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Hepatoprotective Potential of *Astragalus kurdicus* and *Astragalus cinereus* Extracts against Paracetamol Induced Liver Damage in Rats

[1]Hasan Yusufoglu, [1,2]Gamal A. Soliman, [3]Rehab F. Abdel-Rahman and [4]Özgen Alankuş-Calışkan
[1]Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudia Arabia
[2]Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt
[3]Department of Pharmacology, National Research Centre, Cairo, Egypt
[4]Deapartment of Chemistry, Faculty of Science, Ege University, Bornova, İzmir, 35100, Turkey

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Corresponding Author:
Hasan Yusufoglu
Department of Pharmacognosy,
College of Pharmacy,
Prince Sattam Bin Abdulaziz University,
P.O. Box 173, Al-Kharj, 11942,
Kingdom of Saudi Arabia
Tel: 00966-11-588-6012
Fax: 00966-11-588-6001

ABSTRACT
The objective of this study was to investigate the potential hepatoprotective effect of the ethanol extracts of *Astragalus kurdicus* Boiss. var. *kurdicus* (*A. kurdicus*) and *Astragalus cinereus* Willd. (*A. cinereus*) in a rat model of paracetamol (PCM) induced liver damage. Paracetamol administration caused severe hepatic damage in rats as evidenced by elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT) and serum level of total bilirubin (BRN) while decreased serum levels of total protein (TP) and albumin (ALB). In liver homogenates, PCM elevated malondialdehyde (MDA) but decreased glutathione (GSH) levels as well as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities. Administration of *A. kurdicus* and *A. cinereus* extracts (200 and 400 mg kg^{-1}) for 7 days before PCM inhibited the elevation of the serum activities of ALT, AST, ALP and γ-GT enzymes and serum level of BRN. Moreover, they elevated the serum level of TP. Paracetamol-induced lipid peroxidation was also reduced by both extracts. Likewise, both extracts increased the activities of the antioxidant enzymes (GPx, SOD, CAT) in the liver homogenates and reduced GSH concentration. The results of the *in vitro* antioxidant effect revealed marked antioxidant activity for both extracts. The histopathological analysis suggested that both extracts obviously alleviated the degree of liver damage due to PCM administration. The present study suggests that *A. kurdicus* and *A. cinereus* possess hepatoprotective activities that could be partly attributed to their antioxidant effects.

Key words: *Astragalus kurdicus*, *Astragalus cinereus*, paracetamol, hepatotoxicity, Antioxidant, rat

INTRODUCTION

The liver is the initial site of contact for many types of orally ingested therapeutic drugs, alcohol and other xenobiotics from intestinal absorption, thus making this organ particularly susceptible to chemical-induced injury (Gu and Manautou, 2012). Liver ailments have become one of the major causes of morbidity and mortality in various parts of the world. Amongst them, drug induced liver damage is one of the most common causative factor that forms a major clinical challenge. In spite of remarkable scientific progression in the field of hepatology in recent times, inflammation and jaundice are two major hepatic disorders that account for the high death rate (Anbarasu *et al*., 2012). Recently, the use of herbal

natural products has enhanced world-wide considerations. Medicinally, herbal drugs have made a significant contribution to the treatment of hepatotoxicity (Kumar *et al*., 2013).

The genus *Astragalus* belongs to the family Fabaceae of the order Leguminales. In Turkey there are 445 species of *Astragalus*, of which 224 are endemic (Davis, 1970). The dried roots of plants from different *Astragalus* species are used as antiperspirant, diuretic and tonic and for treatment of diabetes mellitus, leukemia and uterine cancer (Hikino *et al*., 1976). They are also famed for their antimicrobial, antiperspirant, cardioprotective and anti-inflammatory effects (Cakilcioglu and Turkoglu, 2010). Chemical studies on *Astragalus* species reported the presence of triterpenoid saponins, which exhibited a wide range of biological properties, including immunostimulant, antiviral, cardiotonic and analgesic activities (Verotta and El-Sebakhy, 2001). Previous studies on Turkish *Astragalus* species resulted in the isolation of a series of oleanane- and cycloartane-type triterpenoidal saponins (Calis *et al*., 1997). The isolated glycosides show interesting biological properties, including immunostimulating, antiprotozoal, antiviral, cytotoxic and wound healing (Nalbantsoy *et al*., 2011). The flavonoid mixture of some species revealed strong antioxidant activity (Ivancheva *et al*., 2006).

