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Acute and Sub-chronic Toxicity of Aqueous Extract of Roots of *Khaya senegalensis* (Desr.) A. Juss. in Mice and Rats Respectively

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Khaya senegalensis* is one of the key medicinal plants used discretionarily in traditional medicine as remedies to several health conditions. This study aimed to establish the safety of *Khaya senegalensis* root aqueous extract in experimental animals with the purpose of optimizing its therapeutic value.
Methodology: A total of 74 animals (20 rats and 54 mice) were randomly assigned into two main groups based on toxicity plan; acute and sub-chronic toxicity. Mice were divided into 9 groups (6

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per group) for the acute toxicity study while rats were divided into 4 groups (5 per group) for sub-chronic toxicity assessment.

Results: The acute concentrations of the extract in mice induced dose-dependent clinical signs severities such as: twitching, increase rate of respiration, sedation, abdominal muscle contractions and increased motor activity. The lethal dose 50 value of the extract was estimated as 320mg/ kg body. The sub-chronic concentrated grades in the rats especially the higher doses elicited significantly increased serum liver enzymes values when compared to the control, while at low dose the values were comparable to that of the control. Also observed were the evidences of renal cellular pathology ranging from mild to severe tubular cell degeneration, tubular cell depletion and congestion of the renal cortex. The liver pathologies such as hepatic portal congestion, cytoplasmic vacuolations and nuclear degeneration were strikingly visible mostly at the higher doses. The lymphocyte and platelet counts were the only haematological parameters that increased significantly more particularly at low dose when compared with the control.

Conclusion: This study has shown that *Khaya senegalensis* seems to be safe only at low doses. However, caution should be taking in its administration for therapeutic purposes especially when long-term usage is desired.

Keywords: LD_{50}; medicinal plant; cellular pathology; haematology; Khaya senegalensis.

1. INTRODUCTION

The use of medicinal plants as therapeutic remedies for various diseases condition is a common norm in traditional medicine especially in African society [1,2]. More than 70% of the African population utilizes medicinal plants to treat various conditions and over 85% of human and animal diseases are cured with herbs or naturally derived compounds [3]. The abundance of secondary metabolites with important therapeutic values and absence of synthetic preservation in medicinal plants also have improved their effectiveness in treating several life-style related disorders [4]. Despite the wide use of medicinal plants in humans and animals as therapeutic remedies for treatment and prevention of diseases, scientific evidence of the quality, efficiency and safety are apparently lacking or not well documented [5] especially in most of the African indigenous therapy where herbs are consumed crude without specific prescription and thoroughly evaluated toxicity profile [6]. Even with their increasing acceptance as alternative to modern drugs for treating several disorders, little is known about their mode of action, safety as well as therapeutic dosage that can be ingested without being toxic to body cells. Thorough evaluation and assessment of crude extracts obtained from medicinal plants is therefore necessary to provide information needed for their safe use and subsequent standardization during drug processing into modern therapy.

Khaya senegalensis (*Desr.) A. Juss* belonging to the family *Meliaceae* [7] is a tree of African origin. It is majorly distributed in some areas in Nigeria and other West Africa countries such as the sub-Saharan savannah from Senegal to Sudan and Uganda. The West African species are commonly referred to as African mahogany [8]. *Khaya senegalensis* is locally known as Ogonwo in Yoruba, Madachi in Hausa, Ono in Igbo [9] and dry zone Mahogany in English (Abubakah *et al.,* 2009).

Khaya senegalensis plant is highly reputed for its numerous medicinal purposes and was often used discretionarily in traditional or popular medicine for the treatment of several disease conditions. The decoction of the stem back, roots and the leaves extracts are found effective against jaundice, dermatoses, malaria, fever, mucous diarrhea and venereal diseases [10,11] The plant extracts also find its application in the treatment of catarrh, epilepsy, hysteria, rheumatic pains, haemorrhoids, painful menstruation, wounds and burns [12]. Previous studies also documented the efficiency of *Khaya senegalensis root* extract in the management of mental illness, leprosy and syphilis [13,14,15]. Other therapeutic potential of *Khaya senegalensis includes* anti-inflammatory effects [16,17] antidiabetic [18,19,20] anti-bacterial [21] anti-cancer [22], antioxidant [23], anti-plasmodial activities [24], Khalid et al. 2016, antisickling [25,26] anti-helminthic [27] and anti-trypanosomal activities [2]

Despite wide use and varieties of health benefit from *Khaya senegalensis* plant, other parts of the plant like leaves, stem and fruit were reported as a remedy for several ailments [11]. However,

there is paucity of information which scientifically confirm the safety of its root extract for therapeutic purposes and human consumption. This study, therefore, evaluated the safety of *Khaya senegalensis* root aqueous extract in experimental animals following acute and prolonged exposure in order to provide guidelines for establishing suitable dose range on further health product development.

