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# Hepatoprotective and nephroprotective activities of *Juniperus sabina* L. aerial parts

[Actividades hepatoprotectora y nefroprotectora de las partes aéreas de *Juniperus sabina* L.]

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**Abstract**

*Context*: Previous studies indicated that *Juniperus* species exhibited promising hepatoprotective activity.

*Aims*: To evaluate the hepatoprotective and nephroprotective activity of the total alcohol extract of the aerial parts of *Juniperus sabina* against carbon tetrachloride ($CCl_4$) induced toxicity using male Wistar rats as experimental animals.

*Methods*: Daily oral administration of two doses (200 and 400 mg/kg) of the total extract of the aerial parts of *J. sabina* for seven days followed by one dose of $CCl_4$ (1.25 mL/kg) at day 6. Liver and kidney functions were monitored via measuring serum and tissue parameters as well as histopathological study. Normal rats, rats treated only with $CCl_4$ and rats treated with $CCl_4$ and silymarin were used as controls.

*Results*: The higher dose showed 47, 50, 38, 17 and 42% decrease in the levels of AST, ALT, GGT, ALP, and bilirubin respectively. Animals received the total extract of *J. sabina* showed a significant dose-dependent recovery of the NP-SH contents, total proteins, and reduction the level of MDA in both liver and kidney tissues. Histopathological study revealed improvement in the architecture of hepatocytes and kidney cells.

*Conclusions*: The hepatoprotective effect offered by *J. sabina* crude extract at the two used doses was found to be significant in all serum parameters. Histopathological study revealed moderate improvement in the architecture of the liver cells that add another indication of protection. Improvement of kidney function was less than liver function.

*Keywords*: biochemical parameters; carbon tetrachloride; histopathology; *Juniperus sabina*; silymarin.

**Resumen**

*Contexto*: Estudios previos indicaron que las especies de *Juniperus* exhibieron actividad hepatoprotectora prometedora.

*Objetivos*: Evaluar la actividad hepatoprotectora y nefroprotectora del extracto alcohólico total de las partes aéreas de *Juniperus sabina* contra la toxicidad inducida por tetracloruro de carbono ($CCl_4$) utilizando ratas Wistar machos como animales de experimentación.

*Métodos*: Administración oral diaria de dos dosis (200 y 400 mg/kg) del extracto total de las partes aéreas de *J. sabina* durante siete días, seguido de una dosis de $CCl_4$ (1,25 mL/kg) en el día 6. Las funciones hepática y renal fueron controladas mediante la medición de los parámetros séricos y tisulares, así como el estudio histopatológico. Las ratas normales, las ratas tratadas solamente con $CCl_4$ y ratas tratadas con $CCl_4$ y silimarina se utilizaron como controles.

*Resultados*: La dosis más alta mostró 47, 50, 38, 17 y 42% de disminución en los niveles de AST, ALT, GGT, ALP y bilirrubina respectivamente. Los animales que recibieron el extracto total de *J. sabina* mostraron una recuperación significativa, dependiente de la dosis, de los contenidos NP-SH, proteínas totales y la reducción del nivel de MDA en tejidos de hígado y riñón. El estudio histopatológico reveló una mejora en la arquitectura de los hepatocitos y las células del riñón.

*Conclusiones*: El efecto hepatoprotector del extracto crudo de *J. sabina* a las dos dosis ensayadas fueron encontradas significativas en todos los parámetros de suero. El estudio histopatológico reveló una mejora moderada en la arquitectura de las células del hígado que añadieron otra indicación de protección. La mejoría de la función renal fue menor que la función hepática.

*Palabras Clave*: histopatología; *Juniperus sabina*; parámetros bioquímicos; silymarin; tetracloruro de carbono.

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## INTRODUCTION

The rate of liver diseases is steadily increasing over the years. They are ranked as the fifth cause of death according to National Statistics in the UK (UK National Statistics, 2013). Among all the digestive diseases, the second leading cause of mortality is the liver diseases in the US (Everhart and Ruhl, 2009). A study among patient suffering from liver diseases in Saudi Arabia showed that 31.8% of them used herbs in addition to the physician-prescribed drugs (Al-Zahim et al., 2013).

