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TOXICOLOGICAL EVALUATION OF TWO FRACTIONS FROM *Annona senegalensis* ROOT BARK

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Abstract

Annona senegalensis root bark is widely used as a medicinal plant for the treatment of a wide array of diseases. This study evaluated the safety of solvent fractions obtained from its aqueous root bark extract. Crude aqueous extract was partitioned into hexane, dichloromethane and ethylacetate fraction by solvent-solvent fractionation. Albino rats were administered 100, 200 and 400 mg/kg b.wt. Dichloromethane and ethylacetate fractions for 14 days. The effect of their administration on liver, kidney, antioxidant enzymes activities, lipid peroxidation and heamatological parameters was investigated. Both fractions significantly increase alanine transaminase activity, Na^+ and K^+ concentration, glutathione peroxidase activity and malondialdehyde concentration. They fraction significantly decreased urea concentration and serum superoxide dismutase activity. Dichloromethane fraction significantly increase the PCV while there was no significant difference in all heamatological parameters of rats treated with ethylacetate fraction from aqueous root bark (EFAR) when compared with the control. The two fractions are toxic and should be used with caution.

INTRODUCTION

Plants are being used for various purposes which include their use for food and medicines[1]. Plants produce various biochemicals that support and promote human health [1,2]. In some communities, medicinal plants are the only therapeutic source [3,4]. Traditional/herbal medicine are being considered as safe because of their natural origin [5]. Despite this belief, cases of their toxicity, contamination and adulteration are being reported [6,7]. Therefore, there is need for toxicological evaluation of plants [2]. Toxicity of plants depends on several factors which include; the quantity of the phytochemicals, the time of exposure, the part of the plant, individual response to the phytochemical, genetic differences in the specie, soil and climate [8]. Information on the safety of several herbs used in developing countries particularly Africa is still scarce [9,10].

Annona senegalensis commonly called "wide custard apple" is a shrub widely distributed in Africa [11,12]. The fruit is consumed as food [13]. The anticonvulsant [14], antibacterial [15,16]; antitrypanosomal [17] and antihyperglycemic [18] activities of the plant have been reported. Specifically, the root bark has been reported to have antidiarrheal property [19,20], anti-inflammatory [21,22], antivenomous [12] and antiplasmodial/antimalarial [23] properties. Flavonoids, alkaloids, anthocyanes, coumarins, glucids, sterols and/or triterpenes are present in the root bark of *Annona senegalensis* [14]. The toxicity of several parts of the plant have been evaluated [14,24,25]. Ilbuodo *et al*. [26] investigated the toxicity of the root wood extract while Okoye *et al*. [24] investigated the toxicity of methanol- methylene chloride root bark extract. No study has been carried out on the aqueous extract of the root bark of *Annona senegalensis* nor on the fractions obtained from the aqueous extract. This study therefore, investigated the toxicity of fractions from the aqueous root bark extract of *Annona senegalensis*.

MATERIALS AND METHODS

Collection and Preparation of Plant

Fresh root barks of *Annona senegalensis* were collected between June, 2019 and August, 2019. The plant was authenticated at the Herbarium unit of the Department of plant biology, University of Ilorin, Ilorin, Nigeria and was assigned a voucher number UILH/001/449. The root bark was washed clean, shredded and air dried under shade to constant weight. It was pulverized using mortar and pestle into powder. The powdered samples were stored in airtight containers and kept at room temperature until required for use.

Extraction of Plant

The powdered root bark was soaked in water in the ratio 1: 10 for 24 hours at ambient temperature (35^0C) with vigorous shaking at 3 hours interval. The crude extract was filtered using Whatman number 1 filter paper. It was evaporated to dryness at 40^0C under reduced pressure.

Fractionation of Aqueous Root Bark Extract of *Annona senegalensis*

The aqueous extract was fractionated by solvent-solvent partitioning as described by the method designed by Kupchan *et al.* [27] and modified by Van-Wagener *et al*. [28]. Crude aqueous extract was successively partitioned with hexane, dichloromethane and ethyl acetate in order of increasing polarity. Dichloromethane fraction (DFAR) and ethylacetate fraction (EFAR) were collected and evaporated to dryness using a rotary evaporator at 4^0C. The dried fractions were stored in an airtight container until required for use.

