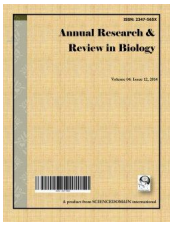

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Phytochemical Analysis and Hepatoprotective Activity of Algerian *Santolina chamaecyparissus* L. Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author DM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HB and ID managed the analyses of the study. Author AS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was designed to evaluate the hepatoprotective activity of *Santolina chamaecyparissus* aqueous and ethanol extracts against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats.
Methodology: Phytochemical analysis of *Santolina chamaecyparissus aqueous and* ethanol extracts was conducted, and then the hepatoprotective activity of *these* extracts was evaluated against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats by assessing serum aspartate aminotransferase, alanine aminotransferase alkaline phosphatasen, lactate dehydrogenase activities and assessing catalase, superoxide dismutase and malonaldialdehyde in liver. Total

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bilirubin, cholesterol and triglycerides were also determined. Moreover, hepatic tissue damage was verified.

Results: Phytochemical analysis revealed the presence of phenolic acids and flavonoids in *aqueous and* ethanol extracts of *Santolina chamaecyparissus* leaves. Both extracts contain chlorogenic acid as significant constituent (1958.21, 2726.57 mg/kg of extract, respectively), while apigenin-7-glycoside was detected as the significant flavonoid (42.44, 66.63 mg/kg of extract, respectively). The intra-peritoneal administration of CCl_4 to rats induced remarkable hepatotoxicity by increasing hepatic damage. However, oral administration of both extracts at 30, 150 and 300 mg/kg during 7 days significantly prevented liver injury by decreasing aspartate aminotransferase, alanine aminotransferase alkaline phosphatase and lactate dehydrogenase activities. Total bilirubin, cholesterol, triglyceride and malondialdehyde were also decreased, while superoxide dismutase activity was restored. On the other hand, aqueous and ethanol extracts protected liver tissue against steatosis and hepatocytic necrosis. The hepatoprotective effect of both extracts was similar to that of 100 mg/kg of silymarin, used as a reference.

Conclusion: The present study revealed that *Santolina chamaecyparissus* aqueous and ethanol extracts are rich in phenolic compounds and exhibit hepatoprotective activity, so they can constitute a promising natural source to develop novel therapeutic drugs for treating liver disorders.

Keywords: Carbon tetrachloride; flavonoids; hepatotoxicity; liver damage; polyphenols; Santolina chamaecyparissus.

1. INTRODUCTION

Liver diseases have become one of the severe health problems and a significant cause of morbidity and mortality all over the world. Major causes of these disorders are the exposure to different environmental pollutants, toxins and chemicals such as mparacetamol, CCl_4 and alcohol [1].

Carbon tetrachloride (CCl_4) accumulates in hepatic parenchyma cells, and it is metabolically activated by cytochrome P450-dependent monooxygenases to form highly reactive radicals, mainly the trichloromethyl radical (CCl_3•). This radical bind covalently to cellular components inhibits the lipoprotein secretion and reacts with the free oxygen to form trichloromethyl peroxyl (•$OOCCl_3$) radicals. Thereby, these radicals induce protein oxidation, DNA damage and lipid peroxidation, and subsequently progress to centro-lobular hepatic necrosis, inflammation and fibrosis [2,3].

Recently, the use of the herbal natural product has gained interest among the world population, and many of the herbs have been developed into herbal supplement which is claimed to assist in a healthy lifestyle. Several studies have been reported the hepatoprotective effect of medicinal plants against damage induced by CCl_4. This hepatoprotective effect is assigned to the content of medicinal plants in polyphenols and flavonoids [2,4,5].

Santolina chamaecyparissus L. (*S. chamaecyparissus*) belongs to Asteraceae family is an aromatic plant, a 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. 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 extracts* yielding a number of secondary metabolites such as essential oils [8], flavonoids [9] and coumarins [10]. The present was conducted to evaluate the hepatoprotective effects of of Algerian *Santolina chamaecyparissus*.

2. MATERIALS AND METHODS

2.1 Chemicals

Folin Ciocalteu reagent, gallic acid, quercetin and sodium carbonate (Na_2CO_3) were purchased from Sigma (Germany). Carbon tetrachloride (CCl_4) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The alanine aminotransferase (ALAT), alkaline phosphate (ALP), aspartate aminotransferase (ASAT), total bilirubin, lactate dehydrogenase (LDH), cholesterol and triglycerides estimated kits were purchased from

Roche (France) COBAS INTEGRA. All other chemicals are from Sigma and were of analytical grade.

