

Effect of Long-term use of *Sida rhombifolia* L. Extract on Haemato-biochemical Parameters of Experimental Animals

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Abstract: *Sida rhombifolia* L. is used as a remedy for inflammatory disorder and some other diseases in African folk medicine. The present study was conducted to evaluate the haematological and biochemical parameters and toxic effects of aqueous acetone extracts of *Sida rhombifolia* L. in mice Swiss and albinos Wistar rats. *Sida rhombifolia* L. is used as a remedy for inflammatory disorder and some other diseases in African folk medicine. In an acute toxicity study, *Sida rhombifolia* extract was administered orally at doses ranging between 1-6 g/kg to experimental mice and observed for any toxic symptoms up to 14 days. In sub-acute toxicity, *Sida rhombifolia* extract was tested at dose levels of 100, 200 and 300 mg/kg on physical (body weight and organ weight), biochemical and haematological parameters in adult albino rats. The *Sida rhombifolia* extract was well tolerated at the acute administration. No mortality was observed even at the highest dose of 6 g/kg (the value of LD₅₀ is greater than 5000 mg/kg). In the sub-acute administration, *Sida rhombifolia* extract significantly ($p < 0.05$) prevented an increase in body weight in a dose dependent manner and this reflected a significant ($p < 0.05$) increase in the relative organs (liver and heart) weights. The biochemical parameters showed a significant ($p < 0.05$) increase in ALT, AST and ALP. However for the other biochemical parameters (glucose, creatinine, urea nitrogen, triglycerides, total bilirubin and direct bilirubin), there is a significant decrease between the control group (10% DMSO) and the other treated groups ($p < 0.05$ and $p < 0.01$). The insipient toxicological effect is more significant in the biochemical rather than in the haematopoietic system of rats. There is a significant decrease for monocytes, basophils, haemoglobin, haematocrit and MCV between the control group (10% DMSO) and the treated groups ($p < 0.05$). At last, one could say that the reported pharmacological and therapeutic effectiveness of *Sida rhombifolia* extract is not without toxicity implication.

Keywords: Biological parameters, LD₅₀, mice, *Sida rhombifolia* L., toxicity, wistar rats

INTRODUCTION

Plant derived products have been used for medicinal purposes since the creation of man, medicinal herbs are an integral part of alternative therapy (Karim *et al.*, 2011). Moreover, plants provide substitution for drugs in some rural areas. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties. There are many herbs have been used for a long time for claimed health benefits (Kayode and Kayode, 2011). Approximately about 80% of the world population today, relies on botanical preparations as medicines to meet their health needs (Polasa and Nirmala, 2008). The problem with the botanical preparations is that most of the plants are

been used indiscriminately without adequate information on associated safety/toxicity risks. Thus for proper knowledge and guidance of these natural products, there is need for scientific documentation on the safety/toxicity profile on these acclaimed medicinal plants (Deng, 1994).

Sida rhombifolia L. (Malvaceae) is a tropical herbaceous found in the West and Central Africa forest. Its widely distributed in various regions of Burkina Faso and other Afro-Asian countries and used in local traditional medicine by people in rural areas as a remedy for skin infections and gastrointestinal disturbances and as an insecticide. Ethnobotanical investigations in the central region of Burkina Faso have shown that this Malvaceae 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 (Nacoulma, 1996). *Sida rhombifolia* L., possess pharmacological properties such as anti-malaria properties, antibacterial, anti-viral activities, hepatoprotective, anti-inflammatory and analgesic properties (Nacoulma, 1996; Konaté *et al.*, 2012). Phytochemical analysis of the aerial parts of *Sida rhombifolia* L., showed the presence of flavonoids, tannins, sterols, triterpenes and volatile oils (Nacoulma, 1996).

The absence of information on the toxicity profile of the aqueous extract of this Malvaceae necessitated the present investigation of its acute and sub-acute toxicity in animal model. Therefore, this study was undertaken to investigate the effects of long-term use of *Sida rhombifolia* on haematological in blood and hepatic related biochemical parameters in blood serum of rats.

MATERIALS AND METHODS

Materials:

Identification of the plant materials: *Sida rhombifolia* L. was collected in August 2008 in Gampela, 25 Km east of Ouagadougou, capital of Burkina Faso. The plant was identified in the Laboratory of Biology and Ecology, University of Ouagadougou, where a voucher specimen was deposited.

