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EFFECTS OF METHANOL EXTRACT OF AZADIRACHTA INDICA LEAVES ON THE HISTOLOGY OF LIVER AND KIDNEY OF WISTER RATS

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ABSTRACT
The plant, *Azadirachta indica* A Juss, family Meliaceae is a native of Asia but has now naturalized in West Africa and is widely cultivated in Nigeria as an ornamental as well as medicinal plant. The plant is used extensively in Nigeria for the traditional treatment of malaria and other associated conditions in form of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects. In the present study, an attempt was made to investigate the effects of methanol extract of the leaves of *A. indica* on the liver and kidney of Wister rats for the period of 28 days. 20 animals were used and grouped into 4 groups of 5 rats each, in which 1, 000 mg/kg, 1, 500 mg/kg and 2, 000 mg/kg were administered to the first 3 groups and referred to as the test groups, the fourth group was administered with an equal volume of distilled water and referred to as the control group. At the end of the experiment, the animals were scarified and their livers and the kidneys excised. These organs were processed for the normal hematoxylin and eosin staining. Histological examination of the livers of the test groups revealed an apoptosis of hepatocytes, ground glass appearance of hepatocytes, presence of inflammatory cells around the portal area and congested blood vessels. Examination of the kidneys also revealed a congestion of vessels in glomerulus, presence of inflammatory cells in the interstitum and congested blood vessels and hyaline globule in collecting tubules. However, the control group revealed normal histological features of both the liver and the kidney. It could therefore be suggested that large dose consumption of the leaves of *A. indica* for long term should be avoided as may cause malfunction of such vital organs.

Key words: *Azadirachta indica*, methanol extract, toxicity, liver, kidney.

INTRODUCTION

Azadirachta indica (Neem tree) A. Juss (Meliaceae) is a native of Asia but has now naturalized in West Africa. The plant is commonly known as Dalbejia or Dogon-yaro in Hausa language, it is widely cultivated throughout Nigeria as an ornamental plant. The plant is drought resistant and therefore grows well even in the arid parts of Nigeria; growing up to 25 m high, but it occurs mostly as a medium sized tree (Oliver, 1959). Various parts of this plant are employed in Nigerian traditional medicine for the treatment of variety of ailments. Dogon-yaro is used extensively in Nigeria for the treatment of malaria using aqueous infusion, decoction or alcoholic extracts of the leaves and stem bark. The effect of Azadirachta extract on methaemoglobin generation and the conversion of glutathione to its oxidized counterpart have also been investigated (Iwu, *et al.,* 1986). The neem plant and its derived products have shown a variety of insecticidal properties on a broad range of insect species

(Isman, 2006). Neem products have shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy, ovicidal activity, fecundity suppression, insect growth regulation and repellency. These activities are frequently attributed to the azadirachtin contents and other constituents of the plant or its products (Modue and Blackwell, 1993). Although neem-based products are considered to be relatively safe towards non-target biota, there was reported risk of direct adverse effects on aquatic macro invertebrates resulting from contamination of water bodies with neem-based insecticides (Goektepe *et al.,* 2004; Fredros, *et al.,* 2007). Phytochemical investigations of neem have established that all part of the plant contain bitter principles, composed of azadirachtin, azadirone, meliacin, nimbin, nimbidin, nimbinin, tiglic acids and fatty acids (Iwu, *et al.,* 1986). Unspecified quantities of neem products are usually consumed locally for a long time, in the course of the treatment of various ailments, without due regards to toxicological and other adverse effects it may cause. The present study is therefore carried out in an attempt to investigate the effects of methanol extract of the leaves of this plant on the liver and kidney of Wister rats.

MATERIALS AND METHODS

Plant Material

The leaves of *A. indica* were collected in the month of August, 2007 from tree growing in Area A, Main Campus of Ahmadu Bello University, Zaria, Nigeria. The plant was identified by Mal M Musa of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, where voucher specimen (No. 900151) was deposited.

Preparation of the Plant Materials

The fresh leaves were allowed to dry under shade and grounded into powder using wooden pestle and mortar. 1 kg of the powdered material was packed into thimble and extracted with 2.5 L of methanol using soxhlet extraction apparatus. The methanol extract was concentrated under reduced pressure to yield 90 g crude methanolic extract, referred to as Azadirachta leaves methanol extract (ALME).

Test Animals

Adult Wistar rats of both sexes, weighing between 201 g and 225 g were purchased from the Animal House Section of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria. The animals were maintained in standard animal cages in the Animal House of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria-Nigeria.

