Asian Journal of Biological and Life Sciences | Original Research

The potential hepatoprotective activity of *Allium paniculatum* and *Capparis spinosa* on thioacetamide induced hepatotoxicity in rats

Hasan Yusufoglu*[1], Gamal A. Soliman[2], Rehab F. Abdel-Rahman[3], Irem Tatli-Çankaya[4], Saleh I. Alqasoumi[5], SerapArabciAnul[4], Galip Akaydin[6]

1 Department of Pharmacognosy, College of Pharmacy, PrinceSattam bin Abdulaziz University, Al-Kharj, KSA.
2 Department of Pharmacology, College of Pharmacy; Prince Sattam bin Abdulaziz University, Al-Kharj, KSA.
3 Department of Pharmacology, National Research Centre, Cairo, Egypt.
4 Department of Pharmaceutical Botany, College of Pharmacy, Hacettepe University, Sihhiye, Ankara, Turkey.
5 Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, KSA.
6 Department of Biology Education, College of Education, Hacettepe University, Beytepe, Ankara, Turkey.

E-mail : hasanagazar@hotmail.com, h.yusufoglu@sau.edu.sa Contact No. : 00966-11-588-6012

Submitted : 01.02.2015 Accepted : 25.03.2015 Published : 30.04.2015

Abstract

The aim of this study was to investigate the hepatoprotective effect of *Allium paniculatum L.* (*A. paniculatum*) and *Capparis spinosa L.* (*C. spinosa*) extracts in rats. Adult rats were divided into seven groups (*n=6*). The 1st (control) and 2nd (hepatotoxic) groups received the vehicle. The 3rd group received silymarin. The 4th - 7th groups received *A. paniculatum* and *C. spinosa* extracts at 2 dose levels (200 and 400mg/kg, respectively). Rats were administered the vehicle, silymarin or extracts orally for 21 days and simultaneously administered thioacetamide (TAA), one h after the respective assigned treatments(except the 1st group), every 72 h. At the end of the experimental period, all animals were sacrificed, blood samples were collected and serum was separated. Livers were dissected out for determination of their antioxidant status and for histopathological examination.

Injection of thioacetamide elevated serum activities of liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyltransferase (γ-GT) in hepatotoxic group compared to normal controls. In the liver, significantly elevated level of malondialdehyde (MDA), lowered levels of reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were observed in hepatotoxic group. Treatment of rats with both extracts displayed hepatoprotective effect as evident by reduced serum activities of liver enzymes, as well as higher CAT, GPx, SOD activities and GSH concentration. The histopathological analysis suggested that both extracts obviously alleviated the degree of liver damage induced by TAA. In conclusion, *A. paniculatum*and *C. spinosa* attenuate hepatotoxicity induced by TAA.

Key words : *Allium paniculatum, Capparis spinosa,* thioacetamide, Hepatoprotective, Hepatotoxicity.

INTRODUCTION

Liver ailments have become one of the major causes of morbidity and mortality in various parts of the world. Among them, drug induced liver damage is one of the most common causative factor that forms a major clinical challenge[1]. Inflammation and jaundice are two major hepatic disorders that account for the high death rate [2]. In recent days, the use of herbal natural products has enhanced world-wide attentions. Many herbal supplements are claimed to assist in healthy lifestyle. Medicinally, herbal drugs have made a significant contribution to the treatment of hepatotoxicity [3].

Allium is the largest and the most important representative genus of the *Alliaceae* family, comprising some 750 species [4]. The genus is naturally distributed only in the Northern Hemisphere, mainly in regions that are seasonally dry. For many centuries, several *Allium* species have been used as vegetables and spices, and as folk medicines for curing various diseases. *A. sativum* like other medicinal plants has potential medicinal values which include antibacterial, antifungal, antixodiant and cytotoxic activities [5]. The main constituents of *Allium* have been demonstrated by Benkeblia and Lanzotti[6] to be thiosulfinates and organosulfur derivatives, steroidal saponins and terpenes.Studies have also revealed the health benefits of saponins, present in *Allium* genus, as lowering the cholesterol levels [7].

