



## **The Potential Hepatoprotective and Antioxidant Activities of *Astragalus davisii* against Paracetamol Induced Liver Damage in Rats**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author AIF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MHA and GAS managed the analyses of the study. Authors RFAR, OAC, MAG and HY managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

The current study aimed to estimate *in vitro* antioxidant effect of *Astragalus davisii* (*A. davisii*) extract. Further, the possible protective effect of *A. davisii* against paracetamol (PCM)-induced liver injury was assessed in rats. *A. davisii* was tested for its antioxidant activity using DPPH radical scavenging assay. The hepatoprotective potential of the extract was assessed in rats following oral

administration for 7 days. Liver injury was induced in rats following oral administration of PCM overdose. Hepatic biomarkers; alanine-aminotransferase, aspartate-aminotransferase, alkaline-phosphatase,  $\gamma$ -glutamyl transferase and bilirubin were increased, while total protein and albumin were reduced in PCM control animals. Additionally, the activities of superoxide dismutase (SOD), catalase and glutathione-peroxidase (GPx) and the levels of glutathione were significantly declined, while levels of hepatic malondialdehyde (MDA) were significantly elevated in PCM alone treated rats. Oral administration of *A. davisii* (400 mg/kg) prior to PCM inhibited the elevation in the levels of liver damage markers in serum and protected against oxidative stress. Histopathological remarks confirmed the hepatoprotective potential of the extract. The results suggest that *A. davisii* extract at 400 mg/kg protects liver against injury induced by PCM overdose.

**Keywords:** Hepatotoxicity; leguminosae; DPPH; liver markers.

## ABBREVIATIONS

*A. davisii*: *Astragalus davisii*; *ALB*: Albumin; *ALP*: Alkaline-Phosphatase; *ALT*: Alanine-Aminotransferase; *AST*: Aspartate-Aminotransferase; *BRN*: Bilirubin; *CAT*: Catalase; *DPPH*: 2,2-Diphenyl-1-picrylhydrazyl Radical; *GPx*: Glutathione peroxidase; *GSH*: Reduced Glutathione; *H&E*: Hematoxylin and Eosin; *LPO*: Lipid Peroxidation; *MDA*: Malondialdehyde; *NAPQI*: *N*-acetyl-*p*-Benzoquinoneimine; *NC*: Normal Control; *PCM*: Paracetamol; *ROS*: Reactive-Oxygen Species; *SOD*: Superoxide Dismutase; *TP*: Total Protein;  $\gamma$ -GT :  $\gamma$ -glutamyl-transferase.

## 1. INTRODUCTION

The liver is a complex organ that is necessary for survival due to its role in the coordination of the metabolic activities, including glucose homeostasis, xenobiotic metabolism and detoxification. Industrial toxins, drugs, free radicals, food additives and alcohol are the risk factors for developing liver diseases [1]. PCM, (also known as acetaminophen, *N*-acetyl-*p*-aminophenol or APAP) is a commonly used mild analgesic and antipyretic available in numerous prescription and over-the-counter formulations [2]. PCM is widely used as a safe analgesic and antipyretic medication. However, its high dosages can lead to serious hepatic damage. Toxicity of PCM-overdose is attributed to the deleterious effect of its reactive metabolite known as *N*-acetyl-*p*-benzoquinoneimine (NAPQI). Elevated level of NAPQI-mediates oxidative damage, and therefore promotes tissue injury and organ dysfunction; including liver [3]. Currently available synthetic medications indicated for the management of liver disorders are inadequate and known for several adverse effects. Hinson et al., 2010 suggested that hepatotoxicity induced by paracetamol is mediated by oxidative damage [4].

Further, previous studies reported that, the use of plants with antioxidant activities minimizes the danger of liver disorders [5]. The genus *Astragalus* is the largest in the Leguminosae family and is cosmopolite, with 2000–3000 species grouped in 100 subgenera [6]. Some species of *Astragalus* has a history of being widely used in traditional medicine as antiperspirants, diuretics, and tonics for a wide array of diseases such as nephritis, diabetes mellitus and hypertension [7]. Others are a source of the economically important natural product, gum tragacanth. It is reported that some of *Astragalus* plants are well-known for their hepatoprotective, antiviral and immunostimulant activities [8]. Previous reports showed *Astragalus* plants to be a great source of saponins, flavonoids, and polysaccharides [9]. Total flavonoids, obtained from *Astragalus* demonstrated potent antioxidant effect and blocked the lipid peroxidation caused by O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> [10]. Growing evidence indicates that PCM enhances the excessive production of reactive-oxygen species (ROS), which resulted in massive lipid peroxidation and subsequent hepatic damage. Therefore, a substance with antioxidant effect may successfully prevent the development of liver damage due to PCM-overdose. Hence, this study aimed to assess the potential hepatoprotective potential of *A. davisii* extract against PCM- induced liver injury in rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

Roots of *A. davisii* were collected from Van: Çatak, Tellikaya köyü, 1940 m altitude, 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 13708).

## 2.2 Plant Extraction

The collected roots were shade dried, grinded to fine powders and extracted with 80% aqueous ethanol (2×3 L) under reflux. Ethanol extract of *A. davisii* was subjected to rotary evaporation (70±2°C) and lyophilized using freeze drier to yield 20.4 g and 15 g, respectively [11].

## 2.3 Estimation of *in vitro* Antioxidant Effect

In an attempt to estimate the antioxidant potential of *A. davisii* extract, the DPPH free-radical scavenging assay was used [12]. A solution of 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) in 0.004% methanol solution was prepared. Different concentrations of the extract were prepared in methanol. In a 96-well plate, 20 µl of each extract concentration was added to 180 µl DPPH solution. The resultant mixture was incubated for 30 min at room temperature and the absorbance was estimated at 520 nm. DPPH solution was used as a control while methanol was served as a blank. In addition, pyrogallol was used as a positive control. The scavenging activities were calculated according to the equation:

$$\text{Scavenging activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where *A control* is absorbance of control (without the extract or Standard), and *A sample* is absorbance of sample. A plot between % inhibition verses concentration was used for the calculation of EC<sub>50</sub> of samples or standards.

## 2.4 Experimental Animals

In the current study, Wistar rats (both male and female, 180-200 g) were used. Animals were bred in Lab Animal-Care Unit, College of pharmacy, Prince Sattam-bin Abdulaziz University, KSA. Animals were kept under standard situation (at, 23 ± 2°C and 12-h light-dark cycle). Rats were fed standard-pellets and water *ad libitum*.

