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Toxicity Studies on Aqueous Stem Bark of *Khaya senegalensis* Extract of Kidneys and Its Biochemical Parameters in Wistar Rats

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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

Introduction: *Khaya senegalensis* is a genus of seven species of trees in the mahogany family Meliaceae, native to tropical Africa and Madagascar. Mahogany in English, Aganwo in Yoruba, Madachi in Hausa and Ono in Igbo. All species become big trees 30–35m tall, rarely 45m, with

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a trunk over 1 m trunk diameter, often buttressed at the base. The leaves are pinnate, with 4-6 pairs of leaflets, the terminal leaflet absent; each leaflet is 10–15 cm long abruptly rounded toward the apex but often with an acuminate tip.

Aim: The aim of the study was to determine the Toxic effect of prolonged oral administration of the aqueous stem bark of *Khaya senegalensis* extract on the histology of Kidneys and its biochemical parameters in wistar rats.

Methods: This work is an experimental research. A total of 20 wistar rats were randomly divided in to 5 groups each of which consist of 4 rats. Group 1 received distilled water to serve as control while group 2, 3, 4, and 5 received 500 mg/kg bw, 1000 mg/kg bw 2000 mg/kg bw and 4000 mg/kg bw of the aqueous extract respectively for 60 days after which they were sacrificed, processed in Automatic Tissue Processor machine, Sectioned and stained with H &E.

Results: There was statistical significant increase in urea and potassium in all the test groups but is not dose dependent. The creatinine was significantly increased in groups 2, 4 and 5. While other parameters such as sodium, chloride and bicarbonate no significant difference when compared to the control group. The kidney sections showed normal structure in group 1 when compared with the test groups. However, there was significant infiltration of inflammatory cell across all the groups which were suggestive of kidney damage or injury. Similarly phenomenon was noticed in group 5 with additional congestion in the glomerulus and more polymorphs seen.

Conclusion: The LD_{50} was found to be greater than 5000 mg/kg bw, therefore, 400 mg/kgbw was used as higher dose in the experimental wistar rats. There were statistical significant increases in some parameters groups while some groups not significant. The kidney section showed significant infiltration of polymorphs across all the groups more marked in group 5 with distension and damaging of the glomerulus indicating renal injury.

Keywords: Khaya senegalensis; aqueous stem bark extract; kidneys; biochemical parameters.

1. INTRODUCTION

Alternative medicine comprises of medical knowledge system that is developed over generations within various societies before the era of modern medicine [1]. It involves the use of natural things (mostly plants) to treat various diseases. There are synthetic or artificial additives in traditional drugs. Furthermore, increasing reliance or on the use of medicinal plants in the industrialized societies has been traced to extraction and development of several drugs and chemotherapeutic from this plant as well as traditionally used rural herbal remedies [1]. The use of medicinal herbs in traditional system of medicine is a common practice in many cultures around the world, especially in African society. This practice has gained widespread acceptance in developing as well as in developed nations. Researchers are also beginning to appreciate the role of medicinal plants in health care delivery. This is as a result of the effectiveness, low cost and the availability of these herbal medicines. It is noteworthy that some orthodox medicines in use today were developed from the biochemical templates obtained from medicinal plants. However, the widespread use and popularity of herbal medicines do not guarantee their efficacy and safety et al. [2]. Therefore, there is need for detailed scientific analyses and adequate information on the toxicity of commonly used herbal drugs [3]. The way to determine the safe or unsafe use of a medicinal plant is the assessment of how it affects hematological and biochemical parameters et al. [4,5]. Changes from normal physiological levels of these parameters after administration of a chemical agent to the experimental animals is an indication of adverse effects of such agent on living organisms [6].

