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# Isolation of Astragaloside-IV and Cyclocephaloside-I from *Astragalus gummifera* and Evaluation of Astragaloside-IV on $CCl_4$ Induced Liver Damage in Rats

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## ABSTRACT

Two Cycloartane saponins, astragaloside-IV and cyclocephaloside-I, were isolated from the roots of *Astragalus gummifera*. Their structures were determined by utilizing 1D and 2D NMR experiments. Hepatoprotective effects of astragaloside-IV against carbon tetrachloride ($CCl_4$) induced nephrotoxicity and hepatotoxicity in rats was studied. The rats (200-230 g) of either sex were divided into 6 groups (6 rats each). Injection of a single dose of $CCl_4$ increases the level of serum AST, ALT, GGT, ALP and total bilirubin activities, decreases non-protein sulfhydryls (NP-SH) and total proteins (TPs), increases malondialdehyde (MDA) and produces changes in hepatic and nephritic tissue structure. Administration of doses of 25, 50 and 75 mg $kg^{-1}$ of astragaloside-IV reversed significantly ($p<0.001$) the liver diagnostic blood markers as well as stricture of nephrotoxicity and hepatotoxicity of the treated groups when compared to the control group. The present finding indicated that astragaloside-IV is an active hepatoprotective agent that restores the affected biochemical markers and nephritic and hepatic tissues.

**Key words:** *Astragalus gummifera*, astragaloside-IV, cyclocephaloside-I, nephrotoxicity, hepatotoxicity

## INTRODUCTION

The genus *Astragalus* (Fabaceae) is represented by 380 species in the Turkish flora (Davis, 1970) among which *Astragalus gummifera* is used economically for the production of gum tragacanth (Calis and Sticher, 1996). In the Turkish folk medicine the root of these plants are used as diuretic, antiperspirant and tonic drugs and for the treatment of diabetes mellitus, leukemia, uterine cancer and nephritis (Li and Fitzloff, 2001). *Astragalus* species has been used as an analgesic, antihypertensive, antioxidative, antiseptic, antisudorific, antiviral, hepatoprotective, immune-stimulant, tonic and for wound healing. Cycloartanes from the genus *Astragalus* are found to possess the same activities (Hikino *et al*., 1976; Rios and Waterman, 1997; Bedir *et al*., 2000). Cycloartanes are produced by photosynthesizing organisms only hence it occupy a special position among low molecular bioregulators and serves as key link in the biosynthesis of different phytosterols. It is weakly polar derivatives hence widespread in the plant kingdom. Genus *Astragalus* is the richest source of cycloartane compounds. About 400 cycloartane-type saponins were isolated and 160 of them were isolated from genus *Astragalus* (Mamedova and Isaev, 2004).

Different technology has been applied to the isolation of cycloartane-type saponins (Li and Fitzloff, 2001; Sun *et al*., 2005). The cycloartane-type saponins, astragaloside-IV and cyclocephaloside-I, are the principal constituents found in the most of *Astragalus* species. Both of compounds were recently evaluated as valuable wound healing agents (Sevimli-Gur *et al.*, 2011). *Astragaloside*-IV has been recently evaluated, in China, for the treatment of cardiovascular diseases possibly due to its antioxidative and nitric oxide-induction and as anti-tuberculosis diseases due to promoting effects on the phagocytosis and the secretion of interleukin-l beta- interleukin-6 and tumor necrosis factor-alpha (Xu *et al*., 2007). The scientific evaluation of related *Astragalus* root extract already showed antioxidant as well as hepatoprotective activities (Zhang *et al*., 2006; Allam *et al*., 2013; Yusufoglu *et al*., 2014). The present study aimed to the isolations and characterization of cycloartane-type saponins and evaluation of their biological activities. Due to the strong supportive evidences of astragaloside-IV here, the present study focus on studying the hepatoprotective and nephroprotective activities of this compound.

## MATERIALS AND METHODS

**Experimental material:** Column chromatography was performed using a silica gel (70-230 mesh, Merck, Darmstadt, Germany) and Thin Layer Chromatography (TLC) was performed using pre-coated silica-gel 60 F254 and RP-18 F254 plates (both 0.25 mm, Merck). Proton ($^{1}H$) and carbon 13 ($^{13}C$ NMR) spectra were recorded using Bruker VX500 NMR spectrometer operating at 500 or 125 MHz, respectively. All the chemicals and reagents for hepatoprotective studies procured were of analytical grade. Silymarin, Lipid Peroxidation (MDA) Assay Kit, EDTA, Trichloroacetic acid (TCA) and DNTB were purchased from Sigma Aldrich.

**Plant collection, authentication and extraction:** The plant *Astragalus gummifera* Labill (Fabaceae) was collected from Elazig-Hazargolu road, 23 km southeast of Elazig, East Anatolia, Turkey, June 2000. The plant has been authenticated by Prof. Dr. Zeki Aytac, Gazi University, Department of Biology, Faculty of Science and Art, Ankara, Turkey. A voucher specimen has been deposited in Herbarium of the Pharmaceutical Botany Department, Faculty of Pharmacy, Hacettepe University, Ankara Turkey (HUEF 00-23). The collected roots were singly chopped into small pieces, dried in shade. The air-dried powdered roots (900 g) was extracted with 80% aqueous ethanol (2×3L) under reflux. The ethanolic extract was combined and subjected to rotary evaporation (70±2°C). The thick solution was lyophilized using freeze drier and the obtained 100 g was used for present studies.

**Isolation and identification of cycloartane-type saponins:** Ten grams of the extract was applied onto silica gel column (200 g, Merck) packed in chloroform. Gradient elution was performed using solvent mixtures of chloroform, methanol and water starting with 100% chloroform, then $CHCl_3$-MeOH-$H_2O$ at the ratios (90:10:0 to 60:40:4), yielded different fractions, fraction C was subjected to medium pressure liquid chromatography reverse phase C-18 column by using methanol: water mixture (0:100-30:70). The effluent was monitored by TLC (pre-coated silica-gel 60 $F_{254}$ plates, Merck) and the pure compounds were identified by NMR spectroscopy.