## 2.5 Acute Toxicity Study in Rats

To test the acute toxicity of *A. davisii* extract, two groups of healthy Wistar-rats (female) were used (n = 3). The study was carried out as per OECD guidelines-423, acute toxic class method OECD [13]. The extract was suspended in the vehicle

(3% v/v Tween 80). Animals of the 1st group received the ethanol extract of *A. davisii* at oral dose of 2000 mg/kg (1 mL/100 g). Rats of the 2<sup>nd</sup> group (control) medicated with the vehicle and kept under the same situations. The rats were observed for signs of toxicity, mortality, and morbidity for 24 h. They were also examined for the changes in neurological, autonomic, and behavioral profile. These rats were further observed for a period of 14 days.

## 2.6 Hepatoprotective Activity

### 2.6.1 Experimental design

Thirty mature male Wistar rats (180-200 g body weight) were divided into five equal groups as follows:

Group I – Normal control (NC) rats received the vehicle at 5 mL/kg b.wt.

Group II – PCM control rats received the vehicle at 5 mL/kg b.wt.

Group III – Rats received silymarin (reference drug) at 100 mg/kg b.wt.

Group IV – Rats received *A. davisii* at 200 mg/kg b.wt.

Group V – Rats received *A. davisii* at 400 mg/kg b.wt.

The vehicle and the test extract were administered for 7 days using oral tube. Liver injury was produced on the 5<sup>th</sup> day via oral dosing of PCM-overdose (2 g/kg) to all groups except rats in Group I.

At day-7, two h after last dosing, blood was collected from each animal through retro-orbital sinus plexus. Sera were taken out after centrifugation of blood samples at 4000 rpm for 15 min and maintained at -20°C until further biochemical evaluation.

### 2.6.2 Assessment of liver damage markers in serum

Serum levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline-phosphatase (ALP), γ-glutamyl-transferase (γ-GT), bilirubin (BRN), total protein (TP) and albumin (ALB) were evaluated according to the instructor manual of commercially available kits (Chema Diagnostica, Italy).

### 2.6.3 Assessment of oxidative stress-related markers

Liver specimens were homogenized using Heidolph DiAx 900 homogenizer, Germany, in 0.1 M phosphate buffer (25 mM, pH 7.4) and centrifuged at 18,000 rpm for 30 min. The supernatants were taken and stored at  $-80^{\circ}\text{C}$  till analysis. The levels of superoxide-dismutase (SOD), glutathione peroxide (GPx), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) were evaluated via the assay kits obtained from Cayman Chemical Company (Michigan, USA) according to steps described by the manufacturer's guidelines.

### 2.6.4 Histopathological evaluation

Liver samples were kept in 10% buffered formalin, embedded in paraffin, and then serial sections of  $5\mu\text{m}$  thickness were cut. The sections of the livers were stained with hematoxylin and eosin (H & E) and evaluated for the pathological changes of hepatotoxicity [14].

### 2.7 Statistical Analysis

The results are expressed as mean  $\pm$  standard error of six observations in each group. Data were analysed via one-way analysis of variance (ANOVA) followed by Dunnett's test using SPSS

ver. 14.0. P-value of  $<0.05$  was considered as the desired level of significance.

## 3. RESULTS

### 3.1 Estimation of *in vitro* Antioxidant Effect

The antioxidant effect of *A. davisii* extract was illustrated in Fig. 1. The *A. davisii* extract showed *in vitro* antioxidant effect in the DPPH assay in a concentration-dependent way. At concentrations of 625, 2500 and 10000  $\mu\text{g/mL}$ , the scavenging activities of *A. davisii* were 18.8, 29.1 and 39.9 %, respectively. The  $\text{EC}_{50}$  values for *A. davisii* and pyrogallol were calculated to be 76748 and 0.0001037  $\mu\text{g/mL}$ , respectively.

### 3.2 Acute Toxicity Study

Two out of three animals were showed good tolerance to the ethanol extract of *A. davisii* at 2000 mg/kg. For these animals, the dose of 2000 mg/kg was found to be non-lethal and did not show any noticeable signs of toxicity and mortality up to 14 days of observation. In addition, one of the animals exposed to 2000 mg/kg of *A. davisii* extract showed piloerection, lethargy and diarrhea and died 7 days after dosing. According to OECD-423 guidelines, LD50 value of *A. davisii* was calculated to be  $> 2000 - 5000$  mg/kg (category 5).

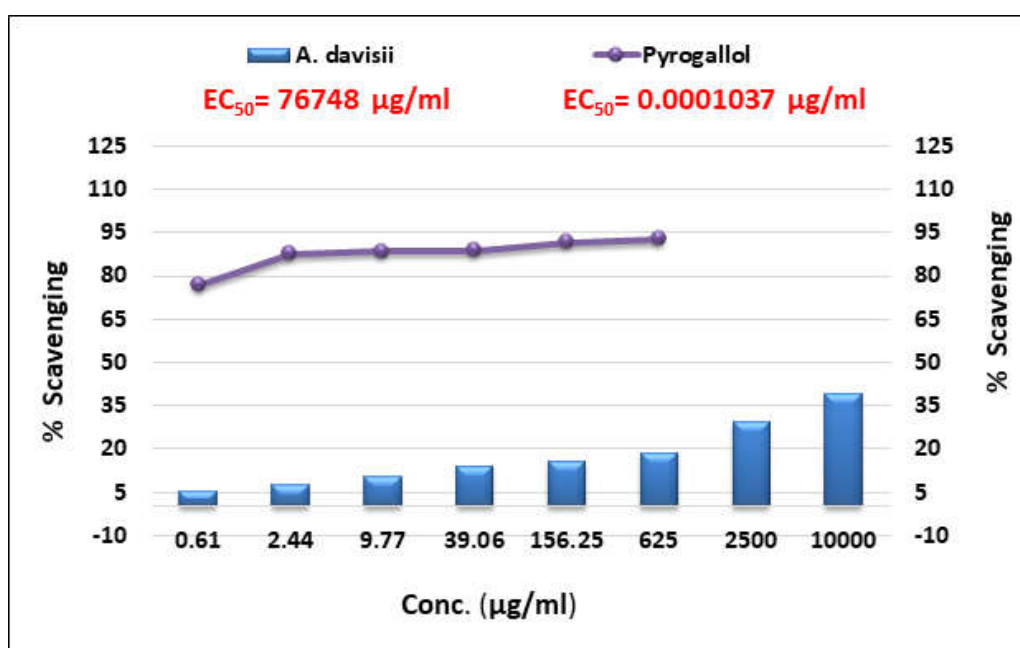


Fig. 1. *In vitro* antioxidant activity of *A. davisii* and pyrogallol, using DPPH radical scavenging activity method