2.2 Plant Material

Santolina chamaecyparissus was harvested during the flowering season in mid-May 24 2012, from Hammam Essoukhna, Sétif region in the 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.

2.3 Animals

Adult male *Albino Wistar* rats weighing 150-180 g were obtained from Pasteur Institute of Algiers, Algeria. All animals were kept to acclimatize under the laboratory conditions for one week and were provided with standard rodent diet and water *ad libitum*. All procedures were performed following European Union Guidelines for Animals Experimentation (2007/526 /EC).

2.4 Preparation of *Santolina chamaecyparissus* Extracts

Santolina chamaecyparissus aqueous and ethanol extracts were prepared as described previously [11]. Briefly, aqueous extract (AE) was prepared by decoction method. 100 g of the powdered aerial part of the plant was boiled in 1 L of distilled water for 20 min. After filtration, the filtrate collected was centrifugated at 3000 rpm for 10 min. The obtained supernatant was lyophilised to give a pale brown powder (yield: 15.77%).

S. chamaecyparissus ethanol extract (EE) was prepared by maceration of 100 g of the powdered aerial part of the plant with 80% ethanol at room temperature for 24h, under continuous shaking. After filtration, the filtrate was concentrated under reduced pressure at 40°C. The residue was lyophilized using a lyophilizator (PHYWE chrisa) to give a bright brown powder (yield: 15.07%). Extracts were stored at -32°C until use.

2.5 Estimation of Total Phenolic and Flavonoid Contents

Total phenolic content of AE and EE 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 and all tests were performed in triplicate. The concentration of total phenolic compounds in both extracts was determined as microgram of gallic acid equivalent per mg of SCA (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 (1ml) 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 extract).

2.6 HPLC-TOF/MS Analysis

HPLC-TOF/MS analysis of *S. chamaecyparissus* ethanol extract was carried out as described elsewhere. This HPLC method was developed and validated to analyse phenolic acids and flavonoids in the plant extracts. Agilent Technology of 1260 Infinity HPLC System was coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x 100 mm, 3.5 µm) column. Mobile phases A and B were ultra-pure water with 0.1% formic acid and acetonitrile, respectively. The flow rate was 0.6 mL min-1 and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0-1 min 10% B; 1-20 min 50% B; 20-23 min 80% B; 23-25 min 10% B; 25-30 min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325°C, nitrogen gas flow of 10.0 L min-1, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered through a PTFE (0.45 µm) filter by an injector to remove particulates [14].

2.7 Hepatoprotective Activity Determination

2.7.1 Determination of serum ALT, AST, ALP, LDH, total bilirubin, cholesterol and triglycerides

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 of the first and the second group were administered daily for 7 days a single dose of water (10 ml/kg, p.o.), prior to olive oil (10 ml/kg i.p.), 50% CCl_4 in olive oil (1 ml/kg i.p.) respectively. The third group received 100 mg/kg p.o. of silymarin, as reference drug for seven days prior to CCl_4 intoxication. Rats of groups 4, 5 and 6 received respectively 30, 150 and 300 mg/kg daily of aqueous extract for 7 days. While rats of groups 7, 8, 9 received orally respectively 30, 150 and 300 mg/kg daily of ethanol extract for 7 days. Three hours after the last treatment, 1 ml/kg of CCl_4 (50%) was administered intraperitoneally.

Blood samples collected in heparinised tubes from all the groups by cardiac puncture 24 h after administration of the hepatotoxic agent. 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. Biochemical parameters ALT, AST, ALP, LDH, total bilirubin, cholesterol and triglycerides were measured using the COBAS INTEGRA Automatic Chemical Analyser.

Liver was quickly removed, cleaned and washed in ice-cold saline solution.

2.7.1.1 *Measurement of catalase, superoxide dismutase and malonaldialdehyde in liver*

Liver tissue were homogenated using an automatic homogenizer: 0.5 g of each tissue was homogenated in 10-fold (w/v) cold phosphate buffer (0.1 M; pH 7.4), and centrifuged at 4000 rpm for 20 min at 4°C then we used the supernatant for the MDA, SOD and CAT assays.