2. MATERIALS AND METHODS

2.1 Preparation of the Plant Extract

The roots of *Khaya senegalensis* were collected from the locality of Ibadan in Oyo State, Nigeria. The plant was identified and authenticated as *Khaya senegalensis* at the Herbarium Unit of the Department of Botany, University of Ibadan, Oyo State, Nigeria where the voucher specimen number UIH- 22873 was deposited. The roots were washed in water, allowed to dry in the open laboratory air to a constant weight and grinded into powdery form with a milling machine. 250g of the pulverized root was soaked with 2L of water for 72 h with constant agitation on a shaker, the preparation was then filtered using a muslin bag and cotton wool. The filtrate was concentrated to dryness in vacuum by rotary vacuum evaporation (Bibby Sterling®, Germany) and then lyophilized with a freeze dryer. The percentage yield of the extract was calculated. The lyophilized powder was scraped into an air tight container and kept at 4°C till needed.

2.2 Experimental Animals

Twenty apparently healthy Wistar male rats weighing between 100 – 120 g and fifty-four male mice (weighing between 20 – 25 g) used in this study were obtained from the Animal House of Ladoke Akintola University of Technology, Mercy land Campus, Osogbo. The experimental animals were maintained in well-ventilated cages, under hygienic condition. They were subjected to mandatory acclimatization in the animal house for two weeks before the commencement of the experiment and were fed with pelletized animal feed and water provided ad libitum. All rats and mice received humane care in accordance to the "Guide for the care and use of laboratory animals" (National Academic Press, Washington DC, USA, 1996).

2.3 Preliminary Phytoconstituents Analysis

Standard methods were used to detect the phytochemical constituents present in the aqueous root extract of *Khaya senegalensis* [28]. The alkaloid and phenol content percentages were calculated using the techniques outlined in the study conducted by Krishnaiah and colleagues in 2009. To assess the flavonoid content, the aluminum trichloride method was employed with quercetin as the benchmark compound, as detailed by Zhishen et al. [29]. The findings were presented in terms of milligrams of quercetin per gram of dry leaves, denoting them as quercetin equivalents. The percentage yield of the alkaloid and phenol content was calculated as

$$\text{Percentage yield (\%)} = \frac{\text{Yield (difference)}}{\text{Weight of plant sample used (g)}} \times 100$$

2.4 Acute Toxicity Study

The modified experimental procedure reported by Folarin et al. [30] on *Vitellaria paradoxa,* was adopted for the determination of acute toxicity of *Khaya senegalensis*. Fifty-four male mice, weighing between 20 and 25 g, were used for acute toxicity study. The mice were sorted randomly into eight treatment groups and a control group of six animals (n=6) per group. The animals were kept in well-ventilated wired cages as seen in Fig. 1. Sequel to an overnight fast, the control group received physiological saline, and each of the exposed groups received aqueous extract of *K. senegalensis* at doses of 10, 20, 40, 80,160, 320, 640 and 1280 mg/kg, administered through oral gavage with a suitable intubation cannula. Animals were constantly monitored for changes in general behavior continuously for 30 minutes, after 3h and 24h post extract administration. Observations were focused on parameters such as piloerection, sensitivity to sound and touch, locomotion, aggressiveness and appearance of faeces. The number of survivors was noted after 24 h. All surviving animals were humanely euthanized at the end of the study by administering ketamine (100mg/kg) and xylazine (10mg/kg) combination intraperitoneally.