**Hepatoprotective study of astragaloside-IV:** The procedure for hepatoprotective experiment was approved by the Ethical Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA. In brief, Wistar albino rats (180-210 g) of either sex were used for

present study. The obtained rats from Lab Animal Care Unit, Pharmacy College, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA, were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, supply with standard rodent diet and water *ad libitum*. All animals were kept for one week before commencement of the experiment to acclimatize to the laboratory condition. The $CCl_4$ induced liver injury method was used for present study (Kadir *et al*., 2013). The rats were divided into six groups (six animals, each). Group-1 (normal control) administered normal saline, Group-II administered $CCl_4$ as hepatotoxic, Groups III, IV and V administered $CCl_4$ and doses of 25, 50 and 75 mg $kg^{-1}$ and Group-VI administered $CCl_4$ and silymarin 10 mg $kg^{-1}$ (control group). Blood was collected in plain tubes by cardiac puncture. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min. All animals were sacrificed under light ether anesthesia, 24 h after the last dose after blood collection. Liver and kidney were detached, rinsed in icy saline, blotted with filter paper and weighted and small pieces were fixed in 10% formalin and kept for histopathological assessment. About 1 g of liver and kidney tissues was weighed. Liver was homogenate in 10% (w/v) and was prepared in 0.25 M sucrose solution and centrifuged at 7000 rpm for 10 min at 4°C while kidney tissue was homogenized in 10 volumes of ice cold buffer 100 mM $KH_2PO_4$ buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 8,000 g for 30 min at 4°C and both of the supernatant was used for used for protein and oxidative stress assessment.

**Assessment of liver function:** Reitman and Frankel (1957) method was used for estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) while King and Armstrong (1934) method was used for determination of serum alkaline phosphatase (ALP). Szasz (1969) method was used for determination of gamma glutamyl transpeptidase (GGT), whereas Walters and Gerarde (1970) method modified by DMSO used for the determined of bilirubin concentration.

**Assessment of kidney function:** Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, UK) was used for estimation of serum creatinine and blood urea while kits from Biodiagnostic, Egypt was used for estimation of serum calcium, potassium and uric acids. Henry *et al*. (1974) method was used to photometric determination of sodium level.

**Assessment of lipid profile:** Roche diagnostic kits (Roche Diagnostics GmbH, Mannheim, Germany) were used for estimation of total cholesterol, triglycerides, high-density lipoproteins (HDL), VLDL (very low-density lipoprotein) and LDL (low-density-lipoprotein) in the serum.

**Assessment of tissue total protein:** The protein was determined by using standard Bovine Serum Albumin (BSA) according to Lowry *et al*. (1951) method.

**Assessment of oxidative stress:** Malondialdehyde (MDA) was determined by Ohkawa *et al*. (1979) method and non-protein sulfhydryls (NP-SH) was determined by according to the Sedlak and Lindsay (1968) method in liver tissue. The measurement of Malondialdehyde (MDA) and non-protein sulfhydryls (NP-SH) were used for assessment of oxidative stress. In brief, take 0.2 mL of both tissues sample separately in a different test tube and then incubated at 37°C for 1 h and then add 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid and boil on water bath at 95°C for 5 min. The tube was cooled in ice and centrifuged at 4°C, 3500 rpm for 10 min. The supernatant was collected and was measured at 532 nm. For NP-SH assay, 0.1 mL of

the supernatant was suspended in tris buffer, 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB) and observance was measured within 5 min at 412 nm against reagent blank with no homogenate. The result was expressed as MDA and NP-SH nmol/mg in liver and kidney separately.

**Assessment of liver and kidney histopathology:** A small fragment of liver tissues from the right lobe and kidney tissue from the right kidney were immersed in 10% formalin, overnight at 37°C. Tissue samples were washed for 7-8 h with tap water and dehydrated with graded series of isopropyl alcohol (80%) for 12 h and 100% isopropyl alcohol for 1 h then the tissues were cleared with two changes of xylene (1 h) each. Finally, the tissues were then infiltrated with paraffin wax (4×1 h). After complete infiltration both the tissues were separately fixed in the paraffin wax. The tissue section (3 μm) was made by rotary microtome (Leitz 1512). The sections were mounted on slides and then placed in an oven with a temperature of 60°C for 10-15 min and then stained with Mayer's hematoxylin solution (15 min), washed (15 min) in lukewarm running tap water, distilled water (2 min) and finally with ethyl alcohol (80%) then counter stained with eosin-phloxine solution (2 min). Histological observations were made under light microscope.

**Statistical analysis:** All the statistical work was analyzed by using Graph Pad Prism 5.01 Software (GraphPad software, San diego, CA, USA). For each analysis, comparison between the groups were made by means of one way analysis of variance (ANOVA) and followed by Student's t-test, the $p<0.001$ regarded as significant. The data was expressed in Mean±Standard Error of Mean (SEM).

## RESULTS

**Isolation and identification of cycloartane-type saponins:** Chromatographic sepration of the chemical constituents of the hydroalcohlic extract of *Astragalus gummifera* using silica gel, reversed-phase C18 and Sephadex LH-20, yielded two compounds 1 and 2. The $^{1}H$ NMR (500 MHz, $CDCl_3$) and $^{13}C$ NMR and DEPT experiments (125 MHz, $CDCl_3$) of both compounds are cited in Table 1 and 2. COSY, HSQC and HMBC spectra were run using the same machines (Table 1-4) and the final structure was established and explain in Fig. 1a-b, respectively for astragaloside-IV and cyclosephaloside-I.

