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Research Article

**CARDIOPROTECTIVE AND NEPHROPROTECTIVE ACTIONS OF  
METHANOLIC EXTRACT OF *PULICARIA SOMALENSIS* HERBS  
AGAINST CARBON TETRACHLORIDE INDUCED TOXICITY IN RATS****Hasan S. Yusufoglu<sup>1,\*</sup>, Mohd Nazam Ansari<sup>2</sup>, Gamal A. Soliman<sup>2,3</sup>, Ahmed I. Foudah<sup>1</sup>,  
Mohammed H. Alqarni<sup>1</sup>, Aftab Alam<sup>1</sup>, Mohammad Ayman Salkini<sup>1</sup>**<sup>1</sup>Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA.<sup>2</sup>Department of Pharmacology, College of Pharmacy; Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA.<sup>3</sup>Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt.**Abstract:**

Previous studies reported that *Pulicaria somalensis* (Asteraceae) and related members of this family have long been used in folk medicines and because of its antioxidant activity, has an effective role in protection of biomarkers of cardiac and renal dysfunction. The aim of the present study was to evaluate the protective effects of *Pulicaria somalensis* methanolic extract (PSME) on carbon tetrachloride (CCl<sub>4</sub>)-induced changes in cardio- and nephro-toxicity. For the cardio-protective activity, biochemical parameters such as LDH, creatine kinase and albumin in serum, total protein, MDA and NP-SH levels in myocardial tissues were estimated. For the evaluation of nephro-protective activity, biochemical parameters such as creatinine, urea, uric acid, sodium, potassium and calcium in serum, total protein, MDA and NPSH levels in renal tissues were assessed. The results of the present finding showed a significant (P<0.01-0.001) protection of cardiac and renal biochemical markers in serum and tissues, at both the doses of PSME (250 and 500 mg/kg). The cardiac and renal protective markers were further confirmed by the histopathological examination of cardiac and renal tissues. On the basis of present findings, it can be concluded that CCl<sub>4</sub>-intoxication induced cardiac and renal biochemical markers dysfunction reversed by the PSME treatment.

**Keywords:** *Pulicaria somalensis*, cardioprotective, nephroprotective, Carbon tetrachloride, Histopathology**\* Corresponding Author:****Prof. Dr. Hasan Soliman Yusufoglu,**

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## INTRODUCTION:

Kidneys are responsible for regulating homeostasis function via balancing of electrolytes in the blood, while heart maintain homeostasis via pumping blood throughout the body. However, various side effects are seen in kidney and heart tissues in spite of the growth in the concern synthetic medicine. Traditional herbal drugs due to their safety and efficacy are become popular for the treatment of nephrotoxicity and cardiac dysfunctions. There are various lines of indication which implicated oxidative stress in the etiology of cardiovascular and kidney diseases. [1,2] *P. somalensis*, a shrub or woody herb reported the presence of diterpenes and flavonoids. [3] The in vitro DPPH and FRAPS antioxidant model showed marked antioxidant properties of MEPS, due to presence of active constituents (phenol, tannins and flavonoids). [4] The related *Pulicaria* species are reported for several actions such as anti-inflammatory, antileukemic and chemopreventive agents. [5] Some of the closed *Pulicaria* species are tested for analgesic, antipyretic, anti-inflammatory, hepatoprotective, nephritic, antimicrobial, antidiarrheal, antischistosomiasis, antifungal, antimalarial and insecticidal activities. [6-8] Antimicrobial and anticancerous studies of the isolated guaianolide sesquiterpenes of *Pulicaria* species showed antibacterial activity against *M. phlei* with potent cytotoxic activities. [9] Phytochemical studies of one of the *Pulicaria* species have shown that, it has a rich source of sesquiterpene and diterpenes. [10,11] Antioxidants flavonoids [12] and the presence of a 6-hydroxyflavone was identified in *P. burchar*. [13] Carbon tetrachloride (CCl<sub>4</sub>), which produces reactive free radicals when metabolized, has been widely used as a solvent for the induction of hepatic damage with simultaneous responsible for cardiovascular and kidney disfunction. [14] CCl<sub>4</sub>-intoxication in animals increases LDH, CK and decreases albumin levels in cardiac damage [15] while increases creatinine, Na, K, Ca, Urea and Uric acid in kidney disfunction. [16] To the best of our knowledge, there was no scientific reports in support of protective nature of *P. somalensis* in cardiac and kidney disfunction despite of its tremendous antioxidative potential. Therefore, the present study was aimed to investigate the possible cardioprotective and nephroprotective effects of *Pulicaria somalensis* mthanolic extract (PSME) on CCl<sub>4</sub>-induced toxicity in the Wistar albino rats.

