

Hepatoprotective Effect of Aqueous Acetone Extract of *Sida alba* L. (Malvaceae) Against Alcohol Induced Liver Damage in albinos Wistar Rats

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Abstract: The present study was conducted to evaluate the hepatoprotective effects of aqueous acetone extract of *Sida alba*. (Malvaceae) an herbal plant used in Burkina Faso to treat traditionally hepatic affections in albinos Wistar rats. Animals were treated by gavage during 28 days with different doses of aqueous acetone extracts of *Sida alba*. (75, 100, 150 mg/kg) suspended in 35% ethanol. Control groups received alcohol 35% and water. In vivo administration of 35% ethanol for 28 days results an activity of liver marker enzymes (AST, ALT, ALP), glucose, triglycerides, total cholesterol, total bilirubin and direct bilirubin in serum as compared with rats which received water (control water). However, administrations of 35% ethanol along with aqueous acetone extract decreased the activities of liver markers enzyme in serum comparatively to the control water group ($p<0.05$ or $p<0.01$). We noticed that extract at a dose of 150 mg/kg was highly effective than 75 and 100 mg/kg body weight compared to the control water group (ALT and ALP; $p>0.05$ and $p<0.05$). This study revealed that *Sida alba* presents a hepatoprotective potential and this plant could be traditionally exploited in the treatment of affection hepatic.

Key words: Albinos Wistar rats, biochemical parameters, hepatoprotective potential, *Sida alba*

INTRODUCTION

Liver is a key organ of the body playing major role in maintaining homeostasis. It is involved in almost all the metabolic pathways of the body related to growth, immunity, energy supply and reproduction. Liver is one of the most important organs in the biotransformation of food, drugs, endogenous and exogenous substances. It also plays a significant role in detoxification of variety of exogenous materials. Therefore, the maintenance of a healthy liver is vital to overall health and well being. Unfortunately, liver is exposed to variety of toxins in our day-to-day life leading to severe liver injuries. The hepatic carcinoma, jaundice and hepatitis are the major liver disorders that account for a high death rate. In spite

of tremendous scientific achievements in the field of hepatology in recent years, there is not a single drug that stimulates liver functions, offer protection to the liver from damage or help regeneration of hepatic cells (Chaterjee, 2000) and (Sherlock and Dooley, 2002). Modern medicinal agents used in liver disorders are also associated with some severe side effects. Plant extracts have been used by traditional medical practitioners for the treatment of liver disorders for centuries (Schuppan *et al.*, 1999).

In add, 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 drugs available for the treatment of liver disorders (Karan *et al.*, 1999;

Chaterjee, 2000). Therefore, many folk remedies from plant origin evaluated for its possible hepatoprotective effects against different chemical-induced liver damage in experimental animals. Alcohol-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts. The changes associated with alcohol-induced liver damage are similar to that of acute viral hepatitis (Passmore and Robinson, 1973).

Sida alba L. has great reputation, as miracle medicinal plant reported in various texts of indigenous system of medicine in Burkina Faso. This Malvaceae is found in several parts of the country is very widely used in traditional medicine in Burkina Faso. The aqueous extracts were used in hepatitis B virus cure in the central region of Burkina Faso and various kinds of diseases such as malaria, hepatic disorders and alcohol properties. Phytochemical analysis of this plant demonstrated the presence of saponosides, coumarins, steroids, polyphenols and alkaloids (Nacoulma, 1996). But still no scientific and methodical investigation has so far been reported in literature regarding its action on liver. Therefore, the present investigation has been designed to study the possible mechanism of aqueous acetone extract of *Sida alba* L., on the biochemical parameters against alcohol induced hepatic damage in rats.

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 plants were 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.

Animals: We used male and female adult albinos Wistar rats (260-265 g) 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 and water *ad libitum*. The animals were feed with pellets of the cattle food of Libreville (Gabon), containing 20% of proteins and water.

Experimental design for hepatoprotection potential: The rats were divided randomly into five groups of six rats each. The first and second groups served as control, received water and 35% ethanol. The remaining groups (group 3; group 4; group 5) received three dose levels of the *Sida alba* extracts (75, 100 and 150 mg/kg) suspended in 35% of ethanol, 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.