Table 1: $^{1}H$ NMR spectral data of astragaloside IV

| H | δ (ppm) |
|---|---|
| 3α | 3.69 dd |
| 5α | 1.94 d |
| 6β | 3.86 ddd |
| 16α | 5.00 m |
| 17α | 2.52 d |
| $H_3$-18 | 1.40 S |
| 19 a | 0.20 d |
| 19 b | 0.58 d |
| $H_3$-21 | 1.30 s |
| 22 a | 1.58 m |
| 22 b | 3.14 dd |
| 24 | 3.89 m |
| $H_3$-26 | 1.58 s |
| H3- 27 | 1.30 s |
| H3-28 | 2.03 s |
| H3-29 | 1.36 s |
| H3-30 | 0.94 s |
| H-1' (xylose) | 4.88 d |
| H-1'' (glucose) | 4.97 d |

Table 2: $^{13}C$ NMR astragaloside-IV in pyridine-$d_5$ at 100 MHz

| Carbon No. | δ (ppm) | Carbon No. | δ (ppm) |
|---|---|---|---|
| C1 | 32.6 | C22 | 35.0 |
| C2 | 30.6 | C23 | 26.9 |
| C3 | 89.0 | C24 | 82.1 |
| C4 | 43.1 | C25 | 71.7 |
| C5 | 52.9 | C26 | 28.6 |
| C6 | 79.5 | C27 | 29.0 |
| C7 | 35.3 | C28 | 29.3 |
| C8 | 46.1 | C29 | 17.1 |
| C9 | 21.2 | C30 | 20.3 |
| C10 | 29.4 | β-D-Xyl (1C) | 108.1 |
| C11 | 26.9 | β-D-Xyl (2C) | 76.0 |
| C12 | 26.6 | β-D-Xyl (3C) | 78.5 |
| C13 | 45.5 | β-D-Xyl (4C) | 71.7 |
| C14 | 46.6 | β-D-Xyl (5C) | 67.4 |
| C15 | 46.6 | β-D-Glu (1C) | 105.7 |
| C16 | 73.8 | β-D-Glu (2C) | 76.0 |
| C17 | 58.6 | β-D-Glu (3C) | 79.7 |
| C18 | 21.5 | β-D-Glu (4C) | 72.2 |
| C19 | 28.6 | β-D-Glu (5C) | 79.0 |
| C20 | 87.7 | β-D-Glu (6C) | 63.4 |
| C21 | 27.5 | | |

Table 3: $^{1}H$ NMR spectral data of cyclocephaloside-I

| H | δ (ppm) |
|---|---|
| 3α | 3.47 dd |
| 5α | 1.89 d |
| 6β | 3.72 dt |
| 16α | 4.80 m |
| 17α | 2.06 d |
| $H_3$-18 | 1.62 s |
| 19 a | 052 d |
| 19 b | 0.17 d |
| $H_3$-21 | 1.52 s |
| 22 a | 2.28 m |
| 22 b | 1.82 m |
| 24 | 3.62 br s |
| $H_3$-26 | 1.41 s |
| H3- 27 | 1.27 s |
| H3-28 | 2.04 s |
| H3-29 | 1.30 s |
| H3-30 | 0.88 s |
| H-1' (xylose) | 4.76 d |
| H-1'' (glucose) | 4.82 d |

**Assessment of liver function of astragaloside-IV in intoxicated rats:** Table 5 lists the liver function parameters profile of astragaloside-IV and silymarin in $CCl_4$-intoxicated rats. ALT, AST and GGT were significantly ($p<0.001$) elevated in the $CCl_4$ intoxicated rats (1.25 mL $kg^{-1}$) when compared to the normal animals. Administration of astragaloside-IV at doses of 25, 50 and 75 mg $kg^{-1}$ prior to $CCl_4$ treatment significantly protected against the elevation of transaminases levels. The serum AST, ALT and GGT in rats treated with astragaloside-IV at a dose of 25 mg $kg^{-1}$ +$CCl_4$ were 241.16±9.06, 240.33±8.58 and 17.46±0.65 IU $L^{-1}$, respectively were not significant when compared to the normal groups. Treatment with a dose of 50 mg $kg^{-1}$+$CCl_4$ were 199.00±5.01, 203.66±5.06 and 13.86±0.50 IU $L^{-1}$, respectively and 75 mg $kg^{-1}$+$CCl_4$ were 166.16±7.96, 133.66±5.19 and 9.96±0.31 IU $L^{-1}$, respectively. These results were significant when compared with the intoxicated control rats (249.83±6.09, 238.00±7.87 and 18.50±0.69 IU $L^{-1}$, respectively). Similarly, the eminent levels of serum ALP, bilirubin and LDH were decreased significantly with higher dose of astragaloside-IV.

Table 4: $^{13}C$ NMR cyclocephaloside-I spectral data in pyridine-$d_5$ at 100 MHz

| Carbon No. | δ (ppm) | Carbon No. | δ (ppm) |
|---|---|---|---|
| C1 | 32.7 | C22 | 27.1 |
| C2 | 30.6 | C23 | 24.4 |
| C3 | 89.0 | C24 | 69.2 |
| C4 | 43.1 | C25 | 75.9 |
| C5 | 53.0 | C26 | 29.5 |
| C6 | 76.0 | C27 | 29.1 |
| C7 | 35.2 | C28 | 20.5 |
| C8 | 46.4 | C29 | 29.9 |
| C9 | 21.5 | C30 | 17.1 |
| C10 | 29.2 | β-D-Xyl (1C) | 108.0 |
| C11 | 26.7 | β-D-Xyl (2C) | 75.0 |
| C12 | 34.6 | β-D-Xyl (3C) | 78.4 |
| C13 | 46.5 | β-D-Xyl (4C) | 72.1 |
| C14 | 47.5 | β-D-Xyl (5C) | 67.3 |
| C15 | 47.7 | β-D-Glu (1C) | 105.4 |
| C16 | 74.5 | β-D-Glu (2C) | 75.9 |
| C17 | 61.2 | β-D-Glu (3C) | 79.7 |
| C18 | 21.3 | β-D-Glu (4C) | 71.6 |
| C19 | 30.6 | β-D-Glu (5C) | 79.5 |
| C20 | 79.9 | β-D-Glu (6C) | 63.3 |
| C21 | 28.4 | | |