Paracetamol also known as acetaminophen is an effective analgesic/antipyretic drug when used at therapeutic doses. However, the overdose of PCM can cause severe liver injury and liver necrosis that can be fatal in experimental animals and humans (Hinson *et al*., 2010). The hepatotoxicity of PCM has been attributed to the formation of the highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI). In the absence of intracellular antioxidants such as glutathione, NAPQI can covalently bind to a number of intracellular target proteins, which leads to a variety of cellular dysfunctions, including mitochondrial damage, ATP depletion and mitochondrial oxidant stress (Gujral *et al*., 2002). The present study aimed to assess the potential hepatoprotective effect of *A. kurdicus* and *A. cinereus* extracts in a rat model of hepatic damage caused by PCM.

MATERIALS AND METHODS

Animal model: Adult male Wistar rats (180-200 g) were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. All animals were housed under constant temperature and 12 h light/dark cycle. They were fed a standard rodent diet and given fresh purified potable water *ad libitum*. After 1 week of acclimatization, the animals were randomly allocated into 7 experimental groups. 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.

Plant material: Roots of *Astragalus kurdicus* Boiss. var. *kurdicus* and *Astragalus cinereus* Willd. were collected from Van: Gevaş, Alacabük mountain southern slopes, 2850 m altitude and Van: Catak, direct fromVan to Catak 4 km from Kızıltaş village to Catak 2000-2100 m, respectively during summer 2010. altitude, East Anatolia, Turkey in June 2010 and identified by Associate Professor Dr. Fevzi ÖzgökCe (Department of Biology, Faculty of Science and Art, Yüzüncü Yıl University, Van, Turkey). Voucher specimens have been deposited in the Herbarium of Yüzüncü Yıl University, Van, Turkey, Herbarium no: VANF 13805 and VANF 13703, respectively.

Preparation of plant extract: The collected plants were shade dried and then grinded to fine powders. The air-dried powdered roots (100 g) were extracted with 80% aqueous ethanol (2×3 L) under reflux. The ethanolic extract was combined and subjected to rotary evaporation (Büchi Rotavapor R-215, vacuum pump V-700) (70±2°C). The thick solution was lyophilized using freeze drier give the total extracts of *A. kurdicus* (16.7 g) and *A. cinereus* (19.8 g). Both extracts were stored in the refrigerator and aliquot of the concentrations were prepared immediately before use (Yusufoglu *et al*., 2015).

DPPH radical scavenging activity: The DPPH assay was performed according to the method followed by Amic *et al*. (2003) and Phang *et al*. (2011). A solution of 2, 2-Diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) in methanol (0.004% solution) was prepared and stored in dark until use. Preparations of the tested extracts at different concentrations were done in methanol. In a 96-well plate, addition of 20 µL of each concentration to 180 µL DPPH solution was carried out. Negative controls were done to correct for colored extracts. The resultant reaction mixtures vortex-mixed and incubated at room temperature for 30 min. The absorbance of the reaction mixtures was measured at 520 nm. Methanol was used as blank and DPPH solution without addition of the extracts was used as control. Ascorbic acid was used as positive control. The scavenging activity was calculated by the following formula:

$$\text{Scavenging activity}\,(\%) = \frac{A_1 - A_0}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A1 is the absorbance of the extract. EC_{50} values were determined from the graph of percentage of inhibition plotted against the concentration of the tested extract. EC_{50} is defined as the amount of extract needed to scavenge 50% of DPPH radicals.

Acute oral toxicity test in rats: Acute oral toxicity study was performed in rats according to OECD-423 guidelines (OECD., 2001). Two groups of rats (n=6) were fasted overnight then

treated orally with *A. kurdicus* and *A. cinereus* extracts, respectively at a dose of 2000 mg kg^{-1}. Another control group received the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions. All animals 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^{-1} and animals were observed for another 48 h.