Khaya senegalensis is a genus of seven species of trees in the mahogany family Meliaceae, native to tropical Africa and Madagascar. Mahogany in English, Aganwo in Yoruba, Madachi in Hausa and Ono in Igbo. All species become big trees 30–35 m tall, rarely 45 m, with a trunk over 1 m trunk diameter, often buttressed at the base. The leaves are pinnate, with 4-6 pairs of leaflets, the terminal leaflet absent; each leaflet is 10–15 cm long abruptly rounded toward the apex but often with an acuminate tip. The leaves can be either deciduous or evergreen depending on the species. The flowers are produced in loose inflorescences, each flower small, with four or five yellowish petals and ten stamens. The fruit is a globose four or five-valved capsule 5–8 cm diameter, containing numerous winged seeds [7].

Khaya senegalensis (KS) is a tree belonging to the Meliaceae family. It has numerous medicinal applications, including anti-malarial and antibacterial effects. The stem bark extract has been shown previously to be toxic to Plasmodium falciparum et al. [8]. Moreover, it is well known that the stem bark of KS possesses anti-sickling et al. [9], anti-hyperglycemic et al. [10], antimicrobial et al. [11], antifungal et al. [12], antiprotozoal et al. [13], anthelmintic effects; et al. [14] and anti-cancer effects et al. [15], as well as free radical scavenger activities et al. [16]; [17]. Furthermore, both hepatoprotective hepatotoxic et al. [18] effects of the stem bark of KS in rats have been described et al. [19].

2. METHODOLOGY

2.1 Study Location

The study was carried out at Laboratory in of the Department of Histopathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto and Animal House Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

2.2 Plant Identification

The plant taxonomic identification and assigning of specimen Voucher Number was carried out by Malam Abdulazeez Salihu from the Botany Unit, Department of Biological Sciences Usmanu Danfodiyo University Sokoto, and a voucher specimen (UDUS/ANS/0143) was assigned and deposited in the herbarium of the same department.

2.3 Plant Extraction and Authentication

The stem bark of *Khaya senegalensis* was collected along Government House Area Sokoto washed with clean water and dried under the shade in the Histopathology laboratory, School of Medical Laboratory Sciences to avoid destruction of the active components by the sun light. Dried materials were pounded in the laboratory by the use of pistle and mortar into powder. About 1000 g of the pounded plant was weighed and dissolved in 3000 ml of distilled water, the solution was stirred with the use of stirrer for two hours and left to stay over 24hours. This was filtered with fine cloth to remove large particles and debris then filtered with filter paper. The filtrates were evaporated to dryness at 40°C in water bath as it was done according to the method modify by et al. [20].

2.4 Toxicity Studies (Lethal Dose)

The Lethal Dose (LD_{50}) was carried out using Lorke's method, in the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. The Lorke's method of LD50 consists of two phases that is first phase and second phase.

2.5 Experimental Animals

A total of 20 healthy wistar rats, weighing approximately between the ranges of 100-200 g were Purchased from animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. They were allowed to acclimatize for a period of 14 days. They were maintained on rat feeds and water in sufficient quantities throughout the experimental period; and kept in a metal cage in well ventilated environment at conducive temperature.

2.6 Dose Formulation

Khaya senegalensis aqueous extract was dissolved in 5 mls distilled water as 500 mg/kg body weight per animal, 1000 mg/kg body weight per animal, 2000 mg/kg body weight per animal and 4000 mg/kg body weight per animal.

2.7 Experimental Design

A total of 20 healthy experimental animals (Wistar rats) were randomly divided into five groups with 4 rats in each group, the group one serve as control which receive 2 mls of distilled water, while the other groups received 500 mg/kgbwt, 1000 mg/kgbwt, 2000 mg/kgbwt and 4000 mg/kgbwt respectively as shown in the Table 1.

2.8 Sacrifice of the Animals and Samples Collection

The wistar rats were anaesthesized using chloroform vapour in an enclosed transparent plastic jar. Blood sample was collected through the cardiac puncture dispensed into the plain test tubes for biochemical analysis. The animals were then dissected by longitudinal abdominal incision with aid of surgical blade to harvest the Kidneys washed with normal saline and then fixed immediately in 10% formol saline for histopathological investigations. Staining procedure for Kidney was carried out using Haematoxylin and Eosin staining method modify by [21].