## MATERIALS AND METHODS:

### Animals

Healthy male Wistar albino rats (180-200 g) were used for the present study. Total of 30 animals were obtained from Lab Animal Care Unit, College of

Pharmacy, King Saud University. The rats were randomly divided into 5 groups of 6 animals each and were kept in standard plastic animal cages separately with 12-hour light and dark cycle under standard environmental conditions of temperature. The rats were provided with standard rat chow diet and tap water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days, prior to experiments. All animals were approved by Institutional Animal Ethics Committee of College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia.

### Plant Collection and Extract Preparation

The aerial parts of *Pulicaria somalensis* was collected in early march 2014 from the new industrial area 17 km south west Alkharj City. The collected plant was authenticated by taxonomist Dr. M. Atiqur Rahman, from College of Pharmacy, Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University, Riyadh. A voucher specimen (PSAU-CPH-3-2014) is maintained in the herbarium of College of Pharmacy, Prince Sattam Bin Abdulaziz University. The shed dried herbs (500 g) were coarsely powdered and macerated in 3 liters of 90% Methanol for 72 h using percolation method. The methanol was then removed at 40°C under reduced pressure in a rotary evaporator. The *Pulicaria somalensis* mthanolic extract (PSME) was then suspended in distilled water just before its administration to the Wistar albino rats.

### Experimental Procedure

Wistar albino rats were arbitrarily divided into five groups. Group I (normal control) received saline (1 mL/kg, p.o.) only for seven days, Group II (toxic control) received normal saline (1 mL/kg, p.o.) for 7 days. Group III (positive control) was prophylactically treated with silymarin (10 mg/kg, p.o.) for 7 days. Groups IV and V (Test groups) were prophylactically treated with PSME at doses of 250 and 500 mg/kg, p.o. respectively. On the 8th day, except Group I (normal), all were injected intraperitoneally (i.p.) with 0.4 ml/kg of CCl<sub>4</sub>, as a 20% solution in paraffin oil.

### Assessment of cardiac and renal function markers in serum

After 24 h of CCl<sub>4</sub> injection, blood samples from all the rats were collected from retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm for 15 minutes and was transferred to pre-labeled eppendorf tubes for assessment of various biochemical parameters for the cardiac and renal function tests. For the cardiac function, the biochemical parameters such as lactate

dehydrogenase (LDH), creatinine kinase (CK) and Albumin were analyzed. For the renal function, the biochemical parameters such as creatinine, urea, uric acid and electrolytes (calcium, sodium and potassium) were estimated. All parameters were analyzed at College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia using different diagnostic kits.

Immediately, after blood collection, all animals were sacrificed by light ether anaesthesia, then, heart and kidney samples were collected, washed with chilled normal saline, followed by processing for biochemical estimations in tissues and histopathological studies.

#### **Preparation of kidney and heart homogenate**

Collected heart and kidney samples were homogenized in ice cold 0.15 M KCl solution using motor driven Teflon pestle. Homogenized tissues were treated with ethylene-diamine tetra acetic acid (EDTA, pH 7.4) followed by centrifugation at 12000 rpm for 20 minutes. The supernatant was used for the estimation of total protein, NP-SH and malondialdehyde (MDA).

