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RESEARCH PAPER

HEPATOPROTECTIVE ACTIVITY OF N-HEXANE AND ETHYL ACETATE FRACTIONS OF *SIDA ACUTA* ON THIOACETAMIDE INDUCED LIVER INJURY IN RATS

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ABSTRACT

This study investigated the hepatoprotective effects of n-hexane and ethylacetate fractions of *Sida acuta* against thioacetamide (TAA) induced hepatotoxicity in rats. Thirty-five adult male albino wistar rats, grouped into seven groups (n=5 each) were used. Groups A-D served as the tests, while E-G served as control. The rats were fed as discussed in the methodology. Phyto-chemical analysis of *Sida acuta* was done using standard methods. Acute toxicity test revealed an oral LD50 of >3000 mg/kg. Test administration was oral for 7 days prior to TAA intoxication. On the 8th day, after an 18-hour fast, TAA (200mg/kg) was injected subcutaneously to all the groups except group G (normal control). Blood samples were collected 24hrs after TAA administration to assess the liver function of the rats (ALT, AST, ALP and Albumin levels). Results revealed a significant increase in the serum levels of ALP, AST and ALT, as well as a significant decrease in the serum Albumin levels due to TAA induced liver damage. Pre-treatment with both n-hexane and ethyl acetate fractions of *Sida acuta* reduced the toxic effect of TAA as indicated by a decrease in the levels of previously elevated liver parameters and an increase in the level of Albumin.

Keys words: Hepatoprotective, n-Hexane, Ethyl acetate, Sida acuta, Thioacetamide, liver function

INTRODUCTION

Ethno-medical studies are today recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for their potentials based on their bioactive constituents (Farnsworth, 1996). Traditional herbal medicines contain naturally occurring plant substances with minimal or no industrial processing but are used in treating illnesses (Zaslowski, 2005). In recent times, traditional herbal medicine has gained optimum attention in global health debates (Chong, 2006) and *Sida acuta* have been recognized amongst them. The plant *Sida acuta* (Malvaceae) is an erect branched small perennial herb or shrub that grows abundantly in Nigeria. It is commonly known as wire weed because of the resilience of the plant. In the southern part of Nigeria, the plant is used to hasten delivery, treat malaria, jaundice and as anti-inflammatory agent (Okwuosa *et al.*, 2011).

On the other hand, the human liver is a major organ involved in the metabolism of drugs, xenobiotics and toxins. During the metabolism, excessive free radicals are generated that may cause liver damage (Chu *et al.*, 2002). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999) consequently presenting liver diseases amongst the fatal diseases in the world today. They pose a serious challenge to international public health (Ashan *et al.*, 2009). Majority of these diseases are not always easy to alleviate therapeutically in spite of the advances in modern medicine but are mostly managed (Cotran *et al.*, 2005).

Liver transplantation seems to be the only effectual remedy for liver failure. Unfortunately, this technique is expensive and is inaccessible in most developing countries of which Nigeria is not an exception. Even in the developed country there is always a challenge in finding a compatible donor.

Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in western countries (Grattagliano *et al.*, 2009). Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz *et al.*, 2002). It is not surprising that hepatoprotective action against liver toxic injury remain one of the major challenges for clinical therapy. Of the drugs/substances that can induce hepatotoxicity is thioacetamide.

Thioacetamide is an organo-sulfur containing compound (Stankova *et al.*, 2010). It was first reported as a hepatotoxic agent by Fitz-hugh and Nelson (1948). A single dose of this toxin in animal can produce centrilobular necrosis with subsequent regenerative response (Mangipudy *et al.*, 1995; Okuyama *et al.*, 2003). Chronic administration can lead to liver cirrhosis and hepatocarcinoma (Low *et al.*, 2004). The advantage of Thioacetamide as a model hepatotoxin consist in its high specificity for the liver, regiospecificity for the perivenous area and a large window of time between its necrogenic effect and liver failure. The injury is characterized by marked elevations in ALT and AST values with minimally elevated ALP. Bilirubin levels invariably are increased, and the prognosis often is worse when hepatocellular necrosis is accompanied by jaundice (Bahrami-Karkevandi *et al.*, 2005).