C. spinosa L. (Caper) belonging to the family Capparidaceae is one of the most common aromatic plants growing in wild in the dry regions around the west or central Asia and the Mediterranean basin [8]. From ancient times, the floral buttons of the caper were employed as a flavouring in cooking and are also used in traditional medicine for their diuretic, antihypertensive, poultice and tonic properties [9]. The plantwas reported to have a number of potentially useful medicinal attributes including anti-oxidative [10] and anti-inflammatory [11]. The antioxidant principles in *C. spinosa* plant have been identified to be flavanols and hydroxycinnamic acid [12].

The aqueous extract of *C. spinosa* demonstrated hypolipidaemic[13] and antihyperglycemic[14] activities. Other activities comprised antiviral, immunomodulatory[15], antiallergic[16], and antimicrobial [17] activities.

TAA (CH3-C(S)NH2) is a hepatotoxin that is widely used as a model to induce acute and chronic liver damage in rats [18]. Metabolic activation of TAA to a toxic metabolite which is responsible for its hepatotoxicity and/or its carcinogenicity has been proposed by a number of investigators [19]. Against this background, the present study was undertaken to assess the effect of ethanolic extracts of *A. paniculatum* and *C. spinosa* in a rat model of hepatic damage caused by TAA.

MATERIALAND METHODS

Animal model

Adult male Wistar rats (180-200 g) were obtained from Animal House of the National Research Centre, Cairo, Egypt. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and given fresh purified potable water *ad libitum*. Commercially obtained sawdust was used as bedding material. The cages were washed once a week. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment.Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Plant material

Fresh flowering aerial parts of *A. paniculatum* and *C. spinosa* were collected from the A4 Ankara: Kızılcahamam, Çeltikçi way and B1 İzmir: Surrounding Urla, respectively during summer, 2012. Taxonomic identifidcation was determind by Prof. Dr. Galip Akaydin and a voucher specimen from each plant (Akaydın 13484& Akaydın 5720, respectively) were deposited at the Herbarium of Faculty of Education (HEF), Hacettepe University, Ankara, Turkey.

Acute oral toxicity test in rats

Acute oral toxicity study was performed in rats according to OECD-423 guidelines [20]. Two groups of rats (n=6) were fasted overnight then treated orally with *A. paniculatum* and *C. spinosa* extracts, respectively at a dose of 2000 mg/kg. Another control group received the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions. Each animal was observed for clinical signs of toxicity and/or mortalities for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 48 h. Since, there was no mortality at this level; the dose of both extracts was increased to 4000 mg/kg and animals were observed for another 48 h.

Justification for dose selection

A. paniculatum and *C. spinosa* extracts were nontoxic at the dose of 4000 mg/ kg so, 1/20th and 1/10th of this dose (200 and 400 mg/kg, respectively) were selected for the study.

Preparation of plant extract

The collected plants were shade dried and then grinded to fine powders. the dried powders of each plant (100g) were extracted by percolation in 70% aqueous ethanol with occasional shaking for 48 h. Percolation was repeated three times, and then the ethanolic extracts of each plant were combined, filtered and concentrated to dryness under reduced pressure at 60 ± 1°C in rotary evaporator to give the total extracts of *A. paniculatum* (29.4g) and *C. spinosa* (23.5g). Both extracts were stored in the refrigerator and aliquot of the concentrations were prepared immediately before use.

Experimental Protocols

Forty two adult male Wistar albino rats were randomly divided into seven groups of six animals, each. Rats of the 1st group (normal control) were received the vehicle (3% Tween 80) orally through an intragastric tube at a dose of 1mL/kg for 3 weeks and injected subcutaneously (sc) with sterile distilled water (2mL/kg) thrice weekly for 3 weeks. Rats of the 2nd group (TAA-intoxicated control) were orally received the vehicle at a dose of 1mL/kg for 3 weeks and injected with TAA (50mg/kg, sc) thrice weekly for 3 weeks[21]. Animals of the 3rd group (reference) were received silymarin at an oral dose of 50 mg/kg for 3 weeks and injected with TAA (50mg/kg, sc) thrice weekly for 3 weeks. The 4th and 5th groups were treated orally with *A. paniculatum* extract (200 and 400mg/kg, respectively) for 3 weeks and injected with TAA (50mg/kg, sc) thrice weekly for 3 weeks. Rats of the 6th and 7th groups were treated orally with *C. spinosa* extract (200 and 400mg/kg, respectively) for 3 weeks and injected with TAA (50mg/kg, sc) thrice weekly for 3 weeks.