#### **Estimation of biochemical markers in heart and kidney homogenate**

Total protein contents, [17] Malondialdehyde (MDA) [18] and non-protein sulfhydryls (NP-SH) [19] were estimated in tissue homogenates by the previously described methods. The estimation of malondialdehyde (MDA) and non-protein sulfhydryls (NP-SH) were used for oxidative stress. In brief, for MDA, 0.2 mL of tissues sample separately kept in a different test tube and then incubated at 37°C for one hour and then added, one mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA) and then boil for five minutes at 95°C. The tube was cooled and then centrifuge. The absorbance of supernatant was measured at 532 nm. For the estimation of NP-SH, 0.1 mL of the supernatant was suspended in tris buffer, 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB) and absorbance was

measured instantly at 412 nm against blank. The result of both MDA and NP-SH were expressed as nmol/mg.

#### **Histopathological Assessment**

For microscopic assessment, heart and kidney tissues were fixed in a graded series of solution (absolute alcohol 60%, formaldehyde 30%, glacial acetic acid 10%) and embedded in paraffin wax. The tissue sections (3  $\mu$ m) were made by rotary microtome (Leitz 1512), mounted on slides and then, placed in an oven with a temperature of 60°C for 15 minutes and then stained with hematoxylin/eosin dye subsequently and observed under a light microscope.

#### **Statistical analysis**

Graph Pad Prism 7.0 Software (GraphPad software, San diego, CA, USA) was used for statistical analysis. The comparison between the groups were done by means of one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, the  $P < 0.001$  was considered as significant.

#### **RESULTS:**

##### **Effect of PSME on cardiac function markers in serum**

Table 1 shows the effects of PSME on cardiac function markers in CCl<sub>4</sub>-intoxicated rats. LDH and Creatin kinase were significantly ( $p < 0.001$ ) elevated in the CCl<sub>4</sub> intoxicated rats ( $310.66 \pm 9.38$  and  $416.00 \pm 13.29$  U/L, respectively) when compared to the normal animals ( $152.50 \pm 4.10$  and  $235.66 \pm 14.32$  U/L, respectively). Administration of PSME at doses of 250 and 500 mg/kg prior to CCl<sub>4</sub> treatment, significantly ( $p < 0.05-0.001$ ) protected the elevated LDH and Creatin kinase levels. The serum albumin levels were reduced in rats treated with CCl<sub>4</sub> ( $1.93 \pm 0.08$  mg/dl) when compare to normal group animals ( $5.16 \pm 0.14$  mg/dl). Administration of PSME at doses of 250 and 500 mg/kg prior to CCl<sub>4</sub> treatment, significantly ( $p < 0.001$ ) maintain the normal albumin levels.

**Table 1: Effect of PSME on cardiac function markers in CCl<sub>4</sub>-intoxicated rats**

Groups	Dose (mg/kg)	LDH (U/L)		Creatine kinase (U/L)		Albumin (mg/dl)	
		Mean ± SEM	% Change	Mean ± SEM	% Change	Mean ± SEM	% Change
Normal control		152.50 ± 4.10		235.66 ± 14.32		5.16 ± 0.14	
Toxic control (CCl <sub>4</sub> )		310.66 ± 9.38*** a		416.00 ± 13.29*** a		1.93 ± 0.08*** a	
Silymarin + CCl <sub>4</sub>	10	180.66 ± 3.98*** b	42	257.66 ± 9.57*** b	38	4.10 ± 0.16*** b	107
PSME + CCl <sub>4</sub>	250	268.16 ± 13.05* b	14	316.33 ± 12.31*** b	24	2.68 ± 0.09*** b	39
PSME + CCl <sub>4</sub>	500	214.50 ± 9.37*** b	40	265.83 ± 10.44*** b	36	3.70 ± 0.16*** b	91

All values are Mean ± SEM (n = 6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, ANOVA, followed by Dunnett's multiple comparison test. Where a denote comparison with normal control group and b denotes comparison with toxic control group.