Experimental Animals

Male adult rats weighing between 120-150 g were obtained from the Animal breeding unit of the department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in well ventilated aluminium cages and given standard laboratory diet and water adlibitum. The rats were handled according to the guidelines for the protection and handling of laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the ethical committee of the department of Biochemistry, University of Ilorin, Ilorin, Nigeria and was given an approval number UERC/ASN/2018/1216.

Experimental Design

A total of thirty five adult albino rats were divided into 7 groups. Group I served as control, groups II, III and IV received 100, 200 and 400 mg/kg b.wt of DFAR V, VI and VII received 100, 200 and 400 mg/kg b.wt of EFAS for 14 days respectively. The feed intake of each rat was recorded on a daily basis. The body weight of the rats were taken on the first day of the experiment and (initial weight) and prior to sacrifice (final weight). Change in body weight was calculated as the difference in the final weight and initial weight. The rats were observed for signs of toxicity and mortality throughout the experiment. The rats were sacrificed under anaesthesia on the 15^{th} day. Blood samples were collected into non-anti-coagulated and ethylenediaminetetraacetic acid (EDTA) sample bottles. The non-anti-coagulated blood was then centrifuged at 3,000 rpm for 10 min and serum was collected and used for biochemical assay. The blood in the EDTA sample bottles were used for heamatological analysis. The rats were quickly dissected. The heart, kidneys, liver and stomach were removed, washed clean and weighed. The relative organ body weight ratio (ROW) of each rat was calculated as:

ROW = weight of organ (g)/body weight of animal (g)

Biochemical Analysis

The serum collected from the rats were used for biochemical analysis namely; ALT, ALP, AST, ALP, albumin, conjugated and unconjugated bilirubin, urea, creatinine, uric acid, Na^+, K^+, glutathione peroxidase, superoxide dismutase, catalase and malondialdehyde as described by Reitman and Frankel [29], Wright *et al.* [30], Reitman and Frankel [29], Doumas *et al.* [31], Jendrassik and Golf [32], Fawett and Scott [33], Bartels and Brohmer [34], Fossati *et al.* [35], Wooten and Freeman [36], Wooten and Freeman [36], Flohe and Guenzle [37], Sun and Zigma [38], Aebi [39] and Kunchandy and Rao [40] respectively.

Heamatological Analysis

The packed cell volume, differential white blood cell count and platelet count was done as described by Ochei and Kolhatkar [41].

Statistical Analysis

The computation of the mean and statistical analysis was done using SPSS software version 24.0. Data was expressed as the mean ± SEM of group of five animals which was statistically analyzed with one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). For all the tests, results with p values < 0.05 was taken to imply statistical significance.

RESULTS AND DISCUSSION

Table 1 shows the change in weight, total feed intake and mortality of rats administered dichloromethane fraction from aqueous root bark extract (DFAR) and ethylacetate fraction

from aqueous root bark extract (EFAR) of *A. senegalensis*. There was no significant difference ($p > 0.05$) in the change in body weight of the rats administered 100, 200 and 400 mg/kg. b.wt. of DFAR when compared to the normal control, while there was a significant increase ($p < 0.05$) in the total feed intake of rats administered all test doses of DFAR when compared with normal control. The change in body weight of rats administered 400 mg/kg b.wt. EFAR was not significantly different ($p > 0.05$) from the control whereas it significantly increased ($p < 0.05$) the total feed intake when compared with the normal control. In contrast, 100 mg/kg b.wt. EFAR significantly increased ($p < 0.05$) the change in body weight when compared with its control while there was a significant decrease ($p < 0.05$) in total feed intake of rats administered 100 mg/kg b. wt. EFAR when compared with its control. Body weight gain is an important indicator of gross toxicity. The significant increase in feed intake with no significant difference in weight gain as exhibited by DFAR and EFAR treated rats indicates that the fractions contain antinutrient that interfered with nutrient reabsorption. Drastic toxicity or interference with absorption of nutrients usually reflects in body weight reduction [42]. Antinutrients such as tannins have been reported to be responsible for decrease in feed intake, feed efficiency, protein indigestibility and growth rate [43]. They combine with either nutrients in foods such as proteins and iron or proteins of the organism such as digestive enzymes [44,45]. Inhibition of the absorption of nutrients and the decrease in the activity of digestive enzymes results in reduction of nutritional efficiency [26].

