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Effect of *Senna mimosoides* leaf aqueous extract on the lactase activity, total protein and the electrolyte-inducing potentials of carbon tetrachloride induced hepatoxicity in Wistar albino rats

NWORAH Florence Nkechi, NKWOCHA Chinelo Chinenye and NWACHUKWU Justus Nmaduka

Biopharmaceutical and Medicinal Plant Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.

Aqueous extract of *Senna mimosoides* leaves (ASML) (a common weed in Savannah region of the African Continent) has been used in management of lactose intolerance and breast toxicity by locals. This study evaluated phytoconstituent of the aqueous extract of *S. mimosoides* leaves and the effect on lactase activity and subsequent electrolyte-inducing potentials. Thirty female albino rats were used for this study and were divided into groups of five rats each: Groups A rats were normal control. Hepatotoxicity was induced with the use of carbon tetrachloride on Groups B, C, D, E and F. Groups B, C and D were treated with extract, administered orally at three dose level (50, 100 and 250 mg/kg body weight of the extract respectively). Group E was treated with 25 mg/kg body weight of Silymarin (standard drug) while Group F served as the negative control. Phytochemical analyses, activities of Intestinal Lactase activity, Total Protein Analysis and the serum ion concentrations of Iron (Fe), Selenium (Se), Zinc (Zn), Calcium (Ca), Phosphate (HPO_4^-), Magnesium (Mg), Sodium (Na) and Potassium (K) were determined using standard methods. Phytochemical analysis revealed the presence of phenols, flavonoids, alkaloids, tannins, reducing sugars, steroids and cyanide. Lactase activity increased significantly (P<0.05) with increasing concentrations of ASML. Total protein content of Groups B, C and D increased significantly (P<0.05) when compared to Group F. Apart from Fe and PO_3^{2-} which showed significant (P<0.05) highest values for Group F, all other ions (Mg, K, Ca, Zn, Se and Na) showed significant (P<0.05) increases in serum levels of Groups B, C and D as compared to Group F. Increased doses of Group D were significantly (P<0.05) higher than Group E for the later ions. The result of the study suggests that the aqueous extract of *S. mimosoides* leaves contain some bioactive compounds that possess electrolyte-inducing/restorative properties and since it increases lactase activity in Wistar rats, may be a viable claim in the management of lactose intolerance.

Key words: *Senna mimosoides*, hepatoxicity, lactase, electrolyte inducing potentials.

INTRODUCTION

Biological reactions especially aging is time-dependent and as one's biological clock ticks some activities of enzymes begin to dwindle. In the US alone, over 50million people suffer from lactose intolerance; a partial

inability of the intestinal lactase enzyme (beta-galactosidase) to completely digest lactose (milk sugar) into its simpler monosaccharaides: glucose and galactose (Bury et al., 2001; Jokar and Karbassi, 2009). These enzymes are located in the epithelial cells of the small intestine. The end-products resulting from the activities of these enzymes are actively translocated from the intestine to the blood by ATPases. The consumption of milk and dairy products by these people could lead to cramp, flatulence, and vomiting. The imperativeness of ensuring a better functioning of the lactase enzyme in humans stems from the widespread use of milk and milk products in food industries involved in baking, yoghurt and cheese production (Vasiljevic and Jelen, 2001; Dilworth et al., 2005).

Homeostasis and osmoregulation are vital metabolic processes needed for continual functioning of the body. These processes though regulated by the brain most times depend on electrolytic-ionic potentials of individual cells which proffers cumulative net-charge difference. These ions include phosphorus, selenium, calcium, magnesium and a host of others whose functions are hugely dependent on their availability to an appreciable extent in the body (Soetan et al., 2010). Practically, every form of energy exchange inside living cells involves the forming or breaking of high-energy bonds that link oxides of phosphorus to carbon or to carbon-nitrogen compounds (Murray et al., 2008). Vitamin D is probably involved in the control of phosphorus absorption and serum levels are regulated by kidney reabsorption. Calcium is one of the most important ions in the body utilised in bone and structural organisation, enzyme function, blood coagulation, in osmotic pressure and maintenance of fluid balances, and is also essential in muscle activity. Disruption of calcium homeostasis may result in the activation of many membrane damaging enzymes like ATPases, phospholipases, proteases and endonucleases, disruption of mitochondrial metabolism and ATP synthesis and damage of microfilaments used to support cell structure (Singh et al., 2011). Sodium is the primary ion in extracellular fluid. It regulates plasma volume and acid-base balance, is involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function and is involved in Na+/K+-ATPase, maintenance of membrane potentials, transmission of nerve impulses and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts (Ekwueme et al., 2011). Potassium, the principal electrolyte (cation) present in intracellular fluid, functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na+/K+-ATPase. It helps in the transfer of phosphate from ATP to pyruvic acid, is required during glycogenesis, inhibits free radical formation and is critically important to maintaining normal heart rhythm and blood pressure (Huang and Kuo, 2007). Selenium, an essential micronutrient for animals and humans plays an important role in antioxidant defence systems (Singh and Gandhi, 2011). It plays a key role in the redox regulation and antioxidant function through glutathione peroxidases that remove excess of potentially damaging radicals produced during oxidative stress. It is present in the active center of glutathione peroxidase (GPx), which protects lipid membranes and macromolecules from oxidative damage produced by peroxides and also permits the regeneration of a membrane lipid molecule through reacylation (Heikal et al., 2012). Iron, the most important transition element of the body, is found in functional forms in haemoglobin, myoglobin, the cytochromes, enzymes with iron sulphur complexes, and other iron-dependent enzymes (Sakar et al., 2012). Iron exists in the blood mainly as haemoglobin in the erythrocytes and as transferrin in the plasma. It is transported as transferrin, stored as ferritin or haemosiderin and is lost in sloughed cells and by bleeding.