#### Effect of PSME on kidney function markers and electrolytes in serum

Table 2 and 3 shows the effects of PSME on renal function markers and electrolytes levels in the CCl<sub>4</sub>-intoxicated rats. The kidney function markers such as creatinine, urea and uric acid in CCl<sub>4</sub> intoxicated group of rats were 3.50 ± 0.23, 224.33 ± 11.59 and 6.36 ± 0.19 mg/dl, respectively when compare to normal control group 0.92 ± 0.03, 37.63 ± 1.86 and 2.08 ± 0.12 respectively. The elevated level of renal function markers were significantly (p < 0.05-0.001)

maintain in the PSME and silymarin groups of animals (Table 2). The serum of CCl<sub>4</sub> injected rats showed a significant (p < 0.001) increase in the levels of sodium (151.49 ± 1.64 mEq/L), potassium (11.53 ± 0.49 mEq/L) and calcium (13.85 ± 0.51 mg/dl) when compared with normal control group. Administration of PSME (250 and 500 mg/kg) significantly (p < 0.001) improved the levels of sodium, potassium and calcium at higher dose (Table 3).

**Table 2: Effect of PSME on Kidney function markers in CCl<sub>4</sub>-intoxicated rats**

Groups	Dose (mg/kg)	Creatinine (mg/dl)		Urea (mg/dl)		Uric Acid (mg/dl)	
		Mean ± SEM	% Change	Mean ± SEM	% Change	Mean ± SEM	% Change
Normal control		0.92 ± 0.03		37.63 ± 1.86		2.08 ± 0.12	
Toxic control (CCl <sub>4</sub> )		3.50 ± 0.23 *** a		224.33 ± 11.59*** a		6.36 ± 0.19*** a	
Silymarin + CCl <sub>4</sub>	10	1.80 ± 0.10 *** b	49	108.78 ± 6.08*** b	52	3.11 ± 0.37*** b	51
PSME + CCl <sub>4</sub>	250	3.15 ± 0.11 <sup>b</sup>	10	178.16 ± 5.65*** <sup>b</sup>	21	4.80 ± 0.23*** <sup>b</sup>	25
PSME + CCl <sub>4</sub>	500	2.75 ± 0.13* <sup>b</sup>	21	164.66 ± 4.49*** <sup>b</sup>	27	3.88 ± 0.09*** <sup>b</sup>	39

All values are Mean ± SEM (n = 6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, ANOVA, followed by Dunnett's multiple comparison test. Where a denote comparison with normal control group and b denotes comparison with toxic control group.