At the end of the experimental period, all the animals were sacrificed by cervical decapitation, blood samples were collected and sera were separated and analyzed for various biochemical parameters. Livers were dissected out and divided into two parts. One part was kept in liquid nitrogen for determination of antioxidant status and the other part was immediately fixed in buffered formalin 10% and was used for histopathological examination.

Assessment of serum and liver biochemical markers

The levels of ALT, AST, ALP, γ-GT, total protein (TP), albumin (Alb) and BRN were determined in serum of all rats. Hepatic tissues from all livers were sampled from the same site of the left lobe, but away from the portal system. One gram of the sampled tissue was placed in 10mL (10% w/v) of PBS (phosphate buffer solution with pH 7.4), then homogenized, centrifuged at 4000rpm for 10min at −4°C and the supernatant was kept in a −80°C freezer. The activities of the antioxidant enzymes such as SOD, GPx and CAT, were assayed in the hepatic tissue homogenate of the control and experimental rats[21-23], respectively. Moreover, levels of MDA were assessed in the liver tissue homogenate as a measure for lipid peroxidation according to [24], while GSH tissue content was measured according to the method described by Moron *et al*. [25].

Histopathological analysis

Liver specimens from all rats were fixed in 10% buffered formalin and embedded in paraffin using automated tissue processing machine. Sections were sliced at 5µm thickness and stained with haematoxylin and eosin (H&E) for histological evaluation.

Statistical analysis

The values are expressed as mean ± standard error of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Dunnett'stest to determine the intergroup variability by using SPSS ver. 14.0. A comparison was made with the normal control and TAA-hepatotoxic groups. We took a P-value of <0.05 as our desired level of significance.

RESULTS

Acute oral toxicity test in rats

There were no behavioral alterations or mortalities recorded in the rats administered *A. paniculatum* and *C. spinosa* extracts at doses of 2 and 4g/kg. Physically, they appeared normal and no abnormal signs were observed in their skins, eyes, and mucus membranes. These findings provided sufficient evidence to conclude that *A. paniculatum* and *C. spinosa* extracts were safe and did not cause extract-related toxicity. The oral LD_{50}values for

Table 1: Effect of the ethanol extracts of *A. paniculatum* and *C. spinosa* on the serum activity of liver marker enzymes in rats with TAA- induced hepatotoxicity.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	γ-GT (U/L)
Normal Control	126.0±3.92[b]	96.5±3.01[b]	249.8±5.41[b]	1.8±0.04[b]
TAA-hepatotoxic Control	423.5±9.57[a]	313.7±8.34[a]	408.7±9.08[a]	5.5±0.16[a]
Silymarin (50 mg/kg)+TAA	177.0±5.62[ab]	138.7±5.94[ab]	278.7±7.15[ab]	2.3±0.08[ab]
***A. paniculatum* (200 mg/kg)+TAA**	285.3±6.91[ab]	214.6±6.92[ab]	344.3±8.21[ab]	3.9±0.08[ab]
***A. paniculatum* (400 mg/kg)+TAA**	218.8±6.73[ab]	187.3±6.84[ab]	317.5±8.64[ab]	3.2±0.09[ab]
***C. spinosa* (200 mg/kg)+TAA**	277.2±7.72[ab]	205.2±5.32[ab]	328.7±8.76[ab]	3.5±0.06[ab]
***C. spinosa* (400 mg/kg)+TAA**	210.7±7.58[ab]	176.7±5.77[ab]	305.2±8.15[ab]	3.0±0.09[ab]

The results are expressed as mean±S.E.M., n =6 rats/group.
a indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
b indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

Table 2: Effect of the ethanol extracts of *A. paniculatum* and *C. spinosa* on the serum levels of TP, Alb and BRN in rats with TAA - induced hepatotoxicity.

