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Safety evaluation of hydroethanolic fruit extracts of *Duranta erecta* Linn

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Abstract

Background: *Duranta erecta* is used in traditional medicine for the treatment of myriad of diseases in most developing countries. The potential safety concerns associated with the administration of hydroethanolic fruit extracts of the plants were investigated in rats.

Materials and Methods: Extracts were screened for their possible antioxidant activities by the DPPH scavenging activity. Bioactive compounds present in methanolic extracts of ripe and unripe fruits of *D. erecta* were identified using Gas chromatography–mass spectrometry (GC–MS). Male and female rats were grouped taking their body weights into consideration to achieve approximately equal conditions among the groups. A freshly prepared solution of DRR or DRU extract was administered orally at 100, 250 and 500 mg/kg b.wt. to different groups while normal group received distilled water daily for 28 days. Toxicity assessment was done using relative organ weight, haematological, and biochemical parameters and histological assessment.

Results: The administration of extracts resulted in overall body weight increase, significant change in relative organ weight of the liver, changes in haematological index such as platelet and biochemical parameters namely ALT, AST, ALP, TBil, DBil, IBil, creatinine and urea of the tested group relative to the normal. Histological observations showed normal hepatocytes.

Conclusion: The findings suggest that hydroethanolic *Duranta erecta* fruit extracts is safe but its prolonged use may have some level of adverse effect on the liver.

Keywords: Toxicity; *Duranta erecta*; Fruit extracts; Gas chromatography–mass spectrometry

1. Introduction

Duranta erecta L. is a plant of many economic values. It serves as a source of essential oils, teas, herbal medicines, fruits, gums, tannins, and ornamentals [1]. It is cultivated as hedge in most African countries because of its tendency to form impenetrable barrier. Phytochemical investigation has revealed the presence of various secondary metabolites such as tannins, glycosides, saponins, alkaloids, flavonoids, and triterpenoids in different parts of the plant [2,3]. Various phytoconstituents with pharmacological activities have been isolated from extracts of *D. erecta*. Isolated triterpenes, β-

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Amyrin and 12-Oleanene 3β, 21β-diol from chloroform fraction with anti-shigellosis and cytotoxic potency has been reported [4]. Previous studies have isolated flavonoids namely acacetin, diosmetin, apigenin, luteolin and quercetin from the leaves [5]. Two C-alkylated flavonoids, two C-tropane type of triterpenes, and flavonoid as 3,5,4'trihydroxy-6,7-dimethoxyflavone produced from the plant has been reported by Ahmad et al. [6]. Hiradate et al. [7] isolated durantanin-I, II, and III from the leaves of *D. erecta* which are plant growth inhibitors. Coumarins isolated from fruits has been reported to have thrombin inhibitory effect [8]. The biological significance of *D. erecta* is due to the presence of various bioactive compounds.

In traditional medicine, the plant has been used to treat many diseases. The fruits and leaves are used as vermifuge and diuretic. The fruits and leaves are used in the treatment of intestinal worms [9] and abscess [10] respectively. The ethyl acetate soluble fraction of *D. erecta* methanol extract showed antiviral activity [11]. The antioxidant [2], antifungal [12] activities of *D. erecta* have been previously reported. Further, the acute and subacute hepatoprotective effect of leaves and ripe fruit extract has been demonstrated in animals [13,14]. In spite of its medicinal value, the safe use of this plant has been engrossed in controversies since a variety of the plant was reported to be poisonous to pigs [15].

The safety evaluation of hydroethanolic leaves extract of *D. erecta* has been reported previously and demonstrated to be safe in animals [13]. There is paucity of information on toxicological screening of fruits of the plant and considering its use in traditional medicine, there is the need to carry out extensive toxicological investigation on its crude fruit extracts. Therefore, the aim of this study was to evaluate the acute and subacute toxicity of hydroethanolic extracts of unripe and ripe fruits of *Duranta erecta* in animals (Figure 1).

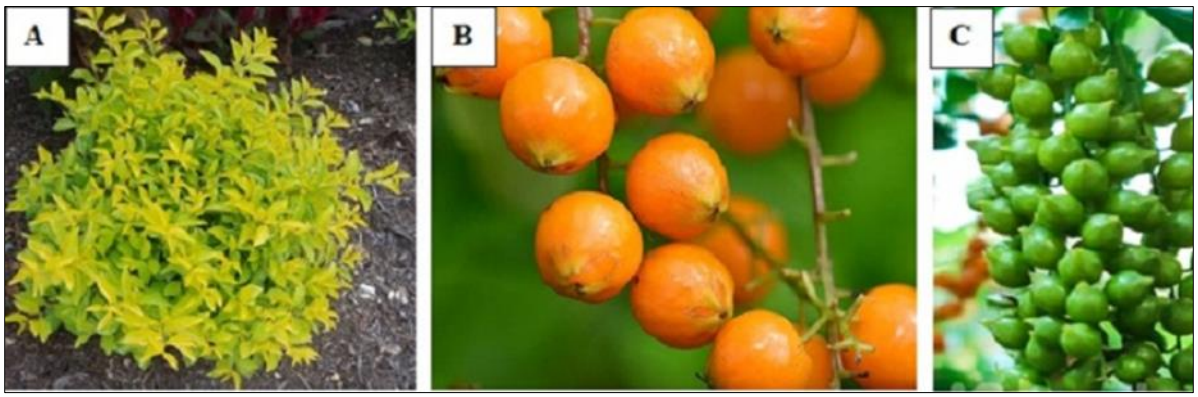

Figure 1 *Duranta erecta* (A) Whole plant (B) ripe fruits (C) Unripe fruits

2. Material and methods

2.1. Plant preparation and extraction

Ripe and unripe fruits of *Duranta erecta* were collected from KNUST campus and authenticated at the Department of Herbal Medicine, Faculty of Pharmaceutical Sciences, KNUST (voucher number KNUST/HM1/2017/L011) and treated as described earlier [2]. Ripe and unripe fruits samples (60 g) were milled and extracted with 50% ethanol (600 ml) for 48 hours. The mixtures were filter and evaporated under reduced pressure using rotary evaporator (Buchi R-205. Switzerland). The extracts were dried using vacuum freeze dryer (Heto PowerDry LL3000, UK) to obtain hydroethanolic extracts of *D. erecta* ripe (DRR) and *D. erecta* unripe (DRU). The qualitative phytochemical composition was determined and reported earlier using standard methods [2,16]. The total phenolic. total tannin, total flavonoid contents and antioxidant activities were evaluated as previously described [2]. Data are reproduced as Tables 1 and 2.