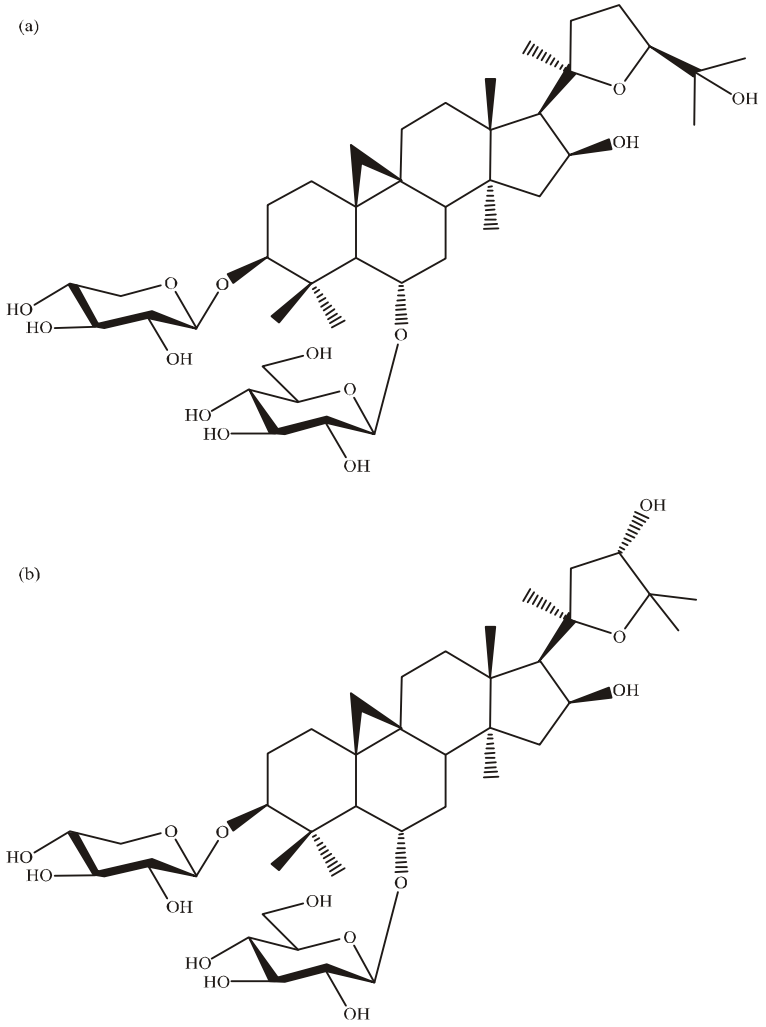


Fig. 1(a-b): Structure of (a) Astragaloside-IV and (b) Cyclocephaloside-I

Table 5: Assessment of liver function of astragaloside-IV and silymarin in $CCl_4$-intoxicated rats

| Treatments | AST (IU $L^{-1}$) | ALT (IU $L^{-1}$) | GGT (IU $L^{-1}$) | ALP (IU $L^{-1}$) | Bilirubin (mg $dL^{-1}$) | LDH (IU $L^{-1}$) |
|---|---|---|---|---|---|---|
| Normal (1 mL) | 71.28±3.56 | 31.20±2.50 | 5.73±0.25 | 261.66±10.18 | 0.50±0.01 | 96.10±2.94 |
| $CCl_4$ (1.25 mg $kg^{-1}$) | 249.83±6.09*** | 238.00±7.87[a]*** | 18.50±0.69*** | 445.00±14.53*** | 2.29±0.07*** | 163.15±4.70*** |
| Astragaloside-IV (25 mg $kg^{-1}$) | 241.16±9.06 | 240.33±8.58 | 17.46±0.65 | 385.16±15.30* | 2.29±0.07 | 160.60±3.23 |
| Astragaloside-IV (50 mg $kg^{-1}$) | 199.00±5.01*** | 203.66±5.06** | 13.86±0.50*** | 360.33±8.60*** | 1.68±0.06*** | 123.88±4.67*** |
| Astragaloside-IV (75 mg $kg^{-1}$) | 166.16±7.96*** | 133.66±5.19*** | 9.96±0.31*** | 358.66±4.94*** | 1.33±0.04*** | 113.02±4.21*** |
| Silymarin (10 mg $kg^{-1}$) | 118.55±6.37*** | 133.33±7.03*** | 7.75±0.46*** | 327.83±6.16*** | 0.77±0.03*** | 104.40±6.30*** |

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001, statistically significant when compare to $CCl_4$ intoxication to normal control group. When compared astragaloside-IV+$CCl_4$ (25, 50 and 75 mg $kg^{-1}$, b.wt.) and $CCl_4$ with silymarin+$CCl_4$ showed statistically significant when the result was compared by Student's t-test

Table 6: Assessment of kidney function of astragaloside-IV and silymarin in $CCl_4$-intoxicated rats

| Treatments | Creatinin (mg $dL^{-1}$) | Urea (mg $dL^{-1}$) | Uric acid (mg $dL^{-1}$) | Calcium (nmol $L^{-1}$) | Sodium (nmol $L^{-1}$) | Potassium (nmol $L^{-1}$) |
|---|---|---|---|---|---|---|
| Normal (1 mL) | 3.92±0.13 | 33.95±1.95 | 1.36±0.78 | 6.11±0.54 | 58.59±1.38 | 3.98±0.31 |
| $CCl_4$ (1.25 mg $kg^{-1}$) | 15.42±0.43*** | 102.51±3.76*** | 7.05±0.18*** | 21.55±0.61*** | 102.86±1.06*** | 13.85±0.51*** |
| Astragaloside-IV (25 mg $kg^{-1}$) | 13.79±0.47* | 92.73±2.24 | 6.57±0.29 | 17.11±0.92** | 100.13±1.79 | 12.20±0.44* |
| Astragaloside-IV (50 mg $kg^{-1}$) | 9.75±0.58*** | 62.90±2.14*** | 6.19±0.25 | 13.16±0.69*** | 96.39±1.58* | 10.78±0.57** |
| Astragaloside-IV (75 mg $kg^{-1}$) | 5.90±0.26*** | 44.15±2.19*** | 2.66±0.20*** | 8.88±0.40*** | 76.81±2.52*** | 7.86±0.39*** |
| Silymarin (10 mg $kg^{-1}$) | 8.57±0.50*** | 72.96±2.35*** | 2.16±0.07*** | 14.05±0.77*** | 73.23±1.68*** | 9.21±0.19*** |

