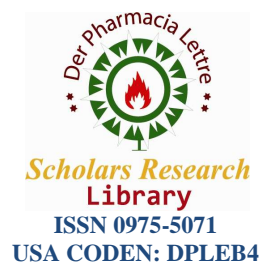




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Evaluation of hepatoprotective effect of Algerian *Santolina chamaecyparissus* against acute exposure to paracetamol

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ABSTRACT

The aim of this study was to evaluate the effect of *Santolina chamaecyparissus* ethanol (SCE) and aqueous (SCA) extracts against paracetamol-induced liver damage in male rats. In this study, SCA and SCE (30, 150 or 300 mg/kg body weight) was administered daily for 7 days in experimental animals. Liver injury was induced chemically, by paracetamol administration (3 g/kg b.w.). The hepatoprotective activity was assessed using biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and histopathological study. Furthermore, polyphenols and flavonoids content was determined by colorimetric methods. Obtained results demonstrated that the treatment with SCA and SCE significantly ($P < 0.01$) prevented chemically induced increase in serum levels of hepatic enzymes. The inhibitory effect was close to that of silymarin 100 mg/kg, used as standard. Histopathological examination showed that SCE and SCA protected against hepatocytic necrosis. Phytochemical analysis revealed that SCE and SCA are rich in polyphenols and flavonoids. The present study revealed that *S. chamaecyparissus* exhibits hepatoprotective activity and it can constitute a promising natural source to develop novel therapeutic drugs for treating liver disorders.

Key words: hepatotoxicity, liver damage, paracetamol, flavonoids, polyphenols

INTRODUCTION

Liver damage is always associated with cellular necrosis, increase serum levels of many biochemical markers like serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, triglycerides, cholesterol, bilirubin, alkaline phosphatase [1].

Paracetamol (acetaminophen) is one of the most widely used drugs, due to its analgesic and antipyretic properties. It is converted by cytochrome P-450 to a high reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) which is responsible for paracetamol-induced hepatotoxicity. Detoxification of NAPQI occurs through its binding to the sulfhydryl group of glutathione (GSH) to form paracetamol-GSH, which is ultimately excreted in the urine as cysteine and mercapturic acid conjugates (acetaminophen-cys)[2]. At therapeutic doses, paracetamol is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, oxidation of proteins, lipid peroxidation, mitochondrial dysfunction and centrilobular necrosis and even death in humans and experimental animals when taken in overdose [3].

Hepatic cells are involved in a variety of metabolic events; therefore the establishment of liver protective/therapeutic agents is of paramount importance in the protection from liver damage. Recently, natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Several studies have been reported the

hepatoprotective effect of medicinal plants against damage induced by paracetamol. This hepatoprotective effect is assigned to the content of medicinal plants in polyphenols and flavonoids [2,4].

Santolina chamaecyparissus L. (*S. chamaecyparissus*) belongs to Asteraceae family is an aromatic plant, small evergreen shrub growing to 50 cm tall and board, widespread in the Mediterranean region. Most commonly, the flowers and leaves are made as a decoction and used to expel intestinal parasites. Aerial part of *S. chamaecyparissus* has antioxidant and anti-inflammatory properties [5]. Flowers are also used for their analgesic, antispasmodic, bactericidal and digestive and vulnerary properties [6,7]. In herbal medicine, *S. chamaecyparissus* is used to treat different types of dermatitis [6]. The essential oil from the aerial parts of this plant has antifungal properties and is used in perfumery and cosmetics. Phytochemical studies of *S. chamaecyparissus* yielding a number of secondary metabolites such as essential oils [8], flavonoids [9] and coumarins [10]. A single article that treated this subject briefly [11]. Therefore the purpose of our work was to evaluate in more detail the effectiveness of aqueous and ethanol extracts of the aerial part of Algerian *S. chamaecyparissus* as a hepatoprotector in experimental liver damage induced by paracetamol in rats.

MATERIALS AND METHODS

Chemicals

Gallic acid, quercetin, folin Ciocalteu reagent, sodium carbonate (Na_2CO_3), were purchased from Sigma (Germany). Paracetamol (PCM) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) were purchased from Roche (France). All other chemicals are from Sigma and were of analytical grade.

