

Hepatoprotective activities of *Astragalus persicus* and *Astragalus tournefortii* ethanolic extracts against paracetamol induced liver damage in rats and their *in vitro* antioxidant effects

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Hepatoprotective activities of Astragalus persicus and Astragalus tournefortii ethanolic extracts against paracetamol induced liver damage in rats and their in vitro antioxidant effects

Astragalus persicus ve Astragalus tournefortii etanol ekstralarının, sıçanlarda, parasetamol ile indüklenmiş karaciğer hasarına karşı hepatoprotektif aktivileri ve in vitro antioksidan etkileri

SUMMARY

The aim of this study was to investigate the potential hepatoprotective effect of the ethanol extracts of *Astragalus persicus* (DC.) Fisch. & C.A.Mey (*A. persicus*) and *Astragalus tournefortii* Boiss (*A. tournefortii*) in a rat model of paracetamol (PCM) induced liver damage. PCM administration caused severe hepatic damage in rats as evidenced by elevated serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyl transferase (γ -GT) and serum level of total bilirubin (BRN), while decreased serum level of total protein (TP) and albumin (ALB). In liver homogenates, PCM elevated malondialdehyde (MDA) but decreased glutathione (GSH) level as well as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities. Administration of *A. persicus* and *A. tournefortii* extracts (200 and 400 mg/kg) for 7 days before PCM inhibited the acute elevation of the serum activities of AST, ALT, ALP and γ -GT enzymes and serum level of BRN. PCM-induced lipid peroxidation was likewise attenuated by both extracts. Similarly, both extracts increased the activities of the antioxidant enzymes (GPx, SOD, CAT) and reduced GSH concentration in the liver homogenates. The results of the *in vitro* antioxidant effect revealed considerable antioxidant activity for both extracts. The median effective concentration (EC₅₀) values of *A. persicus* and *A. tournefortii* extracts and ascorbic acid for DPPH radical scavenging activities were 6455, 6199 and 75.62 μ g/ml, respectively. It was concluded that *A. persicus* and *A. tournefortii* possess hepatoprotective activities that could be partly attributed to their antioxidant effects.

Key Words: *Astragalus persicus*, *Astragalus tournefortii*, paracetamol, antioxidant, hepatoprotective activity.

ÖZET

Bu çalışmanın amacı *Astragalus persicus* (DC.) Fisch. & C.A.Mey (*A. persicus*) ve *Astragalus tournefortii* Boiss (*A. tournefortii*) etanol ekstralarının parasetamol (PCM) ile indüklenmiş karaciğer hasarı olan sıçan modelinde potansiyel hepatoprotektif etkisinin araştırılmasıdır. PCM uygulaması, sıçanlarda, yükselmiş aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkaline fosfataz (ALP), γ -glutamyl transferaz (γ -GT) ve total bilirubin (BRN) serum düzeyleri ve azalmış toplam protein (TP) ve albumin (ALB) serum düzeyleri ile kanıtlanmış çok ciddi karaciğer hasarına sebep olmaktadır. Karaciğer homojenatlarında, PCM malondialdehit (MDA) düzeyinde artışa sebep olurken, glutatyon (GSH), glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitelerinde azalmaya sebep olmaktadır. *A. persicus* ve *A. tournefortii* ekstralarının (200 ve 400 mg/kg) PCM'den önce 7 gün boyunca uygulanması, AST, ALT, ALP ve γ -GT enzimlerinin serum aktiviteleri ve BRN'in serum düzeyinin akut yükselmesini önlemiştir. PCM ile indüklenmiş lipit peroksidasyonu her iki ekstre tarafından da benzer şekilde azaltılmaktadır. Buna benzer olarak, karaciğer homojenatlarında her iki ekstre antioksidan enzimlerinin (GPx, SOD, CAT) aktivitelerini arttırmakta ve GSH konsantrasyonunu azaltmaktadır. *In vitro* antioksidan çalışmanın sonuçları, her iki ekstre'nin de önemli bir antioksidan aktivitesi olduğunu göstermektedir. DPPH radikal süpürücü aktivite için, *A. persicus* ve *A. tournefortii* ekstralarının ve askorbik asitin maksimum etkisinin %50'sini oluşturan konsantrasyonu yani EC₅₀ değeri sırasıyla 6455, 6199 ve 75.62 μ g/ml'dir. Bu sonuçlardan, *A. persicus* ve *A. tournefortii*'nin hepatoprotektif aktiviteye sahip olduğunu, bu aktivitenin de kısmen büki ekstralarının antioksidan etkilerine bağlı olabileceği sonucuna varılmıştır.