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001, statistically significant when compare $CCl_4$ intoxication to normal control group. When compared Astragaloside-IV+$CCl_4$ (25, 50 and 75 mg $kg^{-1}$, b.wt.) and $CCl_4$ with silymarin+$CCl_4$ showed statistically significant when the result was compared by Student's t-test

Table 7: Assessment of lipid profile after treatment with astragaloside-IV and silymarin in $CCl_4$-intoxicated rats

| Treatments | Cholesterol (mg $dL^{-1}$) | Triglycerides (mg $dL^{-1}$) | HDL (mg $dL^{-1}$) | VLDL (nmol $L^{-1}$) | LDL (nmol $L^{-1}$) |
|---|---|---|---|---|---|
| Normal | 115.27±3.67 | 49.31±2.55 | 73.06±1.95 | 9.86±0.51 | 32.34±4.45 |
| $CCl_4$ (1.25 mg $kg^{-1}$) | 251.38±9.70*** | 142.92±4.71*** | 31.05±1.78*** | 28.58±0.94*** | 191.75±8.73*** |
| Astragaloside-IV (25 mg $kg^{-1}$) | 212.50±7.91* | 111.06±3.47 | 33.79±2.20 | 23.01±0.69** | 155.69±7.16 |
| Astragaloside-IV (50 mg $kg^{-1}$) | 158.33±9.62*** | 99.54±3.63*** | 47.95±2.30 | 19.90±0.72*** | 90.47±11.49* |
| Astragaloside-IV (75 mg $kg^{-1}$) | 130.55±4.00*** | 90.36±3.20*** | 57.99±2.69*** | 16.07±0.64*** | 56.48±3.36*** |
| Silymarin (10 mg $kg^{-1}$) | 154.86±8.64*** | 108.67±4.21*** | 44.75±2.00*** | 21.73±1.11*** | 88.37±9.11*** |

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001, statistically significant when compare $CCl_4$ intoxication to normal control group. When compared astragaloside-IV+$CCl_4$ (25, 50 and 75 mg $kg^{-1}$, b.wt.) and $CCl_4$ with silymarin+$CCl_4$ showed statistically significant when the result was compared by Student's t-test

**Assessment of kidney after treatment with astragaloside-IV:** Table 6 lists the result of renal function parameters profile after treatment with astragaloside-IV. Serum creatinine, uric acid and urea in $CCl_4$ injected rats (15.42±0.43, 102.51±3.76 and 7.05±0.18 mg $dL^{-1}$, respectively) were significantly increased ($p \leq 0.001$) while in astragaloside-IV group, levels of creatinine and urea at higher dose were significantly decreased ($p \leq 0.001$). The $CCl_4$ injected rats also showed a significant ($p<0.001$) increase in the levels of serum calcium (21.55±0.61 nmol $L^{-1}$), sodium (102.86±1.06 nmol $L^{-1}$) and potassium (13.85±0.51 nmol $L^{-1}$) when compared with normal control group. Administration of 25-75 mg $kg^{-1}$ of astragaloside-IV significantly ($p<0.05$) improved the level of calcium while sodium and potassium only improved with higher concentration.

**Assessment of lipid profile:** The result of serum lipid profile is tabulated in Table 7. The total cholesterol (251.38±9.70), triglycerides (142.92±4.71), VLDL (28.58±0.94) and LDL (191.75±8.73) levels were increased in $CCl_4$ induced group as compared to that of normal group of rats ($p<0.001$) but HDL (31.05±1.78) level was decreased from the normal (73.05±1.95). Whereas treatment of $CCl_4$ induced group of rats with silymarin (10 mg $kg^{-1}$) and astragaloside-IV at a higher dose (50 or 75 mg $kg^{-1}$) inverted the levels towards normal range.

Table 8: Assessment of oxidative stress and total protein profile of liver and kidney tissue after administration of astragaloside-IV and silymarin in $CCl_4$-intoxicated rats

| | Organs tissue | | | | | |
|---|---|---|---|---|---|---|
| | Kidney | | | Liver | | |
| Treatments | Total protein (g $dL^{-1}$) | MDA (nmol $g^{-1}$) | NP-SH (nmol $g^{-1}$) | Total protein (g $dL^{-1}$) | MDA (nmol $g^{-1}$) | NP-SH (nmol $g^{-1}$) |
| Normal (1 mL) | 96.19±4.05 | 0.97±0.06 | 9.28±0.69 | 100.59±5.81 | 0.99±0.03 | 9.48±0.58 |
| $CCl_4$ (1.25 mg $kg^{-1}$) | 53.48±2.08*** | 4.93±0.27*** | 5.04±0.39*** | 51.89±1.92*** | 5.74±0.40*** | 5.47±0.34*** |
| Astragaloside-IV (25 mg $kg^{-1}$) | 59.07±2.08 | 4.27±0.22 | 3.98±0.41 | 49.89±2.95 | 6.05±0.50 | 6.13±0.36 |
| Astragaloside-IV (50 mg $kg^{-1}$) | 64.26±1.63** | 3.88±0.31* | 4.84±0.41 | 62.27±3.19* | 4.35±0.41* | 7.91±0.43** |
| Astragaloside-IV (75 mg $kg^{-1}$) | 79.43±4.90*** | 2.00±0.10*** | 6.90±0.41*** | 80.23±3.42*** | 2.62±0.24*** | 7.85±0.53** |
| Silymarin (10 mg $kg^{-1}$) | 80.23±2.31*** | 2.22±0.13*** | 6.86±0.48*** | 79.83±3.93*** | 2.09±0.12*** | 8.52±0.41*** |