Plant material

Santolina chamaecyparissus was harvested during the flowering season in mid-May 2012, from Hammam Essoukhna, Sétif region in east of Algeria. The plant was identified, authenticated taxonomically by Pr. H. Laouer (Laboratory of Botany, University of Setif 1, Algeria) and a voucher specimen (No. S.c. 2009-1) was preserved in the local Herbarium of Botany, Department of Botany, University of Sétif for future reference. The aerial part was air dried at room temperature and then reduced to powder.

Extraction

Santolina chamaecyparissus ethanol and aqueous extract (SCE, SCA) was prepared according to Messaoudi et al [5]. Briefly, *Santolina chamaecyparissus* ethanol extract (SCE) was prepared by maceration of 100 g of the powdered aerial part of the plant with 80% ethanol for 24h, under continuous shaking at room temperature. After filtration, the filtrate was concentrated under reduced pressure at 40°C and the residue was lyophilized to give a bright brown powder (yield: 15.07%).

Santolina chamaecyparissus aqueous extract (SCA) was prepared according to the traditional method. Briefly, 100g of powder of the aerial part of the plant is boiled in 1 L of distilled water (1/10: W/V) for 20 min. After filtration, the filtrate collected undergoes centrifugation (3000 rpm) for 10 min. The supernatant obtained was lyophilized to give a pale brown powder (yield: 15.77%). Extracts was stored at -32 °C until use.

Animals

Adult male Wistar albinos rats weighing 150-180 g were used. Ethical approval has been obtained from the Animal Ethics Committee, Universiti Putra Malaysia (reference number UPM/FPSK/PADS/BR-UUH/00382). Rats were maintained under standard environmental conditions and were fed with standard diet and had free access to tap water.

Estimation of total phenolic and flavonoid content

Total phenolic content of SCA and SCE was determined using the Folin Ciocalteu assay [12]. Samples (100 μl) were introduced in test tubes followed by 500 μl of Folin-Ciocalteu reagent 10%. After 4 min, 400 μl of 7.5% Na_2CO_3 was added. The mixture was shaken for 2 h at room temperature and the absorbance was measured at 765 nm. Gallic acid was used as a standard. The concentration of total phenolic compounds in SCE was determined as microgram of gallic acid equivalent per mg of extract (μg GAE/mg extract).

The total flavonoid content was determined by the aluminium chloride (AlCl_3) method [13], using quercetin as standard. The sample solution (1 mL) was mixed with 1 mL of 2% AlCl_3 . After 10 min of incubation at room temperature, the absorbance was measured at 430 nm. Total flavonoid content was expressed as microgram of quercetin equivalent per mg of extract (μg QE/mg).

Acute toxicity

The acute oral toxicity study of *S. chamaecyparissus* extract was carried out using the method of Patrick-Iwuanyanwu *et al.*[14] to evaluate any possible toxic effects. A single dose of aqueous or ethanol extract (2000 mg/Kg) was orally administered. The behavior changes, toxic symptoms and deaths were observed for 4 h after oral intake of the extract and then further observation was conducted for 14 consecutive days.

Determination of serum ALT, AST, ALP and total bilirubin

Hepatoprotective effects of *S. chamaecyparissus* extracts were evaluated according to Kamisan *et al.*[15]. Briefly, rats were divided into 9 groups of six rats each. Animals in group 1 (control) were administered only tap water (10 mL/kg, p.o.) throughout the duration of the experiment. Those in group 2 (paracetamol treated) received tap water for 7 days followed by a single dose of 3 g/kg of paracetamol on day 7. The third group received 100 mg/kg p.o. of silymarin, as a reference drug for seven days prior to paracetamol intoxication. Rats of groups 4, 5 and 6 received respectively 30, 150 and 300 mg/kg daily of aqueous extract for 7 days. The groups 7, 8 and 9 received respectively 30, 150 and 300 mg/kg daily of ethanol extract also for 7 days. Three hours after the last treatment, 10 mL/kg of paracetamol 3 g/kg was administered orally.

Blood samples collected in heparinized tubes from all the groups by cardiac puncture 24 h after administration of the hepatotoxic agents. Plasma was separated by centrifugation at 2500 rpm for 10 min; and then was used for determination of biochemical parameters to assess the functional state of the liver. Liver enzymes ALT, AST and ALP were measured using the COBAS integra Automatic Chemical Analyser.