*Juniperus* species are presented by 50-67 members widely distributed in the Northern Hemisphere (Hampe and Petit, 2010). *Juniperus* are evergreen with a needle or scale-like leaves. They vary from shrubs ranging from low, ground-hugging, medium and large, pyramidal forms up to 20-40 m tall trees (Ogren, 2015).

Previous studies of *Juniperus* species resulted in the isolation of diterpenes some with cytotoxic activity, sesquiterpenoids, lignans, phenylpropanoid, flavonoids, coumarins (Seca et al., 2007; Seca and Silva, 2007; 2010; Moujir et al., 2008; 2011; Chang et al., 2008; Iida et al., 2010; Woo et al., 2011; Inatomi et al., 2013).

Both *J. phoenicea* and *J. procera* were subjected to a detailed phytochemical study directed by hepatoprotective activity resulted in the identification of hinokiflavone, 4-epi-abietol, sugiol as the most active components (Alqasoumi and Abdel-Kader, 2012; Alqasoumi et al., 2013). The hot water extract of *J. chinensis* showed promising anti-obesity effect (Kim et al., 2008). Other reported biological activities for *Juniperus* species include antimicrobial, anti-inflammatory, hypoglycemic, diuretic and lipoxygenase inhibitor activities (Seca et al., 2007).

*J. sabina* described in folk medicine as abortifacient, diuretic, emetic, emmenagogue and irritant (Lust, 1982; Chiej, 1984). Essential oil of the plant reported possessing antimicrobial activity correlated to terpene contents (Akimov et al., 1977). Chemical study of *J. sabina* resulted in the isolation of α-cedrol, coumasabin, isocupressic acid, skimmin, undulatoside A, hypolaetin 7-O-β-D-xylopyranoside and quercetin 3-O-α-L-rhamnoside, syringin (Zhao et al., 2008), junaphtoic acid, (-)3-O-demethylyatein (San Feliciano et al., 1991), siderin, coumarsabin and 8-methoxy coumarsabin (De Pascual et al., 1981).

Literature data lacks any study for the hepatoprotective and nephroprotective effects of *J. sabina* a plant known in Saudi Arabia as "Al Abhal". The aim of the current study was the evaluation of the hepatoprotective and nephroprotective potentials of *J. sabina* extract.

## MATERIAL AND METHODS

### Chemicals

Silymarin, ethylenediamine tetraacetic acid (EDTA), Folin reagent, Mayer hematoxylin, eosinphloxine, trichloroacetic acid, 5, 5`-dithio-bis-(2-nitrobenzoic acid), 2-thiobarbituric acid and paraffin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used were of analytical grade.

### Plant material

Aerial parts of *Juniperus sabina* L. (Cupressaceae) were purchased from the local market in Riyadh, Saudi Arabia. Plant material was identified by the taxonomist Dr. Mohammad Atiqur Rahman, MAPPRC, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#18364) was kept at the herbarium of this center.

### Extraction

The dried ground aerial parts (1000 g) were extracted to exhaustion by percolation with 95% ethanol (10 L) at room temperature. The extract was evaporated *in vacuo* to leave dark green viscous residue with an aromatic odor.

### Animals

Male Wistar albino rats (150-200 g) of approximately the same age (8-10 weeks) were acquired from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh. Animals were kept under controlled conditions: temperature (22 ± 2°C), humidity (55%) and light/dark conditions (12/12 h) and provided with Purina chow (LabDiet, St. Louis, USA) with free access to drinking water *ad libitum* (Abdel-Kader et al., 2009). The experiment and procedures used in this study were approved in a single protocol by the Ethical Com-

mittee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University.

### Hepatoprotective and nephroprotective activities

Male Wistar rats were divided into five groups' five animals each. Group I received normal saline (1 mL, p.o.) and was kept as a control group. Groups II-V received a single dose of $CCl_4$ (1.25 mL/kg body weight). The negative control group II received $CCl_4$ only. The positive control group III was administered silymarin at a dose of 10 mg/kg p.o. (20.7 µmol/kg). Groups IV-V were treated with 200 and 400 mg/kg of the total extract of *J. sabina* aerial parts. Treatment started five days before $CCl_4$ administration and continued until day six. After 24 h, following $CCl_4$ administration in day seven the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated for evaluating the biochemical parameters.