Animal Treatment

The animals were kept in plastic cages at room temperature and moisture, under naturally illuminated environment of 12:12 – hour day and night cycle. The animals were kept in the cages for one week prior to the experiment, to allow them acclimatize with the laboratory conditions. They were allowed free access to drinking water and standard livestock feed (Vital Feed Growers) from Brand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria. Their experimental usage was according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 2002 Publication, No. 83 - 23), (Revised 1978). These guidelines are consistent with guidelines of Ahmadu Bello University for animal handling. The experiments were conducted between the hours of 9: 00 am and 12: 00 noon daily.

Acute Toxicity Determination

The acute toxicity of the test drug was determined by evaluating its median lethal dose (LD_{50}) orally (p.o.) using the method described by Lorke (Lorke, 1983) in two phases. In the first phase, nine rats were divided into three groups each containing three rats and were administered the test drug (ALME) at doses of 10 $mgkg^{-}1$, 100 mg/kg

and 1000 mgkg^{-}1 body weight; and observed for signs of toxicity and death for 24 hours. In the second phase, three groups each containing one rat were treated with the drug (ALME) at doses of 1600, 2900 and 5000 mgkg^{-}1 and observed for 48 hours for signs of toxicity and death.

Sub-chronic Toxicity Determination

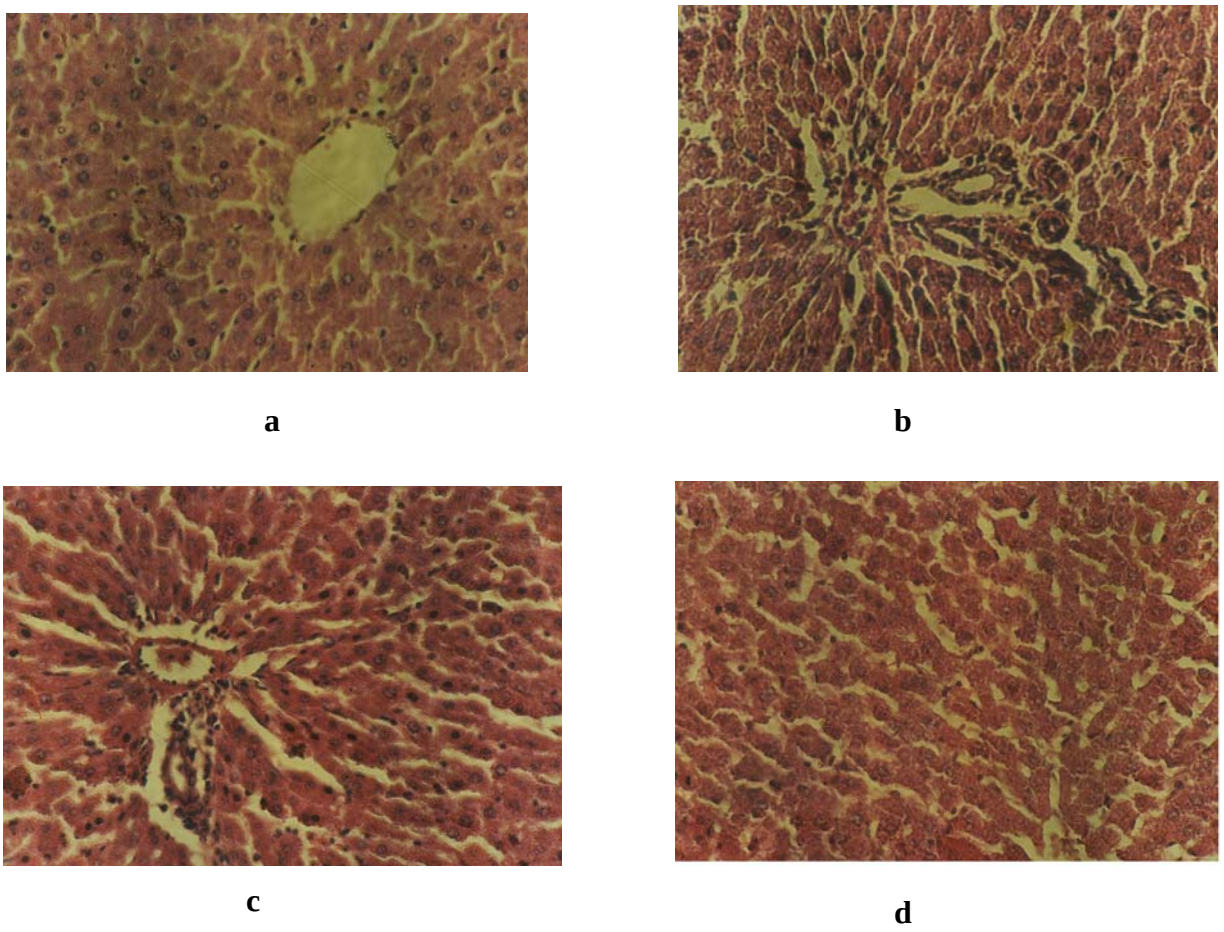
Twenty Wister rats divided in to four groups of five animals each were used for this study. Group I was administered 500 mgkg^{-}1day^{-}1 (p.o.), group II was administered 1, 000 mgkg^{-}1day^{-}1 (p.o.) and group III was administered 2, 000 mgkg^{-}1day^{-}1 (p.o.), whereas group IV was administered distilled water and served as control. The treatment continued daily for 28 days (four weeks). The physical appearance of the animals was observed throughout the period of the study.

