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Pharmacological and Toxicological Effects of Aqueous Acetone Extract of *Sida alba* L. (Malvaceae) in Animals Model

K.Konaté[1], M.Ouédraogo[2], J. F. Mavoungou[3], A.N.Lepengué[4], A.Souza[5], B.M'Batchi[4] O.G.Nacoulma[1]

*Corresponding author:

K. Konaté

[1]Laboratoire de Biochimie et Chimie Appliquées (LABIOCA), UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso.
[2]Laboratoire de Physiologie Animale, UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso.
[3]Institut de Recherche en Ecologie Tropicale (IRET/CENAREST). BP : 13354 Libreville-Gabon.
[4]Laboratoire de Phytopathologie, UFR Agrobiologie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, BP 943 Franceville-Gabon.
[5]Laboratoire de Physiologie Animale, Electrophysiologie et de Pharmacologie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, Franceville-Gabon.

Abstract

The present study was conducted to evaluate the pharmacological and toxic effects of aqueous acetone extract of *Sida alba* L. a Malvaceae species, in mice Swiss and albinos Wistar rats. In acute toxicity test, mice received doses of this extract by intraperitoneal route with LD_{50} value of 3200 mg/kg. In sub-acute toxicity test, albinos Wistar rats were treat by gavage during 28 days with different doses of aqueous acetone extracts of *Sida alba* L., (75, 100 and 150 mg/kg). About to the pharmacological properties, the results varied widely in dose of extract and weight of rats and did not show clinical correlations. We undertook this study of extracts in order to provide a scientific basis for the traditional use of *Sida alba* L., in traditional medicine particularly to treat hepatitis B. Our results of this study appeared to show the safety of acute and sub-acute toxicities of extract from *Sida alba* L., which can therefore be continuously used with safety in traditional medicine. Statistical studies revealed that there is a low significant difference in body and organ weights, and biological parameters between control group and the treated assay groups ($p<0.01$ or $p<0.05$).

Keywords: *Sida alba*, LD_{50}, toxicity, Biological parameters, mice and Wistar rats.

Introduction

Sida alba L., is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world. It belongs to the family Malvaceae and is very common in central and western Africa where it has a wide range of uses [1]. Several secondary metabolites such as tannins, saponins, alkaloids and flavonoids have been reported to be present in the extracts of the plant [2].

Sida alba is widely used in traditional medicine by various cultures worldwide to another. In Burkina Faso, recent investigations in the central region have shown that Sida alba., is used frequently and widely in traditional medicine to treat various kinds of diseases such as infectious diseases in children and is very widely used for the treatment of liver diseases for many years in Burkina Faso particularly in hepatitis B virus treatment, malaria, fever, pain, variola, antibacterial, anti-viral activities and hepatoprotective [1].

Although studies showed that the toxicity of the secondary metabolites [3], however it should be noted that no test of toxicity was carried out on the extracts of *Sida alba*. Despite the extensive use of this plant in traditional health care, the literature provides little information regarding their toxicity so that the toxicities effects of the plant very used are unknown. So, the present study was carried out to investigate the toxicological study and evaluation of pharmacology properties of aqueous acetone extract of *Sida alba*.

Materials and methods

Plants material

Sida alba L. was collected in August 2008 in Gampela, 25 Km east of Ouagadougou, capital of Burkina Faso. The plant was botanically identified by Prof. Millogo-Rasolodimby from the plants Biology

Department of the University of Ouagadougou. A voucher specimen was deposited at the Herbarium of the Laboratoire de Biologie et d'Ecologie Végétale, UFR/SVT of University of Ouagadougou.

Preparation of extracts

Fifty grams (50g) of powdered plant material was extracted with 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) at approximately 40°C and freeze-dried by a being Telstar Cryodos 50 freeze-dryer. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use.

Animals

We used male mice and female Swiss NMRI (20-30g) and adult albinos Wistar (209-249g) coming from University of Yaoundé (Cameroun). The animals were housed in cage under controlled conditions of 12-h light/12-h dark cycle and 25°C. They all receive pellets food enriched with protein 20% and water ad libitum.

Toxicity studies

Acute toxicity study in mice

Healthy male and female Swiss mice (20-30g) were randomly divided into 6 groups (1 control group and 5 treated assay groups) of 6 animals (3 male and 3 female). They deprived of food, but not water 15 h prior to the administration of the test suspension. The control group received water containing 10% dimethylsulfoxide (DMSO) administered by intraperitoneally. The aqueous extract acetone of *Sida alba* suspended in 15% DMSO was administered intraperitoneally at doses of 1800, 2000, 2500, 3000 and 6000 mg/kg. The general behavior of the mice was observed at 120 min after the treatment. The animals were observed for morbidity and mortality once a day for up 14 days, with food and water ad libitum. The number of survivors after the 14 days period was noted. The toxicological effect was assessed on the basis of mortality, which was expressed as the median lethal dose (LD_{50}). The LD_{50} (Lethal Dose 50 estimated from the regression of log-probit mortality rate according to the natural logarithm of the dose of extract administered) was determined [4].

