PharmacologyOnLine August 30, 2014

Archives • 2014 • vol.2 •38-47

PROTECTIVE EFFECT OF *AUTRANELLA CONGOLENSIS* AND *SAPIUM ELLIPTICUM* STEM BARK EXTRACTS AGAINST HEPATOTOXICITY INDUCED BY THIOACETAMIDE

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Abstract

Autranella congolensis and *Sapium ellipticum* are two medicinal plants used in traditional medicine for the treatment of several ailments including liver injuries. In this study, the hepatoprotective activities of these extracts at two different doses (100 and 200 mg/kg) on rat model of thioacetamide induced hepatotoxicity was assessed by investigating biochemical and histopatological markers after 29 days of treatment.

Elevated serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, total bilirubin and direct bilirubin observed in thioacetamide toxic groups were restored toward the normal values, when animals received extract treatment at 100 and 200 mg/kg. Administration of thioacetamide lowered significantly the level of liver antioxidants markers superoxide dismutase, catalase, glutathione, and increased the level of lipid peroxidation, which were moderated in group of animals treated with both extracts at 100 and 200 mg/kg. The activity of liver microsome CYP2E1 was significantly high when animals received thioacetamide treatment; both extracts reduced the increase in enzyme activity. The histological and biochemical changes exhibited by *Autranella congolensis* and *Sapium ellipticum* extracts on toxic animals were comparable to those obtained with standard silymarin.

The hepato-protective effects of SE and AC extracts observed in this study showed that these two medicinal plants are potential sources of anti-hepatotoxic drugs.

Key words: Liver, thioacetamide, CYP2E1, *Autranella congolensis* and *Sapium ellipticum*

Introduction

Liver diseases, generally caused by infectious agents or ingestion of toxic foods, chemicals and overdose of drugs (1-4), are considered as a major health problem worldwide. The susceptibility of the liver to chemical attack is mainly due to its role as the primary organ which comes in close contact with many harmful substances, since it is continuously exposed to environmental toxins and infectious agents; thus, maintaining a healthy liver is a challenge for overall health and well-being. The liver is a vital organ in the body with several biochemical and physiological functions. In the liver, all major metabolic pathways involved in growth, fight against disease, nutrient supply, energy provision and reproduction occur (5). Liver is involved in energy metabolic processes, defence, detoxification and excretion of many harmful metabolites (6). Liver is equipped with a battery of microsomal enzymes, which catalyse the conversion of xenobiotics and others unwanted metabolites. The biotransformation of metabolites by the liver improves their solubility and therefore their excretion. Nevertheless, some of these metabolite derivatives are toxic to the cell, and in some cases can induce hepatocellular carcinoma (7).

Thioacetamide (TAA) is hepatotoxican agent that is converted by the liver enzymes to highly reative toxic S-oxide derivatives which cause centrolobular necrosis (8). These derivatives interfere with the movement of RNA from the nucleus to the cytoplasm which leads to membrane injury, therefore they reduce the number of viable hepatocytes as well as rate of oxygen consumption. Moreover, TAA decreases the volume of bile and its content (bile salts, cholic acid and deoxycholic acid). Rodents intoxicated with TAA have been validated as good experimental model of liver cirrhosis and fibrosis, and have been used for evaluation of antihepatotoxic drugs (3, 9, 10). It is well known that the mechanism of liver toxicity by TAA involved free radical chain reactions. Proteomic analysis of TAA induced hepatotoxicity and cirrhosis in rat livers have been revealed that administration of TAA down-regulates the enzymes of the primary metabolic pathways such as fatty acid beta-oxidation, branched chain amino acids and methionine breakdown, whereas it up-regulates proteins on the other hand, that are related to oxidative stress and lipid peroxidation (11). Under oxidative stress conditions, the cascade of reactions induced by pro-oxidant leads to the degeneration of macromolecules such as nucleic acids, proteins and lipids. In the liver, the peroxidation of membrane lipids is the start point of tissue necrosis. The liver markers such as transaminases, γ-glutamyltransferase, bilirubin, proteins and lipids are released in the blood, as result of cell leakage, and therefore the measure of the specific serum markers of the liver can be used for diagnosis of liver injuries (12).

