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Acute Toxicity Effect of Aqueous Extract of Stem Bark of *Diospyrus Mespiliformis* on Kidneys and Biochemical Parameters of Wistar Rats

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Abstract

Diospyros mespiliformis is a large evergreen tree found mostly in the savannas, tall and growing 12-15 meters tall in drier areas of its range and 25 meters or more in the wetter areas. The bole which is sometimes fluted at the base can be up to 60cm in diameter. The Jackalberry tree is found throughout Africa, from Senegal and the Sudan to Namibia and the Northern Transvaal. It is most commonly found on Savannas or Savanna woodlands where it can be found growing on termite mounds. In heavy soil the termite mound provides the tree with aerated soil, and a source of moisture. The root provides protection for the termite that doesn't eat living wood. Jackalberry wood is almost termite resistant after it has been cut. The aim of this study was to determine the histomorphologic effect of oral administration of aqueous extract of *diospyros mesipiliformis* on kidney and its biochemical parameters. A total 12 of adult Wistar rats were randomly divided into four groups as A, B, C and D. Each group consist of three rats, group A served as control which was administered with equivalent milliliters of distilled water daily, group B was administered with low dose daily of the aqueous extract stem bark of *diospyros mesipiliformis*, group C medium dose daily of the aqueous extract stem bark of *diospyros mesipiliformis*, then group D was administered with the highest dose daily of the aqueous extract stem bark of *diospyros mesipiliformis,* all for the period of 28 days (4 weeks). *Diospyros mesipiliformis* was found to be safe at dose up to *5000mg/kg and the biochemical parameters of kidney which include sodium, potassium, chloride, urea and creatinine showed no significant difference as compared with the control.* The histological result showed normal tissue architecture of kidney with well-preserved glomerulus, Bowman's capsules and tubules. No significant changes in the biochemical parameters and architecture of the kidney histology were observed. Also, the LD50 was found to be above 5000mg/kg. Therefore, the plant can be said to be safe for human consumption.

Keywords: Acute Toxicity Effect, *Diospyrus Mespiliformis,* Kidneys, Biochemical Parameters

Introduction

Medicinal plants include a various type of plants used in herbalism and some of these plants have some medicinal activities. These medicinal plants considered as a rich resource of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world (Hassan, 2012). Moreover, some plants are considered as important source of nutrition and as a result of that these plants are recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants and their derivatives considered as important source for active ingredients which are used in aspirin and toothpaste (Hassan, 2012). Plants have been used for medicinal purposes long before recorded history. Primitive men observed and appreciated the great diversity of plants available to them. Plants provide food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to be developed through observations of wild animals, and by trial and

error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias (Manuchair, 2002). Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. The opium poppy yields morphine, codeine and paregoric, a remedy for diarrhoea. Laudanum, a tincture of the opium poppy, was the favored tranquilizer in Victorian times. Even today, morphine the most important alkaloid of the opium poppy remains the standard against which new synthetic pain relievers is measured (Manuchair, 2002).

