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The Potential Antioxidant and Hepatoprotective activities of *Astragalus subrobustus* and *Astragalus woronowii* ethanolic Extracts against Paracetamol induced Liver Damage in rats

Hasan Yusufoglu^{1,*}, Gamal A. Soliman^{2,3}, Rehab F. Abdel-Rahman⁴, Özgen Alankuş- Çalışkan⁵, Saleh I. Alqasoumi⁶

¹Department of Pharmacognosy, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA. ²Department of Pharmacology, College of Pharmacy; Prince Sattam bin Abdulaziz University, Al-Kharj, KSA.

³Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt.

⁴Department of Pharmacology, National Research Centre, Cairo, Egypt.

⁵Department of Chemistry, Faculty of Science, Ege University, Bornova, İzmir, 35100, Turkey.

⁶Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, KSA.

Corresponding Author's e-mail: hasanagazar@hotmail.com, h.yusufoglu@sau.edu.sa

ABSTRACT

Present study was aimed to investigate in vitro antioxidant and hepatoprotective activities of the ethanolic extracts of *Astragalus subrobustus* (*A. subrobustus*) and *Astragalus woronowii* (*A. woronowii*) on PCM induced liver damage in rats. The antioxidant activities of both extracts were assayed and their activities were compared to standard antioxidants, ascorbic acid and pyrogallol. Liver injury was induced by PCM administration (2 g/kg, orally) as a single dose. The results revealed that the EC₅₀ values of *A. subrobustus* and *A. woronowii* extracts, ascorbic acid and pyrogallol were calculated to be 2535, 0.8408, 75.62 and 0.0000248 µg/mL, respectively. PCM administration showed hepatic damage and oxidative stress in rats as indicated 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). At the same time, PCM decreased the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes, content of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver homogenates. *A. subrobustus* (400 mg/kg) and *A. woronowii* (200 and 400 mg/kg) extracts or silymarin administration prevented the toxic effect of PCM on the above parameters. Liver histopathology supported the biochemical findings. The data obtained in the present study suggests that *A. subrobustus* and *A. woronowii* have potent antioxidant activities and afford significant hepatoprotective activity against PCM induced hepatotoxicity.

Keywords: *Astragalus subrobustus*, *Astragalus woronowii*, paracetamol, antioxidant, hepatotoxicity.

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INTRODUCTION

The liver plays an important role in detoxification and elimination of toxic materials. The liver is often influenced by many of the environmental pollutants and drugs, all of them can damage it leading to hepatitis or cirrhosis [1]. PCM, the widely used analgesic antipyretic drug, though considered a safe drug; it produces hepatic necrosis when given in high doses [2]. A part of PCM is metabolized by cytochrome P450 [3] to form the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). PCM hepatotoxicity is induced by its reactive metabolite, which causes oxidative stress and GSH depletion. Induction of cytochrome or depletion of liver GSH is a required for PCM-induced hepatotoxicity [4].

In spite of enormous steps in modern medicine, the therapy of hepatic disorders is inadequate and many formulations containing herbal materials are used for protection of the liver against damage or to regenerate hepatocytes [5]. 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 [6]. 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 [7]. They are also famed for their

antimicrobial, antiperspirant, cardioprotective and anti-inflammatory effects [8]. Phytochemical studies on *Astragalus* plants indicated the presence of triterpenoid saponins, which exhibited a wide range of biological activities, including immunostimulant, antiviral, cardiogenic and analgesic activities [9]. Previous investigations on Turkish *Astragalus* species resulted in separation of a series of oleanane- and cycloartane-type triterpenoidal saponins [10]. The isolated glycosides show interesting biological properties, including immunostimulating, antiprotozoal, antiviral, cytotoxic and wound healing [11]. The flavonoid mixture of some species revealed strong antioxidant activity [12]. Moreover, products of *Astragalus* species, such as gum tragacanth, are widely used in the preparation of pharmaceuticals and as thickening agents in certain foods [13]. The present study investigates the potential hepatoprotective and antioxidant activities of *A. subrobustus* and *A. woronowii* extracts against PCM-induced toxicity in comparison with silymarin a well-known antihepatotoxic agent.