Tissue collection and histopathology

Livers were excised immediately after the animals were sacrificed and cleaned in normal saline. The histopathological study was performed according to Suzuki and Suzuki [16]. In brief, each fresh tissue sample was divided into pieces, and each piece was fixed in 10% natural formalin during 48h. After fixation and dehydration using a series of ethanol solutions, tissue specimens were embedded in paraffin, and from each block, 5 µm-thick sections were cut and stained with hematoxylin and eosin for the estimation of morphological changes, hepatocyte necrosis and steatosis. The slides were examined and photographed under a Leica DM1000 Microscope with Leica DFC495 Digital Camera 3 and PC System with Leica LAS Software (V 3.8).

Statistical analysis

Data are expressed as mean \pm SEM. Results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett test for multiple comparisons using the Prism 5.01 computer software (GraphPad, San Diego, USA). Statistical differences were considered to be significant at $P < 0.05$.

RESULTS**Total phenolics and flavonoids content**

Phytochemical screening indicated that SCE and SCA extract of *S.chamaecyparissus* contained high amounts of polyphenols (108.61 ± 2.55 , 86.14 ± 2.30 µgGAE/mg extract respectively) and flavonoids (23.29 ± 1.59 , 17.10 ± 0.76 µgQE/mg extract respectively).

Acute toxicity

No toxic symptoms or mortality was observed after oral administration of SCA and SCE at 2000 mg/kg body weight. Treated animals did not display any drug related changes in behavior, breathing, skin effects, water consumption and impairment in food intake. Therefore, the extracts seem to be safe at a dose level of 2000 mg/kg body weight.

Effect of *S. chamaecyparissus* extract on serum ALT, AST and ALP

The results of hepatoprotective effect of SCA and SCE extract on paracetamol intoxicated rats was shown in table 1 and figure 1. Rats intoxicated with paracetamol showed a significant increase in serum ALT, AST and ALP compared to normal control animals, which reflecting the liver injury. However, the treatment with 30, 150 and 300 mg/kg body weight of SCA and SCE attenuated significantly the increase activity of ALT, AST and ALP compared with control group intoxicated by paracetamol, suggesting the hepatoprotective potential of extracts (Figure 1). Inhibitory effect exerted by both extracts of *S. chamaecyparissus* on ALT was very close to that of silymarin, used as a standard hepatoprotective drug. It seems that the dose of 150 mg/kg was more potent on serum ALT level (Figure 1A), serum AST level (Figure 1B) and serum ALP level (Figure 1C).

Table 1. Inhibition percentages exerted by *S. chamaecyparissus* aqueous extract (SCA) and *S. chamaecyparissus* ethanol extract (SCE) on hepatic enzymes

	ALT	AST	ALP
PCM (3g/kg)	/	/	/
SCA 30 mg/kg + PCM	70.45 ± 9.03 ***	36.69 ± 10.96 *	21.13 ± 10.76 ns
SCA 150 mg/kg + PCM	83.55 ± 2.18 ***	67.78 ± 2.72 ***	34.48 ± 5.91 **
SCA 300 mg/kg + PCM	79.99 ± 3.90 ***	64.30 ± 6.24 ***	24.82 ± 3.59 ns
SCE 30 mg/kg + PCM	77.92 ± 5.25 ***	34.17 ± 13.20 *	18.01 ± 4.46 ns
SCE 150 mg/kg + PCM	78.13 ± 5.99 ***	65.29 ± 5.10 ***	23.61 ± 4.32 ns
SCE 300 mg/kg + PCM	73.40 ± 9.08 ***	57.81 ± 9.65 ***	27.58 ± 7.27 *
Silymarin 100 mg/kg + PCM	74.28 ± 3.58 ***	70.77 ± 6.25 ***	26.62 ± 6.38 *

Values are mean ± SEM of (4-6) rats in each group. * compared with paracetamol-induced liver toxicity. ***: $P < 0.001$, **: $P < 0.01$, #: $P < 0.05$.