Groups	TP (g/dL)	Alb (g/dL)	BRN (mg/dL)
Normal Control	7.8±0.14[b]	3.9±0.07[b]	1.3±0.03[b]
TAA-hepatotoxic Control	5.0±0.11[a]	2.1±0.04[a]	3.5±0.06[a]
Silymarin (50 mg/kg)+TAA	7.2±0.12[ab]	3.3±0.09[ab]	1.8±0.04[ab]
***A. paniculatum* (200 mg/kg)+TAA**	6.0±0.12[ab]	2.9±0.06[ab]	2.5±0.05[ab]
***A. paniculatum* (400 mg/kg)+TAA**	6.3±0.11[ab]	3.0±0.08[ab]	2.4±0.07[ab]
***C. spinosa* (200 mg/kg)+TAA**	6.2±0.15[ab]	3.0±0.05[ab]	2.5±0.06[ab]
***C. spinosa* (400 mg/kg)+TAA**	6.7±0.11[ab]	3.1±0.06[ab]	2.1±0.03[ab]

The results are expressed as mean±S.E.M., n =6 rats/group.
a indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
b indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

both extracts were indeterminable being in excess of 4 g/kg b. wt. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic.

Serum and liver biochemical markers

The activities of ALT, AST, ALP, and γ-GT and the levels of TP, Alb and BRN in rat's serum were shown in Tables(1 and 2). Significant increases in the serum ALT, AST, ALP, γ-GT and BRN but strongly lower TP and Alb levels were detected in TAA-hepatotoxic ratsas compared to normal control group. Silymarin+ TAA therapy was effective and restored the activities of these serum biochemical toward their normal values. Levels of ALT, AST, ALP,γ-GT and BRN increased by TAA treatment were reduced significantly by treatment of rats with *A. paniculatum* and *C. spinosa* for 3 weeks. Moreover, the significant decrease of TP and Alb in serum, which was observed in animals with TAA treatment, was significantly reversed by both extracts. These results demonstrated the capacity of *A. paniculatum* and *C. spinosa* extracts to protect against the abnormal hepatic biochemical activities initiated by TAA.

Table 3 lists the activities of enzymes involved in the hepatic antioxidant defense system. It is recorded that SOD, CAT, and GPx enzyme activities were decreased in the liver homogenates of TAA-hepatotoxic controls when compared with the normal control rats. These results indicated the occurrence of liver damage in TAA-hepatotoxic group. Activities of SOD, CAT, and GPx enzymes were significantly increased following medication of rats with silymarin + TAA, *A. paniculatum*+ TAA and *C. spinosa*+ TAA for 3 weeks.Liver homogenates of the TAA-hepatotoxic group had significantly high levels of the lipid peroxidation (MDA) and low levels of GSH than the normal

Table 2: Effect of the ethanol extracts of *A. paniculatum* and *C. spinosa* on the serum levels of TP, Alb and BRN in rats with TAA - induced hepatotoxicity.

Groups	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	GSH (µmol/ g tissue)	MDA (nmol/g tissue)
Normal Control	45.4±1.37^{b}	2.95±0.11^{b}	13.8±0.24^{b}	9.2±0.24^{b}	48.8±1.50^{b}
TAA-hepatotoxic Control	21.6±0.53^{a}	1.10±0.07^{a}	7.2±0.15^{a}	5.6±0.14^{a}	112.9±3.84^{a}
Silymarin (50 mg/kg)+TAA	40.3±1.12^{ab}	2.44±0.10^{ab}	10.8±0.22^{ab}	8.3±0.13^{ab}	57.4±1.68^{ab}
***A. paniculatum* (200 mg/kg)+TAA**	31.4±0.74^{ab}	1.95±0.06^{ab}	9.1±0.15^{ab}	7.1±0.12^{ab}	78.6±2.53^{ab}
***A. paniculatum* (400 mg/kg)+TAA**	34.5±0.77^{ab}	2.14±0.12^{ab}	9.4±0.12^{ab}	7.3±0.10^{ab}	70.4±2.49^{ab}
***C. spinosa* (200 mg/kg)+TAA**	33.0±0.64^{ab}	2.04±0.13^{ab}	9.2±0.11^{ab}	7.3±0.15^{ab}	75.3±2.83^{ab}
***C. spinosa* (400 mg/kg)+TAA**	36.7±0.71^{ab}	2.21±0.15^{ab}	9.6±0.16^{ab}	7.4±0.13^{ab}	68.6±2.15^{ab}

The results are expressed as mean±S.E.M., n =6 rats/group.
a indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
b indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