MATERIALS AND METHODS

Plant material

Fresh flowering aerial parts of *A. subrobustus* and *A. woronowii* were collected from Bitlis: Tatvan, south of Obuz village and Van: Hoşap, Van Güzeldere road, respectively during summer, 2012. Taxonomic identification was determined by Dr. Fevzi Özgökçe and a voucher specimen from each plant (VANF 13726 & VANF 13746, respectively) were deposited at the Herbarium of Department of Botany, Faculty of Science (VANF), Yüzüncü Yıl University, and Van, Turkey.

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% 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 $60 \pm 1^\circ\text{C}$ in rotary evaporator to give the total extracts of *A. subrobustus* (9.85 g) and *A. woronowii* (8.5 g). Both extracts were stored in the refrigerator and aliquot of the concentrations were prepared immediately before use.

In vitro anti-oxidant activity

The DPPH assay was performed according to Phang et al. [14]. 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 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:

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

Where A_0 is the absorbance of the control and A_1 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.

Animals

Adult male albino rats of Wistar strain (120-130 g) were obtained from the Animal House of the National Research Centre, Cairo, Egypt. The animals were housed in polypropylene cages and maintained at $22 \pm 2^\circ\text{C}$ and light/dark (12/12 h) cycles. They were allowed free access to standard pellet diet and water *ad libitum*. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment.

Acute toxicity study

Acute toxicity study for *A. subrobustus* and *A. woronowii* extracts was carried in adult male Albino rats according to OECD-423 guidelines [15]. Animals were kept fasting providing only water, after which *A. subrobustus* and *A. woronowii* extracts were administered orally by gastric tube in different gradual doses (1000-4000mg/kg), and observed for any toxic symptoms and mortality for 72 h.

Hepatoprotective activity

Male rats were divided into 7 groups (n=6). Group I (normal control) and Group II (hepatotoxic control) received the vehicle (1 mL/kg b.wt). Group III received the reference drug, silymarin (50 mg/kg). Groups IV and V received the ethanolic extract of *A. subrobustus* (200 and 400 mg/kg, respectively). Groups VI and VII received the ethanolic extract of *A. woronowii* (200 and 400 mg/kg, respectively). Rats of groups II-VII received PCM (2 g/kg) as a single dose after 30 min of 7-day administration of the tested extracts. The vehicle, extracts and PCM were administered orally by suspending in 3% Tween 80. After 48 h of PCM

administration, rats were anesthetized with diethyl ether. Blood of each rat was collected by puncturing retro-orbital plexus in sterilized dry centrifuge tube and allowed to coagulate for 30 min at 37°. The clear sera obtained after centrifugation (3000 rpm for 15 min) were used for further biochemical estimation. After blood collection, all rats were sacrificed by cervical decapitation and livers were dissected out and divided into two portions. The first portion was kept in liquid nitrogen for estimation of the antioxidant status and the second part was fixed in buffered formalin 10% for histopathological examination.

Preparation of liver homogenate

Hepatic tissues were homogenized in 10% w/v 0.1 M phosphate buffer or 0.1 M tris buffer (pH 7.0) and centrifuged at 12,000·g for 10 min. The supernatant was used for the measurement of liver enzymatic and non-enzymatic antioxidants.

Estimation of serum liver enzymes

The serum activities of ALT, AST, ALP and γ -GT were estimated according to the instructor manual of commercially available kits.

Estimation of biochemical parameters

The biochemical parameters, such as total protein (TP) albumin (ALB) and BRN were estimated according to the instructor manual of commercially available kits.

Determination of *in vivo* antioxidant status

Enzymatic antioxidants were determined by estimating SOD, GPx and CAT activities and non-enzymatic antioxidants by estimating GSH and lipid peroxidation (MDA) in the hepatic tissue homogenates of all rats using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the supplier.