Malonaldialdehyde content

Concentrations of MDA in liver, an index of lipid peroxidation, were determined spectrophotometrically according to Mihara and Uchiyama [16]. Briefly, 125 µl of liver supernatant was added to 125 µl of Trichloroacetic acid (20%) and 250 µl of Thiobarbituric acid (0.67%). The reaction mixtures were incubated at 100°C for 15 min. The tubes were cooled and 1 ml of n-butanol was added and centrifuged at 3000 rpm for 15 min. The supernatant was used and the absorbance was read at 530 nm in a spectrophotometer. MDA content was calculated using MDA - TBA extinction coefficient (ε= 1.56 x 10^5 $mol^{-1}.L.cm^{-1}$).

Catalase activity

Catalase activity was determined by the method of Aebi et al. [17]. Changes in absorbance were recorded at 240 nm. Briefly, 34 µl of liver supernatant was added to 966 µl of H_2O_2 at 19.5 mM prepared in phosphate buffer (50 mM, pH 7). Changes in absorbance were recorded at 240 nm using spectrophotometer UV/VIS (SPECORD 210 PLUS) against a blank that contained liver supernatant and phosphate buffer. Catalase activity was calculated in terms of µmol H_2O_2 consumed/min/mg of protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to Marklund and Marklund [18]. Competition between pyrogallol auto-oxydation by $O2^{-}$• and dismutation of these free radicals by SOD constitute the purpose of this assay. Briefly, 25 µl of liver supernatant was added to 935 µl of Tris buffer (50 mM Tris, 1 mM diethylenetriaminepentacetic ; pH must be between 8.6 and 8.7 using cacodilic acid). After homogenisation, 40 µl of pyrogallol (10 mM) prepared in HCl 0.01N was added to the reaction medium. The mixture was shaken vigorously and absorbance was recorded after 45 s. just after pyrogallol addition at 420 nm for 1 min. SOD activity was calculated in terms of unit/protein. Where unit was calculated as:

U = (inhibition percentage/50) x dilution factor

Inhibition percentage of pyrogallol auto-oxydation was calculated using the following formula:
Inhibition (%) = (Ac-As)/Ac

Where Ac is the absorbance of control (maximal absorbance; pyrogallol alone), and As is the absorbance of sample (pyrogallol with liver supernatant).

2.7.1.2 *Histopathological assessment of hepatic tissue damage*

Livers were excised immediately after the animals were sacrificed and cleaned in normal

saline. The histopathological study was performed according to Suzuki and Suzuki [19]. 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, steatosis and inflammatory cell infiltration. The slides were examined and photographed under a Leica DM1000 Microscope with Leica DFC495 Digital Camera and PC System with Leica LAS Software (V 3.8).

2.8 Statistical Analysis

In vivo, data are expressed as mean ± 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 $P < .05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Phytochemical screening indicated that AE and EE of *S. chamaecyparissus* contained high amounts of polyphenols (86.14 ± 2.30; 108.61 ± 2.55 µg GAE/mg extract) and flavonoids (17.10 ± 0.76; 23.29 ± 1.59 µg QE/mg extract) respectively.

HPLC-TOF/MS analysis revealed the presence of phenolic acids and flavonoids in AE and EE of *S. chamaecyparissus* leaves (Fig. 1). Both extracts of *S. chamaecyparissus* contain chlorogenic acid as major constituent (1958.21, 2726.57mg/kg of extract, respectively), while apigenin-7-glycoside was detected as the major flavonoid (42.44, 66.63 mg/kg of extract, respectively) in these extracts (Table 1).

The extraction yield calculated for ethanol and aqueous extracts of aerial part of *S. chamaecyparissus* showed that ethanol extract registered higher percentage of yield. It may be due to high polarity of ethanolic solvent which can draw high variety of plant constituents than the other solvents did [20]. However, flavonoids and polyphenols were rich in ethanolic extract than the aqueous one, which indicate that the polarity level is playing major role in extracting the secondary metabolites [21].