An excess of free radicals contribute to the failure of the antioxidant defence system of the body, and therefore initiate the oxidative damage. Targeting the origin of the pathology by stopping the free radical cascade reactions, and/or stimulating cell regeneration seem to be an appropriate therapeutic intervention. Under normal conditions, hepatocytes have the ability to regenerate themselves; so that normal liver tissue is replaced by scars and areas of regenerating liver cells (13). The liver has many cell units responsible for the same task. Therefore, if one area is injured, other cells will perform the functions of the injured section indefinitely or until the damage has been repaired. Up to date, development of a safe synthetic product which can solve the problem of liver injury is hallmark to pharmaceutical industries, despite advances in modern medicine. Many product available commercially are from herbal origin. These products are not accessible to all patients, and because the efficacy of several other plants against injuries has been proved in traditional medicine, their standardization may boost or supplement the existing products. *Sapium ellipticum* (Euphorbiaceae) and *Autranella congolensis* (Sapotaceae) are examples of plants which are used in Cameroon by Grassfield and Forest people respectively for the treatment of various ailments, including liver diseases. The scientific basis of their utilisation is not yet proved. Their phytochemcal investigation has shown the presence of phenolic derivatives and high antioxidant content. This study aimed to evaluate *in vivo*, their hepatoprotective activity, using thioacetamide model.

Methods

Collection of plant materials and preparation of the extracts

Based on ethno-botanical surveys, *Autranella congolensis* (AC) and *Sapium ellipticum* (SE) were harvested in East (Forest) and West (Grassfield) regions of Cameroon respectively, and were

identified at Cameroon National Herbarum where voucher specimens were kept. Fresh plant materials were collected and dried at room temperature in an aerated room. Then they were grinded to yield a powder. Five hundred grammes of the bark powder were macerated with 2.5 L of methanol for 48 hours. After filtration, the filtrate was evaporated, and the crude extracts were completely dried. The extraction yields were 11.4 % and 16 % for AC and SE respectively. For acute toxicity and hepatoprotective activity, the stock solutions were freshly prepared by dissolving the dried extract of each plant in 0.5 % gum acacia.

Acute toxicity

Acute toxicity study was performed for plant extracts according to the acute toxic classic method as per OECD guidelines-425 (14). This test also helped to select the dose for evaluation of hepatoprotective activity on rats. Nine female *Swiss* mice were kept fasting for overnight providing only water, after which each extracts was administered orally to 3 of them at a single dose of 2000 $mg.kg^{-1}$ body weight (b.w.) and observed for 24 hours. The extra 3 animals served as normal control and received 0.5 % gum acacia solution. After the administration of the extracts, food was withheld for further 3 to 4 hours. Animals were observed individually once during the first 30 mn after dosing, periodically during the first 24 hours (with more attention during the first 4 hrs), and daily for a period of 14 days. For both extracts, no behaviour changes, neither mortality, nor toxicity sign were recorded. The extracts were considered safe at this dose. Then, one tenth (1/10) and one twenty (1/20) of this maximum experimental dose (i.e. 200 and 100 mg/kg b.w. respectively) were selected for evaluation of hepatoprotective activity.

***In vivo* screening of hepatoprotective activity**

Experimental animals and ethical consideration

Adult *Wistar* albino rats (four weeks) of either sex weighing between 150-200g were used for the study. They were housed in cleaned animal room, in temperature control environment (25±1^{0}C), relative humidity, with regular 12 h light/12 h dark cycle. All animals were fed with standard rat diet, and water *ad libitum*.

The protocol of experimentation was approved by the Institutional Animal Ethical Committee (Ref:SSCPT/IAEC.Clear/141/2012-13) of Sree Siddganga College of Pharmacy, Tumkur, Karnataka, India.

Study design

The hepatoprotective activity of plant extract and product were tested using thioacetamide as hepatotoxican. A total of 45 animals were divided into nine (09) groups of five (05) animals each, and they were treated as indicated below.

Group 1(G1) served as a normal control, and received 1 ml of 0.5% gum acacia (p.o.) alone daily for 29 days. Group 2 and 3 (G2 &G3) served as extract controls and were treated with SE and AC extract alone respectively (200 mg/kg, p.o.), daily for 29 days. Group 4(G4) served as toxic control, and were treated alone with 1 ml of 0.5% gum acacia (p.o.) daily for 29 days. Group 5 and 6 (G5 & G6) served as SE treated groups. They received *Sapium ellipticum* extract at low (100 mg/kg, p.o.) and high (200 mg/kg, p.o.) dose respectively daily for 29 days. Group 7 and 8 (G7 & G8) served as AC treated groups. They received *Autranella congolensis* extract at low (100 mg/kg, p.o.) and high (200 mg/kg, p.o.) dose respectively daily for 29 days. Group 9 known as standard drug were treated with Silymarin (100 mg/kg, p.o.) daily for 29 days. Groups 1, 2 and 3 were also treated alone with normal saline (0.5 ml, s.c.), 1 hour after treatment with extract or gum acacia every 72 hours for 29 days, whereas groups 4, 5, 6, 7, 8 and 9 were treated with thioacetamide (s.c., 50 mg/kg) under the same conditions.

