Original Research Article

Anti-inflammatory and hepatoprotective potentials of the aerial parts of *Silene villosa* Caryophyllaceae methanol extract in rats

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Abstract

Purpose: To explore the anti-inflammatory and hepatoprotective potentials of Silene villosa Caryophyllaceae methanol extract in rats.
Methods: Toxicity of S. villosa extract was evaluated in rats. Inflammation was induced in rats by injection of 0.1 mL carrageenan (1 %) in the left hind paws. Carbon tetrachloride (CCl_4) was used to induce liver damage. Five groups of rat were used. The 1st (normal control) and 2nd (hepatotoxic) groups received the vehicle. The 3rd, 4th, and 5th groups received silymarin, 250 and 500 mg/kg of S. villosa extract, respectively, for 7 days. Liver injury was induced on the 7th day by intraperitoneal administration of 1 mL/kg of CCl_4 to rats in groups 2 - 5.
Results: The results showed that S. villosa is safe. It significantly reduced carrageenan-induced edema compared to normal (p < 0.01) and standard (p < 0.01) groups. The extract protected (p < 0.01) rats against the deleterious effect of CCl_4. It decreased (p < 0.01) the elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT) and alkaline phosphatase (ALP) as well as elevated serum levels of bilirubin (BRN), compared to CCl_4 control rats. Reduced activities of the antioxidant enzymes were significantly increased (p < 0.01) in rat liver, compared with CCl_4 control group. The results were confirmed by histological findings in rat liver as the extract reduced necrosis and hydropic degeneration of hepatic tissue compared to CCl_4 control group.
Conclusion: The results suggest that S. villosa possesses anti-inflammatory and hepatoprotective activities in rats, and therefore, has therapeutic potentials in humans.

Keywords: S. villosa, anti-inflammatory, carrageenan, CCl_4, antioxidant, hepatotoxicity.

INTRODUCTION

The liver is one of the major organs in the human body. It carries out the ordinary metabolic homeostasis of the body as well as the processes of detoxification and excretion of many compounds [1]. According to Nazeema and Brindha [2], hepatitis is the major hepatic

disorder that accounts for high death rate. Mostly, liver damage is considered a result of exposure to ecological poisons, which are linked with metabolic dysfunctions, ranging from temporary increase of liver enzymes to life-threatening liver cirrhosis [3].

CCl_4 is widely used to induce experimental hepatotoxicity in laboratory animals [4]. The adverse effects of CCl_4 are mediated by the highly reactive free trichloromethyl radical ($CCl_3\bullet$). The free radical of CCl_4 reacts with oxygen to generate trichloromethylperoxyl radical ($CCl_3OO\bullet$), which in turn reacts with polyunsaturated fatty acids of the membranous system leading to oxidative injuries such as lipid peroxidation [5]. Accordingly, the free radical mediated lipid peroxidation (LPO) is one of the major mechanisms of hepatic damage by CCl_4 [6].

The family, Caryophyllaceae, is widely known as gardening herbs but the medicinal importance of its members is sparsely known. Among 2,630 species of the Caryophyllaceae family, only a small fraction (~50 – 90 species) is known to have medicinal properties [7]. The ethnopharmacological studies of the Caryophyllaceae family indicate that members of this family possess anticancer, antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties [7]. The genus *Silene* (family Caryophyllaceae) involves more than 700 species of annuals, perennials, and biennials [8].

Some of *Silene* species have been used traditionally for treatment of cold, bronchitis, inflammations, and infections or as a diuretic, emetic, analgesic, and antipyretic [9]. The roots of some species, such as *S. kumaonensis*, *S. latifolia*, *S. conoidea*, and *S. acaulis* rich in saponins, have been traditionally used as a soap substitute for washing clothes [10]. The herb; *S. villosa* grows wildly in Saudi Arabia and is well known as Terba [11]. One of the studies on wild Egyptian *S. villosa* suggested its immune-modulation and antioxidant effects [12]. However, information about the pharmacological activities of *S. villosa* is very little. The purpose of the current study is to explore the anti-inflammatory and hepatoprotective potential of *S. villosa* extract in rats.