Histopathological examination.

Liver samples were fixed in buffered formalin 10%, processed routinely, and embedded in paraffin. 5 μ m thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation. The stained sections were examined and photographed under a light microscope.

Statistical analysis

The results are expressed as mean \pm standard error of six observations in each group. Our obtained results 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

***In vitro* anti-oxidant activity**

The free radical scavenging activity of both extracts against DPPH radicals was shown in Figure 1. *A. subrobustus* and *A. woronowii* extracts showed *in vitro* antioxidant activities at concentrations of 625, 2500 and 10000 μ g/mL. At these concentrations, the scavenging activities of *A. subrobustus* were 17.7, 48.5 and 84.9%, respectively while those of *A. woronowii* were 109.1, 135.0 162.3%, respectively. The EC₅₀ values for *A. subrobustus* and *A. woronowii* extracts, ascorbic acid and pyrogallol were calculated to be 2535, 0.8408, 75.62 and 0.0000248 μ g/mL, respectively. Effectiveness of the antioxidant properties of a compound is inversely correlated with its EC₅₀ value.

Acute toxicity study

The oral acute toxicity test showed no lethality or signs of toxicity for *A. subrobustus* and *A. woronowii* extracts up to a dose level of 4000mg/kg. LD₅₀ of both extracts was estimated to be >4000 mg/kg. Hence, 1/20 and 1/10 of the LD₅₀, (200 and 400 mg/kg) were selected for the present study.

Hepatoprotective activity

It is found that ethanolic extracts of *A. woronowii* are more effective than *A. subrobustus* extract after 7 days of treatment. No statistical difference in any parameters between hepatotoxic rats medicated with *A. subrobustus* at 200mg/ kg and PCM control rats was observed.

Effect on serum liver enzymes and biochemical parameters:

The activities of liver marker enzymes (ALT, AST, ALP and γ -GT) and BRN levels after 48 h of oral administration of PCM at the dose of 2.0 g/kg of body weight are depicted in Tables 1&2. As can be seen, PCM medication resulted in marked hepatic damage as indicated by a significant elevation in the activities of liver marker enzymes and level of BRN in serum in comparison to the normal control animals. Dosing of ethanolic extracts of *A. subrobustus* at 400mg/ kg and *A. woronowii* at 200 and 400 mg/kg for 7 days prior to PCM treatment restored the activities of ALT, AST, ALP and γ -GT (Table 1) along with BRN levels (Table 2). Pretreatment with the standard hepatoprotective agent-Silymarin also decreased all measured serum biochemical activities towards normalness.

In our study, it was noted that administration of PCM to rats reduced the levels of TP and ALB in their sera, compared with the normal control animals. Pretreatment with *A. subrobustus* (400 mg/kg) and *A.*

woronowii (200 and 400mg/kg) extracts showed a marked reversal of these parameters toward the 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.

Effect on *in vivo* antioxidant status

Table 3 shows the effect of *A. subrobustus* and *A. woronowii* extracts on the antioxidant biomarkers and lipid peroxidation in rat's liver homogenates. Significant reduction in the activities of SOD, GPx and CAT enzymes in the liver homogenates were recorded in PCM-hepatotoxic rats as compared to normal control group. Pretreatment of rats with *A. subrobustus* (400 mg/kg) and *A. woronowii* (200 and 400 mg/kg) extracts, for 7 days showed significant elevation in the activity of SOD, enzymes GPx and CAT that confirms the hepatoprotective effect of both extracts. At 400 mg/kg of *A. subrobustus* and *A. woronowii*, the recovery of enzymes activities recorded 74.7% and 80.9% (for SOD), 84.9% and 90.46% (for GPx) and 61.8% and 75.7% (for CAT), respectively of the normal control values.

In the present investigation, we have observed that reduced GSH level was depleted significantly in the liver homogenate of PCM-treated rats compared to normal control group. The reduced levels of GSH which were observed in the liver homogenates of PCM-exposed rats were significantly restored towards normal values by treatment with *A. subrobustus* (400 mg/kg) and *A. woronowii* (200 and 400 mg/kg) extracts.

