Preliminary Phytochemical Analysis and Toxicological Effects of Aqueous Stem Bark Extract of *Albizia Chevalieri* on Experimental Animals (Albino Rats)

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Abstract: *Albizia chevalierihas been reported to be used in treatment of wide range of aliments. The effect of oral administration of aqueous stem bark extract was studied on some organ body ratio, hematological, biochemical and histopathological parameters in male albino rats. A total of thirty (30) male albino rats were grouped into five (six rats each) based on their weight as: Group I (receive 25mg/kg), Group II (receive 50mg/kg), Group III (receive 100mg/kg), Group IV (receive 200mg/kg) and Group V (receive 1 ml of saline) respectively. The phytochemical analysis revealed the presence of Alkaloids, Tanins, flavonoids, saponins and phytate while the organ body weight ratio indicates a decrease with increase concentration of the extract. The result of this work also indicate low counts of WBC, RBC,HCT and PLT while AST, ALT, ALP and Bilirubin tend to increase with increase concentration of the extract. Similar trend was also observed for the other biochemical parameters determined in this work. The histology of the organ from the albino rats indicates some levels of damage with concentration*

of the extract. Therefore from the results of this work it is clear that, the aqueous stem bark extract of Albizia chevalieri may contain toxic compound that is responsible for the toxicity detected.

***Key words**: phytochemicals, toxicity, liver function, renal function, histology*

Introduction

Albizia is among subfamily Mimosoideae which were reported to have flowers with radial symmetry, small, inconspicuous corollas and numerous, showy stamens. The flowers of this plant flowers are similar to those found in many-flowered heads or spikes. The subfamily is reported to include *Acacia* (wattle), *Albizia* (silk tree), *Samanea* (monkey pod), *Prosopis* (mesquite) and *Calliandra* (powder puff) respectively. The genus *Albizia* is comprises of approximately 150 species, most of which trees and shrubs native to tropical and subtropical regions of Asia and Africa[1].

Albizia (A.) chevalieri, member of subfamily acacia,the shrub is native to tropical and subtropical regions of Asia and Africa including Nigeria. The plant leaves are bipinnate with leaflets in

numerous pairs or larger in fewer pairs and thepetiolar glands are conspicuous. Flowers are in globose heads or spikes and the Stamens are usually white and elongate. Its corolla is funnel-shaped, connate beyond the middle and the fruit is broadly linear indehiscent or 2-valved, valves not twisted [1]. There existseveral other species of the *Albizia* and are grown as ornamentals.

The leaf extract of *A. chevalieri* is used in the treatment of diabetes mellitus by traditional healersin some parts of Niger republic and Sokoto State, Nigeria. The leaves extract was reported literature to possessa significant hypoglycemic effect [2]. The aqueous stem bark extract was been used by traditional healers in Wudil town Kano Nigeria as cold water decoction or dried, ground and sieved stem bark (additive to local foods) to treat hypertension and several form of gastrointestinal tract cancers.

The traditional uses albizia for treatment of diseases may be due to the presence of different secondary metabolites. It was reportedby Karuppannan*et al.*[1] that, the species of Albiziacontain different classes of secondary metabolites such as saponins, terpenes, alkaloids and flavonoids. Also some bioactive compounds were isolated and identified from genus *Albizia* were e.g. triterpenoid saponins.Few of these compounds may tends be toxic, and thus the plants tend containing them, consumption of extract from such plant could therefore, confer different levels of toxicity to the individual. There are some plants that are inherently dangerous, because they naturally contains toxins and often with cytotoxic, carcinogenic effects, or some other toxic properties[3].

In this work, the *in vivo* toxicity effects of the crude aqueous stem bark extract of *A. chevalieri* on organ-body weight, liver function, renal function, hematological variables and histopathological changes in albino rats were assessed.

Materials and Methods

Collection of Plant Materials and Extraction

The stems bark of *Albizia chevalieri* (Fabaceae, minosaceae) were collected in the month of January from farm in Wudil town, Kano state, Nigeria. The identity of the plant was authenticated by Mr. Namadi Sunusi, Department of botany, Ahmadu Bello University Zaria, Kaduna Nigeria. A voucher spacemen of the plant (voucher no. 900247) was deposited for future reference in the Departmental Herbarium.