Assessment of hepatoprotective potential: 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 30000 rpm for 5 min to obtain plasma or serum. Plasma was used to determine glucose (Burn and Price, 1985; Trinder, 1969) and the serum for other biochemical parameters such as aspartate aminotransferase (AST) determined according (Schumann *et al.*, 2002a) and alamine aminotransferase (ALT) determined according (Schumann *et al.*, 2002b), alkaline phosphatase (ALP) estimated by (Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974), German Society for Clinical Chemistry, 1972) total bilirubin and direct bilirubin determined according (Sherwin and Thompson, 2003), triglycerides (Fossati and Prencipe, 1982), total cholesterol (Allain *et al.*, 1974). All these biochemical parameters were measured by Selectra XL Vital Scientific (Elitech Group Company).

Organ and animals 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

Body weight: Indeed, one does not notice a significant difference between the weights of the animals of the group controls and the group test ($p > 0.05$) the first day of the treatment. However, one notes an increase in the weight of the animals according to the processing time expressed in week. However, during the four weeks, one notices a significant difference between the weight of the animals

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

Groups	1st day	1st week	2nd week	3rd week	4th week
Group 1	264.5±0.55	275.5±0.55	290.5±0.55	299.00±1.10	304.00±1.10
Group 2	264.00±2.19	254.00±1.10	259.5±1.64	266.5±1.64	267.33±5.13
Group 3	264.5±0.55 ^{ns}	243±2.7**++	261.5±1.64**	268.5±8.22**	267.00±1.10**
Group 4	265.5±0.55 ^{ns}	262±1.10**++	271±1.10**++	279±1.10**++	278.5±1.64**++
Group 5	265.00±1.10 ^{ns}	265±0.55**++	287.5±2.7++	290.5±0.55**++	289.0±1.10**++

Values are mean±S.E.M. (n=6) one-way ANOVA followed by Dunnett's t-test: Compare all vs. control; *: p<0.05; **: p<0.01 compared with control water; +: p<0.05 with control alcohol

Group 1: control water, rats received water

Group 2: control alcohol, rats received 35% ethanol

Group 3: rats received 35% ethanol with extract (75 mg/kg body weight)

Group 4: rats received 35% ethanol with extract (100 mg/kg body weight)

Group 5: rats received 35% ethanol with extract (150 mg/kg body weight)

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

Groups	Left kidney	Right kidney	stomach	Lungs	Liver	Heart
Group 1	0.96±0.09	0.95±0.0	5.003±0.05	1.87±0.068	10.96±0.00	1.31±0.00
Group 2	0.76±0.05	0.73±0.01	4.99±0.007	1.867±0.007	7.278±0.00	1.25±0.06
Group 3	0.73±0.00**	0.74±0.00**	4.97±0.00 ^{ns}	1.848±0.14 ^{ns}	8.98±0.01**++	1±0.03**++
Group 4	0.77±0.00**++	0.79±0.00**++	4.92±0.07 ^{ns}	1.846±0.12 ^{ns}	8.69±0.00**++	1±0.07**++
Group 5	0.86±0.00**+	0.82±0.00**+	4.97±0.04 ^{ns}	1.860±0.01 ^{ns}	8.19±0.00**++	1±0.01**++

Values are mean±S.E.M. (n = 6) one-way ANOVA followed by Dunnett's t- test: Compare all vs. control; *: p<0.05, **: p<0.01 compared with control water; +: p<0.05 with control alcohol

Group 1: control water, rats received water

Group 2: control alcohol, rats received 35% ethanol

Group 3: rats received 35% ethanol with extract (75 mg/kg body weight)

Group 4: rats received 35% ethanol with extract (100 mg/kg body weight)

Group 5: rats received 35% ethanol with extract (150 mg/kg body weight)

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

Weights/biochemical parameters	Group 1	Group 2	Group 3	Group 4	Group 5
AST (UI/L)	101.5±1.64	134.0±1.38	123±2.19**++	119.5±0.55**++	109±2.1**++
ALT (UI/L)	48.00±2.19	63.5±0.02	54.00±1.1**++	51.2±3.29**++	50.5±2.74**++
ALP (ALP/L)	102.25±0.82	127.5±0.55	123.5±1.64**	120.88±0.14*	105.84±6.39++
GGT (UI/L)	Nd	Nd	Nd	Nd	Nd
Triglyceride (mmol/L)	2.21±0.11	2.29±0.01	1.41±0.10**++	1.16±0.05**++	1.12±0.13**++
Total cholesterol (mmol/L)	3.10±0.10	2.51±0.04	1.52±0.01**++	1.8±0.22**++	2.25±0.21**++
Total bilirubin (mmol/L)	0.28±0.00	0.32±0.00	0.32±0.00**	0.184±0.02**++	0.133±0.03++
Direct bilirubin (mmol/L)	0.08±0.00	0.066±0.00	0.022±0.00**	0.014±0.00**++	0.013±0.00**++
Glucose (mmol/L)	8.26±0.00	9.77±0.01	10.10±0.11**+	9.95±0.00**++	8.14±0.18**++