3.2 Effect of *S. chamaecyparissus* Aqueous and Ethanol Extracts on Liver Enzymes

3.2.1 Effect of AE and EE on ALT, AST, ALP, LDH, total bilirubin, cholesterol and triglycerides

Results indicated that intraperitoneal injection of CCl_4 induce significant ($P < .001$) increase in the serum levels of AST, ALT, ALP and LDH (2004.2, 764.10, 407.24 and 2660.18 U/L, respectively) when compared to control group (232.08, 92.35, 147.93, and 365.5 U/L, respectively). The pretreated rats with 30, 150 and 300 mg/Kg of *S. chamecyparissus* AE and EE showed an important decrease in the level of these enzymes compared to the rats intoxicated with CCl_4 (Fig. 2).

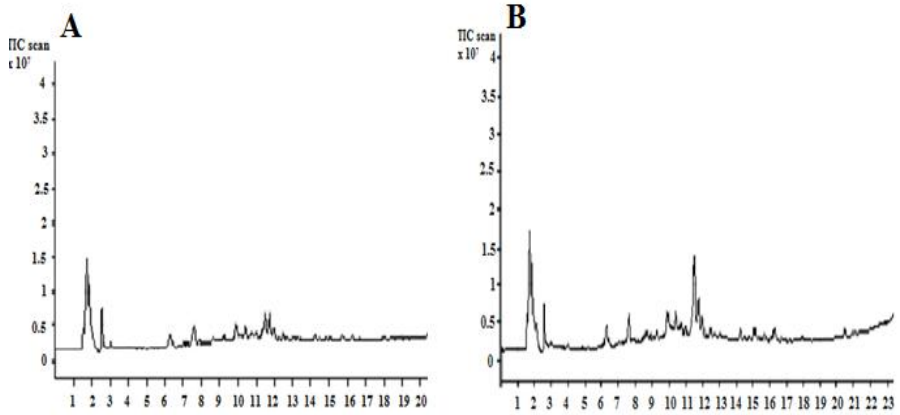

Fig. 1. HPLC-TOF/MS chromatogram of *S. chamaecyparissus* aqueous (A) and ethanolic (B) extracts

Table 1. Phenolic and flavonoid compounds identified by HPLC–TOF/MS in *S. chamaecyparissus* aqueous and ethanol extracts

Compound	Retention time (min)	mg/Kg of plant	
		AE	EE
Gallic acid	2.644	02.72	03.60
Gentisic acid	4.297	33.18	19.52
Chlorogenic acid	6.228	1958.21	2726.57
4-hydroxybenzoic acid	6.463	28.09	51.91
Protocatechic acid	6.891	03.29	01.87
Caffeic acid	7.667	31.68	43.64
Vanillic acid	7.741	10.19	/
Ferulic acid	10.838	03.02	00.96
Acide cichorique	10.088	/	02.22
Acide salicylique	13.534	/	02.33
Rutin	9.972	11.84	10.28
Apigenin-7-glycosid	11.678	42.44	66.63
Quercetin	15.165	03.66	11.95
Kampférol	17.727	/	01.05