Table 1: Influence of administration of DFAR and EFAR on weight gained, total feed intake and mortality of rats

Grps	Weight gained (g)	Total feed intake (g)	Mortality (%)
Control	26.00 ± 0.36^{a}	423.33 ± 1.86^{a}	0
100 mg/kg b.wt DFAR	24.67 ± 0.90^{a}	438.00 ± 1.00^{b}	0
200 mg/kg b.wt DFAR	16.00 ± 1.22^{a}	458.00 ± 2.90^{c}	0
400 mg/kg b.wt DFAR	23.67 ± 0.97^{a}	479.00 ± 2.52^{d}	0
Control	26.00 ± 0.13^{a}	423.33 ± 1.86^{c}	0
100 mg/kg b.wt EFAR	56.00 ± 1.51^{b}	398.33 ± 2.19^{a}	0
200 mg/kg b.wt EFAR	14.67 ± 0.67^{a}	419.33 ± 1.20^{b}	0
400 mg/kg b.wt EFAR	22.33 ± 1.60^{a}	429.00 ± 1.23^{d}	0

Values are mean of five replicates ± S.E.M. Values with different superscript down the column in each category are significantly different ($p < 0.05$).

Table 2 shows the organ to body weight ratio of rats administered DFAR and EFAR. There was a significant increase ($p < 0.05$) in organ to body weight ratio of kidney, heart and stomach of rats administered DFAR. Out of all of the organs assessed in albino rats administered EFAR, only the stomach and heart increased significantly when compared to the normal control. The significant increase in organ to body weight ratio of kidney, heart and stomach of rats administered DFAR and stomach of rats administered EFAR suggest toxic exposure to the organs. Alteration in body weight and or organ weight has been linked to toxic events arising from exposure to a toxicant [46, 47]. Adverse interaction of plant extract with major organs can cause inflammation and cellular constriction which usually reflects in the organ to body ratio [48].

Table 2: Organ to body weight ratio of rats administered DFAR and EFAR from *Annona senegalensis* root barks ($\times 10^{-3}$)

	Kidney	Liver	Heart	Stomach
Control	3.0 ± 0.0^{a}	33.0 ± 1.0^{a}	2.0 ± 0.0^{a}	44.0 ± 1.0^{a}
100 mg/kg.b.wt DFAR	6.0 ± 0.5^{c}	38.0 ± 2.0^{a}	4.0 ± 0.0^{b}	77.0 ± 1.3^{b}
200 mg/kg b.wt DFAR	6.0 ± 0.4^{c}	34.0 ± 3.0^{a}	4.0 ± 0.1^{b}	81.0 ± 5.0^{c}
400 mg/kg b.wt. DFAR	4.0 ± 0.2^{b}	35.0 ± 3.2^{a}	3.0 ± 0.3b	86.0 ± 4.0^{c}
Control	3.0 ± 0.00^{a}	33.0 ± 1.0^{a}	2.0 ± 0.0^{a}	44.0 ± 1.0^{a}
100 mg/kg b.wt. EFAR	2.0 ± 0.20^{a}	30.0 ± 2.3^{a}	2.0 ± 0.2^{b}	75.0 ± 6.0^{b}
200 mg/kg b.wt. EFAR	5.0 ± 0.40^{a}	43.0 ± 3.0^{a}	5.0 ± 0.4^{b}	63.0 ± 2.7^{b}
400 mg/kg b.wt EFAR	5.0 ± 0.45^{a}	42.0 ± 2.0^{a}	5.0 ± 0.3^{b}	99.0 ± 1.1^{c}

Values are mean of five replicates ± S.E.M. Values with different superscript down the column in each category are significantly different ($p < 0.05$).