Lipid peroxidation was increased in the PCM control rats, as revealed by elevated MDA levels in their liver homogenate, in comparison to the normal control group. Pretreatment with *A. subrobustus* (400 mg/kg) and *A. woronowii* (200 and 400 mg/kg) extracts significantly reduced the MDA level in the liver tissues of PCM-medicated rats indicating their protective role during oxidative damage.

Histopathological analysis

Histological investigation of liver tissue of the normal control rats showed normal hepatocytes with central vein and sinusoidal dilation (Figure 2-A). Histopathological observations revealed that the normal architecture of liver was completely lost in rats treated with PCM with the appearance of focal hepatic necrosis, destruction of the lobular architecture and collections of inflammatory cells (Figure 2-B). The hepatoprotective effects of the tested extracts were confirmed by histopathological examination of the liver tissue. Pretreatment of *A. subrobustus* and *A. woronowii* reduced the severity of PCM induced liver intoxication Fig. 2-C&D.

Table 1: Effect of the ethanol extracts of *A. subrobustus* and *A. woronowii* 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	34.5±0.98†	61.4±1.51†	116.4±3.72†	13.8±0.16†
PCM-hepatotoxic Control	62.2±2.02*	89.8±2.87*	336.2±5.20*	29.6±0.19*
Silymarin (50 mg/kg) +PCM	41.6±1.14*†	69.2±2.20*†	144.4±3.17*†	16.0±0.17*†
<i>A. subrobustus</i> (200 mg/kg) +PCM	60.7±1.91*	84.2±2.05*	307.8±4.62*	27.2±0.18*
<i>A. subrobustus</i> (400 mg/kg) +PCM	51.4±1.64*†	77.4±2.24*†	265.4±4.55*†	23.0±0.14*†
<i>A. woronowii</i> (200 mg/kg) +PCM	46.6±2.73*†	73.8±2.81*†	227.0±3.47*†	21.8±0.16*†
<i>A. woronowii</i> (400 mg/kg) +PCM	45.5±2.54*†	70.3±2.25*†	202.6±3.99*†	21.1±0.18*†

Values are expressed as mean ± S.E.M., n =6 rats/group.

* indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

† indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

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

Groups	BRN (mg/dL)	TP (g/dL)	ALB (g/dL)
Normal Control	0.64±0.03†	7.5±0.10†	3.7±0.09†
PCM-hepatotoxic Control	1.14±0.09*	5.0±0.09*	2.4±0.17*
Silymarin (50 mg/kg) +PCM	0.71±0.04*†	7.2±0.12*†	3.4±0.16*†
<i>A. subrobustus</i> (200 mg/kg) +PCM	1.06±0.03*	5.3±0.11*	2.6±0.15*
<i>A. subrobustus</i> (400 mg/kg) +PCM	0.90±0.04*†	6.0±0.12*†	3.0±0.17*†
<i>A. woronowii</i> (200 mg/kg) +PCM	0.82±0.05*†	6.6±0.13*†	3.0±0.13*†
<i>A. woronowii</i> (400 mg/kg) +PCM	0.78±0.03*†	6.9±0.12*†	3.1±0.14*†

Values are expressed as mean ± S.E.M., n =6 rats/group.

* indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

† indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

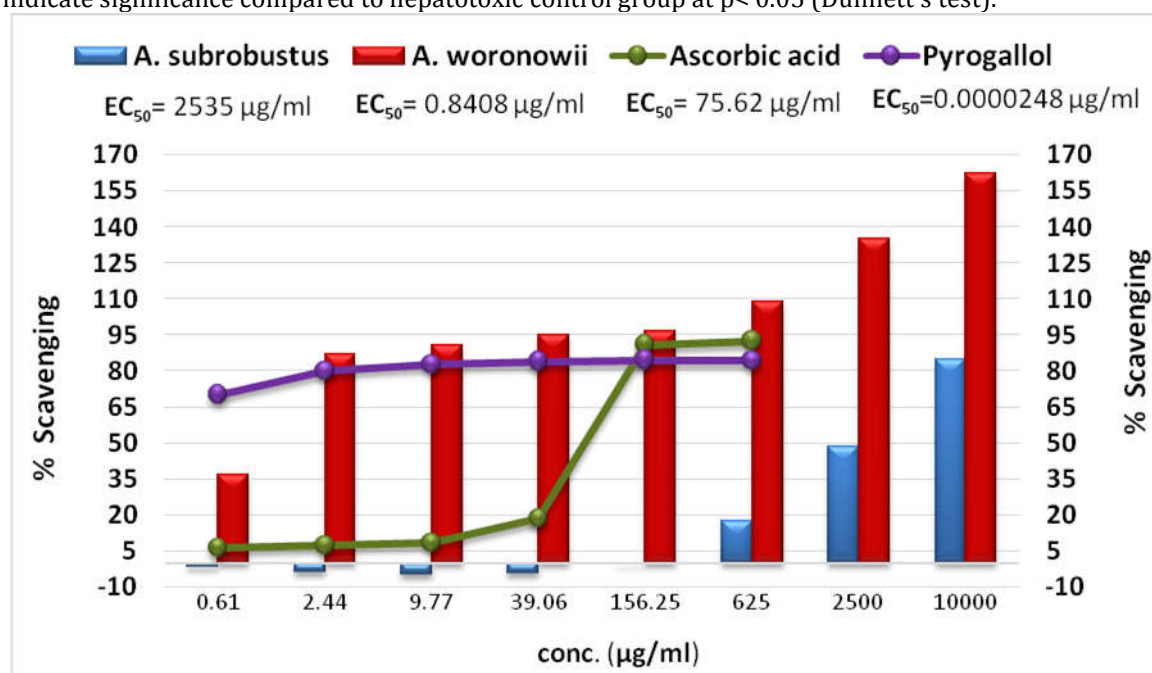
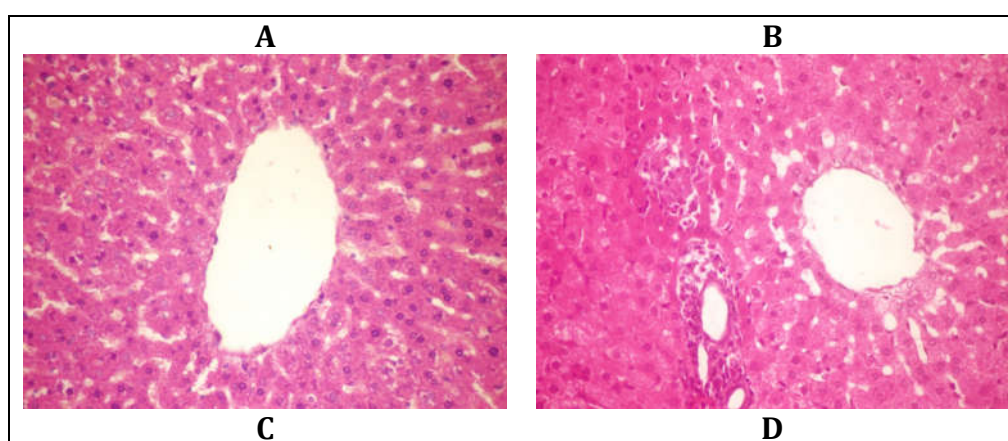
Table 3: Effect of the ethanol extracts of *A. subrobustus* and *A. woronowii* on hepatic antioxidant profile, glutathione (GSH) and lipid peroxidation (MDA) in liver homogenate of rats with PCM- induced hepatotoxicity.