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001, statistically significant when compare $CCl_4$ intoxication to normal control group. When compared astragaloside-IV+$CCl_4$ (25, 50 and 75 mg $kg^{-1}$, b.wt.) and $CCl_4$ with silymarin+$CCl_4$ showed statistically significant when the result was compared by Student's t-test

**Assessment of tissue total protein and oxidative stress:** Total protein and oxidative stress profile are tabulated in Table 8. The MDA (5.74±0.40) levels was increased in $CCl_4$ induced group as compared to that of normal group (0.99±0.03) of rats liver tissue ($p<0.001$) but total protein (51.89±1.92) and NP-SH (5.47±0.34) level were decreased from the normal (100.59±5.81 and 9.48±0.58), respectively. Similarly MDA (4.93±0.27) level was increased in $CCl_4$ induced group as compared to that of normal group (0.97±0.06) of rats kidney tissue ($p<0.001$) but total protein (53.48±2.08) and NP-SH (5.04±0.39) level were decreased from the normal (96.19±4.05 and 9.28±0.69), respectively. Whereas, treatment of $CCl_4$ induced group of rats with silymarin (10 mg $kg^{-1}$) and astragaloside-IV at a dose (50 or 75 mg $kg^{-1}$) inverted the levels towards normal range.

**Assessment of liver and kidney histopathology:** The brief histopathological studies of liver and kidney are given in Fig. 2 and 3, respectively. The normal hepatic architecture (Fig. 2a) showed the normal hepatic lobule, where the hepatocytes (Fig. 2b) showed portal infiltration with massive mononuclear cells. Treatment with astragaloside-IV 25 mg $kg^{-1}$ (Fig. 2c), 50 mg $kg^{-1}$ (Fig. 2d) and 75 mg $kg^{-1}$ (Fig. 2e) and Silymarin (Fig. 2f) exhibited reversal of these changes. Similarly, the normal structure of renal parenchyma is shown in Fig. 3a, where the hepatocytes (Fig. 3b) showed vacuolization of endothelial lining of glomerular tuft. Treatment with astragaloside-IV 25 mg $kg^{-1}$ (Fig. 3c), 50 mg $kg^{-1}$ (Fig. 3d) and 75 mg $kg^{-1}$ (Fig. 3e) and silymarin (Fig. 3f) exhibited reversal of these changes.

## DISCUSSION

Compound 1 was identified as astragaloside-IV by comparing its $^1$H NMR (Table 1) and $^{13}$C NMR (Table 2) spectra with those reported for 20,24-epoxy-3β-(β-D-xylopyranosyl)-oxy-6α-(3-β-D-xylopyranosyl)-oxy-9,19-cycloartane-16 β, 25 β-diol (astragaloside-IV). Compound 2 was identified as cyclosephaloside-I by comparing its $^1$H NMR and $^{13}$C NMR (Table 3 and 4) spectra with those reported for 20,25-epoxy-3β-(β-D-xylopyranosyl)-oxy-6α-(3-β-D-xylopyranosyl)-oxy-9,19-cycloartane-16β,25α-diol (cyclosephaloside-I) as well as through COSY, HSQC and HMBC experiments (Peng *et al*., 2008).

Both compounds belong to cycloartane-type triterpene, a common metabolites isolated from the genus *Astragalus* (Bedir *et al*., 1998). The $^{13}$C spectrum contained 48 signals; 30 of them are attributed to the aglycon moiety and the rest of the signals are assigned to the sugar moiety.