Sub-acute toxicity study in albinos Wistar rats

Animals were divided into 4 groups of 6 animals (3 males and 3 females). Body weight was (250-255g). The first group served as control, and received water containing DMSO 10%. The remaining groups (group 2; group 3; group 4) received three dose levels of the *Sida alba* extract (75, 100 and 150 mg/kg) suspended in 10% of DMSO, administered orally by gavage daily for a period of 28 days. Body weight was measured weekly, and the animals were observed daily for signs of abnormalities throughout the study. At the end of a 28 day period, the animals were deprived of food for 15 h. Blood samples were collected by cardiac puncture for biochemical examinations, and selected organs were carefully dissected and removed for weighing.

Blood analysis

Blood samples were collected by cardiac puncture in three tubes for haematology, glucose and serum biochemistry. The blood samples with heparin and without anticoagulant were centrifuged at 3000 rpm for 5 min to obtain plasma or serum. Plasma was used to determine glucose by [5, 6] methods and the serum for other biochemical parameters such as aspartate aminotransferase (AST) and alamine aminotransferase (ALT) determined according [7] and according [8], alkaline phosphatase (ALP) estimated by [9, 10] methods, creatinin [11], uric acid [12], blood urea nitrogen (BUN) according [13], triglycerides [14], total cholesterol [15], total bilirubin and direct bilirubin determined according [16]. All these biochemical parameters were measured by Selectra XL Vital Scientific (Elitech Group Company).

Animal weights

The body weights of animals were measured weekly and at the end of a 28 day period, the animals were deprived of food for 15 h. After the collection of blood samples by cardiac puncture for biochemical examinations, organs such as heart, lungs, stomach, liver, kidneys in rats were carefully dissected and removed for weighing.

Statistical analysis

The data were expressed as Mean±Standard deviation (SD) of six determinations (n=6). Results were analyzed by one-way ANOVA followed by Dunnett's t-test using Prism 4 software. The level of significance was accepted at $p \leq 0.05$.

Results

Acute toxicity study in mice

The effect of intraperitoneal treatment of the aqueous acetone extract from *Sida alba* on mortality, LD_{50} value. The value of LD_{50} is 3200 mg/kg for intraperitoneal administration. No significant difference in body weight gain of the treated assay groups over the period of observation. No statistical difference was observed between the organ weights in the control and the intraperitoneal route groups.