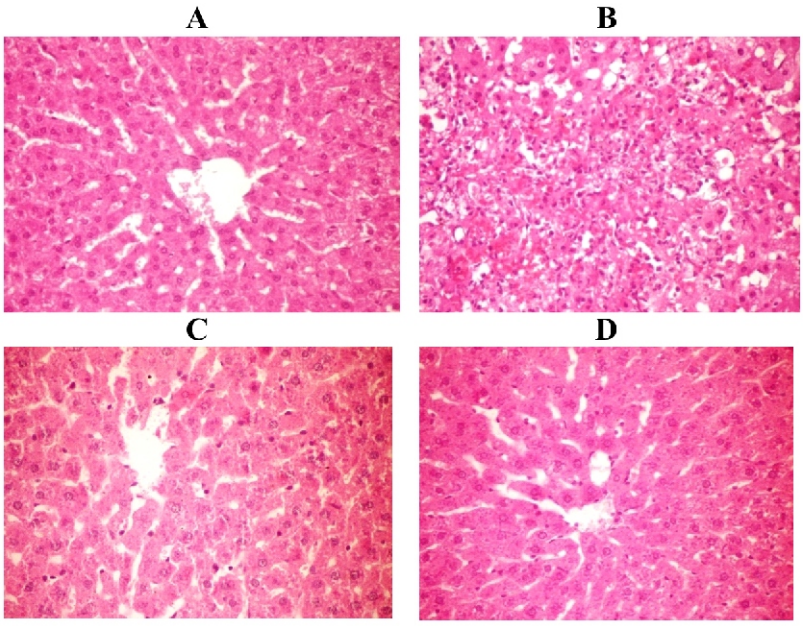

Figure 1: Photomicrographs of rat liver (H & E stain) under low power (X200), (A) Normal control group showing normal parenchymal architecture; (B) TAA-hepatotoxic group showing hepatocytic necrosis, lymphocytic infiltration and proliferation of fibrous connective tissue and the hepatocytes showed vacuolated cytoplasm; (C) *A. paniculatum* (400 mg/kg) + TAA showing normal hepatic cords and absence of lymphocytic infiltration; (D) *C. spinosa* (400 mg/kg) + TAA showing appreciable levels of normal parenchymal architecture with lesser amounts of cytoplasmic vacuolization.

control rats. The level of liver MDA was significantly declined while the GSH activity was significantly elevated following silymarin + TAA, *A. paniculatum*+ TAA and *C. spinosa*+ TAA-medication of rats for 3 weeks as compared with those of TAA-hepatotoxic group. This set of data indicates that *A. paniculatum* and *C. spinosa* extracts are antioxidants and protect the liver through this property.

Histopathological analysis

Histopathological examination of the liver sections from normal rats showed hepatocytes with normal parenchymal architecture and normal liver lobular structure with well-preserved cytoplasm and prominent nucleus (Fig. 1-A). Liver sections of TAA-hepatotoxic rats showed hepatocytic necrosis, lymphocytic infiltration and proliferation of fibrous connective

tissue and the hepatocytes showed vacuolated cytoplasm (Fig. 1-B). The hepatoprotective effect of silymarin, *A. paniculatum* and *C. spinosa* was confirmed by histopathological examination of the liver tissue. Silymarin administration prevented lymphocytic infiltration, hepatic necrosis, and fibrous connective tissue proliferation induced by TAA. Consequently, the liver tissue preserved its nearly normal hepatic lobular architecture with central veins and radiating hepatic cords.Liver sections of rats treated with *A. paniculatum* extract in a dose of 400 mg/kg showed normal hepatic cords (Fig. 1-C) and absence of lymphocytic infiltration and proliferation of fibrous connective tissue indicating pronounced protection of hepatocytes against TAA-induced hepatic damage. *C. spinosa* extract appeared to reduce the TAA-induced toxicity as evidenced by less inflammatory changes and no necrosis (Fig. 1-D). The livers had appreciable levels of normal parenchymal architecture with lesser amounts of cytoplasmic vacuolization.

DISCUSSION

In the current study, oral administration of *A. paniculatum* and *C. spinosa* extracts to rats at doses up to 4g/kg did not produce any symptom of acute toxicity and none of animals died during 48 h of observation. Accordingly, it suggested that oral values of LD_{50} of both extracts were higher than 4g/kg. Therefore, *A. paniculatum* and *C. spinosa* can be categorized as highly safe, since substances possessing LD_{50} higher than 50 mg/kg are non-toxic[26].According to Clarke and Clarke [27], any compound with the oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe.