Similarly, tetrahydrocannabinol (THC), the component of Cannabis sativa responsible for the CNS effect, has also been found to reduce nausea associated with cancer chemotherapy. Another therapeutic area where natural products have had a major impact on longevity and quality of life is in the treatment of cancer. In fact, most of the major anticancer drugs are natural products either from plants or micro-organisms. Examples include important anticancer drugs such as Bleomycin, Doxorubicin, Vincristine, Vinblastine, and now the recent addition of Paclitaxel (Taxol), Ironotecan (a camptothecin derivative) and Etoposide and Tenoposide (Podophyllotoxin derivatives) (Manuchair, 2002). *Diospyros mespiliformis*, the Jackal berry (also known as African ebony and by its Afrikaans name Jakkalsbessie), is a large evergreen tree found mostly in the savannas of Africa. Jackals are fond of fruit, which are rounded, dense crown it varies considerably with height, and tall, growing 12-15 meters tall in drier areas of its range and 25 meters or more in the wetter areas. The bole which is sometimes fluted at the base can be up to 60cm in diameter (Merwe, 2001). The Jakalberry tree is found throughout Africa, from Senegal and the Sudan to Namibia and the Northern Transvaal. It is most commonly found on Savannas or Savanna woodlands where it can be found growing on termite mounds. In heavy soil the termite mound provides the tree with aerated soil, and a source of moisture. The root provides protection for the termite that doesn't eat living wood. Jakalberry wood is almost termite resistant after it has been cut (Merwe, 2001)**.** The kidneys are a pair of organs found along the posterior wall of the abdominal cavity. The left kidney is located slightly more superior than the right kidney due to larger size of the liver on the right side of the body. Unlike the other abdominal organs, the kidney lies behind the peritoneum that lines the abdominal cavity thus are considered to be retroperitoneal organs. The ribs and the muscles of the back protect the kidney from external damage. Adipose tissue known as perirenal fat surrounds the kidneys and acts as protective padding (Taylor, 2014). Each kidney is bean-shaped with convex laterally and concave medially with a medial indentation called the hilum. Blood vessels, lymphatic's vessels, nerves, and the ureter enter or exit at the hilum. An adult kidney is about 12 cm long, 7 cm wide and 2.5 cm thick (Volker, 2016). A thin layer of fibrous connective tissue forms the renal capsule surrounding each kidney. The renal capsule provides stiff pouter shell to maintain the shape of the inner tissues. Deep to the renal capsule is soft, dense, vascular renal cortex. Seven cones shaped renal pyramid form the renal medulla deep to the renal cortex. The renal pyramids are aligned with their apexes point inward toward the center of the kidney. Each apex connects to minor calyx, a small hollow tube that collects urine. The minor calyces merge to form 3 larger calyces, which further merge to form the hollow renal pelvis at the center of the kidney. The renal pelvis exits the kidney at the renal hilum, where urine drains into ureter (Taylor, 2014).

Materials and Method

Study Location

The study was conducted at the Department of Histopathology, School of Medical laboratory science, Usmanu Danfodiyo University, Sokoto.

Plant Collection

The plant was collected in Northern Nigeria, Zamfara State, Gusau Local Government Area.

Plant Identification and Authentication

The plant was identified and authenticated in Faculty of Biological Sciences, Department of

Botany, Usmanu Danfodiyo University, Sokoto by Malam Musa Magaji, with the specimen voucher number PCG/UDUS/Eben/0001 and deposited in the same Department.

Plant Extraction Procedure

The dried stem bark of *Diopyros mespiliformis* was grinded in to powder using mortar and pestle. About 100g was measured and dissolved in 3000 ml of distilled water. It was then stirred and allowed to stay for 24 hours. It was filtered using fine cloth to removed large debris and then filtered with Whatman filter paper then evaporated to dryness using hot air oven set at 40°c. The resulting evaporate was used for the study. Each evaporate was subsequently weighed and the percentage yielded calculated.

3.7 Acute Toxicity Study (LD_{50})

The LD_{50} was carried out in Animal House, Faculty of Pharmaceutical Science using Lorke's method (1983). It consists of two phases, in phase I, nine Wistar rats were used and randomly divided into three groups with 3 rats in each group. The first group was administered 10mg/kg body weight of the extract, group 2 and 3 were administered 100mg/kg and 1000mg/kg respectively. The animals were then observed for 24 hours to monitor their behavior as well as toxicity signs and mortality. In phase II, 3 rats were used and randomly divided in to three groups of 1 rat each. The rats were administered doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. They were observed for 24 hours to monitor their behavior as well as toxicity signs and any mortality.

Experimental Design

A total 12 of adult wistar rats were randomly divided into four groups as A, B, C and D. Each group consist of three rats, group A served as control which was administered with distilled water daily, group B was administered with low dose daily, group C medium dose daily, then group D was administered with the highest dose which is daily all for the period of 28 days (4 weeks).

2.8.1 Summary of Experimental Design.

Groups	Dose/kgbw/ day	Route of administration	Duration
A (control)	2 ml Distilled water	Oral	28 days
B (low)	500mg	Oral	28 days
C (medium)	1000mg	Oral	28 days
D (high)	1500mg	Oral	28 days

Procurement of Experimental Animals

Twenty (20) adults male and female Wistar rats with an average weight of 150g was purchased from Ahmadu Bello University (ABU) Zaria, Faculty of Pharmaceutical Science, Department of Pharmacology and Toxicology and transported to Sokoto, Usmanu Danfodiyo University, and Faculty of Pharmaceutical Science in Animal house. The rats were housed in a metal cage with 12 hours dark/light cycle. They were fed with standard pellets [grower mash] and tap water *ad libitum*. The animals were allowed to acclimatized for 2 weeks before proceeding with the study/. Before the commencement of the study, physical examinations of the animals were carried out and were found to be in a very good state of health. Ethical approval was obtained from the ethical committee for the use of laboratory animal of Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

Sample Collection

At the end of the study, the Animals were sacrificed, twenty-four hours after the last dose administration of the extract. The animals were weighed and scarified following mild anesthesia with chloroform inhalation in an enclosed transparent plastic jar. The blood samples for biochemical studies were collected in plain containers via cardiac puncture; the kidney was carefully harvested and washed with normal saline then fixed in 10% formal saline.