Justification for dose selection: *Astragalus kurdicus* and *A. cinereus* extracts were nontoxic at the dose of 4000 mg kg^{-1} so, 1/20th and 1/10th of this dose (200 and 400 mg kg^{-1}, respectively) were selected for the study.

Hepatoprotective activities: Paracetamol induced hepatotoxicity model was adopted for the study as previously cited (Dash *et al*., 2007). The rats were divided into 7 groups (n=6). Group I (normal control) and group II (hepatotoxic group) received the vehicle (1 mL kg^{-1} b.wt., for 7 days). Group III received the reference drug, silymarin (50 mg kg^{-1} b.wt., for 7 days). Groups IV and V received the ethanolic extract of *A. kurdicus* (200 and 400 mg kg^{-1} b.wt., respectively for 7 days). Groups VI and VII received the ethanolic extract of *A. cinereus* (200 and 400 mg kg^{-1} b.wt., respectively for 7 days). On the 7th day, paracetamol suspension was given orally (2 g kg^{-1} b.wt), to all the rats except those in group I.

After 48 h of hepatotoxicity induction, blood samples were withdrawn from the retro-orbital vein of each animal under light anesthesia by diethyl ether. The blood was allowed to clot and centrifuged at 3500 rpm for 10 min. The serum was separated and analyzed for various biochemical parameters. Livers were dissected out and divided into two parts. One part was kept in -80ºC freezer 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, BRN, TP and ALB were determined in serum of all rats. Hepatic tissues from all livers were sampled from the same site of the left lobe. One gram of the sampled tissue was placed in 4 mL (20% w/v) of PBS (phosphate buffer solution with pH 7.4), then homogenized, centrifuged at 4000 rpm for 10 min at 4°C and the supernatant was kept in a -80°C freezer. The activities of the antioxidant enzymes: SOD (Sun and Zigman, 1978), GPx (Mohandas *et al*., 1984) and CAT (Chance and Maehly, 1955) were assayed in the hepatic tissue homogenate of all rats according to the methods previously described. The levels of MDA were assessed in the liver homogenate as a measure for lipid peroxidation according to Ohkawa *et al*. (1979), while GSH tissue content was measured according to the method of Moron *et al*. (1979). Another liver specimen from each rat was fixed in 10% buffered formalin and embedded in paraffin using automated tissue processing machine. Sections were sliced at 5 μm thickness and stained with haematoxylin and eosin (H and 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's test to determine the intergroup variability by using SPSS 14.0. A comparison was made with the normal control and PCM-hepatotoxic groups. p-value of <0.05 was considered as our desired level of significance.

RESULTS AND DISCUSSION

Acute toxicity test: *Astragalus kurdicus* and *A. cinereus* extracts were well tolerated by rats. Both extracts were found to be nontoxic when administered orally to rats at doses up to 4000 mg kg^{-1}. No mortalities observed with oral administration of both extracts during 48 h of observation even at the highest dose. The oral LD_{50} values for both extracts were indeterminable being in excess of 4 g kg^{-1} b.wt. Therefore, the extracts were considered non-toxic, since substances possessing LD_{50} higher than 50 mg kg^{-1} are non-toxic (Buck *et al*., 1976).

***In vitro* DPPH radical scavenging activity:** The free radical scavenging activity of *A. kurdicus* and *A. cinereus* extracts against DPPH radicals was shown in Fig. 1. Both the plant

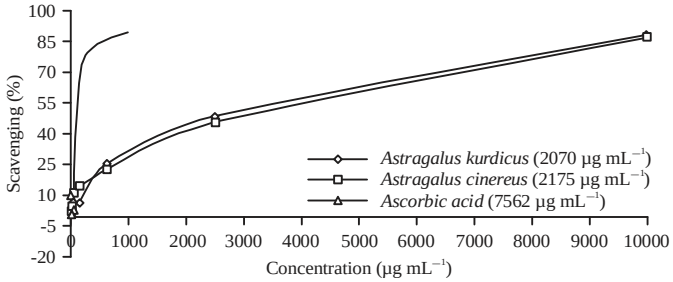

Fig. 1: *In vitro* antioxidant activity of *Astragalus kurdicus, Astragalus cinereus* and ascorbic acid using DPPH radical scavenging activity method