Table 3 shows the effect of administration of DFAR and EFAR on rat liver function parameters. There was significant increase ($p < 0.05$) in alanine aminotransferase (ALT) activity of rats administered 100, 200 and 400mg/kg b.wt. DFAR and EFAR when compared to the normal control. There was no significant difference ($p > 0.05$) in aspartate aminotransferase (AST) activity and albumin concentration at all test doses of DFAR when compared with the normal control while conjugated and unconjugated bilirubin significantly decrease ($p < 0.05$) when compared with the control. There was no significant difference ($p > 0.05$) in serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities of rats administered 100, 200 and 400 mg/kg b.wt of EFAR when compared with the normal control. The concentration of conjugated and unconjugated bilirubin significantly decreased ($p < 0.05$) when compared with the normal control. There was significant increase ($p < 0.05$) in the concentration of serum albumin at 200 and 400 mg/kg b.wt EFAR. This increase was not dose dependent. Alanine aminotransferase (ALT) is an enzyme predominantly present in the liver. Elevated levels in serum is an indicator of liver damage. Aspartate aminotransferase (AST) is an enzyme present in the cell of the liver, skeletal muscles, kidney, heart and pancreas. Alanine aminotransferase is the primary pathological marker of hepatic dysfunction while aspartate transaminase is used as supplementary marker to substantiate the degree of liver damage [49]. The significant increase in the level of serum alanine aminotransferase (ALT) of rats administered EFAR and DFAR indicates liver damage. Alanine aminotransferase (ALT) is a cytosolic enzyme predominantly present in the liver. Damage to the plasma membrane will lead to leakage of cytosolic contents including the cytosolic enzymes (ALT) into the serum [50]. Therefore, elevated levels of ALT in the serum is an indicator of hepatocellular injury. The increase in serum ALT of rats administered EFAR and DFAR might be as a result of assault of the toxic constituents of these fractions on the plasma membrane of the liver leading to the leakage of these enzymes from the cytosol into the blood. Albumin is synthesized in the liver. Albumin levels are used to evaluate the synthetic capacity of the liver. With progressive liver disease, serum albumin levels fall reflecting decrease synthesis [51]. The non-significant difference in serum albumin level in rats administered DFAR observed in this study suggest that the fraction did not affect the synthetic function of the liver. Bilirubin is a useful index of the excretory function of the liver [52]. The significant decrease in the serum levels of conjugated and unconjugated bilirubin of rats administered DFAR and EFAR suggest that administration of these sub-fractions did not affect the excretory function of the liver. This finding therefore indicates that EFAR and DFAR caused hepatocellular injury at 100, 200 and 400 mg/kg. In contrast, Okoye *et al.* [24] reported that *A. senegalensis* root was not hepatotoxic at low concentrations (50 and 100 mg/kg b.wt.) but was hepatotoxic at 400 mg/kg b.wt. Ilboudo *et al*. [26] also reported that aqueous *A. senegalensis* root bark at doses less than 300 mg/kg b.wt. was not hepatotoxic. This suggests that the hepatotoxic constituent of the root has been fractionated into these fractions.

Table 3: Effect of administration of DFAR and EFAR on rat liver function parameters