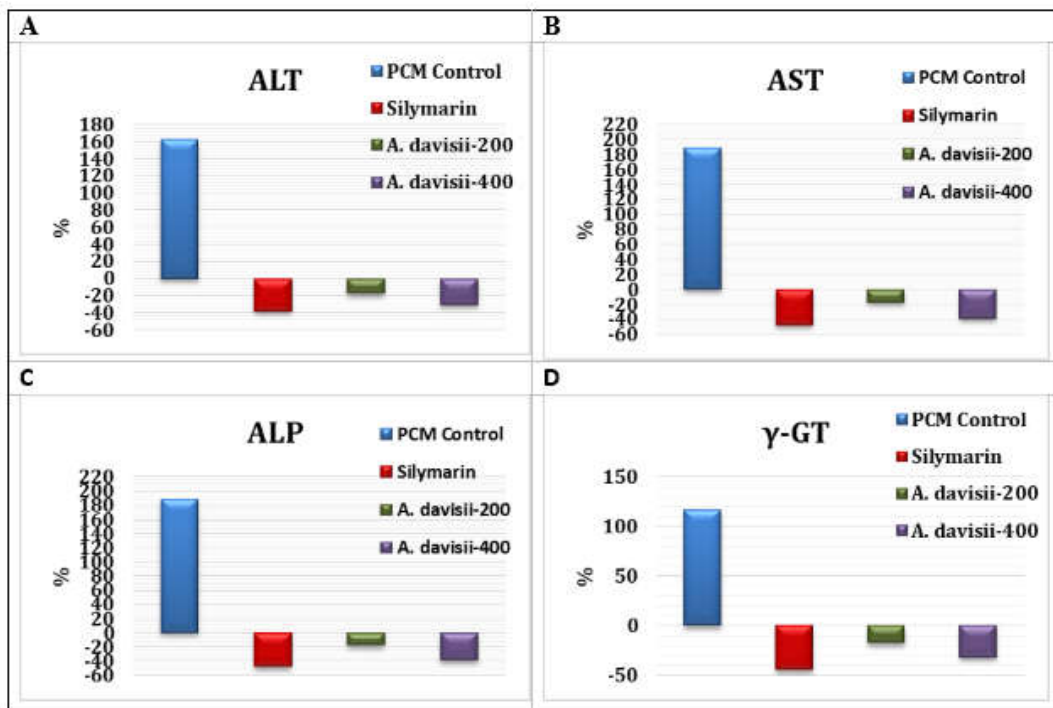
### 3.3 Hepatoprotective Activity

#### 3.3.1 Assessment of liver damage markers in serum

The effects of *A. davisii* extract on the activities of ALT, AST, ALP and  $\gamma$ -GT in serum of rats with PCM- induced hepatotoxicity are summarized in Table 1 and their % changes are depicted in Fig. 2. Compared with the normal control, serum levels of liver damage markers including ALT, AST, ALP and  $\gamma$ -GT were significantly ( $P<0.05$ ) increased in PCM control rats (162.13%, 188.96%, 205.17% and 116.67%, respectively), indicating a serious hepatic injury caused by PCM. Pre-treatment of PCM-exposed rats with silymarin showed significant ( $P<0.05$ ) improvement in the levels of these biomarkers (60.7 $\pm$ 3.88 U/L, 148.4 $\pm$ 8.72 U/L, 185.7 $\pm$ 9.85 U/L and 27.4 $\pm$ 1.83 U/L, respectively) compared to the PCM group (98.3 $\pm$ 5.69 U/L, 282.6 $\pm$ 16.64 U/L, 348.5 $\pm$ 19.38 U/L and 49.4 $\pm$ 2.83 U/L, respectively). Low dose of *A. davisii* extract showed only 17.40%, 16.95%, 15.95% and 17.41% decreases in the serum activities of ALT, AST, ALP and  $\gamma$ -GT, respectively in comparison

with the PCM control group. Moreover, pretreatment of the PCM-exposed rats with *A. davisii* extract at 400 mg/kg significantly ( $P<0.05$ ) inhibited the elevations in the activities of liver enzymes in which 30.62%, 38.57%, 35.38% and 31.78% reductions were found in the serum activities of ALT, AST, ALP and  $\gamma$ -GT, respectively.

The effects of *A. davisii* extract on the serum levels of BRN, TP and ALB in rats with PCM-induced hepatotoxicity are depicted in Table 2 and Fig. 3. PCM-control rats demonstrated marked elevation in serum level of BRN and decrease in total protein and albumin values in comparison with normal group. Fig. 3 depicts that pretreatment of PCM-exposed rats with silymarin or *A. davisii* extract at 400 mg/kg exhibited 38.73% and 32.39% reduction in the PCM-induced increase in the level of BRN, respectively. In addition, they markedly increased the level of TP and ALB, resulting in significant restoration towards their control values. BRN, TP and ALB were insignificantly ( $P<0.05$ ) changed in the group of rats given the low dose of *A. davisii* extract.



**Fig. 2. Effect of the ethanol extract of *A. davisii* on the activities of ALT, AST, ALP and  $\gamma$ -GT in serum of rats with PCM- induced hepatotoxicity**

% change values of PCM-control group were calculated in relation to the corresponding values of normal control group. % change values of silymarin and *A. davisii*-treated groups were calculated in relation to the corresponding values of PCM- control group

**Table 1. Effect of the ethanol extract of *A. davisii* on the activities of ALT, AST, ALP and  $\gamma$ -GT in serum of rats with PCM- induced hepatotoxicity**