Anahtar kelimeler: *Astragalus persicus*, *Astragalus tournefortii*, parasetamol, antioksidan aktivite, hepatoprotektif aktivite

Received: 04.06.2015

Revised: 11.08.2015

Accepted: 20.08.2015

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INTRODUCTION

The liver, the second largest organ after the skin, has anabolic, detoxifying and storage functions and is indispensable for life. Liver toxicity is a common cause of severe metabolic disorders and even death. The manifestations of xenobiotic-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to culminate hepatic failure. Malnutrition, anemia, infection, and availability of over-the-counter hepatotoxic drugs are the most frequent causes of liver damage in developing countries (1). It is well recognized that free radicals are critically involved in various pathological conditions including liver diseases. Unfortunately most of the synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious adverse effects. In view of severe undesirable side effects of synthetic agents, there is growing interest in herbal remedies because of their minimal side effects and relatively low cost. Accordingly, efforts have been made to find suitable agents for the treatment of liver diseases among natural products.

The genus *Astragalus* L. belongs to the family Fabaceae of the order Leguminales. In Turkey there are 445 species of *Astragalus*, of which 224 are endemic (2). 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 (3). They are also famed for their antimicrobial, antiperspirant, cardioprotective and anti-inflammatory effects (4). 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 (5). Earlier investigations on Turkish *Astragalus* species resulted in the isolation of a series of oleanane- and cycloartane-type triterpenoidal saponins (6). The isolated glycosides show interesting biological properties, including immunostimulating, antiprotozoal, antiviral, cytotoxic and wound healing (7). The flavonoid mixture of some species revealed strong antioxidant activity (8).

PCM 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 in experimental animals and humans. It is mainly metabolized by hepatic cytochrome P450 to excretable glucuronide and sulphate conjugates (9). The hepatotoxicity of PCM has been attributed to the formation of the highly reactive me-

tabolite N-acetyl-P-benzoquinone imine (NAPQI) (10). 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. Against this background, the present study was undertaken to assess the potential hepatoprotective effect of *A. persicus* and *A. tournefortii* extracts in a rat model of hepatic damage caused by PCM.

MATERIAL AND METHODS

Plant material

Root parts of *Astragalus persicus* (DC.) Fisch. & C.A.Mey and *Astragalus tournefortii* Boiss were collected from Van: Çatak, 3 km from Görentaş to Çatak and Siirt: Pervari, southern east of Gözlü (Erkis) town, East Anatolia, Turkey in June 2010, and identified by Assoc. Prof. Dr. Fevzi Özgökçe (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 13702 & VANF 13812, respectively).

Preparation of plant extract

The collected roots were shade dried and then grinded to fine powdered. The air-dried powdered roots (150 g) was extracted with 80% aqueous ethanol (2×3 L) under reflux. The ethanolic extract was combined and subjected to rotary evaporation (70±2°C). The thick solution was lyophilized using freeze dryer to give the total extracts of *A. persicus* (22.4 g, yield 14.9% w/w) and *A. tournefortii* (19.5 g, yield 13% w/w). Both extracts were stored in the refrigerator and aliquot of the concentrations were prepared immediately before use.