Preparation of samples for biochemical and histological analysis

On the day 30th, the overnight fasted animals were anesthetized with ether and the blood was collected by puncturing the orbital plexus and was allowed to coagulate on ice for 30 min. Serum was separated by centrifugation at 3500 rpm for 10 min and analyzed immediately for various biochemical markers. Next day later, rats were scarified by decapitation; the livers of all animals were perfused with ice cold phosphate buffer saline (PBS), removed and processed for other investigations. The hepatoprotective activity was evaluated biochemically and histopathologically.

Estimation of serum biochemical parameters

The total serum protein (TP), total Bilirubin (TB), direct bilirubin (DB), serum albumin (ALB), Serum Glutamate Oxaloacetate Transaminase (GOT) and

serum Glutamate Pyruvate Transaminase (GPT) were quantified using the sero-diagnostic kit (ERBA Diagnostics Mannheim, Germany). Assays were carried out according to the protocol indicated by the manufacture, and analysed using a semi-automatic analyser (QUALISYSTEM).

Estimation of liver antoxidant markers

Liver homogenates were prepared. A piece of 500 mg of the fresh liver was cut and homogenized with ice cold PBS. The homogenates were stored at -20 ºC for not more than 4 days, and they were used to assess the antioxidant contents.

Estimation of liver proteins

Liver proteins were estimated by the method of Lowry (15). The working reagent called copra-alkaline solution was prepared by mixing 48 ml of 2% Na_2CO_3, 1 ml of 1% potassium sodium tartrate and 1 ml of 0.5% copper sulfate. This solution was freshly prepared and kept for 2 hours before use. The reagent (2 ml) was mixed with 100 µl of homogenate and the tubes were incubated for 10 minutes at room temperature. Then, 200 µl of 1N Folin reagent was added, and the tubes were vortex immediately, incubated at room temperature for 30 minutes. The absorbance was read at 600 nm using a UV / visible double beam spectrophotometer (LABINDIA). The standard protein Bovine Serum Albumin was used to prepare the calibration curve.

Estimation of reduced glutathion (GSH)

The reduced glutathione was estimated by the method described by Moron *et al.* (16). GSH react with DTNB to give a yellow coloured complex with maximum absorption at 412nm. To 0.5 ml of (10%) homogenate, 125 µl of 25% TCA was added to precipitate protein. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 0.6 ml of TCA, centrifuged for 10 minutes at 1500 rpm and 0.3ml of resulting supernatant was taken for GSH estimation. The volume of the aliquot was made up to l ml with 0.2 M phosphate buffer. Then 2 ml of freshly prepared 0.6mM DTNB (HIMEDIA) was added to the tubes and intensity of yellow colour formed was read at 412 nm. Standard curve of GSH (Himedia) was prepared using concentrations varying from 5 to 100 nM in 5% TCA for assay. Values were expressed as nmol/mg protein.

Estimation of lipid peroxidation (LPO)

The LPO was assessed by estimating the thiobarbitturic acid reactive substances (TBARS), equivalent to the end product of lipid peroxidation Malone dialdehyde (MDA) according to the method of An aliquot of 1.0 ml of the sample homogenate was combined with 2.0 ml of the reagent (equivalent volumes of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25N Hydrochloric acid) and mixed thoroughly. The solution was then heated on a boiling water bath for 15 min. The resultant flocculent precipitate was removed by centrifugation. The absorbance of the supernatant was read at 532 nm against a blank. The concentration of the TBARS was determined using a molar extinction coefficient of MDA ($1.56\times10^5.M^{-1}.cm^{-1}$) and the results were expressed as nmoles MDA/mg protein.