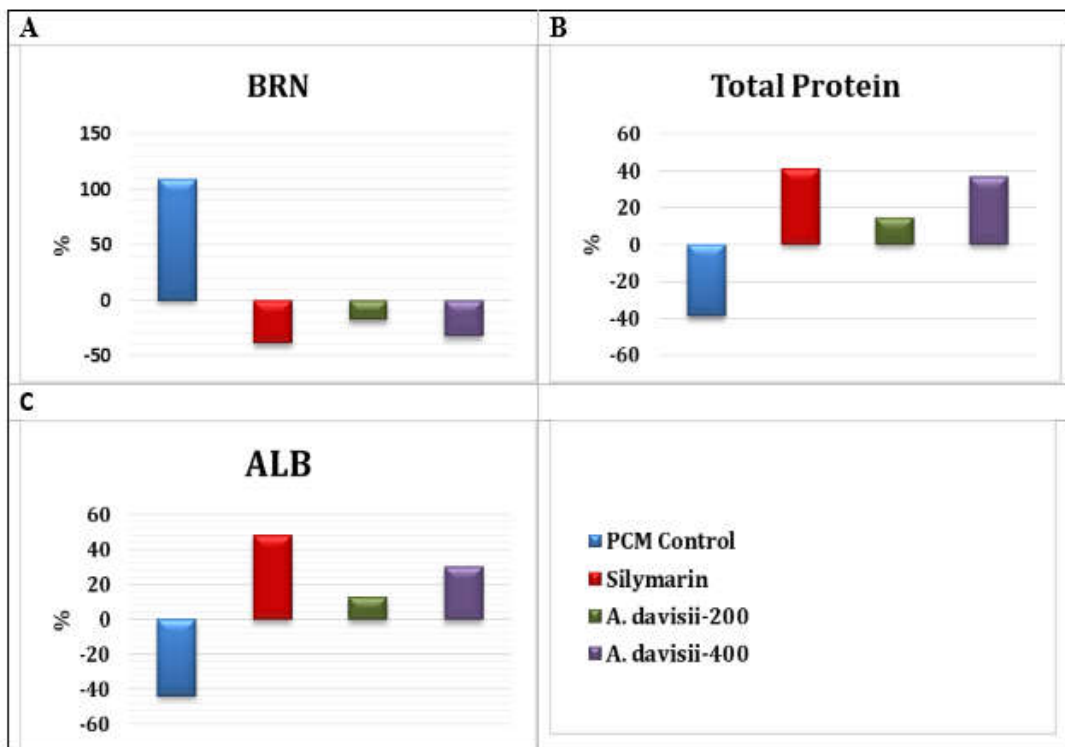
Groups	ALT (U/L)	AST(U/L)	ALP (U/L)	$\gamma$ -GT (U/L)
Normal Control	37.5±1.84 $\phi$ #	97.8±5.17 $\phi$ #	114.2±6.21 $\phi$ #	22.8±1.45 $\phi$ #
PCM Control	98.3±5.69*#	282.6±16.64*#	348.5±19.38*#	49.4±2.83*#
Silymarin (50 mg/kg) +PCM	60.7±3.88* $\phi$	148.4±8.72* $\phi$	185.7±9.85* $\phi$	27.4±1.83* $\phi$
<i>A. davisii</i> (200 mg/kg) +PCM	81.2±5.27*#	234.7±15.60*#	292.9±17.53*#	40.8±2.82*#
<i>A. davisii</i> (400 mg/kg) +PCM	68.2±3.15* $\phi$	173.6±9.45* $\phi$	225.2±15.27* $\phi$	33.7±2.57* $\phi$

Values explain the mean  $\pm$  S.E. of six animals per group, values between brackets means % changes

\*  $P < 0.05$ : Statistically significant from normal control (Dunnett's test)

$\phi P < 0.05$ : Statistically significant from PCM-hepatotoxic control (Dunnett's test)

# $P < 0.05$ : Statistically significant from Silymarin-treated group (Dunnett's test)



**Fig. 3. Effect of the ethanol extract of *A. davisii* on the levels of BRN, TP and ALB in serum of rats with PCM- induced hepatotoxicity**

% change values of PCM-control group were calculated in relation to the corresponding values of normal control group. % change values of silymarin and *A. davisii*-treated groups were calculated in relation to the corresponding values of PCM- control group

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

Groups	BRN(mg/dL)	TP(g/dL)	ALB(g/dL)
Normal Control	0.68±0.04 $\phi$ #	6.7±0.31 $\phi$ #	4.1±0.21 $\phi$ #
PCM Control	1.42±0.09*#	4.1±0.27*#	2.3±0.16*#
Silymarin (50 mg/kg) +PCM	0.87±0.07* $\phi$	5.8±0.22 $\phi$	3.4±0.23 $\phi$
<i>A. davisii</i> (200 mg/kg) +PCM	1.17±0.09*#	4.7±0.25*#	2.6±0.18*#
<i>A. davisii</i> (400 mg/kg) +PCM	0.96±0.08* $\phi$	5.6±0.30* $\phi$	3.0±0.21* $\phi$

Values explain the mean  $\pm$  S.E. of six animals per group, values between brackets means % changes

\*  $P < 0.05$ : Statistically significant from normal control (Dunnett's test)

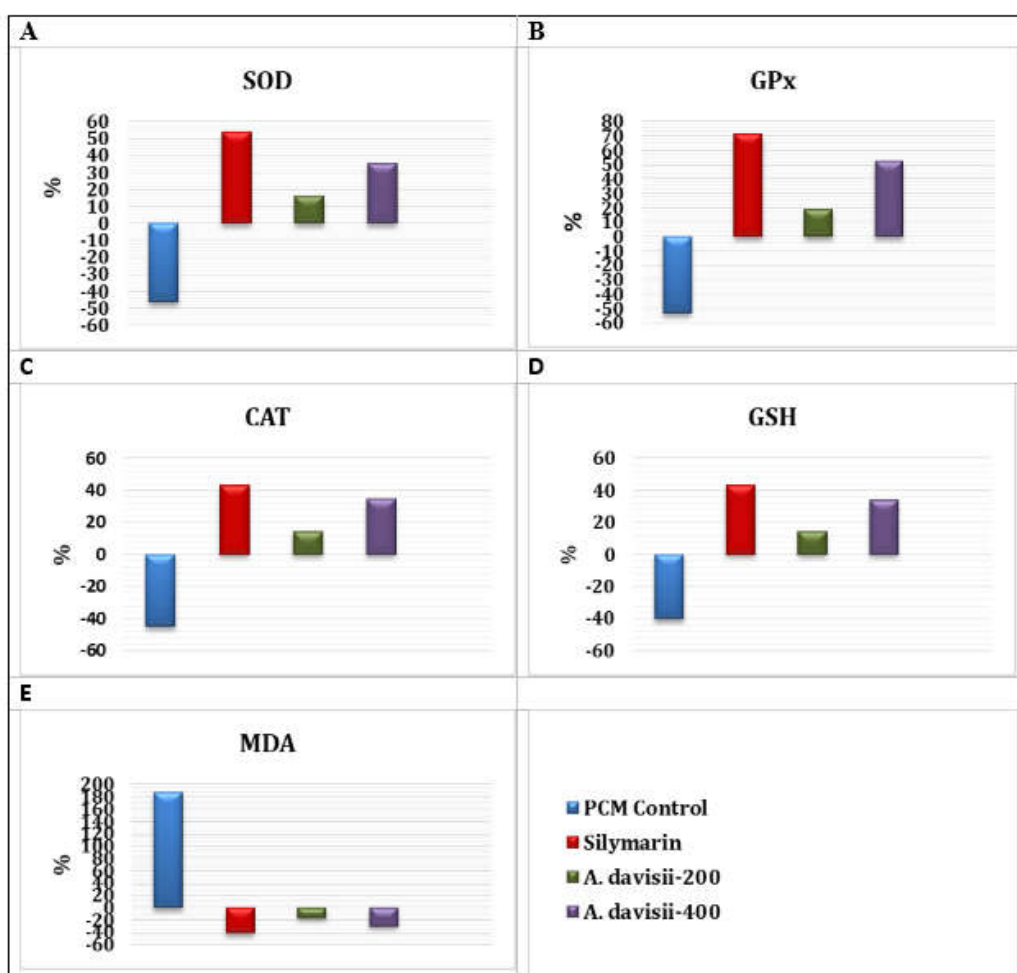
$\phi P < 0.05$ : Statistically significant from PCM-hepatotoxic control (Dunnett's test)