crude extracts and ascorbic acid (as standard for antioxidant) showed antioxidant activity in a concentration-dependent manner. *Astragalus kurdicus* and *A. cinereus* extracts showed the highest *in vitro* antioxidant activities at a concentration of 10000 μg mL^{-1} with scavenging activities of 88.9 and 87.3%, respectively. The EC_{50} values for *A. kurdicus*, *A. cinereus* and ascorbic acid were calculated to be 2070, 2175 and 7562 μg mL^{-1}, respectively. This finding is correlated with previous studies that the antioxidant activity of *Astragalus* is due to its content of bioactive compounds, such as flavonoids (Asgarpanah *et al*., 2011). Moreover, it was reported that if the EC_{50} value of an extract is less than 10 mg mL^{-1}, it indicates that the extract is an effective antioxidant (Lee *et al*., 2008). In the current study, the EC_{50} values of both extracts were less than 10 mg mL^{-1}, demonstrating that both extracts are effective antioxidants.

Serum and liver biochemical markers: Liver injury induced by PCM is the best characterized system of xenobiotic induced hepatotoxicity and is commonly used model for screening hepatoprotective drugs. It is well established that the formation of the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) by hepatic cytochrome P_{450} is the initial step in the development of PCM-induced liver toxicity (Yang *et al*., 2013). In overdose conditions, NAPQI leads to depletion of hepatic GSH, which permits excess NAPQI to covalently bind cellular macromolecules such as protein. These events are followed by oxidative stress and mitochondrial damage, both of which have been thought to be the major mechanisms for PCM-induced liver injury. When liver cell is injured, a variety of liver marker enzymes (ALT, AST, ALP and γ-GT) located normally in cytosol is released into the blood. Accordingly, the serum activities of ALT, AST, ALP and γ-GT in addition to serum BRN level are largely used as most common biochemical markers to evaluate liver injury (Girish *et al*., 2009).

In the present study, serum biochemical parameters in the control and various experimental groups are presented in Table 1-3. Rats treated with an overdose of PCM (2 g kg^{-1}) developed significant hepatic damage as indicated by a significant increase in the activities of serum marker enzymes (ALT, AST, ALP, γ-GT) and BRN levels compared to normal control rats. Elevation of the activities of those serum marker enzymes is indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Added to that, the serum of PCM control group showed decrease in TP and Alb levels in comparison to the normal control rats. Treatment of rats with *A. kurdicus* and *A. cinereus* extracts (200 or 400 mg kg^{-1}) for 7 days before PCM administration restored serum level of ALT, AST, ALP, γ-GT, BRN, TP and ALB towards their normal values. The efficacy of both extracts was comparable with that of the standard drug silymarin and their activities were found to be dose dependent. The lowered levels of TP and ALB in the serum reveal the intensity of hepatopathy (Aniya *et al*., 2005). The majority of ALB is synthesized in the liver. Therefore, variation of serum TP or ALB concentrations can reflect liver health status (Wan *et al*., 2014). In the current study, a marked decrease of serum TP and ALB levels were observed in PCM-hepatotoxic rats compared with the normal control animals. Moreover, PCM may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins such as ALB in the liver (Yakubu *et al*., 2013). Besides, reduction of serum albumin in PCM treated group may be due to binding of the reactive metabolite of PCM (NAPQI) to the amino acid cysteine in proteins, forming PCM protein adducts (Cohen and Khairallah, 1997). Pretreatment with *A. kurdicus* and *A. cinereus* extracts showed a significant reversal of these parameters toward the normal. This assures the hepatoprotective activity of both extracts against PCM-hepatotoxicity.