The stem bark was dried under shade in the absence of sunlight for two weeks and grinded to powdered using mortar and pestle. The powdered plant material (400g) was mixed with 200ml of 70°C hot distilled water and was kept for 2 days with regular stirring. The mixture was filtered using Whatman no 1 filter paper. The filtrate was collected and concentrated using rotary evaporator. The concentrated extract was then freeze dried and powder of aqueous extract was obtained. The powdered form of the extract was kept in refrigerator at -20°C beforeto use.

Preliminary Phytochemical Screening

The quantitative determination of total Alkaloid, Saponin, Flavonoid, Tannin and Phytate were carried out using standard procedure.

Animals

A total of 30 adult male albino rats aged 6-8 weeks and weighing 100-160g were purchased from animal house of department of zoology, Bayero University Kano, Nigeria. The animals were housed under a standard laboratory living conditions with 12 hours dark/light and with full access of standard animal feed (Pelletised growers feed, Zawan round about, Jos, Plateau State, Nigeria) and provided water ad libitum. The animals were divided into five groups based on weight and concentration of extract to be administered and were used for this studies after one week of acclimatization.

The animals were fasted for 15 hours (5.00pm- 8.00 am) before the beginning of the experiments but were all allowed for free access to water. These albino rats were handled with strict compliance to international guidelines as reported by the Canadian council on the care and uses of experimental animals (1993).

Toxicity Studies

The albino rats were divided into five groups of six animals each, group one received 25mg/kg body weight, group two received 50mg/kg, group three 100mg/kg and group four received 200mg/kg while the fifth group received only 1ml of physiological saline which serves as control.

Extract Administration and Samples Collection

The aqueous stem bark extract of A. chevalieri was found to dissolve completely in water and corresponding dose was orally administered daily to the rats for a period of six weeks. At the end of the experimental period, all the rats were sacrificed under a mild chloroform anesthesia. A total of 5ml blood samples were collected with aid of cervical decapitation from each experimental animal. One ml of the blood was immediately taken out and mixed with anticoagulant (EDTA) for hematological analysis. The remaining 4 ml were allowed to clot and then centrifuged at3000 rpm for 10 minutes. The serum was separated from the cells and was used to determine the liver and renal function's parameters (biochemical analysis). The liver, kidney, pancreas, spleen, lungs and heart were carefully dissected from the animal and the remnant's blood was wiped out with filter paper and weighed. At the end of dissection the liver and kidneys were immediately preserved in 10% neutral buffered formalin for histopathological analysis.

Parameters Investigated

Relative organ weight

The organ weight in relation to body weight of the animal (relative organ weight) is the percentage of the ratio of absolute organ weight to the body weight of animal on sacrifice day[4].

$$\text{Relative organ weight} = \frac{\text{Absolute weight of the organ}}{\text{Body weight of the animal on sacrifice day}}^{x}100$$

Hematological Parameters

The percentage hematocrits (HCT%) was determined according to method described by Sanderson [5], while the RBC, Total WBC and platelet counts were determined according to standard procedure.

Liver and Renal Function Indices (Biochemical parameters)

The liver function indices such as aspartate amino transferase (AST), Alanine amino tranferase (ALT), and Alkaline phosphatase (ALP) were determine using Randox kits as described by manufacturer while the total and direct bilirubin were determined by method described by Jendrassik and Grof [6].

The renal function parameters determined include the serum concentration of chloride (Schoenfeld and Lowellen [7]), Bicarbonate (van slyke method[8]), urea (Veniamin andVakirtzi

[9]) andCreatinine(Jaffe [10])while the sodium and potassium ion concentrations were determined by flame photometry.

Histopathology Test

The histopathological analysis was carried out one rat per each experimental group. The rats were dissected according to procedure adopted by Mikel[11] and only liver and kidneys were taken for this analysis. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned to approximately 5mm thin, stained with hematoxylin and eosin, and finally the general structure was examined under electron microscope [12].

Statistical Analysis of Data: The results of this work were all express as Mean±SD with exception for histopathological test and the statistical variations among the groups were evaluated by one way analysis of variance (ANOVA) at p value of less than 0.05.

Results

The phytochemical constituents of *A. chevalieri* stem bark aqueous extract was determined using standard method. The result was recorded as mean and standard deviation as depicted on table 1. The percentage alkaloids, tannins, flavonoids, saponins and phytate were found to be 2.1, 3.1, 89, 10 and 0.1 % respectively.

The organ body weight ratio of the albino rats used in this work was found to decrease with increase extract concentration as shown on Table 2.

The hematological parameters determined include the count for WBC, RBC HCT and PLT, the result indicate significant decrease for the test as compare to the control group as shown on table 3 respectively.