# $P < 0.05$ : Statistically significant from Silymarin-treated group (Dunnett's test)

### 3.3.2 Assessment of oxidative stress-related markers

The effect of *A. davisii* extract on the oxidative stress-related markers of PCM-exposed rats was investigated via assessment of the levels of SOD, GPx, CAT and GSH in their liver tissue (Table 3 & Fig. 4). The antioxidants; SOD, GPx, CAT and GSH were significantly ( $P < 0.05$ ) declined in the liver of PCM control rats ( $33.5 \pm 2.13$  U/mg protein,  $2.1 \pm 0.12$  U/mg protein,  $10.7 \pm 0.35$  U/mg protein and  $9.7 \pm 0.39$   $\mu\text{mol/g}$  tissue, respectively) when compared with the normal group ( $62.7 \pm 3.75$  U/mg protein,  $4.5 \pm 0.19$  U/mg protein,  $19.4 \pm 0.73$  U/mg protein and  $16.3 \pm 0.45$   $\mu\text{mol/g}$  tissue, respectively). In addition, animals in PCM group exhibited a remarkable increase in the hepatic MDA

( $187.22\%$ ), a marker of lipid peroxidation (LPO) as compared to normal controls. In PCM-exposed rats, pre-treatment with *A. davisii* extract at 200 mg/kg did not improve the activities of hepatic antioxidant enzymes compared to the PCM control group. However, pre-treatment with silymarin or *A. davisii* extract (400 mg/kg) significantly bolstered the antioxidant status and reduced MDA level in PCM-exposed rats. The high dose of *A. davisii* extract resulted in significantly higher levels of SOD (35.52%), GPx (52.38%), CAT (34.58%) and GSH (34.02%) in comparison with PCM control group. Interestingly, there were no significant differences in the tested oxidative stress biomarkers between *A. davisii* (400 mg/kg)-treated rats and silymarin treated animals.



**Fig. 4. Effect of the ethanol extract of *A. davisii* on the oxidative stress-related markers in liver homogenate of rats with PCM- induced hepatotoxicity**

% change values of PCM-control group were calculated in relation to the corresponding values of normal control group. % change values of silymarin and *A. davisii*-treated groups were calculated in relation to the corresponding values of PCM- control group

**Table 3. Effect of the ethanol extract of *A. davisii* on the oxidative stress-related markers in liver homogenate of rats with PCM- induced hepatotoxicity**

Groups	SOD (U/mg protein)	GPx(U/mg otein)	CAT (U/mg protein)	GSH (µmol/ g tissue)	MDA(nmol/g tissue)
Normal Control	62.7±3.75 $\phi$ #	4.5±0.19 $\phi$ #	19.4±0.73 $\phi$ #	16.3±0.45 $\phi$ #	22.7±0.86 $\phi$ #
PCM Control	33.5±2.13*#	2.1±0.12*#	10.7±0.35*#	9.7±0.39*#	65.2±3.25*#
Silymarin (50 mg/kg)+PCM	51.6±3.22* $\phi$	3.6±0.10* $\phi$	15.3±0.42* $\phi$	13.9±0.35* $\phi$	38.7±2.53* $\phi$
<i>A. davisii</i> (200 mg/kg)+PCM	38.9±2.48*#	2.5±0.15*#	12.2±0.66*#	11.1±0.33*#	54.6±3.87*#
<i>A. davisii</i> (400 mg/kg)+PCM	45.4±2.75* $\phi$	3.2±0.16* $\phi$	14.4±0.36* $\phi$	13.0±0.31* $\phi$	45.7±2.84* $\phi$

Values explain the mean  $\pm$  S.E. of six animals per group, values between brackets means % changes

\*  $P < 0.05$ : Statistically significant from normal control (Dunnett's test)

$\phi P < 0.05$ : Statistically significant from PCM-hepatotoxic control (Dunnett's test)

# $P < 0.05$ : Statistically significant from Silymarin-treated group (Dunnett's test)

### 3.3.3 Histopathological study

The histopathological study added a supportive confirmation for biochemical analysis is depicted in Fig. 5. Sections from liver of the normal rats manifested typical lobular architecture and common hepatocytes with well-preserved cytoplasm (Fig. 5-A). additionally, PCM-control rats revealed congestion, severe necrotic changes, inflammatory cell infiltration in many areas (Fig. 5-B). Treatment with *A. davisii* extract at 400 mg/kg showed marked liver protection against PCM hepatotoxicity, which is manifested by the presence of normal liver cells and reduced inflammatory infiltration and necrosis (Fig. 5-C).

## 4. DISCUSSION

### 4.1 Estimation of *in vitro* Antioxidant Effect

Many antioxidants from natural origin oftentimes have potent free radical scavenging activities. The protection against the diseases by antioxidants is considered to be due to their free radical scavenging capabilities in the biological systems. The DPPH free radical scavenging ability has exceedingly gained importance to establish the antioxidant potential of plant extracts. In our study, *A. davisii* extract showed *in vitro* antioxidant effect. In similar earlier reports, *Astragalus squarrosus* showed free radical scavenging effect that might be due to its phenolic and flavonoid constituents [15]. Moreover, *Astragalus sinicus* showed antioxidant capacity measured by DPPH assay, and is correlated to its total phenolic content [16]. The antioxidant effect of phenolic compounds is predominantly due to their redox characteristics

[17]. Accordingly, the present study indicated that the antioxidant effect of *A. davisii* extract is due to the presence of phenolic and flavonoid contents. It has earlier been reported that among most important constituent of the genus *Astragalus* are phenolic and flavonoid compounds [18].

*In vitro* antioxidant potential of a compound is inversely proportional to its EC50 value. In this regard, Lee et al., 2008 reported that EC50 value of  $< 10$  mg/mL suggests that *A. davisii* has effective antioxidant activity [19]. In our study, the EC50 values of *A. davisii* extract was found to be less than 10 mg/mL that confirm the effectiveness of the extract as antioxidant.