Animals handling: Swiss NMRI mice (25-30 g) and adult albinos Wistar rats (160-180 g) of both sexes were used for this study. All animals were housed in cages under controlled conditions of 12-h light/and 12 h without light and 25°C. They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. Experiments on the animals were performed according to the protocols already approved by the Institute of Health Sciences Research/University of Ouagadougou (Burkina Faso) and met the international standards for animal study (Konaté *et al.*, 2012).

Methods:

Preparation of extracts: Fifty grams of powdered plant materials (dried in laboratory condition) was extracted with 500 mL of acetone 80% (400 mL acetone +100 mL water) 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, Rotavapor R-200, Switzerland) at approximately 40°C and freeze-dried (Telstar Cryodos

50 freeze-dryer). The extracts were weighed before packing in waterproof plastic flasks and stored at 4°C until use. The yields of the extractions were measured with precision balance (Adventurer).

Toxicity studies:

Acute toxicity: Swiss mice (male and female) were randomly divided into 7 groups (1 control group and 6 treated groups) of 6 animals (3 males and 3 females). The control group received water containing 10% dimethylsulfoxide (DMSO) administered intraperitoneally. The aqueous acetone extract of *Sida rhombifolia* L., suspended in 10% DMSO was administered intraperitoneally at doses of 1, 2, 2.5, 3, 4, 5 and 6 g/kg, respectively. The general behavior of the mice was observed for 120 min after the treatment. The animals were observed for morbidity and mortality once a day for 14 days. The number of survivors after the 14 days period was noted. The toxicological effect was assessed on the basis of mortality for 14 days, which was expressed by the median lethal dose value (Lethal Dose 50 or LD₅₀) estimated from the regression of log-probit mortality rate (Miller *et al.*, 1944).

Subchronic toxicity study: Wistar rats were divided into 5 groups of 6 animals (3 males and 3 females) for each type of extract. The first groups served as control, and they received water containing DMSO 10%. The second, the third and the fourth group of rats received daily and orally (gavage) for 28 days respectively 100, 200 and 300 mg/kg, respectively of each of two type of extract (suspended in 10% of DMSO). Body weight was weekly taken, and the animals were daily observed to detect any signs of abnormalities throughout the study period. At the end of the 28 days period, the animals were deprived of food for 15 h. Then blood samples were collected by cardiac puncture for biochemical and hematological tests, and selected organs were carefully removed and weighed.

Collection of blood samples: Blood samples were collected by cardiac puncture in three tubes for haematology, glucose and serum analysis. The blood samples with heparin and those without anticoagulant were centrifuged at 3000 rpm for 5 min to obtain plasma and serum respectively. Plasma was used to determine glucose (Trinder, 1985; Burn and Price, 1985) and the serum for other biochemical parameters such as Aspartate aminotransferase (AST) (Schumann *et al.*, 2002a), Alanine aminotransferase (ALT) (Schumann *et al.*, 2002b), Alkaline Phosphatase (ALP) (German Society for Clinical Chemistry, 1972; Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974), total bilirubin and direct bilirubin (Sherwin and Thompson, 2003), creatinine (Fossati and Prencipe,

Table 1: Animal weights (g) with time of treatment

Groups	First day	First week	Second week	Third week	Fourth week
Group 1	174.33±1.20	180.63±2.60	189.27±1.40	200.31±3.19	210.33±3.67
Group 2	174.12±3.12 ^{ns}	175.17±1.02*	182.17±1.03*	200.13±0.10 ^{ns}	203.13±2.20*
Group 3	174.33±1.60 ^{ns}	177.00±0.23*	179.01±2.23*	201.37±1.20 ^{ns}	202.17±1.01*
Group 4	175.37±2.73 ^{ns}	172.67±1.53*	181.67±1.51*	204.63±3.53*	207.23±1.67*

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 (100 mg/kg body weight)

Group 3: Rats received 10% DMSO with extract (200 mg/kg body weight)

Group 4: Rats received 10% DMSO with extract (300 mg/kg body weight)

Table 2: Effects of aqueous acetone extract of *Sida rhombifolia* L. on the weights (g) of organs of rats