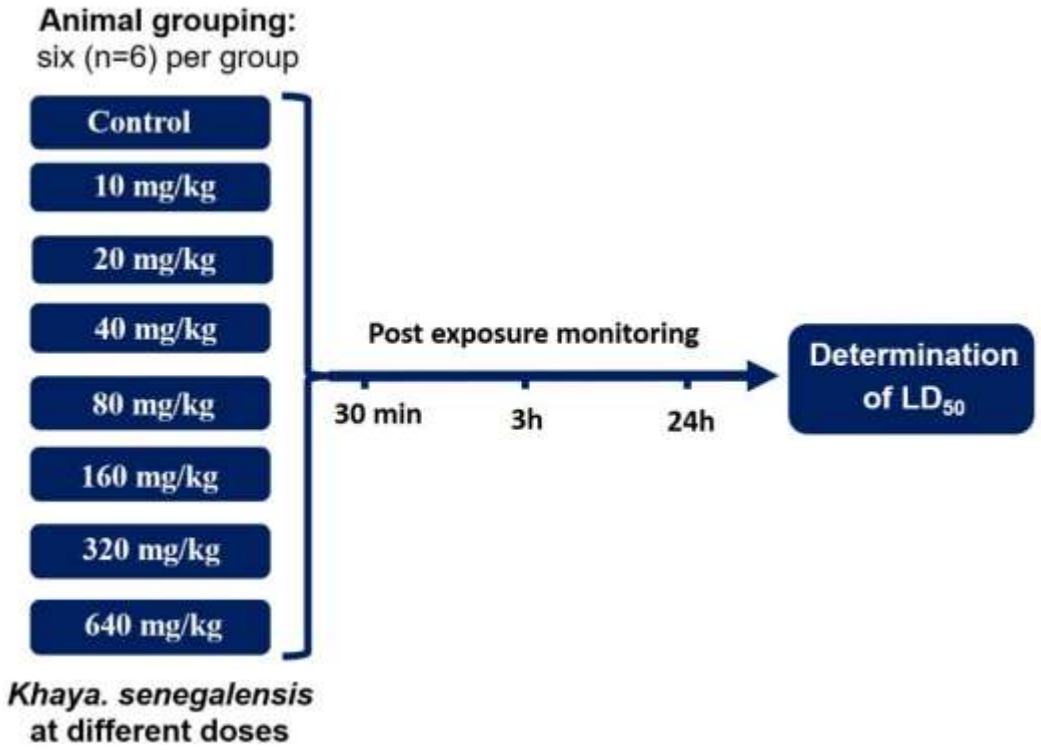

Fig. 1. Experimental schedule for acute toxicity study in mice

2.5 Median Dose Calculation

This was predicated on the approach of Hodge and Sterner (2005). The Dose that kills 50% of the population of the animal was calculated as 320mg/kg and from this dose the low, median and high doses to be administered in the sub-chronic toxicity study were calculated as highlighted below:

Median Dose= 1/10th of the LD_{50} i.e., 320; Median dose = 32mg/kg

Low Dose = $^{1}/_{2}$ of median dose = 16mg/kg

High dose = 2 × median dose = 64mg/kg

2.6 Sub-Chronic Toxicity Protocols

Sub-chronic toxicity study was also conducted as described by Folarin et al. [30]. Briefly, twenty healthy male Wistar rats were divided into four groups of five animals per group (n=5) as represented in Fig. 2. The treatment groups received low (16 mg/kg), medium (32 mg/kg) and high (64 mg/kg) doses of the aqueous root extracts of *Khaya senegalensis* derived from the LD_{50}. The extract was administered to the rats for a period of 28 consecutive days while the control group received the physiological saline. The animals were observed daily for deviation from normal behavioral signs.

2.7 Sample Collection

Sequel to the termination of the experiment, blood was collected into EDTA and plain sample bottles via orbital sinus venipuncture, for haematological and biochemical analyses, respectively. Thereafter, the animals were humanely euthanized by administering the combination of ketamine (100mg/kg) and xylazine (10mg/kg) intraperitoneally. The liver and kidneys were subsequently excised for histopathological assessments.

2.8 Haematological Analysis

The blood samples collected in the EDTA sample bottles were analyzed immediately after collection using automatic haematological analyzer Cell Dyn®3500 (Abbot Laboratories Ltd, USA). The haematological parameters evaluated include: Packed Cell Volume (PCV), haemoglobin count, Red Blood Cell Count (RBC), Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration

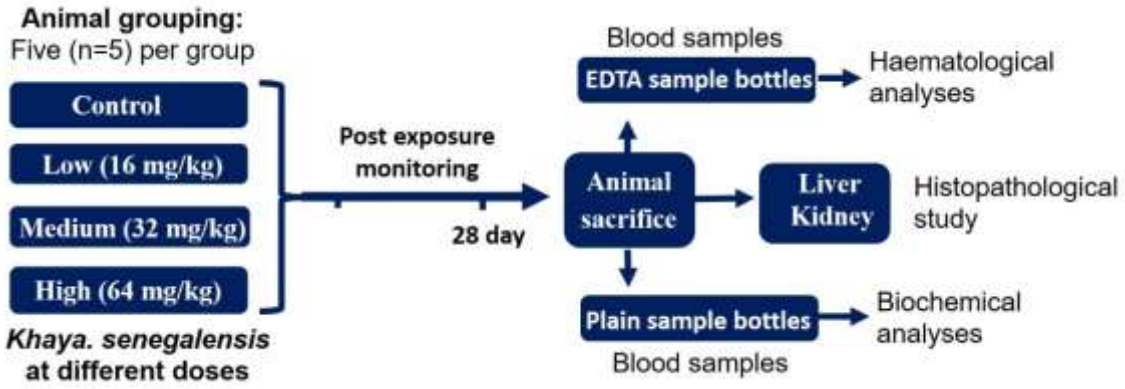

Fig. 2. Experimental schedule for sub-chronic toxicity assessment in rats

(MCHC), White Blood Cell Count (WBC) and differential (neutrophil, eosinophil, lymphocyte, monocyte, basophil and platelet) counts.

2.9 Biochemical Analysis

The blood samples collected in plain sample bottles were allowed to stand on laboratory bench in an inclined position for 15min and then centrifuged at 3000 rpm for 10min. The resultant sera obtained were transferred into an appropriately labeled Eppendorf tube and kept at -20°C before the analysis was conducted. The automatic chemistry analyzer Cobas®Integra 400 plus (Roche Diagnostics Ltd., Switzerland) was used to assay biochemical parameters such as alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin and total protein.

2.10 Histopathological Preparations of the Tissues

The excised liver and kidneys tissues were fixed in 10% neutral buffered formalin. The tissues were furthered subjected to routine histological processing technique (dehydration, clearing, infiltration and embedment in paraffin wax). Sections of 5 µm were obtained by using Leica RM 2115-rotatory microtome. The obtained sections were subsequently stained with Haematoxylin and Eosin (H&E) for light microscopy. Photomicrographs were taken at ×400 magnification with the use of Olympus, China. The photomicrographs were evaluated for the presence of histopathological lesions.

2.11 Statistical Analysis

Results were expressed as a mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance and followed by Dunnett test to evaluate significant differences between groups. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using GraphPad Prism version 5.00 for Windows (Graph Pad Software, San Diego, CA).