2.2. GC-MS analyses of methanolic extracts

The methanolic extract of ripe and unripe fruits of *D. erecta* was prepared by extraction 1 g of plant material with HPLC grade methanol and analysed using the GC-MS to identify the major compounds present. GC-MS analyses of the samples was performed using a PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm DF). The oven temperature was programmed from 100°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C and holding for 22 min at 280°C. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.9999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 1 µL was employed. The injector temperature

was maintained at 250°C, the ion-source temperature was 220 °C. Mass spectra were taken at 70 eV; a scan interval of 1 s and fragments from 50 to 500 Da. The solvent delay was 0 to 3 mins, and the total GC-MS running time was 50 min respectively. The mass-detector used in this analysis was Turbo-Mass, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-6.1.0. Interpretation of mass-spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns.

2.3. Animals

The experiment was performed using Sprague-Dawley rats (65 – 110 g) of either sex. The animals were sourced from University of Ghana Medical School, Korle-Bu, Ghana and acclimatized in the animal holding facility of the Department of Biochemistry and Biotechnology, KNUST, Kumasi for 14 days prior to commencement of experiments. They were housed in aluminum cages, suitably bedded with wood shaving. They were maintained under standard laboratory conditions of temperature and humidity with 12-hour light and dark cycle. The animals had free access to standard feed (Mash, AGRICARE, Kumasi-Ghana) and tap water *ad libitum* except an overnight fast prior to commencement and at termination. The rats were grouped taking their body weights into consideration to achieve approximately equal conditions among the groups. 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 National Research Council's Guide for Care and Use of Laboratory Animals [17]. All animals were humanely handled during the experiment and study protocol reviewed and approved by a veterinarian from the School of Veterinary Medicine, KNUST, Kumasi.

2.4. Acute Toxicity Assessment

Albino mice of either sexes were used for the acute oral toxicity studies. The animals were put into four groups with three animals each: one control group and three treated groups. After an overnight fast, the control group received sterile distilled water while each treated group received 100, 1000, and 2500 mg/kg body weight (b.wt) administered orally with the aid of a feeding needle connected to syringe at stated doses dissolved in appropriate volume of normal saline. Doses were selected based on the fixed dose method [18]. The animals were observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days. The oral median lethal dose (LD_{50}) was calculated as the geometric mean of dose that caused 0% and 100% mortality, respectively. This was used to guide the selection of three doses (100, 250, and 500mg/kg b.wt) for subacute toxicity studies [19].

2.5. Design of subacute toxicity study

Twenty-one male and female rats (n=3) were used for the study. For each sex and extract, group 1 served as normal control and received normal saline daily for 28 days. Groups 2, 3 and 4 received 100, 250 and 500 mg/kg b.wt. respectively orally. Extracts were freshly prepared each day by dissolving in appropriate volume of normal saline in order not to exceed an administrable volume of 1 mL. The animals were observed daily for general signs of toxicity and mortality. The body weights of rats were taken at the first day (D0) and every 4 days afterwards to day 28. The percent change in body weight was calculated as a function of the initial body weight of the rats on day 0 using the formula:

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

Where Wn = weight of animals on respective days and Wo = weight on the first day (D0).

2.6. Blood collection and haematological profiling

At the end of the experimental period of 28 days, animals were fasted and sacrificed by ether anaesthetization. Incisions were made in the cervical region with the aid of a sterile blade and blood samples collected into EDTA bottles for haematological analyses using Sysmex Haematology Systems (USA). The haematological profile included red blood cell count (RBC), white blood cell count (WBC), 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).

2.7. Biochemical analysis

Five milliliters of blood were 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 Selectra E (Vital Scientific, Japan) and reagents from ELITECH (France). Parameters determined included alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γGT), total, direct and indirect bilirubin, proteins, creatinine, urea, total cholesterol (TC) and total triglycerides (Trigs).

2.8. Isolation and assessment of organs

The internal organs of the animals namely liver, kidney, heart, stomach, spleen, testes, or uterus were carefully dissected out, defatted, gently washed with normal buffered saline, and blotted dry with clean tissue paper. The organs were weighed to obtain absolute organ weight (AOW). The relative organ weights (ROW) were calculated for each of the rat using the formula:

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

Liver sections were fixed in 10% normal buffered formalin, dehydrated in graded alcohol and embedded in paraffin. 4 µm thick sections were obtained, mounted on coded glass slides, and stained with haematoxylin and eosin (H&E). The specimens were cover-slipped for light microscopic examination (at 100x and 400x) by a histopathologist in a blinded manner.

2.9. Statistical analysis

Statistical analysis was done using GraphPad Prism 8.0 (CA, USA). 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. Statistical significance was considered at $p<0.05$.

3. Results

3.1. Phytochemical Constituent

Table 1 shows the phytochemical data of unripe and ripe fruits of *D. erecta*. The fruits were all deficient in coumarins while the ripe fruits were deficient in alkaloids. Table 2 shows the quantitative phytochemical constituent (phenols, tannins, and alkaloids) and DPPH scavenging activity of extracts. Both ripe and unripe fruits showed similar levels ($p>0.05$).

Table 1 Phytochemical constituents of *D. erecta*

Test	Unripe fruits	Ripe fruits
Tannins	+	+
Glycosides	+	+
Alkaloids	+	-
Coumarins	-	-
Saponins	+	+
Flavonoids	+	+
Triterpenoids	+	+
Sterols	+	+

Present (+) or Not detected (-)

Table 2 Antioxidant activities of extracts

Simples	Total phenolic Content (TPC mg GAE/100g)	Total flavonoid content (TFC µg QE/100 g)	Total tannins content (TTC mg GAE/100g)	(DPPH %)
DRR	10.43±0.34	218.01±5.98	12.01±0.21	77.02
DRU	9.31±0.33	248±4,39	8.28±0.11	71.53

(Data shown in Tables 1 & 2 were previously published; Donkor et al. [2])

3.2. Chemical composition of methanolic extracts by GC-MS

The various compounds were identified by comparing with the National Institute of Standard Technology (NIST) database (Table 3 and 4). Figure 2 shows the spectra of the methanolic extract of fruits.