EXPERIMENTAL

Plant material

The herb of *S. villosa* was collected in early March 2016 from Al-shadeedah village - 9 km north of Al-Kharj region of Saudi Arabia. The collected plant was authenticated by taxonomist Dr. M. Atiqur Rahman, Pharmacy College, Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University, Riyadh. A voucher specimen (no. PSAU – CPH - 2 - 2016) is maintained in the herbarium of Pharmacy College, Prince Sattam bin Abdulaziz University Al-Kharj, Kingdom of Saudi Arabia. The shed dried herbs (500 g) were coarsely powdered and macerated in 3 liters of Methanol for 72 h using percolation method. The methanol was then removed at 50 °C under reduced pressure in a rotatory evaporator.

Animals

Adult male Wistar albino rats (180 – 200 g) were obtained from Lab Animal Care Unit at Pharmacy College, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA, and were used for the experiments. Rats were preserved under standard situations of temperature (23 ± 1.0 °C) and 12 h light/12 h dark cycle. Rats were fed with standard diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee, Pharmacy College, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia (protocol no. PHARM-02-5-2016), and followed the Guide for the Care and Use of Laboratory Animals [13].

Acute toxicity study

Acute toxicity of *S. villosa* extract was studied following the guidelines of the Organization for Economic Co-operation and Development [14]. Twelve rats were fasted overnight and divided to 2 equal groups. Animals of the first group received a single oral dose of *S. villosa* extract (5000 mg/kg). Rats of the second group received an oral dose of the vehicle (3 % v/v Tween 80 in distilled water) and kept as control. Rats were observed for the signs of toxicity and/or deaths for 30 min and periodically during 24 h, then every 24 h for a period of 14 days.

Anti-inflammatory activity

The anti-inflammatory potential of *S. villosa* extract was estimated in rats according to Winter *et al* [15]. Four groups of animals (n = 6) were used. Groups I (normal control) and II (reference) orally received the vehicle (2 mL/kg) and phenylbutazone (PBZ) at 5 mg/kg, respectively. Groups III and IV received *S. villosa* extract at 250 and 500 mg/kg, respectively by oral route. One hour later, the rats were subcutaneously injected with 100 µL of 1 % suspension of carrageenan (Sigma chemical co, St. Louis MO, USA) in normal saline into the plantar side of the

left hind paw. The volume of erach paw was estimated directly after carrageenan injection (0 h) and then after 3 h using a plethysmograph apparatus.

Hepatoprotective activity

The hepatoprotective activity of *S. villosa* extract was evaluated in adult rats by using CCl_4 as a hepatotoxic agent [4]. Five groups of male rats were used (n = 6). Animals of the 1st and 2nd (normal and hepatotoxic controls, respectively) groups administered the vehicle (Tween 80, 3 % in distilled water) at 2 mL/kg. The 3rd group kept as reference and was prophylactically treated with silymarin at 50 mg/kg. Rats of the 4th and 5th groups administered *S. villosa* extract at doses of 250 and 500 mg/kg, respectively. The vehicle (Tween 80), silymarin and *S. villosa* extract were dosed orally for 7 days. On the 7th day, hepatotoxicity was induced in rats of groups II - V using 1 mL/kg of CCl_4 (50 % solution in olive oil) by intraperitoneal route [4], while rats of Group I were injected with an equal volume of olive oil.

Autopsy schedule

After 48 h of CCl_4-induced hepatotoxicity, blood samples (3 mL) were obtained from all rats by puncturing their retro-orbital plexus under mild ketamine anesthesia. Serum was separated following centrifugation of blood samples at 3000 rpm for 15 min. Liver of each rat was removed, washed with cold normal saline and blotted. Half of each liver was frozen (at -70 °C) for biochemical analysis and the remaining half was fixed in buffered formalin 10 % for histological study.

Assessment of biochemical parameters

The serum activities of liver marker enzymes (ALT, AST, γ-GT, and ALP) and serum levels of BRN, total protein (TP), and albumin (ALB) were evaluated according to the instructor manual of commercially available kits from Sigma-Aldrich (United States of America).