3. RESULTS

3.1 Phytochemical Studies

The percentage yield of the aqueous root extract of *Khaya senegalensis* was 8.46% (w/w), in respect of the dried powder. Qualitative phytochemical analysis of the aqueous root extract of *Khaya senegalensis* revealed the presence of saponins, tannins, flavonoids, alkaloids, terpenoids, sterols and cardiac glycosides while, phlobatannins were, however, absent as presented in Table 1. Alkaloids had the highest yield of 48.68% compared to flavonoids and tannins with 22.53% and 3.14% respectively Table 2.

3.2 Acute Toxicity Study

The acute toxicity study results of root extract of *K senegalensis* was presented in Table 3. This study showed that severe toxicity of the aqueous root extract of *K senegalensis* was observed at the dose of 1280 mg/kg body weight of the treated animals. This particularly dose was the established LD_{100} dose Table 1 and was typified by complete mortality after 12 h of extract administration. Also, the experimental animals displayed remarkable changes in behavioral signs such as severe twitching, increase respiratory rate, sedation, abdominal muscle contractions, elevated motor activity, bradypnea, cyanotic (purplish appearance) mucous

membranes of the tail and nail and piloerection, comma and death. Next to this dose in term of severity is 640 mg/kg which caused more than two-third of mortality. The varied doses of 40, 80 and 160 mg/kg elicited in the exposed animals mild to moderate twitching, salivation, abdominal muscle contraction, piloerection and minimally reduced mortality relative to the higher doses. Of note is the normal appearance of the survival animal after 24 h of extract administration. On the contrary, the lower limit doses of 10 and 20 mg/kg body weight of *K senegalensis* revealed no remarkable changes in the behavioral signs in the surviving animals and more importantly there was no mortality.

Table 1. Qualitative phytochemical analysis of aqueous root extract of *Khaya senegalensis*

S/N	Phytochemicals	Detection
1	Saponins	+
2	Tannins	+
3	Flavonoids	+
4	Alkaloid	+
5	Sterol	+
6	Terpenoids	+
7	Cardiac glycoside	+
8	Phlobatannins	-
9	Carbohydrates	+

Key: + Present; - Absent

3.3 Determination of LD_{50} from Acute Toxicity Study

The LD_{50} value of aqueous root extract of *Khaya senegalensis* was found to be 320mg/kg body weight Table 3 based on the animal's observation and calculation by Karber. (1931). According to Hodge and Sterner (2005) toxicity scale, the LD_{50} of *Khaya senegalensis* aqueous root extract was classified to be moderately toxic Table 1.

3.4 Haematological Profile

The effect of sub chronic exposure of rats to graded doses (16 mg/kg, 32 mg/kg and 64 mg/kg) of *Khaya senegalensis* on the haematological parameters is shown in Table 4. With the exception of exclusive significant increase ($p<0.05$) in the lymphocyte and platelet counts in rats administered low dose (16mg/kg) of the extract compared to others, there was no significant difference ($p>0.05$) in the values of the entire haematological parameters (WBC and its differentials, RBC, Hb Conc., PCV, MCV, MCH, MCHC) of the various groups when compared with their respective controls Table 4.

3.5 Biochemical Parameters

The effect of sub chronic exposure of rats to graded doses (16 mg/kg, 32 mg/kg and 64 mg/kg) of *Khaya senegalensis* on the hepatic enzymes is shown in Table 5. There was no significant difference ($p>0.05$) in the level of the albumen and total protein of rats exposed to the graded doses of *Khaya senegalensis* when compared to the control. However, the serum hepatic enzymes (ALT, ALP and AST) were significantly elevated in the rat groups administered the medium (32 mg/kg) and high (64 mg/kg) doses of the extract of *Khaya senegalensis* when compared to the control Table 5.

3.6 Histopathology of the Liver and Kidneys of Rats Exposed to Graded Doses of Ethanolic Extract of *Khaya senegalensis*

3.6.1 Liver

The results of the impact of sub-chronic exposure of grades of ethanolic extract of *Khaya senegalensis* on the liver of rats are presented in Plate 1A-D. The hepatic parenchyma of rats in the control groups showed no visible lesion and is characterized by nearly roundish nuclei within cytoplasmic hepatocytes with regular outline and intact central vein Plate 1A. Similarly, the parenchyma of the liver of rat exposed to sub-chronic low dose (16 mg/kg) of *Khaya senegalensis* extract appeared to be devoid of histopathological lesions. However, the liver of rats that were chronically exposed to median (32 mg/kg) and high (64 mg/kg) doses of aqueous root extract of *Khaya senegalensis* showed mild to severe degrees of hepatic histoarchitectural alterations (portal congestion, cytoplasmic vacuolations, nuclear degeneration and necrotic cells). The liver of rats that was exposed to the highest dose of *Khaya senegalensis* (64 mg/kg) displayed markedly severe hepatic damage Plate 1B-D.

