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Research Paper

ANTI-DIARRHOEAL AND TOXICOLOGICAL PROPERTIES OF STEM BARK EXTRACT OF *Khaya senegalensis*

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Abstract

The anti-diarrrhoeal activity of the stem bark extracts of *Khaya senegalensis* were evaluated in castor oil -induced diarrhoea and gastrointestinal transit model in rats. The crude methanol extract was partitioned using hexane, chloroform, ethylacetate and saturated butanol . Hexane and chloroform did not yield any fractions. Phytochemical and toxicological studies were carried out using standard methods. The methanol extract, ethylacetate and saturated butanol fractions were screened for anti-bacterial activity against *Salmonella spp. Esherichia coli* and *Pseudomonas aeruginosa* using agar diffusion bioassay. The precipitation rate of the total tannins in crude methanol extract and saturated butanol fraction were also evaluated. The effect of the fractions on biochemical indices of the liver, kidney, haematological and histopathological studies were carried out. The phytochemical constituents detected were steroids, tannins, alkaloids, saponins, volatile oils, glycosides, saponin glycosides, anthraquinones, balsams and cardiac glycosides. Protein precipitation rate on the crude methanol extract and saturated butanol fraction increased as the concentration of the tannins increased. The medium lethal dose (LD_{50}) of the crude extract was greater than 5000 mg kg^{-1} (LD_{50} >5000 mg kg^{-1}) and no adverse effects were observed. Sub-chronic administration of the methanol extract at 25-150 mg ml^{-1} had no effect on the body weight of the rats but there was significant ($p<0.05$) difference in the organ body weight ratio and organ body index. There were no significant ($p>0.05$) differences in the effect of ethylacetate and saturated butanol fractions on most of the liver and renal function indices. Non significant ($p>0.05$) differences of the fractions were also observed for haematological parameters. Histopathological studies indicated that the liver architecture, kuffer cells and bile duct were preserved. The crude methanol extract, ethylacetate and saturated butanol fractions at doses of 25-150 mg kg^{-1} significantly ($p<0.05$) reduced the diarrhoea droppings and also showed a significant ($p<0.05$) reduction in gastrointestinal motility on charcoal meal test. The crude methanol extract, ethylacetate and saturated butanol fractions also indicated significant ($p<0.05$) anti-bacterial activity at 25-150

mg/ml. This result suggests that the stem bark extract of *Khaya Senegalensis* is relatively safe and could be used in the management of diarrhoea.
Key words: *Khaya Senegalensis,* anti-diarrrhoeal activity, castor oil, toxicity studies, Protein precipitation, phytochemicals.

INTRODUCTION

Diarrhoea is defined by the World Health Organization as having three or more loose or liquid stools per day or as having more stools than is normal for that person [1]. It is primarily a symptom of gastrointestinal infection [2]. This condition can be a symptom of injury, disease or food borne infection and is usually accompanied by abdominal pain, nausea and vomiting [3]. Diarrhoea is a common cause of death in developing countries and the second most common cause of infant mortality worldwide [4]. In 2009, diarrhoea was estimated to have caused 1.1 million deaths in people aged 5 and above, 1.5 million deaths in children under the age of 5 [1]. The incidence of diarrhoea in children still remains high, despite the efforts of many governments and international organization to curb it [5]. In order to combat the problem of diarrhoea, World Health Organization has inaugurated the Diarrhoea Disease Control (DDC) which includes the studies of traditional medical practices together with evaluation of health education and preventive approaches [6]. Some medicinal plants have been used for management of diarrhoea without much information on their pharmacology and toxicological properties.

Khaya senegalensis is a medicinal plant growing mainly in sub- Sahara savannah forest from Senegal to Uganda. Dried stem bark is used externally for treating skin infections and blennorrhagra. It is very bitter and is extensively used for the treatment of ulcers on the back of sheep, camels and horses. The oil from the seed is used for treatment of leprosy, syphilis and ulcer in humans [7]. In Adamawa State, Nigeria, the stem bark and the leaves of this plant have been used in form of decoction and concoctions for the cure of mucous diarrhoea, syphilis and malaria fever [8]. Stem bark extract of *K. senegalensis* is widely used in Northern Nigeria for management of diarrhoea. There is no much information on the anti-diarrhoeal and toxicological properties of the stem extract of the plant.

MATERIALS AND METHODS

Animals: Wistar strains albino rats of both sexes were obtained from the Faculty of Pharmaceutical Sciences Ahmadu Bello University Zaria (ABU). The rats were fed with pellet feeds (Vital Feeds Bukuru, Jos, Nigeria) and water *ad libitum*. They were kept in wire mesh cages at room temperature for two weeks to acclimatize. The study was conducted between March 2012 and June 2013. Animal treatment and handling were done according to the ethical guideline [9] and in accordance with US guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 18-23).

Collection of plant material:

The stem bark of *K. senegalensis* was obtained in March 2012, from a mature tree of *K. senegalensis* around Usmanu Danfodiyo University campus, Sokoto, Nigeria. The plant was taxonomically identified and authenticated at the Botany unit of the Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen was thereafter deposited at the Herbarium of the same department for future reference.

Preparation of stem bark extract:

The stem bark collected was open-air-dried under shade, pulverized into coarse powder (with a wooden pestle and mortar) and sieved with a 1 mm^2 and stored in a plastic container until required.

Preparation of the crude methanol extract:

Five hundred and fifty (550g) grammes of the powdered stem bark were socked in 2500ml of methanol at room temperature for 72 hours. After, the extract was sieved and 2500ml of methanol was added to the sieved extract and allowed to stand for another 72 hours. The mixtures were later filtered using a clean, sterile, white muslin cloth and then re-filtered again using a whatman filter paper No1. The filtrate obtained was then evaporated at room

temperature leaving the residue. The gumming residue was then kept at 4°C until usage. The methanol extract obtained was used for anti-diarrhoeal and toxicological studies. The extract was stored in sealed plastic containers until needed. The dried powdered residue of the extract was further reconstituted in distilled water at different concentrations for oral administration to albino rats.

Partition

The crude methanol extract was partitioned using hexane, chloroform, ethylacetate and saturated butanol. Distilled water was added to the methanol extract to pre-dissolve it and then 300 mls of hexane were added and shaken vigorously. After 30min, the hexane phase was collected and the process was repeated thrice. The extract was similarly treated with chloroform, ethylacetate and saturated butanol The fractions were used to carry out anti-diarrhoeal studies, liver and renal function indices and haematological parameters.