Determination of antioxidant status

A portion of liver was homogenized in ice-cold 1.15 % w/v KCl in a Potter Elvehjem Teflon-glass homogenizer for one minute to obtain a 10 % w/v liver homogenate. The activities of the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the levels of glutathione (GSH) and malondialdehyde (MDA) were estimated in liver tissue homogenates of rats

according to the instructor manual of commercially available kits from Sigma-Aldrich (United States of America).

Histopathological examination

Liver specimens were fixed in buffered formalin 10 %, processed routinely, and embedded in paraffin. 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation. The stained sections were examined and photographed under a light microscope.

Statistical analysis.

Data are expressed as the mean ± standard error (n = 6). Results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test using SPSS ver. 14.0. $P < 0.05$ and $p < 0.01$ were considered significant, respectively.

RESULTS

Acute toxicity

The result indicates that *S. villosa* extract has a high safety profile. No mortalities were observed in rats following oral dosing of the extract at 5000 mg/kg and none of them showed signs of toxicity. Hence, doses of 250 and 500 mg/kg of *S. villosa* extract were selected for further investigation.

Anti-inflammatory activity

The anti-inflammatory activity of *S. villosa* is presented in Table 1 and Figure 1. Subcutaneous injection of carrageenan (100 µL of 1 % suspension) created an inflammatory edema in the foot pad of rat hind paw. At the 3rd hour following carrageenan injection, the paw swelling was increased by 178.09 % compared to the initial volume. As shown in Table 1, the volume of swellings induced by carrageenin in hind paws of rats (1.78 mL) were markedly reduced ($p < 0.05$) by 250 and 500 mg/kg of *S. villosa* extract, dose-dependently (1.14 mL and 0.95 mL, respectively) and by PBZ (0.60 mL).

Hepatoprotective activity

Tables 2 and 3 show the effect of *S. villosa* extract on some liver function biomarkers of CCl_4-exposed rats. Exposure of rats to CCl_4 was observed to cause significant increase ($p < 0.01$) in the serum activities of ALT, AST, γ-GT, and ALP in comparison with normal rats. The results showed that *S. villosa* extract at 250 and 500 mg/kg were able to retain the serum liver

function biomarkers of CCl₄-exposed rats toward the normal levels. The higher dose of the extract (500 mg/kg) displayed the highest suppression ($p < 0.01$) in percentage of serum ALT, AST, γ-GT and ALP, when compared to CCl₄ control group. Silymarin showed more significant and better inhibition.

In addition, CCl₄-exposed rats demonstrated significant elevation ($p < 0.01$) in serum level of BRN and decrease in TP and ALB values in comparison with the normal control group. Pretreatment with *S. villosa* extract exhibited a marked reversal of BRN, TP and ALB toward their normal values (Table 3). The efficacy of the tested extract was found to be dose dependent.

Table 4 shows the effect of *S. villosa* extracts on biochemical variables indicative of liver oxidative stress in rats. The results indicated that CCl₄ induced oxidative stress with a significant decrease ($p < 0.01$) in the activities of liver SOD, CAT and GPx in comparison with the normal control values. In addition, hepatic GSH showed a significant ($p < 0.01$) decrease while MDA

levels significantly ($p < 0.01$) increased in CCl₄ exposed rats relative to the normal group.

Pretreatment with both doses of *S. villosa* extract decreased the oxidative stress, as evidenced by increased activities of SOD ($p < 0.05$), CAT ($p < 0.05$), and GPx ($p < 0.01$) in liver tissues when compared with those of CCl₄-exposed rats.