Estimation of catalase (CAT)

Catalase activity was estimated according to the method of Beers and Sizer (17) with slight modifications. The working reagent was 10.3 mM hydrogen peroxide solution in phosphate buffer (pH 7). In a cuvete were immediately mixed 950 µl of reagent and 50 µl of diluted homogenate. The absorbance was recorded at 240 nm during the first 30 seconds with 10 second interval. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2. A Unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH = 7.0 at 25° C.

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was estimated using the enzyme inhibition of the oxidation of epinephrine (18). The superoxide anion (O_2^-) substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O_2^- builds in the solution, the formation of adrenochrome accelerates because O_2^- also reacts with epinephrine to form adrenochrome. To 925 µl of 50 mM sodium carbonate buffer (pH 10.25, with 0.1 mM EDTA) were added 50 µl of homogenate and 25 µl of 9.74 mM adrenaline bitartrate (freshly prepared in 10 mM HCl). The absorbance was immediately recorded at 480 nm within 5 min with 30 seconds interval. The initial rates were computed from the graph Abs vs time and the percentage of inhibition was computed. A unit of SOD activity is defined as that amount of SOD

required to cause 50% inhibition of the oxidation of the epinephrine (SOD_{50}).

Estimation of CYP2E1 activity

The CYP2E1 activity was estimated in liver microsomal fractions prepared as described below.

Preparation of the microsomal fraction

Microsomal fractions of the liver were prepared by calcium precipitation and low speed centrifugation methods (19). A piece of 1 g on the liver was cut and washed with ice cold PBS. The excess of the liquid was absorbed with tissue paper. The tissue was cut into small pieces and homogenized with 10 ml of ice cold PBS, then centrifuged at 1,000 g for 10 min at 4ºC. The supernatant was further centrifuged at 12,000 g for 15 min at 4ºC, and the second supernatant known as post mitochondrial fraction (PMF) was collected. A volume of 8mM calcium chloride solution equivalent to 7.5 times the total volume of the PMF was added dropwise, to PMF upon agitation. The mixture was further centrifuged at 8000 g for 10 min at 4ºC. The pellet was collected after decantation and dissolved in 0.3 ml of sodium phosphate buffer (pH 6.8), then homogenized completely. The protein content was assessed and adjusted to 2 mg/ml by diluting with buffer.

Assessment of CYP2E1 activity

Activity of microsomal CYP2E1 enzyme was estimated by kinetic Assay for p-nitrophenol hydroxilase described by Allis *s* (1994) with slight modifications. To 1 ml of PNP (0.25 mM) in 1oo mM sodium phosphate buffer (pH 6.8) was added 25 µl of microsome fraction. The mixture was incubated at 37ºC for 5 min, and the reaction was initiated by adding 1 ml of NADPH (2.5 mM) in sodium phoasphate buffer, then incubated for another 5 min. Measurement of absorbance was performed for 12 min with 3 min interval at 480 nm.

Statistical analysis

The results were expressed as mean value ± SEM. Each parameter was analyzed separately using ANOVA followed by Dunnets 't' test for statistical significance.

Results and discussion

Administration of the toxic dose of TAA to animals altered significantly serum and liver morphological and biochemical markers of hepatotoxicity.

The methanolic extract of SE and AC were observed to exhibit hepatoprotective effect by inducing significant changes in liver function markers and by preventing liver histopathological changes in rats treated with TAA, therefore sustaining the safety of these extracts pre-established in acute toxicity tests.

Results of relative weight of the liver and serum markers of hepatotoxicity are indicated in table 1. It was found that the s.c. injection of TAA to animal results in significant increase of relative weight of the liver, and serum level of GPT, GOT, ALP, TB and DB. However, significant decrease in serum level of TP and ALB in another hand. These changes were not surprising, since it is well known that liver cell damage induced by TAA and other toxicants lead to the release of livers specific markers such as ALP, GOT and GPT, impaired metabolism and excretion of bilirubin and impaired synthesis of albumin (12, 20, 21). Serum GOT, GPT, ALP and billirubin are sensitive markers of hepatocellular injury. When the liver cell is injured or dies, these proteins can leak through the liver cell membrane into the circulation and this result in the rise of their serum level, as observed in the toxic group. The rises in transaminases, ALP and billirubin observed in TAA treated animals of this study were comparable to those obtained by previous authors (22-24), indicating that the study were carried out on proper cirrhosis and fibrosis model.