3.6.2 Kidneys

The renal histological results of rats sub-chronically exposed to graded doses of ethanolic extract of *Khaya senegalensis* are presented in

Table 2. Quantitative phytochemical analysis showing percentage yield of phytochemicals

Phytoconstituents	Yield	% Yield
Alkaloids	0.972 ± 0.004	48.60 ± 2.04
Flavonoids	0.456 ± 0.001	22.53 ± 1.01
Tannins	0.063 ± 0.002	3.14 ± 0.07

Table 3. Acute toxicological profile of the different doses of aqueous extract of *Khaya senegalensis* administered orally in mice

Groups	Dose (mg/kg)	Number of mice	Number of deaths	Percentage (%) death
A	Saline (10ml/kg)	6	0	0
B	10	6	0	0
C	20	6	0	0
D	40	6	1	16.7
E	80	6	1	16.7
F	160	6	2	33.3
G	320	6	3	50
H	640	6	5	83.3
I	1280	6	6	100

Plate 2A-D. The renal histological appearance was devoid of lesion as typified by intact glomerulus within the Bowman's capsule and distinct normal renal tubule Plate 2A. With the exception of normal histology observed in kidneys of rat exposed to 16 mg/kg of *Khaya senegalensis* Plate 2B, the kidneys' parenchyma in other exposed groups (32 mg/kg and 64 mg/kg) displayed mild to moderate range of renal histo-architectural alterations including cortical congestion, tubular cell degeneration and tubular cell depletion Plate 2C and 2D.

4. DISCUSSION

The phytochemical screening of aqueous crude extracts from roots samples of *Khaya senegalensis* used in this study revealed that the crude extracts contained tannins, saponins, flavonoids, sterols, alkaloids and terpenoids which represent essential metabolites of the plant. These findings align with the research by Kankia and Zainab [31], who also identified these phytonutrients as the primary bioactive compounds in various crude root extracts of *Khaya senegalensis.* These compounds are found to have numerous therapeutic purposes [32,12], for example flavonoid groups exhibited high antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic activities Saponins and tannins play roles in the plant's defense system. However, having precise knowledge of the phytochemicals in plant extracts is crucial for determining appropriate dosages, avoiding toxicities, and understanding potential antagonistic effects. These bioactive compounds in plants are also important for the manufacture of therapeutic drugs and several report are available on their efficacy against varieties of health conditions, Nevertheless, some of them which are classified as major part of the secondary metabolites have been found to be toxic to the body cells (Jamloki *et al*., 2022), especially when ingested in excess amount, example of such metabolites that exhibit specific kinds of toxicity are pyrrolizidine-alkaloid found in Comfrey and Dryopteris, the thiocyanates present in brassica vegetables and lectins of many pulses including soya and red kidney beans [33]. The right dosage of the plant's bioactive compounds acts as medicine and differentiates it from the poison, low doses often found to be beneficial while overdose can induce toxicity (Botha and Penrith, 2008) therefore, quantity is often an important consideration in herbal therapy [34].

In the present study, the LD_{50} of aqueous root extract of *Khaya senegalensis* in mice gavaged at doses ranging from 10 to 1280 mg/kg was found to be 320 mg/kg body weight. The dose falls within the range categorized as moderately toxic dose by Hodge and Sturner. (2005). This finding is in agreement with the toxic concentration earlier reported for the root extract of *Vitellaria paradoxa* (Folarin *et al*., 2020). Although, [35] observed nontoxic effect of stem bark extract of the same plant after oral administration in male Wistar rats. Onu et al. [11] and Hermine *et al*. (2018) also reported zero toxicity following short-term oral administration of stem bark and leaf extracts of *Khaya*

senegalensis in rats. These discrepancies may be due to the effects of some toxic metabolites that may occur in different plant parts or variations in photochemical constituents from different plant sources.

Blood parameters provide insight into the health of blood and organs involved in blood production in animals exposed to toxic substances and other agents [36,37,38]. Additionally, the evaluation of blood parameters in laboratory animals can offer valuable information for extrapolating potential toxicity in the human population. On this premise, the lack of significant differences observed in most of the haematological parameters suggests that sub-chronic exposure of rats to *Khaya senegalensis* appeared not to elicit alteration in their haematological profile. This finding on haematological profile is similar to reports of Adebayo et al. [39] on *Chrysophyllum albidum*, Ilodigwe et al. [40] on *Spathodea campanulata* and Folarin *et al*. (2020) on *Vitellaria paradoxa*. This haemato-protectant potential could be linked to the presence of antioxidants in the root of *Khaya senegalensis* (Atawodi *et al*., 2014); though, this was not covered in the present scope of this study.

In contrast to the finding above, there was a significant increase in the lymphocyte count and the marked rise in the platelet counts in rats administered 32mg/kg group of the plant extract. These elevated white blood cell differentials were suggestive of the plant inherent immune enhancing potential which could offer a degree of protection against infections. This finding lends its credence in its local usage for treating various infections as earlier observed by Lompo et al. [16], Onu et al. [11] and Abdelgaleil et al. [12].