**Table 3: Effect of PSME on serum electrolyte levels in CCl<sub>4</sub>-intoxicated rats**

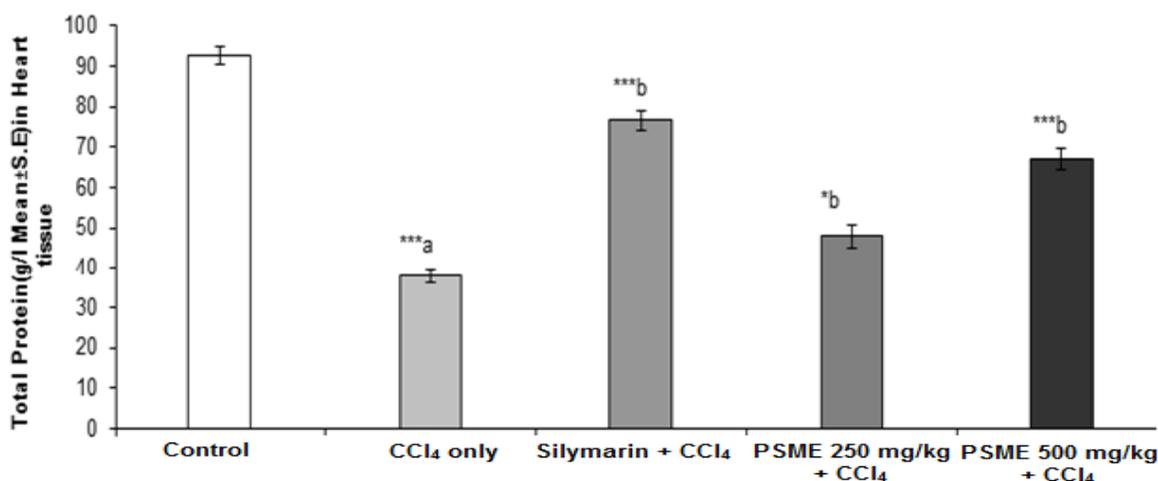
Groups	Dose (mg/kg)	Sodium (mEq/L)		Potassium (mEq/L)		Calcium (mg/dl)	
		Mean ± SEM	% Change	Mean ± SEM	% Change	Mean ± SEM	% Change
Normal control		111.17 ± 1.28		4.30 ± 0.22		3.76 ± 0.13	
Toxic control (CCl <sub>4</sub> )		151.49 ± 1.64*** <sup>a</sup>		11.53 ± 0.49*** <sup>a</sup>		18.85 ± 0.65*** <sup>a</sup>	
Silymarin + CCl <sub>4</sub>	10	123.40 ± 3.16*** <sup>b</sup>	19	6.53 ± 0.25*** <sup>b</sup>	43	9.14 ± 0.34*** <sup>b</sup>	51
PSME + CCl <sub>4</sub>	250	146.10 ± 2.44 <sup>b</sup>	4	9.90 ± 0.41* <sup>b</sup>	14	16.76 ± 0.71 <sup>b</sup>	11
PSME + CCl <sub>4</sub>	500	137.33 ± 2.28*** <sup>b</sup>	9	8.00 ± 0.30*** <sup>b</sup>	31	13.80 ± 0.35*** <sup>b</sup>	27

All values are Mean ± SEM (n = 6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, ANOVA, followed by Dunnett's multiple comparison test. Where a denote comparison with normal control group and b denotes comparison with toxic control group.

#### Effect of PSME on myocardial oxidative stress markers

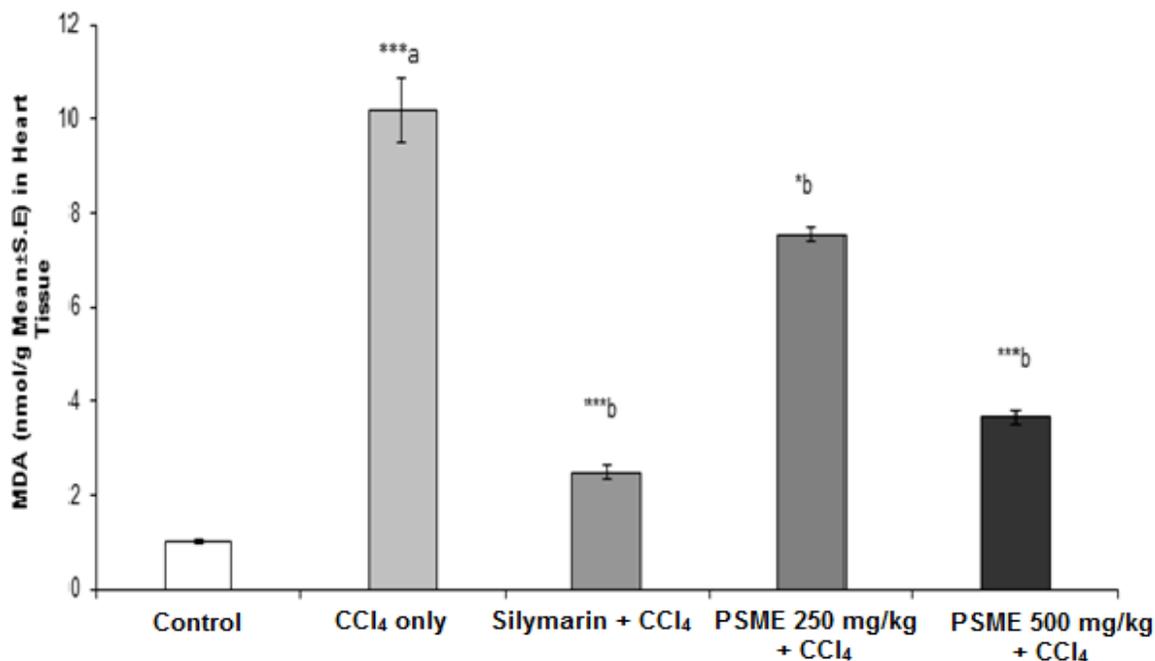
Total protein, MDA and NPSH (oxidative stress profile) of heart tissues were shown in Figure 1-3. The level of total protein (Figure 1) in CCl<sub>4</sub> intoxicated rats was significantly decreased (p < 0.001) when compare to the normal group. The level of total proteins in PSME (250 and 500 mg/kg) and silymarin (10 mg/kg) groups were showed the significant (p < 0.05-0.001) improvement in the total protein level. The MDA (≈10.18 nmol/g) was elevated in CCl<sub>4</sub> intoxicated group when compared to

