



Chronic Toxicity Study Aqueous Stem Bark of *Khaya senegalensis* Extract on the Histology of the Liver 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.

Article Information

Editor(s):

(1) Dr. Fidanka Trajkova, Goce Delcev University of Stip, North Macedonia.

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(2) Shailja Puri, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/52704>

Original Research Article

Received 12 December 2019

Accepted 20 February 2020

Published 17 March 2020

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 30–35 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.

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Aim: The study aimed to determine the chronic toxicity on the histological effect of chronic oral administration of the aqueous stem bark of *Khaya senegalensis* extract on the liver and its biochemical parameters in Wistar rats.

Methods: This project dissertation work is experimental research. A total of 20 Wistar rats were randomly divided into 5 groups each of which contains 4 rats. Group 1 received distilled water 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.

Results: There was a significant increase in Aspartate transaminase and Alanine aminotransferases in group 5 compared with group 1 (control), while no significant increase in the other groups, but Alkaline phosphatase there was a decrease in group 3, other groups not significant. The total protein there was an increase in groups 3, 4 and 5 but group 2 no significant increase. Similarly, albumin there was an increase in groups 2 and 4 while other groups not significant. The total bilirubin and direct bilirubin in all test groups were increased. The liver section has normal histology in group 1(control), after administration of distilled water. The test groups showed increase infiltration of polymorphs across all the groups, more marked in group 5 indicating inflammation of the liver.

Keywords: *Khaya senegalensis*; liver and biochemical parameters of liver.

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 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 [2]. Therefore, there is a 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 haematological and biochemical parameters [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* [8]. Moreover, it is well known that the stem bark of KS possesses anti-sickling (Fall et al., 1999), anti-hyperglycemic [9], antimicrobial [10], antifungal [11], antiprotozoal [12], anthelmintic effects [8,13] and anti-cancer effects [14,15] as well as free radical scavenger activities [16,17]. Furthermore, both hepato protective [18] and hepatotoxic [19], Kolawole et al., 2011 effects of the stem bark of KS in rats have been described [20].

2. METHODOLOGY

2.1 Study Location

The study was carried out at Department Histopathology, School of Medical Laboratory Science, 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 prepared 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 and dried under the shade in the Histopathology Laboratory, School of Medical Laboratory Sciences to avoid the destruction of the active components by the sunlight. Dried materials were pounded in the laboratory by the use of pestle and mortar into powder. 1000 g of the pounded plant was weighed and dissolved in 3000ml of distilled water; the solution was stirred with the use of stirrer for two hours and left to stay over 24 hours. This was filtered with a fine cloth to remove large particles and debris then filtered with filter paper. The filtrates were evaporated to dryness at 40°C in a water bath as it was done according to the method described by Majekodunmi et al. [21].

2.4 Toxicity Studies (Lethal Dose)

The Lethal Dose (LD₅₀) was carried out using Lorke's method, in the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. Lorke's method of LD50 consists of two phases.

2.5 Experimental Animals

A total of 20 healthy Wistar rats, weighing approximately between the range of 100-200 g were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. They were allowed to acclimatize for 14 days. They were maintained on rat feeds and water in sufficient quantities

throughout the experimental period and kept in a metal cage in the well-ventilated environment at conducive temperature.

2.6 Dose Formulation

The stem bark of *Khaya senegalensis* aqueous extract was dissolved in 5 mls distilled water as dilution factor for 500 mg/kg body weight / animal, 1000 mg/kg body weight/animal, 2000 mg/kg body weight/animal and 4000 mg/kg body weight/ animal all the groups respectively.

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 serves as control which receives 2 mls of distilled water, while the other groups received 500 mg/kgbw, 1000 mg/kgbw, 2000 mg/kgbw and 4000 mg/kgbw respectively as shown in the Table 1.

2.8 Sacrifice of the Animals and Samples Collection

The Wistar rats were anaesthetized using chloroform vapour in an enclosed transparent plastic jar. Blood samples were collected with the aid of 5mls syringe and needle through the cardiac puncture into the plain test tubes for biochemical analysis. The animals were then dissected by longitudinal abdominal incision with aid of the surgical blade to harvest the liver washed with normal saline and then fixed immediately in 10% formol saline for histopathological investigations. Staining procedure for the liver was carried out using Haematoxylin and Eosin staining method [22].

2.9 Laboratory Analysis

The serum sample for liver function test was used for determination of serum activities of transaminases (AST and ALT) which was carried out using the colorimetric method of Reitman and Frankel (1957) and determination of serum alkaline phosphatase was done using modified Bowers and McComb Method [23]. Total protein was determined using the Biuret method of Henry et al. [24]. The concentration of albumin was determined as described by Grant and Kachman [25]. All measurements were done using Spectronic and spectrophotometer (Bausch and Lomb, NY).