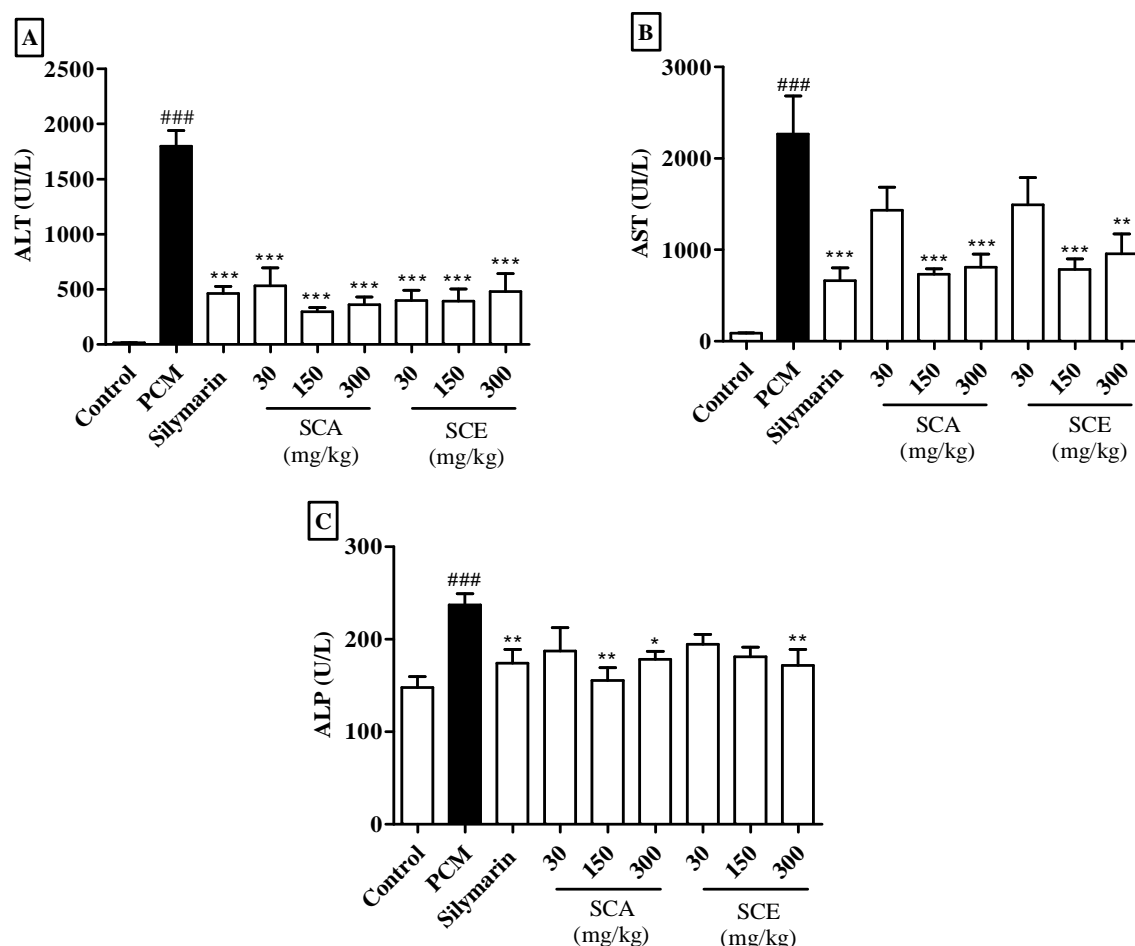


Figure 1. Effect of aqueous and ethanol extract of *Santolina chamaecyparissus* (SCA and SCE) on ALT, AST and ALP in paracetamol-induced liver toxicity in rats. Values are mean ± SEM of 6 rats in each group. # compared with the normal control group, *** $P < 0.001$ compared with paracetamol (PCM) induced liver toxicity in control group

Histopathological effects of *S. chamaecyparissus* extracts

The hepatoprotective effect of SCA and SCE observed in hepatic transaminase and ALP levels was confirmed by histological study of the liver. Indeed, livers of rats in normal group showed normal histological appearance (Figure 2A), while paracetamol 3 g/kg induced histopathological changes, which caused severe liver damage (Figure 2B). The most pronounced change was necrosis. However, histological examination of the liver from groups pretreated with 30, 150 and 300 mg/kg of SCA and SCE before the induction of hepatotoxicity reduced necrotic zones. Indeed, high reduction in perivascular necrotic areas was observed. The above changes were also reduced in the liver of rats pre-treatment with 100 mg/kg of silymarin (Figure 2C-I).