Test Animals

Adult male Wistar rats (180-200 g) were obtained from the 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 In-

stitutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

DPPH radical scavenging activity

The DPPH assay was performed according to Phang *et al.* (11). A solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) 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 μ l of each concentration to 180 μ l DPPH solution was carried out. Negative controls were done to correct for colored extracts. The resultant reaction mixtures were 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:

Where A0 is the absorbance of the control and A1 is the absorbance of the extract. EC₅₀ values were determined from the graph of percentage of inhibition plotted against the concentration of the tested extract. EC₅₀ 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 (12). Two groups of rats ($n=6$) were fasted overnight then treated orally with *A. persicus* and *A. tournefortii* 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. persicus and *A. tournefortii* 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.

Hepatoprotective activities

PCM induced hepatotoxicity model was adopted for the study (13). The rats were divided into 7 groups

($n=6$). Group I (normal control) and Group II (hepatotoxic group) received the vehicle (1 mL/kg b.wt, for 7 days). Group III received the reference drug, silymarin (Sigma Chemical Company, USA) at 50 mg/kg, for 7 days. Groups IV and V received the ethanolic extract of *A. persicus* (200 and 400 mg/kg, respectively for 7 days). Groups VI and VII received the ethanolic extract of *A. tournefortii* (200 and 400 mg/kg, respectively for 7 days). On the 7th day, PCM (Sigma Chemical Company, USA) was suspended in 0.5% Tween-80 and administered orally (2 g/kg b.wt), to all the rats except those in Group I.

After 48 h of PCM administration, animals were anaesthetized using ether and 1 mL of blood was collected by cardiac puncture. The blood was allowed to clot and centrifuged at 3000 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 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, 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 10 mL (10% 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, GPx and CAT were assayed in the hepatic tissue homogenate of all rats according to the methods of Sun and Zigman (14), Mohandas *et al.* (15) and Chance and Maehley (16), respectively. Moreover, levels of MDA were assessed in the liver homogenate as a measure for lipid peroxidation according to Ohkawa *et al.* (17), while GSH tissue content was measured according to the method described by Moron *et al.* (18). 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 μ m thickness and stained with haematoxylin and eosin (H&E) for histological evaluation.

Statistical analysis

The values are expressed as mean \pm 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 ver. 14.0. A comparison was made with the normal control and PCM-hepatotoxic groups. We took a P-value of <0.05 as our desired level of significance.

RESULTS AND DISCUSSION

Acute oral toxicity test in rats

Evaluation of the potential toxicity of natural products is usually an initial step in screening for their pharmacological activities. The non-toxic nature of *A. persicus* and *A. tournefortii* extracts is evident by the absence of mortality of rats following oral doses up to 4000 mg/kg. Accordingly, it suggested that their LD₅₀ values were higher than 4 g/kg b.wt. In general, the higher the LD₅₀ value, the lower toxic the compound. Therefore, the tested extracts can be categorized as highly safe since substances possessing LD₅₀ higher than 50 mg/kg are non-toxic (19). In addition, substances with LD₅₀ values higher than 4000 mg/kg by oral route are regarded as being safe or practically nontoxic (20).

DPPH radical scavenging activity

In the present study, *A. persicus* and *A. tournefortii* extracts showed *in vitro* antioxidant activities at concentrations of 625, 2500 and 10000 µg/mL (Figure 1). At these concentrations, the scavenging activities of *A. persicus* were 2.62, 21.18 and 64.41%, respectively while

those of *A. tournefortii* were 1.97, 25.11 and 64.41%, respectively. The EC₅₀ values for *A. persicus*, *A. tournefortii* and ascorbic acid were calculated to be 6455, 6199 and 75.62 µg/mL, respectively.

Serum and liver biochemical markers

Hepatic damage induced by overdoses of PCM is the best characterized system of xenobiotic induced liver toxicity and is commonly used model for screening hepatoprotective agents. It is well established that the formation of the highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) by cytochrome P450 of the liver is the initial step in development of PCM-induced hepatotoxicity. In PCM overdose, NAPQI leads to depletion of hepatic GSH, which allows excess NAPQI to 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 damage (21).