TAA administration is an established technique for generating a model of experimental hepatotoxicity in rats. Hepatic microsomal cytochrome P450 enzymes are known to metabolize TAA into a toxic reactive metabolites; TAA-S-oxide and TAA-S-dioxide [28]. Neal and Halpert[29]postulated the mechanism of toxicity of TAA to be due to the formation of TAA-S-oxide that may causes damage to the biological membranes leading to serious cellular injury and leakage of liver marker enzymes like ALT, AST, ALP and γ-GT. The prevention of this phenomenon can be considered as hepatoprotective activity [30].In this study, TAA-hepatotoxic rats developed hepatic damage as manifested by a significant increase in the serum activities of ALT, AST, ALP and γ-GT. Raised activity of liver enzymes in intoxicated rats can be attributed to the damaged structural integrity of the liver. Treatment of rats with *A. paniculatum*+ TAA and *C. spinosa*+ TAA effectively protected rats against TAA-induced hepatic damage, resulting in reduction in serum activities of liver enzymes in a dose dependent manner, where 400mg/kg was more effective than 200mg/kg of dose.The reduction seen in the levels of these enzymes in the treated rats suggested that *A. paniculatum* and *C. spinosa* extracts have stabilized the hepatocytes membranes and interrupted the release of enzymes from liver into blood.

The lowered levels of TP and Alb in the serum reveal the intensity of hepatopathy[31]. The majority of Alb is synthesized in the liver. Therefore, variation of serum TP or Alb concentrations can reflect liver health status [32]. In the current study, a marked reduction of serum TP and Alb levels were observed in TAA-hepatotoxic rats compared with the normal control animals. The decrease in serum TP and Alb may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein. Perez *et al*. [33] explained that TAA decreases the protein contents in the liver by inhibiting incorporation of amino acids into liver protein. Administration of *A. paniculatum* + TAA and *C. spinosa* + TAA showed a significant reversal of these parameters toward the normal. The effects of these extracts were comparable to that of standard silymarin. This assures the hepatoprotective activity of *A. paniculatum* and *C. spinosa* extracts against damage by TAA.Further, high serum level of BRN is used as a measure of hepatotoxicity and hence reflects the necrotic conditions of hepatocytes [34]. In this study, TAA treatment increased serum BRN concentration in rats. The rise in the serum level of BRN could be attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction [35].The high serum level of BRN was reversed significantly in groups treated with *A. paniculatum*+ TAA and *C. spinosa*+ TAA. The ability of both extracts to reduce the level of BRN in the serum of intoxicated rats reconfirmed their potential hepatoprotective effect.

Hepatic antioxidant enzymes (SOD, GPx and CAT) represent one protection against oxidative tissue-injury [36]. SOD is an important defense enzyme that catalyzes the dismutations of superoxide radicals to hydrogen peroxide while GPx and CAT metabolize H2O2 to non-toxic products. Since TAA induced hepatotoxicity is due mostly to oxidative stress [37], antioxidant mediated protective role of *A. paniculatum* and *C. spinosa* extracts has been assessed. In the present investigation, scinjection of TAA to rats was shown to cause oxidative stress in liver that was manifested by reduced activities of the antioxidant enzymes as well as GSH depletion in the liver homogenate. The reduced levels of the hepatic antioxidant enzymes and GSHin the liver homogenates of TAA-hepatotoxic rats were reverted back toward normal levels after the treatment with *A. paniculatum* and *C. spinosa*. Accordingly, the possible mechanism of the antihepatotoxic effect of both extracts may be, in part, attributed to their antioxidant activities.

MDA, a lipid peroxidized product, can reflect the extent of lipid peroxidation induced by oxidative stress. A significant increase in MDA level was observed in the liver homogenate of TAA-hepatotoxic rats compared with the normal control animals. Several studies have suggested that part of hepatocellular injury induced by TAA is mediated through oxidative stress caused by the action of cytokines through lipid peroxidation [38].*A. paniculatum* and *C. spinosa* extracts at 200 and 400 mg/kg, significantly decreased the level of lipid peroxidation induced by TAA. This decrease could be attributed to the increase in GPx activity in rats treated with both extracts since GPx has been known to inactivate lipid peroxidation reactions [39]. Increased activities of the antioxidant enzymes with concomitant increase in GSH level and reduced lipid peroxidation product are indications that *A. paniculatum* and *C. spinosa* extracts offered significant protection.