At the dose 100 and 200 mg/kg, both extracts exhibited significant decrease in GPT and GOT level compare to toxic group. Serum GPT (liver cytosolic enzyme) levels are very high in patients of viral hepatitis and hepatic necrosis, 10 to 200 fold higher in patients of post hepatic jaundice, intrahepatic cholestasis and below 10 fold in patients of metastatic carcinoma, cirrhosis and alcoholic hepatitis. GOT is a mitochondrial enzyme released from heart, liver, skeletal muscles and kidney. The serum levels are 10 to 200-fold elevated in patients with acute hepatic necrosis, viral hepatitis.

Administration of AC and SE extracts to intoxicated animals decreased significantly the serum level of these enzymes in toxic groups of animals, showing a protective effect of those extracts on the studied model. Significant increase of TP and ALB observed in the extract treated groups compared to toxic group suggested that some components of these extracts may induce the protein synthesis.

Liver cells synthesize albumin, fibrinogen,

prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha foetoproteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage.

Serum alkaline phosphatase (ALP) is another non-specific marker produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. Elevated ALP levels in toxic group of rats may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. The protective effect of SE and AC extract was expressed by significant decrease of the level of ALP in serum.

Estimation of bilirubin, metabolic product of the breakdown of heme is one of the better liver function tests. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in hemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilbert's disease. The capacity of both extract to reduce serum TB compared to toxic group was an additional prove of their hepatoprotective activity.

Liver enzymatic and non-enzymatic antioxidants were also investigated and results are illustrated in figure 1. All oxidative stress markers of the liver were significantly altered in group of animals treated with TAA. Compared to normal control, significant increase of GSH, CAT and SOD as well as significant decrease of LPO were observed on toxic control animals. These changes were progressively restored on toxic group which were treated with SE and AC extract.

The methanolic extract of AC and SE enhanced the activities of antioxidant enzymes (SOD, CAT) and GSH. They also reduced the strength of lipid peroxides against TAA-induced hepatotoxicity in these animals, suggesting the reduction of oxidative stress in this scenario plays a role in mechanism of their hepatoprotective effect.

Free radicals are reactive molecules involved in many physiological processes and human diseases such as cancer, aging, arthritis, Parkinson syndrome, ischaemia, toxin induced reaction, alcoholism, liver injury etc. The damage to hepatic parenchymal cells, leading to hepatic injury, is due to oxidative stress within the cells caused by Reactive oxygen species (ROS).

The direct estimation of free radicals is not a routine laboratory work due to their transient nature.

This was done indirectly by measuring the levels of LPO, antioxidant enzymes (CAT and SOD) and metabolite (GSH) which are the markers of the defence status of the livers. The elevation of LPO levels observed in toxic group during the liver damage can be link to enhanced production of free radicals and decreased scavenging potential of the cells. The later was illustrated by the low levels of antioxidant enzymes (SOD, CAT) and metabolites (GSH). It is important to remember that a variety of intrinsic antioxidants such as GSH, SOD, GST and peroxydases are present in the organism, and their role is to protect the cells from oxidative stress. However, the protective effect of both extracts on liver damage was expressed by a decrease of the LPO level as well as increase of antioxidants enzymes and metabolites levels in extract treated groups. This observations suggested that AC and SE extracts protect the liver against oxidative damage, either directly by scavenging the reactive oxygen species or indirectly by inducing the endogenous antioxidant system pathways.

The injection of TAA to animals also induced a significant increase in the amount of CY2E1 enzymes compare to control. It has been shown that this enzyme contribute to the activation of TAA to its toxic metabolite. Studies investigated by Kang *et al.*(25) on CYP2E1 knockout mice showed that CYP2E1 mediates TAA-hepatotoxicity in wild type mice as a result of increased oxidative stress. It has been postulated that TAA mediates liver injuries by interfering with RNA translocation from the nucleus to the cytoplasm. Biotransformation of chemical toxicants and carcinogens such as TAA is mediated by enzymes belonging the cytochromes P450 (CYP) system. TAA is bioactivated by CYP systems to sulfine (sulfoxide) and sulfene (sulfone) metabolites (26, 27). CYP is a heme containing enzyme that catalyzes the oxidation of a wide variety of endogenous and exogenous compounds (28). CYP2E1 induction is associated with elevated hepatotoxicity (29). Experiment had shown that treatment of rodents with thioacetamide (TAA) induced liver cell damage, fibrosis and/ or cirrhosis, associated with increase of oxidative stress and activation of hepatic stellate cells (27, 30). Against a significant decrease of the level of CYP2E1 was found after treating the animals with crude extracts of AC and SE, which could explain the reduction of TAA toxicity in these group of

animals. The above observations were also confirmed on tissue slices (See Figure 2).