In the present investigation, serum biochemical parameters in the control and various experimental groups are depicted in Tables (1-2). Rats treated with an overdose of PCM (2 g/kg) developed significant hepatic damage as indicated by a significant increase in the serum activities of ALT, AST, ALP, γ-GT and level of BRN by 230.42% 334.09%, 67.98%, 212.50% and 111.76%, respectively compared to normal controls. The elevated

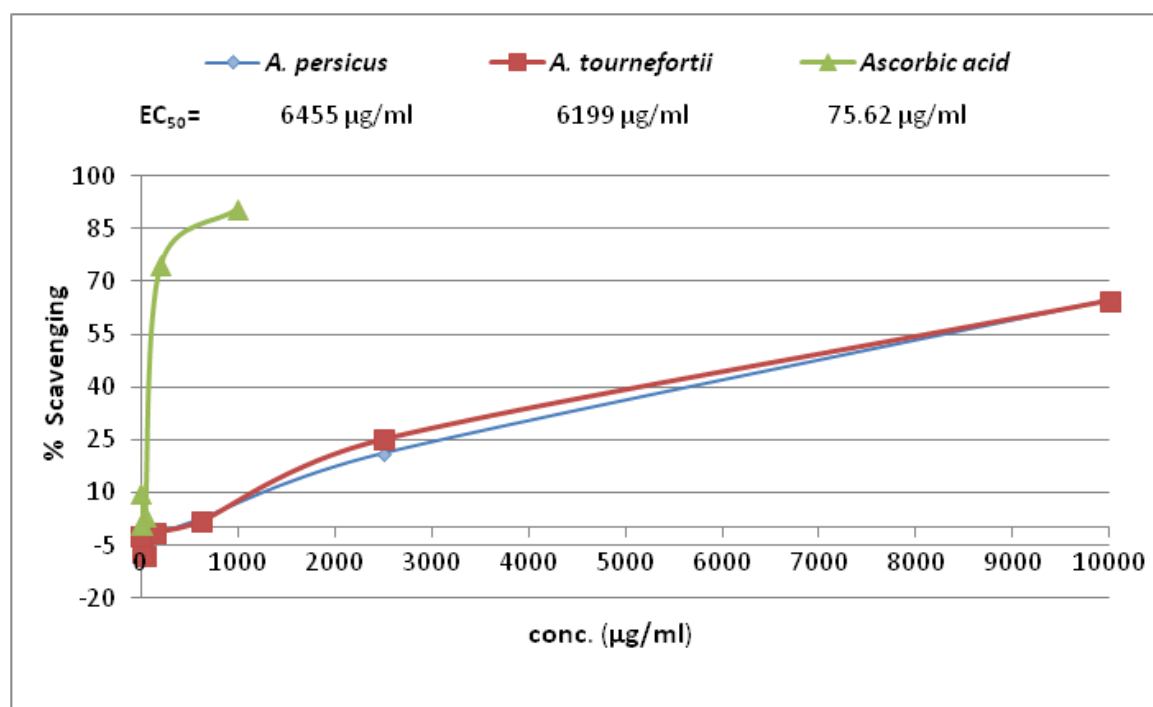


Figure 1. *In vitro* antioxidant activity of *A. persicus*, *A. tournefortii* and ascorbic acid, using DPPH radical scavenging activity method.

levels of liver marker enzymes in serum of PCM-intoxicated rats can be attributed to the damaged structural integrity of the liver because these are cytoplasmic in nature and are released into the circulation after cellular damage (22). In addition, the abnormal level of BRN in serum of PCM-intoxicated rats could be attributed to

impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction (23).