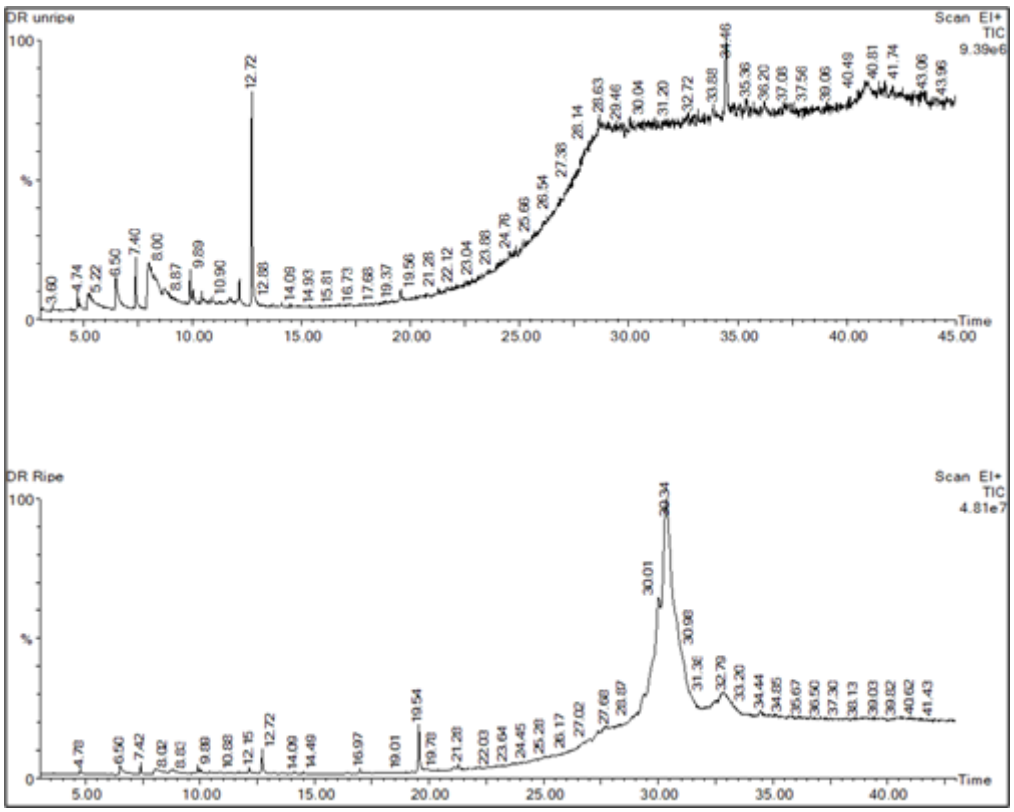

Figure 1 GC-MS spectra of methanolic extract of ripe and unripe fruit extract of *D. erecta*

Table 3 GC-MS analyses showing compounds in methanolic ripe fruit extract of *D. erecta*

SN	RT	Area	% Area	% Norm	SI	Chemical Constituent
1	4.777	88,163.7	3.069	17.47	85.33	1-Undecene, 8-methyl-
2	6.500	326,422.8	11.363	64.70	95.35	Ethanone,1-(2-hydroxy-5-methylphenyl)-
3	7.417	88,100.8	3.067	17.46	87.54	3-Tetradecene (E)-
4	8.077	319,763.9	11.132	63.38	91.87	d-Glycero-d-ido-heptose
5	8.756	234,240.9	8.154	46.43	77.39	D-Glycopyranose, 1,6-anhydro-
6	9.892	68,748.4	2.393	13.63	88.76	3-Hexadecene (Z)-
7	10.039	77,732.7	2.706	15.41	84.75	2,3-Dihydro-2-methylthiazzolo(3,2-a) benimidazole
8	10.424	26,576.2	0.925	5.27	91.98	Benzenepropanoic acid, 4-[2,4-dinitrophenyl)azo] methyl ester
9	10.883	26,023.4	0.906	5.16	73.07	3-(2,4-Dimethoxyphenyl)buta-2-one
10	11.689	26,954.6	0.938	5.34	97.80	4-methyl-3-3oxo-(1-oxa-4-dyl]- diacetate (5a) Pregnane-3,20a-diol
11	12.148	66,464.2	2.314	13.17	97.52	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-
12	12.716	268,461.4	9.346	53.21	99.43	4,7-Dimethoxy-2-methylindian-1-one
13	14.091	35,290.8	1.229	6.99	77.37	n-Hexadecanoic acid

14	16.420	29,891.6	1.041	5.92	94.79	8,14-Secor-3, 19-epioxyandrostane-8, 14-dione, 17-acetoxy-3a-methoxy-4-4-dimethyl
15	16.970	58,782.5	2.046	11.65	85.62	Octadecanamide
16	17.098	31,859.1	1.109	6.31	99.03	Carotene,3,3',4-4'-tetrahydro-1',2'-dihydro-1-hydroxy-1-methoxy
17	19.537	504,535.6	17.564	100.00	98.78	9-Octadecenamide (Z)-

Table 4 GC-MS analyses showing compounds in methanolic unripe fruit extract of D. erecta

SN	RT	Area	% Area	% Norm	SI	Chemical Constituent
1	4.740	29,266.6	1.109	4.11	83.98	3-Tetradecene, (E)
2	4.814	22,464.5	0.851	3.16	95.03	Oleic acid, ecosyl ester
3	5.272	168,546.0	6.384	23.68	91.55	Benzofuran, 2,3-dihydro
4	5.730	35,544.2	1.346	4.99	88.0	4-Peperidineaceic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-a-methy, methylester
5	6.500	193,942.2	7.346	27.24	96.18	2-Methoxy-4-vinylphenol
6	7.399	82,732.3	3.134	11.62	87.87	3-Trifluoroacetoxypentadecane
7	8.004	711,875.1	26.963	100.00	97.24	2-Propenoic acid, 3-phenyl-
8	8.719	302,598.3	11.461	42.51	58.18	-
9	9.892	57,444.3	2.176	8.07	84.47	Hexadecen-1-ol
10	10.039	47,170.8	1.787	6.63	81.11	6-Methoxt-3-methyl-2-2benzofuran carbaldehyde
11	10.424	33,793.6	1.280	4.75	90.57	1H-Inden-1-dihydro-5,6-dimethoxy-3-methyl-
12	10.553	18,519.5	0.701	2.60	94.77	Olean-12-ene-3-15,16,21,22,28-hexol
13	10.901	21,986.1	0.833	3.09	77.00	1-(4-Nitrophenyl)pienazine
14	11.726	30,089.3	1.140	4.23	91.89	4-Hydroxy-4-(1-methoxycyclopropyl)-3,35,8,10.10-hexamethyltricyclo[6,2,2(2,7)]dodeca-5,11-die
15	12.148	51,218.3	1.940	7.19	95.40	1-Tricosanol
16	12.716	425,049.8	16.099	59.71	100	4,7-Dimethyoxy-2-methylindan-1-one
17	19.555	35,214.3	1.334	4.95	84.84	9-Octadecenamide, (Z)

3.3. Acute Toxicity of DRR and DRU

Hydroethanolic fruit extracts of *D. erecta* had no mortality or significant behavioural changes up to 2500mg/kg b.wt. in mice. Therefore, the LD_{50} is estimated at $LD_{50} \geq 2500$ mg/kg b.wt. in mice. It is therefore considered as safe.