The hepatoprotective activity of AC and SE extracts was well illustrated by histopathological changes (Figure 2). The histoarchitecture of normal control group (Figure 2, G1), AC and SE control groups (Figure2, G2 & G3) animals showed normal cells. Histopathological analysis of the H & E-stained liver tissue sections of the TAA-treated group revealed cloudy swelling and fat cells and degeneration of hepatocytes (Figure 2, G4). Periportal necrosis of cells was also observed with complete degeneration of liver cells, broken cell pieces, irregular appearance due to oozing of cell materials and cell death. In the group of animals treated with TAA with AC or SE extract (100 & 200 mg/kg), normal and affected areas of liver in patches adjacent to each other, was observed (Fig 2, G5, G6, G7 & G8). The degenerative changes, necrosis, were less observed and consequent mild leucocytes infiltration noted in the groups treated with these SE and AC extracts which is essential for self-immunity confirming the hepatoprotective effect. These results provide evidence to SE and AC as hepatoprotective plants, are in conformity with the previous reports that demonstrated the potential hepatoprotective of plant extracts on TAA model (24, 31-39).

The protective effect of AC and SE methanol crude extract observed in the present study justify scientifically the use of these two plants by traditional healers for the treatment of various ailments involving. Further studies required for the identification of actives ingredients.

Acknowledgments

The authors thank the Centre of International Co-operation in Sciences (CICS), Chennai, India, to provide Research Training Fellowship for developing Country Scientists (RTFDCS) to one of the authors (AJN). They also thank the Sri Siddaganga College of Pharmacy, Tumkur, Karnataka, India for providing research infrastructures, and to the pathologist Dr. Vishl for his expertise in histopathological studies. They are greatfull to the African Phytomedcicine Centre “Alango Foundation”, Dschang, Cameroon for the partially funding of the study.

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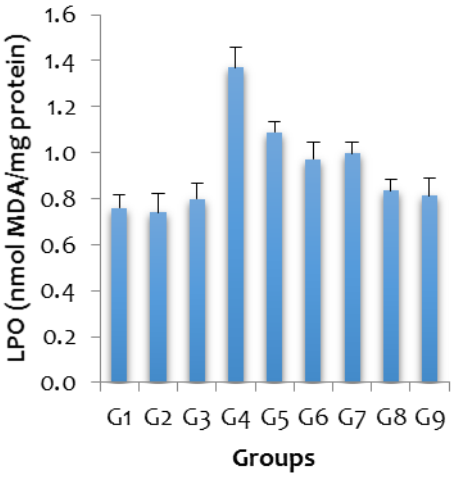
Table 1: Relative liver weight (RLW) and serum parameters of animals after 30 days of treatment

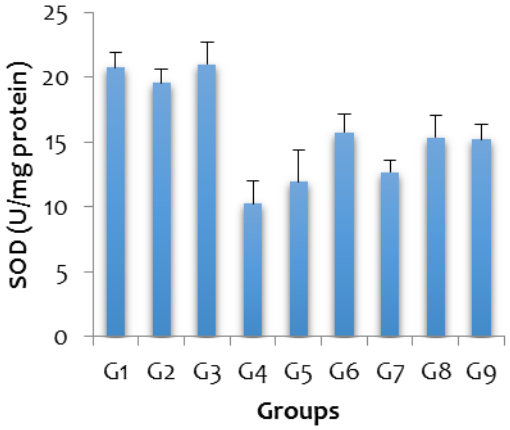
Groups	Parameters						
	RLW (g/100 g b.w.)	GPT (UI/l)	GOT (UI/l)	ALP (UI/l)	TP (mg/dl)	ALB (mg/dl)	TB (mg/dl)
G1	3.27± 0.19[b]	67.05±8.67[b]	167.60±15.04[b]	143.25±6.59[b]	7.56±0.07[b]	3.57±0.13[b]	0.24±0.02[b]
G2	2.89±0.12[b]	61.02±3.41[b]	154.57±12.82[b]	142.12±11.82[b]	8.18±0.07[b]	3.57±0.17[b]	0.27±0.02[b]
G3	3.06±0.22[b]	63.87±9.19[b]	146±17.01[b]	148.56±18.65[b]	7.89±0.11[b]	3.47±0.09[b]	0.26±0.03[b]
G4	3.75±0.27[a]	222.94±13.64[a]	372.28±9.45[a]	300.68±25.51[a]	6.38±0.20[a]	2.62±0.09[a]	0.68±0.07[a]
G5	2.00±0.42[b]	105.50±11.75[b]	291.30±24.64[b]	396.88±28.28[b]	7.48±0.15[b]	3.56±0.07[b]	0.41±0.04[b]
G6	3.59±0.41	81.58±5.75[b]	190.10±13.93[b]	292.73±41.85	7.98±0.18[b]	3.55±0.27[b]	0.48±0.06[b]
G7	3.00±0.18[b]	131.77±14.66[b]	247.02±11.30[b]	318.98±36.29	7.73±0.18[b]	3.42±0.10[b]	0.38±0.03[b]
G8	3.13±0.29	102.56±10.66[b]	211.34±8.39[b]	247.40±15.40[b]	7.63±0.30[b]	3.54±0.19[b]	0.43±0.02[b]
G9	3.08±0.35	81.38±9.85[b]	161.20±13.34[b]	254.07±31.84[b]	7.76±0.18[b]	3.63±0.25[b]	0.51±0.03[b]