Drugs and chemicals: Loperamide (standard reference anti-diarrhoeal drugs), Castor-oil (laxative) charcoal meal (activated carbon) all of standard grades were used.

Phytochemical analysis: This was done using the procedures of Harborne [10], Trease and Evans, [11] and El-Olemyl *et al*., [12].

Acute toxicity studies (determination of LD_{50}): Crude methanol extract of *K. senegalensis* (5000 mg kg^{-1} body weight) was administered to 5 groups (one rat per group) of rats one after the other at a grace observation period of 48 h in a single oral dose using a feeding syringe. The control group received distilled water. Observations of toxic symptoms (such as increased motor activity, arching and rolling, writhing, depression, salivation, loss of hair and death) if any, were made and recorded systematically at 1, 2, 4 and 6 h after administration. The number of survivors was noted after 48 h for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated by using the limit test dose of up and down procedure of OECD [13].

Castor oil-induced diarrhoea:

Thirty rats were fasted for 18 h and were divided into 6 groups of 5 animals each. The animals in group 1 served as negative control and received distilled water orally. Group 2 was positive control and received 5 mg kg^{-1} (b.wt.) of Loperamide. Groups 3, 4, 5 and 6 received 25, 50, 75 and 150 mg kg^{-1}, respectively of the crude methanol extract of the plant. After 1hr of the drug pre-treatment, castor oil (10 mL kg^{-1}) was administered orally to all the groups. The animals were then placed in observation cages singly over clean filter paper and observed for defecation for up to 6 h. The total number of watery stools was counted [14].The above procedure was repeated for ethylacetate and saturated butanol fractions.

Gastrointestinal motility (small intestinal transit time):

Thirty rats were fasted for 24 h and then placed in 6 groups of 5 animals each. Group 1 was administered with distilled water. Group 2 received Loperamide 5 mg kg^{-1} (b.wt.). Groups 3, 4, 5 and 6 received 25, 50, 75 and 150mg kg^{-1} (b.wt.) respectively of the crude methanol extract of *K. senegalensis.* After 30 min of the extract administration, each animal was orally administered 1 mL of charcoal meal (10% activated charcoal in distilled water). The rats were sacrificed thirty minutes later and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of the total length of the intestine [15,16].The above procedure was repeated for ethylacetate and saturated butanol fractions.

Sub-acute toxicity:

Thirty rats were divided into 5 groups. The rats in group 2, 3, 4 and 5 were orally administered graded doses of the LD_{50} of the extract i.e. (20; 40, 60 and 80%) respectively of the LD_5o translating to the corresponding mg kg^{-1} once daily for 28 days. Animals in group 1 served as control group (0.00 mg kg^{-1}) and received distilled water for the test period. The body weights of all the animals were then recorded before and after treatment weekly within the days of the treatment. After 28 days, the animals were sacrificed and their liver and kidney were weighed.

Protein precipitation test of total tannins

A 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.4 and 1.6ml of 100mg/ml total tannins (TT) solution were respectively added to the test tube in which 2ml of 2mg/ml bovine serum albumin solution has

been previously added, and distilled was added to each tube to make the total volume up to 4ml. As a result, the final concentration of bovine serum albumin was 1mg/ml and TT were respectively 0.5, 10, 15, 20, 25, 30, 35 and 40mg/ml with three test tubes designated for each level of concentration. After it was well shaken and mixed, each test tube was kept in the dark at 4°C for 12 hrs. The protein content of the supernatant from each tube was determined by Coomassie brilliant blue kit. Precipitation rate of bovine serum albumin by the crude methanol extract was calculated according to the formula [17]. The above procedure was repeated using the total tannins content of the saturated butanol fraction.

$$\text{Precipitation rate (\%)} = \frac{C_1 - Cn}{C_1} \times 100$$

Where C_1 is the protein content of supernatant before precipitation (in No.1 tube) and C_n is the protein content of supernatant after precipitation (n= 2-9).

Collection of blood sample and Tissues

Blood samples were collected from the jugular vein by sacrificing the animal. The blood was allowed to clot in a test tube and then centrifuged using a table- top centrifuge to obtain the serum. Kidney and liver function tests were carried out using the serum samples. The liver and the kidney were collected by dissecting the animal and these organs were then removed and preserved in formalin saline solution.

Biochemical parameters:

Animals were sacrificed on the 29th day and blood samples were collected, allowed to clot and centrifuged to obtain sera. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined using Randox assay kit by Reitman and Frankel, [18]. Alkaline phosphatase activity was estimated by the randox kit (Colorimetric) of Rec, [19]. Total Protein was determined by the biuret method as described by Doumas [20]. Total Bilirubin (TBL) were analyzed (Randox kits) using the methods of Jenderassik and Grof, [21] and Sherlock [22]. Albumin (Bromocresol green) and Urea (Diacetylmonoxime) were done by the methods of Cheesbrough [23]. 5'-nucleotidase of Reider and Otero [24] and Subhani *et al.,* [25]. Uric acid was estimated by the method of Collins and Diehl, [26]; Morin, and Prox [27]. Urea Wygbenga *et al.,* [28]. Creatinine were estimated by the methods of Henry [29]. Potassium and sodium ions by Ranjna, [30]; Uriyo and Singh [31].

Antibacterial Activity

The antibacterial activity was assessed by utilizing the hole-in-plate bioassay procedure as reported by Hugo and Russel,[32] and Vlietinck *et al.,* [33]. Pure culture of the organisms were inoculated into Muller-Hinton nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of $9x10^8$ cfu mL^{-1} equivalent to MC-Farland test tube number 3. The suspension was used to streak for confluent growth on the surface of Muller-Hinton agar plates with sterile swab. Using a sterile cork-borer of 6 mm diameter, four holes were made on to the set agar in Petri dishes containing the bacterial culture. Various concentrations of the extracts (25, 50, 75 and 150 mg mL^{-1}) were poured in to the wells. Ciprosan (10 mg mL^{-1}) (a product of May and Baker Ltd) was used as reference or positive control. The plates were placed in the incubator at 37°C overnight. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm.

Haematological Parameters

After sacrificing the animals, the blood was collected for determination of some haemotological parameters. The parameters were determined using an automated hematology analyzer (specifically SYSMEX KX 21 N) as described by Kakkar and Makkara,[34].

Histopathological Assessment:

Histopathological examinations were carried out on the liver of the rats. They were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin. Sections (4-6 μM thick) were prepared and then stained with

hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light microscope at high power magnifications (x200 objective) Wasfi *et al.,* [35]; Rao *et al.,* [36].