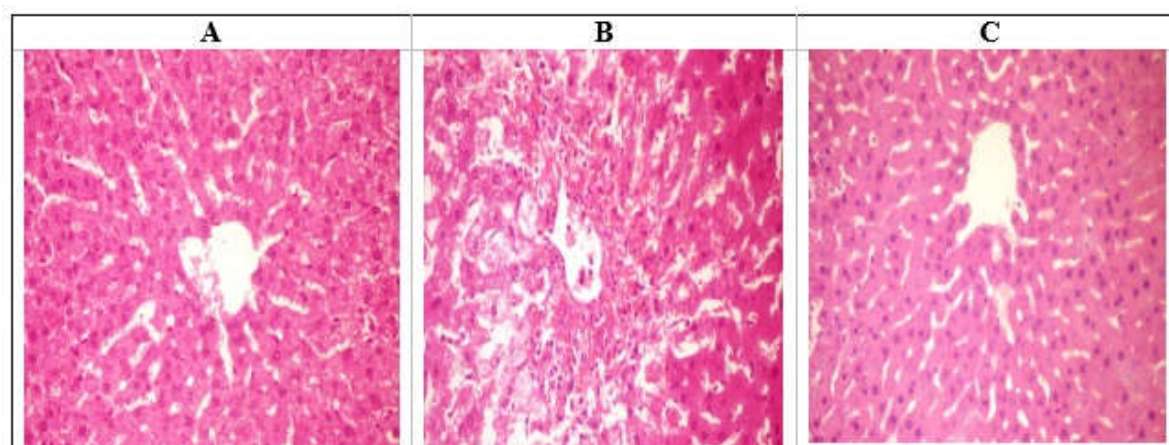
### 4.2 Acute Toxicity Study

In this study, *A. davisii* extract at 2000 mg/kg had no adverse effect on 2 out of 3 rats during 14 days of observation. At 2000 mg/kg, there was a case of mortality observed in only one rat throughout the experimental period. According OECD-423 guidelines, *A. davisii* extract was related to category 5 with LD50  $> 2000 - 5000$  mg/kg. In a previous study, ethyl acetate extract from the aerial parts of *Astragalus corniculatus*, containing flavonoids, was found to be practically nontoxic as its acute oral toxicity value was  $> 5$  g/kg in mice [6].

### 4.3 Hepatoprotective Activity

Liver is among the most important organs involved in metabolic activities. Hepatocellular injury induced by PCM overdose has been documented by a number of studies [19-20]. In this study, the potential hepatoprotective effect of





**Fig. 5. Photomicrographs of rat liver (H & E stain, X400), (A) Normal control group showing normal histological structure of hepatic lobule; (B) PCM-hepatotoxic group showing necrosis and dissociation of centrilobular hepatocytes; (C) *A. davisii* (400 mg/kg) + PCM showing no histopathological changes**

*A. davisii* extract was evaluated in PCM-overdose rat model and compared with the reference drug; silymarin. The serum levels of some remarkable biochemical parameters are used as characteristic markers of liver damage. One of the most diagnostic signs of liver damage is the liberation of intracellular enzymes, such as ALT, AST, ALP and  $\gamma$ -GT. The increased activities of liver marker enzymes are suggestive of cellular infiltration and the loss of the functional integrity of hepatocyte membranes that are constantly associated with hepatonecrosis [21].

In our study, the oral administration of PCM-overdose showed drastic elevation in the level of serum marker enzymes when compared with normal rats, indicating PCM mediated hepatic damages. Prescott et al., 1977 described the biochemical changes of PCM hepatotoxicity to be a considerable elevation in serum ALT and AST levels [22]. The elevation of these markers in serum following overdose of PCM could be referred to the damaged hepatocytes as these enzymes are located in cytoplasm and are released into circulation after hepatocyte injuries. The pretreatment of PCM-exposed rats with silymarin or *A. davisii* extract at 400 mg/kg exhibited inhibition of PCM-induced increase in the serum levels of ALT, AST, ALP and  $\gamma$ -GT enzymes, resulting in significant restoration towards their control values. In this investigation, the activity of *A. davisii* extract at 400 mg/kg was comparable with that of the standard drug silymarin. In addition, the reduction in the activities of liver marker enzymes in serum by the pre-treatment of PCM-exposed rats with *A.*

*davisii* extract (400 mg/kg) is a marker of repair of hepatic tissue damage caused by PCM.

The protective potential of *A. davisii* extract at 400 mg/kg might be a result of the improvement of oxidative stress and preservation of the antioxidant capacity conferred by the extract. This suppresses the leakage of ALT, AST, ALP and  $\gamma$ -GT enzymes into blood circulation. Moreover, the mechanism by which *A. davisii* extract reduced serum activities of liver enzymes may be referred to their capacity to inhibit the cytochrome P450 so decrease the generation of the reactive metabolites of PCM.

Serum BRN is considered as one of the important tests of liver functions. The increased concentrations of serum BRN are a sign of biliary obstruction, hepatic injury and alterations in binding, conjugation and excretory functions of hepatocytes [22]. The increased serum concentration of BRN in alone PCM-intoxicated rats suggests PCM-induced liver injury.

The liver is the main source of most of blood proteins as the liver cells are responsible for production of fibrinogen, albumin and most of the  $\alpha$ - and  $\beta$ -globulins [23]. Therefore, the marked decrease in serum levels of total protein and albumin in PCM-control rats indicates cellular damage produced. In the current study, the marked decrease in serum levels of albumin and total protein in PCM-control animals might be a result of a reduction in the number of liver cells responsible for the albumin synthesis through necrosis or might be due to the functional failure

of endoplasmic reticulum of hepatocytes [24]. 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 [25]. Pretreatment with *A. davisii* extract at 400 mg/kg reduced the increased serum level of BRN in rats due to PCM overdose, suggests the stabilization of biliary function as well as repair of damaged liver tissue of rats exposed to PCM [26]. The significant elevation in serum levels of total protein and albumin in *A. davisii*-medicated rats indicated the stabilization of the ER (Endoplasmic reticulum) that is responsible for the synthesis of protein. This assures the hepatoprotective activity of *A. davisii* extract against PCM-hepatotoxicity.

PCM hepatotoxicity is characterized by an extensive oxidative stress [27]. A key mechanism of the toxicity is cytochrome P450-catalyzed metabolic activation of PCM, which generates the highly reactive metabolite; NAPQI [28]. This reactive metabolite is responsible for oxidative stress and glutathione depletion and liver damage [29]. However, several cellular mechanisms defend the cells against the toxic effects of ROS generated by PCM and other toxicants. In this respect, hepatocytes have possess an antioxidant defense system including enzymatic and non-enzymatic antioxidants to protect themselves against free radicals and oxidative stress. Physiologically, the levels of free radicals are maintained within normal concentrations by the enzymatic antioxidants, mainly SOD, GPx and CAT and non-enzymatic antioxidants such as GSH [30]. Oxidative stress leads to toxicity when there is an increased free radicals generation than the cell's capacity for their removal. Monitoring of the levels of the cellular antioxidants in biological samples is commonly used to determine the state of oxidative stress.