3.4. Effect of treatment on body weight

The effect of crude extract in the subacute study on the body weight of animals is shown in Figures 3 and 4 and Table 5. 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 with 100 mg/kg DRR. However, there were significant increase ($p<0.05$) in percent body weight changes in male rats treated with 250 mg DRR on day 24 and 28. For female rats, significant increases were observed at 100 mg DRR on day 12 ($p<0.05$), 16 ($p<0.01$), as well 20, 24, and 28 ($p<0.0001$) compared with the normal (Figure 3). Animals gained weight and appeared active and normal.

Sub-acute treatment with DRU caused significant difference in the pattern of weight gain in both sexes of the rats when compared with normal. There were significant increases in percent body weight changes in male rats treated with 100 mg DRU on day 8, 12, 16, 20, 24 and 28 *p<0.0001*); 250 and 500 mg DRU on day 12, 16, 20, 24 and 28 (*p<0.0001*). In the female rats, significant increases were recorded at 100 mg DRU on day 12 (*p<0.01*), 16, 20, 24 and 28 (*p<0.0001*); 250 mg on day 20 (*p<0.05*), 24 (*p<0.01*) and 28 (*p<0.05*) (Figure 4).

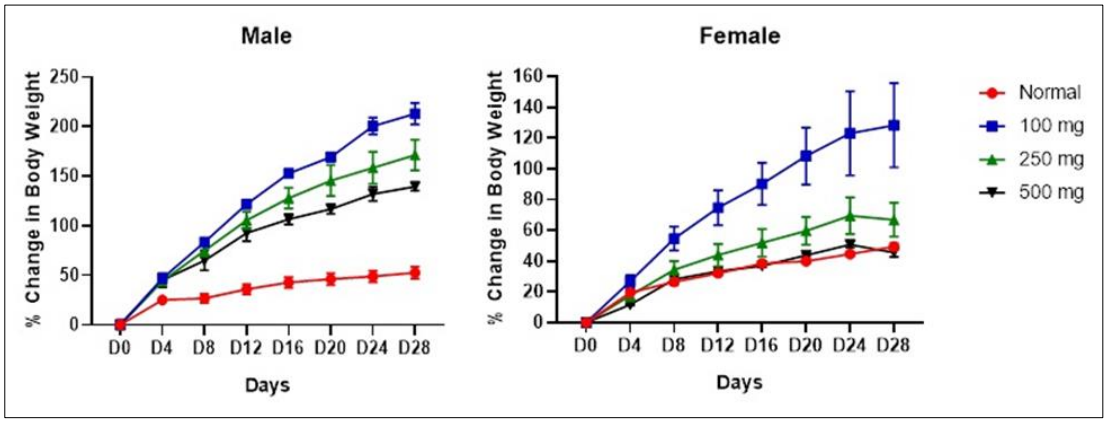

Figure 3 Percent change in body weight of normal and DRR treated animals. Each point represents a mean ± standard error of mean (SEM)

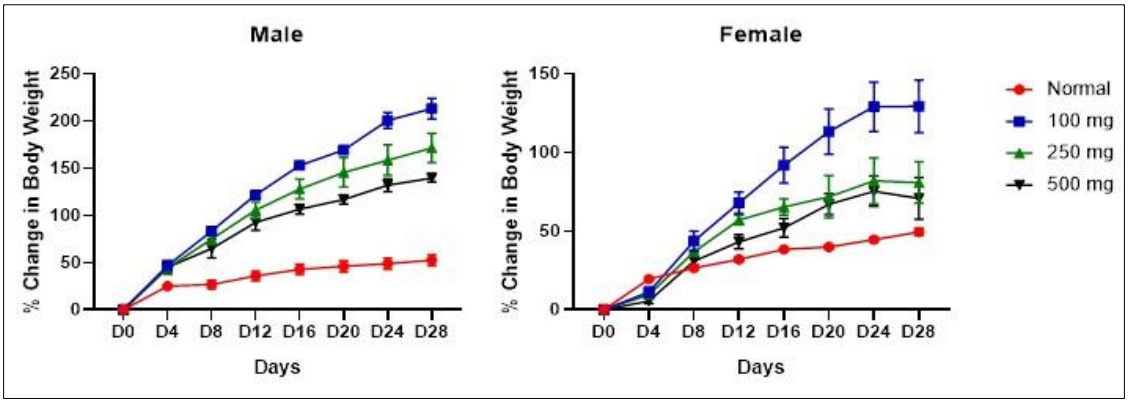

Figure 4 Percent change in body weight of normal and DRU treated animals. Each point represents a mean ± standard error of mean (SEM)

Table 5 Effect of treatment of percent change in body weight at termination (D28)

		Male	Female
Normal		52.64±5.85	49.40±2.24
DRR	100 mg	82.91±21.61	128.38±27.43[d]
	250 mg	90.62±12.89[a]	66.93±11.00
	500 mg	51.01±4.94	45.50±2.82
DRU	100 mg	213.02±10.74[d]	129.30±16.64[d]
	250 mg	171.43±15.41[d]	80.84±13.10[a]
	500 mg	139.57±4.13[d]	70.76±13.16

Statistical difference: a (p<0.05), and d (p<0.0001) from Normal control

3.5. Effect of treatments on relative organ weight (ROW) of animals

Treatment with DRR at all doses did not cause any significant (*p<0.05*) changes in the ROW of the kidney, lung and heart of male rats compared with the normal control. However, treatment with 100 mg/kg DRR caused significant increases in liver (*p<0.0001*), spleen (*p<0.05*), testes (*p<0.05*); 250 mg/kg DRR on liver (*p<0.05*); 500 mg/kg DRR on liver *(p<0.0001*) and spleen (*p<0.05*) relative to normal control. Similarly, in the female rats, significant increases were observed in 100 mg/kg DRR on liver (*p<0.0001*), womb (*p<0.01*), 250 mg/kg DRR liver (*p<0.0001*), spleen (*p<0.05*) and 500 mg/kg DRR liver (*p<0.01*) versus normal control (Table 6). The effect of DRU on ROW is shown in Table 6. No significant change in relative weights of testes, lung, stomach, heart, testes, kidney, uterus, and spleen of rats was observed. There was, however, significant increase (*p<0.0001*) in the relative liver weight of treated male rats at all doses when compared with that of the normal control. Treatment with DRU at all doses on female rats produced significant increase (*p<0.0001*) in the relative liver weight compared with normal control.