Dose	AST (U/L)	ALP (U/L)	ALT(U/L)	Uncon bil. (mg/dL)	Con. BiI (mg/dL)	Alb (g/L)
Control	34.37 ± 0.43^{a}	163.67 ± 1.16^{c}	36.33 ± 0.14^{a}	2.92 ± 0.23^{c}	2.47 ± 0.00^{c}	0.86 ± 0.05^{a}
100 mg/kg b.wt. DFAR	23.13 ± 0.76^{a}	101.36 ± 0.65^{a}	75.44 ± 0.18^{b}	2.71 ± 0.13^{b}	2.27 ± 0.12^{b}	1.17 ± 0.15^{a}
200 mg/kg b. wt.. DFAR	24.67 ± 0.58^{a}	119.60 ± 0.85^{a}	106.67 ± 1.04^{c}	2.53 ± 0.11^{a}	2.12 ± 0.09^{a}	1.27 ± 0.17^{a}
400 mg/kg b.wt. DFAR	22.07 ± 1.14^{a}	132.69 ± 0.11^{b}	143.00 ± 0.20^{d}	2.42 ± 0.03^{a}	2.03 ± 0.03^{a}	0.98 ± 0.06^{a}
Control	34.37 ± 1.03^{a}	163.67 ± 1.16^{a}	36.33 ± 0.14^{a}	2.92 ± 0.02^{b}	2.47 ± 0.00^{b}	0.84 ± 0.05^{a}
100 mg/kg b.w. EFAR	22.33 ± 2.16^{a}	153.43 ± 1.52^{a}	99.33 ± 0.88^{d}	2.46 ± 0.15^{a}	2.12 ± 0.09^{a}	0.91 ± 0.07^{a}
200 mg/kg b.w. EFAR	16.20 ± 3.10^{a}	161.43 ± 1.18^{a}	77.33 ± 0.26^{c}	2.48 ± 0.13^{a}	2.09 ± 0.11^{a}	1.18 ± 0.09^{c}
400 mg/kg b.w. EFAR	33.73 ± 1.11^{a}	168.93 ± 2.11^{a}	54.67 ± 0.14^{b}	2.40 ± 0.14^{a}	2.02 ± 0.12^{a}	0.98 ± 0.04^{b}

Values are mean of five replicates ± S.E.M. Values with different superscript down the column in each category are significantly different ($p < 0.05$).
AST- aspartate aminotransferase, ALP – alkaline phosphatase, ALT – alanine aminotransferase. Unconj. Bil.- unconjugated bilirubin, Conj. Bil.- conjugated bilirubin, Alb. - albumin

The effect of administration of dichloromethane fraction of aqueous root bark extract (DFAR) and ethylacetate fraction of aqueous root bark extract (EFAR) of *A. senegalensis* on rat kidney function parameters is presented in Table 4. There was significant decrease ($p < 0.05$) in serum urea concentration of rats administered all test doses of DFAR and EFAR when compared with the normal control. The decrease was dose dependent. Serum Na^{+} and

K^+ concentration of rats administered 100, 200, 400 mg/kg b.wt DFAR and EFAR significantly increased ($p < 0.05$) when compared to the normal control. There was a significant reduction ($p < 0.05$) in the concentration of creatinine of rats treated with 100, 200, 400 mg/kg b.wt. of EFAR when compared with the normal control. The kidney is responsible for removal of metabolic wastes such as urea, ions, and creatinine. The concentration of these metabolites are used to assess the normal functioning of the nephrons. The significant decrease in serum urea concentration of rats administered EFAR and DFAR observed in this study, suggest kidney damage. A reduction in urea may be due to high rate of urea excretion (i.e. inhibition of urea reabsorption in the renal tubule of the nephron) [53]. Urea is freely filtered into the glomerulus but reabsorbed by urea pumps in the collecting ducts of the nephron [54]. The decreased serum urea concentration in rats administered EFAR and DFAR observed in this study, therefore suggests that these fractions contain substance capable of inhibiting urea pumps with a resultant decrease in reabsorption of urea and a decrease in urea concentration [54]. A major role of the kidney is the maintenance of electrolytes balance [55]. Chronic renal failure result in sodium retention in the blood [56]. Increased serum sodium concentration in rats administered DFAR and EFAR, treated rats observed in this study indicates chronic renal failure. These sub-fractions may contain substances capable of stimulating any of the sodium pumps responsible for active sodium transportation out of the nephron. Aldosterone has been reported to cause an increase in the tubular reabsorption of Na^+ by stimulating the electrogenic sodium channel (ENaC) leading to decrease in the excretion of sodium in urine and a concomitant increase in the blood [57]. This suggests that they two fractions contains aldosterone.