The liver was grossed, processed, cut and stained with Haematoxylin and Eosin method for histological findings.

2.10 Data Analysis

The data analysis was performed using Graphpad prism 6.0 as mean \pm SD. Statistical comparison between groups was 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.

3. RESULTS

Table 2 shows 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%.

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 the aqueous extraction procedure. However, it was in contrast to the value obtained by Huzaifa et al., [27] this could be attributed to a different method of extraction procedure used.

The acute toxicity test or lethal dose effect of aqueous stem bark of *khaya senegalensis* 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 senegalensis* 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}), is greater than 5000 mg/kg bw, was thought to be safe as suggested 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 liver function tests parameters there was a significant increase in enzymes such as an Aspartate-transaminase (AST) and Alanine-amino-transaminase (ALT) in group 5 only compared with the control group (control).

Table 1. Experimental design

Experimental groups	Treatment is given/ Kgbwt	Mode of administration	How often given	Duration of treatment
1(Control) (4rats)	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

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

Plant part	Type of extract	% yield	Texture	Colour
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

Table 4. Liver function test parameters of different doses of aqueous stem bark extract of *Khaya senegalensis* administered to the control group and test groups as shown below

Group	Total protein	Albumin	Total bilirubin	Direct bilirubin	AST	ALT	ALP
1 Control	5.78±0.51	3.3±0.62	0.35±0.26	0.08±0.07	505.25±244.47	117.25±44.29	235.5±17.99
2 500 mg	6.15±0.19	4.05±0.21	0.77±0.3*	0.14±0.03*	398.75±249.06	151.5±97.12	355.0±173.5
3 1000 mg	7.75±0.4*	3.95±0.34	0.60±0.4*	0.13±0.06*	446.75±171.29	104.25±43.82	186.25±59.63
4 2000 mg	7.13±0.1*	4.58±0.55	0.43±0.2*	0.09±0.01*	430.5±237.29	103.0±46.2	287.0±46.30
5 4000 mg	7.18±0.6*	3.78±0.22	0.60±0.5*	0.12±0.08*	703.25±34.21*	180.75±12.5*	284.75±59.5

Key: AST = Aspartate transaminase, ALT = Alanine amino transaminases and ALP = Alkaline phosphatase, * = increase and $p < 0.05$ is considered statistically significant using one-way analysis of variance (ANOVA)

P<0.05, this could be an indicator of liver damage by the extract, while the other groups there was no significant increase in them, but ALP there was a significant decrease in group3 while other groups increase could also be attributed to the extract. These findings were not in agreement of findings by Uchegbu et al. [29] and Ali et al. [18], this might be due the doses they used were lower than the doses used in this research. The total protein there was a significant increase in groups 3, 4 and 5 this shows that there was degradation of proteins in the rat but group 2 not significant compared to the control

group. Similarly, Albumin there was a significant increase in groups 2 and 4 while other groups not significant compared to the control group. The total bilirubin and direct bilirubin in all test groups were significantly increased but not dose-dependent when compared with the control group. The above findings were in line with Onu et al., [26], but however, it was in contrast to the values obtained by Uchegbu et al. [29] this could be attributed to the fact that he used different methods of estimation from the method used in this research P<0.05 is considered as statistically significant.

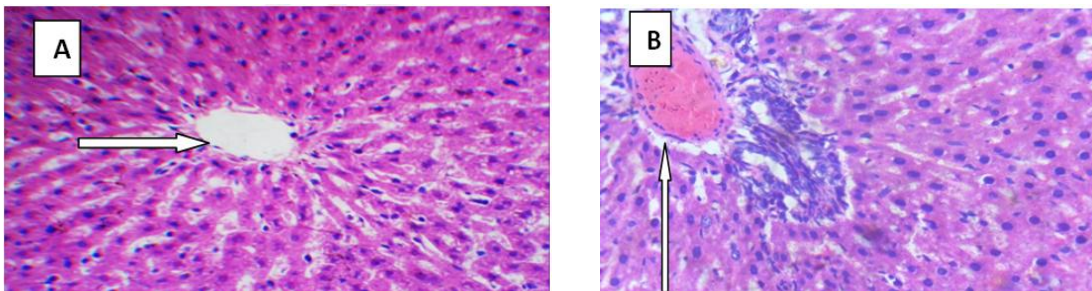


Plate 1. Photomicrograph of the liver section showing normal histology of liver architecture (A) as control administered with 2 ml of distilled water (mg/kg bw). While (B) group 2 administered with 500 mg/kgbw Central vein (white arrow) with mild infiltration of inflammatory cells (Polymorphs) H and E staining technique x400