Table 1. Experimental design

Experimental groups	Treatment given/ Kgbwt	Mode of administration	How often given	Duration of treatment
1 (Control) (4 rats)	Distilled water	Orally	Daily	60 days
2 (4 rats)	500 mg	Orally	Daily	60 days
3 (4 rats)	1000 mg	Orally	Daily	60 days
4 (4 rats)	2000 mg	Orally	Daily	60 days
5 (4 rats)	4000 mg	Orally	Daily	60 days

2.9 Laboratory Analysis

The serum samples were used for the estimation of Electrolytes, Urea and Createnine. Electrolytes determination of serum sodium (Na+) and potassium (K+) concentration which were carried out by use of method described by et al. [22], while the chloride was estimated using mercuric method modify by [23] and determination of serum bicarbonate concentration was also carried out by using back titration method modifyby [24]. The serum urea determination was carried out using Caraway method modify by [25] and serum creatinine was determined using Jaffe-slot alkaline picrate creatinine method modify by [25].

The kidneys was collected, fixed and stained with Haematoxylin & Eosin method for general structure of tissue and photomicrographs were taken and presented as shown in the results below.

2.10 Data Analysis

The data analysis was performed using Graphpad prism 6.0 as mean± SD. Statistical comparison between groups were made using one way analysis of variance (ANOVA) with post hoc Bonferroni Multiple comparison Test to identify differences in means where appropriate. $P<0.05$ was taken as statistically significant. The kidneys were stained with H and E staining technique and photomicrographs were taken and presented.

3. RESULTS

Table 2 showing the physical properties of *Khaya senegalensis* stem bark aqueous extract and also percentage yield of the extract after aqueous extraction procedure which yielded 12.2%.

Table 3 showing the LD_{50} of the aqueous extract stem bark of *Khaya senegalensis* in wistar rats total of 12 rats were used for the procedure. First Phase consist of 9 rats while second Phase consist of 3 rats no toxicity signs or mortality recorded throughout the procedure after administration of higher dose of 5000 mg/kg bwt.

Table 2. Physical properties of *Khaya senegalensis* stem bark aqueous extract

Plant part	Type of extract	% yield	Texture	Color
Stem bark	Aqueous extract	12.2%	Gummy	Red-brown

Table 3. Showing the LD_{50} of the aqueous extract stem bark of *Khaya senegalensis* in wistar rats

Dose (mg)	Observation	
	First phase	Second phase
10	0/3	-
100	0/3	-
1000	0/3	-
1600	-	0/1
2900	-	0/1
5000	-	0/1

Key: mg= milligram, 0/3 non of the wistar rats died out of three wistar rats in a group and 0/1 non of the wistar rats died in each group of one wistar rat after 24 hours of the experiment

The electrolytes, urea and creatinine there was statistical significant increase in urea and potassium in all the test groups but is not dose dependent. The creatinine was significantly increased in groups 2, 4 and 5 while there was significant decrease in group 3 compared to the control group. While other parameters such as sodium, chloride and bicarbonate there were no significant difference when compared to the control group.

Kidney section in group 1 showed normal histology of glomerulus as control. While group 2 showed glomerulus with mild infiltration of inflammatory cells (Polymorphs). The kidney section in group 3 showed moderate infiltration of

polymorphs into glomerulus capillaries. The group 4 showed more infiltration of polymorphs into the glomeruls causing distension of the capillaries and occupying capsular space. The group5 showed distension and damage of the glomerulus causing little capsular space due to marked increase infiltration of polymorphsin glomerulus.