Table 6 Effect of treatment on relative organ weights (ROW) of animals

		DRR			DRU		
ROW (%)	**Normal**	**100 mg**	**250 mg**	**500 mg**	**100 mg**	**250 mg**	**500 mg**
MALE							
Liver	2.93±0.13	3.83±0.34^{d}	3.53±0.17^{b}	4.24±0.18^{d}	3.99±0.10^{d}	3.91±0.18^{d}	4.29±0.15^{d}
Kidneys	0.69±0.04	0.88±0.06	0.71±0.05	0.79±0.04	0.79±0.03	0.76±0.01	0.79±0.03
Spleen	0.34±0.00	0.81±0.06^{a}	0.57±0.05	0.90±0.13^{b}	0.47±0.07	0.48±0.07	0.48±0.04
Lung	0.76±0.04	0.99±0.04	0.930.09±	0.88±0.03	0.82±0.04	0.80±0.02	0.88±0.07
Testes	1.20±0.09	1.67±0.14^{a}	1.25±0.13	1.60±0.02	1.31±0.03	0.99±0.31	1.45±0.11
Heart	0.51±0.16	0.38±0.01	0.32±0.01	0.35±0.02	0.40±0.01	0.36±0.02	0.37±0.01
Stomach	0.75±0.09	0.74±0.07	0.73±0.05	0.82±0.09	0.85±0.07	0.81±0.01	0.93±0.09
FEMALE							
Liver	2.77±0.05	3.97±0.33^{d}	3.74±0.32^{d}	3.47±0.28^{b}	3.39±0.27^{c}	3.35±0.20^{c}	3.47±0.30^{d}
Kidneys	0.71±0.01	0.74±0.01	0.79±0.01	0.87±0.05	0.87±0.04	0.86±0.02	0.84±0.05
Spleen	0.40±0.03	0.62±0.15	0.90±0.07^{a}	0.62±0.05	0.59±0.15	0.46±0.02	0.57±0.11
Lung	0.82±0.10	0.74±0.08	1.04±0.06	1.07±0.03	1.12±0.02	1.00±0.10	0.97±0.08
Womb	0.31±0.02	0.94±0.31^{b}	0.63±0.12	0.44±0.05	0.42±0.01	0.43±0.06	0.48±0.05
Heart	0.38±0.02	0.36±0.01	0.38±0.01	0.37±0.00	0.38±0.02	0.44±0.01	0.37±0.00
Stomach	0.74±0.05	0.77±0.02	0.81±0.06	0.83±0.06	0.86±0.02	0.83±0.06	0.77±0.02

Statistical difference: a (p<0.05), b (p<0.01), c (p<0.001) and d (p<0.0001) from Normal control

3.6. Effect on haematological indices

Significant decreases (*p<0.0001*) were observed in platelet levels in the male rats at all doses whilst significant decrease (*p<0.0001*) and increase (*p<0.0001*) were recorded in female rats treated with DRR at doses 250 mg and 500 mg/kg respectively (Table 7). Treatment with DRU at all doses resulted in significant decline (*p<0.0001*) in the platelet levels in the male rats, whilst significant increase (*p<0.0001*) was observed at high dose 500 mg/kg in the female rats. However, there was no significant effect on other parameters at all doses tested. All other determinations showed no significant difference from normal control except some decreases in RBC levels.

3.7. Effect on biochemical indicators

The effect of treatment on biochemical indicators is summarized is in Table 8. Male and female animals treated with DRR and DRU showed significant increases in liver functions parameters (ALT, AST, ALP and Bilirubin) at all doses. Also, dose-unrelated decreases (p<0.05) were recorded for Creatinine and Urea from both male and female animals receiving DRR and DRU. Extract treatments however had no significant effects on the serum proteins and lipid profile.