Groups	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	GSH ($\mu\text{mol/g}$ tissue)	MDA (nmol/g tissue)
Normal Control	52.6 \pm 2.17†	3.25 \pm 0.12†	16.5 \pm 0.48†	14.6 \pm 0.39†	23.1 \pm 0.81†
PCM-hepatotoxic Control	22.5 \pm 0.85*	1.27 \pm 0.07*	7.8 \pm 0.19*	8.6 \pm 0.28*	60.6 \pm 1.17*
Silymarin (50 mg/kg) +PCM	49.7 \pm 1.53*†	3.04 \pm 0.12*†	14.3 \pm 0.25*†	12.9 \pm 0.30*†	31.4 \pm 0.52*†
<i>A. subrobustus</i> (200 mg/kg) +PCM	25.8 \pm 1.28*	1.53 \pm 0.14*	8.8 \pm 0.28*	9.7 \pm 0.29*	56.0 \pm 0.70*
<i>A. subrobustus</i> (400 mg/kg) +PCM	39.3 \pm 1.35*†	2.67 \pm 0.18*†	10.2 \pm 0.24*†	10.8 \pm 0.21*†	43.7 \pm 0.77*†
<i>A. woronowii</i> (200 mg/kg) +PCM	42.0 \pm 1.15*†	2.82 \pm 0.13*†	11.7 \pm 0.27*†	11.5 \pm 0.27*†	40.5 \pm 0.68*†
<i>A. woronowii</i> (400 mg/kg) +PCM	42.6 \pm 1.27*†	2.94 \pm 0.15*†	12.5 \pm 0.25*†	12.0 \pm 0.29*†	38.1 \pm 0.65*†

Values are expressed as mean \pm S.E.M., n =6 rats/group.

* indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

† indicate significance compared to hepatotoxic control group at p< 0.05 (Dunnett's test).

**Figure 1:** *In vitro* Antioxidant activity of *A. subrobustus*, *A. woronowii*, ascorbic acid and pyrogallol, using DPPH radical scavenging activity method.

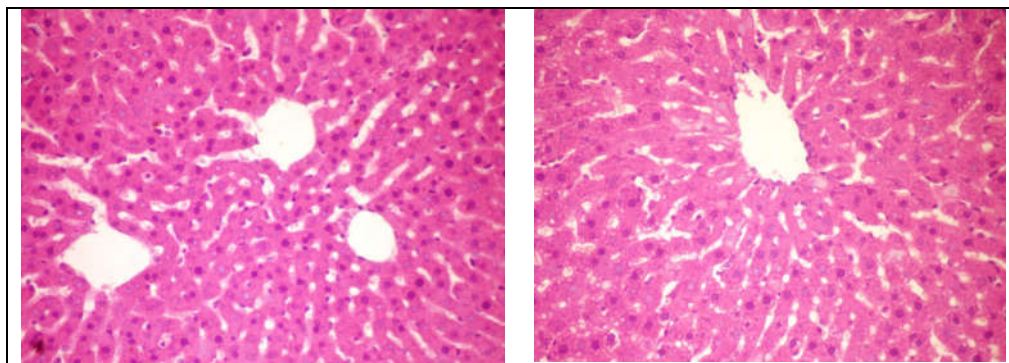


Figure 2: Photomicrographs of rat liver (H & E stain) under low power (X400), (A) Normal control group showing normal parenchymal architecture; (B) PCM-hepatotoxic group showing focal hepatic necrosis, destruction of the lobular architecture and collections of inflammatory cells; (C and D) *A. subrobustus* (400 mg/kg) + PCM and *A. woronowii*(400 mg/kg) + PCM, respectively showing normal histological structure of hepatic tubules.