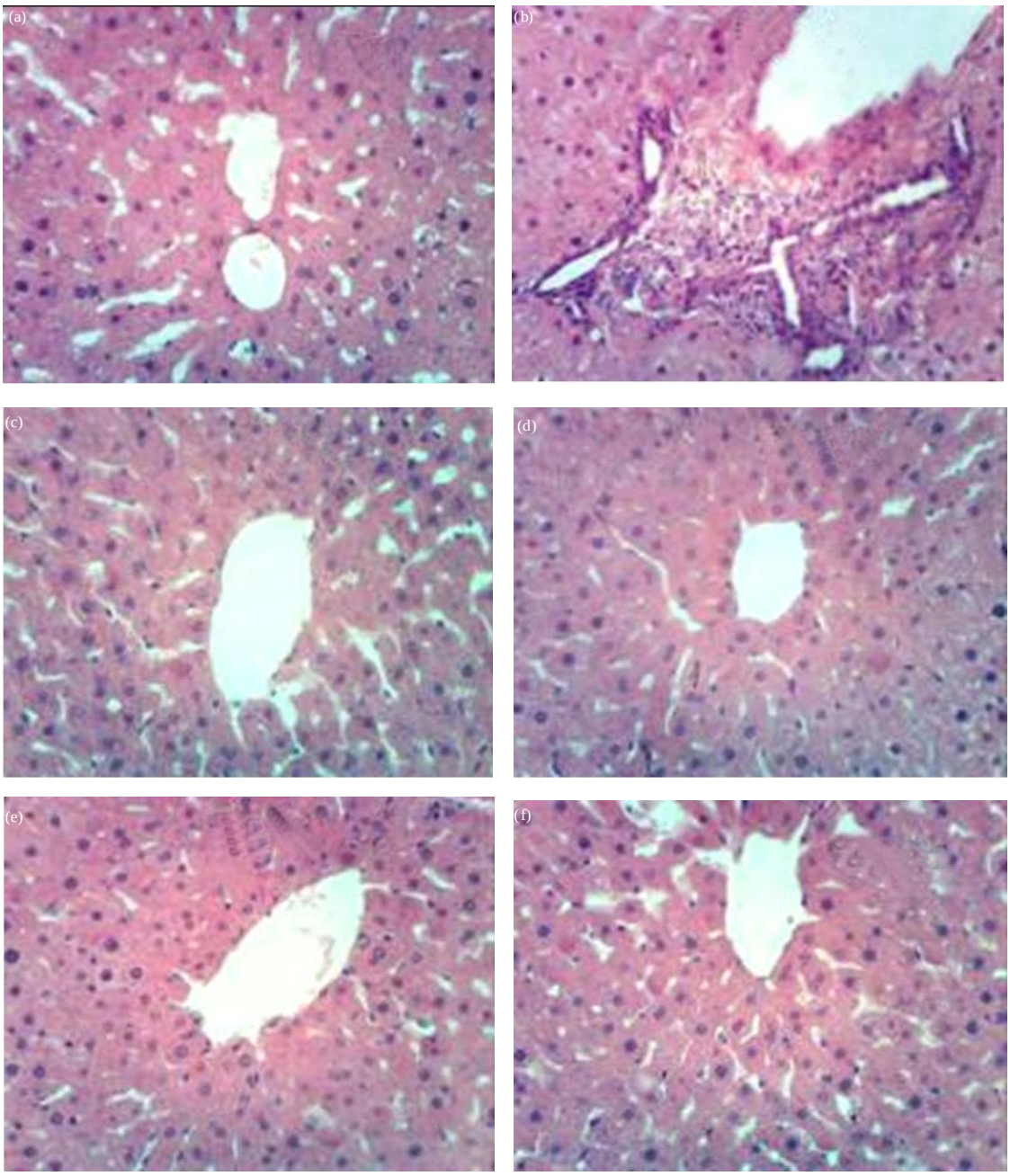


Fig. 2(a-f): Histopathological section of liver tissues (H and E×400), (a) Section of normal (Group-1) rat, showing the normal histological structure of hepatic lobule, (b) Section of $CCl_4$ induced renal toxicity (Group-II) showing portal infiltration with massive mononuclear cells, (c) Astragaloside-IV (25 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-III) showing no histopathological changes, (d) Astragaloside-IV (50 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-IV) showing binuclear in hepatocytes, (e) Astragaloside-IV (75 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-V) showing slight activation of kupffer cells and (f) Silymarin (100 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-VI) showing slight activation of kupffer cells

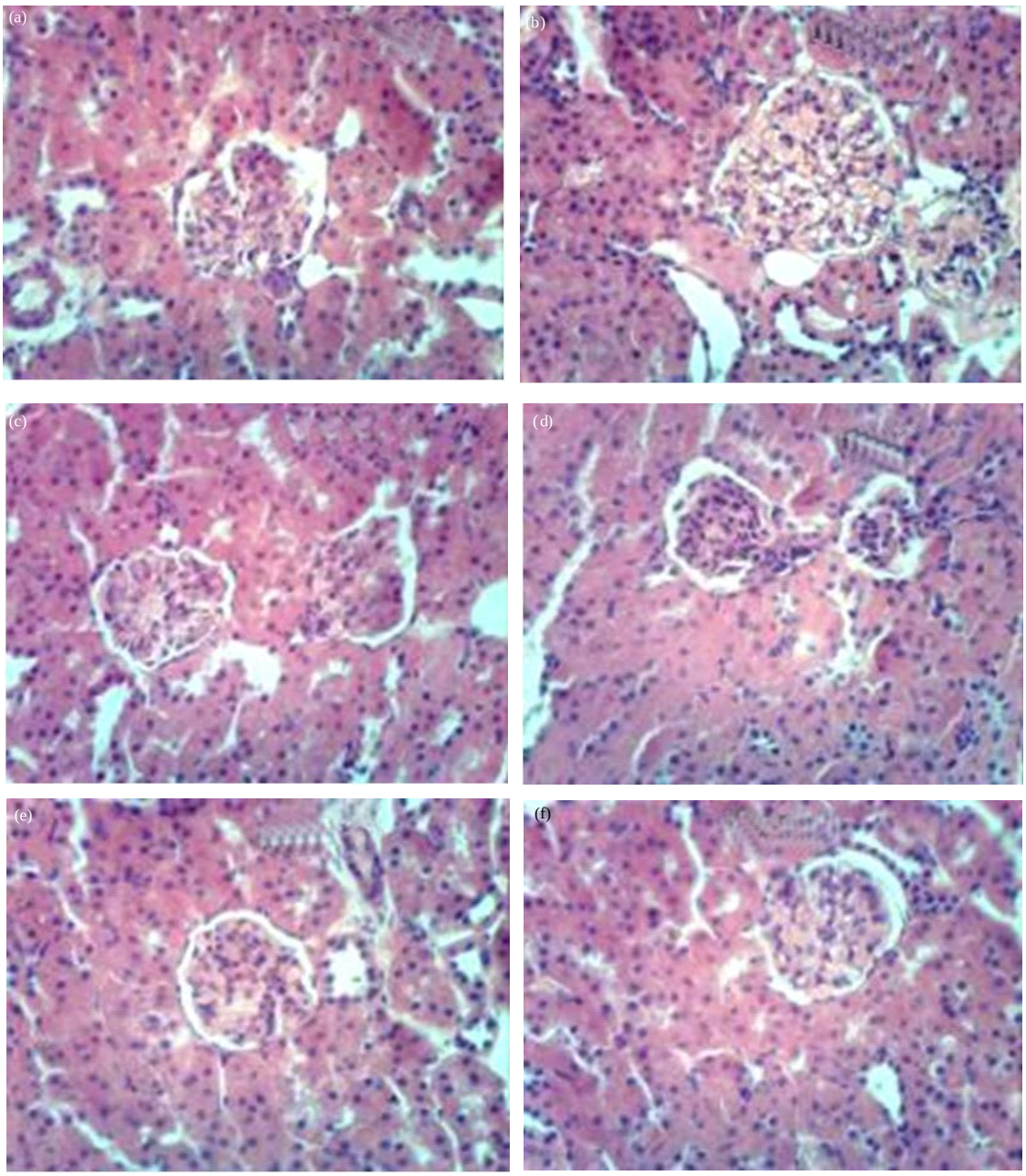


Fig. 3(a-f): Histopathological section of renal tissues (H and E×400), (a) Section of normal (Group-1) rat, showing the normal histological structure of renal parenchyma, (b) Section of $CCl_4$ induced renal toxicity (Group-II) showing vacuolization of endothelial lining of glomerular tuft, (c) Astragaloside-IV (25 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-III) showing slight vacuolization of endothelial lining glomerular tufts, (d) Astragaloside-IV (50 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-IV) showing no histopathological changes, (e) Astragaloside-IV (75 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-V) showing no histopathological changes and (f) Silymarin (100 mg $kg^{-1}$ b.wt.)+$CCl_4$ (Group-VI) showing normal renal parenchyma

The aglycon signals were in full agreement with those reported for cycloastragenol (Kitagawa *et al*., 1983). The hepatic injury induced by carbon tetrachloride is commonly used as an experimental method for the evaluation of hepatoprotective drugs (Zimmerman, 1964). Generally, the extent of hepatic damage is assessed by histopathological evaluation and the level of cytoplasmic enzymes released into the circulation.