All these ions drop in concentrations way below levels that are advisable for the body especially in cases of hepatoxicity, as abnormal functioning of the liver would deter the metabolic processes which ensure adequate increases in both protein and ion capacity.

Senna mimosoides, formerly known as C*assia mimosoides* belongs to the family Caesalpinaceae and the genus *Senna* (Young, 2000). *S. mimosoides* is a weed common in wastelands, roadsides and fallows in savannah zone and is widespread in West Africa. Generally, the leaves of some *Senna* are useful in treating constipation, abdominal disorder, leprosy, skin diseases, leucoderma, splenomegaly, hepathopathy, jaundice, helminthiasis, dyspepsia, cough, bronchitis, typhoid fever, anaemia, tumors (Joy et al., 1998). Not much research has been done on *S. mimosoides*. However, it is known to be a natural lipase inhibitor, that is, it prevents the absorption of fats from digested food and is therefore used as a slimming agent. It also has a laxative effect, which implies that it is used to relieve constipation and support normal body function (Robbins, 2000).The aqueous leaf extract of *S. mimosoides* exhibits anti-inflammatory effects by stabilizing membrane, inhibiting phospholipase A_2 activity and prostaglandin

*Corresponding author. E-mail: Florence.nworah@unn.edu.ng

Table 1. Experimental groups.

Groups	Description	Treatment
A	Normal Control	Administered with normal saline
B	Treatment group 1	CCl_4 administered and treated with 50 mg/kg body weight of the extract
C	Treatment group 2	CCl_4 administered and treated with 100 mg/kg body weight of the extract
D	Treatment group 3	CCl_4 administered and treated with 250 mg/kg body weight of the extract
E	Standard Control	CCl4 administered and treated with 25 mg/kg body weight of silymarin (standard control)
F	Experimental control	Induced with CCl_4 and left untreated

Extract: Aqueous extract of *S. mimosoides* leaves, number of rats per group (n) =5.

synthase activity (Ekwueme et al., 2011)

This study is aimed at validating the traditional use of *S. mimosoides* leaves in folk medicine for the treatment of oedema and breastmilk toxicity in neonates and in boosting ionic potentials in liver damaged individuals. Table 1

METHODOLOGY

Plant material

Fresh leaves of *S. mimosoides* were collected and used in this study.

Animal material

Wistar albino rats of either sex weighing between 130-250 g were obtained from the Animal House, Faculty of Biological Sciences, University of Nigeria, Nsukka. These animals were given standard feeds (vital) for at least one week after purchase to acclimatize them to their new environment before use.

Exact duration and year of study

This study was conducted in 2017 for a period of approximately 6 months

Collection and authentication of plant material

Fresh leaves of *S. mimosoides* were obtained from Orba, Nsukka L.G.A of Enugu State, Nigeria. The leaves were identified and authenticated by the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Voucher specimens were deposited in the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for reference purpose.

Reagents and chemicals

All chemicals used in this study were of analytical grade and were obtained from commercial dealers in Nsukka and Onitsha, both in Nigeria. They include products of May and Baker, England; BDH Chemical Limited, Poole, England and Merck, Germany. Reagents used for all the determining concentration were commercial kits and products of Randox Laboratories Limited, United Kingdom and TECO Diagnostics, USA.

Equipment and instrument

Equipment and instruments used for this study were obtained from the Laboratory Unit of Department of Biochemistry, University of Nigeria, Nsukka and Shalom Research Laboratory, Nsukka. Others were purchased from commercial dealers in Nsukka and Onitsha, both in Nigeria. They include: Spectrophotometer (model SPM721-2000, Biodiagnostic Inc., USA), centrifuge (model 800D), micropipette (volume range 0-100 µL) (Swastic Scientific Instrument Private Ltd., Mumbai, India) and test tubes (Pyrex, England).

Extraction of plant material

The leaves of *S. mimosoides* plant were collected and dried under atmospheric temperature (25 to 45^{o}C) for two weeks. Thereafter, the leaves were sliced into small bits and ground into coarse form. The coarse form was then soaked in distilled water in a 3,000-ml conical flask and the top sealed with foil and masking tape to prevent evaporation. The system was shaken vigorously and allowed to stand for forty-eight h, after which the aqueous extract was filtered out with the help of a cotton wool and a white filter cloth. The resulting aqueous extract was concentrated and evaporated to dryness using a rotary evaporator at an optimum temperate to avoid denaturation of the active ingredients. The extract was put under a fan and allowed to dry completely. A known weight of the dry extract was determined. The container was sealed and store in a refrigerator.

Experimental design

Thirty (30) Wistar rats were housed in separate cages, acclimatized for seven days and then divided into six (6) groups of four (5) rats each.

Qualitative phytochemical analysis of extract

The method described by Harbone (1998) was used.