Preparation of the Kidney and Liver

After four weeks (on 29th day of the treatment), the animals were sacrificed by cervical dislocation, the livers and kidneys were removed and preserved in buffered 10 % formalin saline solution for histopathological processing. These organs were first gross-examined for any observable lesion before they were processed using the automatic tissue processor. The technique involved dehydrating the fixed tissues placed in tissue baskets with their respective labels and passing them through graded alcohol (70 %, 90 %, 95 % and 100 %). The tissues were removed after dehydration and moved into xylene solution baths to clear the alcohol and facilitate molten wax impregnation. The tissues were finally sectioned using rotary microtome (at 5µ thickness), stained with haematocytocin and eosin (H&E) and then examined microscopically following standard procedures (Arthur and John, 1978; Igale *et al.,* 1995). The slides were allowed to dry and examined under a light microscope.

RESULTS AND DISCUSSION

The animals in groups II to III were observed to start defecating very soft feaces on the fifth day of the experiment, which could be due to the sloughing of the gastrointestinal tract. The possible toxic effects of the extract could have brought about an increased gastrointestinal motility, resulting in the diarrhea. The two vital organs, (livers and kidneys) removed from the test groups at the end of the sub-chronic study were carefully observed macroscopically and revealed no any observable gross lesions when compared with the control group. Microscopically however, the two organs of the test groups revealed some histological changes when compared with the control group, which shows normal histological appearance. The livers of the rats administered with the highest dose of the extracts revealed very clear pathological changes, such as congested vessels channels; periportal inflammation; apoptosis; ground glass hepatocytes and kupffer cell prominence (Fig. 1). Evidence of liver damage, usually manifest as a result of architectural disarray, vascular congestion, hepatocytes necrosis, apoptosis, or inflammatory cell infiltration in either acute or chronic conditions. Some of these features were observed in the rats administered with the highest dose of the extract. Generally, cells died as a result of necrosis or apoptosis when they are challenged with toxins, noxious agents or injuries (Eroschenko, 2000).