Statistical analysis:

Results are expressed as Mean±Standard error of mean. The data collected were subjected to one way Analysis of Variance (ANOVA), using Graph Pad Instat, Bonferroni post hoc test to compare all columns (means) and significance level was considered at P<0.05.

RESULTS

The crude methanol extract residue was 98.32g and percentage yield was 17.88% (W/W). Phytochemical screenings showed the presence of steroids, tannins, alkaloids, saponins, volatile oils, glycosides, saponin glycosides, anthraquinones, balsams and cardiac glycosides. Table 1shows mean body weight of rats before and after administration of crude methanol stem bark extract of *Khaya senegalensis*. All values are not significantly (P>0.05) different when compared with control.Table 2 shows the effects of oral administration of methanol stem bark extract of *K. senegalensis* on the weight of organs (kidney and liver) and organ body weight. There were significant (p<0.05) increase in kidney weight compared to the control group. There were no significant (p>0.05) difference in the weight of liver but the group administered with 75mg kg-1 of the extract (4.65g) showed significant difference when compared with the control group (3.58g). The liver body weight had no significant (p>0.05) changes when compared to the control group while there were significant (p<0.05) differences in kidney body weight of the groups 50 and 75mg kg-1 with kidney body weight ratio of 0.0079 and 0.0082 respectively when compared with the control (0.0049). The saturated butanol fraction was the most effective at high and lower doses when compared with the crude methanol extract and ethylacetate fraction with percentage inhibition of 87.5 and 62.5% respectively (table 3). Table 4 shows that, the crude methanol extract, ethylacetate and saturated butanol fraction of *Khaya senegalensis* (25, 50, 75 and 150mg/kg) have inhibited intestinal propulsion in charcoal-treated rats**.** The crude methanol had the lowest inhibition of 18.5% (25mg/kg) when compared to ethylacetate and saturated butanol fraction.

Table 1 : Mean body weight of rats before and after administration of crude methanol stem bark extract of *Khaya senegalensis*

Dose (mg kg-1)	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	% weight change
0.0	130.2 ± 1.10	130.9 ± 1.11	131.0 ± 1.08	136.2 ± 1.41	141.5 ± 1.33	7.99
25.0	156.9 ± 4.71	158.2 ± 4.49	159.4 ± 4.65	162.9 ± 5.04	167.4 ± 4.41	6.27
50.0	157.4 ± 13.40	160.1 ± 13.22	163.7 ± 13.01	165.9 ± 13.19	170.9 ± 12.08	7.90
75.0	162.3 ± 23.07	164.2 ± 23.33	168.6 ± 24.04	170.5 ± 22.30	174.8 ± 21.79	7.20
150.0	174.4 ± 19.25	175.8 ± 20.00	177.2 ± 20.47	180.4 ± 18.86	188.9 ± 18.56	7.68

Values are expressed as mean ± SEM (n=5) = All values are not significantly (P>0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software (Sandiego USA).

Table 2: Effect of oral administration of crude methanol stem bark extract of *Khaya senegalensis* on the ration of organ weight and organ body index

Dose (mg kg-1)	Kidney weight (g)	Liver weight (g)	Kidney: body weight	Liver : body weight	Kidney organ body index (%)	Liver organ body index (%)
0.00	0.74 ± 0.02	3.58 ± 0.22	0.0049 ± 0.0002	0.026 ± 0.0004	0.49 ± 0.02	2.60 ± 0.04
25.0	1.32 ± 0.03*	4.34 ± 0.14	0.0079 ± 0.0002*	0.025 ± 0.0009	0.79 ± 0.02*	2.50 ± 0.09
50.0	1.15 ± 0.06*	4.21 ± 0.10	0.0069 ± 0.0005	0.025 ± 0.0013	0.69 ± 0.05	2.50 ± 0.13
75.0	1.45 ± 0.06*	4.65 ± 0.30*	0.0082 ± 0.0008*	0.030 ± 0.0022	0.82 ± 0.08*	0.30 ± 0.22
150.0	1.30 ± 0.13*	3.84 ± 0.24	0.0074 ± 0.0006	0.021 ± 0.0018	0.74 ± 0.01	2.10 ± 0.18

Values are expressed as mean ± SEM (n=5) = * significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software (Sandiego USA).

There were no significant differences in most of the liver function indices observed (Table 5). There were slight increases in ALT and AST in ethylacetate fraction at dose of 150mg/kg. Renal Function indices of rats administered with ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis*, shows most of the values were not significantly different (P>0.05) when compared with the control (Table 6). For haematological parameters, all values were not significantly different (P>0.05) when compared with the control (Table 7). When the total tannin was added to the bovine serum albumin solution, the flocculent precipitation emerged immediately. There were significant (p<0.05) increases in precipitation rate in both methanol crude extract and saturated butanol fraction when compared with control. The maximum value of precipitation rate was 81.0 and 85.1% for the methanol crude extract and saturated butanol fraction as seen in tables 8 and 9 respectively. There were significant (p<0.05) inhibitory activities against *Salmonella spp, Pseudomonas aeruginosa,* and *Esherichia coli* with crude extract, ethylacetate and saturated butanol fractions at 25 to 150mg/ml (Table 10). Histopathological studies indicated that the liver architecture, kuffer cells and bile duct were preserved.

Table 3: Effect of methanol extract, ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis* on castor oil -induced diarrhoea in rats

Fractions/ Drug	Dose ($mgkg^{-1}$)	Diarrhoea Droppin	%Inhibition
Methanol	25	1.80 ± 0.38*	40.0
	50	1.60 ± 0.25*	46.7
	75	1.40 ± 0.25*	53.3
	150	0.80 ± 0.20*	73.3
Ethylacetate	25	1.60 ± 0.89*	52.9
	50	1.20 ± 1.09*	64.7
	75	0.80 ± 0.84*	76.5
	150	0.60 ± 0.89*	82.4
Saturated butanol	25	1.20 ± 0.85*	62.5
	50	1.00 ± 0.71*	68.8
	75	0.60 ± 0.89*	81.3
	150	0.40 ± 0.55*	87.5
Loperamide	5	0.00 ± 0.00*	100.0
Water	-	3.20 ± 0.31	-

Values are expressed as mean ± SEM (n=5) = * significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software (San Diego USA).