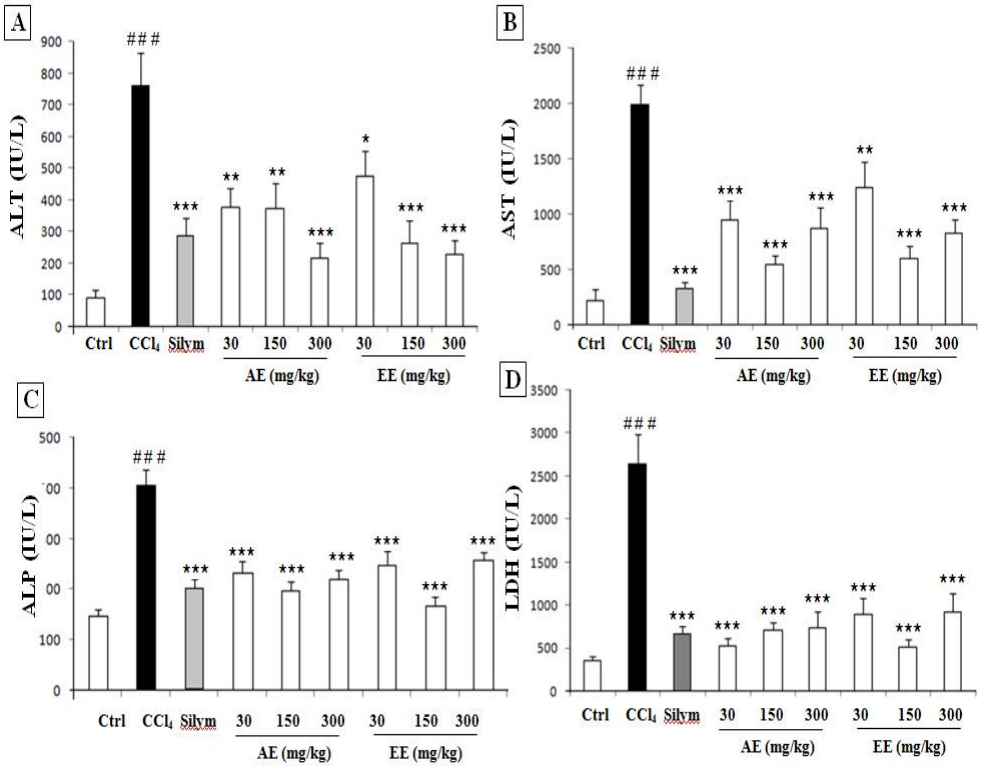

Fig. 2. Effect of *S. chamecyparissus* aqueous (AE), ethanolic (EE) extracts and silymarin (Silym) **on ALT (A), AST (B), ALP (C) and LDH (D) in CCl_4-induced liver toxicity in rats.** Values are mean ± *SEM, (n=6). # compared with the normal control group (Ctrl), *P < .05, ** P < .01, *** P < .001 compared with CCl_4-induced liver toxicity*

Carbon tetrachloride is a potent environmental hepatotoxin, it is metabolized to free radicals, which react with cellular macromolecular proteins and fatty acids present in the cytoplasmic membrane phospholipids. Consequently, cause acute and chronic liver toxicity, resulting to steatosis, cellular necrosis, fibrosis, and cirrhosis [22]. Moreover, CCl_4 is known to reduce

antioxidant enzymes leading to oxidative stress, which is an important factor in acute and chronic injuries in various tissues [23].

CCl_4 intoxication damaged structural integrity of the liver and caused disturbance in the hepatocytes functions [24,25,26]. The significant increase in ALT, AST, ALP, LDH activities and MDA content subsequent to CCl_4 intoxication is due to an increase in hepatic cell membrane fragility that led to enzyme release into circulation. High levels of ALT, AST, ALP, LDH activities and MDA content are sensitive indicators of liver cell injury and are most helpful in recognizing hepatic diseases [27]. Serum LDH is the well known indicator of cell and tissue damage by toxic substances. Peroxidation of membrane lipids also leads to the release of transaminases (ALT, AST), ALP, bilirubin and LDH [28,29].

The treatment of intoxicated rats with *Santolina chamaecyparissus* aqueous and ethanol extracts reduced the rate of these enzymes. This result indicates that the studied extract prevented the leakage of intracellular enzymes by stabilizing the membrane and repairing hepatic tissue damage caused by CCl_4 exposure. Several studies have been reported the hepatoprotective activity of medicinal plants. Indeed, it has been reported that extracts of *Sesbania grandiflora* flower decrease AST, ALT, ALP after CCl_4 administration [30]. Furthermore, Dey et al. [31] showed that the biochemical indicators AST, ALT, ALP, glucose, protein, albumin, globulin, bilirubin, urea, LDH and, cholesterol were overexpressed due to CCl_4 administration, which were significantly normalized by *Nerium indicum* leaf extract treatment. The effects exerted by *Santolina chamaecyparissus* extracts were similar to that exerted by silymarin, used as standard hepatoprotective drug. The ability of silymarin in preventing hepato-toxicity is associated with its ability to act as a radical scavenger, thereby protecting membrane permeability [32].

When rats were intoxicated with CCl_4, the level of total bilirubin, cholesterol and triglyceride increased (11.52 mg/l, 82.80 mg/dL and 205.20 mg/dL, respectively) compared to control group (0.45 mg/L, 46.75 mg/dL and 61 mg/dL, respectively). The treatment with 30, 150 and 300 mg/Kg of AE and EE clearly reduced these levels compared to intoxicated rats (Table 2).

The elevation of total bilirubin in the bloodstream could be attributed to high synthesis, increased hemolysis, decreased conjugation or impaired transport [33]. Therefore, bilirubin is used as an indicator to evaluate the secretory function of hepatocytes [34].