The result of the serum liver and renal function parameter analysis was shown in table 4 and 5 respectively. There was significant difference in all the parameters for the test as compared to the control group. The AST, ALT, ALP and bilirubin were significantly increased with dosage as compared to that of control group.

The liver section for the control group showed normal hepatic design and architecture. The section showed normal hepatocytes with no sign of inflammation or necrosis (Figure 6e). The histology of the test liver was found with some degenerative changes which increase with the dosage (Figure 6a-d).

The histology of the control group kidney shows normal appearance with no change in its renal tubules and glomerulus (Figure 7 e) while the section of the test kidney shows areas of renal damage and dilatation of renal tubules (Figure7a-e).

Table 1: The quantitative phytochemical constituents of*A. chevalieri*stem bark aqueous extract

Phytochemicals	**Mean±SD**
Phytate (%)	0.13±0.01
Alkaloids (%)	2.10±0.20
Tanins (%)	3.11±0.29

Flavonoids (%)	88.94±0.04
Saponins (mg/L)	10.65±0.08

Values are mean±SD, SD=standard deviations of mean

Table 2: Effect of aqueous stem bark extract of *Albizia chevalieri* on organ body weight ratio of the albino rats

Organ	Group I (25mg/kg)	Group II (50mg/kg)	Group III (100mg/kg)	Group IV (200mg/kg)	Control Group
Liver	3.50*	3.41*	3.23*	3.44*	4.01
Kidney	0.66*	0.70	0.66*	0.64*	0.74
Spleen	0.43*	0.37*	0.60*	0.43*	0.46
Pancreas	0.23*	0.26*	0.30*	0.33*	0.38
Lungs	1.25*	0.93*	1.23*	1.09*	2.06
Heart	0.43*	0.37*	0.42*	0.47	0.48

Key: Organ body ratio, sign *represent significant decrease and increase in the ratio

Table 3: Effect of oral administration of aqueous leaves extract of *Albizia chevalieri* on Hematological parameters of albino rats

Groups/ Extract concentration	WBC $x10^9/L$	$RBCx10^{12}/L$	HCT %	$PLTx10^9/L$
Group I (25 mg/kg)	0.1±0.03*	0.1±0.01*	0.7±0.07*	375.5±1.41*
Group II (50 mg/kg)	0.9±0.06*	5.5±0.09*	35.8±0.71*	375.5±4.95*
Group III (100 mg/kg)	1.5±0.96*	5.0±0.06*	32.1±0.71*	313.5±0.70*
Group IV (200 mg/kg)	30.8±0.65**	5.8±0.34*	38.6±0.28*	216.5±2.12*
Group V (Control)	3.2±0.21	8.4±0.03	63.6±0.78	942.5±0.71

Sign* stance for significant increase and decrease when compared with control group at $p<0.05$; the values are mean±SD. SD= standard deviation of the mean, WBC = white blood cell count, RBC= red blood cell count, HCT =hematocrit and PLT= platelet count respectively

Table 4:Effect of oral administration of aqueous leaves extract of *Albizia chevalieri* on Liver function parameters of control and test groups of albino rats

Conc. Extract (mg/kg)	AST (U/L)		ALT (U/L)	ALP (IU/L)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)
Group I (25 mg/kg)	23.0 4.24	±	25.5±0.71*	57.5±0.71*	11.2 ±1.13*	2.15±0.21*
Group II (50 mg/kg)	36.0 2.83*	±	34.0±4.24*	61.2±0.35*	8.3 ± 1.41*	3.0±0.28*
Group III (100 mg/kg)	51.5± 0.07*		34.0±7.07*	64.0±1.41*	12.7±3.25*	4.5±3.54*
Group IV (200 mg/kg)	51.5 2.12*	±	43.0±1.41*	66.5±0.71*	23.0±1.41*	5.0±0.14*
Group V (Control)	22.9 0.14	±	69.5±3.54	46.0±5.66	12.1±0.07	1.20±0.28

Sign* stance for significant increase and decrease when compared with control group at $p<0.05$; the values are mean±SD. SD= standard deviation of the mean, AST = Aspartateaminotransferase, ALT= Alanine aminotransferase, ALP=Alkaline phosphatase.