ALT and AST are the most sensitive markers of hepatocellular injury and their elevation in serum is indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Yang *et al*., 2012). The ALP is a membrane bound enzyme involved in active transport across the capillary wall. The increased level of ALP is, also, a reliable marker of liver damage (Muriel and Escobar, 2003). The GGT is important in transport of amino acids required for the synthesis of GSH in cells (Sweiry *et al*., 1995). Bilirubin is an important degradation product of hemoglobin and is normally excreted into the bile. Increase in total serum bilirubin concentration after $CCl_4$ administration might be attributed to the failure of normal uptake, conjugation and excretion by the damaged hepatic parenchyma. The level of bilirubin is related to the function of hepatic cell. A high concentration of bilirubin in serum is an indication for erythrocytes degradation rate caused due to liver injury when treated with hepatotoxin (Singh *et al*., 1998). Exposure of hepatocytes to $CCl_4$ results in a significant increase in the leakage of LDH enzyme leakage (El-Tawil and Moussa, 2006). Treatment of hepatocytes with astragaloside-IV or silymarin ameliorated the effects of $CCl_4$ on LDH enzyme leakage. Some studies support nephrotoxicity that can be generated by $CCl_4$ (Al-Sowayan and Mousa, 2014). Administration of $CCl_4$ causes nephrotoxicity as indicated by elevation in urine and serum level of urea, creatinine and urobilinogen while it decreased the creatinine clearance. These pathological changes signify the potential damage to liver and kidney cells induced with $CCl_4$ treatment (Ogeturk *et al*., 2005). From the present study it is evident that elevation in plasma urea and creatinine levels can be attributed to the damage of nephron structural integrity (Khan and Siddique, 2012). It is well documented $CCl_4$ increased creatinine liver and kidney tissues (Simko *et al*., 1992). The increase in serum creatinine after $CCl_4$ treatment suggested the abnormal renal function in cirrhotic rats (Natarajan *et al.,* 2006). It is well documented that uric acid in blood is the most important antioxidant and impairment in kidney function that can result from oxidative stress (Khan *et al*., 2009). The nutritional homeostasis controlled by liver abnormalities in nutrient metabolism caused various liver diseases (Bavdekar *et al*., 2002). To check for renal function, we looked at levels of creatinine, urea, calcium, sodium and potassium in the rat serum. The hypothesis that calcium chelation may be beneficial in $CCl_4$-induced necrogenesis. Various studies proved that it partially prevent the necrogenic process induces by $CCl_4$ and other hepatotoxin and increase of potassium in $CCl_4$ intoxication (Meral and Kanter, 2003). The hyperkalemia in patients with hepatic disease may be related to the reduction of K in urinary excretion and impaired K entry into cells (Milionis and Elisaf, 1999). The activity of serum lipid profile such as total cholesterol, triglycerides, LDL and VLDL were elevated and HDL lowered this indicated deterioration in hepatic function due to the damage caused by $CCl_4$ administration. Whereas treatment with astragaloside-IV significantly declined the effect of $CCl_4$ induced damage and it was evidenced by the decreased level of total cholesterol, triglycerides, LDL and VLDL and increased level of HDL. This may be explained on the basis that astragaloside-IV has strong antioxidant ability (Gui *et al*., 2012). The lowering serum total cholesterol (TC) and triglycerides (TG) levels with astragaloside-IV were also reported in another study (Lv *et al*., 2010). Decrease in total protein level reflecting the liver and kidney injury caused by $CCl_4$ (Al-Yahya *et al*., 2013). In the

present experiments, major decrease in total protein by $CCl_4$ reversed by treatment with astragaloside-IV. Non-protein sulfhydryl (NP-SH) level was measured in the liver as an indicator of oxidative stress (Hussein and Abdel-Gawad, 2010). The NP-SH concentration in the liver and kidney tissue, decreased significantly in the $CCl_4$-treated rats while astragaloside-IV administration prevented the decrement in NP-SH induced by $CCl_4$. astragaloside-IV contributed significantly to the intracellular antioxidant defense system by acting as a powerful consumer of singlet oxygen and hydroxyl radicals. Obviously, the reduced level of NP-SH groups is due to the involvement of reduced glutathione in rendering the harmless estradiol metabolites by the formation of conjugates. The present study revealed that $CCl_4$ treatment in rats markedly changed the activity of antioxidant enzymes, which was reverted by the co-administration of astragaloside-IV. Thiobarbituric Acid Reactive Substances (TBARS), the final metabolites of peroxidized polyunsaturated fatty acids, are considered as a late biomarker of oxidative stress. In present experiments, major decrease in lipid peroxidation and consequent reduction in TBARS were obtained by treatment with astragaloside-IV. The increment in lipid peroxidation, as assessed by the elevated levels of TBARS following $CCl_4$ administration, has been well documented (Khan *et al*., 2009).

Histopathological observations after $CCl_4$-administration showed severe damage in liver and kidney. The prevention of liver and kidney cells texture account protective nature of astragaloside-IV in hepatotoxic conditions.

## CONCLUSION

The protective nature of astragaloside-IV was probably due to protection of marker enzymes, lipid and protein, antioxidative and histological texture of both kidney and liver tissue. These parameters account for the protective nature of astragaloside-IV in various liver and kidney ailments.

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