Histopathologic studies also supported the evidence of biochemical analysis. Histological examination of rat liver treated with TAA shows significant hepatotoxicity characterized by necrosis of hepatocytes. There was extensive infiltration of the lymphocytes around the central vein and loss of cellular boundaries. However, in animals treated with ethanolic extracts of *A. paniculatum* and *C. spinosa* the severity of hepatic damage was decreased when compared with TAA-hepatotoxic rats. In accordance with these results, the protective effect of both extracts against TAA may be attributed to the presence of phytochemicals.Therefore, the beneficial effects of *A. paniculatum* can be attributed to the presence of non-enzymatic antioxidants such as selenium and copper metals, vitamin C and

other phytochemicals such as organosulphur compounds [40]. In addition,the protective activity of *C. spinosa* extract may be due to its flavonoids that display a remarkable role in various pharmacological activities including anti-inflammatory and antioxidant effects [41].

CONCLUSION

In conclusion, the result of the present study demonstrates that the ethanolic extracts of *A. paniculatum* and *C. spinosa* extracts showed a significant hepatoprotective effect when orally administrated in doses of 200 and 400 mg/kg, and the protective effect was dose-dependent. The hepatoprotective effect of both extracts might be due to direct antioxidant mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification.

REFERENCES

1. Russmann S, Gerd A, Grattagliano I. Current concepts of mechanism in drug induced hepatotoxicity. Curr Med Chem 2009: 16: 3041-3053.

2. Nazeema T, Brindha V. Antihepatotoxic and antioxidant defense potential of *Mimosa pudica*. Int J Drug Discov 2009: 1: 14.

3. Manoj S, Mohanty P, Jaliwala Y. Hepatoprotective activity of fruits of *Prunus domestica*, Int JPharma Bio Sci 2011: 2: 439-453.

4. Stearn W. How many species of Allium are known? *Kew Mag* 1992: 9: 180-182.

5. Park J, Park Y, Park E. Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. Plant Foods Hum Nutr 2009; 64: 244 - 249.

6. Benkeblia N,Lanzotti V. Allium Thiosulfinates: Chemistry, Biological Properties and their Potential Utilization in Food Preservation. Food 2007:1: 193-201.

7. Matsuura H. Saponins in garlic as modifiers of the risk of cardiovascular disease. *J Nutr*2001; 131: 1000 - 1005.

8. Sultan A, Çelik T.Genotoxic and antimutagenic effects of *Capparis spinosa* L. on the *Allium cepa* L. root tip meristem cells. Caryologia 2009: 62: 114-123.

9. Çaliş I, Kuruüzüm A, Rüedi P.1H- indole-3- acetonitrile glycosides from *Capparis spinosa* fruits. Phytochemistry 1999: 50: 1205-1208.

10. Germano M, De Pasquale R, D'Angelo V, Catania S, Silvari V, Costa C.Evalution of extracts and isolated fraction from *Capparis spinosa* L. buds as antioxidant source. J. Agric. Food Chem 2002: 50: 1168-1171.

11. Rossi T, Melegari M, Bianchi A, Albasini A,Vampa G. Sedative, anti-inflammatory and anti-diuretic effects induced in rats by essential oils of varieties of *Anthemis nobilis*: A comparative study. Pharm Res Commun 1988: 20: 71-74.

12. Bonina F, Puglia C, Ventura D, Aquino R, Tortora S, Sacchi A, Saija A, Tomaino A, Pellegrino M, De Caprariis P. In vitro antioxidant and in vivo photoprotective effects of a lyophilized extract of *Capparis spinosa* L. buds. J Cosmet Sci 2002: 53: 321-335.

13. Eddouks M, Lemhadri A, Michel J. Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. J Ethnopharmacol 2005:98: 345-350.

14. Eddouks M, Lemhadri A, Michel J. Caraway and caper: potential anti-hyperglycaemic plants in diabetic rats. J Ethnopharmacol 2004: 94: 143-148.

15. Arena A, Bisignano G, Pavone B, Tomaino A, Bonina F, Saija A, Cristani M, D'Arrigo M, Trombetta D. Antiviral and immuno modulatory effect of a lyophilized extract of *Capparis spinosa* L. buds. Phytother Res 2008: 22: 313317.