### Determination of liver and kidney serum parameters

The biochemical serum parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin were estimated by reported methods (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron®, Roche, Basel, Switzerland) and were read using Reflotron® Plus instrument (Roche, Basel, Switzerland). Serum creatinine and blood urea were determined using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) applying the reported method (Varley and Alan, 1984). Creatinine kinase and uric acid were estimated by Reflotron, Roche kit and LDH was estimated using Human diagnostic kit with UV-VIS Spectrophotometer (Shimadzu, Japan) (Merdes et al., 1985; Braun et al., 1987). Potassium concentration was determined using diagnostic strips (Reflotron®, ROCHE). Sodium was estimated by photometric determination using Mg-uranyl acetate method (Henry et al., 1974). Calcium was determined colorimetry using cresol phthalein method (Moorehead and Biggs, 1974).

### Determination of tissue parameters

The livers and kidneys samples were separately cooled in ice bath. The tissues were homogenized in 0.02 M EDTA in a Potter-Elvehjem type C homogenizer (Sigma-Aldrich, St. Louis, MO, USA). Homogenate equivalent to 100 mg tissues was used for the measurements.

For the determination of non-protein sulfhydryl groups (NP-SH) tissue homogenate was mixed with 4 mL distilled water and 1 mL of 50% trichloroacetic acid (TCA). The mixtures were shaken intermittently for 10-15 min followed by centrifugation for 15 min at 3000 rpm. Two mL of the supernatant were mixed well with 0.1 mL of 0.01 *M* DTNB [5, 5`-dithio-bis-(2-nitrobenzoic acid)] and 4 mL of 0.4 *M* Tris buffer pH 8.9 and measured spectrophotometrically at 412 nm (Sedlak and Lindsay, 1968).

For the level of MDA, aliquots of homogenate were incubated at 37°C for 3 h in a metabolic shaker and then mixed with 1 mL of 10% aqueous TCA and centrifuged at 800 rpm for 10 min. One milliliter of the supernatant was mixed with 1 mL aqueous solution of 0.67% 2-thiobarbituric and placed in a boiling water bath for 10 min. After cooling the mixture was diluted with 1 mL distilled water and the absorbance was then measured at 535 nm. The content of MDA (nmol/g wet tissue) was then calculated from the standard curve of MDA solution (Utley et al., 1967).

For the TP determination, parts of the homogenate were treated with 0.7 mL of Lowry's solution, mixed and incubated for 20 min in the dark at room temperature. Diluted Folin's reagent (0.1 mL) were then added and samples were incubated at room temperature in the dark for 30 min. The absorbance of the resulted solutions was then measured at 750 nm (Lowry et al., 1951).

### Histopathology

The liver and kidney samples were fixed, placed in cassettes and mounted into automated vacuum tissue processor (ASP 300 S, Leica Biosystems, Nussloch, Germany). The samples were embedded and blocked in paraffin wax, and thin sections (3 µm) were made using microtome (TBS SHUR/cut 4500, Triangle Biomedical Sciences, Durham, North Carolina, USA). Sections were stained with Mayer's

hematoxylin solution and counterstained in eosin-phloxine solution (Prophet et al., 1994). Slides were examined using Slide Scanner (SCN 400 F Leica Microsystems, Wetzlar, Germany) for slide observation and imaging on the magnification of 10x and 20x objectives.

### Statistical analysis

Results are expressed as mean ± standard error of mean (SEM). Statistical analysis was performed, using one-way analysis of variance (ANOVA). When the F-value was found statistically significant ($p<0.05$), further comparisons among groups were made using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS software 17.0 (Released Aug. 23, 2008), Chicago, USA.

## RESULTS AND DISCUSSION

Carbon tetrachloride is converted in the endoplasmic reticulum into trichloromethyl free radical ($\cdot CCl_3$) and $\cdot Cl_3COO$ that by the aid of oxygen conjugated with essential cellular macromolecules, cellular lipids, and proteins to induce lipid peroxidation (Snyder and Andrews, 1996). Lipid peroxidation form reactive aldehydes that can form adducts with proteins (Weber et al., 2003). These reactions increase endoplasmic reticulum and other membranes permeability to $Ca^{2+}$ resulting in severe disturbances of calcium homeostasis and consequently necrotic cell death (Weber et al., 2003). Treatment of the animals with the hepatotoxic agent carbon tetrachloride resulted in significant increase of transaminases (AST and ALT) and alkaline phosphatase (ALP) levels due to hepatocytes damage (Zafar and Ali, 1998). Severe jaundice was reflected by the elevated levels of serum bilirubin (Lin et al., 1997) (Table 1).