Table 5: Effect of aqueous leaves extract of *Albizia chevalieri* on Renal Function indices of Control and Test groups of albino rats

Conc. Extract (mg/kg)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	Urea (mg/dl)	Creatinine (mg/dl)
Group I (25mg/kg)	153.5±0.85*	10.8±0.35*	74.5±3.53*	12.3±5.73*	7.3±0.92*	22.8±6.36*
Group II (50mg/kg)	154±0.71*	11.6±0.07*	74±5.66*	9.25±1.77*	7.8±1.70*	26.5±2.83
Group III (100mg/kg)	157.5±2.12*	12.5±0.63*	73±1.41*	8.85±1.20*	5.7±1.00	26.6±2.12*
Group IV (200mg/kg)	162.5±3.54*	13.6±1.00*	72±1.41*	9.9±1.70*	5.0±0.35*	36.5.0±4.24*
Group V (Control)	164.5±0.71	9.7±0.21	65.5±2.12	12.5±0.71	5.5±0.50	25.1±0.17

Sign* stance for significant increase and decrease when compared with control group at $p<0.05$; the values are mean±SD. SD= standard deviation of the mean

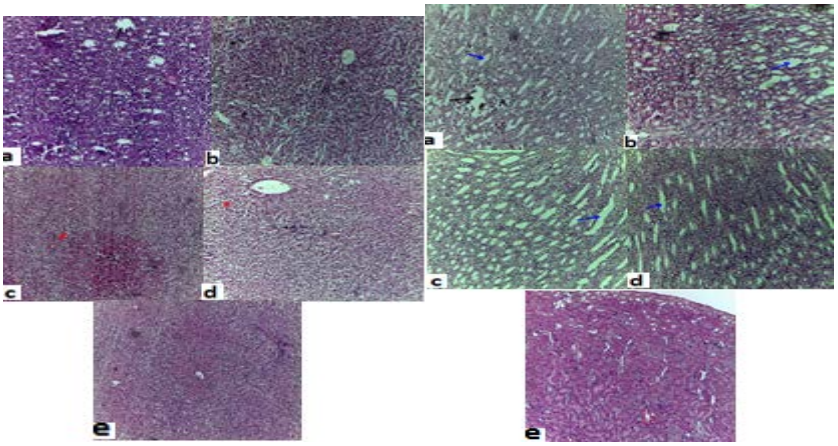

Figure 6-7: Effect of aqueous leaves extract of *A. chevalieri* on the histology of liver and kidney of albino rats

Discussion

Traditional medicine plays an important role in the world primary health care program because of the general belief that herbal drugs natural and are always safe [12]. The oral consumption of any herbal preparations without any prescription or standard dosage coupled to shortage of available scientific studies on the safety of the herbal drugs raised an alarm concerning toxicity [13]. The findings from this work clearly indicate that aqueous stem bark extract of A. chevalieri have negatively affect the liver and kidney of the albino rats. Phytochemicals are non-nutrient bioactive natural compounds that are produced by plants [14] in small quantity and were found to be protective against some degenerative diseases in human. The phytochemical analysis result of this work shows that, the aqueous stem bark contains Alkaloids(2.1%), Tanins (3.1%), flavonoids (89%), saponins (10.7%) and phytate (0.13%). This could be the reason for using the plant in treating some diseases. This result is in line with that reported by Evans [15] that, alkaloids and their synthetic derivatives are used as anti-analgesic, anti-malaria, antiseptic and antibacterial. This is also in line with that reported by Okwu and Emenika[16] that, saponins are used as anti-fungal and anti-bacterial. Therefore presence of alkaloids, saponins and tannins can make the bark to have as natural antibiotic properties [17] as well as anti-inflammatory [18].

The aqueous tem bark extract of A. chevalieri was found based on the result of this work to unfavorably interact with organs of the albino rats as indicated by the significant decrease in the organ body weight ratio with dosage (Table 2). This may be due to cellular constriction and inflammation of rat organs. This result does not tally with that reported by Devaki*et al*.[19]and Schmidst*et al*. [20]that the aqueous *Passifloraedulis*is not toxic to experimental animals.

The effect of aqueous stem bark extract of A. chevalieri on WBC, RBC, HCT and PLT counts were studied, the result shown on Table 3, that, there is significant difference or decrease in the count for both WBC, RBC, HCT and PLT for the test groups when compares with the control group. The trend was found to decrease with increase in the extract concentration for all the hematological parameters determined in this work. These indicate that, at low dosage of the extract both parameters were significantly decrease which may be due to the toxic effects of the extract on the bone marrow of the rats. The result tallies with that reported by Appidi*et al*. [21] that, administration of leaves extract of *Hermaniaincana* results in mild changes in hematological index in rats. This result was found not in line with that reported by Devaki*et al*. [19], that aqueous extract of *Passifloraedulis* produce no any effect on the hematological parameters of the rats.