The toxicity of Thioacetamide results from bioactivation by a mixed function oxidase system, particularly by CYP2E1 and FAD monooxygenases (Zaragoza *et al.*, 2000). Metabolic activation of thioacetamide then leads to the formation of reactive metabolites that are represented by radicals derived from thioacetamide-s-oxide (TASO) and by reactive oxygen species (ROS) generated as intermediate. TASO is responsible for the changes in cell permeability, increase intracellular concentration of Ca⁺⁺, increase in nuclear volume, and also inhibits mitochondrial activity which leads to cell death and severely affecting those cells which are located in the perivenous acinus region (Amad *et al.*, 2002).

Fortunately for man, nature has bestowed some plants with the property to protect, treat and cure hepatic disturbances with interception of fewer side effects. Such substance(s) or drugs is said to be hepatoprotective and is thus referred to as hepatoprotective agent (Copping, 1996). Herbal drugs have more advantages than allopathic drugs as hepatoprotective agents because they are inexpensive, culturally acceptable, and compatible with the human body and possess minimal side effects. Many studies have been conducted for the efficacy of *Sida acuta*. Hence, this study was undertaken to verify the folkloric claim on the use of *Sida acuta* as a hepatoprotective agent, using n-hexane and ethyl acetate fractions of *Sida acuta* on Thioacetamide induced liver injury in Wistar rats.

MATERIALS AND METHODS

Location and duration of study: This work was carried out in a laboratory at the University of Teaching Hospital, Enugu State.

Experimental Animals/Housing Conditions: Thirty-five adult male Albino Wistar rats of comparable weights (150 to 180g) were used for this study. They were purchased from the animal house of the College of Medicine, University of Nigeria Teaching Hospital, Enugu, Enugu State, and allowed to acclimatize for two weeks. During the period of acclimatization, the animals were housed under standard temperature condition on a 12 hour light/12 hour dark cycle and fed with standard pellets (vital^R starter) in accordance with the institutional guidelines for the care and use of laboratory animals (Richard and Crawford, 2012).

Groupings: The experimental animals were divided into seven (7) groups (A, B, C, D, E, F and G) of five (5) rats each. Group A - D served as test groups, while group E - G served as control groups.

Study duration: The animal's acclimatization, substance procurement/fractionation, actual animal experiment and chemical analysis lasted for a period of seven months.

Substance of study/Plant collection and identification: The leaves of *Sida acuta* used for this study were collected from their natural habitat in and around Enugu between the months of January and February, 2013. A specimen of the plant was authenticated by a taxonomist at the Herbarium section of the Department of Botany, University of Nigeria, Nsukka. A voucher specimen was deposited at the Herbarium for reference (UNH/82b).

Plant Extraction: The leaves were air dried under the shade to avoid the decomposition of phytochemical constituents. They were dried for about seven days after which were observed to be dried and brittle. They were ground into fine powder with a gasoline powered grinding machine. The dry powder was stored until needed for the fractionation process.

Fractionation of Plant Materials: The solvent fractionation was carried out using the cold maceration method in order of increasing polarity from n-hexane to methanol. The powdered leaves (1000g) of *Sida acuta* was weighed out and macerated in 2.5Liters of crude methanol for 24 hours. The mixture was sieved through muslin cloth and filtered using whatman No 1 filter paper. The Marc were spread out on McIntosh and allowed to air dry. The dried marc was reweighed and macerated in 2.5 liters of n-hexane for 24 hours. After 24 hours, the extract was sieved using muslin cloth and filtered through whatman No1. The filtrate were evaporated to dryness on a rotary evaporator (model 349/2 corning Ltd England) and labeled n-hexane fraction. The marc was spread out on a McIntosh and allowed to air dry.

The dried marc was reweighed and further macerated in 2.5 liters of Ethyl acetate for 24 hours after which it was sieved using muslin cloth and filtered through whatman No1 filter paper. The filtrate was evaporated to dryness on a rotary evaporator and labeled the Ethyl acetate fraction. The percentage yield of the plant fractions were 0.64% and 1.27% (^w/_w) respectively for n-hexane fraction of *Sida acuta* (HFSA) and ethyl acetate fraction of *Sida acuta* (EAFSA).

6.2g of the residue of n-hexane fraction was dissolved in 2.5% aqueous suspension of tween-80 and then made up to 62ml with the same solvent producing a concentration of 100mg/ml of HFSA. 10g of the residue of ethyl acetate fraction was dissolved in 2.5% aqueous suspension of tween-80 and then made up to 100ml with the same solvent to produce a concentration of 100mg/ml of EAFSA.