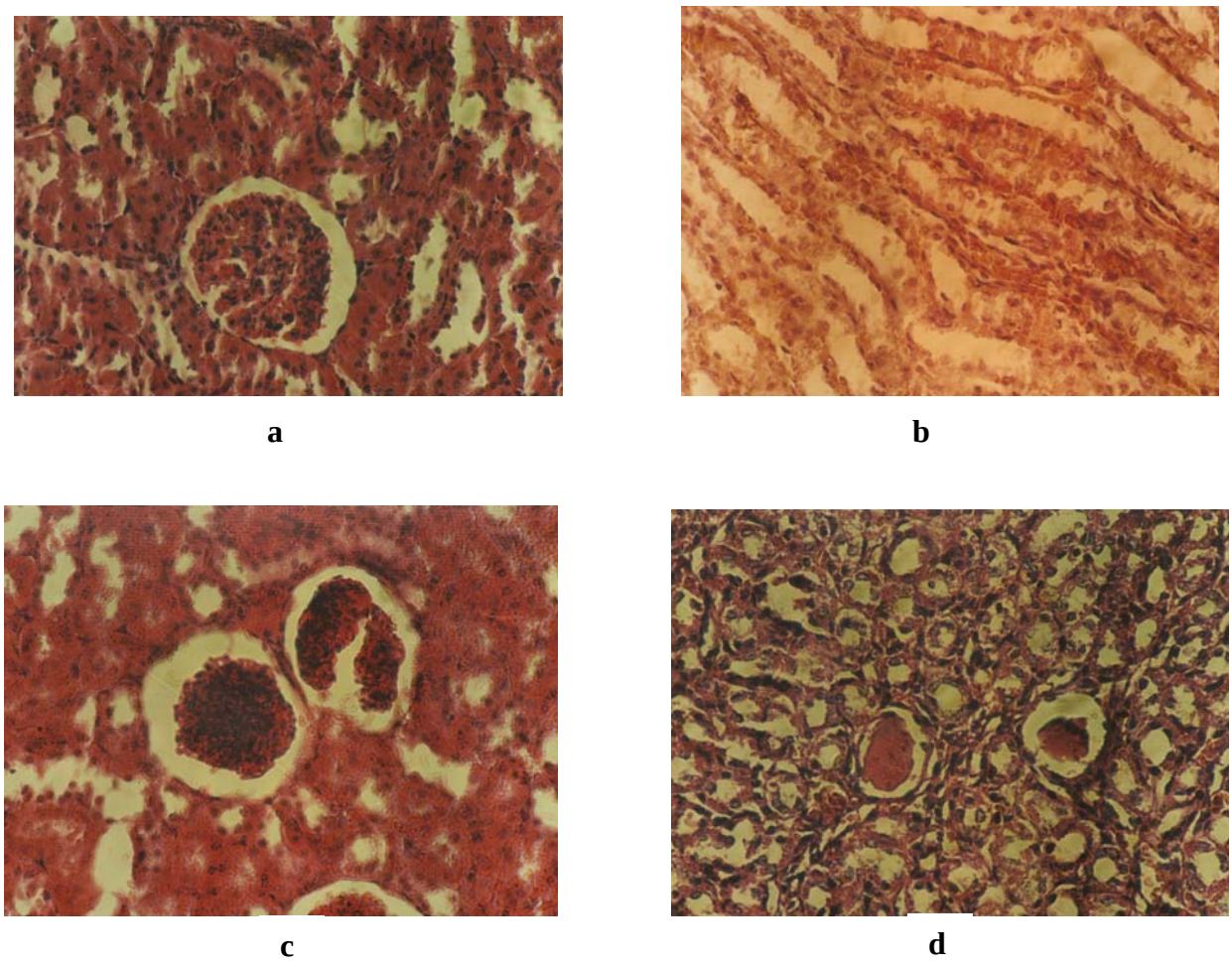


a **b**

c **d**

Figure 1: Cross Section of the Liver Showing (**a:** normal central vein from control rats **b:** normal portal triad from control rats, **c:** periportal inflammation from GII, **d:** apoptosis from GIII); Stained in H & E. X250

The architectural appearance of the kidneys from the rats in the control group, presented a normal histological appearance, as against the kidneys from the rats in the test groups, which presented different degrees of vessels congestion, cells inflammation and presence of hyaline globules in collecting tubules (Fig. 2). Damages to kidney caused either by chemical agents or drugs could be manifested as vascular congestion (glomerulus), inflammatory cell infiltration and hyaline globule in collecting tubules (Eroschenko, 2000). All these features were clearly observed in the test groups, for example, the presence of hyaline globules in collecting tubules, as observed in the group III, is an indication of accumulation of proteins in glomerular filtrate, which could lead to decreased filtration rate, urine formation and/or nephritis. Infiltration of kidney with chronic inflammatory cells (lymphocytes) was conspicuously seen in group II. Interstitum is the commonest site for infiltration with inflammatory cells as observed in variety of diseases (Young and Heath, 2000). Toxic agents can cause all these changes observed in the kidneys of the rats in the test groups, which means that the active constituents in the neem leaves, could cause damages to liver and kidney at high doses.



a **b**

c **d**

Figure 2: Cross Section of the Kidney Showing (**a:** normal renal coruscles from conrol rats **b:** normal medullary region from conrol rats, **c:** vascular congestion in glomerulus from GIII, **d:** hyaline globules in collecting tubules GIII); Stained in H & E. X250

CONCLUSION

The methanol extract of Azadirachta indica leaves was found to cause liver and kidney histopathological damages of Wister rats, when the animals were administered with the extract at doses of 1, 000 and 2, 000 $mgkg^{-}$ $1day^{-}1$ (p.o.) for 28 days. Oral dosing of the extract was considered in this study, to replicate the ethnomedical method of administration by the traditional medical practitioners and the likely route of administration during clinical evaluation. Methanol was considered as solvent for extraction in this present study, since it is easy to handle without destruction of most of the active components by heat or hydrolysis, and it is very close to the solvent used by the local people, which is mostly water.

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