Table 4: Effects of administration of DFAR and EFAR on serum kidney function parameters in rats

Group	Urea (mmol/L)	Creatinine (mg/mL)	Na^+ (mmol/L)	K^+ (mg/dL)	Ca^{2+} (mg/dL)	Uric acid (mmol/L)
Control	155.26 ± 1.46^c	0.93 ± 0.09^a	226.00 ± 0.01^a	9.62 ± 0.17^a	3.20 ± 0.07^a	4.78 ± 0.06^a
100 mg/kg b.wt. DFAR	78.48 ± 2.70^b	0.96 ± 0.01^a	1950.00 ± 1.99^b	14.56 ± 1.32^b	3.39 ± 0.16^a	5.05 ± 0.24^a
200 mg/kg b.wt. DFAR	74.28 ± 1.29^a	0.64 ± 0.06^a	2586.36 ± 3.76^b	13.80 ± 0.89^b	2.96 ± 0.18^a	4.41 ± 0.25^a
400 mg/kg b.wt. DFAR	53.35 ± 7.27^a	0.41 ± 0.03^a	2047.72 ± 1.63^b	14.96 ± 0.60^b	3.23 ± 0.32^a	4.81 ± 0.49^a
Control	155.26 ± 1.46^c	$0.83 \pm .005^c$	226.00 ± 0.05^a	9.62 ± 1.18^a	3.20 ± 0.04^a	4.78 ± 0.06^a
100 mg/kg b.wt EFAR	115.15 ± 5.25^b	$0.50 \pm .002^b$	1963.0 ± 1.16^b	13.11 ± 0.26^b	3.02 ± 0.08^a	4.50 ± 0.11^a
200 mg/kg b.wt EFAR	125.11 ± 5.73^b	$0.23 \pm .002^a$	1925.0 ± 3.34^b	14.12 ± 0.84^b	3.20 ± 0.09^a	4.60 ± 0.03^a
400 mg/kg b.wt EFAR	64.09 ± 2.71^a	$0.55 \pm .003^b$	1763.6 ± 5.12^b	14.02 ± 0.34^b	3.13 ± 0.14^a	4.67 ± 0.20^a

Values are mean of five replicates ± S.E.M. Values with different superscript down the column are significantly different ($p < 0.05$).

Table 5 shows the effect of administration of DFAR and EFAR on rat serum antioxidant enzyme activities. Serum glutathione peroxidase (GPx) activity significantly increased ($p < 0.05$) at all test doses of DFAR and EFAR in a dose dependent manner while there was a corresponding significant decrease ($p < 0.05$) in the activity of superoxide dismutase (SOD) when compared to the control. There was a significant decrease ($p < 0.05$) in catalase activity at all test doses of DFAR when compared with the control. In contrast, administration of EFAR to rats significantly increased ($p < 0.05$) serum catalase activity. The increase in catalase and glutathione peroxidase (GPx) activities in rats administered EFAR observed in this study might have been induced in response to increased generation of reactive oxygen species particularly hydrogen peroxide (H_2O_2). Catalase and glutathione peroxidase catalyzes the conversion of H_2O_2 to O_2 and H_2O [58]. Reactive oxygen species are usually produced during biotransformation of xenobiotic in the liver [59]. Usually, the antioxidant defense systems including the antioxidant enzymes are induced in response to increased reactive oxygen species production [60]. Superoxide dismutase (SOD) catalyzes the formation of hydrogen peroxide from superoxide radicals. Its levels are directly related to catalase activity [61]. Thus, decreased superoxide dismutase activity should lead to a decrease in catalase activity. This trend was not observed in this study. Decreased activity of superoxide dismutase did not lead to decrease in catalase activity of rats administered DFAR. The decreased activity of serum superoxide dismutase may be attributed to inactivation by hydrogen peroxide. Hydrogen peroxide can inactivate superoxide dismutase in a feedback mechanism [62]. It is therefore possible that overproduction of hydrogen peroxide inactivated superoxide dismutase. The activities of the antioxidant enzymes suggest that they fraction induced generation of reactive oxygen species which may be responsible for the observed hepatocellular injury.