Phyto-chemical Test: Chemical tests were carried out on the Hexane and Ethyl acetate fractions of *Sida acuta* for the qualitative determination of phytochemical constituents as described by Harborne (1998), Trease and Evans (1989) and Sofowora (1993) which are:

Alkaloids: 0.5 g of each plant fraction was diluted with 10 ml of acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendoff's reagent) was regarded as positive for the presence of alkaloids.

Saponins: To 0.5 g of each of the plant fraction was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubble indicated the presence of saponins.

Tannins: About 0.5 g of each of the plant fractions was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration indicating the presence of tannins.

Flavonoids: About 0.5 g of each of the plant fraction was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicated the presence of flavonoids.

Steroids: About 0.5g of each of the plant fraction was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Cardiac glycosides (Keller-Killiani test): To 0.5 g of each of the plant fraction was diluted to 5 ml in water. 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Chemicals and Drugs: The methanol, n-hexane and ethyl acetate used for the extraction was manufactured by BDH chemicals, England. Also, the Silymarin tablets (Silybon-140 RX) used was manufactured by Micro Lab. Limited, India and the Thioacetamide by Sigma Chemical Co, USA. All the chemicals were of analytical grade and used without further purification.

Drug Preparation and Reconstitution: Silymarin (SILYBON 140) tablets (1400mg) was dissolved in normal saline (8.5g/l) and made up to 100ml with normal saline to produce a final concentration of 14mg/ml. This was stored in a refrigerator. Thioacetamide (TAA): Thioacetamide (10g) was dissolved in distilled water and made up to 100ml with distilled water, producing a final concentration of 100mg/ml. This was stored at room temperature and marked toxic.

Acute Toxicity Test: A total of six of the rats were used for the acute study. The animals were divided into two groups A and B. After an overnight fast, group A received a single oral dose of (3000 mg/kg) of hexane fraction of *Sida acuta* and group B received a single oral dose (3000 mg/kg) of ethyl acetate fraction of *Sida acuta*. After administration of the fractions, food was withheld for further 3 - 4 hours. The animals were observed individually at least once during the first 30 minutes post-administration; periodically during first 24 hours (with special attention during the first 4 hours); and daily thereafter for period of 3 days. All the animals did not show lethal or toxic signs at the dose of 3000 mg/kg.

Substance administration: Oral daily administration of the fractions was done between 8am- 10am at different doses as follows:

1. Group A (n=5) and B (n=5) received 150mg/kg HFSA and 300mg/kg HFSA respectively, alongside normal feed and water *ad libitum*.
2. Group C (n=5) and D (n=5) received 150mg/kg EAFSA and 300mg/kg EAFSA respectively, alongside normal feed and water *ad libitum*.
3. Group E (n=5) served as the Silymarin (positive control) group and received 100mg/kg Silymarin, alongside normal feed and water *ad libitum*.
4. Group F (n=5) served as the thioacetamide (negative control group) and received thioacetamide, alongside normal feed and water *ad libitum*.
5. Group G (n=5) was the normal control group and received only normal feed and water *ad libitum*

The administration of the HFSA, EAFSA and Silymarin was for a period of seven (7) consecutive days. On the 8th day, after an 18-hour fast, 200mg/kg thioacetamide (TAA) was administered subcutaneously to groups A, B, C, D, E and F. Group G was not given thioacetamide (TAA) as they served as the normal control (NC).

Sample collection: Twenty four (24) hours after TAA administration, the animals (group A to G) were bled through the retro-orbital sinus into labeled plain tubes under ether anesthesia. The blood samples were allowed to retract and clot undisturbed and the sera expressed after centrifugation at 3000r.p.m for 10 minutes. The sera were transferred into correspondingly labeled serum containers and stored frozen for biochemical analysis.

Biochemical Analysis/methods: The sera were analyzed for liver maker enzymes, Aspartate transaminase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP) and Albumin (ALB). The transaminases (ALT/AST) and the ALP were determined by end point colorimetric method as described by Reitman and Frankel (1957) and King and King (1954) respectively. Albumin (ALB) estimation was determined by dye binding method as described by Doumas and Biggs (1972).