The use of silymarin, at a dose of 10 mg/kg (20.7 μmol/kg) before the administration of $CCl_4$ resulted in a significant decrease ($p<0.001$) in the elevated AST, ALT, GGT, ALP and bilirubin levels in rats. (Table 1). Silymarin acts as an antioxidant by scavenging prooxidant free radicals and by increasing the intracellular concentration of GSH. It also enhances the cellular membrane permeability to protect against xenobiotics injury. It also promotes the synthesis of ribosomal RNA by stimulating DNA polymerase-I as well as steroid like action in regulating DNA transcription and stimulation of protein synthesis leading to regeneration of liver cells (Dehmlow et al., 1996; Saller et al., 2007).

Treatment of rats with the total extract of the aerial parts of *J. sabina* before the administration of $CCl_4$ resulted in a significant ($p<0.01$; 0.001) decrease in the elevated levels of AST, ALT, GGT, ALP, and bilirubin. The higher dose showed 47, 50, 38, 17 and 42% reduction in AST, ALT, GGT, ALP, and bilirubin respectively. The total extract of *J. sabina* at 400 mg/kg reduced the concentration of MDA to about half of that in the $CCl_4$ treated group (Table 2). The levels of NP-SH and Total protein were also significantly ($p<0.05$) restored to levels closer to those measured in silymarin treated group (Table 2). Histopathology of the liver of rats treated with *J. sabina* at 400 mg/kg showed some degree of protection especially the absence of central vein congestion (Fig. 1). The total extract of *J. sabina* was superior in lowering the levels of AST, ALT, and bilirubin when compared with *J. procera* and *J. phoenicea* previously studied in our group. Its effect on the elevated level of ALP was almost equal to that of *J. phoenicea* and higher than that of *J. procera* (Abdel-Kader et al., 2009; Alqasoumi et al., 2009). The effect of *J. phoenicea* on NP-SH was studied, and 74.3% increase in these groups were observed at 500 mg/kg (Alqasoumi et al., 2009). Treatment with *J. sabina* total extract at 400 mg/kg resulted in 77% increase in the NP-SH in comparison with the $CCl_4$ treated group.

In addition to regulation of ionic concentration in plasma such as sodium, potassium, calcium, magnesium, chloride the kidney also plays the major role in the removal of nitrogenous metabolic waste products such as urea, creatinine and uric acid (Pocock and Richards, 2006). Nephrotoxicity can be demonstrated via the increase in the levels of serum electrolytes, urea, and creatinine (Adelman et al., 1981).

The nephrotoxicity of $CCl_4$ is reflected as the elevation of the serum levels of LDH, creatinine-kinase, urea, uric acid, creatinine, sodium, potassium and calcium (Tables 3, 4). In kidney tissues, the level of MDA was increased, while NP-SH and total protein levels were decreased (Table 5). Silymarin treatment improved these disturbances with different degrees.

**Table 1.** Effect of *J. sabina* total extract (EJS) on serum marker enzymes of control and experimental rats.

| Treatment | Dose (mg/kg) | AST (IU/L) | % | ALT (IU/L) | % | GGT (IU/L) | % | ALP (IU/L) | % | Bilirubin (mg/dL) | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1 mL | 100.20 ± 6.89 | - | 30.07 ± 3.82 | - | 3.57 ± 0.26 | - | 363.75 ± 13.44 | - | 0.54 ± 0.01 | - |
| $CCl_4$ | 1.25 mL/kg | 314.75 ± 13.30***,a | - | 277.75 ± 8.91***,a | - | 10.72 ± 0.37***,a | - | 657.00 ± 21.06***,a | - | 2.57 ± 0.17***,a | - |
| Sil + $CCl_4$ | 10 | 136.00 ± 10.00***,b | 57 | 94.27 ± 6.10***,b | 66 | 5.82 ± 0.33***,b | 46 | 416.00 ± 8.72***,b | 37 | 0.89 ± 0.08***,b | 65 |
| EJS + $CCl_4$ | 200 | 201.25 ± 9.14***,b | 36 | 202.50 ± 5.26***,b | 27 | 8.27 ± 0.48**,b | 23 | 575.75 ± 12.36**,b | 12 | 1.81 ± 0.07**,b | 30 |
| EJS + $CCl_4$ | 400 | 166.75 ± 6.40***,b | 47 | 138.25 ± 4.87***,b | 50 | 6.65 ± 0.26***,b | 38 | 545.75 ± 11.98***,b | 17 | 1.50 ± 0.05***,b | 42 |

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

[a]As compared with Control group (administered with a solution of 0.9% sodium chloride). [b]As compared with $CCl_4$ only group.