Serum inorganic ion determination

Serum concentrations of inorganic ions, Sodium ion (Na^{+}), Calcium

Table 2. Phytochemical constituents of aqueous extract of *S. mimosoides* leaves.

Constituent	Bioavailability	Amount (%)
Alkaloids	+	8.719
Flavanoids	+	6.787
Terpenoids	+	2.212
Tannins	++	12.074
Phenolics	++	18.912
Saponins	+	5.008
Reducing Sugar	+++	21.124
Carbohydrates	++	11.896
Steriods	+	2.186
Cyanide	+	4.093
Glycosides	+	6.99

+++: High content, ++: Moderate content, +: Low content. Values are mean of a single run.

ion (Ca^{2+}), Iron ion (Fe^{2+}), Magnesium ion (Mg^{2+}), Potassium ion (K^{+}), Selenium ion (Se^{2+}), phosphate ion (HPO_4^{-}) and Zinc ion (Zn^{2+}) were determined using AOAC (2005) method.

Determination of serum protein concentration

Serum total protein was determined using Biuret method as described by Lubran (1978).

Histopathological study

For histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Thereafter, representative blocks of liver tissues from each lobe will be taken and possessed for paraffin embedding using the standard microtechnique. Sections (5 µM) of livers stained with hemotoxyline and eosin will be observed microscopically for histopathological studies.

Determination of the effect of the extract on the activity of lactase

The intestinal disaccharidases inhibitory activity was assayed for the extract under investigation based on the method of Dahlqvist (1968).

Preparation of Intestinal homogenate

Rats were decapitated, and the intestine was removed by cutting of both the upper end of the duodenum and the lower end of the ileum. The entire intestine was homogenized with four parts of cold distilled water by ultrasonic probe (frequency, at 10 KHz) for 1 min. The tube was chilled with crushed ice before and during homogenization. The homogenate was centrifuged at 4000 rpm for 10 min and the supernatant was used to determine the activity of lactase.

Procedure

A known volume (10, 20, 30 and 40 µl) of the extract solution (0.1%) was added to 50 µl of diluted supernatant (dilution factor = d). The solution were mixed well and incubated at 37°C for 30 min. 100 µl of substrate-buffer solution (0.056 M lactose in 0.1 M sodium maleate buffer, pH 6.0) was added and mixed well. After exactly 60 min, the reaction was interrupted by incubating at 100°C for 5 min, and then 200 µl of distilled water was added and mixed well. The liberated glucose was determined by glucose kit. While activity of lactase was calculated from the following equation, the inhibitory activity was calculated by the difference.

$$\text{Lactase activity} = \frac{a.d}{n.1080}$$

Where a is amount of glucose (µg) liberated in 60 min (sample-blank), d is dilution factor for the enzyme solution (supernatant, 50 µl) and n is the number of glucose molecule per molecule of lactose. Also,
Test tube 1: 50 µl of boiled enzyme and 10 µl of extract
Test tube 2: 50 µl of boiled enzyme and 20 µl of extract
Test tube 3: 50 µl of enzyme (not boiled) only
Test tube 4: 50 µl of enzyme (not boiled) and 10 µl of ectract
Test tube 1: 50 µl of enzyme (not boiled) and 20 µl of extract
Test tube 1: 50 µl of enzyme (not boiled) and 30 µl of extract
Test tube 1: 50 µl of enzyme (not boiled) and 40 µl of extract
Test tube 1: 50 µl of enzyme (not boiled) and 50 µl of extract.

Statistical analysis

The results were expressed as mean ± SD and test of statistical significance was carried out using one way analysis of variance (ANOVA). The data obtained were analysed using Statistical Product and Service Solutions (SPSS), version 18. $P < 0.05$ will be considered significant.

RESULTS AND DISCUSSION

The mechanism of the hepato-protective action of the plant is uncertain but may be related to the ability of the plant to inhibit lipid peroxidation in the liver. Results from Table 2 shows an appreciable level of reducing sugar, phenols, tannins and flavonoids. Flavonoids, tannins and microelements have been suggested to act as antioxidants and exert their antioxidant activity by scavenging the lipid peroxidation and their presence in this plant might be responsible for its potential effect in combating lipid peroxidation (Middleton et al., 2000). Therefore, the present work provides conclusive evidence for the hepato-protective effect of *S. mimosoides* against CCl_4-induced hepatotoxicity.

Table 3 shows a significant decrease of calcium ion in the serum of rats that received CCl_4 when compared to the normal control group. This decrease might be because CCl_4 causes hepatic injury resulting from peroxidation of membrane lipids which resulted in the alteration of structural and functional characteristics of

Table 3. Electrolytic potential of experimental rats.