Groups	Left kidney	Right kidney	Stomach	Lungs	Liver	Heart
Group 1	0.72±1.01	0.70±0.01	2.26±0.05	1.51±1.20	5.64±2.20	0.68±0.02
Group 2	0.70±0.03 ^{ns}	0.68±0.03 ^{ns}	2.17±1.33 ^{ns}	1.37±0.01 ^{ns}	5.36±1.01 ^{ns}	0.63±1.01 ^{ns}
Group 3	0.67±1.06 ^{ns}	0.69±0.08 ^{ns}	2.23±2.20 ^{ns}	1.41±1.17 ^{ns}	5.30±1.67 ^{ns}	0.60±0.01 ^{ns}
Group 4	0.66±0.02 ^{ns}	0.67±0.02 ^{ns}	2.18±0.13 ^{ns}	1.39±1.53 ^{ns}	4.53±1.01*	0.55±0.06*

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

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Group 4: Rats received 10% DMSO with extract (300 mg/kg body weight)

1982), uric acid (Fossati and Prencipe, 1982), Blood Urea Nitrogen (BUN) according (Fawcett and Scott, 1960) and triglycerides (Fossati and Prencipe, 1982), total cholesterol (Allain *et al.*, 1974). All these biochemical parameters were measured with a laboratory automat (Selectra XL Vital Scientific, Elitech Group Company).

Hematological analyses were performed on the whole blood using an automatic counter (Mindray Auto hematology Analyser BC-5500) to evaluate the following parameters: total Red Blood Cells (RBC), hemoglobin, hematocrit, platelet count, leukocytes (WBC), neutrophils, basophils, eosinophils, lymphocytes, monocytes, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration).

Statistical analyses: The data were expressed as Mean±Standard Deviation (SD) of six determinations (n = 6). Results were analysed by one-way ANOVA followed by Dunnett's t-test using Prism 4 software. The level of significance was accepted at p≤0.05.

RESULTS

Acute toxicity study of the plant extracts: For the acute toxicity study in mice, the value of LD₅₀ is greater than 5000 mg/kg. No significant difference in body weight gain of the treated assay groups over the period of observation.

Subchronic toxicity study in rats:

Body weights: No significant difference in body-weight gain between control group and the test groups was observed in the first days of treatment (p>0.05).

However, with four weeks, a significant decrease in body weight was noticed between the test groups and the control groups (p<0.05). The results are summarized in Table 1.

Relative organ weights: Table 2 shows the effects of extracts on weight of some vital body organs of rats. No significant difference for kidneys, stomach and lungs (p>0.05); but we noticed a significant decrease about the liver weight and heart between the treated groups and the control group (p<0.05).

Haematological analysis: The effects of extracts on the haematological parameters are summarized in Table 3. There is a significant decrease for monocytes, basophils, haemoglobin, haematocrit and MCV between the control group (10% DMSO) and the treated groups (p<0.05).

Biochemical analyses: Table 4 shows the effects of extracts on the biochemical parameters. For certain biochemical parameters (AST, ALT, ALP) one observed a significant increase (p<0.05); however for the other biochemical parameters (glucose, creatinine, urea nitrogen, triglycerides, total bilirubin and direct bilirubin), there is a significant decrease between the control group (10% DMSO) and the other treated groups (p<0.05 and p<0.01).

DISCUSSION

Acute oral administration of the aqueous extract of *Sida rhombifolia* produced no mortality in experimental rats. In effect, in acute toxicity testing doses higher than 5 g/kg body weight are generally not considered as dose

Table 3: Effect of aqueous acetone extract of *Sida rhombifolia* L. on haematological parameters on whole blood of rats

Haematological parameters	Group 1	Group 2	Group 3	Group 4
WBC ($10^3/\mu\text{L}$)	13.36±1.12	13.42±1.10 ^{ns}	13.41±2.02 ^{ns}	13.42±0.01 ^{ns}
RBC ($10^6/\mu\text{L}$)	7.31±1.01	7.33±0.02 ^{ns}	7.36±1.10 ^{ns}	7.38±1.13 ^{ns}
Eosinophil (%)	2.23±1.67	2.27±1.20 ^{ns}	2.18±0.21 ^{ns}	2.20±1.53 ^{ns}
Lymphocyte (%)	79.05±0.02	78.75±1.53 ^{ns}	78.52±1.03 ^{ns}	78.67±0.20 ^{ns}
Neutrophil (%)	26.52±0.01	26.63±1.33 ^{ns}	26.62±1.53 ^{ns}	26.78±1.20 ^{ns}
Monocyte (%)	1.23±0.20	1.12±1.10*	1.02±1.33*	1.18±1.20 ^{ns}
Basophil (%)	0.12±1.03	0.12±0.01 ^{ns}	0.12±1.33 ^{ns}	0.12±1.20 ^{ns}
Haemoglobin (g/dL)	14.73±1.10	14.54±1.15 ^{ns}	13.97±1.63 ^{ns}	14.01±2.53 ^{ns}
Haematocrit (%)	43.30±1.20	43.05±1.01 ^{ns}	42.84±2.23 ^{ns}	41.93±1.01*
MCV (μm^3)	58.15±1.10	57.86±1.20 ^{ns}	57.87±1.10 ^{ns}	56.05±1.33*
MCH (pg)	18.56±1.10	18.64±0.43 ^{ns}	18.81±0.22 ^{ns}	18.80±1.02 ^{ns}
MCHC (g/dL)	33.62±1.10	33.43±2.20 ^{ns}	33.32±1.20 ^{ns}	33.60±0.05 ^{ns}
Platelet ($\times 10^3/\mu\text{L}$)	981.05±2.10	973.15±4.10 ^{ns}	964.02±1.33 ^{ns}	964.33±1.53 ^{ns}