Sil: Silymarin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltranspeptidase; ALP: alkaline phosphatase.

% Represents % of change respect to $CCl_4$ group.

**Table 2.** Effect of *J. sabina* total extract (EJS) on MDA, NP-SH and total protein in liver tissue of control and experimental rats.

| Treatment | Dose mg/kg | MDA (nmol/g) | NP-SH (nmol/g) | Total protein (g/L) |
|---|---|---|---|---|
| Control | 1 mL | 0.40 ± 0.03 | 7.78 ± 0.66 | 112.57 ± 4.68 |
| $CCl_4$ | 1.25 mL/kg | 4.26 ± 0.58*** | 3.90 ± 0.39*** | 60.47 ± 4.62*** |
| Sil + $CCl_4$ | 10 | 1.09 ± 0.07***,b | 6.71 ± 0.67** | 88.62 ± 4.68**,b |
| EJS + $CCl_4$ | 200 | 2.92 ± 0.19*,b | 4.46 ± 0.70b | 70.65 ± 3.16b |
| EJS + $CCl_4$ | 400 | 2.12 ± 0.16**,b | 5.06 ± 0.18*,b | 80.23 ± 5.31*,b |

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

[a]As compared with Control group (administered with a solution of 0.9% sodium chloride). [b]As compared with $CCl_4$ only group.

NP-SH: Non-protein sulfhydryl groups; MDA: Malondialdehyde; Sil: Silymarin.

