

Determination of Toxicity Level of *Sclerocarya birrea* Stem bark Ethanol Extract against Wistar Rats

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Abstract

Many plants are highly poisonous when ingested. The study was aimed to study the toxicity effects of ethanol extract *Sclerocarya birrea* stem bark extracts against liver and kidney of wistar rats. The stem bark of *S. birrea* was extracted using ethanol by Soxhlet extraction method. Twenty-one (21) adult wistar rats were used in the study. Lorke's method was used to test the acute toxicity of the extracts. The animals were divided into seven groups with three rats in each group. Animals in groups were orally administered with the extract of 62.5, 125, 250, 500, 1000 and 2000 mg/Kg body weight once daily for 10 days respectively. The result showed that there is no mortality in wistar rat after oral administration of the extract at a dose up to 2000 mg/Kg. Other clinical sign such as salivation, loss of hair, decrease in respiration rate (pulsating) and diarrhea were absent. However, the animals experienced restlessness at a concentration of 1000 and 2000 mg/Kg which disappeared within 24 hours. The histopathological analysis of the liver and kidney following administration of different concentration of the extract showed little loss of hepatocellular boundaries and vacuolation in the hepatocytes at 1000 and 2,000 mg/Kg in the liver and mild tissue degeneration and glomerulus shrinkage at 1000 and 2,000 mg/Kg in the kidney, but there is no visible degeneration at lower concentration. It is concluded that the ethanol extract of *S. birrea* may be safe for human consumption when use at low or moderate concentrations.

Keywords: toxicity level; *sclerocarya birrea*; stem bark; wistar rat

Introduction

Since many years, human population across the world utilized elements of their environment, in particular plants, to treat themselves. Most pharmaceutical products depend on plants as the main source of material for their production. Plant derived medicines can serve as a basis for the manufacture of different more active drugs [1]. Most plants are made up of bioactive compounds such as lipids, phytochemicals, pharmaceuticals, flavons, fragrances and pigments. To date, even with the spectacular progress accomplished in the field of science, an estimate of 66% to 85% of the world's population, especially from developing countries, depend directly on plants as medicines in treating all sorts of diseases [2,3]. Various studies have reported that medicinal plants contain numerous biologically active compounds such as flavonoids, terpenoids, carotenoids, steroids, simple phenolic, glycosides, tannins, saponins, polyphenols, to mention a few, which have shown medicinal activities [4]. Most of these phytochemicals, commonly referred as secondary

metabolites, were reported to act as antimicrobials [5]. According to World Health Organization, even developed countries are beginning to turn to plants for their medicine source due to the increased resistance of most existing antimicrobial drugs [6]. *Sclerocarya birrea* (commonly called marula) is a savannah tree, belongs to the family Anacardiaceae, with a plum-like pale yellow fruit of 3-4 cm in diameter with a juicy mucilaginous flesh. *Sclerocarya birrea* is deciduous and mainly dioecious, although there have been reports of monoecious trees [7]. It is a medium sized tree reaching heights of between 7 to 17 m, with grey fissured bark, stout branch lets and pale foliage. The leaves are compound, pinnate and the flowers greenish-white or reddish. The fruits are yellow, resembles a mango. Rough stems-bark is flaky, with a mottled appearance due to contrasting grey and pale brown patches [8]. The leaves are divided into 10 or more pairs of leaflets, each about 60 mm long, dark-green above, and sharp point. The flowers are borne in small, oblong clusters [7]. In some African countries, the stems-bark, roots and leaves of

Sclerocarya birrea are used for an array of human ailments, including: malaria and fevers, diarrhea and dysentery, stomach ailments, headaches, sore eyes, toothache, backache and body pains, infertility, schistosomiasis, constipation, abdominal cramps and some other unspecified gastro-intestinal problems, toothaches and swollen or infected gums, cough, hypertension, arthritis, proctitis, epilepsy, diabetes mellitus, sores, boils, carbuncles, abscesses and certain other bacterial infections [9]. Traditionally the stem bark is used for the treatment of various gastrointestinal disorder especially dysentery/diarrhea, hemorrhoid, stomach ulcers and pain, sore throat/mouth and toothache [10]. Both the ethanolic and aqueous extract of this plant was found to be anti-inflammatory in rats paw induced edema, and with some antioxidant activity [11]. Despite the medicinal uses of plants extracts, the plants can be toxic when used at certain concentrations. Hence, the study was aimed to study the toxicity effects of *Sclerocarya birrea* stem bark ethanolic extracts against the liver and kidney of wistar rats.