Almost all organisms possess antioxidant defense systems including antioxidant enzymes (SOD, GPx and CAT) and nonenzymatic antioxidants. However, these systems are insufficient to prevent the damage entirely in some cases (Simic, 1988). The reduction in the activity of these enzymes may results in number of deleterious effect due to accumulation of highly toxic metabolites and hydrogen peroxide on PCM administration, which can induce oxidative stress in the cells (Pauli-Magnus *et al*., 2005). Oral administration of PCM, at the dose of 2 g kg^{-1} to rats showed a significant decrease in the activity of SOD, GPx and CAT in their liver homogenates, comparative to the normal control group. The suppression in the activity of these enzymes may results in a number of deleterious effects due to the

Table 1: Effect of the ethanol extracts of *Astragalus kurdicus* and *Astragalus cinereus* on the serum activity of liver marker enzymes in rats with PCM-induced hepatotoxicity

Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	γ-GT (U L^{-1})
Normal control	35.9±1.85‡	65.4±1.45‡	122.4±3.45‡	13.4±0.13‡
Hepatotoxic control	59.8±1.31*	87.8±2.87*	328.2±7.56*	27.0±0.35*
A. kurdicus (200 mg kg^{-1})+PCM	56.2±1.62*	84.3±2.35*	316.8±6.23*	24.2±0.27*
A. kurdicus (400 mg kg^{-1})+PCM	48.4±1.82*‡	74.8±2.04*‡	226.8±5.94‡	18.2±0.07‡
A. cinereus (200 mg kg^{-1})+PCM	45.4±1.15*‡	71.0±2.31‡	215.5±3.04‡	19.6±0.18‡
A. cinereus (400 mg kg^{-1})+PCM	42.8±1.72*‡	68.6±1.54‡	199.4±3.16‡	16.6±0.11‡
Silymarin (50 mg kg^{-1})+PCM	41.0±1.61‡	66.2±2.58‡	148.4±3.25‡	15.6±0.15‡

Values are expressed as Mean±SEM, n = 6 rats/group, *p<0.05: Statistically significant from normal control (Dunnett's test), ‡ p< 0.05: Statistically significant from hepatotoxic control (Dunnett's test), ALT: Alanine amino transferase, AST: Asparate aminotransferase, ALP: Alkaline phosphatase, γ-GT: γ-glutamyl transferase

Table 2: Effect of the ethanol extracts of *Astragalus kurdicus* and *Astragalus cinereus* on the serum levels of total protein, albumin and bilirubin in rats with PCM- induced hepatotoxicity

Groups	TP (g dL^{-1})	ALB (g dL^{-1})	BRN (mg dL^{-1})
Normal control	7.3±0.11‡	3.5±0.07‡	0.52±0.02‡
Hepatotoxic control	5.2±0.10*	2.2±0.06*	1.16±0.06*
A. kurdicus (200 mg kg^{-1})+PCM	5.5±0.08*	2.4±0.05*	1.04±0.04*
A. kurdicus (400 mg kg^{-1})+PCM	6.4±0.09*‡	2.8±0.07*‡	0.74±0.02*‡
A. cinereus (200 mg kg^{-1})+PCM	6.6±0.11*‡	2.9±0.09*‡	0.67±0.03*‡
A. cinereus (400 mg kg^{-1})+PCM	6.7±0.10*‡	3.1±0.10*‡	0.63±0.03*‡
Silymarin (50 mg kg^{-1})+PCM	7.0±0.09‡	3.3±0.11‡	0.61±0.02‡

Values are expressed as Mean±SEM, n =6 rats/group, *Significance compared to normal control group at p<0.05 (Dunnett's test), ‡Significance compared to hepatotoxic control group at p<0.05 (Dunnett's test), TP: Total protein, ALB: Albumin, BRN: Bilirubin

Table 3: Effect of the ethanol extracts of *Astragalus kurdicus* and *Astragalus cinereus* on hepatic antioxidant profile, glutathione and lipid peroxidation in liver homogenate of rats with PCM-induced hepatotoxicity