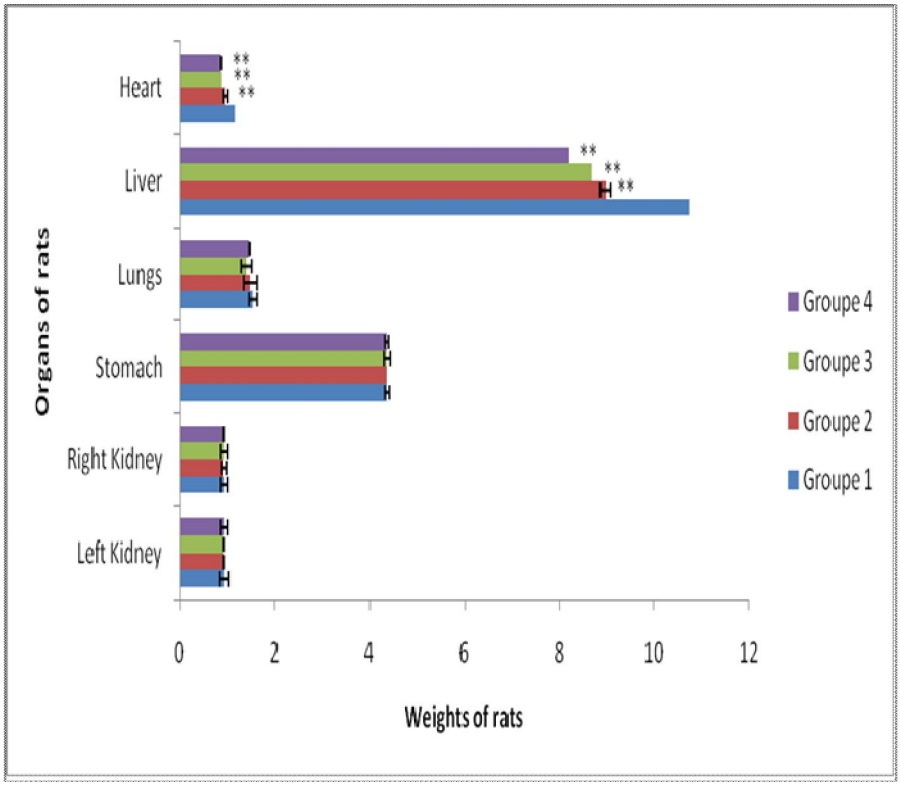

Figure 1: Animal weights (g) with time of treatment
Values are mean ± S.E.M. (n=6) one-way ANOVA followed by Dunnett's t- test:: Compare all vs. control: $^{ns}p>0.05$,*p<0.05, **p<0.01 compared with control
Group 1: control, rats received 10% DMSO
Group 2: rats received 10% DMSO with extract (75 mg/kg body weight)
Group 3: rats received 10% DMSO with extract (100 mg/kg body weight)
Group 4: rats received 10% DMSO with extract (150 mg/kg body weight)

Sub-acute toxicity study in rats

Body weight:

We noticed no significant difference in body weight gain between control group and the test groups ($p>0.05$). However, there is also an increase in animal weight as a function of treatment time (in weeks). In the fourth week, there was a significant difference in body weight gain between the test groups and the control group ($p<0.01$). We note a decrease in weight of animals. The results are summarised in Figure 1.

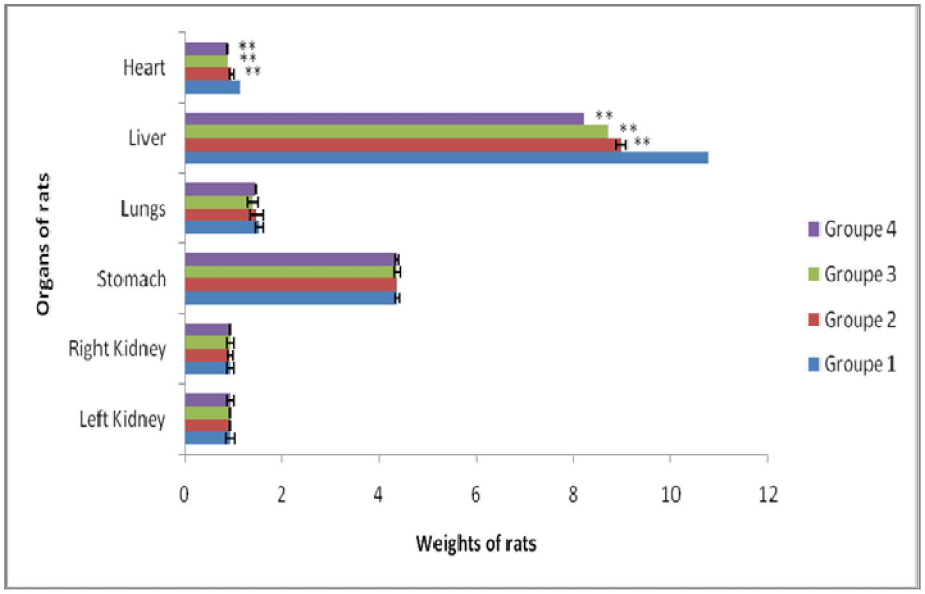

Figure 2: Effects of Aqueous Acetone Extract of *Sida alba* on the weights (g) of organs of rats
Values are mean ± S.E.M. (n=6) one-way ANOVA followed by Dunnett's t- test:: Compare all vs. control: $^{ns}p>0.05$,*p<0.05, **p<0.01 compared with control
Group 1: control, rats received 10% DMSO
Group 2: rats received 10% DMSO with extract (75 mg/kg body weight)
Group 3: rats received 10% DMSO with extract (100 mg/kg body weight)
Group 4: rats received 10% DMSO with extract (150 mg/kg body weight)

Organ weights:

Figure 2 shows the effects of Sida alba. extract on the weights of some vital body organs in rats. The weights of liver (75; 100 mg/kg and 150 mg/kg; p<0.01) and heart (75; 100 and 150 mg/kg; p<0.01) decreased significantly compared to the control group (DMSO 10%). However there is no significant difference between the other vital body organs weights of the treated assay groups and the control group (p>0.05).