DISCUSSION

In vitro anti-oxidant activity

A. subrobustus and *A. woronowii* extracts and ascorbic acid and pyrogallol (as standards) showed antioxidant activity in a concentration-dependent manner. The antioxidant activity of both extracts, suggesting that they could scavenge the free radicals generated during PCM metabolism. This finding is consistent with the results of Ivancheva et al. [12] who mentioned that the antioxidant activities of *Astragalus* are related to its bioactive compounds, such as flavonoids. The EC₅₀ values for *A. subrobustus* and *A. woronowii* extracts, ascorbic acid and pyrogallol were calculated to be 2535, 0.8408, 75.62 and 0.0000248 µg/mL, respectively. Effectiveness of the antioxidant properties of a compound is inversely correlated with its EC₅₀ value. In this respect, Lee et al. [16] reported that if the EC₅₀ value of an extract is less than 10 mg/mL, it indicates that the extract is an effective antioxidant. In this study, the EC₅₀ values of both extracts were less than 10 mg/mL, and this indicates that both extracts are effective antioxidants.

Acute toxicity study

In our study, oral administration of *A. subrobustus* and *A. woronowii* extracts at doses up to 4000 mg/kg did not produce any sign of acute toxicity and none of animals died during 72 h of observation. The LD₅₀ of PCM in rats following oral administration was calculated to be >4000 mg/kg. In general, the higher the LD₅₀ value, the lower toxic the compound. Consequently, the tested extracts are considered safe since agents having LD₅₀ higher than 50 mg/kg are nontoxic[17].

Hepatoprotective activity

Liver participates in a variety of enzymatic metabolic activities. In our study, we have tested the hepatoprotective potential of *A. subrobustus* and *A. woronowii* extracts (200 and 400 mg/kg) in PCM-induced hepatotoxic rats. PCM is a common analgesic and antipyretic drug, known to possess hepatotoxic effect at very high doses. It has been used as a common experimental model to evaluate the hepatoprotective activity of agents [18]. The formation of the highly reactive metabolite (NAPQI) by the hepatic cytochrome P450 is the first step in development of PCM hepatotoxicity [19]. 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 that has been thought to be the major mechanism for PCM-hepatotoxicity.

Effect on serum liver enzymes and biochemical parameters:

The liver marker enzymes (ALT, AST, ALP and γ-GT), which are present in higher level in hepatocytes have still remained the gold standards for the assessment of liver injury [20]. When there is hepatopathy, liver marker enzymes infiltrate into the blood stream in conformity with the severity of hepatic injury [21]. Elevated levels of these enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. In addition, the elevated serum level of BRN is the usual indicator of hepatotoxicity [22].

Administration of PCM causes elevation of serum ALT, AST, ALP and γ-GT and BRN levels in rats, indicating that PCM may induce hepatocellular damages which in turn alter the structure and function of liver cells. The elevated serum activities of liver marker enzymes observed in PCM-control rats in our study can be related to the injured structural integrity of the hepatocytes because these are cytoplasmic in nature and are liberated into blood stream after cellular damage [23]. 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 [24]. This study demonstrated that *A. subrobustus* and *A. woronowii* extracts and silymarin had reduced levels of serum marker enzymes and BRN level which were elevated by PCM administration. The return of ALT, AST, ALP and γ -GT activities toward normal may be due to the inhibitory effect of the tested extracts on cytochrome P450 to reduce the production of the reactive metabolite (NAPQI) of PCM. This may result in stabilization of cellular membrane so preserving the structural integrity of hepatocytes as well as the restoration of hepatic injury induced by PCM. Silymarin is a standardized extract of the milk thistle (*Silybum marianum*) chiefly contains flavonoid [25]. It offers good protection in various toxic models of experimental liver diseases in laboratory animals. It functions through mechanisms of antioxidative, anti-inflammatory, membrane stabilizing and liver regenerating [26].

ALB is the most abundant plasma protein produced by hepatocytes. Therefore, variation of serum TP or ALB levels can reflect liver health status [27]. In our study, the reduction in serum TP and ALB in PCM-control rats may be due to binding of the reactive metabolite of PCM (NAPQI) to the amino acid cysteine in proteins, forming PCM protein adducts [28]. Pretreatment with *A. subrobustus* and *A. woronowii* extracts showed a significant reversal of TP and ALB toward their normal levels and suggested the stabilization of endoplasmic reticulum that is responsible for protein synthesis. This assures the protective activity of both extracts against PCM-hepatotoxicity.