In this investigation, a marked decrease in activities of the antioxidant enzymes in liver homogenate was induced by PCM overdose. The results suggested that administration of *A. davisii* extract at 400 mg/kg had successfully restored the activities of the hepatic antioxidant enzymes of PCM-overdosed rats. Improvement in the actions of the antioxidant enzymes does provide evidence to an improvement in the endogenous antioxidant defense system [31]. Accordingly, the beneficial effect of the extract could relate to its ability to maintain the activities of the hepatic antioxidant enzymes of PCM-

overdosed animals. Following treatment of PCM-exposed rats with *A. davisii* extract at 400 mg/kg, the hepatic levels of SOD, GPx and CAT were significantly restored to established values that were not different from silymarin-treated group, suggesting the capability of the extract to scavenge and neutralize PCM induced oxidative stress.

GSH is the main non-enzymatic antioxidant that is responsible as the major defense mechanism during oxidative stress. At non-toxic dose, the reactive metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI) of PCM was detoxified by the SH-group (sulfhydryl-group) of GSH forming paracetamol-GSH conjugate. In case of PCM-overdose, NAPQI depleted the stores of GSH in hepatocytes by as much as 80–90% and thereafter bound to cellular proteins leading to necrosis of hepatocytes [32] in this investigation, a marked reduction in hepatic GSH contents in PCM-exposed animals is an indication of tissue injury produced by free radicals. The depletion of cellular-GSH leaves the cell vulnerable to oxidative-stress following PCM-overdose. In our study, *A. davisii* extract (400 mg/kg) preserved the hepatic GSH contents of PCM-overdosed rats. This indicates the antioxidant potential of *A. davisii* extract against PCM-induced oxidative stress in rat liver. Therefore, we can predict that the tested extract may stimulate the synthesis of endogenous GSH that antagonizes the oxidative damage induced by PCM-overdose.

The significantly raised level of MDA, a by-product of lipid peroxidation, generated by PCM overdose indicated free radical stress induced LPO [33]. In this investigation, MDA levels increased markedly in liver homogenate of PCM control rats in comparison with normal control. Subsequent treatment with *A. davisii* extract at 400 mg/kg led to a significant decrease in MDA levels in PCM-exposed rats. LPO is one of the hallmark properties of oxidative stress in PCM-induced liver injury. According to Luqman and Rizvi [34] LPO is known to injure the cells by inactivation of membrane enzymes, decrease fluidity of the membrane and resolve into cytotoxic aldehydes such as MDA. Kanbur et al [35] have reported MDA, a product of LPO, to increase in the liver-tissues of PCM-induced liver injury in mice.

In this investigation, LPO induced by PCM-overdose was confirmed by the increase in hepatic level of MDA. The increased liver

contents of MDA has been regarded as a measure for cellular injury and suggests disability of the antioxidant defense system to protect against the generation of excessive free radicals. The present results showed that 400 mg/kg of *A. davisii* extract ameliorated the increased liver MDA contents of PCM-intoxicated rats toward normalcy. On intergroup comparison, *A. davisii* extract (400 mg/kg) showed an improvement in oxidative status comparable to that obtained by silymarin. The improved antioxidant defense in addition to reduced LPO product in the liver are indicative for the antioxidant effect of *A. davisii* extract. The capability of *A. davisii* extract to block LPO is in agreement with Soliman et al., 2013 who mentioned that some of the *Astragalus* plants have potent LPO inhibition activity [20]. Plants of the genus *Astragalus* are well known to contain biologically active compounds such as polyphenolics, triterpenoid saponins and polysaccharides. Flavonoids represent the largest group of polyphenolic compounds occurring in *Astragalus* species [6]. It is recognized that flavonoids can balance various classes of oxidizing species such as hydroxyl radical, superoxide anion or peroxy radicals. Further, flavonoids may act as quenchers of singlet oxygen [36]. Additionally, the flavonoid mixture and polysaccharides of some *Astragalus* plants are documented to have strong antioxidant effect [37]. Therefore, we believe that the possible mechanism of hepatoprotection offered by *A. davisii* extract is due to its phytochemicals as flavonoids and polysaccharides.

The histopathological findings supported the biochemical results. As demonstrated in this study, PCM-control animals revealed serious histological injury that might be due to the generation of free radicals because of PCM-overdose and subsequent lipid peroxidation (LPO). Many studies demonstrated the induction of necrosis of hepatic cells by high doses of PCM in animals [38]. The histologic alteration were ameliorated in rats that were pre-treated with *A. davisii* extract suggesting marked protection of liver tissues against damage induced by PCM-overdose. It can be referred to the antioxidant effect of the extract that markedly decreased the oxidative stress and thereby reduced the histopathological alterations of the liver.

## 5. CONCLUSION

In conclusion, this study demonstrates that *A. davisii* had a protective effect against liver

damage induced by PCM-overdose in rats. The hepatoprotective potential of *A. davisii* may be explained by increasing levels of antioxidant enzymes and decreasing the lipid peroxidation and liver enzyme on PCM-induced damage in rats. Additional phytochemical studies are in progress to separate and characterize the active compound(s) responsible for the antioxidant and hepatoprotective activities.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not approval.

## ETHICAL APPROVAL

Experimental procedures were done in accordance with international regulations on animal protection, and/or the Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the Institutional Animal Ethics Committee.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Abirami A, Nagarani G, Siddhuraju P. Hepatoprotective effect of leaf extracts from *Citrus hystrix* and *C. maxima* against paracetamol induced liver injury in rats. Food Sci. Hum. Wellness. 2015;4:35-41.
2. Hayward K, Powell E, Irvine K, et al. Can paracetamol (acetaminophen) be