Pretreatment of rats with *A. persicus* (200 and 400 mg/kg) and *A. tournefortii* (200 and 400 mg/kg) extracts have prevented the increased activities of serum marker

Table 1. Effect of the ethanol extracts of *A. persicus* and *A. tournefortii* on the serum activity of liver marker enzymes in rats with PCM- induced hepatotoxicity.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	γ-GT (U/L)
Normal Control	107.8±3.74 ^b	65.7±2.17 ^b	265.2±6.32 ^b	1.6±0.05 ^b
PCM-hepatotoxic Control	356.2±8.57 ^a (+230.42)	285.2±6.64 ^a (+334.09)	445.5±9.65 ^a (+67.98)	5.0±0.18 ^a (+212.5)
Silymarin (50 mg/kg) +PCM ^c	148.5±4.32 (-58.30)	88.5±2.85 (-68.96)	287.3±7.75 (-35.51)	2.2±0.11 (-56.0)
<i>A. persicus</i> (200 mg/kg) +PCM ^c	256.2±6.35 (-28.07)	125.2±3.49 (-56.10)	357.0±8.54 (-19.86)	3.5±0.17 (-30.0)
<i>A. persicus</i> (400 mg/kg) +PCM ^c	185.3±6.25 (-47.97)	102.7±3.55 (-63.99)	311.8±7.86 (-30.01)	2.9±0.17 (-42.0)
<i>A. tournefortii</i> (200 mg/kg) +PCM ^c	248.4±5.84 (-30.26)	118.2±3.84 (-58.55)	334.2±7.48 (-24.98)	3.2±0.12 (-36.0)
<i>A. tournefortii</i> (400 mg/kg) +PCM ^c	177.4±5.23 (-50.19)	96.5±2.95 (-66.16)	310.8±7.56 (-30.23)	2.7±0.11 (-46.0)

The results are expressed as mean ± S.E.M., values between brackets means % changes, 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).

(+) represents percentage of increase and (-) represents decrease in each value when compared to either vehicle or PCM

^c Compared to PCM as hepatotoxin

Table 2. Effect of the ethanol extracts of *A. persicus* and *A. tournefortii* on the serum levels of TP, ALB and BRN in rats with PCM- induced hepatotoxicity.

Groups	TP (g/dL)	ALB (g/dL)	BRN (mg/dL)
Normal Control	7.5±0.18 ^b	3.5±0.05 ^b	1.7±0.07 ^b
PCM-hepatotoxic Control	5.4±0.13 ^a (-28.00)	2.2±0.08 ^a (-37.14)	3.6±0.13 ^a (+111.76)
Silymarin (50 mg/kg) +PCM ^c	6.8±0.15 (+25.92)	3.1±0.11 (+40.90)	2.1±0.10 (-41.66)
<i>A. persicus</i> (200 mg/kg) +PCM ^c	6.2±0.13 (+14.81)	2.8±0.09 (+27.27)	2.8±0.09 (-22.22)
<i>A. persicus</i> (400 mg/kg) +PCM ^c	6.3±0.17 (+16.66)	2.9±0.08 (+31.81)	2.5±0.10 (-30.55)
<i>A. tournefortii</i> (200 mg/kg) +PCM ^c	6.3±0.18 (+16.66)	2.8±0.08 (+27.27)	2.6±0.08 (-27.77)
<i>A. tournefortii</i> (400 mg/kg) +PCM ^c	6.5±0.18 (+20.37)	3.0±0.12 (+36.36)	2.3±0.07 (-36.11)

The results are expressed as mean ± S.E.M., values between brackets means % changes, 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).

(+) represents percentage of increase and (-) represents decrease in each value when compared to either vehicle or PCM

^c Compared to PCM as hepatotoxin

enzymes and BRN level in a dose-dependent manner. The hepatoprotective effect of *A. persicus* and *A. tournefortii* extracts may be the result of stabilization of plasma membrane so preserving the structural integrity of hepatocytes as well as the repair of hepatic tissue damage caused by PCM. These findings are in agreement with the documented fact that serum levels of ALT, AST, ALP and γ -GT return to normal following healing of hepatic parenchyma and regeneration of hepatocytes (24). In addition, the return of ALT, AST, ALP and γ -GT activities toward normal may be due to the inhibitory effects of the tested extracts (200 and 400 mg/kg) on cytochrome P450 or/and promotion of its glucuronidation (25).