16. Trombetta D, Occhiuto F, Perri D, Puglia C, Santagati N, De Pasquale A, Saija A, Bonina F. Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds. Phytother Res 2005: 19: 29-33.

17. Eddouks M, Maghrani M, Lemhadri A, Ouahidi M, Jouad H. Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). J Ethnopharmacol 2002: 82: 97-103.

18. Ramaiah SK, Apte U, Mehendale HM. Diet restriction as a protective mechanism in noncancer toxicity outcomes: a review. Int J Toxicol 2000: 19: 413-424.

19. Hunter AL, Holscher MA, Neal RA. Thioacetamide-induced hepatic necrosis. I. Involvement of the mixed-function oxidase enzyme system. J Pharmacol Exp Ther 1977:200:439-448.

20. OECD (Organization for Economic Cooperation and Development) (2001) The OECD 423 Guideline for Testing of Chemicals Acute Oral Toxicity-Acute Toxic Class Method, Paris, France.

21. Anbarasu C, Rajkapoor B, Bhat K, John G, Amuthan A, Satish K; Protective effect of *Pisoniaaculeata* on thioacetamide induced hepatotoxicity in rats. Asian Pac J Trop Biomed 2012: 2: 511-515.

22. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Bioch 1978: 247: 81-89.

23. Mohandas J, Marshall J, Duggin G, Horvath J, Tiller D. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res 1984: 44: 5086-5091.

24. Chance B, Maehley A. Assay of catalases and peroxidases. Meth Enzymol 1995: 2: 764.

25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979: 95: 351.

26. Moron M, Diperre J, Mannerv K. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lungs and liver. BiochemBiophysActa 1979: 582: 67-71.

27. Buck W, Osweiter G, Van Gelder A. Clinical and Diagnostic Veterinary Toxicology. Iowa: Kendall Hunt Publishing Co, Iowa pp. 1976; 521-534.

28. Clarke EG, Clarke ML. Landers veterinary toxicology in London. London: BailliereTindall 1997.

29. Chilakapati J, Shankar K, Korrapati MC, Hill RA, Mehendale HM. Saturation toxicokinetics of thioacetamide: role in initiation of liver injury. Drug MetabDispos 2005; 33: 1877-

1885.

30. Neal RA, Halpert J.Toxicity of thionosulfur compounds. Ann Rev PharmcolToxicol 1982: 22: 3219.

31. Mak D, Li P, Poon M, Ko K. Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. Mol Cell Biochem 1996: 162: 153158.

32. Aniya Y, Koyama T, Miyagi C, Miyahira M, Inomata C, Kinoshita S, Ichiba T. Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands. Biol Pharm Bull 2005: 28: 1923.

33. Wan T, Liu Y, Duann L, Yu K, Chen C, Lin L, Sakata R. Effects of animal liver and bile extracts on biochemical values of rat ethanol-induced fatty liver. Animal Science Journal 2014: 85: 75-80.

34. Perez MJ, Sanchez-Medina F, Torres M, Gil A, Saurez A. Dietary nucleotides enhance the liver redox state and protein synthesis in cirrhotic rats. J Nutr 2004: 134:25048.

35. Gressner O, Weiskirchen R, Gressner A. Biomarkers of liver fibrosis: clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests. Clinica Chimica Acta 2007: 381: 107-113.

36. Blanckaert N, Schmid D. Zakim, T.D. Boyer (Eds.), Hepatolog A Textbook of Liver Disease, W.B. Saunders, Philadelphia 1982: pp. 246-296

37. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine, Oxford University Press; New York, 1999. P. 246.

38. Okuyama H, Shimahara Y, Nakamura H, Araya S, Kawada N, Yamaoka Y, Yodoi J. Thioredoxin prevents thioacetamide-induced acute hepatitis. Comparative Hepatology 2004; 3: S6.

39. Levy U, Zaltzber H, Ben-Amotz A, Kanter Y, Aviram M. β-Carotene affects antioxidant status in non-insulin dependent diabetes mellitus; Pathophysiology 1999: 6: 157-161.

40. Prasad K, Laxdal V, Yu M, Raney B. Antioxidant activity of allicin and active principal in garlic. Mol. Cell. Biochem 1995: 148: 183-189.

41. Harborne J. Williams C. Advances in flavonoid research since 1992. Phytochemistry 2000:55: 481-504.