Statistical analysis: The data analysis was achieved using SPSS evaluation package version 15. Results were expressed where appropriate as mean \pm standard error of mean. Differences between mean values were determined with the one-way analysis of variance followed by Tukey's post-hoc comparisons with $p < 0.05$ considered as significant.

RESULTS

Table 1 shows the mean values of serum liver hepato-specific enzymes and Albumin levels in control and experimental rats. A significant increase in serum ALP, AST and ALT with mean values of 157.40 ± 0.97 IU/L, 216.80 ± 2.80 IU/L, 327.80 ± 5.54 IU/L respectively were observed in thioacetamide treated group when compared with the normal control (group G) with mean levels of 128.00 ± 5.30 IU/L, 81.25 ± 9.20 IU/L, 65.00 ± 10.86 IU/L for ALP, AST and ALT respectively ($p < 0.001$). The mean serum Albumin level of the thioacetamide group was 2.56 ± 0.12 g/dl and this was significantly lower than that of the normal control (5.13 ± 0.18 g/dl) ($p < 0.05$). The positive control (group E) which received 100mg/kg dose of Silymarin showed a marked increase in mean values of serum ALP, AST and ALT of

151.80±3.36IU/L, 225.20±1.71IU/L and 307.40±12.83IU/L respectively when compared with the normal control (p<0.001).

The prophylactic administration of 150mg/kg HFSA in Group A produced a significant decrease in the serum mean level of ALT (184.40±15.68IU/L) when compared with the Positive (group E) and Negative (group F) controls respectively (p<0.001). The Albumin level of the 150mg/kg HFSA treated group also showed a significant increase with mean values of 5.60±0.24g/dl when compared with the Positive control (2.75±0.85g/dl) and thioacetamide control (2.56±0.12g/dl) (p<0.01). However, there was a significant increase in ALP, AST and ALT with mean values of 155.40±2.20IU/L, 255.40±9.50IU/L and 184.40±15.68IU/L respectively in group A when compared to the normal control with mean values of 128.00±5.30IU/L, 81.25±9.20IU/L and 65.00±10.86IU/L for ALP, AST, ALT respectively (p<0.001; p<0.05).

Furthermore, Group B which received 300mg/kg HFSA had a significant decrease in mean ALT levels (133.60±30.85IU/L) with respect to the Positive control, and Negative control, mean values of 307.40±12.83IU/L and 372.80±5.54IU/L respectively (p<0.001). A significant increase in mean Albumin level was observed (5.40±0.40g/dl) when compared with mean levels in Positive and Negative controls with mean values of 2.75±0.85g/dl and 2.56±0.12g/dl respectively (p<0.01). A significant increase was also seen in ALP and AST with mean values of 151.80±3.36IU/L and 236.60±30.00IU/L with respect to the mean values in Normal control with mean values of 128.00±5.30IU/L and 81.25±9.20IU/L for ALP and AST respectively (p< 0.001).

Table 1: Effect of the fractions of *Sida acuta* on some hepatospecific enzyme and albumin levels of rats intoxicated with thioacetamide.

Groups	Grades administered	ALB(g/dl)	ALP(IU/L)	AST(IU/L)	ALT(IU/L)
A	150mg/kg HFSA	5.60±0.24 ^{e,h}	155.40±2.20 ^c	255.40±9.50 ^c	184.40±15.68 ^{a,t,h}
B	300mg/kg HFSA	5.40±0.40 ^{e,h}	151.80±3.36 ^c	236.60±30.00 ^c	133.60±30.85 ^{f,i}
C	150mg/kg EAFSA	4.80±0.48 ^{d,g}	147.80±1.74 ^b	245.80±9.14 ^c	199.80±39.97 ^{b,f,g}
D	300mg/kg EAFSA	4.20±0.37	142.20±3.33 ^{a,d}	247.40±11.88 ^c	307.40±12.83 ^c
E	100mg/kg Silymarin Positive control	2.75±0.85 ^a	151.80±3.36 ^c	225.20±1.71 ^c	307.40±12.83 ^c
F	200mg/kg Thioacetamide, Negative Control	2.56±0.12 ^a	157.40±0.97 ^c	216.80±2.80 ^c	372.80±5.54 ^c
G	Normal control	5.13±0.18	128.00±5.30	81.25±9.20	65.00±10.86
p-value	Ratio	7.477	9.910	16.194	23.158
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