Nd: Not determined; Values are mean ± S.E.M. (n=6) one-way ANOVA followed by Dunnett's t- test: Compare all vs. control; *: p<0.05; **: p<0.01 compared with control Water; +: p<0.05, ++: p<0.01 compared with control alcohol

Group 1: control water, rats received water

Group 2: control alcohol, rats received 35% ethanol

Group 3: rats received 35% ethanol with extract (75 mg/kg body weight)

Group 4: rats received 35% ethanol with extract (100 mg/kg body weight)

Group 5: rats received 35% ethanol with extract (150 mg/kg body weight)

of the group controls and the group test (p<0.01). The results are consigned through Table 1 (animals treated with the extract hydroacetonic of *Sida alba* L.).

Organ weights: Table 2 shows the effects of the extract hydroacetonic of *Sida alba* on the weights of the vital bodies of the rats. It is noted that the effect of the extracts to the concentrations 75 and 100 mg/kg entrained a significant decrease of the weights of the liver (p<0.05), kidneys (p<0.01 and p<0.05) and heart (p<0.01) compared to the group controls (DMSO 10%). However, there is no significant difference between the effect of the extracts to the various concentrations on the variation of the weights of the other bodies and the group controls (p>0.01).

Biochemical analyses: Table 3 shows the effects of the extracts hydroacetonic of *Sida alba* on the parameters of the rats. Respectively, it is noted that: Glucose (75, 100 and 150 mg/kg; p<0.01; p<0.01 and p<0.01), acid uric (75, 100 and 150 mg/kg; p<0.01; p<0.01 and p<0.01), the urea nitrogen (75, 100 and 150 mg/kg; p<0.01; p<0.01 and p<0.01), creatinin (75, 100; p<0.01; p<0.01 and 150 mg/kg; p>0.01), AST (75, 100 and 150 mg/kg; p<0.05; p<0.01 and p<0.01), ALT (75, 100 and 150 mg/kg; p>0.01; p<0.05 and p<0.01), ALP (75, 100 and 150 mg/kg; p>0.01 and p<0.01), triglycerides (75, 100 and 150 mg/kg; p>0.01 and p<0.01), bilirubine total (75, 100 and 150 mg/kg; p<0.01) and the direct bilirubin (75, 100 and 150 mg/kg; p<0.01). These parameters of the tests groups compared change to a significant degree to the

group controls (10% DMSO). For the other biochemical parameters however, there is no significant difference between the control group (10% DMSO) and the other treated assay groups ($p>0.05$).

DISCUSSION

The diseases of the liver are some of the fatal diseases in the world of today. They pose a serious challenge for the international public health. The drugs available in the modern system of medicine are corticoids and which ensure a symptomatic relief without treatment and their use is associated the risk of relapses and the side effects dangerous. Miss therapeutic agents of modern medicine and use of a vast range of plants to the investigation justify the traditional practices of these plants by using modern techniques to validate the allegations hepatoprotective and in the search of new molecules to use them against hepatic toxicity. A review of the literature shows that the plants will prove to be the most fertile ground to find drugs useful of liver (Handa *et al.*, 1986).

Concerning the effect of the extracts on the body weight of the rats, the results consigned in (Table 1) make us say that there is a bond between the effect of the extracts, the body mass of the rats and the duration of the treatment. Statistical studies revealed that there is a weak significant difference of the profit of body weight between the groups of control and the groups of proportioning treated during the four weeks ($p<0.05$ or $p<0.01$).The results also underline an increase in the weight of the animal according to the period of treatment. However, in the fourth week, there was a significant difference of the profit of body weight between the tests groups and the group of alcohol control ($p<0.01$).In this fact, we noted a reduction in the weight of the animals.