Group	Ionic concentration (mg/dl)							
	Sodium	Magnesium	Iron	Potassium	Phosphate	Calcium	Zinc	Selenium
A	1.07±0.07^{a}	1.03±0.07^{a}	0.58±0.01^{a}	0.14±0.01^{a}	0.53±0.02^{a}	2.52±0.08^{a}	1.12±0.03^{a}	0.91±0.04^{b}
B	0.59±0.04^{b}	0.69±0.05^{b}	0.76±0.01^{b}	0.07±0.00^{b}	0.75±0.03^{c}	1.82±0.08^{b}	0.73±0.02^{c}	0.69±0.04^{a}
C	0.81±0.05^{c}	0.79±0.05^{b}	0.62±0.01^{b}	0.08±0.00^{b}	0.66±0.05^{c}	2.18±0.08^{bc}	0.89±0.03^{a}	0.79±0.02^{c}
D	0.97±0.07^{c}	0.98±0.06^{ac}	0.53±0.01^{ab}	0.12±0.01^{ac}	0.53±0.04^{b}	2.42±0.08^{c}	1.03±0.07^{b}	0.89±0.03^{b}
E	0.90±0.03^{d}	0.90±0.03^{c}	0.61±0.02^{a}	0.10±0.01^{c}	0.56±0.05^{a}	2.28±0.08^{a}	0.99±0.09^{b}	0.81±0.04^{c}
F	0.33±0.04^{b}	0.49±0.03^{d}	0.95±0.01^{c}	0.05±0.00^{b}	0.91±0.05^{d}	1.44±0.05^{b}	0.59±0.04^{d}	0.50±0.03^{d}

Data are Mean±SEM (n = 5). Values with different superscripts are significant at $p<0.05$.

the membrane or due to impaired intestinal absorption of Calcium. It may be that CCl_4 or its metabolite, chelate calcium ion thereby preventing its absorption in the intestine. Also, impaired intestinal absorption may result from impairment in the conversion of vitamin D to the active form, 1, 25- dihydroxyvitamin D3, which has been reported to be the primary hormone that mediates calcium absorption in the intestine (Pradeep et al., 2007). This then affects the activities of membrane bound ATPases, that is, Na+/K+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase which are responsible for the transport of Ca^{2+} ions across cell membrane at the expense of ATP. These enzymes have been reported to be extremely sensitive to hydroperoxides and superoxide radicals (Pradeep et al., 2007). Moreover, the decrease in Ca^{2+} concentration must have been due to the inability of the enzymes in the liver to convert 7-dehydrocholesterol to 1, 25-dihydroxy cholecalciferol, a hormone known to regulate Ca uptake in the intestine and calcium level in kidney and bone. Therefore, the ability of the extract to increase Ca^{2+} concentration suggest that the extract was able to restore the integrity of the hepatocyte membrane which consequently lead to restoration of the functions of these enzymes. However, the dose dependent increase in Ca^{2+} concentration of rats that were treated with *S. mimosoides* extract may be due to membrane stabilizing effect of the extract which then prevents peroxidation of the membrane (Ekwueme et al., 2011). This could be due to the antioxidant effect of the extract. Consequently, the altered activity of Ca^{2+} ATPase is restored and this normalizes the metabolism of Calcium. It could also be possible that the mechanism of action of the extract in treating breast milk toxicity in neonates may be that it increases Ca^{2+} which binds to phosphoserine groups present in casein - a breast milk protein efficiently providing casein to the suckling young.

From Table 3, there was a decrease in the K^+ level in serum of rats that were treated with CCl_4. The membrane bound enzyme, that is, Na^+/K^+ ATPase is responsible for the transport of K^+ across cell membrane at the expense of ATP. Studies have shown that hepatic injury resulting from peroxidation of membrane lipids results in the alteration of structural and functional characteristics of the membrane, which affects the activities of this membrane, bound ATPase. This enzyme is extremely sensitive to hydroperoxides and superoxide radicals. Table 3 shows that the extract at different concentration was able to reverse the effect of CCl_4 on K+ level. This could be attributed to the membrane stabilizing effect of the extract reported by Ekwueme et al. (2011). The extract was able to reverse the altered structural and functional characteristics of the membrane, thereby leading to an increase in the activity of Na^+/K^+ ATPase.

The increase in the K^+ level depict that there was increase in the activity of ATPases on posttreatment with silymarin which could be due to the membrane stabilizing activity by preventing peroxidation of membrane lipids. Studies have shown that antioxidants inhibit CCl_4 induced hepatotoxicity (Galisteo et al., 2000). Therefore, silymarin by virtue of its antioxidant role could have prevented damage to the hepatocytes and thereby maintained the membrane in a healthy state. The results of the present investigation shows silymarin to be effective in scavenging the free radicals released during treatment with CCl_4. The mechanism of action of the extract might be that it uses its antioxidant potential to mop up the free radicals generated by treating with CCl_4.

From Table 3, there was a reduction in Mg level of the rats that were treated with CCl_4 when compared with the normal control. A reduction in extracellular magnesium lowers the threshold levels of excitatory amino acids (e.g., glutamate) needed to activate N-methyl-D-aspartate (NMDA), thereby allowing the influx of calcium into the cell (Cole and Morgan, 2003). The importance of the NMDA receptor in the inflammatory response to magnesium deficiency is indicated by the finding that NMDA receptor blockade decreased pro-inflammatory prostaglandin E2 in plasma and inhibited cardiac inflammation indicators in the heart, which were induced by magnesium deficiency in the rat. Tyrosine kinase activity is inhibited by Mg deficiency resulting in insulin resistance and impaired secretion. This resistance leads

to release of more insulin, causing more Mg (and K) to be transported from blood into cells. Glycosuria is the most significant mechanism for urinary magnesium wasting and this leads to membrane instability (decreased magnesium phospholipid complexes) and pump dysfunction (defective Ca/Mg ATPase and Na/K ATPase pumps), causing more Mg loss and more insulin resistance. Therefore, the ability of the extract to increase the level of Mg shows it could restore the activity of tyrosine kinase thereby alleviating the impaired secretion of insulin. Moreover, the extract due to its membrane stabilizing potential, stabilized the membrane and restored pump, that is, Ca/Mg ATPase function. On the other hand, increase in the level of Mg leads to a concommitant increase in Mg phospholipid complexes which lead to the restoration of the integrity of the membrane and consequently increased activity of ATPases (Zimmer and Doyle, 2006). Furthermore, the ability of the extract to increase Mg level shows its possible potential in increasing vitamin D3, that is, 1,25-dihydroxycholecalciferol (cholecalciferol = Vitamin D3) because the formation of 1,25-dihydroxycholecalciferol involves a magnesium dependent hydroxylase enzyme (Coron et al., 2000).