DISCUSSION

Liver diseases in humans become one of the serious health problems worldwide. There are few conventional drugs that can stimulate liver function and offer hepatic protection, but they are proved to be hepatotoxic at a particular

dose. Natural treatments from medicinal plants are considered to be effective and safe for hepatotoxicity [17]. The current investigation was carried out to study the hepatoprotective activity of aqueous and ethanol extract of *S. chamaecyparissus* aerial part by using paracetamol-induced toxicity in rats, as this experimental model are the most widely used for assessing hepatoprotective activity of plant extracts.

At high doses, paracetamol is converted by the drug-metabolizing enzymes to a toxic product, N-acetyl-pbenzoquinone imine, which binds covalently to the intracellular protein (adducts) and then lead to hepatic necrosis centrolobular [18], disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress and may eventually lead to cellular damage and death [19,20].

The significant increase in ALT, AST and ALP activities subsequent to paracetamol intoxication is due to an increase in hepatic cell membrane fragility that led to enzyme release into circulation. These cytoplasmic enzymes are released into the circulation after the damaged structural integrity of the liver and the disturbance caused in the hepatocytes functions [21,22].

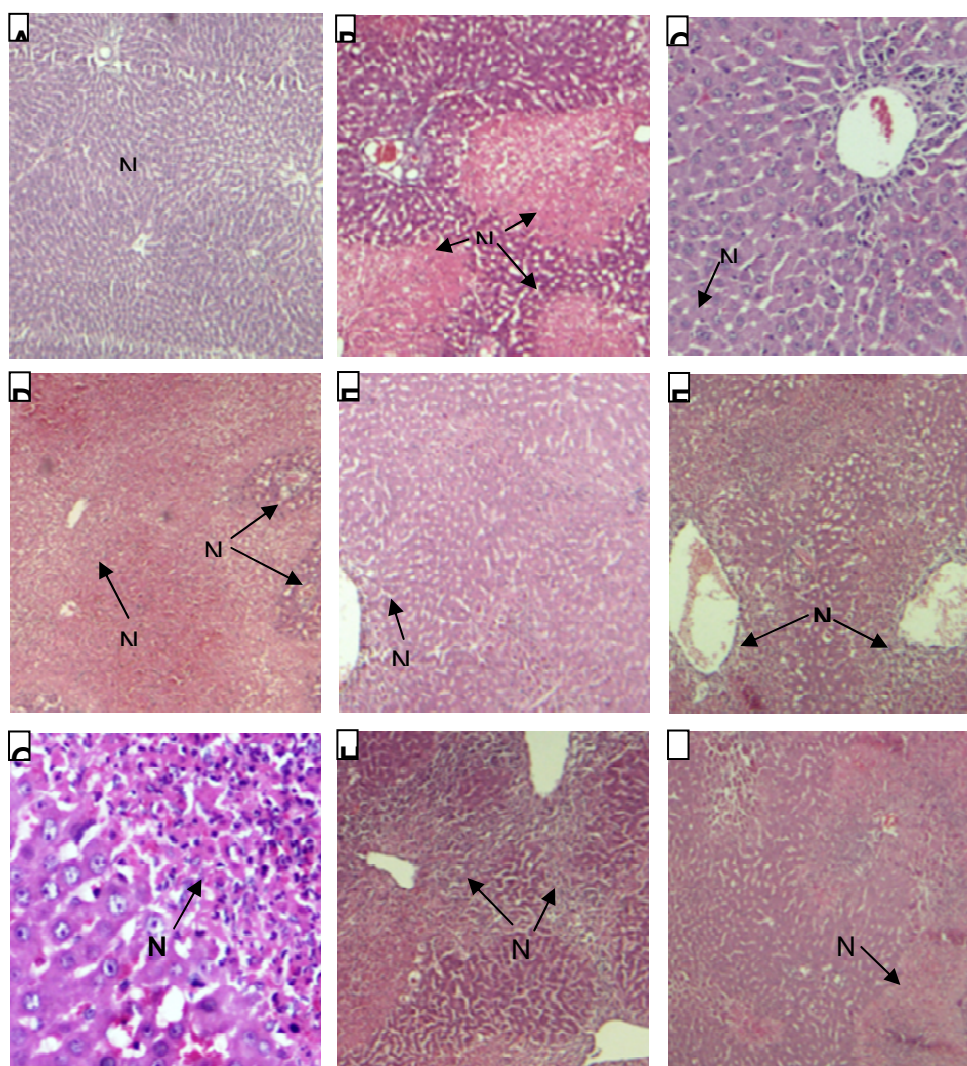


Figure 2. Photomicrographs of hematoxylin and eosin stained histological sections (x 100) of normal, paracetamol (PCM), SCA, SCE and silymarin treated rat liver. A: Normal control, B: PCM intoxicated rat liver, C: PCM intoxicated rat liver treated with 100 mg/Kg Silymarin, D: PCM intoxicated rat liver treated with 30 mg/Kg SCA, E: PCM intoxicated rat liver treated with 150 mg/Kg SCA, F: PCM intoxicated rat liver treated with 300 mg/Kg SCA, G: PCM intoxicated rat liver treated with 30 mg/Kg SCE, H: PCM intoxicated rat liver treated 150 mg/Kg SCE, I: PCM intoxicated rat liver treated with 300 mg/Kg SCE, (NP, Normal Parenchyma; Nc, Necrosis)

Santolina chamaecyparissus aqueous and ethanol extracts reduced the rate of these hepatic transaminases during liver damage by paracetamol. This result indicates that the studied extract prevented the leakage of intracellular enzymes by their membrane stabilizing activity as well as repair of hepatic tissue damage caused by paracetamol.

Several studies have been reported the hepatoprotective activity of medicinal plants. Indeed, it has been reported that extracts of *Boerhaavia diffusa* decrease AST, ALT, ALP, bilirubin and LDH after paracetamol administration [23]. Furthermore, Tiwari *et al.