Table 7 Effect of treatment on haematological parameters of animals

		DRR			DRU		
	Normal	**100 mg**	**250 mg**	**500 mg**	**100 mg**	**250 mg**	**500 mg**
MALE							
WBCx103/µL	10.13±1.65	16.43±4.15	18.57±1.59	21.83±2.03	9.80±1.32	16.63±2.34	13.40±2.62
RBCx106/µL	8.37±0.51	6.99±0.52	7.52±0.17	6.45±0.73	6.53±0.54	7.76±0.16	8.20±0.06
HGB g/dL	14.00±0.96	12.10±0.57	13.03±0.43	12.63±1.13	11.93±1.19	14.03±0.30	15.17±0.33
HCT %	48.73±3.84	44.33±2.23	46.50±1.40	43.20±4.39	41.90±4.43	48.70±0.81	52.90±2.21
MCV/ Fl	58.07±1.09	63.63±1.59	61.90±1.07	67.20±0.85	63.90±1.70	62.63±0.81	64.50±0.51
MCHC g/Dl	16.70±0.12	27.33±0.75	28.07±1.05	29.10±0.36	18.23±0.39	18.03±0.23	18.50±0.40
MCH pg	28.77±0.33	17.37±0.65	17.40±0.78	19.60±0.45	28.53±0.19	28.83±0.13	28.70±0.50
PLT 10^3/µl	1014.67 ±80.42	517.00 ±76.70[d]	768.00 ±20.22[d]	389.00 ± 79.13[d]	388.00 ±21.22[d]	764.67 ±112.67[d]	679.67 ±131.56[d]
LYM%	86.67±2.23	77.10±3.18	69.33±5.49	78.47±0.45	76.60±2.50	77.17±1.13	75.20±2.39
LYMx103/µl	8.77±1.33	12.90±3.73	13.00±2.01	17.10±1.50	7.53±1.13	12.83±1.79	10.20±2.32
RDW-SD/Fl	39.53±1.27	39.47±1.84	34.33±0.44	39.43±1.19	38.70±1.51	37.00±1.68	36.23±0.58
RDW-CV/%	19.47±0.58	16.50±1.64	14.10±0.21	15.60±0.36	15.67±1.19	15.33±0.94	14.60±1.27
P-LCR/%	11.70±0.58	9.73±1.68	6.43±0.26	10.50±1.28	9.73±0.30	8.63±0.18	8.33±0.07
PDW/Fl	8.33±0.12	9.33±0.30	8.30±0.12	9.67±0.28	7.70±0.15	7.80±0.70	7.23±0.07
MPV/Fl	16.23±1.23	7.73±0.26	7.07±0.03	7.57±0.20	9.50±1.47	7.40±0.96	7.17±0.39
PCT (%)	0.85±0.08	0.40±0.06	0.49±0.03	0.29±0.06	0.30±0.02	0.56.07±0.01	0.50±0.09
FEMALE							
WBCx103/µL	11.53±0.58	16.97±2.20	17.73±2.65	17.40±2.00	13.90±3.33	9.23±1.14	12.60±0.25
RBCx106/µL	8.64±0.06	6.97±0.70	5.76±0.49	6.97±0.49	6.87±0.21	7.26±0.54	7.45±0.26
HGB g/dL	15.20±0.12	13.73±0.74	11.83±0.97	13.55±0.95	13.67±0.35	14.13±0.86	14.13±0.26
HCT %	50.30±0.17	45.63±3.14	40.07±3.74	43.15±2.45	44.83±1.19	46.933.27 ±3.27	46.37±0.73
MCV/ Fl	58.20±0.46	65.93±2.25	69.53±3.00	61.95±0.85	65.27±0.46	64.70 ±0.38	62.20±0.45
MCHC g/Dl	17.60±0.06	30.13±0.42	29.60±0.40	31.40±0.40	19.90±0.10	19.53±0.72	19.00±0.32
MCH pg	30.20±0.31	19.90±0.91	20.60±0.76	19.40±0.00	30.47±0.22	30.20±0.96	30.47±0.32
PLT 10^3/µl	661.33 ±36.54	606.67 ±93.49	427.33 ±35.71[d]	791.50 ±119.50[d]	603.00 ±7.02	645.00 ±182.98	928.00 ±47.25[d]
LYM%	82.70±3.98	74.27±4.19	78.40±0.15	74.20±3.10	77.00±2.41	70.77±1.18	65.73±3.03
LYMx103/µl	9.53±0.71	12.77±2.22	17.47±0.12	12.95±2.05	10.73±2.74	6.57±0.86	8.30±0.55
RDW-SD/Fl	36.77±2.42	36.13±0.84	37.50±2.71	32.05±0.55	33.30±0.58	34.40±0.10	34.30±0.61
RDW-CV/%	17.63±1.31	13.77±0.52	13.63±0.70	12.55±0.41	12.50±0.40	13.23±0.29	14.10±0.50
P-LCR/%	11.77±0.77	8.47±1.45	10.80±1.20	6.35±0.35	8.87±0.18	8.97±0.18	8.80±0.17
PDW/Fl	8.07±0.03	8.97±0.34	9.37±0.07	8.30±0.40	7.47±0.09	7.47±0.09	7.33±0.12
MPV/Fl	13.97±0.26	7.43±0.18	7.80±0.20	7.05±0.35	8.60±0.56	8.77±0.71	8.23±0.88
PCT (%)	0.53±0.03	0.45±0.08	0.34±0.04	0.56±0.05	0.45±0.01	0.48±0.13	0.68±0.03

Statistical difference: d ($p<0.0001$) from Normal control

3.8. Effect of Treatments on Liver Histology

There were no observable lesions in all the groups. They exhibited normal hepatocytes with some cells showing higher numbers of vacuoles in the cytoplasm (Figure 5).

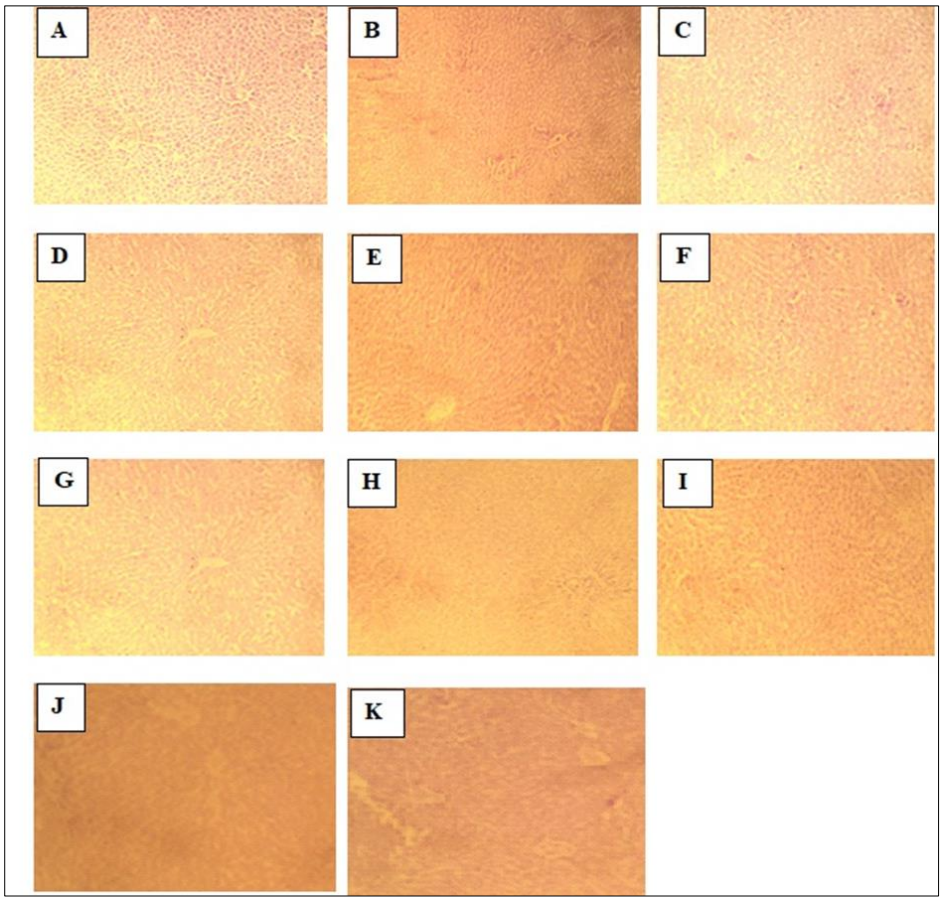

Figure 5 Photomicrographs of liver from rats administered orally for 28 days with: (A) distilled water (control), (B) 100 mg/kg b.wt of ripe (male), (C) 250 mg/kg b.wt of ripe (male), (D) 500 mg/kg b.wt of ripe (male), (E) 100 mg/kg b.wt of ripe (female), (F) 250 mg/kg b.wt of ripe (female), (G) 100 mg/kg b.wt of unripe (male), (H) 250 ml/kg b.wt. of unripe (male), (I) 500 mg/kg b.wt of unripe (male), (J) 250 mg/kg b.wt of unripe (female), (K) 500 mg/kg b.wt of unripe (female). Photomicrograph (A) – (K) shows normal hepatocytes with no observable lesion (H&E x 400)