CCl_4 metabolism leads to the formation of free radicals that attack membrane lipids of the endoplasmic reticulum. These radical attacks are responsible for the decrease of metabolic functions such as protein synthesis and inhibition of cholesterol storage [35]. Cholesterol level was increased by CCl_4-induce liver intoxication. This increase might be due to the increased esterification of fatty acids, inhibition of fatty acid β-oxidation and decreased excretion of cellular lipids [36]. In addition, CCl_4 increases the synthesis of triglyceride and enhances lipid esterification [22]. The accumulation of triglyceride in liver might occur due to the inhibition of lysosomal lipase activity and VLDL secretion [37]. Pretreatment by both extracts of *S. chamaecyparissus* modulated lipid profiles. Among the antioxidant compounds, phenolic compounds are the strongest in inhibiting lipid peroxidation [38,37,39].

Table 2. Effect of *S. chamecyparissus* aqueous (AE) and ethanolic (EE) extracts on ALT, AST, ALP, LDH, total bilirubin, cholesterol and triglycerides

	Total bilirubin (mg/L)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Negative control	0.45 ± 0.06	46.75 ± 3.01	61.00 ± 11.11
Positive control	11.52 ± 2.11 ###	82.80 ± 12.02 ###	205.20 ± 29.67 ###
Silymarin 100 mg/Kg	0.86 ± 0.15 ***	46.20 ± 1.32 ***	66.17 ± 6.16 ***
AE 30 mg/Kg	1.18 ± 0.13 ***	49.60 ± 4.37 ***	49.60 ± 3.99 ***
AE 150 mg/Kg	0.64 ± 0.05 ***	40.00 ± 1.14 ***	50.00 ± 2.72 ***
AE 300 mg/kg	1.02 ± 0.19 ***	43.67 ± 3.25 ***	43.50 ± 9.07 ***
EE 30 mg/kg	1.58 ± 0.28 ***	56.83 ± 4.26 **	72.67 ± 14.01 ***
EE 150 mg/kg	0.98 ± 0.15 ***	43.00 ± 3.57 ***	63.33 ± 6.43 ***
EE 300 mg/kg	1.04 ± 0.16 ***	42.67 ± 2.19 ***	69.83 ± 6.10 ***

*Values are means ± SEM, (n=6). # compared with the negative control (no intoxicated with CCl_4), *P < .05, ** P < .01, *** P < .001 compared with positive control (intoxicated with CCl_4 and untreated)*

On the other hand, the intraperitoneal injection of CCl_4 induced a significant ($P < .001$) rise in malondialdehyde concentration. Pretreatment with *S. chamaecyparissus* extracts (30, 150 and 300 mg/kg) significantly reduced ($P < .001$) the MDA level compared to the rats intoxicated with CCl_4 groups (Table 3). An inhibition of 53.57 ± 5.03% was observed with 150 mg/kg of AE. The activity of SOD decreased by 78.12 ± 4.99% under the effect of CCl_4, while the treatment with AE and EE extracts of *S. chamecyparissus* and silymarin restored SOD activity. Unexpected, CCl_4 intoxication does not induce any significant change in catalase activity.

Free radicals produced during the metabolism of this hepatotoxin reduce levels of antioxidant enzymes leading to oxidative stress, which is an important factor in liver injury [23]. In this study, catalase and superoxide dismutase (SOD) activities were reduced by CCl_4 administration. Treatment with *S. chamaecyparissus* extracts restored the activity of these antioxidant enzymes. These results are in agreement with those of Shanmugam et al. [40] who showed that the treatment of rats with the acetone extract of *Passiflora subpeltata* leaves restores the level of antioxidant enzymes (SOD, catalase, and GPX). This restoration leads to the reduction of lipid peroxidation and consequently to the reduction of liver lesions. In fact, the decrease in the activity of these enzymes is at the origin of the increase in lipoperoxidation, that the final product is the MDA, which is a marker of lipid peroxidation.

Hepatic MDA concentration increased following CCl_4 intoxication. This increase reflects a strong oxidative stress that leads to tissue damage and failure of antioxidant defense mechanisms. It can penetrate the membrane lipid bilayer, leading to loss of fluidity and membrane integrity and ultimately damage or loss of hepatocyte function [41]. This dysfunction increases the permeability of the plasma membrane, thereby inducing swelling and cell necrosis [42]. The level of hepatic MDA decreased significantly by administration of *S. chamaecyparissus* extracts. These results prove that EA and even EE can protect the liver against free radical attacks, and this protection would be due to inhibition of lipid peroxidation and inhibition of chain oxidation reactions [43].