ALB is the most abundant plasma protein produced by hepatocytes. Therefore, variation of serum TP or ALB concentrations can reflect liver health status. In the current study, marked reductions of serum levels of TP and ALB were observed in PCM-hepatotoxic rats compared with the normal control animals. The reduction in serum TP and ALB may be due to binding of the reactive metabolite of PCM (NAPQI) to the amino acid cysteine in proteins, forming PCM protein adducts. Pretreatment with *A. persicus* (200 and 400 mg/kg) and *A. tournefortii* extracts (200 and 400 mg/kg) showed a significant reversal of these parameters toward the normal and suggested the stabilization of endoplasmic re-

ticulum that are responsible for protein synthesis. This assures the protective activity of both extracts against PCM-hepatotoxicity.

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Most organisms possess antioxidant defense systems including antioxidant enzymes (SOD, CAT and GPx) in addition to nonenzymatic antioxidants. The reduction in the activity of these enzymes may results in a number of adverse effects due to the accumulation of superoxide radicals and hydrogen peroxide. Since PCM induced hepatotoxicity is due mostly to toxic metabolite NAPQI that causes oxidative stress, antioxidant mediated protective role of *A. persicus* and *A. tournefortii* extracts has been assessed.

The activities of SOD, CAT, and GPx and the levels of MDA and GSH in rat's liver homogenates were shown in Table 3. Significant decline in the activities of SOD, CAT, and GPx in the liver homogenates were estimated in PCM-hepatotoxic rats as compared to normal control group. The activities of these enzymes were significantly restored towards their normal values in rats pre-medicated with silymarin, *A. persicus* or *A. tournefortii* for 7 days prior to PCM administration. Attainment of near normal level of SOD, CAT, GPx, MDA and GSH in *A. persicus* (200 and 400 mg/kg) and *A. tournefortii* (200 and 400 mg/kg)-treated rats confirms the hepatoprotective

Table 3. Effect of the ethanol extracts of *A. persicus* and *A. tournefortii* on hepatic antioxidant profile, glutathione (GSH) and lipid peroxidation (MDA) in liver homogenate of rats with PCM- induced hepatotoxicity.

Groups	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	GSH (μ mol/ g tissue)	MDA (nmol/g tissue)
Normal Control	52.6 \pm 2.16 ^b	3.26 \pm 0.15 ^b	13.5 \pm 0.30 ^b	9.8 \pm 0.18 ^b	41.3 \pm 1.36 ^b
PCM-hepatotoxic Control	27.2 \pm 0.85 ^a (-48.28)	1.28 \pm 0.06 ^a (-60.73)	7.7 \pm 0.13 ^a (-42.96)	5.7 \pm 0.12 ^a (-41.83)	97.6 \pm 2.55 ^a (+136.31)
Silymarin (50 mg/kg) +PCM ^c	46.5 \pm 1.57 (+70.95)	2.84 \pm 0.12 (+121.87)	11.2 \pm 0.28 (+45.45)	8.8 \pm 0.15 (+54.38)	51.2 \pm 1.83 (-47.54)
<i>A. persicus</i> (200 mg/kg) +PCM ^c	38.4 \pm 1.15 (+41.17)	2.16 \pm 0.11 (+68.75)	9.6 \pm 0.18 (+24.67)	7.8 \pm 0.11 (+36.84)	67.7 \pm 2.74 (-30.63)
<i>A. persicus</i> (400 mg/kg) +PCM ^c	39.5 \pm 1.14 (+45.22)	2.24 \pm 0.18 (+75.00)	9.7 \pm 0.16 (+25.97)	8.0 \pm 0.13 (+40.35)	63.1 \pm 1.75 (-35.34)
<i>A. tournefortii</i> (200 mg/kg) +PCM ^c	39.8 \pm 1.22 (+46.32)	2.35 \pm 0.15 (+83.59)	10.0 \pm 0.19 (+29.87)	8.0 \pm 0.18 (+40.35)	61.5 \pm 2.36 (-36.98)
<i>A. tournefortii</i> (400 mg/kg) +PCM ^c	41.7 \pm 1.42 (+53.30)	2.55 \pm 0.19 (+99.21)	10.6 \pm 0.17 (+37.66)	8.3 \pm 0.17 (+45.61)	58.8 \pm 1.46 (-39.75)