WBC: Leucocyte count; RBC: Erythrocyte count; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; 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 (100 mg/kg body weight)

Group 3: Rats received 10% DMSO with extract (200 mg/kg body weight)

Group 4: Rats received 10% DMSO with extract (300 mg/kg body weight)

Table 4: Effect of aqueous acetone extract of *Sida rhombifolia* L. on biochemical parameters in the plasma and the serum of rats

Biochemical parameters	Group 1	Group 2	Group 3	Group 4
Glucose (mmol/L)	6.37±1.01	4.62±1.33*	3.28±1.53**	3.45±0.01**
Uric acid (mmol/L)	0.22±1.10	0.17±0.01 ^{ns}	0.14±0.01 ^{ns}	0.15±1.53 ^{ns}
Urea nitrogen (mmol/L)	10.01±0.10	9.82±0.02 ^{ns}	9.66±0.03 ^{ns}	9.52±0.01 ^{ns}
Creatinine (mmol/L)	0.056±1.01	0.054±0.02 ^{ns}	0.052±1.01 ^{ns}	0.051±0.02 ^{ns}
AST (UI/L)	70.33±1.10	73.60±1.53*	78.01±0.02*	70.02±1.10 ^{ns}
ALT (UI/L)	43.05±2.21	49.01±1.53*	45.00±1.33*	43.67±0.01 ^{ns}
ALP (UI/L)	73.00±1.18	76.10±2.20*	79.01±0.01*	70.00±1.67*
Triglycerides (mmol/L)	0.80±1.13	0.78±2.02 ^{ns}	0.79±1.53 ^{ns}	0.80±2.10 ^{ns}
Total cholesterol (mmol/L)	2.13±1.02	2.08±0.03 ^{ns}	2.07±1.10 ^{ns}	2.09±1.33 ^{ns}
Total bilirubin (mmol/L)	0.18±0.02	0.16±0.01 ^{ns}	0.14±2.01 ^{ns}	0.16±0.01 ^{ns}
Direct bilirubin (mmol/L)	0.0018±0.01	0.0016±1.33 ^{ns}	0.0017±2.01 ^{ns}	0.0016±1.10 ^{ns}

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

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relative toxicity (Hayes, 1987). Thus the aqueous acetone extract of *Sida rhombifolia* was well tolerated and is relatively not toxic at acute administration. *Sida rhombifolia* can be declared non toxic at acute administration.

Change in body weight is used as an assessment to the response of an individual to therapeutic drugs (Winder *et al.*, 1969) and as an indication of the adverse effect of a drug (Teo *et al.*, 2002; El-Sanusi and El-Adam, 2007). In the present study *Sida rhombifolia* L. had a decreasing effect on the body weight in a dose depend manner which may be associated to the general discomfort which may have led to a low feeding rate in the treated rats as earlier suggested (Adeneye and Agbaje, 2008) or it could be that *Sida rhombifolia* interferes with the lipid metabolism of experimental animals.

The relative organ (liver and heart) decrease lowly in weight gain. This was expected since the relative weight of the organs was based on the weight of experimental animals. That is the weight of organ

divided by decreasing animal weight. Thus a decrease relationship does exist between the organ weight and animal weight.