Table 5: Effect of administration of DFAR and EFAR on some rat serum antioxidant enzyme activities

Group	GPX	SOD	CAT ×10^{-3}
		(U/L)	
Control	0.28 ± 0.02^{a}	211.57 ± 1.25^{c}	17.67 ± 0.01^{b}
100 mg/kg b.wt. DFAR	3.28 ± 0.09^{b}	91.21 ± 4.87^{b}	2.67 ± 0.01^{a}
200 mg/kg b.wt. DFAR	6.50 ± 0.19^{c}	41.46 ± 1.29^{a}	4.35 ± 0.01^{a}
400 mg/kg b.wt. DFAR	6.53 ± 0.28^{c}	33.17 ± 0.29^{a}	5.50 ± 0.06 ^{a}
Control	0.28 ± 0.02 ^{a}	211.57 ± 1.25^{c}	1.77 ± 0.14^{a}
100 mg/kg b.wt. EFAR	1.05 ± 0.07^{b}	41.46 ± 0.58^{b}	41.67 ± 1.27^{b}
200 mg/kg b. wt. EFAR	6.25 ± 0.56^{c}	33.17 ± 0.98 ^{a}	52.33 ± 3.94^{b}
400 mg/kg b. wt. EFAR	5.42 ± 0.17^{c}	33.16 ± 0.89^{a}	80.37 ± 0.15^{c}

Values are mean of five replicates ± S.E.M. Values with different superscript down the column are significantly different ($p < 0.05$).

Figure 1 shows the effects of administration of DFAR and EFAR on serum malondialdehyde concentration in rats. Malondialdehyde concentration in serum of rats administered 100, 200 and 400 mg/kg b.wt of DFAR and EFAR significantly increased ($p < 0.05$) when compared with the control. Malondialdehyde, an oxidative damage product of lipid peroxidation, is the main marker in lipid peroxidation [63]. The significant increase in serum malondialdehyde concentration of rats administered DFAR and EFAR suggests that they fraction initiated lipid peroxidation as a result of oxidative stress. Oxidative stress due to toxic effects is usually indicated by an increase in malondialdehyde [64]. Oxidative stress causes reactive oxygen species to react with unsaturated lipids in the membranes of cells and organelles thus initiating lipid peroxidation [65]. Lipid peroxidation may be responsible for hepatocellular injury which resulted in the increase in ALT observed in this study.

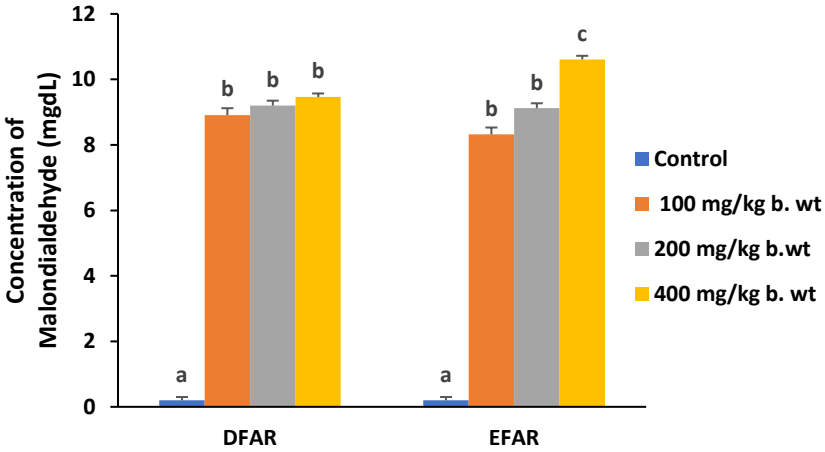

Figure 1: Effect of administration of DFAR and EFAS on serum malondialdehyde concentration in rats. Bars with different superscript in each category are significantly different ($p < 0.05$).

Table 6 shows some heamatological indices of rats administered DFAR and EFAR. There was a significant increase ($p < 0.05$) in percentage packed cell volume (PCV) at all test doses of DFAR when compared to the control. There was no significant difference ($p > 0.05$) in erythrocytes, basophil, neutrophil, platelets, eosinophil and monocytes at all test doses of DFAR when compared with the control. There was no significant difference ($p > 0.05$) in the studied heamatological parameters of rats administered 100, 200 and 400 mg/kg b.wt EFAR when compared to the control. Heamatological parameters are usually assessed to determine the well being of an animal [66]. Heamatological parameters are good indicators of the physiological status of animals [67]. The significant increase in percentage packed cell volume (PCV) in rats administered DFAR indicated that the sub-fractions contain substances that stimulated red blood cell production. Differential white blood cell counts are indicators of an organism's ability to defend the body against invasion from foreign bodies [68]. The significant increase in lymphocyte count by DFAR observed in this study reflects the leukopoietic and immunomodulatory effects of DFAR. Lymphocytes produce, transport or distribute antibodies in immune response [69]. Elevated lymphocyte count in organisms indicate high degree of resistance to invasion from foreign body [70]. The increase in lymphocyte count suggests that DFAR confer high resistance to assault caused by toxic constituents of the sub-fraction.