Table 3 shows an increase in the phosphate level of the rats that that were treated with CCl_4. The phosphate ions released from hydroxyapattite may account for the increase in serum phosphate ion concentration observed in this study. The observed increase in serum phosphate ion concentration suggests that the homeostatic mechanism for phosphate ion concentration has been over-stressed or impaired. However, treatment with the extract decreased the phosphate level. This suggests that the extract might have the potential of influencing metabolic processes especially those involving the buffers in body fluids. Moreover, the decrease in phosphate ion concentration by the extract suggests that the extract induces a heightened use of phosphate ion in the synthesis of phospholipids and phosphoproteins which are all part of the processes involved in liver regeneration (Soetan et al., 2010). This also suggests that the ability of the extract to stabilize membrane might be due to induction of heightened phosphate utilization. The decrease in phosphate ion by the extract suggests that the extract indirectly stimulate 1-alpha hydroxylase enzyme in the kidneys, thereby increasing the conversion of calcidiol to calcitriol, which in turn, increases intestinal absorption of phosphorus that will be used in the synthesis of phospholipids and phosphoproteins.

Serum sodium is a readily available, reproducible, and objective laboratory test that predicts liverrelated mortality and is therefore a reasonable candidate for inclusion in a liver allocation model. The result in Table 3 shows a decrease in the Na^+ level of rats that were treated with CCl_4 when compared with the normal control. Present study also depicts probable inhibition of $Na^+ K^+$ ATPase activity due to experimental hepatic cell destabilization and dysfunction. Hyponatremia has been well described in associations with hepatorenal syndrome,ascites and liver-related mortality (Dale et al., 2008). However, treatment with different concentration of extract showed a dose dependent increase in the serum NA^+ level. This could be attributed to the membrane stabilising effect of the extract which must have reactivated the activity of Na^+ K^+ ATPase. Pretreatment with silymarin also produced significant increase in $Na^+ K^+$ ATPase activity.

Iron, selenium and zinc are essential trace minerals that have a variety of functions. The concentration of these minerals stored in the liver can be measured as an aid in evaluating possible deficiency or toxicity. The present study showed an elevated level of liver iron content in CCl_4 treated rats when compared to normal rats. Iron overload which has been induced by administration of CCl_4 is associated with oxidative stress induced disorders like liver cirrhosis, fibrosis, cancer, anaemia (Sarkar et al., 2012). However, administration of *S. mimosoides* and silymarin reduced the iron level in groups that were treated with the extract and standard drug. The decrease in liver iron deposition induced by treatment with *S. mimosoides* extract shows that it might have iron-chelating potential. There might be possibility that the extract contain constituents that stimulate the proliferation and production of ferritin. Body's iron level is positively correlated with ferritin, a ubiquitous intracellular protein that stores iron in a nontoxic form and also helps prevent iron from mediating oxidative damage to cell constituents. Increase in liver iron is often associated with inflammatory lesions, suggesting that the extract was able to decrease iron concentration using its anti-inflammatory potential as reported by Sakar et al. (2012).

In this research, the serum levels of zinc were significantly lower in rats that were treated with CCl_4 in comparison to controls. Our results confirm findings of decreased serum concentrations of zinc in animal with liver cirrhosis (Schultheiss et al., 2002). A decrease in serum zinc may be the result of inadequate dietary zinc, anorexia, vomiting or various drugs. In addition, a substance known as leucocyte endogenous mediator released from leucocytes during inflammation acts to redistribute the body zinc from the serum to the liver and may produce a drop in serum zinc (Lonnerdal, 2000). However, treatment with different concentration of the extract led to a corresponding increase in the serum level of zinc. This could be attributed to the fact that the extract increases the concentration of protein thereby increasing albumin, transferring and metalloproteins which are known to bind zinc. Proteins generally have positive influence on zinc absorption, because zinc absorption tends to increase with protein intake (McDowell, 2003). Amino acids released from the animal protein keep zinc in solution and protein also binds the phytate known to inhibit zinc bioavailability. Generally, binding of zinc to

Table 4. Total protein content of normal and experimental rats.

Group	Total Protein content (mg/dl)
A	11.64 ± 1.17^{a}
B	8.14 ± 0.74^{c}
C	10.33 ± 0.51^{b}
D	12.74 ± 1.02^{ab}
E	11.27 ± 0.60^{a}
F	4.33 ± 0.67^{d}

Results expressed in mean±SEM (n=5). Values with different letter(s) as superscript across the column are considered significant (P<0.05).

soluble ligands or chelators has a positive effect on zinc absorption as they increase the zinc solubility.