Table 4: Effect of methanol extract, ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis* on normal intestinal transit in rats

Fractions/ Drug	Dose ($mgkg^{-1}$)	IL(cm)	CML (cm)	%IT	%Inhibition
Methanol	25	93.84 ± 1.23	76.48 ± 0.77	81.50 ± 1.21*	18.5
	50	92.94 ± 1.65	73.92 ± 0.77	79.60 ± 1.28*	20.5
	75	93.90 ± 1.99	72.76 ± 0.63	77.68 ± 2.21*	22.5
	150	93.28 ± 2.21	71.68 ± 1.01	77.04 ± 2.39*	23.2
Ethylacetate	25	92.40 ± 2.02	73.80 ± 0.72	79.98 ± 1.40*	20.1
	50	92.68 ± 1.65	72.80 ± 0.71	78.62 ± 1.41*	21.5
	75	89.82 ± 3.47	70.02 ± 1.10	78.26 ± 2.05*	22.0
	150	93.72 ± 1.38	69.42 ± 0.35	74.14 ± 1.28*	26.0
Saturated butanol	25	91.72 ± 1.67	68.46 ± 0.70	75.18 ± 3.48*	25.4
	50	90.94 ± 2.75	67.86 ± 1.75	74.96 ± 1.22*	26.3
	75	89.36 ± 2.39	63.40 ± 1.42	71.10 ± 2.01*	29.1
	150	87.76 ± 2.16	61.77 ± 0.93	69.72 ± 3.20*	30.0
Loperamide	5	91.20 ± 1.33	58.34 ± 0.35	3.98 ± 0.61*	36.0
Water	-	96.18 ± 1.17	85.29 ± 0.89	88.79 ± 0.60	-

Values are expressed as mean ± SEM (n=5) = * significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software, (Sandiego USA). IL: Intestinal Length, CML: Charcoal Meal Length, IT: Intestinal Transit.

Table 5: Liver Function indices of rats administered ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis*

Fractions/ Drug	Dose (mgkg^{-1})	ALT (U/l)	AST (U/I)	ALP(U/l)	TB(mg/dl)	ALB(g/dl)	TP(g/dl)	5N'(U/1)
Saturated Butanol	25	12.3 ± 0.22	18.3 ± 0.45	87.6 ± 0.75	0.31 ± 0.25	2.34 ± 0.13	7.18 ± 0.70	12.6 ± 0.21
	50	12.4 ± 0.37	18.9 ± 0.68	88.8 ± 0.66	0.33 ± 0.03	2.60 ± 0.05*	6.78 ± 0.26	13.0 ± 0.36
	75	12.5 ± 0.38	19.9 ± 0.56*	89.4 ± 0.93	0.29 ± 0.03	2.40 ± 0.06	6.74 ± 0.11	15.0 ± 0.85*
	150	13.9 ± 0.64*	19.0 ± 0.34	89.6 ± 0.40	0.23 ± 0.02	2.50 ± 0.01	6.50 ± 0.39	17.6 ± 1.88*
Ethylacetate	25	11.5 ± 0.22	18.2 ± 0.36	88.6 ± 0.68	0.23 ± 0.03	2.50 ± 0.08	7.02 ± 0.11	12.9 ± 0.38
	50	11.6 ± 0.57	20.3 ± 0.37	89.0 ± 1.00	0.14 ± 0.02	2.40 ± 0.06	7.78 ± 0.05	13.5 ± 0.55
	75	13.0 ± 0.31	20.0 ± 0.38	90.8 ± 1.00	0.33 ± 0.03	2.28 ± 0.18	6.82 ± 0.21	17.8 ± 1.62*
	150	15.2 ± 0.93*	23.3 ± 1.55*	92.0 ± 2.08	0.22 ± 0.03	2.02 ± 0.02	5.78 ± 0.33*`	26.8 ± 2.10*
Loperamide	5	12.1 ± 0.46	18.1 ± 0.38	89.6 ± 0.97	0.24 ± 0.02	2.42 ± 0.06	6.71 ± 0.18	10.7 ± 0.43
Water	-	10.5 ± 0.47	17.2 ± 0.46	88.5 ± 0.25	0.22 ± 0.14	2.29 ± 0.12	6.95 ± 0.25	9.08 ± 0.92

Values are expressed as mean ± SEM (n=5). * significantly ($P<0.05$) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software, (Sandiego USA). ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline phosphatase, ALB: Albumin, TP: Total protein TB: Total bilirubin, 5N':(5' Nucleotidase)

Table 6: Renal Function indices of rats administered ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis*.

Fractions/ Drug	Dose(mgkg^{-1})	Urea (mmol/l)	Creatine (mg/dl)	Uric acid (mg/dl)	Na+ (mmol/l)	K+ (mmol/l)
Saturated Butanol	25	8.3 ± 0.40	0.7 ± 0.04	2.6 ± 0.42*	125.2 ± 2.99	3.9 ± 0.10
	50	7.3 ± 0.38	0.7 ± 0.05	3.0 ± 0.64*	126.8 ± 3.65	4.8 ± 0.48
	75	8.0 ± 0.26	0.7 ± 0.37	2.3 ± 0.42*	127.2 .± 3.84	4.4 ± 0.39
	150	8.0 ± 0.22	0.6 ± 0.04	3.3 ± 0.01*	132.2 ± 1.77	3.9 ± 0.08
Ethylacetate	25	6.3 ± 0.05	0.6 ± 0.05	3.0 ± 0.64*	127.6 ± 1.33	4.5 ± 0.14
	50	7.2 ± 0.07	0.6 ± 0.01	5.3 ± 0.34	134.8 ± 2.27	4.9 ± 0.42
	75	6.5 ± 0.97	0.7 ± 0.07	4.0 ± 0.87	122.2 ± 1.86	3.9 ± 0.15
	150	8.2 ± 0.08	0.8 ± 0.08	4.3 ± 0.68	127.6 ± 3.49	4.0 ± 0.21
Loperamide	5	8.2 ± 0.19	0.6 ± 0.04	4.2 ± 0.68	132.3 ± 1.04	4.0 ± 0.06
Water	-	7.1 ± 0.33	0.6 ± 0.06	6.2 ± 0.52	127.0 ± 2.00	4.9 ± 0.24

Values are expressed as mean ± SEM (n=5). * Significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test using Graph Pad Instant Software, (Sandiego USA).

Table 7: Effect of ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis* on some haematological parameters.