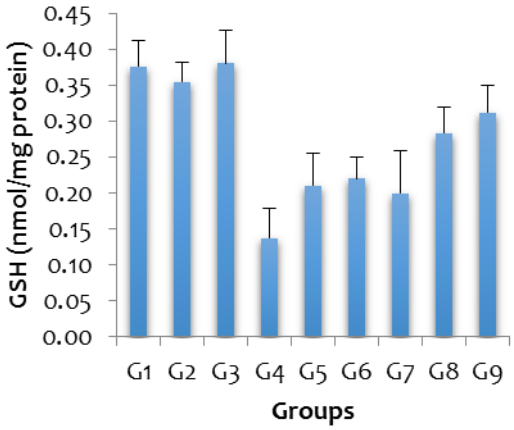
a: significantly different (P<0.05) to normal control (G1)

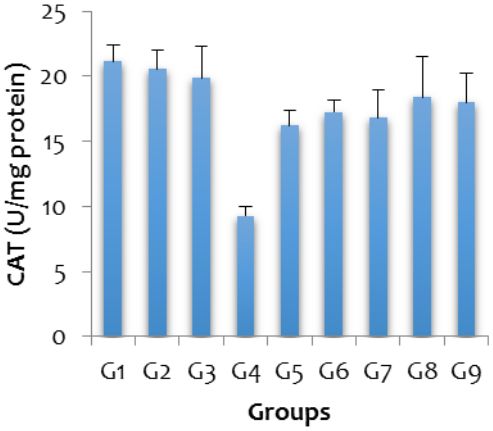
b: significantly different (P<0.05) to toxic control (G4)

G1: Normal control; G2: AC control (200 mg/kg b.w.); **G3**: SE control (200 mg/kg b.w.); **G4**: Toxic control, TAA (50 mg/kg b.w, s.c); **G5**: SE (100 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G6**: SE (200 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G7**: AC (100 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G8**: AC (200 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G9**: Silymarin (100 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c)