Table 8 Effect of treatments on some biochemical parameters of animals

		DRR			DRU		
	Normal	**100 mg**	**250 mg**	**500 mg**	**100 mg**	**250 mg**	**500 mg**
MALE							
ALT (U/L)	45.37 ±2.45	95.87 ±6.12^{b}	72.07 ±5.09	93.87 ±5.25^{b}	72.97 ±3.09^{b}	71.77 ±6.15^{b}	81.97±4.28^{b}
AST (U/L)	296.43 ±16.21	450.63 ±19.66^{d}	320.60 ±13.72	418.70 ±12.66^{d}	327.77 ±39.04	358.83 ±34.69^{c}	345.13±10.25^{a}
ALP (U/L)	237.10 ±12.29	478.17 ±36.10^{d}	494.37 ±55.72^{d}	389.90 ±18.82^{c}	465.63 ±52.22^{d}	424.07 ±11.35^{d}	450.27±14.50^{d}
GGT (U/L)	3.23±0.43	3.07±1.62	4.10±0.35	3.53±0.52	2.40±0.67	2.93 ±0.47	2.10±0.20
TBIL (mmol/L)	3.27±0.66	10.27±1.84^{d}	11.82±0.97^{c}	7.49±0.29^{b}	9.61±0.22^{b}	7.95 ±0.38^{b}	8.72±0.46^{b}
DBIL (mmol/L)	1.37±0.20	1.50±0.34	2.54±0.26	1.71±0.29	1.65±0.47	1.20 ±0.05	1.46±0.09
IBIL (mmol/L)	1.90±0.51	8.57±1.30^{d}	9.30±0.79d	5.80±2.35^{c}	7.97±0.69^{c}	6.73 ±0.33^{c}	7.27±1.24^{c}
TP (mmol/L)	77.30±4.55	69.27±1.30	68.27±0.92	72.90±0.06	64.97±1.01	65.53 ±1.48	66.17±1.24
ALB (mmol/L)	49.37±2.47	32.07±0.47	33.40±0.93	33.73±0.49	34.23±0.98	34.83 ±0.38	34.33±1.52
GLO (mmol/L)	27.93±2.19	37.20±1.43	34.87±0.60	39.17±0.46	30.73±1.92	30.70 ±1.71	31.83±1.03
CREAT (umol/L)	39.77±1.61	25.38±8.59^{a}	32.17±5.44	21.39±2.38^{a}	14.73±5.88^{b}	22.36 ±6.89^{b}	18.15±6.81^{b}

UREA (mmol/L)	7.37±0.18	3.50±0.40[b]	4.36±0.57[a]	2.81±0.75[b]	3.65±1.60[a]	4.32 ±1.79[a]	5.42±1.70[a]
TC (mmol/L)	1.90±0.03	1.27±0.10	1.49±0.07	1.37±0.15	1.12±0.04	1.31 ±0.10	1.30±0.07
TG (mmol/L)	0.64±0.05	0.78±0.14	0.67±0.10	1.04±0.07	0.71±0.14	0.98 ±0.16	0.95±0.09
FEMALE							
ALT (U/L)	77.13±5.68	66.70±2.45	70.53±3.58	68.55 ±5.60	60.50 ±2.55	70.40 ±6.81	81.57±10.49
AST (U/L)	289.37 ±14.19	366.07 ±25.07	392.87 ±9.62[a]	387.60 ±5.60[a]	337.93 ±19.95	324.60 ±6.03	406.67±24.88[a]
ALP (U/L)	232.20 ±5.20	351.27 ±33.91[a]	284.30 ±43.85	281.45±2.85	289.63±54.41	231.43 ±9.36	316.17±73.73[a]
GGT (U/L)	1.23±0.07	2.67±0.23[b]	2.67±0.33[b]	3.50±0.20[b]	3.37±0.69[b]	1.83 ±0.35	3.77±0.03[b]
TBIL (mmol/L)	0.59±0.13	8.00±0.58[d]	8.68±0.37[d]	9.41±0.85[d]	10.84±0.67[d]	8.53 ±0.78[d]	9.63±0.98[d]
DBIL (mmol/L)	0.18±0.05	1.20±0.20[b]	1.69±0.20[b]	0.88±0.11[a]	1.02±0.36[b]	1.02 ±0.04[b]	1.20±0.24[b]
IBIL (mmol/L)	0.41±0.15	7.07±1.00[d]	7.00±0.57[d]	8.55±0.75[d]	9.87±0.32[d]	7.53 ±0.83[d]	8.43±0.74[d]
TP (mmol/L)	66.33±2.76	66.27±1.34	68.93±1.51	68.95±0.25	71.57±1.63	69.07 ±1.36	68.33±1.40
ALB (mmol/L)	33.37±1.71	34.13±0.58	34.60±1.12	31.95±0.55	33.80±0.68	35.00 ±0.15	33.27±0.53
GLO (mmol/L)	32.97±1.27	32.03±0.86	34.33±0.38	37.00±0.80	37.77±2.20	34.17 ±1.11	35.07±1.15
CREAT (umol/L)	46.40±2.43	29.90±4.05[a]	27.61±10.89[a]	31.11±13.81[a]	34.67±3.10[a]	33.34 ±6.85[a]	36.68±9.91[a]
UREA (mmol/L)	6.94±0.27	3.87±0.51[a]	3.24±1.17[a]	3.44±1.23[a]	3.65±0.76[a]	4.74 ±0.87[a]	3.49±0.87[a]
TC (mmol/L)	2.21±0.16	1.67±0.12	1.47±0.22	1.19±0.04	1.46±0.25	1.26 ±0.03	1.44±0.15
TG (mmol/L)	0.83±0.02	0.89±0.08	0.80±0.14	1.22±0.06	0.74±0.11	0.77 ±0.06	0.76±0.05