Fractions	Dose ($mgkg^{-1}$)	WBC ($10^3\mu/l$)	RBC ($10^6\mu/l$)	HGB (g/dl)	HCT (%)	PLT ($10^3\mu/l$)
Saturated Butanol	25	11.7 ± 0.42	7.8 ± 1.09	14.3 ± 0.18	50.9 ± 0.66	6.14 ± 0.20
	50	15.2 ± 0.21	7.2 ± 2.06	14.1 ± 0.20	49.1 ± 1.14	5.44 ± 0.43
	75	14.9 ± 0.32	8.4 ± 1.77	14.7 ± 0.21	51.6 ± 0.97	6.42 ± 0.81
	150	11.1 ± 1.21	7.9 ± 2.53	14.0 ± 0.25	50.6 ± 0.45	6.18 ± 0.24
Ethylacetate	25	16.2 ± 0.54	7.8 ± 3.53	14.5 ± 0.17	50.8 ± 0.45	6.26 ± 0.12
	50	13.0 ± 1.65	7.1 ± 3.26	14.4 ± 0.08	51.8 ± 0.44	5.91 ± 0.34
	75	13.1 ± 1.52	6.8 ± 0.46	13.9 ± 0.34	50.1 ± 1.34	6.06 ± 0.23
	150	11.7 ± 1.06	7.4 ± 1.67	13.6 ± 0.22	50.0 ± 0.27	4.78 ± 0.68
Loperamide	5	15.1 ± 1.09	6.5 ± 2.03	14.2 ± 0.19	50.0 ± 0.34	6.31 ± 0.52
Water	-	12.6 ± 0.41	8.2 ± 1.54	14.7 ± 0.14	51.2 ± 0.33	6.76 ± 1.00

Values are expressed as mean ± SEM (n=5) = All values are not significantly (P>0.05) when compared with control by using analysis of variance, Bonferroni multiple comparisons test using Graph pad Instat software, (Sandiego USA). WBC: White blood cell, RBC: Red blood cells, HGB: Haemoglobin, HCT: Haematocrit, PLT: Platelet.

Table 8: Protein precipitation test of total tannins in crude methanol stem bark extract of *Khaya senegalensis*.

Tubes No	Concentration of tannin (mg/ml)	Absorbance of supernatant after precipitation	Precipitation rate (%)
1	0.0	0.00	0.00 ± 0.00
2	0.2	0.136	32.1 ± 0.61*
3	0.4	0.100	49.5 ± 0.27*
4	0.6	0.088	56.9 ± 0.46*
5	0.8	0.082	59.2 ± 0.12*
6	1.0	0.068	65.5 ± 0.62*
7	1.2	0.058	71.2 ± 1.06*
8	1.4	0.059	70.5 ± 0.03*
9	1.6	0.039	81.0 ± 0.14*

Values are expressed as mean ± SEM (n=3) = * significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instat Software (Sandiego USA).

Table 9: Protein precipitation test of total tannins in saturated butanol fraction of stem bark of *Khaya senegalensis*

Tubes No	Concentration of tannin (mg/ml)	Absorbance of supernatant after precipitation	Precipitation rate (%)
1	0.0	0.00	0.00 ± 0.00
2	0.2	0.069	65.6 ± 0.21 *
3	0.4	0.064	68.2 ± 0.09 *
4	0.6	0.061	69.7 ± 0.23 *
5	0.8	0.054	73.1 ± 0.06 *
6	1.0	0.051	74.6 ± 0.09 *
7	1.2	0.037	81.6 ± 0.18 *
8	1.4	0.034	83.2 ± 0.03 *
9	1.6	0.030	85.1 ± 0.03 *

Values are expressed as mean ± SEM (n=3) = *significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software (Sandiego USA).

Table 10: Antibacterial activity of methanol extract, ethylacetate and saturated butanol fractions of stem bark of *Khaya senegalensis* against some bacteria causing diarrhoea.

Fractions/ Drug	**Concentration ($mgml^{-1}$)**	**Zone of Inhibition (mm)**		
		Salmonella spp	***Esherichia coli***	***Pseudomonas aeruginosa***
Methanol	25	12.4 ± 0.32*	12.1 ± 0.45*	12.3 ± 1.20*
	50	13.0 ± 0.58*	12.7 ± 0.66*	12.7 ± 1.00*
	75	13.7 ± 1.77*	13.9 ± 1.78*	13.0 ± 0.03*
	150	14.7 ± 0.33*	14.0 ± 0.58*	13.8 ± 0.09*
Ethylacetate	25	12.7 ± 0.88*	12.2 ± 0.87*	12.9 ± 0.23*
	50	13.0 ± 1.15*	13.3 ± 0.88*	13.6 ± 0.83*
	75	14.0 ± 0.58*	14.6 ± 0.36*	13.7 ± 0.35*
	150	15.3 ± 0.35*	17.0 ± 1.53*	15.4 ± 0.81*
Saturated butanol	25	14.3 ± 1.33*	13.7 ± 1.21*	14.1 ± 0.64*
	50	16.7 ± 0.70*	15.7 ± 0.68*	15.3 ± 0.46*
	75	18.3 ± 0.33*	18.0 ± 1.00*	16.1 ± 0.12*
	150	20.0 ± 0.58*	19.7 ± 1.53*	18.3 ± 0.55*
Ciprosan	5	23.7 ± 0.33*	24.3 ± 0.88*	24.7 ± 0.33*
Water	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± SEM (n=3). Values greater han 12mm indicated some activity. Zone of inhibition is in mm, *significantly (P<0.05) different when compared with control by using analysis of variance, Bonferroni multiple comparisons test, using Graph Pad Instant Software, (Sandiego USA).

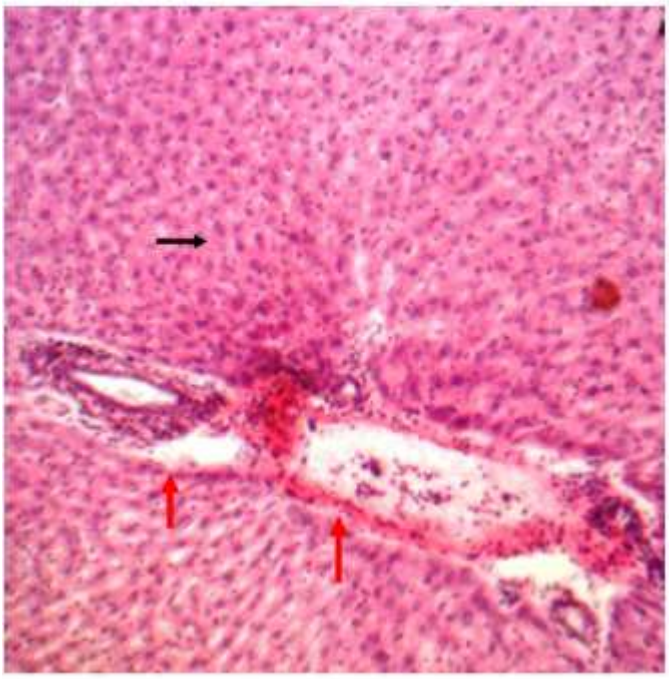

Plate1: liver tissue of control group showing preserved lobular architecture (black arrow) and preserved portal area (red arrow). Hematoxylin and Eosin X100 (ethylacetate group)