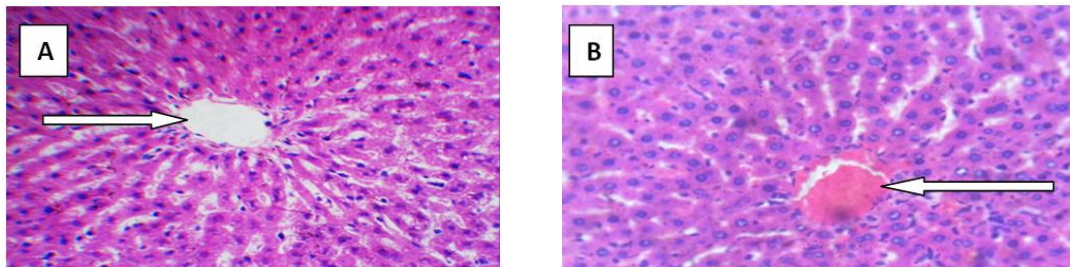


Plate 2. Photomicrograph of the liver section showing A (control) normal central veins (white arrows) and B (group 3) mild infiltration of polymorphs group 2 after oral administration of aqueous stem bark of *Khaya senegalensis* extract 1000 mg/kgbw on Wistar rat H and E staining technique x400

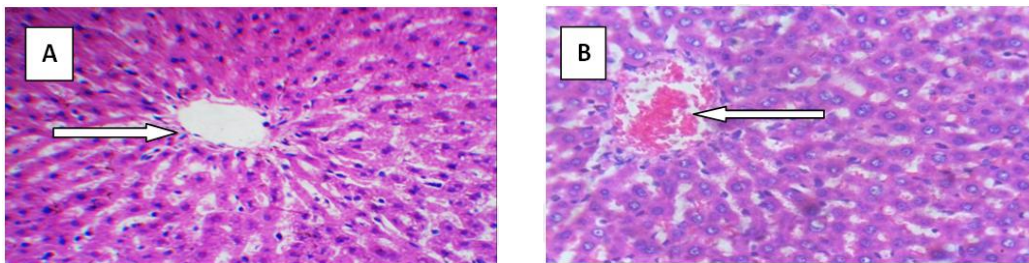


Plate 3. Photomicrograph of liver section showing A (control) normal central vein (white arrow) and B (group 4) increase infiltration of polymorphs group 3 after oral administration of aqueous stem bark of *Khaya senegalensis* extract 2000 mg/kgbw on Wistar rats H and E staining technique x400

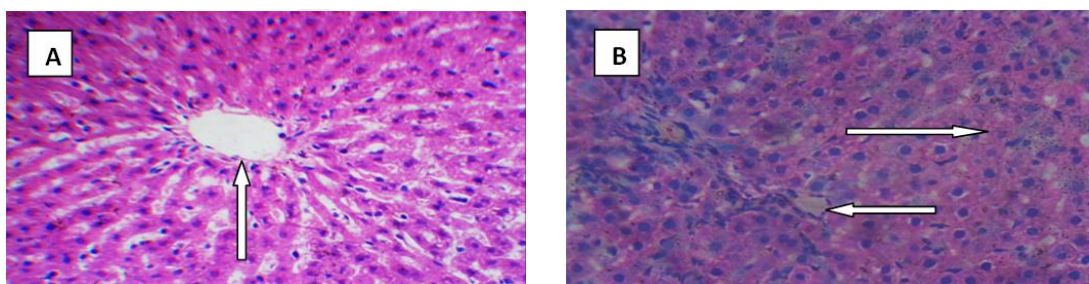


Plate 4. Photomicrograph of the liver section showing A (control) normal central vein (white arrow). B (group 5) congestion of the portal vein (white arrow) and moderate increase infiltration of polymorphs after oral administration of aqueous stem bark of *Khaya senegalensis* extract 4000 mg/kgbw on Wistar rats Hand E staining technique x400

The liver sections showed normal structure in group 1 when compared with the test groups. However, there was significant infiltration of the inflammatory cell across all the groups which were suggestive of liver damage or injury. Similarly, the phenomenon was noticed in group 5 with additional congestion in the central vein and more polymorphs seen these findings were in line with the findings reported by Ali et al. [18].

5. CONCLUSION

The LD₅₀ of this research work, was found to be greater than 5000 mg/kg bw, therefore, 400 mg/kgbw was used as a higher dose in the experimental Wistar rats.

The liver function tests parameters there was a significant increase in enzymes such as AST and ALT in group 5 only compared to control group while the other groups there was no significant increase in them, but ALP there was a significant decrease in group 3 while other groups increase. The total protein there was a significant increase in groups 3, 4 and 5 but group 2 not significant compared to the control group. Similarly, Albumin there was a significant increase in groups 2 and 4 while other groups not significant compared to the control group. The total bilirubin and direct bilirubin in all test groups were significantly increased.

The liver sections shows significant infiltration of polymorphs across all the groups more marked in group 5 with central vein congestion indicating liver injury.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed following the ethical standard laid down in 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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