Table 4. Electrolytes, Urea and Creatinine after administration of aqueous stem bark extract of *Khaya senegalensis* on kidney function parameters of control group and test groups

Group	Na^+	K^+	Cl-	HCO_3^-	UREA	CREAT
Control	135.25±4.57	3.33±0.31	93.25±3.69	19.75±3.5	3.85±0.74	1.25±0.12
500 mg/kg	135.75±1.26	5.93±0.98	97.75±0.95	17.5±1.29	14.88±12.5[b]	1.63±0.53
1000 mg/kg	135.75±3.86	5.88±0.64	96.25±2.06	21.25±3.4	7.78±1.05	0.83±0.45
2000 mg/kg	137.5±2.65	6.03±0.87	97.25±3.20	22.25±2.22	6.15±1.77	1.93±1.47
4000 mg/kg	139.25±2.06	6.23±0.53	98.5±1.29	22.5±1.73	7.95±2.98[a]	4.53±0.45[b]

Key: Na^+=Sodium, K^+= Potassium, Cl-=Chloride, HCO_3^-=Bicarbonate. Therefore, $p<0.05$ is considered statistically significant using one way analysis of variance (ANOVA)

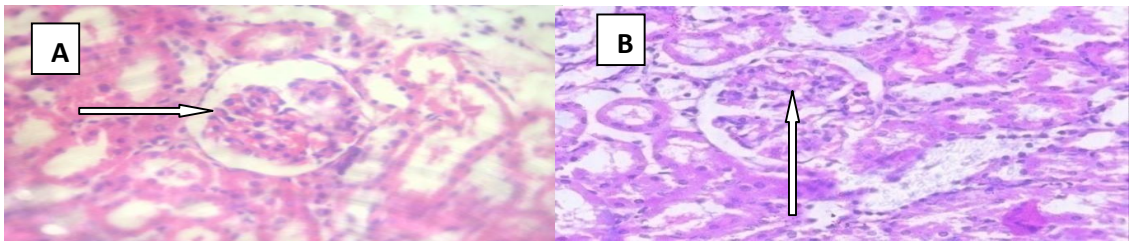

Plate 1. Photomicrograph of kidney section arrow showing normal histology of glomerulus (A) group 1 as control. While (B) group 2 administered with 500 mg/kgbw showing glomeruluswith mild infiltration of inflammatory cells (Polymorphs) H and E staining technique x400

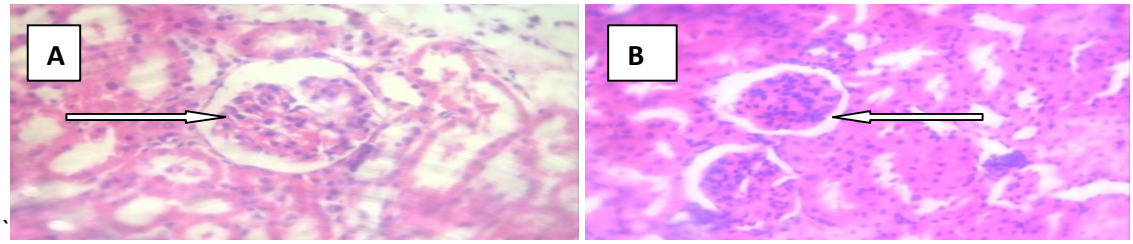

Plate 2. Photomicrograph of kidney section arrow showing normal glomerulus and capsular space A group 1 as control while B group 3 moderate infiltration of polymorphs into glomerulus capillaries after oral administration of aqueous stem bark of *Khaya senegalensis* extract 1000 mg/kgbw. H and E staining technique x400