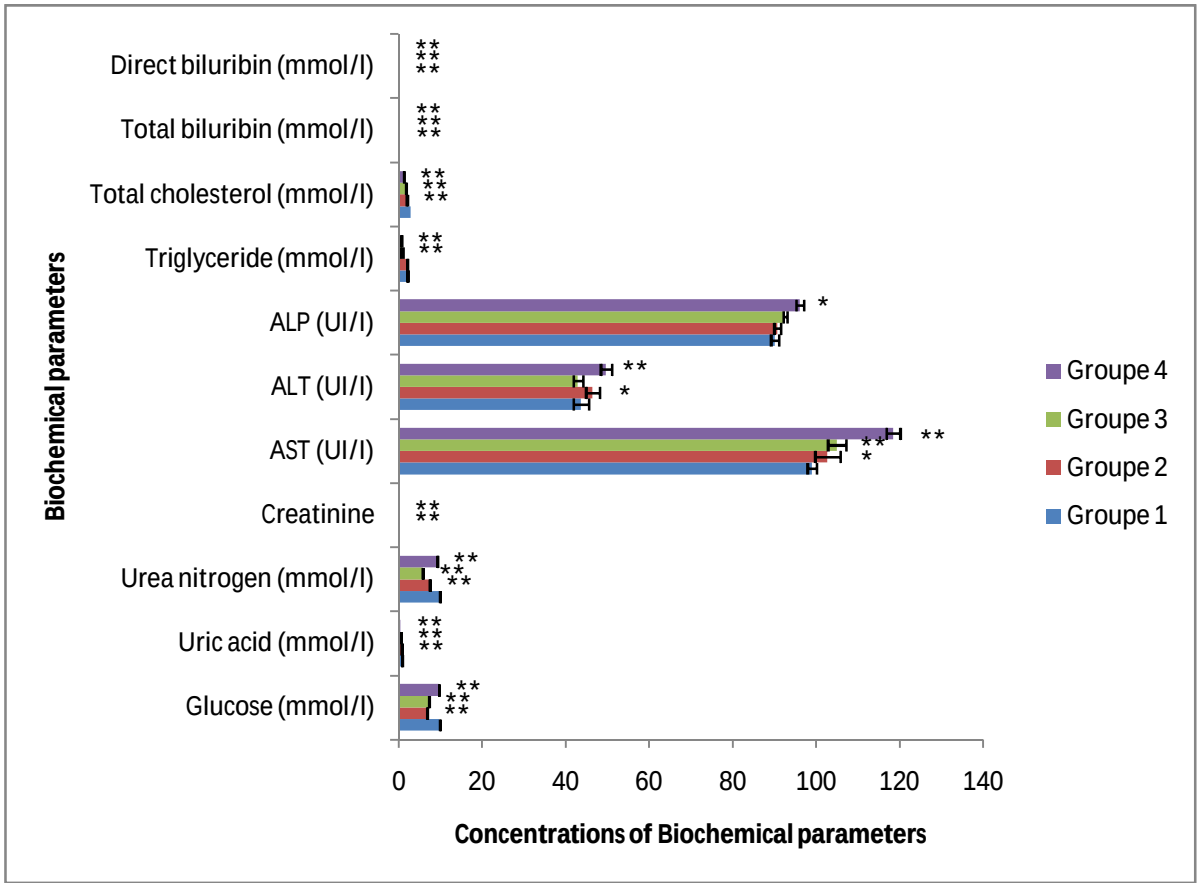

Figure 3: Effects of Aqueous Acetone Extract of *Sida alba* on the biochemical parameters in the plasma and the serum of rats.

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase
Values are mean ± S.E.M. (n=6) one-way ANOVA followed by Dunnett's t- test: Compare all vs. control:
$^{ns}p>0.05$,*p<0.05, **p<0.01 compared with control
Group 1: control, rats received 10% DMSO
Group 2: rats received 10% DMSO with extract (75 mg/kg body weight)
Group 3: rats received 10% DMSO with extract (100 mg/kg body weight)
Group 4: rats received 10% DMSO with extract (150 mg/kg body weight)

Biochemical analyses:

Figure 3 shows the effects of Sida alba. extract on the biochemical parameters. Glucose (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.01; p<0.01 and p<0.01), acid uric (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.01; p<0.01 and p<0.01), the urea nitrogen (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.01; p<0.01 and p<0.01), creatinin (75 mg/kg, 100 mg/kg; p<0.01; p<0.01 and 150 mg/kg; p 0.01), AST (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.05; p<0.01 and p<0.01), ALT (75 mg/kg, 100 mg/kg and 150 mg/kg; p 0.01; p<0.05 and p<0.01), ALP (75 mg/kg, 100 mg/kg and 150 mg/kg; p 0.01 and p<0.01), triglycerides (75 mg/kg, 100 mg/kg and 150 mg/kg; p 0.01 and p<0.01), bilirubin total (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.01) and the direct bilirubin (75 mg/kg, 100 mg/kg and 150 mg/kg; p<0.01). These

parameters of the tests groups compared change to a significant degree to the group control (10% DMSO).