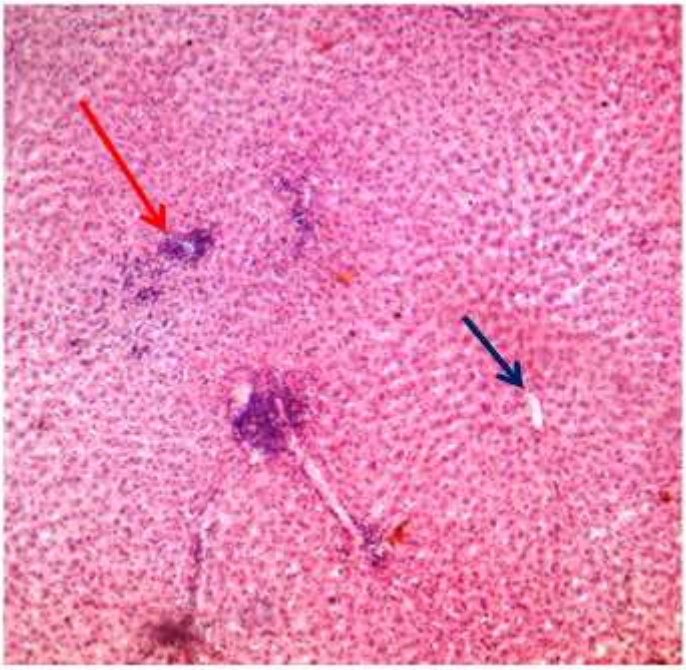

Plate2: Liver tissue of rat administered with 5mg kg-1 of loperamide, arranged in lobules around central veins (black arrow) and mild interstitial inflammation (red arrow). Hematoxylin and Eosin (H & E) X100.

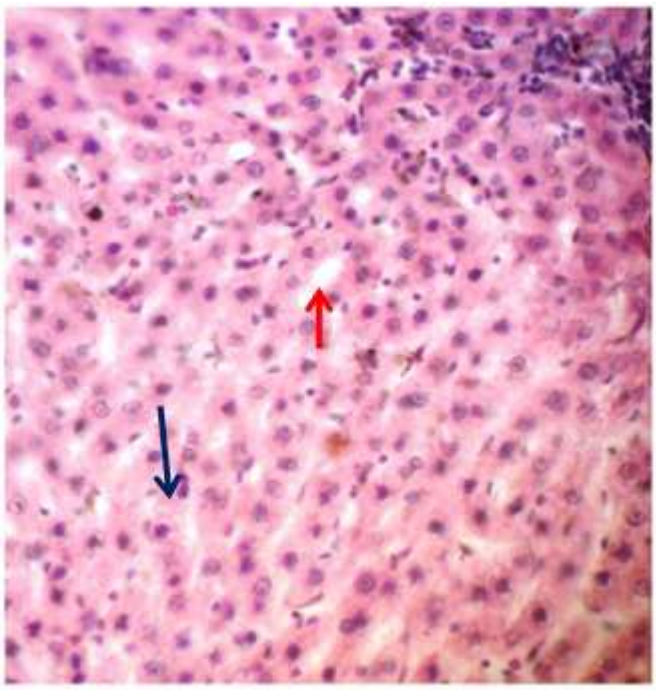

Plate 3: Liver tissue of rat administered with 25mg kg-1 of ethylacetate fraction, hepatocytes with mild hydropic change (black arrow) and dilated sinusoids (red arrow). Hematoxylin and Eosin (H & E) X200

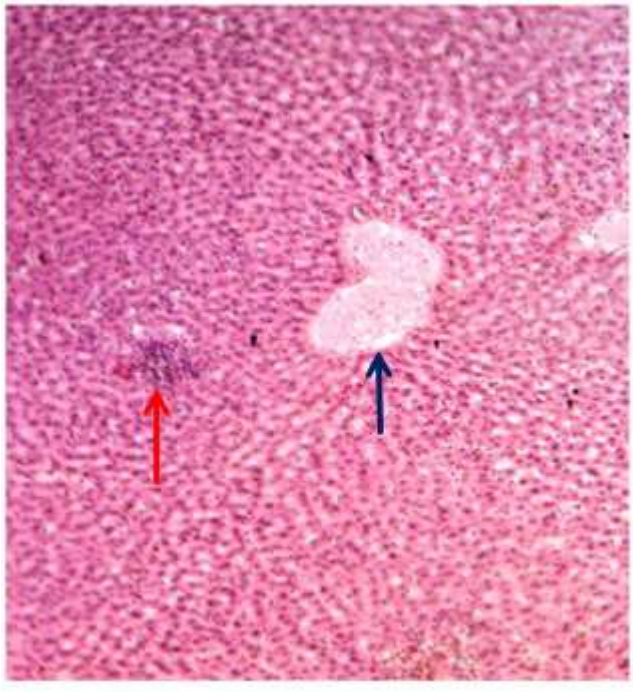

Plate 4: Liver tissue of rat administered with 50mg kg-1 of ethylacetate fraction, with mild inflammation (red arrow) and congested central vein (black arrow). Hematoxylin and Eosin (H & E) X100

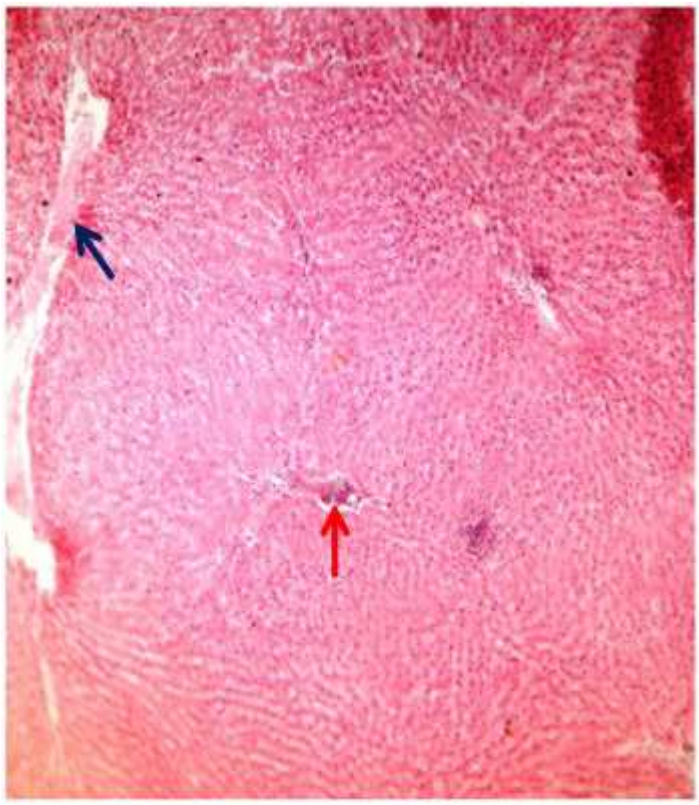

Plate 5: Liver tissue of rat administered with 75mg kg-1 of ethylacetate fraction, preserved liver lobular architecture, congested central vein (black arrow) and mild portal inflammation(red arrow). Hematoxylin and Eosin (H & E) X100