Various medicinal herbs or conventional drugs/chemicals adversely affect certain blood components (King and Kelton, 1984). Haemolytic anaemia and thrombocytopenia is known to be induced by flavonoids from herbs (Gandolfo *et al.*, 1992). However, *Sida rhombifolia* extract also contains polyphenolics glucosides which did not reveal any significant impact on the haematopoietic system. The effects on haematological indices were not significant. This may suggest no adverse effects on bone marrow, a source of reticulocytes. Though, initially an insignificant increase in blood platelet counts were observed, however, this effect was normalized at 28th day of treatment. *Sida rhombifolia* treatment increased WBC counts and slight decrease of lymphocyte and granulocyte counts all of no statistical significance. This observation suggests that the elevation of WBCs caused by *Sida rhombifolia* was compensated

for by the decreased bone marrow production of granulocytes, the precursors of WBCs. Thus, the report of WBC counts could be evidence of the balance between the rate granulocyte production and that of WBC destruction as a result of direct actions of *Sida rhombifolia*.

Creatinine and urea are two serum metabolites that are indicative of the renal function. Though these metabolites are the end products of protein metabolism, their concentrations remain fairly constant under normal conditions unless renal function changes (Whitby *et al.*, 1987). In the present study, the decrease of creatinine and unalteration of urea nitrogen is an indication that *Sida rhombifolia* interferes with the protein oxidation and may affect the functioning of the kidney at high dosage or concentration. Sub-acute administration of *Sida rhombifolia* did not significantly affect the plasma level of the cholesterol and triglycerides. This indicates that the extract did not present a risk of hypercholesterolemia and hypertriglyceridemia. However, the decreasing effect at dose level of is an indication of the plant extract as a hypolipidemic agent and may be interesting in weight loss research. The ability of *Sida rhombifolia* to significantly reduce plasma glucose concentration of normal albinos rats is indicative of a possible hypoglycemic activity.

The liver is one of the most important organs in the body and it is responsible for metabolism and detoxification of all toxins that enter the body. Liver diseases resulting from liver damage is a global problem. Liver function test conducted through blood assays give information about the state of the liver and cellular integrity. Chemical and drugs are known to induced lipid peroxidation, cause the swelling and necrosis of liver cells, resulting to the release of cytosolic enzymes such as ALT, AST, ALP (Agbor *et al.*, 2005). Thus, ALT and AST increases in plasma are an indication of liver damage. ALT is the most sensitive marker of the liver and this enzyme leaks out into the blood stream when there is a liver damage. Toxicants (stanozolol) and drugs (antiretrovirals, Paracetamol) have been reported to increase plasma level of ALT and AST as a measure of hepatotoxicity (Mosallanejad *et al.*, 2011; Basu *et al.*, 2009). An increase in the activity of ALT and AST was observed in this study at high dose administration of the extract compared to the control group.

Some herbal medicines have hepatotoxic and nephrotoxic effects (Lin *et al.*, 2003). Damage to these organs often results in elevation in clinical chemistry parameters (Stonard and Evans, 1995) such as serum enzymes like AST and ALT and analytes like total and conjugated bilirubin, BUN and creatinine (Akdogan *et al.*, 2003). ALP levels may indicate that *Sida rhombifolia* caused a low damage to cardiac or skeletal muscle and impacted on hepatic excretory function

(Stonard and Evans, 1995; Gaw *et al.*, 1998). A typical myocardial infarction gives an AST/ALT ratio greater than 1; however, AST/ALT ratios of less than 1 are found due to the release of ALT from the affected liver (Hawcroft, 1987). Since, the result gave an AST/ALT ratio to be less than 1, the extract is less likely to lead to myocardial infarction if large doses are taken over a long period of time. Moreover, the standard range for plasma ALT levels for rats is 21-52 UI/L (Coimbra *et al.*, 1995), present results provide evidence of no hepatic overload. So, oral administrations of *Sida rhombifolia* at sub-acute dosing do exhibit lowly significant toxicological effects in rats. The insipient toxicological effect is more significant in the biochemical rather than in the haematopoietic system of rats. The reported pharmacological and therapeutic effectiveness of *Sida rhombifolia* extract is not without toxicity implication.

CONCLUSION

The toxicological and biological effects obtained in this study seem be interesting for the therapeutic use of *Sida rhombifolia* L. LD₅₀ value suggests this Malvaceae is not toxic. The extract of *Sida rhombifolia* L. may not be toxic at low dosage in sub-acute administration. *Sida rhombifolia* L. reduces plasma glucose and lipid concentrations; it may find application as antidiabetic and hypolipidemic agent. However, further research is needed to substantiate this claim.

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