Materials and Methods

Collection and Identification of Plant Materials

The stem bark *Sclerocarya birrea* was collected at Kibiya town in Kibiya Local Government Kano. The Identification and authentication of the plant materials was conducted at Herbarium unit in the Department of Plant Science Bayero University Kano and Department of Biological Science, Kano University of Science and Technology, Wudil and the following voucher number was assigned BUKHAN 0435. Voucher specimen was deposited there for future reference. The samples were washed with water to remove dust and rinsed with distilled water, air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by Ali *et al.* [12]. The powdered sample was bagged in a black polythene bag and store in air tight container for further use.

Extraction of Plant Materials

Ethanol was used as solvent in the extraction process. One hundred grams (100g) of the powdered leaves was weighed out and mixed with 500 ml of the solvent in a sterile conical flask and extracted using soxhlet extraction method. The filtrate was evaporated and dried at 40 °C under reduced pressure using rotary vacuum evaporator. The extract yields was weighed, stored in dark air tight container at 4°C [13].

Preparation of Extracts Concentrations

The solid residues (extracts) obtained was weighed and dissolved in 30% DMSO at a stock concentration of 2000mg/mL by dissolving 20 g in 10 mL. Various concentrations (1000, 500, 250, 125 and 62.5mg/mL) were made from the stock solution and stored at 4°C until use [12].

Experimental Animals

Twenty one (21) adult wistar rats were obtained from Department of Pharmaceutical Science, College of Natural and Pharmaceutical Sciences,

Bayero University Kano, Nigeria. The rats were kept in cage and maintained under laboratory condition of light and temperature with free access to food and water. The experimental animals were left for 10 days to adapt to the environment (acclimatization) and the animals were divided into 7 groups (A, B, C, D, E, F and G) of three animals each. The experimental protocol for the experiment was in accordance with OECD guidelines for the testing of chemicals.

Acute Toxicity Testing

Lorke's method was used to test the acute toxicity of the extracts [14]. The animals were divided into seven groups with three rats in each group. Animals in Groups A, B, C, D, E, F and G were orally given extract of 62.5, 125, 250, 500, 1000 and 2000mg/kg body weight once daily for 10 days respectively. Group A was the control group and received only distilled water by the same route. Soon after dosing the rats were observed in an effort to note any changes in behavior. These included noting any changes in behaviour and structure such as salivation, restlessness, pulsating, loss of hair, diarrhea and death.

Histopathological Examination

The liver and one of the kidneys for each rat were fixed and preserved in 10% formaldehyde before subjection to tissue processing procedures for the preparation of permanent mount of each tissue as described by Baker and Silverton [15]. The tissues were dehydrated through various grades of alcohol 30%, 50%, and 70% 95% with a final bath in 100% alcohol (twice) to ensure total elimination of moisture. Clearing was performed in toluene in order to raise its refractive index to that of glass (1.5) to enable transparency of the cellular inclusions. The processes of infiltration and embedding were performed using liquid paraffin and molten paraffin wax using L-shaped mould respectively. Sections were made using Rotary Microtome and the Hot plate methods were used for mounting specimens onto slides. Staining of the tissues was performed using Iron haematoxylin and Eosin. Canada balsam was used in mounting of the tissues. Slides were viewed under X 100 objective of the microscope and photographed using the camera

Results

Acute Toxicity and Lethality Tests

Toxicity and Lethality Profile of *S. birrea* Stem Bark Extract

The toxicity test result of different concentration of *S. birrea* stem bark extract is presented in Table 1 below. The result showed that there is no mortality in wistar rat after oral administration of the extract at dose as high as 2000mg/Kg. Other clinical signs such as salivation, loss of hair, decrease in respiration rate (pulsating) and diarrhea were absent. However, the animals experienced restlessness at a concentration of 1000 and 2000mg/Kg which disappeared within 24 hours.

Dose (mg/Kg)	Salivation	Restlessness	Loss of hair	Pulsating	Diarrhea	Death
Group A (control)	-	-	-	Normal	-	0/3
Group B (62.5)	-	-	-	Normal	-	0/3
Group C (125)	-	-	-	Normal	-	0/3
Group D (250)	-	-	-	Normal	-	0/3
Group E (500)	-	-	-	Normal	-	0/3
Group F (1000)	-	+	-	Normal	-	0/3
Group G (2000)	-	+	-	Normal	-	0/3

Table 1: Toxicity and Lethality Profile of *S. birrea* Stem Bark Extract against wistar rat

Histopathological Examination of Liver

The result of histopathological examination of liver of the experimenting animals is presented in figure 1–5 below. The result showed that there were no significant histopathological changes in the liver of the animal in

group C (125mg/kg) and E (500mg/kg) when compared to control group. However, there some histopathological changes observed on animals in group F (1000mg/kg) and G (2000mg/kg). These changes include: the presence of congested blood vessels, peritoneal inflammation, presence of fatty cell vacuoles, and cell necrosis.