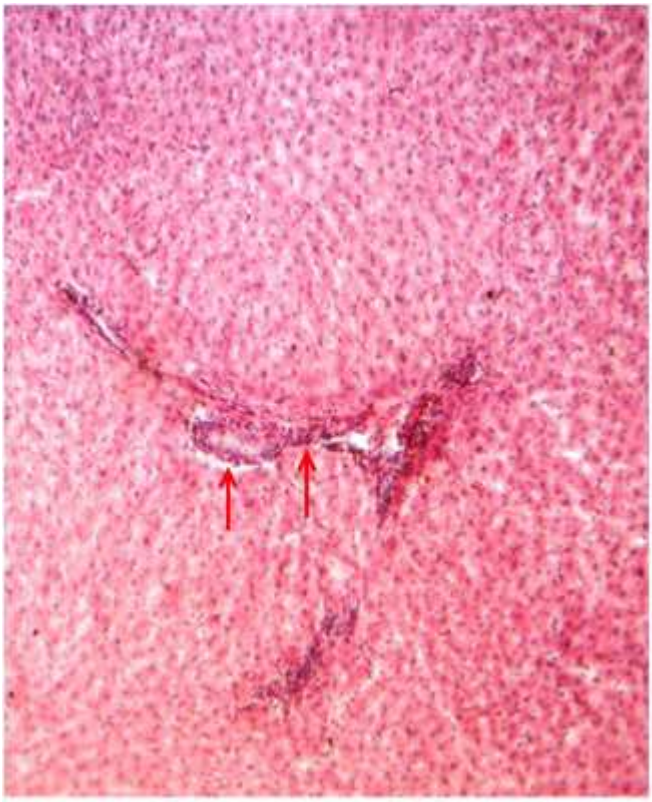

Plate 6: Liver tissue of rat administered with 150mg kg-1 of ethylacetate fraction, with portal inflammation (red arrows). Hematoxylin and Eosin (H & E) X100

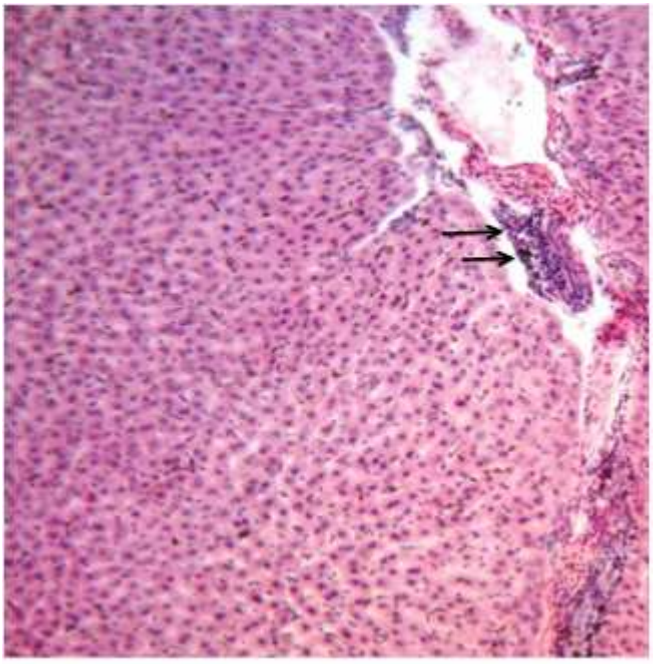

Plate 7: Liver tissue of rat in the control group with mild portal inflammation (black arrows). Hematoxylin and Eosin (H & E) X100

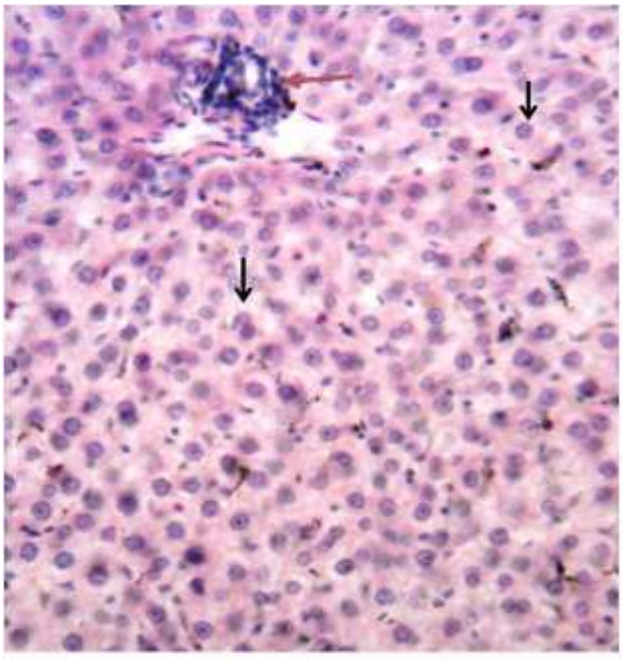

Plate 8: Liver tissue of rat administered with 5mg kg-1 of loperamide, mild hydropic change (black arrows) and portal inflammation (red arrow). Hematoxylin and Eosin (H & E) X100