Laboratory Analysis

Urea Estimation was done using the Diacetyl

Monoxime Method while the Creatinine Estimation was carried out by the alkaline picrate method.

Data Analysis

The result generated was analyzed using Graph Pad Instant Prism software version 7.0. One-way analysis of variance (ANOVA) was used to compare the test and control. Results were expressed as mean plus or minus standard error of mean (SEM

Result

Table 4.1: Physical properties of aqueous extract of the stem bark of *Diospyrus Mespiliformis*

Plant part	Color	Percentage yield	Texture	Consistency
Stem bark	Brown	2.2g	Powder	Smooth

The table above, show the physical properties of the aqueous extract of the stem bark of *Diospyrus Mespiliformis*. The plant part used was stem bark and the color of the extract was brown and yielded a percentage of 2.2g after extraction. The texture was powder and consistency were smooth in nature.

All graded doses of DM administered to the Wistar rats showed no sign of toxicity or behavioral change. After 24hours observation, no death was recorded in both the first phase and second phase of the procedure. The lethal dose (LD_{50}) of DM was 5000mg/kg.

Table 4.2*:* Acute oral toxicity testing that is lethal dose (LD_{50}) of aqueous stem bark extract of *Diospyrus mespiliformis* in wistar rats

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

Table 4.2 above shows the result of acute oral toxicity (LD_{50} determination procedure).

Table 4.3: **Biochemical parameters of the Kidney Function Test**

Parameters	Group A (Control)	Group B	Group C	Group D
Sodium	135.67 ± 2.08	33.33±4.04	134 ± 1.7	141.33±6.66
Potassium	6.57± 0.23	4.9 ±0.35	4.77 ± 0.15	4.83 ± 0.30
Urea	8.93 ± 1.79	10.47 ± 2.51	7.87 ± 1.37	11.53 ± 2.73
Chloride	97.33 ± 10	95.67 ± 20	96 ± 10	101 ± 30
Creatinine	0.6 ± 0.20	0.6 ± 00	0.67± 0.10	0.6 ± 00

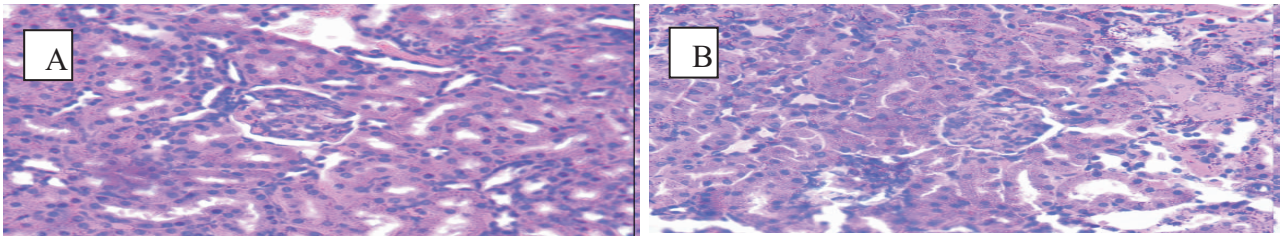

Plate 4.1: Kidney section A=Control and B=Test (500mg) stained with Haematoxylin and Eosin (×400).

Section of kidney tissue showing normal general histological structures. Black arrow showing normal glomerular capillaries and green arrow showing normal capsular spaces in both control and test.