The results are expressed as mean \pm S.E.M., values between brackets means % changes, n = 6 rats/group.

^a indicate significance compared to normal control group at p < 0.05 (Dunnnett's test).

^b indicate significance compared to hepatotoxic control group at p < 0.05 (Dunnnett's test). (+) represents percentage of increase and (-) represents decrease in each value when compared to either vehicle or PCM

^c Compared to PCM as hepatotoxin.

effect of both extracts. The capability of both extracts to protect against PCM-induced hepatic damage in rats might be attributed to the ability of their secondary metabolites to restore the activity of antioxidant enzymes. The genus *Astragalus* is well known to be a rich source of bioactive secondary metabolites. The biologically active constituents of *Astragalus* species are saponin, phenolic and polysaccharide compounds. In this connection, Wu and Chen (26) mentioned that polysaccharides are one of the main efficacious active principles of *Astragalus*, which is reported to have antioxidant activity.

GSH depletion is considered as one of the main biochemical markers for PCM-caused hepatotoxicity. GSH is widely distributed in the cells and plays a major role to protect cells against free radicals, peroxides and other toxic compounds (27). In the present investigation, we have observed that reduced GSH level was depleted significantly in the liver homogenate of PCM-treated rats by 41.83% compared to normal control group. Depletion of GSH in liver homogenate of PCM-hepatotoxic group may be due to conjugation of NAPQI metabolite with GSH to form mercapturic acid. Furthermore, GSH depletion catalyze the endogenous reactive oxygen species (ROS) to bind to cellular macromolecules leading to initiation of lipid peroxidation processes, break-

down of cellular membranes and cell death (28). The decreased levels of GSH which were observed in the liver homogenates of PCM-treated rats were significantly restored towards normal values by treatment with 200 and 400 mg/kg of *A. persicus* and *A. tournefortii* extracts. MDA, a lipid peroxidized product, can reflect the extent of lipid peroxidation induced by oxidative stress. Lipid peroxidation is a common consequence of cell death and its MDA level is elevated due to oxidative stress associated with PCM (29). In the present study, the level of MDA as an index of lipid peroxidation in liver homogenate of PCM-treated rats was significantly increased by 136.31% when compared to normal control animals. Pretreatment with *A. persicus* and *A. tournefortii* extracts significantly reversed these changes in a dose dependent pattern. This result 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. Accordingly, it may be possible that the mechanism of hepatoprotection of both extracts is due to their ability to reduce lipid peroxidation. In this connection, Zhang *et al.* (30) have reported the antioxidant effect of *Astragalus* and its component cycloastragenol-xylo-syl-glucoside on lipid peroxidation, *in vivo* and *in vitro*.