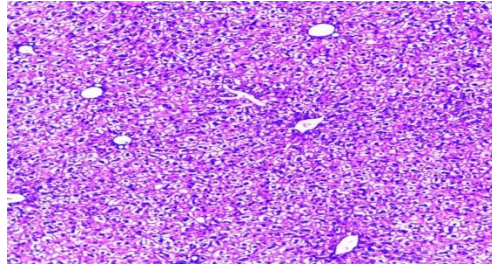


Figure 1: Section of Liver of Control group showing normal Liver tissue/hepatocytes

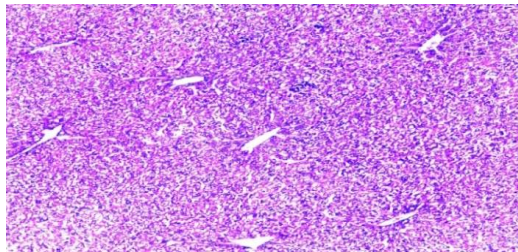


Figure 2: Section of Liver of group (125mg/kg) animal showing normal Liver tissue/hepatocytes

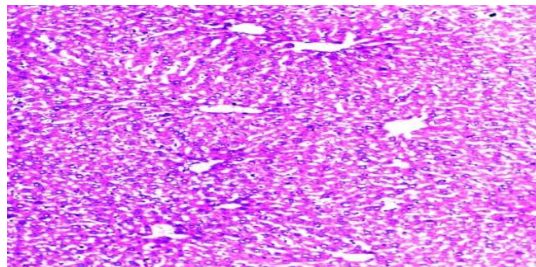


Figure 3: Section of Liver of group E (500mg/kg) animal showing normal Liver tissue/hepatocytes

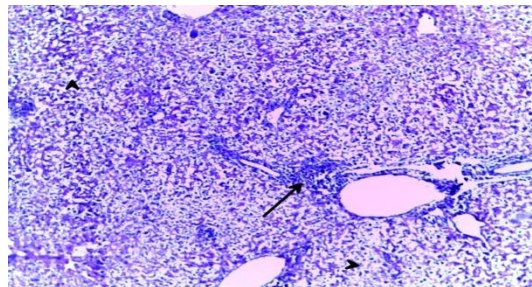


Figure 4: Section of Liver of Group F animal treated with 1000 mg/kg of the extract showing the presence of congested blood vessels, fatty cell vacuoles, and cell necrosis.

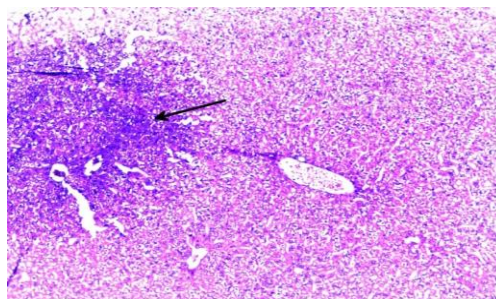


Figure 5: Section of Liver of Group G animal treated with 2000 mg/kg of the extract showing the presence of congested blood vessels, peritoneal inflammation, presence of fatty cell vacuoles, and cell necrosis.

Histopathological Examination of Kidney

The result of histopathological examination of kidney of the experimenting animals is presented in figure 6 – 10 below. The result showed no significant histopathological changes in the liver of the animal

in group B (62.5mg/kg) and E (500mg/kg) when compared to control (Group A). However, there some histopathological changes observed on animals in F (1000mg/kg) and G (2000mg/kg) showing areas of mild tissue degeneration, Glomerulus shrinkage and disintegration.

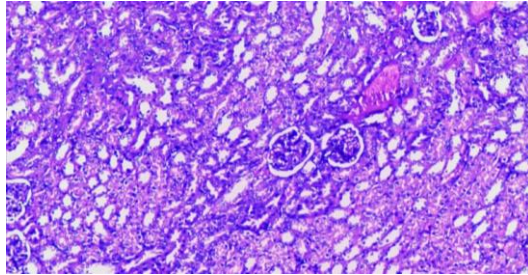


Figure 6: Section of Kidney of Control group showing normal nephron (Bowman capsule, glomerulus and renal tubule)

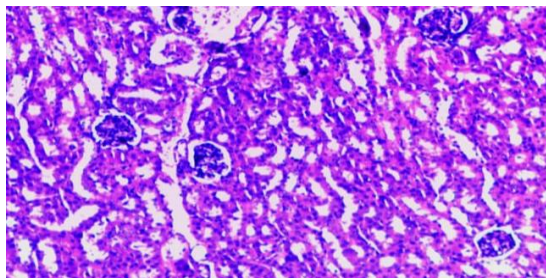


Figure 7: Section of Kidney of Group B animal treated with 62.5 mg/kg of the extract showing normal nephron