Table 1: Anti-inflammatory activity of *S. villosa* on carrageenan- induced paw edema

Group	Dose (mL/kg)	Paw volume (mL)		Difference in paw volume
		0 h	3 h	
Control	0.1	1.05±0.05	2.92±0.11	1.78±0.10
PBZ	5	1.04±0.04	1.67±0.05	0.60±0.05#
S. villosa	250	1.03±0.05	2.21±0.12	1.14±0.11#
S. villosa	500	1.04±0.02	2.03±0.10	0.95±0.07#

Values are expressed as mean ± SEM of six animals in each group; #indicate significance compared to normal control group at $p < 0.05$ (Dunnett's test)

Table 2: Effect of the ethanol extract of *S. villosa* on the serum activity of liver marker enzymes in rats with CCl₄-induced hepatotoxicity

Group	ALT (U/L)	AST (U/L)	γ-GT (U/L)	ALP (U/L)
Normal control	34.01±2.43#	104.68±5.81#	17.16±0.98#	103.19±7.13#
CCl4-hepatotoxic control	116.16±7.69ϕ (241.54%)	284.50±15.25ϕ (171.78%)	38.18±1.21ϕ (122.49%)	309.45±19.54ϕ (199.88%)
Silymarin (50 mg/kg)+CCl4	40.43±2.85# (-65.19%)	126.16±8.57# (-55.65%)	20.13±1.22# (-47.27%)	137.66±8.32*# (-55.51%)
S. villosa (250 mg/kg)+CCl4	61.33±4.66ϕ# (-47.20%)	162.5±9.95ϕ# (-42.88%)	28.49±1.42ϕ# (-25.37%)	188.41±14.49ϕ# (-39.11%)
S. villosa (500 mg/kg)+CCl4	54.16±3.91*# (-53.37%)	148.74±9.28*# (-47.71%)	22.64±1.85*# (-40.70%)	167.96±12.92*# (-45.72%)

*Values are mean ± SEM (n = 6), values in parenthesis indicate % change; *$p < 0.05$: statistically significant from normal control (Dunnett's test); ϕ$p < 0.01$: statistically significant from normal control (Dunnett's test); ▪$p < 0.05$: Statistically significant from CCl₄-hepatotoxic control (Dunnett's test; #$p < 0.01$: statistically significant from CCl₄-hepatotoxic control (Dunnett's test)*

Table 3: Effect of *S. villosa* extract on the serum levels of BRN, TP, and ALB in rats with CCl₄-induced hepatotoxicity

Group	BRN (mg/dL)	Total protein (g/dL)	ALB (g/dL)
Normal control	0.64±0.04#	8.4±0.36#	4.1±0.26#
CCl₄-hepatotoxic control	1.71±0.12ϕ (167.18%)	5.1±0.28ϕ (-39.28%)	2.3±0.10ϕ (-43.90%)
Silymarin (50 mg/kg)+CCl4	0.80±0.07# (-53.21%)	7.8±0.40# (52.94%)	3.6±0.15# (56.52%)
S. villosa (250 mg/kg)+CCl4	1.25±0.10ϕ▪ (-26.90%)	6.5±0.37*▪ (27.45%)	2.9±0.11*▪ (26.08%)
S. villosa (500 mg/kg)+CCl4	1.04±0.08*# (-39.18%)	7.1±0.49▪ (39.21%)	3.2±0.26▪ (39.13%)

*Values are mean ± SEM (n = 6), values in parenthesis indicate % change; * $p < 0.05$: statistically significant from normal control (Dunnett's test); ϕ$p < 0.01$: statistically significant from normal control (Dunnett's test); ▪$p < 0.05$: Statistically significant from CCl₄-hepatotoxic control (Dunnett's test; #$p < 0.01$: statistically significant from CCl₄-hepatotoxic control (Dunnett's test)*

Table 4: Effect of *S. villosa* extract on CCl$_4$-induced oxidative stress in rats

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GSH (µmol/ g tissue)	MDA (nmol/g tissue)
Normal control	60.2±3.57	15.3±0.82	3.85±0.27	10.2±0.87	2.21±0.18
CCl$_4$-hepatotoxic control	34.7±1.35φ (-42.35%)	8.5±0.43φ (-44.07%)	1.54±0.11φ (-60.00%)	5.5±0.35φ (-46.07)	8.65±0.65φ (291.40%)
Silymarin (50 mg/kg)+CCl$_4$	52.2±1.97# (50.43%)	13.7±0.78# (61.17%)	2.87±0.18*# (86.36%)	9.0±0.61# (63.63)	2.87±0.25# (-66.82%)
S. villosa (250 mg/kg)+CCl$_4$	43.7±2.35*▪ (25.93%)	10.9±0.84▪ (28.23%)	2.48±0.11*# (61.03%)	7.7±0.47*▪ (40.00)	4.15±0.34φ# (-52.02%)
S. villosa (500 mg/kg)+CCl$_4$	46.8±2.84*▪ (34.87%)	11.2±0.96*▪ (31.76%)	2.75±0.18*# (78.57%)	8.1±0.59▪ (47.27)	3.84±0.22φ# (-55.60%)