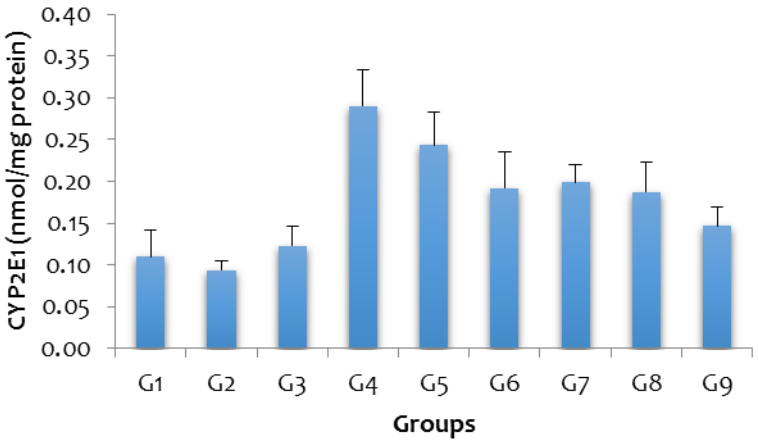
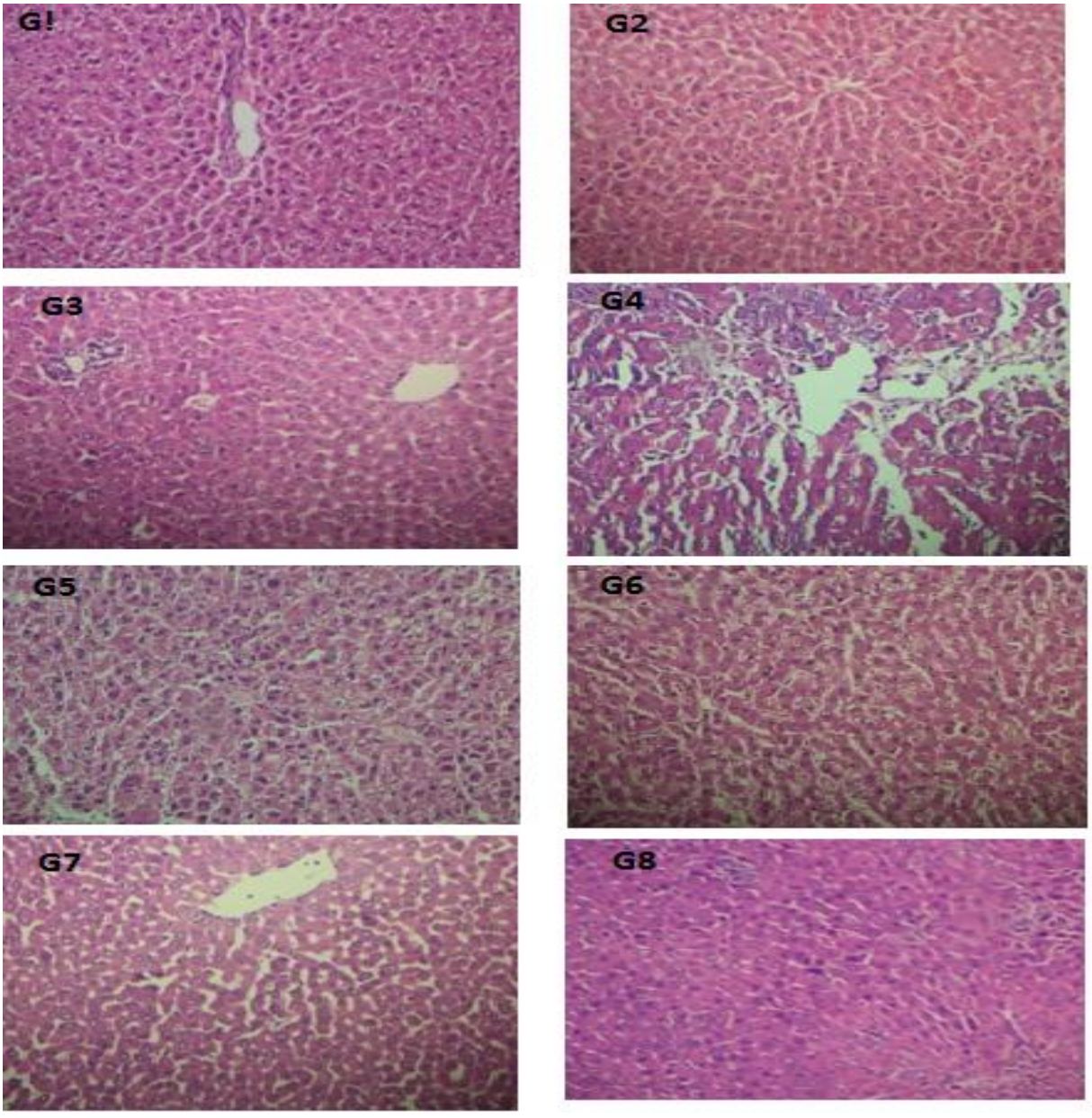


Figure 1: Antioxidant markers of the liver

Figure 2: Histopathological slices of livers stain sections.



G1: Normal control; G2: AC control (200 mg/kg b.w.); **G3**: SE control (200 mg/kg b.w.); **G4**: Toxic control, TAA (50 mg/kg b.w, s.c); **G5**: SE (100 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G6**: SE (200 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G7**: AC (100 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c); **G8**: AC (200 mg/kg b.w.) & TAA (50 mg/kg b.w, s.c).