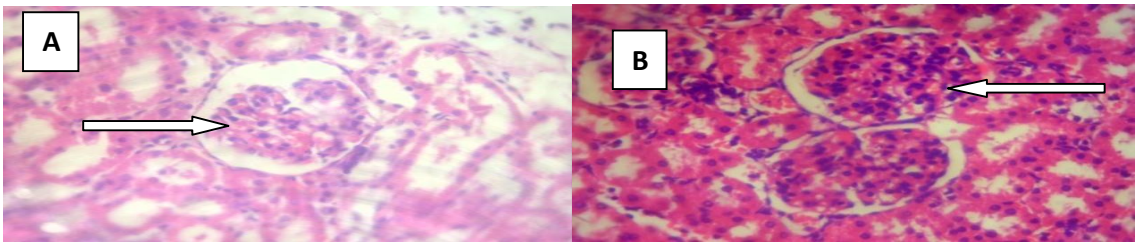

Plate 3. Photomicrograph of kidney section arrow showing normal glomerulus and capsular space A group 1 as control while B group 4 more infiltration of polymorphs into the glomeruls causing distension of the capillaries and occupying capsular space, after oral administration of aqueous stem bark of *Khaya senegalensis* extract 2000 mg/kgbw H and E staining technique x400

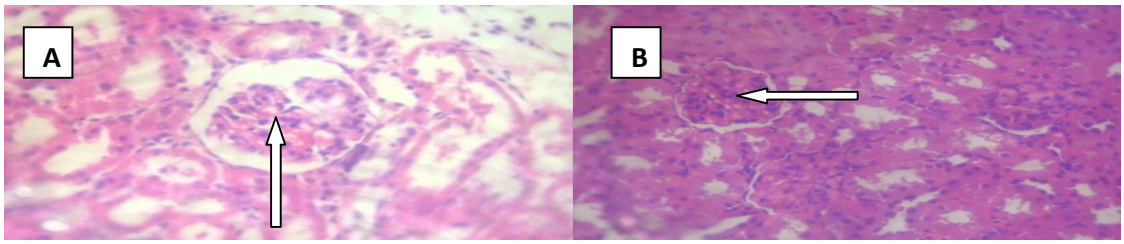

Plate 4. Photomicrograph of kidney section arrow showing normal glomerulus and capsular space A group 1as control while. B group 5 distension and damage of the glomerulus causing little capsular space due to marked increase infiltration of polymorphsin glomerulus, after oral administration of aqueous stem bark of *Khaya senegalensis* extract 4000 mg/kgbw Hand E staining technique x400

4. DISCUSSION

The aqueous extraction procedure used in this research work yielded 12.2%. This was in agreement with the value obtained by Onu et al. [26] using the same method of aqueous extraction procedure. However, it was in contrast to the value obtained by Huzaifa et al. [27], this could be attributed to different method of extraction procedure used.

The acute toxicity test or lethal dose effect of aqueous stem bark of *Khaya sengalensis* extract on wistar rats (Table 1) shows that no animal died within and after 24 hours of the oral administration of aqueous stem bark of *Khaya sengalensis* extract in phase I. Also there were no signs of toxicity noticed within and after 24 hours in phase II. Therefore the Lethal dose (LD_{50}), being greater than 5000 mg/kg bw, was thought to be safe as modify by Lorke [28]. Again, the absence of death among rats in all the dose groups throughout the twenty four hours of the experimental period seems to support this claim.

The significant increase in urea and potassium in all the test groups but is not dose dependent may be caused by the administration of the extract. The creatinine was significantly increase in groups 2, 4 and 5 but there was significant decrease in group 3 compared to the control group. The values were in contrast to value modify by Uchegbu et al. [29], which could be due to different methods of estimation. Therefore, $P<0.05$ is considered statistically significant using one way analysis of variance (ANOVA). While other parameters such as sodium, chloride and bicarbonate no significant difference when compared to the control group.

The kidney sections showed normal structure in group 1 when compared with the test groups. However, there was significant infiltration of inflammatory cell across all the groups which were suggestive of kidney damage or injury. Similarly phenomenon was noticed in group 5 with additional congestion in the glomerulus and more polymorphs seen these finding were in line with the finding s reported by Zhang et al. [30].

5. CONCLUSSION

The LD_{50}, of this research work was found to be greater than 5000 mg/kg bw, therefore, 400 mg/kgbw was used as higher dose in the experimental wistar rats.

The kidney sections shows significant infiltration of polymorphs across all the groups more marked in group 5 with distension and damaging of the glomerulus indicating renal injury.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standard laid down in 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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