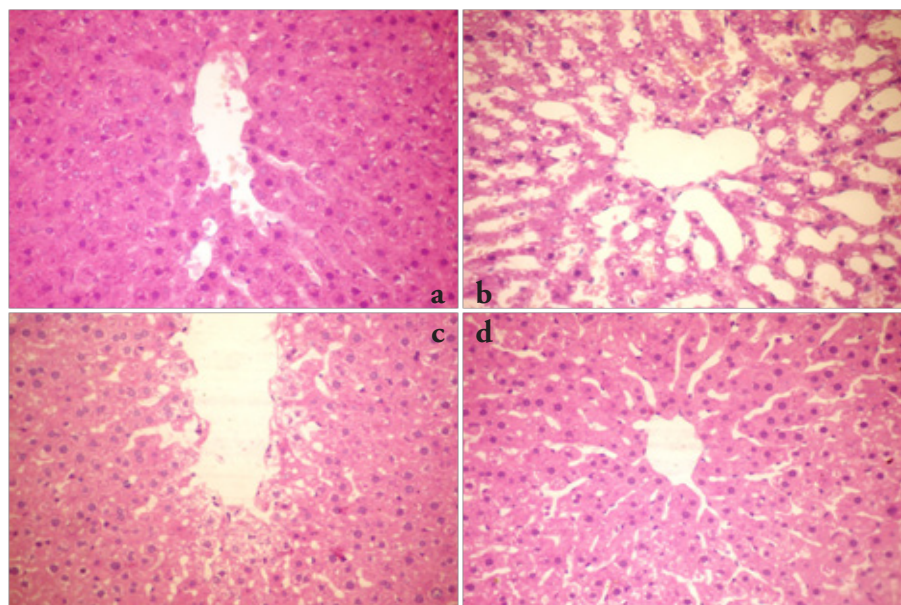


Figure 2. Photomicrographs of rat liver (H & E stain) under low power (X200), (A) Normal control group showing normal parenchymal architecture; (B) PCM-hepatotoxic group showing cytoplasmic vacuolizations of hepatocytes, dilatation of hepatic sinusoids, diffuse necrosis and destruction of the lobular architecture; (C) *A. persicus* (400 mg/kg) + PCM showing normal hepatocytes with cytoplasmic vacuolizations of centrolobular hepatocytes; (D) *A. tournefortii* (400 mg/kg) + PCM showing normal hepatic cells with cytoplasmic vacuolizations of some hepatocytes.

Histopathological analysis

Histopathological studies of liver tissues of the control rats showed normal hepatocytes with central vein and sinusoidal dilation (Figure 2-A). Histological examination of rat livers treated with PCM shows significant hepatotoxicity characterized by cytoplasmic vacuolizations of hepatocytes, dilatation of hepatic sinusoids, diffuse necrosis, and destruction of the lobular architecture and collections of inflammatory cells (Figure 2-B). The hepatoprotective effect of *A. persicus* and *A. tournefortii* extracts was confirmed by histopathological examination of the liver tissue. Histopathological analysis showed that the pathological lesions caused by PCM were very minimal in groups pretreated with ethanolic extracts of *A. persicus* and *A. tournefortii*. Normal hepatocytes with cytoplasmic vacuolizations of centrolobular hepatocytes were observed in groups treated with ethanolic extract of *A. persicus* (Figure 2-C). Liver tissue from *A. tournefortii* group had normal hepatic cells with cytoplasmic vacuolizations of some hepatocytes (Figure 2-D). In accordance with these results, the protective effect of both extracts against PCM may be attributed to the presence of phytochemicals. Therefore, the beneficial effects of both *Astragalus* extracts can be attributed to the presence of flavonoids and/ or polysaccharides that exhibit anti-inflammatory, antioxidant and hepatoprotective properties (31).

CONCLUSION

Based on the dose dependent ability of *A. persicus* and *A. tournefortii* extracts to restore the biochemical parameters like AST, ALT, ALP, BRN, SOD, CAT, GPx, GSH and MDA in PCM-hepatotoxic rats in addition to their *in vitro* antioxidant activities, it was presumed that their hepatoprotective mechanism was largely due to their antioxidant effects. The results suggest that *A. persicus* and *A. tournefortii* can be used as safe and effective alternative protective agents in the management of PCM-related liver injuries therefore, further phytochemical studies are in progress to isolate and identify the active compound(s) responsible for the antioxidant and hepatoprotective activities.

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