Table 6: Effect of administration of DFAR and EFAR on selected heamatological parameters in rats

Group	PCV (%)	Eryt×10^6µL	Baso×10^6 µL	Neu×10^6 µL	Lym×10^6 µL	Eos×10^6 µL	Mon×10^6 µL	plate×10^6 µL
Control	41.0 ± 0.58[a]	0.33 ± 0.03[a]	0.33 ± 0.03[a]	2.33 ± 0.20[a]	30.0 ± 0.58[a]	0.3 ± 0.03[a]	0.33 ± 0.0[a]	2.33±0.17[a]
100 mg/kg DFAR	45.7 ± 0.88[c]	1.00 ± 0.09[a]	1.33 ± 0.12[a]	1.67 ± 0.16[a]	29.3 ± 0.95[a]	0.0 ± 0.00[a]	0.67 ± 0.03[a]	1.67± 0.16[a]
200 mg/Kg DFAR	42.3 ± 2.03[b]	1.00 ± 0.10[a]	1.00 ± 0.09[a]	0.00 ± 0.00[a]	26.7 ± 1.47[a]	0.0 ± 0.00[a]	1.00 ± 0.06[a]	2.33 ± 0.19[a]
400 mg/kg DFAR	44.7 ± 1.20[b]	2.67 ± 0.17[a]	2.67 ± 0.19[a]	0.67 ± 0.06[a]	16.0 ± 0.66[b]	0.0 ± 0.00[a]	1.00 ±0.06[a]	1.67 ±0.13[a]
Control	41.0 ± 0.58[a]	0.33 ± 0.03[a]	0.33 ± 0.03a	2.33 ± 0.22[a]	30.0 ± 0.58[a]	0.33 ± 0.03[a]	0.33 ± 0.03[a]	2.33 ± 0.23[a]
100 mg/kg b. wt. EFAR	42.3 ± 2.03[a]	0.33 ± 0.02[a]	1.33 ± 0.13[a]	0.67 ±0 .06[a]	10.7 ± 0.43[a]	0.33 ± 0.03[a]	0.67 ± 0.06[a]	0.67 ± 0.05[a]
200 mg/kg b. wt. EFAR	48.0 ± 2.51[a]	1.33 ± 0.11[a]	1.33 ± 0.12[a]	2.00 ± 0.19[a]	23.7 ±0.12[a]	0.67 ± 0.03[a]	0.67 ± 0.05[a]	1.67 ± 0.07[a]
400 mg/kg b. wt. EFAR	43.3 ± 2.84[a]	0.00 ± 0.00[a]	0.67 ± 0.05[a]	0.00 ± 0.00[a]	19.3 ± 1.08[a]	0.00 ± 0.00[a]	0.67 ± 0.03[a]	2.00 ± 0.06[a]

Values are mean of three replicates ± S.E.M. Values with different superscript down the column are significantly different ($p < 0.05$)
PCV – packed cell volume, Eryt. – erythrocytes, Baso – basophils, Neu. – neutrophils, lymp. – lymphocytes, Eos. - eosinophils, Mon. – monocytes,Plate.platelet

CONCLUSION

The dichloromethane fraction of aqueous root extract (DFAR) and ethylacetate fraction of aqueous root extract (EFAR) are hepatotoxic and nephrotoxic but DFAR improved packed cell volume (PCV). Therefore, these two fractions from the aqueous root extracts contain phytochemicals that are not safe and should be used with caution.

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CONFLICT OF INTEREST

They authors declare that there is no conflict of interest.

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