KEYS: a=<0.05; b=<0.01; c=<0.001 when compared with Normal Control; d=<0.05; e=<0.01; f=<0.001 with respect to Negative Control; g=<0.05; h=<0.01; i=<0.001 when compared with Positive Control; HFSA - Hexane fraction of *Sida acuta*; EAFSA - Ethyl acetate fraction of *Sida acuta*

Also, pre-treating of rats with 150mg/kg of EAFSA caused a significant decrease in ALT with mean value of 199.80±39.97IU/L when compared with the Positive control and Negative control groups with mean ALT value of 307.40±12.83IU/L and 372.80±5.54IU/L respectively (p<0.05 and p<0.001). In group C, there was also a significant increase in mean Albumin level (4.80±0.48g/dl) when compared with the Positive and Negative controls with mean albumin values of 2.75±0.85g/dl and 2.56±0.12g/dl respectively (p<0.05). Moreover, an increase was also observed in ALP, ALT and AST levels with mean values of 147.80±1.74IU/L, 199.80±39.97IU/L and 245.80±9.14IU/L respectively when compared with the normal control group (p<0.001).

Group D, which received 300mg/kg of EAFSA showed a significant increase in ALP, AST and ALT with mean values of 142.20±3.33IU/L, 247.40±11.88IU/L and 307.40±12.83IU/L respectively when compared with respective mean values in the Normal control, with mean values of 128.00±5.30IU/L ($p<0.05$), 81.25±9.20IU/L and 65.00±10.86 respectively ($p<0.001$). A significant decrease in ALP with the mean value 142.20±3.33IU/L was observed with respect to the Negative control, with the mean value of 157.40±0.97IU/L ($p<0.05$). It was also observed that there were no significant differences in Albumin value when compared with both the Positive and Negative control Groups ($p>0.05$). The preliminary phytochemical analysis of both the HFSA and EAFSA revealed the presence of Alkaloids, Saponins and Tannins. HFSA also contain flavonoids in addition to the afore-mention compounds as presented in table 2 below.

Table 2: Preliminary phytochemical screening of leaf fractions of *Sida acuta*

S/N	Phytochemical	HFSA	EAFSA
1	Saponin	++	+
2	Flavonoids	+	-
3	Tannins	++	+
4	Phenols	-	-
5	Alkaloids	++	+
6	Steroids	-	-
7	Glycosides	-	-

KEY: + = Present; ++ = Strongly Present; - = Absent

DISCUSSION

Thioacetamide (TAA) was chosen for this experiment because it consistently produces various grades of liver damage including nodular cirrhosis, liver cell proliferation, production of pseudo lobules and parenchymal cell necrosis (Mitra *et al.*, 1998). In a number of animal models, TAA induced cirrhosis has a histological appearance that is more akin to human cirrhosis (Li *et al.*, 2002; Mgbemena *et al.*, 2015). Several investigators have reported that a single dose of this hepatotoxin can produce centrilobular hepatic necrosis and chronic administration leads to cirrhosis in rats (Khatri *et al.*, 2009).

Thioacetamide is a known hepatotoxin that induces liver necrosis by producing free radicals (Mohammed *et al.*, 2009). In a short period of time, thioacetamide induces liver damage (Minnady *et al.*, 2010). This toxin is metabolized by detoxification enzymes of p450 cytochrome system (Mohammed *et al.*, 2009; Madani *et al.*, 2006). Thioacetamide metabolites result in the production of thioacetamide-s-oxide that attacks membrane protein and lipids, changes the cell membrane permeability, increase intracellular calcium concentration, increase in nuclear volume and also inhibits mitochondrial activity which leads to cell death and severely affecting those cells which are located in the perivenous acinus region (Minnady *et al.*, 2010).

Reduced hepatic antioxidant function has also been suggested as one of the mechanism of TAA induced hepatotoxicity. Free radicals damage cell membrane such as those of hepatocytes which results in increasing liver enzyme activity. This causes the liver enzymes which are normally located inside cell cytosols to enter blood circulation. Increasing activity of these enzymes indicates the degree and type of liver damage (Taheri *et al.*, 2012).