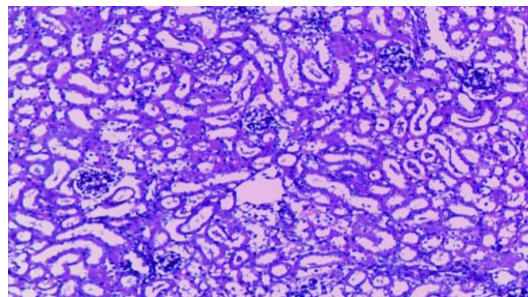


Figure 8: Section of Liver of Group B animal treated with 500 mg/kg of the extract showing normal nephron

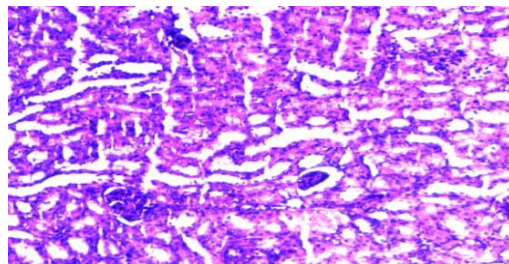


Figure 9: Section of Liver of Group F animal treated with 1000 mg/kg of the extract showing the presence of tissue degeneration and Glomerulus shrinkage

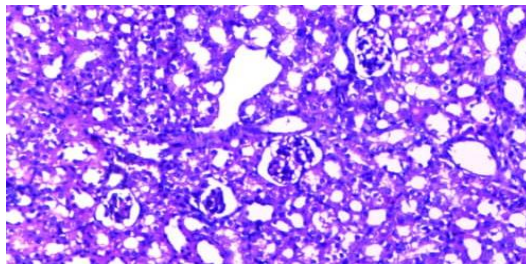


Figure 10: Section of Liver of Group G animal treated with 2000 mg/kg of the extract showing the presence of tissue degeneration, Glomerulus shrinkage and disintegration

Discussion

Toxicity is the degree to which a substance can harm animals [16]. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ (organ toxicity), such as the liver (hepatotoxicity) [17]. A poisonous substance is any substance that produces disease conditions, tissue injury, or otherwise interrupts natural life processes when in contact with or absorbed into the body [18]. Many plants are highly poisonous when ingested. The study was aimed to determine the toxicity level of different concentration of *S. birrea* ethanol extract on wistar rat. In the present study, no mortality was observed in the acute toxicity study at all the concentrations (62.5 – 2000mg/kg) dosages indicating that the LD₅₀ of the ethanol extract of *S. birrea* stem bark is greater than 2,000 mg/kg. Similarly, other clinical sign such as salivation, loss of hair, decrease in respiration rate (pulsating) and diarrhea were absent in all the concentrations. However, the animals experienced restlessness at a concentration of 1000 and 2000mg/kg which disappeared within 24 hours. Finding of this study was in conformity with that of and Thakur and Mengi [19] and Balogun *et al.* [20] who both reported report that the locomotory activity is considered to be an index of alertness, and any decrease in locomotion can indicate sedation. Acute toxicity study results suggest that *S. birrea* might be alerting the animal when high doses (1000 and 2000 mg/kg) are used.

Results obtained from the histopathological analysis of the liver and kidney following administration of different concentration of ethanol extract of *S. birrea* stem bark showed little loss of hepatocellular boundaries and vacuolation in the hepatocytes at 1000 and 2,000 mg/kg respectively indicating mild hepatotoxicity as compared to the 500, 250 and 125 mg/kg dosage groups which showed normal hepatocytes similar to control group. On the other hand, the sections obtained from the kidney showed areas of mild tissue degeneration; glomerulus shrinkage and disintegration at concentration of 1000 and 2,000 mg/kg respectively, but no visible degeneration were observed at lower concentration. Finding of this study supported the finding of Mawoza *et al.* [21] who reported mild degeneration of kidney when administered with 2000 and 5000 mg/kg respectively. Finding of this study showed that *S. birrea* stem bark ethanol extract is considered safe for human use when use at low or moderate concentrations. *S. birrea* is a medicinal plant having vast nutritional and therapeutic potential [22].

Conclusion

Based on the finding of this study, it is concluded that the lethal dose of ethanol extract of *S. birrea* stem bark is higher than 2,000 mg/kg as there is no mortality in the experimental animal. Similarly, clinical sign such as salivation, loss of hair, decrease in respiration rate (pulsating) and diarrhea were absent. However, the animals experienced restlessness at a concentration of 1000 and 2000 mg/Kg which disappeared within 24 hours which shows that the extract is safe for human use when use at low

or moderate concentrations. It is recommended that the extracts from plant should be used at lower concentration.

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