3.2.2 Effect of *S. chamecyparissus* aqueous (AE) and ethanolic (EE) extracts on liver histology

Rat livers in normal group showed normal histological appearance (Fig.3 A), while CCl_4 injection caused severe liver damage (Fig. 3 B). The most pronounced changes are necrosis and steatosis. However, histological examination of the liver from groups pretreated with 30, 150 and 300 mg/kg of AE and EE before the induction of hepatotoxicity reduced necrotic zones. Minimal deposition of fat vacuoles and mild fibrous tissue were apparently observed. A high reduction in perivascular necrotic areas was observed. The above changes were also reduced in the liver of rats pre-treated with 100 mg/kg of silymarin (Fig. 3 C-I).

The hepatoprotective effect of *Santolina chamaecyparissus* aqueous and ethanol extracts was confirmed by histopathological evidences showing less hepatocellular necrosis, inflammation and steatosis in rats treated whith aqueous and ethanol extracts of *S. chamaecyparissus*. Indeed, histological examination of intoxicated liver samples showed massive deformation of hepatic tissue architecture, marked degree of steatosis and necrosis. These severe liver injuries were

Table 3. Effect of *S. chamecyparissus* aqueous (AE) and ethanolic (EE) extracts on hepatic MDA and SOD

	MDA µmol/mg protein	SOD (U/mg protein)
Negative control	4.16 ± 0.35	193.55 ± 22.22
Positive control	15.88 ± 1.88 ###	42.34 ± 9.65 ###
Silymarin 100 mg/Kg	6.43 ± 0.37 ***	157.50 ± 14.93 ***
AE 30 mg/Kg	8.53 ± 1.30 ***	125.83 ± 11.71 **
AE 150 mg/Kg	7.37 ± 0.80 ***	139.01 ± 13.86 ***
AE 300 mg/Kg	9.25 ± 1.46 **	107.18 ± 21.06 *
EE 30 mg/Kg	9.37 ± 1.21 **	74.60 ± 7.62
EE 150 mg/Kg	8.63 ± 0.96 **	127.37 ± 11.22 **
EE 300 mg/Kg	7.16 ± 0.91 ***	123.54 ± 17.60 **

*Values are means ± SEM, (n=6). # compared with the negative control (no intoxicated with CCl_4), *P < .05, ** P < .01, *** P < .001 compared with positive control (intoxicated with CCl_4 and untreated)*

markedly reduced by the treatment with *Santolina chamaecyparissus* aqueous and ethanol extracts. This effect may be due to the membrane stabilizing effect, the antioxidant and the anti-inflammatory activities of these extracts. Indeed, it has been reported recently that *Santolina chamaecyparissus* extracts exhibit ant-inflammatory and immunomodulatory effects by inhibiting neutrophils migration and its other functions [44]. Oliveira et al. [45] reported that the chlorogenic acid, the major constituent of the extract, is positively correlated with anti-inflammatory properties. Furthermore, Hafez et al. [46] reported that rutin, identified in the extract, protects against CCl_4-induced liver injuries in rats and increases levels of endogenous liver antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione -S- transferase, glutathione reductase, and glutathione contents and decreased lipid peroxidation [47].The hepatoprotective effect of plant extracts appears to be attributed to free radical scavenging properties, which leads to inhibition of lipid peroxidation and preservation of the cell membrane.

These properties might be due to the presence of bioactives compounds in the studied extracts. In fact, phytochemical analysis by HPLC-TOF/MS revealed the presence of 12 compounds corresponding to phenolic acids and flavonoids. Several studies have demonstrated the hepatoprotective effect of phenolic and flavonoid compounds such as rutin [46], apigenin-glycosids [48] and chlorogenic acid [49].