Statistical difference: a ($p<0.05$), b ($p<0.01$), c ($p<0.001$) and d ($p<0.0001$) from Normal control

4. Discussion

Medicinal plants which are rich source of diverse bioactive compounds, have been used for the prevention and treatment of myriad of diseases worldwide and thus provide unlimited opportunities for the discovery of novel drugs. Natural products and for that matter herbal remedies have been generally considered safe because of their natural origin. Nevertheless, some natural substances are potentially toxic and may be harmful to human [20]. Therefore, the need to carry out systematic safety studies on medicinal products that are natural based cannot be overemphasized as safety assessment does not only help to identify dosage regimens but also reveal possible adverse effects associated with the substance under investigation. The results of phytochemical screening showed the presence of triterpenoids, sterols, alkaloids, flavonoids, saponins, glycosides, and tannins; the presence of these phytochemicals account for their multipurpose in folk medicine.

GC-MS analysis has been used to identify compounds and compare their concentrations within samples. The GC-MC results revealed the presence of various compounds (Tables 3 and 4) which have synergistic effect in disease management and control. The methanolic ripe fruits extract was principally 9-Octadecenamide (Z)- which is reported to be antiinflammatory, whilst that of the unripe fruits were 3-phenyl- 2-Propenoic acid, reported to have anticoagulant properties. However biological activity of 4,7-Dimethyoxy-2-methylindan-1-one is unknown [21]. Alterations in body weight are used as parameters to evaluate the toxicity of drugs [22]. In this study, the general gain in weight of rats treated with the extracts at all doses, is an indication that the extracts did not interfere with normal metabolism and stability of appetite leading to increase in food and water intake which is responsible for augmentation of body weight gain [23]. The results of no significant changes in the relative organ weight of kidney, heart, lung, spleen, stomach, testes, and womb of control and treated groups which showed that none of the organs were adversely affected the administration of extracts in both sexes. Organ weight changes have been used as a sensitive indicator of chemically induced changes to organs [24]. The decrease of organ indices indicates that the organs are degenerated whilst increase of organ indices possibly show that the organs are proliferative, and hypertrophic [25]. The significant increase in relative liver weight at all doses in both female and male rats is an indication of possible adverse reaction on the liver.

The estimation of haematological indices provides physiological information on a proper blood assessment in the body. The non-significant change observed in almost haematological parameters with the exception platelet (Table 7) showed that extracts are safe on haemopoietic system of the rats which is one of the most sensitive targets of toxic compounds. Interestingly, the significant reduction in number of platelets at almost all doses may be a sign of hepatopathy. However,

significant increase in platelet (*p<0.0001)* in female rats treated with 500 mg/kg means the extracts at high dose can be exploited in the management and treatment of liver fibrosis. The underlying mechanism between platelets and prognosis of liver fibrosis is well documented [26–28]. Platelets improve liver fibrosis by inactivating hepatic stellate cells, which decreases collagen production and deposition of extracellular matrix [29].

Serum biochemical markers provide valuable information on the toxic effect of test substance to the physiological status within the body [30]. The administration of extracts produced some biochemical markers that were altered in the treatment groups compared with normal (Table 8). ALT and AST are the fundamental indicators of liver injury [31] and ALP is also marker for liver and gallbladder diseases, especially the obstruction of common bile duct [32]. Total, conjugated and unconjugated bilirubin is an important biomarker for hepatocellular and secretory functions of the liver and hence hepatic impairment [33]. Creatinine and urea are good indicators for kidney functions [34]. Significant increase in the levels of liver enzymes AST, ALT and ALP indicate the extracts have deleterious effect on the liver and hence liver function impairment. Similarly, biochemical changes observed in total, conjugated and unconjugated bilirubin concentration suggest a possible hepatic injury emanating from failed ability of the hepatocytes to conjugate and clear bilirubin. However, the histopathological studies showed evidence of safety associated with the use of the fruit extracts. Hepatocytes showed normal appearance. Overall, histopathological evidence showed that the fruit extracts were safe, though the biochemical indicators showed some level of adverse reactions. The effect of the extracts on serum creatinine and urea levels showed a remarkable decrease in both male and female compared with normal which suggest possible nephroprotective activity of the extracts especially by improvement of renal filtration mechanism.

5. Conclusion

The results suggest that subacute oral treatment with hydroethanolic fruit extracts of *D. erecta* tend to have adverse effect on the liver and thus for long time usage liver function should be monitored. The fruit extracts also exhibited nephroprotective activity.

6. Abbreviations

- DRR: *Duranta erecta* ripe
- DRU: *D. erecta* unripe
- GC-MS: Gas chromatography–mass spectrometry
- LD: Lethal dose
- AOW: Absolute organ weight
- ROW: Relative organ weight
- RBC: Red blood cell count
- WBC: White blood cell count
- HGB: Haemoglobin concentration
- MCV: Mean corpuscular volume
- MCH: Mean corpuscular haemoglobin
- MCHC: Mean corpuscular haemoglobin concentration
- LYM: Lymphocyte
- PLT: Platelet
- HCT: Haematocrit
- RDW: Red Cell Distribution width
- MPV: Mean platelet volume
- NEUT: neutrophil
- PCT: Plateletcrit
- P-LCR: Platelet large cell volume
- PDW: Plate volume distribution width
- ALT: Alanine aminotransferase
- AST: Aspartate amino transferase
- ALP: Alkaline phosphatase
- γGT: Gamma glutamyl transferase
- TBIL: Total bilirubin
- DBIL: Direct bilirubin
- IBIL: Indirect bilirubin
- TP: Total protein

- GLO: Globulin
- ALB: Albumin
- CREAT: creatinine
- TC: Total cholesterol
- TRIGS: Total triglycerides
- TPC: Total phenolic content
- TFC: Total flavonoid content
- TTC: Total tannins content

Compliance with ethical standards

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Authors' contribution

All authors equally contributed to preparing this article

Disclosure of conflict of interest

None Declared

Statement of ethical approval

The study was conducted following guidelines as stipulated by 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 Animal. All animals were humanely handled per protocol reviewed and approved by a veterinarian at the School of Veterinary Medicine, KNUST.

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