EFFECTS OF THE METHANOLIC LEAF EXTRACT OF *ADENODOLICHOS PANICULATUS* (HUA) ON RAT LIVER FUNCTION

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ABSTRACT

In present study acute oral toxicity value and the effect of the methanolic leaf extract of the *Adenodolichos paniculatus* (MAP) on function status of the liver were investigated in rats after two weeks of daily oral administration of graded doses (250, 500 and 1000 mg/kg body weight) of the extract. The preliminary phytochemical screening of the plant revealed the presence of flavonoids, tannins, steroids, carbohydrates, glycosides, alkaloids, anthraquinones and cardiac glycosides. The oral LD_{50} of the extract in rats was found to be greater than 5000 mg/kg body weight suggest that the extract is relatively non-toxic and safe. Results obtained from the study showed that all values for biochemical parameters (ALT, ALT, TB and CB) for the control and experiment groups were statistically insignificant ($P < 0.05$) in all the graded doses used (250, 500, 1000 mg/kg body weight) except for AST which is statistically significant ($P > 0.05$) when compared with control group in all the graded doses used (250, 500, 1000 mg/kg body weight). The extract does not relatively show a dose dependent alteration in liver enzymes functions. It could be suggest that the methanolic leaf extract of *Adenodolichos paniculatus* had no adverse effects on the functional status of the liver at doses tested and for period of the study. Sub chronic and chronic toxicity study is required to ascertain this claim.

Keywords: *Adenodolichos paniculatus*, $LD_{50,}$ Phytochemistry and liver Function

INTRODUCTION

Liver is an organ in the body where metabolism of drugs and other xenobiotics take place. Amongst other functions, the enzyme systems involved in biotransformation are localized primarily in the liver, and the active metabolites can cause liver damage. So it has a significant role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fights against disease(s), supplies nutrients, provides energy and helps in reproduction[1]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification of poisons, secretion of bile

and storage of vitamins. Thus, to maintain a liver is a crucial factor for overall health and well being. However, liver is continuously exposed to environmental toxins, and abused by poor drug habits, alcohol users and the misused of prescribed and over-the-counter drugs which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease [2,3].

Thus liver diseases are some of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders [4,5].

Adenodolichos paniculatus (HUA) (*Leguminosae*) is a shrub that grow up to 4m high in savanna region of Guinea, Northern Nigeria and across to Sudan. In Nigeria, the leaf is applied topically with butter oil as a dressing for burns[6]. The leaf has been used in toothache[7] and Central Africa Republic, the root –decoction has been used for *blenorrhoea a*nd liver trouble[7].

This study is therefore aimed at evaluating the sub-acute toxicity of the methanolic leaf extract of *Adenodolichos paniculatus* on the liver in experimental or laboratory animals.

MATERIALS AND METHODS:

Plant collection:

The leaves of *Adenodolichos paniculatus* were collected from Kundun village, Birnin Gwari, Kaduna State in June, 2009. The leaves were identified and authenticated by Mallam Umar S. Gallah of the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (Number 3107) was deposited at the herbarium for future reference. The leaves were cleaned, air dried and crushed into powder with pestle and mortar. 200 g of the powdered leaf was cold macerated at room temperature with 70% methanol for 48 hours with occasional shaking. The solvent was evaporated at 100^0C to obtain the percentage yield of the concentrated crude extract. The percentage yield of the extract is 8.45%

Experimental animals:

Swiss albino mice (18 - 30 g) and Wistar rats (160 - 250 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University,

Zaria, Nigeria. The rats were maintained on rodent feed and water *ad libitum*, and housed in propylene cages at room temperature throughout the study. All experimental protocols were approved by the University Animal Ethics Committee.

Phytochemical analysis:
Adenodolichos paniculatus was subjected to phytochemical screening using standard protocol[8].

Acute Toxicity (LD_{50}) determination:
Oral acute toxicity in rats:
The acute toxicity study of the methanolic extract was determined orally in rats using the method described by Lorke[9] (1983). The animals were fasted overnight and the LD_{50} evaluation was carried out in two phases. In the first phase, three (3) groups of three (3) rats each were treated with the MAP at doses of 10, 100 and 1000 mg/kg body weight orally respectively. The rats were monitored for 24 hours for signs and symptoms of toxicity, such as paw licking, salivation, sedation convulsion and mortality. In the second phase, four groups of one (1) rat each were further treated with the MAP at doses of 1600, 2900 and 5000 mg/kg body weight respectively. The rats were also observed for signs and symptoms of toxicity and mortality.

Sub acute toxicity study:
Twenty four (24) Wister albino rats weighing between (160 – 250 g) were used for this study. The rats were randomly divided into four (4) groups of six (6) rats each. The first group served as control was administered normal saline (10ml/kg), while the second, third, and fourth groups were administered with 250, 500, 1000 mg/kg of the MAP orally respectively on daily basis for two weeks, after which the animals were sacrificed after 24hrs, on the fifteenth (15^{th}) day.

Sample collection and preparation:

Rats were anaesthetized in light chloroform and blood samples collected by cardiac puncture into clean, dry centrifuge tubes. Blood samples which were processed individually were allowed to stand for 10mins at room temperature and then centrifuged at 1000 rpm for 15mins on laboratory centrifuge (SM 800B, Surgifriend Medicals, England) and the supernatant (serum) carefully removed with Pasteur pipette and stored frozen until required for enzyme analysis.