In the present study, administration of TAA caused a decrease in albumin level compared with the normal control group ($p<0.05$). This is in agreement with results of Perez *et al.*(2004). Decrease in the albumin level may suggest decrease in the synthetic function of the liver cell. However, the damage caused by TAA was ameliorated with the administration of HFSA and EAFSA. These show the hepatoprotective potential of the plant fractions.

Hepatocellular damages are identified by increase in serum ALT and AST levels in that these enzymes are in the cytoplasm and after cellular damage; they enter blood circulation (Minnady *et al.*, 2010). ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Dhorajiya and Galani, 2012). ALP is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in serum are raised with large bile duct obstruction, intrahepatic cholestasis or infiltrative disease of the liver (Dhorajiya and Galani, 2012). In the present study, administration of TAA caused elevation of serum ALP, AST and ALT activities compared with normal control group ($p < 0.05$). This is in agreement with previous data (Abul *et al.*, 2010), which could be taken as an index of liver injury.

The rise in ALP and ALT levels induced by TAA administration was significantly reduced in the prophylactic groups when compared to the TAA control group ($p < 0.05$ and $P < 0.001$) respectively for EAFSA and HFSA. This view is also supported by the works of Sreedevi *et al.* (2009), who reported that methanolic extract of the root of *Sida acuta* has a hepatoprotective effect on paracetamol induced liver injury. However, low dose of EAFSA produced a stronger hepatoprotective effect than the high dose. The hepatoprotective effect of the plant fractions might be due to their effect against cellular leakage, loss of functional integrity of the cell membrane and the loss of stabilization of plasma membrane in the liver.

Earlier studies have reported the presence of biologically active compounds in *Sida acuta* (Jang *et al.*, 2003) and in support of these studies, phytochemical analysis of the leaf fractions were carried out in the present study and these revealed the presence of alkaloids, saponins, flavonoids and tannins. The observed antioxidant property exhibited *in vitro* by *Sida acuta* in the study by Karou *et al.* (2005) may be related to the presence of some of the afore-mentioned compounds as also demonstrated in previously reported studies (Ekor *et al.*, 2010). The hepatoprotective effect of *Sida acuta* fractions against thioacetamide induced hepatotoxicity as seen in the study could be also as a result of antioxidant activity against the free radicals produced by thioacetamide metabolites. Antioxidants oppose the process of oxidation largely by neutralizing free radicals and they have potential to inhibit the oxidant chain reactions and ultimately reconstitute the damaged membrane (Naik, 2003).

On comparing both fractions, hexane fraction was found to be a stronger hepatoprotective agent than the corresponding ethyl acetate fraction against the toxicant. This may be due to the presence of flavonoids which has an inhibitory effect on cytochrome p450 system and prevents the further metabolism of thioacetamide which eventually lead to the reduction of free radical production (Cordova *et al.*, 2002). Flavonoids also revive cells against glutathione depletion by increasing the capacity of antioxidant enzymes (Bahrami-karkevandi *et al.*, 2003).

The significant increase in AST levels in the prophylactic groups (Groups A-D) may be as a result of toxic effect produced either by TAA or the plant fractions on the heart, brain, kidney, skeletal muscle and red blood cells. This is in agreement with the works of Abbasi *et al.* (2013) who reported that single intra peritoneal injection of TAA leads to the distortion in red cell morphology which is characterized by increased hemoglobin concentration and decline of erythrocyte count. Histopathological examination of sections from rat kidneys treated with TAA showed impaired renal morphology throughout with severe and generalized tubular epithelial cell necrosis associated with diffuse tubular swelling, glomerular congestion and inflammatory cell infiltration (Kadir *et al.*, 2013). However, in the works of Kebe *et al.* (2013), chronic administration of ethanolic extracts of *Sida acuta* to Wistar rats resulted in decreased body weight, decrease bowman space, dilation of renal tubules and cellular degeneration of treated rats.

It can then be concluded that both HFSA and EAFSA have potential hepatoprotective activity and attenuates the hepatotoxic effects of TAA through its antioxidant and membrane stabilizing effects. HFSA was more potent than EAFSA as a hepatoprotective agent. It restored the different liver functions and normal histology better than EAFSA, thus, these results constitute a lead towards discovering a novel herb in traditional and complementary medicine.

Further research/investigations on isolation and characterization of the active compounds responsible for the offered hepatoprotection by the plant fractions as well as chronic toxicity studies on the plant fractions should be carried out.

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