Increase in the concentration of zinc by the extract could cause increased releasing of glutamine from sceletal muscle and also activate glutamine synthetase, which can decrease the level of ammonia and improve hepatic encephalopathy (Choong et al., 2000). That could be explained with the fact that increase in zinc increases the hepatic activity of ornithine transcarbamoylase, a key enzyme of the urea cycle, which consecutively increases urea formation and decreases ammonia levels. Finally, decreased serum concentrations of zinc in rats treated with CCl_4 which have been shown by histopathology to have liver cirrhosis could have an important role in the pathogenesis of this liver cirrhosis and its complications, especially in hepatic encephalopathy (Kumar and Sharma, 2010). Considering all that, the correction of serum trace elements concentrations would have a beneficial effect on some complications of liver cirrhosis and maybe on progression of the disease, so it would be recommendable to provide laboratory analysis of trace elements as a routine.

The data derived from this study indicate a decrease in selenium level in the serum of rats treated with CCl_4 (Table 3). Deficiency of selenium produced experimentally in animals has been reported to result in abnormalities differing among species and varying from defective growth and hepatic necrosis in rats, mice and pigs to myocardial degeneration and muscular dystrophy in sheep, cattle, chickens and horses (Alfrey, 2004). This is due to the fact that Se deficiency is usually associated with increased lipid peroxidation which alters the integrity consequently affecting cell functions. Selenium deficiency is a factor potentiating dietary liver disease presumably by decreased glutathione peroxidase activity. However, treatment with different concentrations of the extract augmented this decrease. This could be attributed to the fact that the extract increases the activity of glutathione peroxidase (a selenium containing enzyme) known to accompany liver impairment. Selenium has been shown to be active in glutathione peroxidase in the red blood cells (Parmar et al., 2010). This also suggests that the extract has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against oxidative injury (Parmar et al., 2010). This decrease in serum selenium and zinc in CCl_4 may be indicative of an interrelationship of these elements and/or their respective metalloenzymes.

Reductions of serum total protein in CCl_4 treated group as shown in Table 4 may be due to formation of protein adduct. It might be that CCl_4 or its toxic metabolites led to covalent modification of cellular target protein, cell death and organ damage (Parmar et al., 2010). These changes may be related to an increased ubiquitin-associated degradation of protein in the hepatocytes induced by CCl_4 toxic stress (Galisteo et al., 2000). This work reports that the plant extract contain antioxidants and hepatoprotective activity through regulatory action on cellular permeability, stability and suppressing oxidative stress. Hypoproteinaemiain CCl_4 treated group might be due to an increase in amino acid oxidative metabolism. This result is in agreement with Shahjahan et al. (2004) who found a decrease in serum total proteins in CCl_4-administered rats and suggested that it was due to the decrease in the functional ability, that is, synthetic role of the liver in CCl_4-administered rats. The pre-treatment of rats with *S. mimosoides* extract and silymarin effectively protected the liver from these CCl_4-induced alterations in serum protein. These data suggest that the constituents of *S. mimosoides* extract reduced protein degradation and afforded the same protection to hepatocyte intermediary metabolism as did silymarin a standard anti-hepatotoxic drug. However, the effect of the extract at higher concentration is more than that of silymarin. Moreover, the increase in protein concentration in the groups that were treated with the extract suggests that the extract might contain constituents that stimulate the regeneration of hepatic tissue which increased protein synthesis in damaged liver and improved the functional statues of the liver cells.

From Figure 1, the extract significantly increased the activity of lactase in a dose dependent manner. This was observed by the increase in the concentration of glucose liberated. This shows that the extract might be useful in the treatment of lactose intolerance. Lactose intolerance is inability to tolerate and digest lactose which arises from lack of lactose (Kumar and Subrahmanyam, 2013). Human milk has the highest concentration of the disaccharide lactose. The extract might have the potential of stimulating the genes that codes for lactase leading to the synthesis of lactase as preproprotein with five domains namely signal sequence, proprotein domain, enzymatic domain, membrane spanning anchor domain and hydrophobic C-terminal domain (Yu and Keeffe, 2002). Moreover, it might be that the extract enhances the activity of a transport protein that is known to bind