Enzyme assays and clinical chemistry:

Alkaline phosphatase activity was assayed using the method described by[10].The

procedure described by [11] was employed for the assay of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Total bilirubin (Randox assay kit) was determined by the methods of [12] and [13]

Statistical analysis:

The data were expressed as mean ± SEM and analyzed statistically using student's t-test. P values less than 0.05 ($P < 0.05$) were considered to be statistically significant.

RESULTS

The phytochemical screening of methanolic leaf extract of *Adenodolichos paniculatus* revealed the presence of flavonoids, tannins, steroids, carbohydrates, glycosides, alkaloids, anthraquinones and cardiac glycosides.

Acute toxicity study:

The median oral lethal dose of *Adenodolichos paniculatus* in rats was found to be greater than 5000 mg/kg,

Enzyme Assays and Clinical Chemistry

The effects of administration of MAP to rats on the activities of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and conjugated bilirubin respectively are shown in **Tables 1.** The administration of MAP for 14 days did not produce significant increase ($P > 0.05$) in serum liver enzymes at doses tested (250, 500 and 1000 mg/kg body weight) respectively.

DISCUSSION

The preliminary phytochemical screening revealed the presence of flavonoids, glycosides, tannins, saponins, steroids, anthraquinones and cardiac glycosides.

The oral LD_{50} of the MAP was found to be greater than 5000 mg/kg which is relatively safe because no death was recorded even at the dose of 5000 mg/kg. The Organization for Economic Cooperation and Development (OECD, Paris, France)[14] (Walum, 1998) recommended chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values as: very toxic, $\leq$ 5 mg/kg; toxic, $> 5 \leq 50$ mg/kg; harmful, $> 50 \leq 500$ mg/kg; and not toxic or harmful, $> 500 \leq 2{,}000$ mg/kg. Based on this classification, the oral LD_{50} up to 5,000 mg/kg established for rats indicated relative oral safety. According to the toxicity scale[15], any compound with an oral LD_{50} of between 500 – 2000 mg/kg should be considered practically non toxic. This also agrees with the findings of[16] .WHO ranks all plants whose LD_{50} figures are less than 25 mg/kg in "very toxic group", between 25 and 200 mg/kg in

"toxic group" and from 200 to 2000 mg/kg in "noxious group". This could be attributed to the fact that orally administered drugs and compounds do undergo some events that potentially decrease the amount reaching systemic circulation for pharmacological effects [17]

In the assessment of liver damage by drugs or any other hepatotoxin, the determination of enzyme levels such as ALT and AST is largely used [18]. Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of AST indicate liver damage, such as that due to viral hepatitis, cardiac infarction and muscle injury. The ratio of AST to ALT is sometimes used in differentiating causes of liver damage[19]. Elevated AST are not specific for liver damage, and AST has also been used as cardiac marker. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury[19]. Serum ALP level on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme, in presence of increasing biliary pressure [20].

In this study, as shown in **table 1**, the MAP did not altered any significant ($P < 0.05$) changes in all the indices of liver function (AST, ALT, ALP, TB, and CB). There was no significant difference between treated groups (ALT, ALP, TB, and CB). ($P < 0.05$) when compared with the control except for AST where there was significant difference ($P > 0.05$) in all doses used (250, 500, 1000 mg/kg) when compared with the control. Thus, these findings provide evidence for the clinical safety of the plant. Conjugating ability of the liver was intact as revealed by total and conjugated bilirubin levels. The plasma ALT and AST activities are markers of hepatocellular damage; their levels in the rat were not affected by the extract intake. It is probable that the presence of Flavonoids which are hepatoprotectives[21].

Therefore, the observed antioxidant and hepatoprotective activity of *Adenodolichos paniculatus* may be due to the presence of flavonoids.

In conclusion, the presence study suggest that all indices for liver functions were not affect by extract intake for a period of fourteen (14) days also established that the extract has no adverse effect on the liver functions and support ethno medicinal uses of the plant in liver trouble. Sub chronic

and chronic toxicity studies would give final answer on the safety of *Adenodolichos paniculatus* as an analgesic and anti-inflammatory agent.

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Table 1. Effects of MAP on the functional status of the liver after 14 days treatment in Rats

		MEAN ± SEM				
Treatment	**Dose mg/kg**	**AST**	**ALT**	**ALP**	**TB**	**CB**
Normal saline	10	255.3 ± 22.3	123.8 ± 3.74	14.5 ± 3.74	26.7 ± 3.37	17.30 ± 3.21
Extract	250	163.2 ± 21.3	111.3 ± 134.16	22.7 ± 1.22	24.5 ± 5.61	13.33 ± 3.30
Extract	500	163.17 ± 7.50	119.33 ± 7.07	25.5 ± 0.66	25.5 ± 5.99	13.33 ± 2.61
Extract	1000	200.8 ± 24.17	122.5 ± 6.42	24.83 ± 1.1 1	32.33 ± 5.9	16.00 ± 2.86

All values for AST, ALT, ALP, TB and CB are statistically insignificant at ($P < 0.05$) compared with control using student's t-test. n = 6