that of the normal group (≈1.03 nmol/g) of rats cardiac tissue (p < 0.001). The significant (p < 0.05-0.001) protective level of MDA (Figure 2) was showed in the both PSME (250 and 500 mg/kg) and silymarin (10 mg/kg) groups. Figure 3 showed the NP-SH (≈2.29 nmol/g) level in CCl<sub>4</sub> was significantly (p < 0.001) decreased in compare to the normal (≈6.46 nmol/g) groups of the rats. Whereas, treatment with PSME (250 and 500 mg/kg) and silymarin significantly (p < 0.05-0.001) protect the heart tissues.



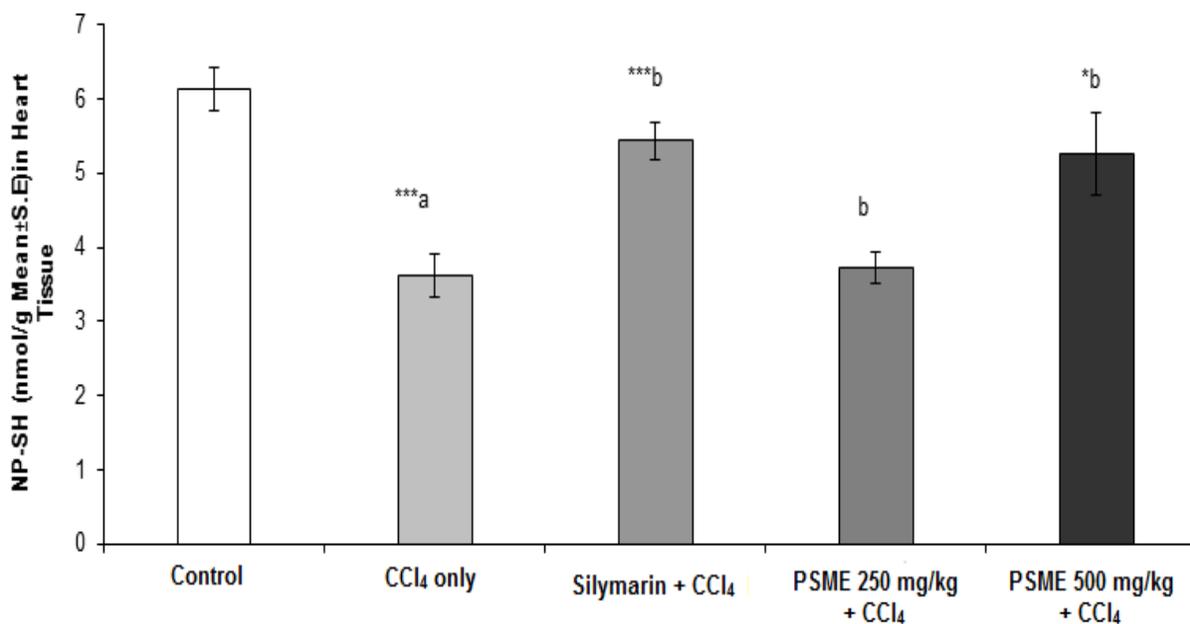
**Figure 1:** Effect of PSME on myocardial total protein concentration in CCl<sub>4</sub>- intoxicated rats.