*[24] showed that the biochemical indicators AST, ALT, ALP, total bilirubin, were overexpressed due to paracetamol administration, which were significantly normalized by *Selaginella lepidophylla* extracts pretreatment. The effects exerted by *Santolina chamaecyparissus* aqueous and ethanol extract were similar to that exerted by the standard hepato-protective drug silymarin. The ability of silymarin in preventing hepatotoxicity is associated with its ability to act as a radical scavenger, thereby protecting membrane permeability [25]. Medicinal plants are good antioxidants and play an important role by their various constituents in the treatment of various diseases. This gives an additional support that *Santolina chamaecyparissus* aqueous and ethanol extract is able to protect hepatocytes from the free radical attacks, accelerate regeneration of parenchyma cells, protects against membrane fragility and then decrease leakage of enzymes into circulation. Indeed our previous work showed that *S. chamaecyparissus* is rich in antioxidant nutrients. Synergistic interactions amongst the various antioxidative components in the aerial part of *S.chamaecyparissus* extracts might be responsible for the relatively high values of antioxidant activity exemplified by the *in vitro* DPPH free radical scavenging activity, reducing power and iron chelation [5]. These properties might be due to the presence of bioactives compounds in this extract. In fact, phytochemical analysis by colorimetric assay revealed that both extract are rich in polyphenols and flavonoids. Several studies have demonstrated the hepatoprotective effect of phenolic and flavonoid compounds such as rutin [26].

These findings were further supported by histopathological evidences showing less hepatocellular necrosis, inflammation in rats treated with aqueous and ethanol extracts of *S. chamaecyparissus*. Indeed, in paracetamol-induced liver damage in rats, histological examination of liver samples showed massive deformation of hepatic tissue architecture, marked degree of inflammation, necrosis and inflammatory cell infiltration. These severe liver injuries were markedly reduced by the treatment with *Santolina chamaecyparissus* extracts and Silymarin. This effect may be due to the membrane stabilizing effect, the antioxidant and the anti-inflammatory activities of the studied extract. Indeed, it has been reported recently that *Santolina chamaecyparissus* extracts exhibit immunomodulatory effect by inhibiting neutrophils migration and its other functions [27]

CONCLUSION

Ethanol and aqueous extracts of the aerial part of *Santolina chamaecyparissus* exhibits hepatoprotective effects against paracetamol-induced liver damage. The phenolic compounds in the extract may be responsible for the hepatoprotective activity. So, the present work provides a scientific evidence for the appropriate use of *S. chamaecyparissus* in folk medicine for the treatment of liver diseases. However, further application in medical practice should be confirmed by conveying pharmacological and clinical studies.

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