity study. These changes usually precede morphological changes. The extract caused negligible changes in relative weight of testes, lung, stomach, heart, kidney and spleen save the liver that showed significant reduction (P<0.001) in weight in male rats. The female rats similarly showed no significant changes in relative organ weight of kidney, heart, uterus and spleen except an increase in the liver and lung, and a decrease in the stomach weights of treated female rats. Nevertheless, these changes do necessarily not indicate organ toxicity [17]. Albeit, decrease in organ weight often indicates organ degeneration or atrophy [18]. However, our data could not be used to explain the observation.

The administration of extract caused non-significant change in percentage haematocrit (HCT %) in the female rats but significant decrease (p<0.05) HCT% amongst male rats. A

lower haematocrit usually results from insufficient supply of healthy blood red blood cells or high number of white blood cells due to long term illness. The reduction in haematocrit amongst the treated rats may have emanated from high white blood cell counts which was reflected in the lymphocyte levels. The observed significant increase in lymphocytes level in the female and male treated rats compared with the normal is suggestive of activation of the immune system by the extract through the stimulation of lymphoid follicle and sinus histiocyte in the spleen or activation of lymphoid aggregate in the lungs. This phenomenon is attributed to normal physiological response of the immune mechanisms following perception of a foreign challenge. The significant decrease in MVC/FI and RDW-SD amongst treated female rats showed that the extract has the potential of inducing anaemic conditions and as such its prolonged use in the female should be investigated. Moreover, the increased MCHC (P<0.001) amongst the male treated compared to the normal is an indication of extract to boost production of red blood cells. Previous research has reported that male rats had a significant higher level of MCHC compared to female rats of the same age [19], however, this is not consistent with the result of this study which recorded non-significant difference between male and female rats. Haematological indices may vary based on the type, genotype and sex of laboratory animals [20]. The extract caused significant reduction in P-LCR% ($p<0.001$) amongst the treated rats. Higher P-LCR values have been associated with increased risk of coronary artery disease [21]. The reduction in P-LCR value associated with the administration of the extract emphasizes the beneficial effect of the extract and can that it can be explored in the fight against cardiovascular diseases.

The subacute toxicity study produced only few biochemical markers that were altered in the treatment groups compared to the normal (Tables 3 and 4). ALT is found primarily found in the liver and kidney. Increased amount of the enzyme in the blood is an indication of liver injury. AST is mainly found in the liver and heart but it is also found in the muscle, red blood cells and pancreas. Damage to these organs results in the elevation of AST in the blood. The observed significant decrease in ALT level at all doses in male rats and non-significant decrease in female rats suggests that extract is not hepatotoxic but rather hepatoprotective. The significant increase of AST level in both male and female rats and AST/ALT in the male rats is an indication that the source of elevated AST level may be nonhepatic. It may be from other sources probably muscle, etc. The non-significant change in the serum level of total and conjugated bilirubin at all doses signify that extract is safe on the liver. Creatinine is a waste product formed at a relatively constant rate in muscle as part of regular everyday activity. Kidney filters creatinine from your blood and send it out of the body in urine. If there is problem with the kidney, creatinine can build up in the blood. Elevated creatinine level in the blood has been associated with renal impairment [22]. In the present study, the elevated creatinine levels observed in the rats may have emanated from renal impairment. This is consistent with the results of other studies which reported elevated level of creatinine associated with the use of herbal extract [10,23]. LDH is found in blood, brain, muscle, kidney, heart, pancreas and liver, Higher levels of LDH in the blood may sign of tissue damage or disease. The general decrease of LDH level in both male female rats at all doses shows that extract had no deleterious effect on most of body organs and therefore lends further credence to the oral safety of the extract. Previous observations in dogs and cat showed adverse effects and mortality following consumption of whole leaves and fruits [24]. The current observations showed the hydroethanolic extract of the leaves of *D. erecta* to be safe in animals for use as medicine. The current study showed 500 mg/kg to be well tolerated in animals. This translates to 25 g for an average human body weight of 50 kg. This high dose cannot be achieved in humans and therefore be safe for use.

5. CONCLUSION

The extract at all doses tested produced no toxic effect on the behavioural response of the rats an indication that the extract is safe. The blood thinning property of the extract can be of benefit in the fight against cardiovascular diseases. However, the prolonged use of the extract at low dosage of 100 mg/kg body weight may have a dire consequence on the kidney.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals were humanely treated following standard International protocols.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/55990