Groups	SOD (U mg^{-1} protein)	GPx (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH (µmol g^{-1} tissue)	MDA (nmol g^{-1} tissue)
Normal control	55.2±2.47‡	3.73±0.09‡	17.7±0.26‡	15.5±0.28‡	21.9±1.13‡
Hepatotoxic control	24.7±0.92*	1.15±0.05*	7.5±0.10*	8.6±0.17*	60.6±1.50*
A. kurdicus (200 mg kg^{-1})+PCM	30.2±1.54*	1.32±0.05*	8.4±0.17*	9.1±0.17*	55.2±2.54*
A. kurdicus (400 mg kg^{-1})+PCM	37.7±1.71*‡	2.48±0.08*‡	11.3±0.22*‡	11.2±0.25*‡	41.7±1.57*‡
A. cinereus (200 mg kg^{-1})+PCM	36.7±1.25*‡	2.52±0.13*‡	10.8±0.21*‡	11.3±0.28*‡	33.2±1.63*‡
A. cinereus (400 mg kg^{-1})+PCM	40.5±2.79*‡	2.96±0.11*‡	12.1±0.18*‡	12.7±0.32*‡	29.8±0.84*‡
Silymarin (50 mg kg^{-1})+PCM	48.3±2.55‡	3.17±0.18‡	14.8±0.29‡	13.9±0.18‡	26.7±0.87‡

Values are expressed as Mean±SEM, n = 6 rats/group, *Significance compared to normal control group at p<0.05 (Dunnett's test), ‡Significance compared to hepatotoxic control group at p<0.05 (Dunnett's test), SOD: Superoxide dismutase, Gpx: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde

accumulation of superoxide radicals and hydrogen peroxide. Pretreatment of rats with *A. kurdicus* and *A. cinereus* extracts increased the activities of SOD, GPx and CAT enzymes in a dose dependent manner to prevent the accumulation of excessive free radicals and protects the liver against PCM intoxication. In this concern, it was mentioned that *Astragalus* polysaccharides are one of the main efficacious principles of *Astragalus*, which is reported to have antioxidant activity (Wu and Chen, 2004).

Paracetamol overdose causes liver injury secondary to the temporary depletion of glutathione stores in the liver. Glutathione conjugation is critical in preventing liver injury from several agents, including acetaminophen, by acting as a free radical scavenger (Sturgill and Lambert, 1997). The GSH depletion is considered one of the main biochemical markers for PCM-caused hepatotoxicity (James *et al*., 2003). The GSH is an intracellular reductant, widely distributed in cells and plays major role to protect cells against free radicals, peroxides and other toxic compounds (Fairhurst *et al*., 1982). Besides, the depletion of GSH causes the endogenous Reactive Oxygen Species (ROS) to bind to cellular macromolecules leading to initiation of processes of lipid peroxidation, membrane breakdown and cell death (Udem *et al*., 1997). Pretreatment of rats with *A. kurdicus* and *A. cinereus* extracts reduced GSH depletion induced by PCM and protected markedly against its hepatotoxicity.

Malondialdehyde (MDA), a lipid peroxidized product, can reflect the extent of lipid peroxidation induced by oxidative stress. In the present investigation, a significant increase in MDA level was observed in the liver homogenate of PCM-hepatotoxic rats compared with the normal control animals. Previous studies have proved that lipid peroxidation has been postulated as being the destructive process in liver injury due to PCM administration (Guillen-Sans and Guzman-Chozas, 1998). Pretreatment with the tested extracts significantly reversed these changes in a dose dependent manner. In this respect, the increase in GPx activity in animals treated with both extracts as GPx has been known to reduce lipid peroxidation reactions (Levy *et al*., 1999). The present results suggest that the possible mechanism of action by extracts could be related to their antioxidants activity. This may be due to the presence of flavonoids and/or polysaccharides that exhibit anti-inflammatory, antioxidant and hepatoprotective properties (Hong *et al*., 2014).

Histopathological analysis: Histopathological studies of liver tissue of the control rats showed normal hepatic architecture of hepatic lobule (Fig. 2a). The histological architecture of PCM treated liver sections showed focal hepatic necrosis associated with inflammatory cells infiltration and destruction of the lobular architecture and collections of inflammatory cells (Fig. 2b). Histopathological analysis showed that the pathological lesions caused by PCM were very minimal in groups pretreated with ethanolic extracts of *A. kurdicus* and *A. cinereus*. Normal hepatocytes with slight activation of Kupffer cells were observed in groups treated with ethanolic extract of *A. kurdicus* (Fig. 2c). Liver tissue from *A. cinereus* group had normal hepatic cells with hydropic degeneration of some hepatocytes (Fig. 2d). Silymarin treated group (50 mg kg^{-1}) showed necrosis of centrolobular hepatocytes (Fig. 2e).