Discussion

In this study, the results indicated that the extract of *Sida alba* is low poisonous. During the 14 day period of acute toxicity evaluation, some signs of toxicity were observed, but they were all quickly reversible. Recent studies showed that pharmacological substances whole LD_{50} is less than 5 mg/kg body weight are classified in the range of highly toxic substances, those with a LD_{50} between 5 mg/kg body weight and 5000 mg/kg body weight are classified in the range of moderately toxic substances and those with the lethal dose is more than 5000 mg/kg body weight not toxic [17]. Consequently, if we refer to this classification we could say that the extract of *Sida alba* is moderately toxic and would be regarded as being safe or of low toxicity [18].

Concerning the sub-acute study at doses of 75, 100 and 150 mg/kg body weight during a period 28 days, we noted any change in animal behaviour or mortality. Changes in body weight and internal organ weights could be due to the adverse side effects. According to [19, 20], weight loss is a simple and sensitive index of toxicity after exposure to toxic substance and this fact was noticed by the low variation between animal weights and their internal organs compared with control group. However, the decrease in body weight observed in the rats treated with the doses of the extract at 4th week may be due to low feed intake and utilization. [21] reported severed growth depression as a consequence of reduce feed intake in rats fed high tannins containing diet. Certainly the tannins would be responsible in this fact; because, according to recent studies, tannins have been known to occur in high concentrations in the leaf extract of *Sida alba* [1].

As for the results of biochemical parameters, we notice a variation between the different doses administered but this variation is low. There is a low significant difference between the control group (10% DMSO) and the other treated assay groups ($p<0.01$). Many research works reported that some factors can be useful in differentiating a significant change from control values, from a treatment-related effect. This difference is less likely to be an effect to treatment if: there is no obvious dose response; it is due to finding in one or more animals that could be considered outlier; it is within normal biological variation. [22]. To this end, such changes do not suggest that the extract of *Sida alba* produced toxicity in the treatment period. Biochemical evaluation is important, because kidney and liver toxicity has been reported the use of phytotherapeutic products [23, 24, 25, 26]. In the present study, creatinin, urea and uric acid determinations were critical as markers of kidney function [27]. There is not much significant differences in uric acid, creatinin and urea comparatively to the control group ($p<0.01$). Among the parameters evaluated, AST, ALT and ALP are considered markers of liver function [28, 29]. There is not much differences in AST, ALT and ALP comparatively to the control group ($p<0.01$). The results revealed relationship between these enzymatic markers and liver function and this was demonstrated by the variation of liver weight. Also, high levels of glucose and uric acid in control group may be explained by the food of rats which contains proteins and some sugar. Several studies have revealed that xanthine oxidase is the enzyme responsible for the formation of uric acid from the purines hypoxanthine and xanthine and is responsible for the medical condition known as gout [30]. As a result, the decrease in uric acid during the treatment because *Sida alba* has xanthine oxidase inhibitory properties [1].

Indeed dose-dependent elevations were observed in serum enzymes in the treated groups. This indicates hepatocellular damage [31]. [32, 33] reported that the increase in the activity of these enzymes in the plasma is often seen following liver damage and it is attributed to the loss of the enzyme from damaged hepatocytes rather than increased production. The elevation in AST in all the treated groups and ALP in the highest dose group suggest that other non-specific tissue damage also occurred as these enzymes have a wider tissue distribution beyond liver [34, 35, 36].

Extract treated groups showed significantly lowered bilirubin, urea and cholesterol levels. The decrease in bilirubin concentration may be attributed to the depressant effect of the extract. [37] observed that some depressant compounds are known to decrease bilirubin concentration while decrease cholesterol concentration in treated rats may indicate hepatocellular damage or malnutrition. It might also be that the extract possesses hypocholesterolemic effect. Serum urea concentration also showed dose dependent decrease following extract administration. Urea is a production of protein metabolism that is excreted in urine and its retention in the body may indicate renal damage [38].

Conclusion

The toxicological and pharmacology effects obtained in this study seem be interesting for the therapeutic use of Sida alba. The low toxicity evidenced by LD_{50} value suggests a wide margin of safety for therapeutic doses. In sub-acute study, some effects were observed but there were no relevance of serious signs or significant changes in animal weights, effect of extract on animal organs and biochemical parameters. Briefly, these toxicity studies suggest that the extract of *Sida alba* is safe.

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