Table 4. Haematological profiles of Wistar rats exposed to sub-chronic administration of different doses of aqueous root extract of *Khaya senegalensis*

Blood Parameters	Doses of *Khaya senegalensis*			
	Control	**16mg/kg**	**32mg/kg**	**64mg/kg**
PCV	37.20 ± 0.66 [a]	34.40 ± 1.97[a]	38.60 ± 1.91[a]	36.60 ± 0.51[a]
Hb	10.86 ± 0.34[a]	10.70 ± 0.47[a]	10.98 ± 0.50[a]	10.34 ± 0.31[a]
RBC	6.260 ± 0.17[a]	5.380 ± 0.47[a]	6.520 ± 0.32[a]	5.980 ± 0.30[a]
MCV	62.00 ± 4.65[a]	58.40 ± 1.21[a]	59.00 ± 1.79[a]	58.20 ± 1.43[a]
MCH	16.10 ± 0.61[a]	15.72 ± 0.21[a]	16.28 ± 0.28[a]	16.56 ± 0.56[a]
MCHC	27.76 ± 0.16[a]	26.92 ± 0.86[a]	28.56 ± 0.43[a]	28.06 ± 0.30[a]
TWBC	3.92 ± 0.27[a]	4.360 ± 0.17[a]	4.080 ± 0.16[a]	3.740 ± 0.28[a]
NEUTRO	20.40 ± 1.54[a]	23.60 ± 2.64[a]	23.60 ± 3.71[a]	20.00 ± 2.983[a]
LYMPHO	65.00 ± 1.79[a]	75.40 ± 2.56[b]	58.20 ± 2.13[a]	59.60 ± 1.03[a]
BASOPHIL	15.20 ±1.16[a]	15.20 ± 1.50[a]	18.20 ± 1.83[a]	15.60 ± 1.50[a]
PLATELET	406.6 ± 25.89 [a]	567.0 ±72.28[b]	464.4 ±16.87[a]	405.8 ±27.92[a]

Values in the same row with different superscripts are significantly different (p < 0.05). WBC- White blood count, MCV- Mean corpuscular volume, RBC- Red blood cell count, MCH - Mean corpuscular haemoglobin, Hb Conc. - Haemoglobin concentration, MCHC - Mean corpuscular haemoglobin concentration, PCV - Pack cell volume, PLT - Platelet count

Table 5. Changes in the biochemical parameters of experimental animals sub-chronically exposed to different doses of aqueous extract of *Khaya senegalensis*

Biochemical Parameters	Doses of *Khaya senegalensis*			
	Control	**16 mg/kg**	**32 mg/kg**	**64 mg/kg**
AST (IU/L)	15.40 ± 0.40[a]	15.20 ± 0.86[a]	43.40 ± 1.21[b]	45.20 ± 0.86[b]
ALP (IU/L)	45.20 ± 1.65[a]	49.60 ± 2.02[b]	68.80 ± 2.71[c]	69.20 ± 2.42[c]
TP (g/dL)	6.58 ± 0.04[a]	6.860 ± 0.12[a]	6.80 ± 0.10[a]	6.68 ± 0.10[a]
ALB (g/dL)	3.48 ± 0.04[a]	3.580 ± 0.03[a]	3.62 ± 0.07[a]	3.72 ± 0.11[a]
ALT (IU/L)	11.60 ± 0.50[a]	12.20 ± 0.86[a]	41.00 ± 1.18[b]	41.00 ± 0.63[b]

Values in the same row with similar superscripts are not significantly different (p > 0.05). AST- Aspartate Aminotransferase, ALP - Alkaline Phosphatase, TP – Total protein, ALB – Albumen, ALT- Alanine Aminotransferase