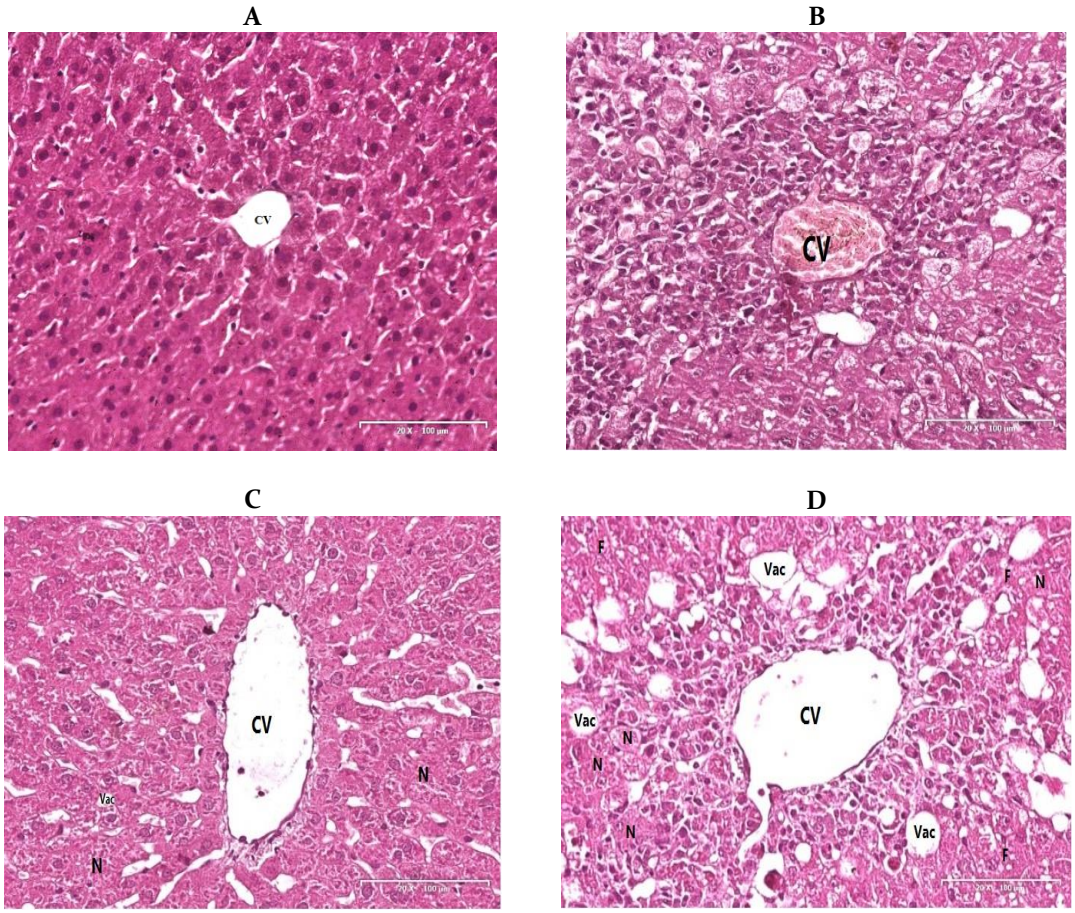



**Figure 1.** Histopathological appearance of liver cells.

**(A)** normal cells; **(B)** Liver cells of rats treated with $CCl_4$ showing multiple areas of necrosis, severe vacuolization of hepatocytes, fatty changes, parenchymal architecture disruption, deposition of collagen fibers, decrease in glycogen content, dilatation of sinusoids, central vein congestion and inflammatory infiltration. **(C)** Liver cells of rats treated with $CCl_4$ and silymarin showing slight necrosis and vacuolization of hepatocytes, portal triage with slight aggregation of nuclei towards endothelial of portal vein. **(D)** Liver cells of rats treated with $CCl_4$ and 400 mg/kg body weight of *J. sabina* total extract with multiple areas of necrosis, vacuolization of hepatocytes, fatty changes and absence of central vein congestion. Magnification 20x, 100 µm.

CV= Central vein; N= Necrosis: dead cells or group of cells within living tissue; Vac= Vacuolization: large empty space within the tissue; F= Fatty change: accumulation of fat in form of small droplets within cells.

**Table 3.** Effect of *J. sabina* total extract (EJS) on LDH, creatinine-kinase, urea, uric acid and creatinine of control and experimental rats.

| Treatment | Dose mg/kg | LDH (IU/L) | % | Creatinine-Kinase (IU/L) | % | Urea (nmol/L) | % | Uric acid (mg/dL) | % | Creatinine (mg/dL) | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1 mL | 121.25 ± 9.69 | - | 164.00 ± 7.22 | - | 37.60 ± 1.77 | - | 1.95 ± 0.14 | - | 0.98 ± 0.06 | - |
| $CCl_4$ | 1.25 mL/kg | 207.25 ± 9.15***,a | - | 275.00 ± 11.94***,a | - | 189.50 ± 7.68***,a | - | 6.32 ± 0.27***,a | - | 3.82 ± 0.18***,a | - |
| Sil+ $CCl_4$ | 10 | 135.00 ± 13.54***,b | 35 | 211.25 ± 6.30***,b | 23 | 119.65 ± 9.48***,b | 37 | 2.42 ± 0.26***,b | 62 | 1.79 ± 0.13***,b | 53 |
| EJS+ $CCl_4$ | 200 | 182.50 ± 10.62b | 12 | 221.25 ± 6.32**,b | 20 | 187.75 ± 6.01b | - | 6.17 ± 0.09b | 2 | 3.8 ± 0.20b | - |
| EJS+ $CCl_4$ | 400 | 153.75 ± 4.98***,b | 26 | 215.75 ± 9.33**,b | 22 | 158.25 ± 3.03**,b | 16 | 5.69 ± 0.24b | 9 | 2.52 ± 0.18***,b | 34 |

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

[a]As compared with Control group (administered with a solution of 0.9% sodium chloride). [b] As compared with $CCl_4$ only group

% Represents % of change respect to $CCl_4$ group.

Sil: Silymarin; LDH: lactate dehydrogenase.