*Values are mean ± SEM (n = 6), values in parenthesis indicate % change; * p < 0.05: statistically significant from normal control (Dunnett's test); φp < 0.01: statistically significant from normal control (Dunnett's test); ▪ p < 0.05: Statistically significant from CCl$_4$-hepatotoxic control (Dunnett's test; #p < 0.01: statistically significant from CCl$_4$-hepatotoxic control (Dunnett's test)*
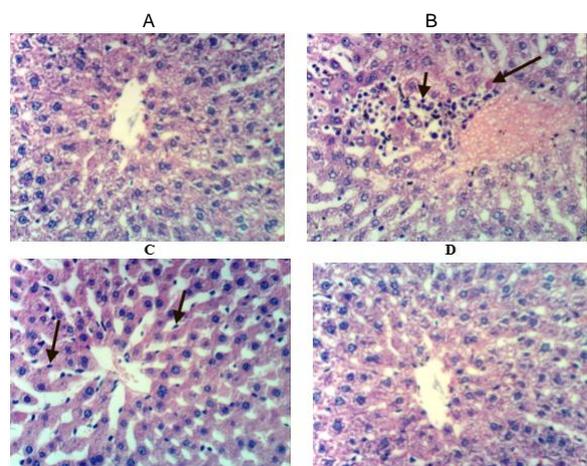
In addition, the protective action of *S. villosa* on the most potent endogenous antioxidants, GSH was estimated in liver tissue of rats (Table 4). Pretreatment with *S. villosa* (250 and 500 mg/kg) was found to protect against GSH depletion in the liver homogenate of CCl$_4$-exposed rats. Further, both doses reduced the increased MDA level in liver, when compared to CCl$_4$-exposed rats.

Figure 1: Liver (H & E X 400) of rats from (A) normal control group, (B) CCl$_4$-exposed group, (C) *S. villosa* (250 mg/kg) + CCl$_4$ group and (D) *S. villosa* (500 mg/kg) + CCl$_4$ group

Histological assessment of the liver of the normal rats showed the typical hepatic architecture of hepatic lobule (Figure 1A). The typical architecture of hepatic tissues was lost in rats exposed to CCl$_4$. Liver sections of CCl$_4$-treated rats showed severe necrosis, infiltration of neutrophils, and hydropic degeneration of the liver tissue (Figure 1B). The hepatoprotective effect of *S. villosa* was confirmed by histological inspection of the liver tissue. Pretreatment of rats with both doses of *S. villosa* reduced the severity of CCl$_4$-induced liver intoxication. Moderate necrotic changes were noticed in hepatic tissues

of animals pretreated with *S. villosa* extract at 250 mg/kg (Figure 1C). Liver tissue from rats pretreated with large dose of the extract had normal hepatic cells with mild necrotic and inflammatory changes of some hepatocytes (Figure 1D).

DISCUSSION

The results of acute toxicity study revealed that *S. villosa* extract is non-toxic. Treatment with 5000 mg/kg of *S. villosa* extract was well tolerated by rats, since there were no toxic symptoms or deaths noted during the experimental period of acute toxicity study. In this investigation, the value of the oral LD$_{50}$ of *S. villosa* extract was not estimated in rats being in excess of 5000 mg/kg. Generally, the lower the value of the LD$_{50}$, the higher the toxicity of the compound. Thus, the methanolic extract of *S. villosa* can be classified as non-toxic as compounds having oral LD$_{50}$ value of more than 4 g/kg are considered as being nontoxic [16].