- administered to patients with liver impairment?. Br. J. Clin Pharmacol. 2016; 81:210-222.
3. Hinson J, Roberts D, James L. Mechanisms of acetaminophen-induced liver Necrosis. Handb. Exp. Pharmacol. 2010;196:369-405.
  4. Jeyadevi R, Ananth D, Sivasudha T. Hepatoprotective and antioxidant activity of *Ipomoea staphylinia* Linn. Clin. Phytosci. 2019;5:18. Available:<https://doi.org/10.1186/s40816-019-0112-4>
  5. Gaur G, Alam M, Jabbar Z, et al. Evaluation of antioxidant activity of *Cassia siamea* flowers. J. Ethnopharmacol. 2006; 108:340-348.
  6. Bratkov V, Shkondrov A, Zdraveva P, et al. Flavonoids from the Genus *Astragalus*: Phytochemistry and Biological Activity. Pharmacogn. Rev. 2016;10:11-32.
  7. Choudhary MI, Jan S, Abbaskhan A, et al. Cycloartane triterpenoids from *Astragalus bicuspic*. J. Nat. Prod. 2008;71:1557-1560.
  8. Linnek J, Mitaine-Offer AC, Miyamoto T, et al. 2011. Cycloartane glycosides from three species of *Astragalus* (Fabaceae). Helv. Chim. Acta. 2011;94:230-237.
  9. Ibrahim LF, Marzouk MM, Hussein SR, et al. Flavonoid constituents and biological screening of *Astragalus bombycinus* Boiss. Nat. Prod. Res. 2013;27:386-393.
  10. Wang D, Shen W, Tian Y, et al. The effects of the three components isolated from *Astragalus mongholicus* bunge on scavenging free radicals. Chin. Pharmacol. Bull. 1994;10:129-32.
  11. Foudah A, Soliman G, Abdel-Rahman R, et al. The antioxidant and hepatoprotective effectiveness of *Astragalus echinops* and *Astragalus logopodioides* ethanolic extracts against liver injury induced by paracetamol in rats. Afr. J. Tradit. Complem. 2017;14: 31-40.
  12. Phang C, Abd Malek S, Ibrahim H, et al. Antioxidant properties of crude and fractionated extracts of *Alpinia mutica* rhizomes and their total phenolic content. Afr. J. Pharm. Pharmacol. 2011;5:842-852.
  13. OECD. OECD guideline for testing of chemicals, acute oral toxicity-acute toxic class method, Guideline No. 423. Organization for Economic Co-operation and Development; 2001:1-14.
  14. Carleton H. Carleton's Histological Technique, 4th ed. Oxford University Press, New York, Toronto; 1976.
  15. Asgarpanah J, Motamed S, Farzaneh A, et al. Antioxidant activity and total phenolic and flavonoid content of *Astragalus squarrosus* Bunge. Afr. J. Biotechnol. 2011;10:19176-19180.
  16. Yeom SH, Kim MK, Kim HJ, et al. Phenolic compounds from seeds of *Astragalus sinicus* and its antioxidative activities. Saengyak Hakhoechi. 2003;34:344-51.
  17. Bagheri S, Keyhani L, Heydari M, et al. Antinociceptive activity of *Astragalus gummifer* gum (*Gum tragacanth*) through the adrenergic system: An *in vivo* study in mice. J. Ayurveda Integr. Med. 2015;6:19-23.
  18. Lee Y, Jian S, Lian P, et al. Antioxidant properties of extracts from a white mutant of the mushroom *Hypsizygus marmoreus*. J. Food Compos. Anal. 2008;21:116-124.
  19. Johnkennedy N, Adamma E. The protective role of *Gongronema latifolium* in acetaminophen induced hepatic toxicity in Wistar rats. Asian Pac. J. Trop. Biomed. 2011;S151-S154.
  20. Soliman G, Abdel-rahman R, Al-saikhan F, et al. Hepatoprotective activities of *Astragalus persicus* and *Astragalus tournefortii* ethanolic extracts against paracetamol induced liver damage in rats and their *in vitro* antioxidant effects. FABAD J. Pharm. Sci. 2013;38:1-9.
  21. Naik SR, Panda VS. Hepatoprotective effect of Ginkgo select Phytosome in rifampicin induced liver injury in rats: evidence of antioxidant activity. Fitoterapia. 2008;79:439-45.
  22. Prescott LF, Park J, Ballantyne A, et al. Treatment of paracetamol (acetaminophen) poisoning with N-acetylcysteine. Lancet. 1977;2:432-434.
  23. Pawlikowska-Pawlega B, Gruszecki WI, Misiak L, et al. Modification of membranes by quercetin, a naturally occurring flavonoid, via its incorporation in the polar head group. Biochim. Biophys. Acta. 2007; 1768:2195-2204.
  24. Ravikumar S, Gnanadesigan, M, Sesherebiah J, et al. Hepatoprotective effect of an Indian salt marsh herb *Suaeda monoica* Forsk. Ex. Gmel against concanavalin: an induced toxicity in rats. Life Sci. Med. Res. 2010;LSMR-2.
  25. Cohen SD, Khairallah EA. Selective protein arylation and acetaminophen-induced hepatotoxicity. Drug Metab. Rev. 1997;29:59-77.

26. Gini K, Muraleedhara K. Hepatoprotective effect of *Spirulina lonar* on paracetamol induce liver damage in rats. Asian J. Exp. Biol. Sci. 2010;1:614-623.
27. Du K, Ramachandran A, Jaeschke H. Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. Redox. Biol. 2016;10:148-156.
28. Vermeulen NP, Bessems JG, Van de streat R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism based prevention. Drug Metab. Rev. 1992;24:367.
29. Shah V, Deva K. Hepatoprotective activity of leaves of *Parkinsonia aculeata* Linn against paracetamol induced hepatotoxicity in rats. Int. J. Pharm. 2011; 1:59-66.
30. Dröge W. Free radicals in the physiological control of cell function. Physiol. Rev. 2002; 82:47-95.
31. Hemabarathy B, Budi nans SB, Feizal V. Paracetamol hepatotoxicity in rats treated with crude extract of *Alpinia galangal*. J. Biol. Sci. 2009;9:57-62.
32. Fontana R. Acute Liver Failure including Acetaminophen Overdose. Med. Clin. North Am. 2008; 92:761–794.
33. Chugh SN, Dhawan R, Kishore K, et al. Glibenclamide vs gliclazide in reducing oxidative stress in patients of noninsulin dependent diabetes mellitus- a double blind randomized study. J. Assoc. Phys. India. 2001;49:803-807.
34. Luqman S, Rizvi SI. Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. Phytother. Res. 2006;20:303- 306.
35. Kanbur M, Eraslan G, Beyaz L, et al. The effects of royal jelly on liver damage induced by paracetamol in mice. Exp. Toxicol. Pathol. 2009;61:123-32.
36. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55:481-504.
37. Ivancheva S, Nikolova M, Tsvetkova R. Pharmacological activities and biologically active compounds of Bulgarian medicinal plants. Phytochemistry: Advances in Research. 2006;87-103.
38. Dash D, Yeligar V, Nayak S, et al. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop. J. Pharm. Res. 2007;6:755-765.

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