**Table 4.** Effect of *J. sabina* total extract (EJS) on sodium, potassium and calcium of control and experimental rats.

| Treatment | Dose mg/kg | Sodium (mEq/L) | % | Potassium (mEq/L) | % | Calcium (mg/dL) | % |
|---|---|---|---|---|---|---|---|
| Control | 1 mL | 98.15 ± 3.53 | - | 4.40 ± 0.21 | - | 4.53 ± 0.34 | - |
| $CCl_4$ | 1.25 mL/kg | 158.39 ± 1.75***,a | - | 13.45 ± 1.09***,a | - | 23.61 ± 1.35***,a | - |
| Sil+ $CCl_4$ | 10 | 129.43 ± 1.73***,b | 18 | 6.45 ± 0.25***,b | 52 | 13.69 ± 1.55**,b | 42 |
| EJS+ $CCl_4$ | 200 | 141.10 ± 1.15***,b | 11 | 9.10 ± 0.36**,b | 32 | 25.00 ± 0.10b | 6 |
| EJS+ $CCl_4$ | 400 | 120.24 ± 3.17***,b | 24 | 8.42 ± 0.25***,b | 37 | 16.84 ± 1.09**,b | 29 |

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

[a]As compared with Control group (administered with a solution of 0.9% sodium chloride). [b]As compared with $CCl_4$ only group.

% Represents % of change respect to $CCl_4$ group.

Sil: Silymarin.

**Table 5.** Effect of *J. sabina* total extract (EJS) on MDA, NP-SH and total protein in kidney tissue of control and experimental rats.

| Treatment | Dose mg/kg | MDA (nmol/g) | NP-SH (nmol/g) | Total protein (g/L) |
|---|---|---|---|---|
| Control | 1 mL | 0.29 ± 0.01 | 6.46 ± 0.25 | 141.91 ± 5.32 |
| $CCl_4$ | 1.25 mL/kg | 4.42 ± 0.28***,a | 3.91 ± 0.19***,a | 64.07 ± 4.82***,a |
| Sil + $CCl_4$ | 10 | 1.24 ± 0.02***,b | 6.41 ± 0.44***,b | 104.19 ± 4.42***,b |
| EJS + $CCl_4$ | 200 | 2.82 ± 0.19***,b | 4.91 ± 0.27*,b | 76.04 ± 3.57b |
| EJS + $CCl_4$ | 400 | 1.93 ± 0.13***,b | 5.09 ± 0.30**,b | 94.01 ± 3.29***,b |

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. [a]As compared with Control group (administered with a solution of 0.9% sodium chloride). [b]As compared with $CCl_4$ only group. NP-SH: Non-protein sulfhydryl groups; MDA: Malondialdehyde; Sil: Silymarin.