Speaking the effects of the extract hydroacetonic of *Sida alba* on the weights of the vital bodies of the rats, Table 2 shows that the effect of the extracts to the concentrations 75, 100 and 150 mg/kg entrained a significant decrease of the weights of the liver ($p<0.05$), kidneys ($p<0.01$ and $p<0.05$) and heart ($p<0.01$) compared to the group controls (DMSO 10%). However, there is no significant difference between the effect of the extracts to the various concentrations on the variation of the weights of the other bodies and the group controls ($p>0.01$).

Indeed, certain studies reported that the weight is a simple and significant index of toxicity after exposure to toxic substances (Teo *et al.*, 2002).There is a close connection between the exposure of alcohol and the

development of the oxydative stress. Alcohol increases not only the generation of ROS, but exhausts also antioxydants, therefore, the creation of a state of oxydative stress (Tuma and Casey, 2003). Also, most of the direct cellular lesion which occurs during the alcohol consumption seems to be caused by the accumulation of acetaldehyde, a toxic by-product of metabolism of alcohol (Tuma and Casey, 2003). Consequently, the biochemical implications of the toxic effects of acetaldehyde and the ROS on the liver are, partly, the signs of affection of the liver. The reduction in the weight in the rat is the direct result of the signs of affection of the liver caused by alcohol.

However, compared to the reference group (water), this weight decrease in the rat is weak (150 mg/kg). In this fact, one could say that *Sida alba* extracted (150 mg/kg) has a potential hepatoprotector even if it is weak. Our results consigned in (Table 2) show this established fact.

Concerning the biochemical parameters, (Table 3) shows the effects of the extracts of *Sida alba* on the biochemical parameters. The AST and ALT are reliable markers of the hepatic function. It is established that the AST can be found in the liver, the cardiac muscle, the skeletal muscle, the kidney, the brain, the pancreas, the lungs, the leucocytes and the erythrocytes where that ALT presence in liver (Rej *et al.*, 1978). The increased levels of serum enzymes like AST and ALT indicate the increased permeability and of the damage and/or one necroses hepatocytes (Goldberg and Watts, 1965).The enzymes related to the membrane of fabrics like ALP and the GGT are released in blood circulation by the pathological phenomenon (Sillanaukee, 1996).

In our study, we noted that the ethanol consumption caused a significant increase in the activities of the AST, ALT, ALP, which could be extremely prejudicial with the membrane of fabrics. But the reduction in these enzymes thus indicates the potential of extract hydroacetonic of *Sida alba* (150 mg/kg).These results could be explained by the presence of biologically active antioxydants in the extract hydroacetonic of *Sida alba* (Nacoulma, 1996). What could be responsible for the reduction of the oxydative stress during toxicity of ethanol. Active components such as flavonols, the oligomers of flavonols and proanthrocyanidins were found in the extract hydroacetonic of *Sida alba* and which is powerful antioxydants (Nacoulma, 1996). The phytochimic analysis carried out on the extract *Sida alba* shows that it is rich in compounds polyphenolic (Nacoulma, 1996).Thus, the restoration of the antioxydant system of defense in this study can be ascribable partly to the antioxydant action of

the compounds polyphenolic (Regi and Kuttan, 2002). One could say that the polyphenols could be probably responsible for the potential hepatoprotector of *Sida alba*.

Moreover, our results revealed a direct correlation between the weight of the liver which decreased significantly compared to the group controls (water and the ethanol 35%), the markers of the hepatic function (AST, ALT), ALP, triglycerides glucose, total cholesterol, total bilirubine and the direct bilirubine ($p<0.01$ or $p<0.05$) of the tests groups compared to the reference groups (water and alcohol 35%). These reports/ratios could be explained on the clinical level. It is well-known that, medically an increase in the cholesterol level also to cause increase in triglyceride, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT is a sensitive indicator of the acute damage of the liver. ALP, but is not a specific enzyme of the liver and liver. Also the levels of increase in cholestase, of the high levels of GGT seem to be indicating of an affection of the liver, bile ducts and pancreas. The increase in the levels of bilirubin in blood thus indicates a hepatic attack. The increase in glucose could also be due to a hepatic attack.

In short one compared notes an increase in the biochemical parameters of the tests groups to the group controls (alcohol). But the reduction in the activities of these same parameters indicates the potential hepatoprotective of the hydroacetonate extract of *Sida alba* (150 mg/kg).

CONCLUSION

At the end of this part of our study it appears that the extract hydroacetonate of *Sida alba* has a potential hepatoprotective even if there is not of relation obvious amount-answer. By doing this, one could then use the extract of *Sida alba* in the care of the hepatic affections of which hepatitis B.

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