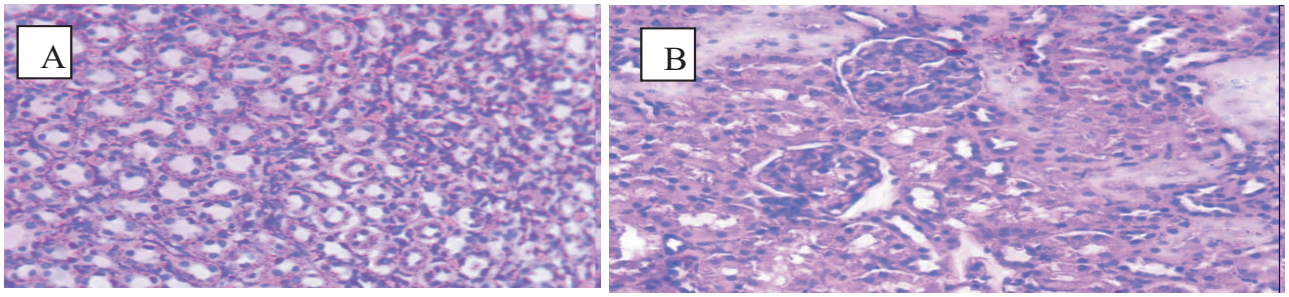

Plate 4.2: Kidney tissue section A=Control and B=Test (1000mg) stained with Haematoxylin and Eosin (×400)

Section of kidney tissue showing normal general histological structures Black arrow showing normal glomerular capillaries and green arrow showing normal capsular spaces in both control and test.

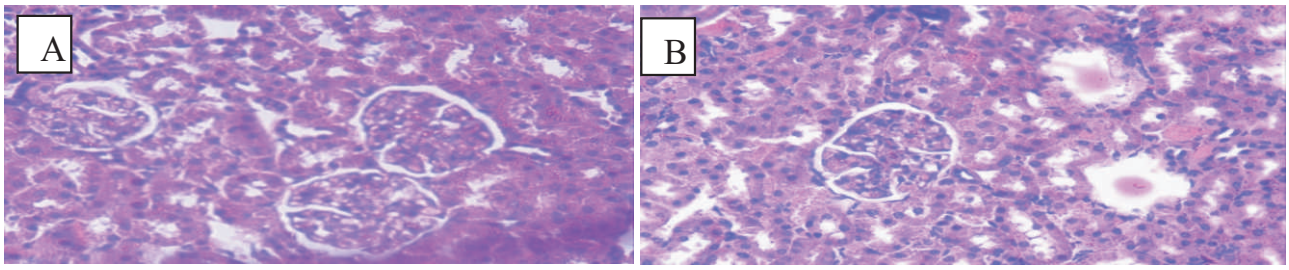

Plate 4.3: Kidney tissue section A=Control and B=Test (1500mg) stained with Haematoxylin and\Eosin (×400).

Section of kidney tissue showing normal general histological structures. Black arrow showing normal glomerular capillaries and green arrow showing normal capsular spaces in both control and test.

Discussion

Diospyros mesipiliformis was found to be safe at dose up to 5000mg/kg signifying its lethal dose is above 5000mg/kg body weight. This observation was in agreement with the previous report (Muhammad, 2014) which indicated that at a dose above 5000mg/kg Diospyros mespiliformis is safe for human consumption. The biochemical parameters of kidney which include sodium, potassium, chloride, urea and creatinine showed no significant effect or findings as compared with the control. Similar finding was observed a previous by Muhammad (2014) which stated that at a 500mg/kg and 1000mg/kg causes only slight but non-significant increase in sodium and a slight decrease in urea.

In this study there was no increase or decrease of both potassium and creatinine levels between subjects and controls. Our finding is contrary with the finding of Muhammad (2014) which stated that, there was a statistically significant decreased in the value of creatinine at dose 500mg/kg and 100mg/kg of the extract when compared with the controls. This might be due to difference in the preparation of the extract. Similarly, our finding is at variance with the work of Oguche et al. (2016) at a medium dose of methanol extract of Diospyros mesipiliformis which indicated a statistically significant increase in the creatinine level.

The histological result showed normal tissue architecture of kidney with well-preserved glomerulus, Bowman's capsules and tubules. Our finding is at variance with previous report by Muhammad (2014) which indicated that at a dose of 500mg/kg, there was moderate degeneration of renal tubules with normal glomeruli. While at the dose of 1000mg/kg, there was a loss of renal tubular architecture and degeneration of renal tubules but with intact glomeruli.

Conclusion

Our study indicates that extract of Diospyros mesipiliformis is safe at dose up to 5000mg/kg and does not have any statistically significant effect of the sodium, potassium, chloride, urea and creatinine. The histological result showed normal tissue architecture of kidney with well-preserved glomerulus, Bowman's capsules and tubules. No significant changes in the biochemical parameters and architecture of the kidney histology were observed. Also, the LD50 was found to be above 5000mg/kg. Therefore, the plant can be said to be safe for human consumption.

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