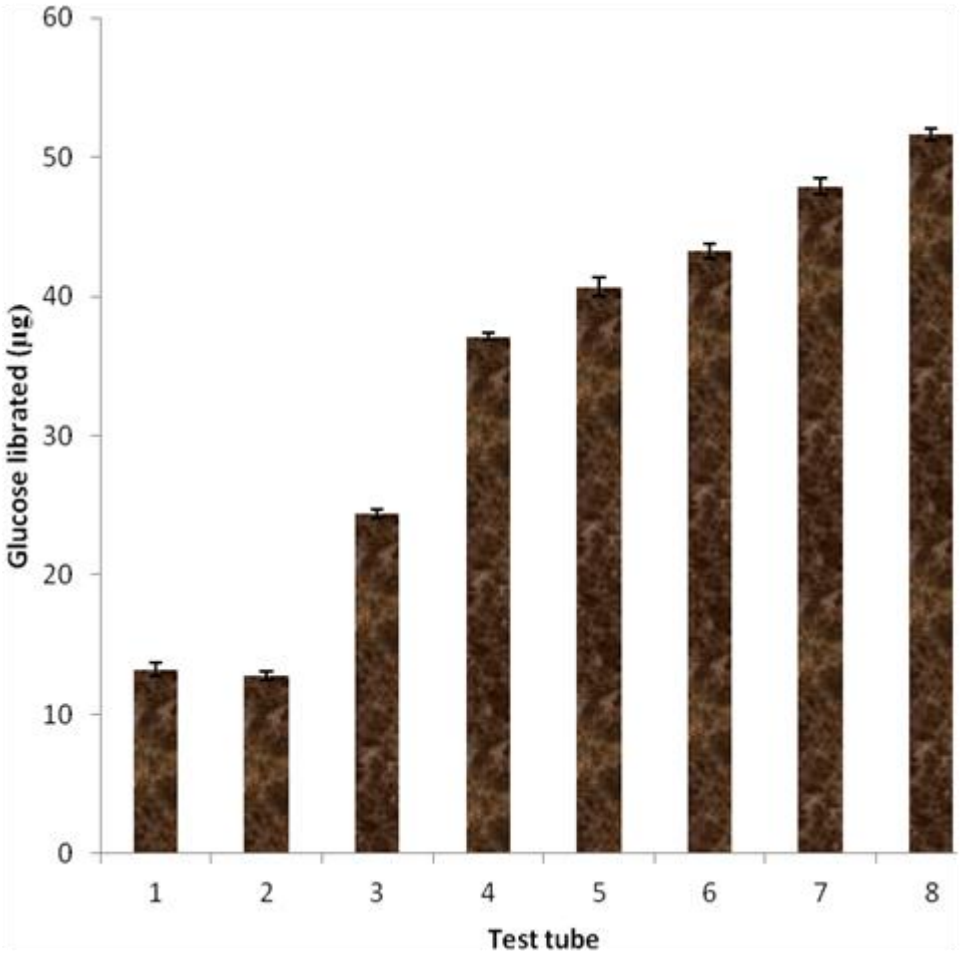

Figure 1. Effect of extract on the activity of lactase. The values are expressed as (Mean±SD) n=5 per group $p<0.05$

sodium, glucose (or galactose) and approximately 260 water molecule; sodium moves down its electrochemical gradient and brings glucose or galactose in against concentration gradient (Yu and Keeffe, 2002). The ability of the extract to increase lactase activity suggests that it might enhance enterocyte maturation as lactase is known to be a marker of enterocyte maturity.

Conclusion

On the basis of the available data in this report, it can be suggested that *S. mimosoides* leaf extract elicit protection against CCl_4-induced hepatic and oxidative damage in rats possibly by acting as an in vivo free radical scavenger or through induction of antioxidant enzymes and drug detoxifying enzymes. *S. mimosoides* extract can also be said to be electrolyte-inducing, as such it stimulates increases in useful ions that are necessary for metabolism especially in cases of hepatoxicity. The ability of the extract to induce electrolytes enabling homeostasis, restore the function of compromised liver and roles in activating lactase justifies its use in folklore medicine in treating breast milk toxicity in neonates.

Further studies need to be conducted to isolate and purify the active principle involved in the hepatoprotective and immunomodulatory activity of this plant; as well as evaluate the potential usefulness of this extract in clinical conditions associated with liver damage.

SIGNIFICANCE STATEMENT

Studies on the leaves of *S. mimosoides* showed presence of alkaloids, phenolics, flavonoids and other bioactive compounds that may possess strong antioxidant properties, which from the results helped in the alleviation of liver toxicity. These studies also showed the potential of *S. mimosoides* to stimulate the increases in elemental ions and its ability to activate lactase activity.

This finding will enable researchers trace the mechanisms of action which may include how these lead extracts function in the human body to alleviate liver and possibly kidney damage as shown in certain liver biomarkers. This research finding is able to further advance the growing interest in the use of natural products as antioxidants in combating oxidative stress. More importantly, these findings serves as a path that would enable researchers and drug markers produce drugs that would more efficaciously combat lactose intolerance and wipe-off the menace of breast milk toxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alfrey AC (2004). The role of abnormal phosphorus metabolism in the progression of chronic kidney disease and metastatic calcification. Kidney International 66:13-17.

Association of Official Analytical Chemists (AOAC), (2005).Official method of analysis of AOAC INTERNATIONAL 18th Edition. AOAC International, Gaithersburg. pp. 49-59.

Bury D, Jelen P, Kalab M (2001).Disruption of Lactobacillus delbrueckiissp. Bulgaricus11842 Cells for Lactose Hydrolysis in Dairy Products: A Comparison of Sonication, High Pressure Homogenization and Bead Milling. Innovative Food Science and Emergency Technology 2(1):23-29.

Cole DS, Morgan BP (2003).Beyond lysis: how complement influences cell fate. Clinical Science 104:455-466.

Choong S, Walkden M, Kirby R (2000).The management of intractable haematuria.Biochemical Journal of Urology 86:951-959.

Coron M, Young C, Beynon H(2000).Calcium metabolism in sarcoidosis and its clinical implications.Rheumatology 39:707-713.

Dahlqvist A (1968). Assay of intestinal disaccharidases. Analytical Biochemistry 22:99-107

Dale DC, Boxer L, Liles CW (2008). The phagocytes: neutrophils and monocytes. Blood 112:935-945.