In the current study, the potential anti-inflammatory activity of *S. villosa* was investigated in rats. Carrageenan-induced paw edema in rats is one of the experimental animal model for exploring anti-inflammatory potential of extracts and other compounds [17]. Subcutaneous injection of carrageenan in the hind paw of rats induced local edema. The inflammatory response against carrageenan includes a stepwise release of vasoactive compounds such as histamine, serotonin, and bradykinin in the early phase and prostaglandins in the acute late stage [18]. These vasoactive compounds produced an increase in the permeability of blood vessels, therefore enhancing fluid accumulation in the tissues resulting in edema.

The present results indicate that *S. villosa* suppressed the volume of edema induced by carrageenan in rat's paws in a dose-dependent manner. The ability of *S. villosa* to decrease the volume of edema proposes that it has chemical constituent(s) that may be active against inflammation.

The hepatoprotective potential of *S. villosa* extract was studied using the model of CCl_4-induced hepatotoxicity in rats. Levels of liver enzymes (ALT, AST, γ-GT, and ALP) in serum have long been considered as a sensitive index of liver damage [19]. As expected, CCl_4 markedly elevated the serum levels of liver marker enzymes in rats. The elevation in the levels of ALT, AST, γ-GT, and ALP in serum may be explained as a result of the damage of hepatic cells or alterations in their membrane permeability denoting drastic liver injury. Pretreatment with *S. villosa* extract at 250 and 500 mg/kg was able to protect against the elevation in the level of these enzymes in serum of CCl_4-exposed animals. These results can be referred to as the ability of the extract to keep the membrane integrity of hepatocytes of the CCl_4-exposed rats by reducing the production of the reactive metabolites of CCl_4.

Serum BRN is considered as one of the most sensitive tests of liver functions. The increased level of serum BRN in CCl_4-exposed rats indicates hepatic parenchymal damage. A possible explanation is that CCl_4 exposure is known to induce the generation of reactive oxygen species that has the possibility to elicit tissue damage. This study demonstrated that *S. villosa* extract and silymarin had reduced the level of serum BRN which was elevated by CCl_4 administration.

ALB is the most abundant plasma protein produced by hepatocytes. Therefore, serum levels of TP or ALB reflect the functional status of the liver [20]. It is well known that exposure to CCl_4 induces a marked reduction in levels of TP and ALB in serum [21].

In the present study, a marked reduction in levels of TP and ALB was observed in serum of CCl_4 control group in relation to the normal control. However, pretreatment with *S. villosa* extract showed a significant reversal of TP and ALB toward their normal levels and suggested the stabilization of endoplasmic reticulum that is responsible for protein synthesis. This assures the protective activity of *S. villos* against CCl_4-hepatotoxicity.

Oxidative stress is reported to represent the main

mechanism in the pathogenesis of CCl_4-induced hepatotoxicity in experimental animals [22]. Oxidative stress induced by CCl_4 is largely attributed to its active metabolite, trichloromethyl radical that plays a key role in producing liver damage [23]. However, cells have diverse defense processes including enzymatic and non-enzymatic antioxidants mechanisms to keep themselves against the adverse effects of free radicals. The oxidative stress gives rise to toxicity when the rate of free radicals generation outweighs the cell's ability for their removal.

The antioxidant enzymes (SOD, CAT and GPx) present the natural first line of antioxidative stress in the liver tissue. They play a substantial function in neutralizing the toxic intermediate of oxidation. SOD and CAT are the most important enzymes to remove the free radicals *in vivo*. SOD stimulates the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), which is then degraded by GPx or by CAT to H_2O. Reduction in the antioxidant enzymes activities may increase the availability of H_2O_2 and O_2^-, which in turn produces hydroxyl radicals that initiate LPO [24].