Effect on *in vivo* antioxidant status

The free radicals of most of the hepatotoxic agents harm hepatocytes mainly by inducing lipid peroxidation and other oxidative damages. The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, GPx and CAT. It is known that SOD converts superoxide anion into H₂O₂ and O₂, where as GPx reduces reactive peroxides to alcohols and water at the expense of GSH, which is oxidized to GSSG. GSSG is recycled to GSH by GSH reductase. CAT reduces H₂O₂ to H₂O, resulting in the detoxification of free radicals [29].

In present study, reduced SOD, GPx and CAT levels were observed in PCM-hepatotoxic control rats. The pretreatment of rats with *A. subrobustus* (400 mg/kg) and *A. woronowii* (200 and 400 mg/kg) extracts for 7 days provided protection against the depletion in activities of SOD, GPx and CAT. This shows that both extracts protects rats against oxidative injury by maintaining the levels of these enzymes even after PCM treatment. The action of *A. subrobustus* and *A. woronowii* extracts to restore the altered antioxidant enzymes in PCM-hepatotoxic rats indicates their free radical scavenging potential.

GSH is widely distributed in the cells and plays a major role to protect cells against free radicals, peroxides and other toxic compounds [30]. Its depletion is considered as one of the main biochemical markers for PCM-caused hepatotoxicity. The liver damage induced by PCM has been related to the development of a highly reactive toxic electrophile, NAPQI by cytochrome P-450 [31]. NAPQI is initially detoxified by conjugation with GSH and excretion in urine [32]. When the level of the reactive metabolite exceeds the detoxifying capacity of GSH, NAPQI oxidizes tissue macromolecules such as lipid or -SH group of proteins. Depletion of GSH in liver homogenate of PCM-hepatotoxic group may be due to conjugation of NAPQI metabolite with GSH to form mercapturic acid [33].

Lipid peroxidation can reduce membrane fluidity, inactivate membrane-bound proteins, and decompose into cytotoxic aldehydes such as MDA [34]. The inhibition of lipid peroxidation is thus a crucial property of antioxidant compounds. In the present study, decline in the levels of antioxidant enzymes SOD, GPx and CAT observed in PCM treated rats is a clear manifestation of excessive formation of hepatic MDA content. Previous studies also reported the decline in the levels of antioxidant enzymes and excessive formation of hepatic MDA in PCM treated rats [35].

Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [36]. The phytochemistry of *Astragalus* plants has been studied by many investigators and is well documented as a good source of biologically active compounds such as saponins, phenolics and flavonoids [37]. The present results suggest that the possible mechanism of action by extracts as antioxidants could be related to their phytochemicals, particularly phenolics and flavonoids. Phenols and flavonoids are considered as most responsible functional groups having antioxidant potential in plant source [38]. More recently, it has become evident that phenolic natural products may reduce oxidative stress by indirect antioxidant action [39]. There is a strong positive relationship between the phenolic content and antioxidant potential of different plant species because of the scavenging ability of hydroxyl groups attached with phenols [38].

Histopathological analysis

Histopathologic studies also supported the evidence of biochemical analysis. Histological examination of rat liver treated with PCM 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. subrobustus* and *A. woronowii* the severity of hepatic damage was decreased when compared with PCM-hepatotoxic rats. In accordance with these results, the protective effect of both extracts against PCM may be attributed to the presence of phytochemicals. Therefore, the protective activity of *A. subrobustus* and *A. woronowii* may be due to their flavonoids that display a remarkable role in various pharmacological activities including antioxidant effect [12].

CONCLUSIONS

In conclusion, the present study demonstrates that *A. subrobustus* and *A. woronowii* have potential hepatoprotective activity which is mainly attributed to their antioxidant property. The antioxidant effect may be attributed, at least in part, to the presence of flavonoids, triterpenoid saponins and glycosides in the extracts. Further detailed investigations are needed in order to identify and isolate the hepatoprotective components in the extracts and to justify their use in the treatment of liver disorders.

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