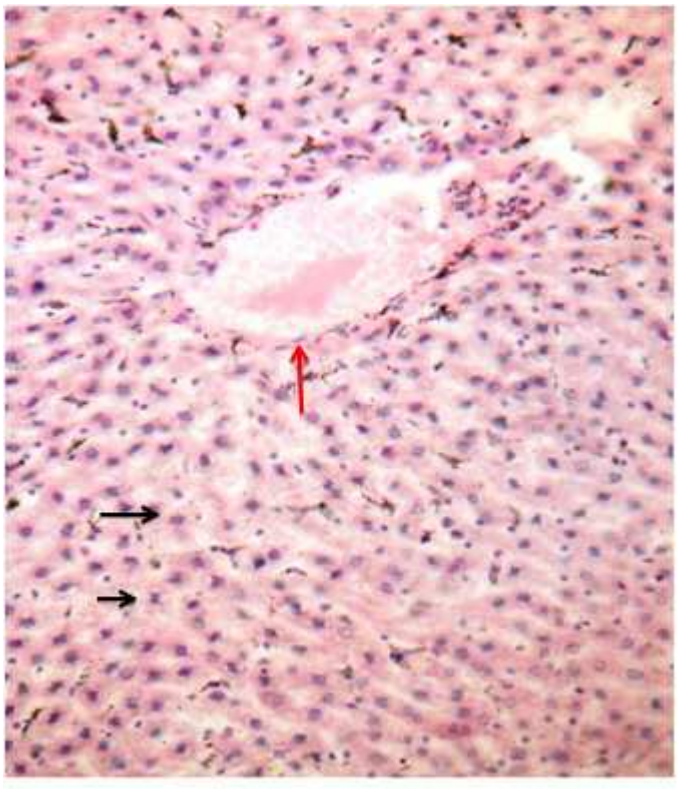

Plate 9: Liver tissue of rat administered with 25mg kg-1 of saturated butanol fraction, mild hydropic change (black arrows) and congested central vein (red arrow). Hematoxylin and Eosin (H & E) X100

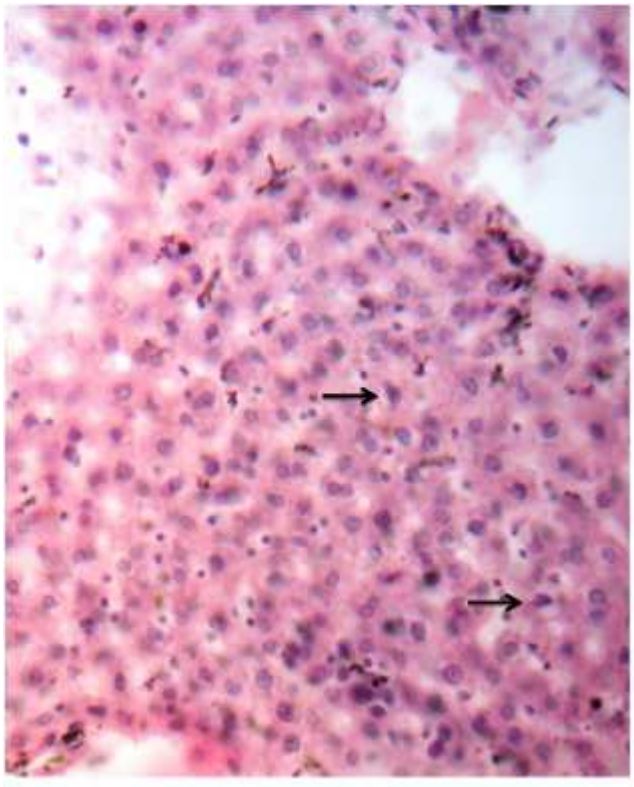

Plate 10: Liver tissue of rat administered with 50mg kg-1 of saturated butanol fraction, mild hydropic change (black arrows). Hematoxylin and Eosin (H & E) X100

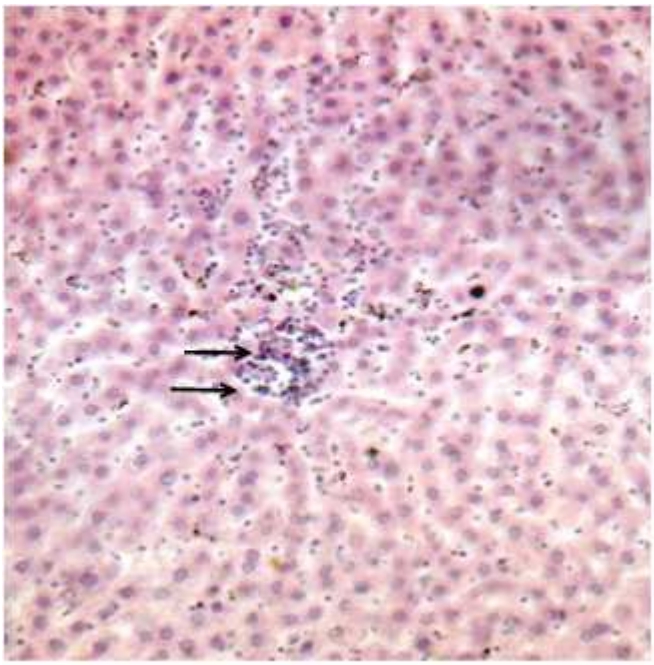

Plate 11: Liver tissue of rat administered with 75mg kg-1 of saturated butanol fraction, mild globular inflammation (black arrows). Hematoxylin and Eosin (H & E) X100

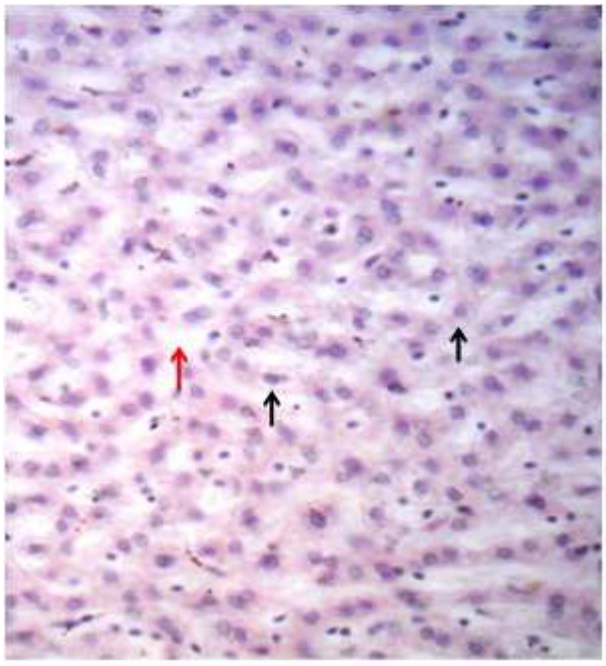

Plate 12: Liver tissue of rat administered with 150mg kg-1 of saturated butanol fraction, mild hydropic change (black arrows) congested sinusoids (red arrows). Hematoxylin and Eosin (H & E) X100

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