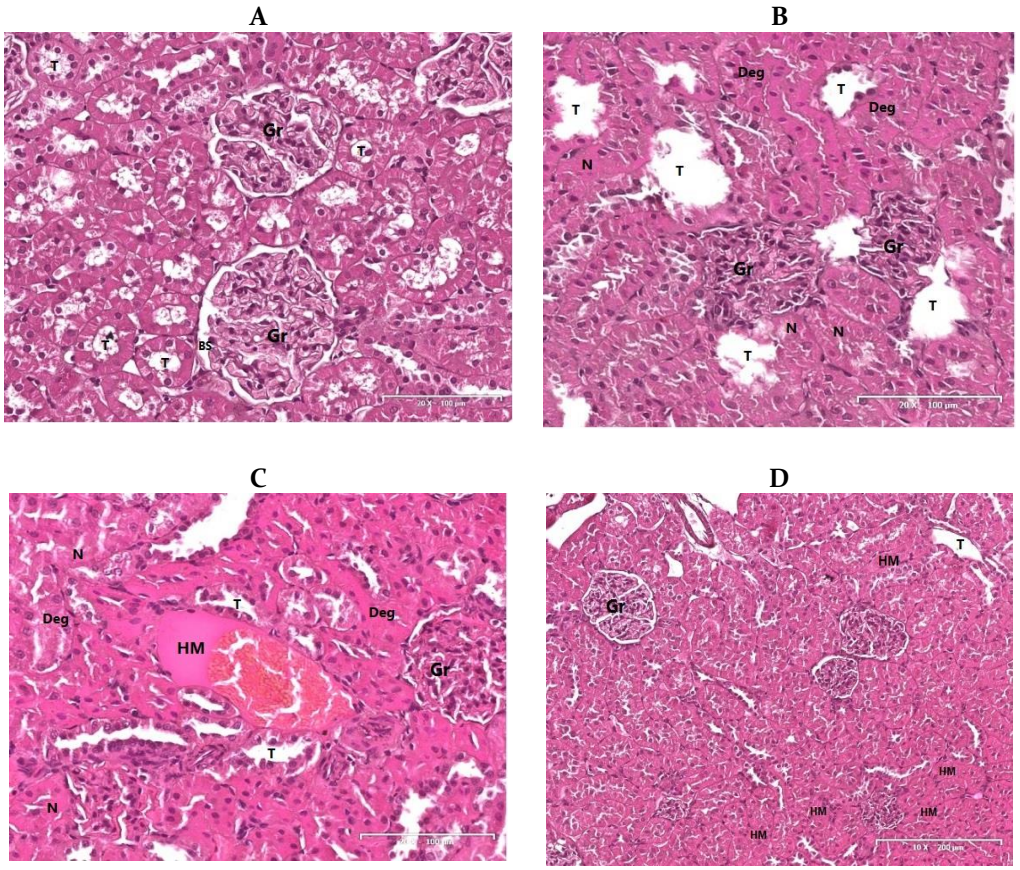



**Figure 2.** Histopathological appearance of kidney cells.

**(A)** normal cells; **(B)** kidney cells of rats treated with $CCl_4$ showing abnormal glomeruli reduced in size with the almost absence of Bowman space, degenerated and convoluted necrotic tubules, abnormal medulla structure with inflammatory infiltrate, abnormal calyx structure obstructed by hyaline and granulated materials. **(C)** Kidney cells of rats treated with $CCl_4$ and silymarin showing glomerulus architecture disruption, reduced in size, reduced Bowman space, degeneration, and necrosis in both glomerulus and tubules, congestion and accumulation of hyaline material in the cortex and presence of inflammatory infiltration. **(D)** Kidney cells of rats treated with $CCl_4$ and 400 mg/kg body weight of *J. sabina* total extract with almost normal corpuscle with few glomeruli having narrow Bowman space, mild degeneration in few tubule cells, few obstructed tubules by hyaline material and deposition of collagen fibers, almost normal calyx with a mild presence of the hyaline material. Magnification 10x, 100 (A-C) or 20x, 200 µm (D).

Gr= Glomerulus; T= Tubule; BS= Bowman space; HM= Hyaline material; Deg= Degeneration; N= Necrosis

Treatment with *J. sabina* total extract showed a dose-dependent, highly significant ($p<0.01$; 0.001) reduction in creatinine-kinase, LDH and creatinine levels (22, 26 and 34%, respectively) (Table 3). Improvement in the levels of sodium, potassium, and calcium was also observed (Table 3). The level of sodium was improved better than the silymarin treated group (Table 4). The level of MDA was reduced significantly ($p<0.001$) to 2.82 ± 0.19 and 1.93 ± 0.13 nmol/g with the use of 200 and 400 mg/kg extract, respectively. Restoring the levels of NP-SH and TP were statistically valid ($p<0.01$; 0.001) and comparable to that of silymarin treated group (Table 4).

Histopathological study revealed the normal renal architecture in control group (Fig. 2-A) while (Fig. 2-B) showed dramatic histopathological changes due to treated with $CCl_4$. Kidney cells of rats treated with $CCl_4$ and *J. sabina* 400 mg/kg showed few signs of toxicity reflected by mild degeneration and obstruction of tubules. Corpuscles and calyx were almost normal in shape (Fig. 2-D).

Diterpene acids, sesquiterpene alcohols, naphthalene derivatives, lignans, flavonoids, and coumarins were reported from *J. sabina* (De Pascual et al., 1981; Barrero et al., 1987; San Feliciano et al., 1991; Zhao et al., 2008). Biologically directed phytochemical study of the total extract is in process aiming to locate the compounds responsible for the activity.

## CONCLUSIONS

The total extract of the aerial parts of *Juniperus sabina* showed as other *Juniperus* species promising hepatoprotective activity against $CCl_4$ induced toxicity in experimental rats. Protection of the liver was measured via serum parameters (AST, ALT, ALP, GGT, and bilirubin), tissue parameters (MDA, NP-SH, and TP) and histopathology of liver section. A moderate degree of protection was also offered on kidney functions. The higher doses of the extract exceeded silymarin in improving sodium level. Further study of the extract fractions and components is highly recommended.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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|---|---|---|---|---|---|
| Concepts or Ideas | X | X | X | X | |
| Design | X | | X | X | |
| Definition of intellectual content | X | | | | |
| Literature search | X | X | | | |
| Experimental studies | X | X | X | X | X |
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