Dilworth LL, Omoruyi FO, Asemota HN (2005). Digestive and absorptive enzymes in rats fed phytic acid extract from sweet potato (ipomoea batatas).Diabetologia Croatica 34(2):58-65.

Ekwueme FN, Oje OA, Ozoemena NF, Nwodo OFC (2011). Anti-inflammatory effect of the aqueous leaf extract ofSenna mimosoides in membrane stabilization and inhibition of phospholipase A_2 activity. Journal of Pharmacy Research 4(7):1973-1976.

Ekwueme FN, Oje OA, Nwodo OFC, Ozoemena NF (2011). Anti-inflammatory capacity of the aqueous leaf extract of Senna mimosoides on inhibition of rat oedema, platelet aggregatory activity and prostaglandin synthase activity. Journal of Medicinal Plants Research 5(14):3028-3036.

Galisteo M, Sua´rez A, Montilla MP, Utrilla MP, Jime´nez J, Gil A Faus MJ, Navarro MC (2000).Antihepatotoxic Activity of Rosmarinustomentosusin a Model of Acute Hepatic Damage Induced by Thioacetamide.Phytotherapy Research 14:522-526.

Harbourne JB (1998). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. 2nd edition London: Chapman and Hall Ltd. P 282.

Heikal TM, El-sherbiny M, Hassan SA, Arafa A, hanem HZ (2012). Antioxidant effect of selenium on epatotoxicity induced by chlorpyrifosinmale rats. International Journal of Pharmacy and Pharmaceutical Sciences 4:603-609.

Huang C, Kuo E (2007).Mechanism of Hypokalemia in Magnesium Deficiency. Journal of American Society of Nephrology 18(10): 2649-2652.

Jokar L, Karbassi A (2009).Determination of Proper Conditions for the Production of Crude Beta-galactosidase Using *Lactobacillus delbrueckii* ssp. bulgaricus. Journal of Agricultural Science and Technology 11:301-308

Joy PP,Thomas J,Mathew S, Skaria BP (1998). Medicinal plants. Keriala publishers. pp 1-211.

Kumar V, Sharma A (2010). Neutrophils: Cinderella of innate immune system. International Immunopharmacology 10:1325–1334.

Kumar GVP, Subrahmanyam SN (2013). Phytochemical analysis, in-vitro screening for antimicrobial and anthelmintic activity of combined hydroalcoholic seed extracts of four selected folklore indian medicinal plants. Der Pharmacia Lettre 5(1):168-176.

Lonnerdal B (2000). Dietary factors influencing zinc absorption. Journal of Nutrition 130:1378-1383.

Lubran MN (1978). The measurement of total serum proteins by the biuret method.American Clinical Laboratory Science 8(2):106-110

McDowell LR (2003). Minerals in Animal and Human Nutrition. 2nd ed. Amsterdam: Elsevier Science. P 660.

Middleton EJR, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacologycal Review 52:673-751.

Murray KF, Hadzic N, Wirth S, Bassett M, Kelly D (2008).Drug-related hepatotoxicity and acute liver failure.Journal of Pediatrics and Gastroenterological Nutrition 47:395-405.

Parmar SR, Vashrambhai PH, kalia K (2010).Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. Journal of Herbal Medicine and Toxicology 4(2):101-106.

Pradeep K, Mohan CVR, Gobianand K, Karthikeyan S (2007).Silymarin modulates the oxidant–antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. European Journal of Pharmacology 560:110–116

Robbins A (2000). Life balance pack. P 1-16. TonnyRobbins.com/inner balance.

Sarkar R, Hazra B, Mandal N (2012). Iron-Overload-Induced Liver Toxicity in Mice.Evidence-Based Complementary and Alternative Medicine 12:1-9

Schultheiss PC, Bedwell CL, Hamar DW, Fettman MJ (2002). Canine liver iron, copper, and zinc concentrations andassociation with histologic lesions. Journal of Vetirinary Diagnostic Investigation 14:396-402

Shahjahan M, Sabitha KE, Jainu M, Devi CSS (2004). Effect of Solanumtrilobatumagainst carbon tetrachloride induced hepatic damage in albino rats. Indian Journal of Medicinal Research 120:94-198

Singh A, Bhat TK, Sharma OP (2011).Clinical Biochemistry of Hepatotoxicity. Journal of Clinical Toxicology 4:1-9.

Singh N, Gandhi M (2011).Altered levels of fibrinogen in relation to the pathophysiology of recurrent spontaneous abortions. Obstetrics and Gynaecology 2:1-14.

Soetan KO, Olaiya CO, Oyewole OE (2010). The importance of mineral elements for humans, domestic animals and plants: African Journal of Food Science 4(5):200-222.

Vasiljevic T, Jelen P (2001).Production of Beta-Galactosidase for Lactose Hydrolysis in Milk and Dairy Products Using Thermophilic Lactic Acid Bacteria.Innovation in Food Science Emergency Technology 2(1):75-85.

Young T (2000). Vegetation list of Mpala Research Center and Conservancy.

Yu AS, Keeffe EB (2002).Nonalcoholic fatty liver disease.Revolution on Gastroenterological Disorder 2:11-19.

Zimmer J, Doyle DA (2006). Phospholipid requirement and pH optimum for the in-vitro enzymatic activity of E.coli P-type ATpaseZntA. Biochimica et Biophysica Acta 1758(5): 645-652.