In this investigation, marked decreases in the activities of SOD, CAT, and GPx enzymes were detected in the liver homogenate of CCl_4-exposed animals. These findings revealed the role of CCl_4 in inducing oxidative stress in the hepatic tissue. In addition, these results corroborate the observations of Manubolu *et al* [22] who explained that the activity of the antioxidant enzymes is decreased in tissues of CCl_4-exposed rats. One probable mechanism for the decreased activities of SOD, CAT and GPx may resulted from generation of the active metabolite, trichloromethyl radical leading to oxidative stress in the tissues. Furthermore, this investigation has established that the administration of *S. villosa* extract (250 and 500 mg/kg) prevented the reduction in the activities of the antioxidants enzymes in liver of CCl_4-exposed animals, showing a probable role of the extract in free radical inactivation and in the antioxidant defense. Therefore, the hepatoprotective effect of *S. villosa* extract could relate to its ability to preserve the antioxidant enzymes activities.

GSH is a major non-enzymatic antioxidants of the liver cells. It keeps the protein thiols of the hepatocyte membrane against the harmful effects of reactive oxygen metabolites [25]. In cases of oxidative stress, GSH is depleted and transformed to glutathione disulfide, leading to lipid peroxidation. Thus, GSH is considered as a

significant marker for the assessment of oxidative stress [26].

In this study, the depletion of cellular GSH following CCl$_4$ exposure leaves the cell vulnerable to oxidative stress. Pretreatment with *S. villosa* extract preserved the GSH contents in the livers of CCl$_4$-exposed rats that further demonstrated the anti-oxidant property of this plant. The increased level of MDA is a marker for cell membrane LPO and cell damage [27]. According to Luqman and Rizvi [28], lipid peroxidation is known to injure the cells by inactivation of membrane enzymes, decrease fluidity of the membrane and resolve into cytotoxic aldehydes such as MDA. In this connection, Recknagel *et al* [26] stated that CCl$_4$ is metabolically activated by CYP2E1to trichloromethyl free radical (CCl$_3$•) interacting with lipids and proteins of the cells in the presence of oxygen to induce LPO.

In this investigation, there were marked elevations in LPO in the homogenate of CCl$_4$-hepatotoxic animals, as measured by MDA formation. The elevated level of hepatic MDA indicates the inability of the antioxidant defense system to protect against the production of excessive free radicals. The present results showed that *S. villosa* extract ameliorated the increased liver MDA contents of CCl$_4$-exposed rats toward normalcy. The decreased amount of MDA in addition to the increased level of GSH in the liver of groups administered *S. villosa* extract further confirms its antioxidant capacity. In addition, hepatoprotective activity against CCl$_4$-induced hepatic damage achieved by interfering with CCl$_4$-mediated LPO via reduction of generation of free radicals [29] or due to the antioxidant effect of the protective agent itself [30].

The levels of TP and ALB would be reduced in cases of hepatotoxicity as a result of defective protein synthesis in the liver. Therefore, the decrease in TP and ALB levels is a further sign of hepatotoxicity in CCl$_4$-exposed animals [31]. In this study, the levels of TP and ALB in the liver tissues were restored close to the normal values, suggesting the protective action of *S. villosa* extract. The possible mechanism behind the protective effect of *S. villosa* extract could be related to the antioxidant property of the phytoconstituents of the plant. Flavonoids, tannins, coumarins, triterpenoids and saponins are among the chemical constituents identified in plants of the genus *Silene* [32]. The antioxidant activity of some *Silene* plants has been attributed to their high levels of phenolics and flavonoids [33]. Therefore, we believe that the possible mechanism of hepatoprotection offered by *S. villosa* extract is due to its phytocomponents as phenolics, triterpenoid, saponins and flavonoids.

Histopathological observations supported the biochemical results. Histological examination of rat liver treated with CCl$_4$ alone showed marked necrosis, neutrophil infiltration and hydropic degenerative changes of the hepatocytes that might be due to the production of free radicals by CCl$_4$ and subsequent LPO. Pretreatment with *S. villosa* extract exhibited considerable hepatic protection in a dose-dependent pattern, which confirmed the results of biochemical studies.

CONCLUSION

The findings of the study reveal the promising anti-inflammatory and hepatoprotective potential of *S. villosa* plant in rats. The hepatoprotective effect may be mediated through its anti-inflammatory and/or antioxidant actions. Accordingly, the plant extract could be developed as an effective herbal drug for protection against chemical-induced hepatic damage. Additional studies are underway to isolate the active compounds responsible for the protective effect of *S. villosa*.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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