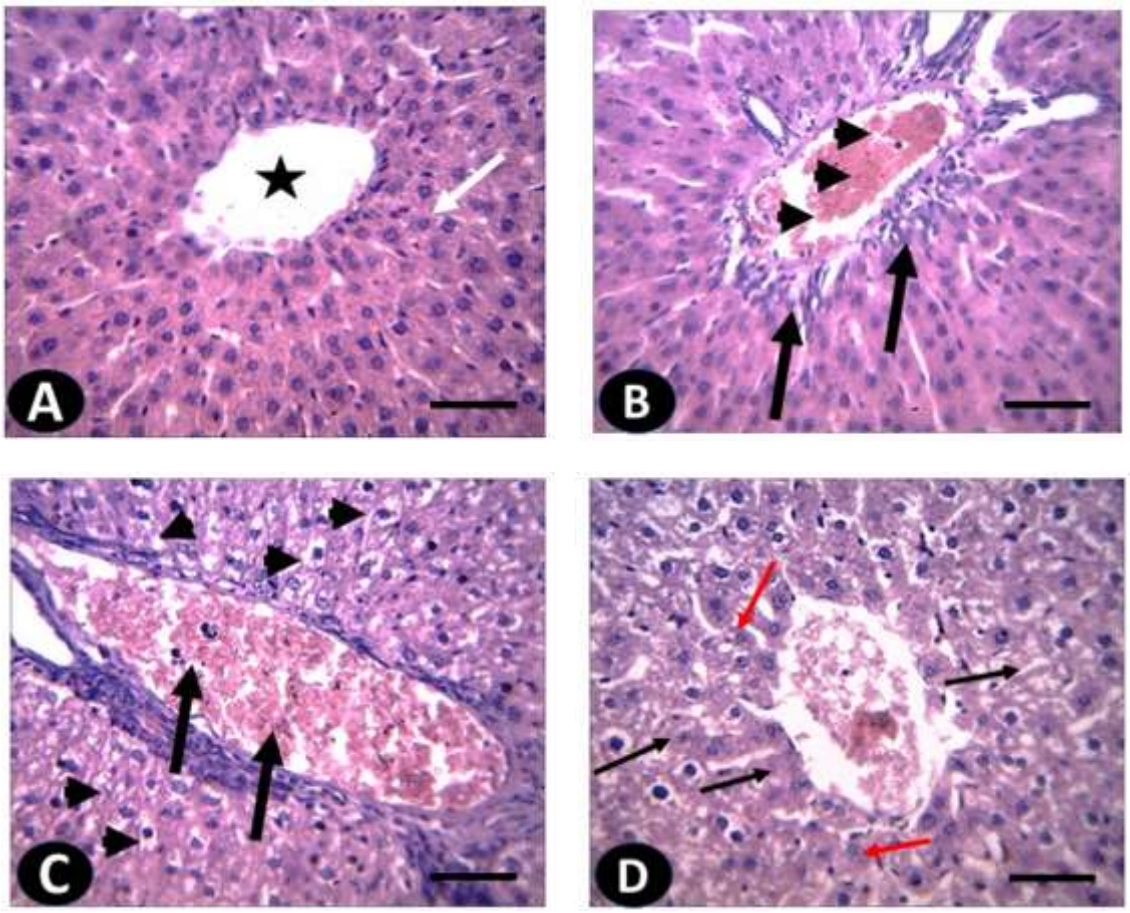

Plate 1. Light micrographs of the liver of rats sub-chronically exposed to graded doses of *Khaya senegalensis*

A. Control: The liver present normal hepatic histoarchitecture characterized by nearly roundish nuclei within cytoplasmic hepatocytes with regular outline (white arrow) and intact central vein (star) B. 16 mg/kg: with the exception of mild portal congestion (arrowheads) and peri-portal cellular infiltration (black arrow), hepatic histo-architecture were similar to the control rats. C. 32 mg/kg: Mild to Moderate portal congestion (black arrows), marked hepatocyte cytoplasmic vacuolations (arrow heads) coupled with diffuse nuclear degeneration (red arrow) D. 64 mg/kg: severe diffuse vacuolar degeneration (black arrow) and necrosis of hepatocytes (red arrow). Stain: Haematoxylin and Eosin; magnification: x400; scale bar = 50 µm.

The elevated hepatic enzymes (ALT, ALP and AST) are reputed serum biochemical markers of liver damage. Generally, increase in serum hepatic enzyme level usually indicate the degree of damage to hepatocellular membrane and subsequent enzyme leakage [41]. Similarly, alteration in serum proteins is an important biochemical pointer to the morphophysiological integrity of the hepatic parenchyma [42]. Therefore, the elevated serum hepatic enzymes occasioned by sub-chronic exposure to higher doses (32 and 64 mg/kg) of *Khaya senegalensis* seemed to establish the deleterious potential of this plant in precipitating hepatic damage especially with advancing dosage of the extract. Interestingly, the serum biochemical profile of rats administered low dose (16mg) of *Khaya senegalensis* appeared to be similar to that of the control. Thus, the serum biochemical profile of the aforementioned group suggested that it is safe when sub-chronically administered. These findings substantiate the similar reports of deranged serum biochemical parameters in prolonged extract administration by Ashafa et al. [43] on *Azadirachta indica* and Folarin et al. [30] on *Vitellaria paradoxa*.

The determination of changes in the serum total protein and albumin levels is important in assessing a wide range of diseases and health disorders and also directly reflects the blood protein synthesis capacity of the liver [44,45,46]. Therefore, the observation of non-significant

difference across all the serum total protein and albumin levels of rats exposed to graded doses of *Khaya senegalensis* extracts portrayed a functionally intact hepatic parenchyma. Although, this is understandable for the low dose but the stable level recorded for the median and high doses could not be explained as virtually all other parameters assessed pointed towards physiological derangements. This finding is similar to the report of Tchuente et al. [42] on Wistar rats exposed to aqueous extract of *Clerodendrum umbellatum.*

Histological examination revealed mild to severe hepatic (portal congestion, cytoplasmic vacoulations and nuclear degeneration) and renal (cortical congestion, tubular cell degeneration and tubular cell depletion) histo-architectural distortions observed in rats exposed to 32 and 64 mg/kg doses of *Khaya senegalensis* seemed to further substantiates the biochemical results from this study. On the contrary, the lack of renal and hepatic lesions in the low dose group appeared to indicate the safeness of *Khaya senegalensis* at this dose during prolong exposure. The hepatic and renal damages seen in this study corroborate the histological findings of Adeyemi et al. [47] on acute and sub-chronic administrations of *Byrsocarpus coccineus* [48,49,50].