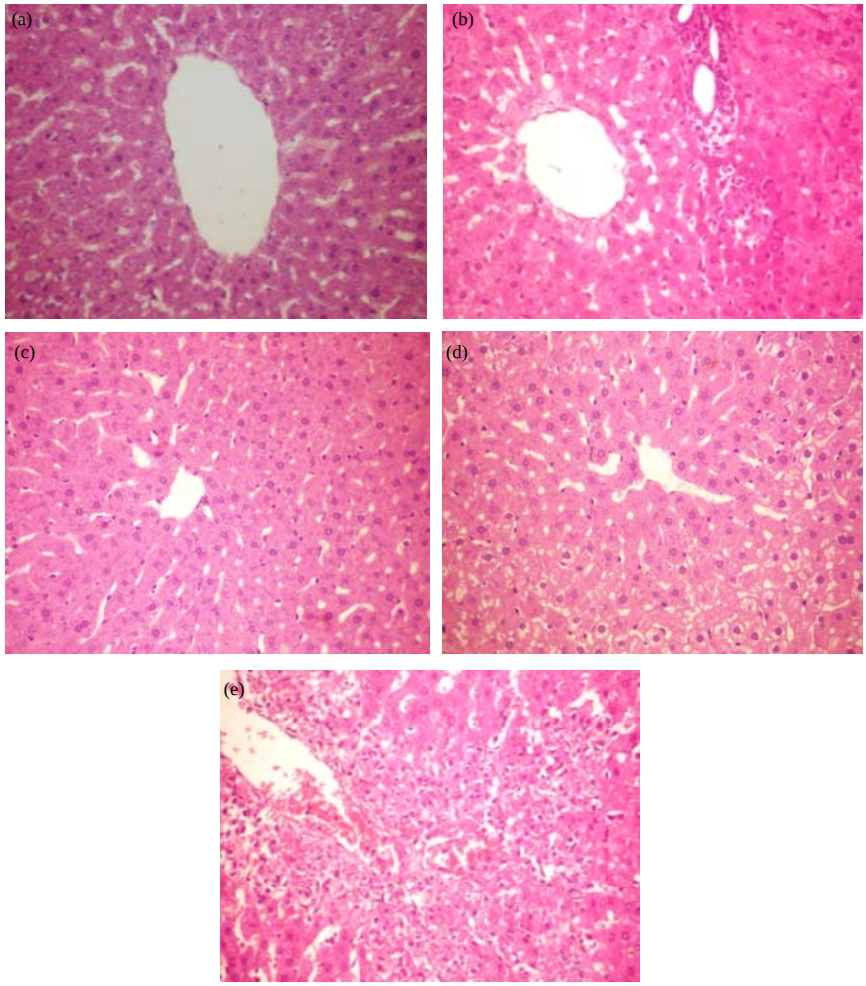

Fig. 2(a-e): Photomicrographs of rat liver, (a) Normal control group showing the normal histological structure of hepatic tubule, (b) PCM-hepatotoxic group showing focal hepatic necrosis associated with inflammatory cells infiltration, (c) *Astragalus kurdicus* (400 mg kg^{-1}) +PCM showing normal hepatocytes with slight activation of Kupffer cells, (d) *Astragalus cinereus* (400 mg kg^{-1}) +PCM showing normal hepatic cells with hydropic degeneration of some hepatocytes and (e) Silymarin (50 mg kg^{-1}) +PCM showing necrosis of centrolobular hepatocytes (H and E, ×400)

CONCLUSION

In conclusion, *A. kurdicus* and *A. cinereus* extracts have a hepatoprotective effect in addition to their *in vitro* antioxidant activities. They restored the antioxidant parameters like SOD, CAT, GPx, GSH and MDA in PCM-hepatotoxic rats. Accordingly, the hepatoprotective effect of both extracts might be attributed to their antioxidant activities. Further phytochemical studies are required to isolate and identify the active compound(s) responsible for the antioxidant and hepatoprotective activities.

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