The serum AST, ALT, ALP and bilirubin are the liver function parameters evaluated in this work as full markers to assess the possible toxic effect of aqueous stem bark extract of *A. chevalier*. A change in these markers is a clear indication of the impairment of liver by the extract toxicity. A valuable tool of diagnostic important that provides information on effect and nature of the pathological damage to the tissue is the measurement of serum enzyme activity. The result of this work clearly shows a significant increase in the activities of AST and ALP for the group that received the extract in comparism with that if control as depicted on Table 4. The increase in the activity was found in correspondence with the increase in the concentration of the extract received which may be due to increased livers membrane damage with concentration. This result is in conformity with the fact that, liver toxication may lead to a compromised membrane integrity [22]. The elevation of serum AST and ALP activity at all the dosage may be caused by leakage from the hepatocyte as a result of destruction that alters membrane permeability to these enzymes [23]. This damage may also have negative effect on the metabolism and regulation of amino acid in the liver. The serum alkaline phosphatase is a major marker for plasma membrane and endoplasmic reticulum ([24] and [25]). The result of these work shows,significant increase in Alp with dosage of the extract as compared to the control, which may be due to increased denovo synthesis of the enzyme by the liver [26]. The serum ALT activity for the groups that received the extract was found to be significantly less than that of control group, this may be

due to the presence in the extract of component that can inhibit the enzyme, and this result is not in line with that reported by Lathe *et al*. [23]. Therefore, the general increased in AST and ALP following the administration ofthe extract could be due to the toxic effect of the extract on liver. This is not in line with Yakubu *et al*.[27] that, serum ALP and AST activity increased following administration of *H. sabdariffa*.

There is significant increase in both total and direct serum bilirubin concentration in the groups that received the extract when compared to that of control group. This may be due to reduction of its uptake by liver arising from liver damage and is line with Ashafa *et al*.[28] that, liver damage impaired it function.

The kidneys are vital to body homeostasis and plays important role in the excretion of toxic/ waste metabolites from the body. Kidney also regulates intracellular volume, electrolyte and acid-base balance [29], toxic insult to the kidneys could generally affect total body metabolism [30]. The result of this work shows significant increases in the serum potassium and chloride for the group that received the various doses of the extract compared to the control but the serum sodium and bicarbonate were found to be significantly less than that of control as depicted on Table 5. This may be as a result that, the extract may be rich in potassium and chloride but low in sodium. The result is not in line with that reported by Mojiminiyi *et al*. [31] that, H. sabdariffa consumption had elevates plasma sodium, chloride

and potassium and also not in line with that reported by Ross and Desai [32] that, water deprivation causes hypernatremia in rats.The significant differences exist in serum chloride and bicarbonate in groups that received the aqueous extract with the control group could be as a result of tubular and glomerular dysfunction. This is in line with the work reported by Ukoha*et al*. [33] that, administration of aqueous extract of *H. sabdariffa* increases the serum chloride and bicarbonate.

Urea is a waste product of protein metabolism while creatinine is a hydrolyzed product of creatine phosphate and were good markers for renal function, the significant increase in the serum urea observed in group I and iii and the increase in creatinine in group ii and iv as compared to the control group suggest that, the extract have compromised the functional integrity of the kidneys. This is in line with findings reported by Orisakwe *et al*. [28] and Abubakar*et al*. [34] respectively.

The histopathological result (Figure 6 and 7) of this work revealed that, administration of different doses of aqueous stem bark extract of A. chevalieri result in degenerative changes in both liver and kidney cells of the albino rats that received the doses. This result is in conformity with that reported by Seyyed *et al*. [12] that, aqueous extract of *Asaferida* causes little degenerative changes in hepatocyte but no prominent effect on the kidney of rats. Also,

the result is in line with that of Ukoha *et al*. [34] that, chronic consumption of aqueous H. sabdariffa calyx might be toxic to kidneys of rats.

Conclusion

The effect of consumption of aqueous stem bark extract of *A. chevalieri* was tested on male albino rats. The result of our work suggest that, consumption of the aqueous extract of *A. chevalieri* might be toxic to both liver and kidneys by causing distortional changes in their architecture as indicated by histological test and by increases in serum liver and renal markers concentrations.

Competing Interests

Authors have declared that no competing interests exist.

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