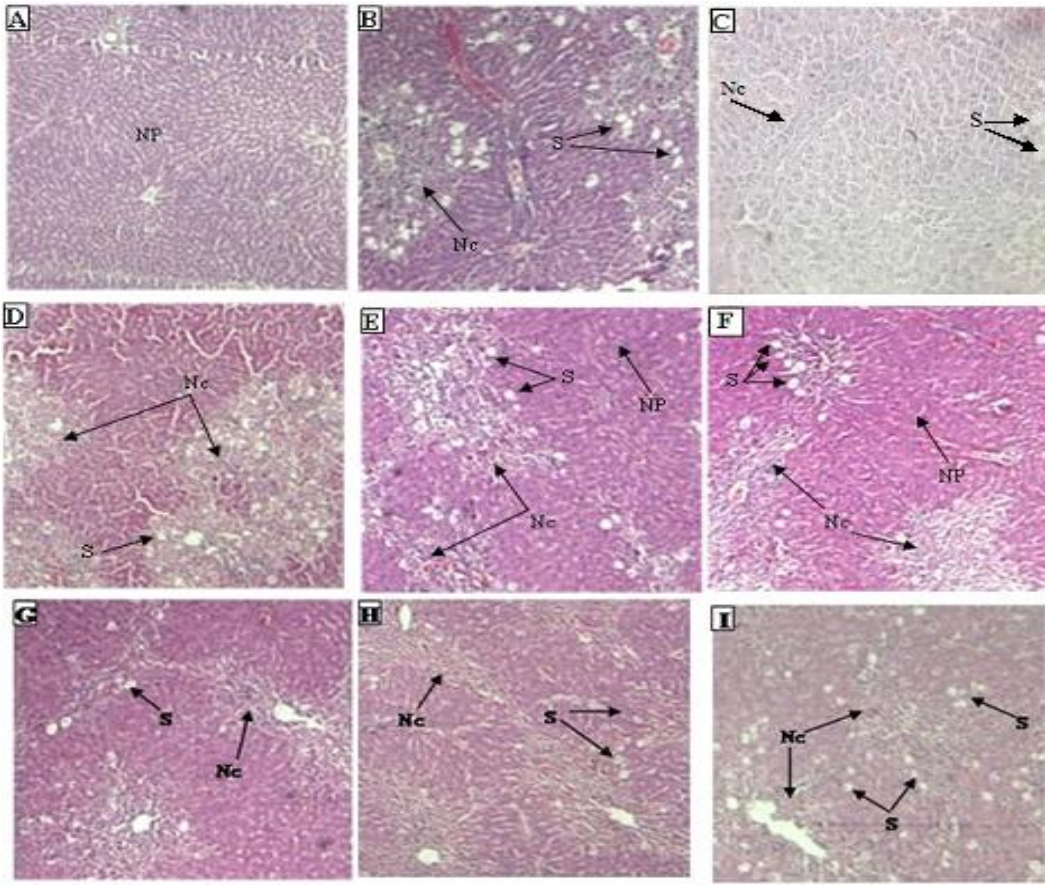

Fig. 3. Photomicrographs of hematoxylin and eosin stained histological sections (x 100) of liver's rats intoxicated with CCl_4

A: liver parenchyma of normal control, B: hepatic parenchyma of CCl4-intoxicated rats, C: hepatic parenchyma of rats pretreated with 100 mg / kg of silymarin, D: hepatic parenchyma of rats pretreated with 30 mg/kg of AE, E: hepatic parenchyma of rats pretreated with 150 mg/kg of AE, F: hepatic parenchyma of rats pretreated with 300 mg/kg of AE, G: hepatic parenchyma of rats pretreated with 30 mg/kg of EE, H: hepatic parenchyma of rats pretreated with 150 mg/kg of EE. I : hepatic parenchyma of rats pretreated with 300 mg/kg of EE (NP: Normal parenchyma, Nc: Necrosis, S: Steatosis)

4. CONCLUSION

Aqueous and ethanol extracts of the aerial part of *Santolina chamaecyparissus* exhibit hepato-protective effects against CCl_4-induced liver damage. Indeed, this study shows that these extracts can protect hepatocytes from the free radical attacks, accelerate the regeneration of parenchyma cells, protect against membrane fragility and then decrease leakage of enzymes into circulation. Medicinal plants are good antioxidants, and they play an essential role by their various constituents in the treatment of various diseases. Phenolic compounds in these extracts may be responsible for the hepatoprotective activity. So, the present work provides scientific evidence for the appropriate use of *S. chamaecyparissus* in folk medicine for the treatment of liver diseases.

ETHICAL DISCLAIMER

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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