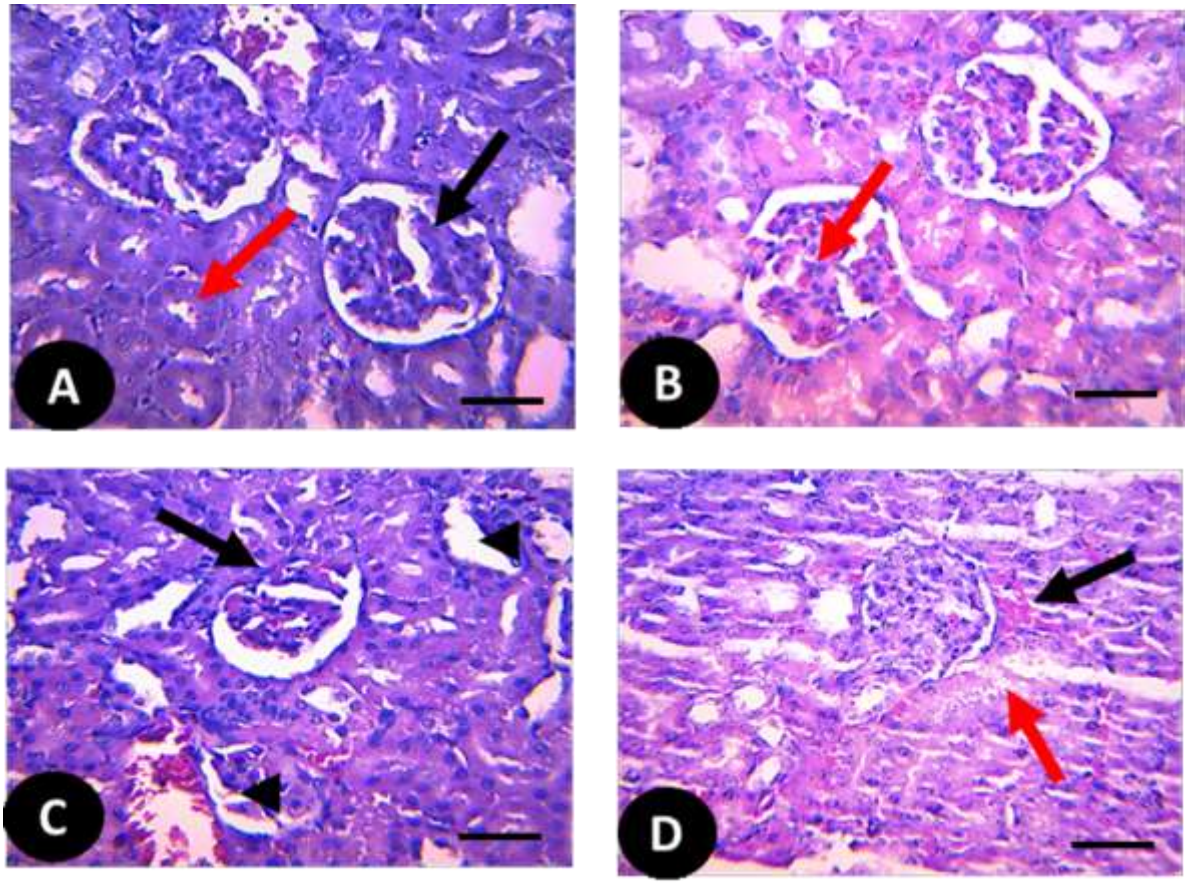

Plate 2. Light micrographs of the kidneys of rats sub-chronically exposed to graded doses of *Khaya senegalensis*

A. Control: Renal parenchyma bears normal histoarchitecture typified by intact glomerulus within the Bowman's capsule (black arrow) and distinct normal renal tubule (red arrow) B. 16 mg/kg: Renal parenchyma bear normal histological appearance of intact glomerulus (red arrow) and renal tubules C. 32 mg/kg: moderate congestion of the renal cortex (black arrow) with intermittent foci of renal tubular depletion (arrowhead). D. 64 mg/kg: mild to moderate renal cortical congestion (black arrow), with moderate diffuse tubular degeneration (red arrow). Stain: Haematoxylin and Eosin; magnification: x400; scale bar = 50 µm

5. CONCLUSION

The study on aqueous crude extracts from Khaya senegalensis root samples has confirmed the presence of vital plant metabolites, such as tannins, saponins, flavonoids, sterols, alkaloids and terpenoids, aligning with prior research. These compounds exhibit promising therapeutic potential for addressing a variety of health conditions, including their role in antioxidant, anti-inflammatory, antimicrobial, anti-angiogenic, anticancer and anti-allergic activities, with flavonoids playing a significant role. However, the precise knowledge of phytochemical composition is imperative to determine safe dosages and avoid potential toxicities. Bioactive compounds derived from plants are integral for developing therapeutic drugs, but their proper dosage is crucial, as excessive consumption can lead to toxicity. Looking at the serum biochemical, haematological and histological data from this study, it is obvious that *Khaya senegalensis* is safe only at 16 mg/kg dose. Therefore, caution should be taking in its usage for therapeutic purposes especially when prolonged administration is desired. Overall, the study emphasizes the need for careful consideration of dosage levels when exploring the potential therapeutic benefits and risks associated with the root of *Khaya senegalensis* and similar plant extracts.

ETHICAL APPROVAL

The study was approved by the animal ethical committee of the laboratory of medical laboratory sciences of the faculty of basic medical sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria (LTH/OGB/EC/2023/348).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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