All values represent mean ± SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with CCl<sub>4</sub> only group



**Figure 2:** Effect of PSME on myocardial MDA concentration in CCl<sub>4</sub>- intoxicated rats.

All values represent mean ± SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with CCl<sub>4</sub> only group



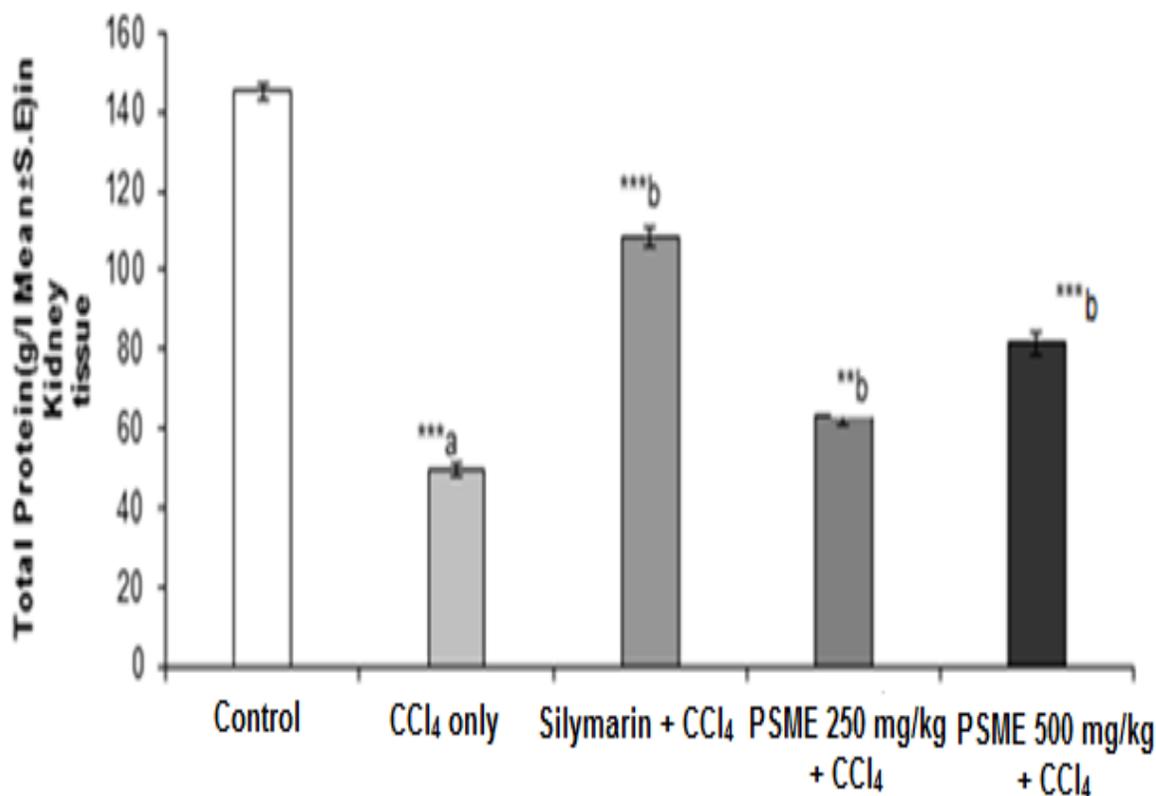
**Figure 3:** Effect of PSME on myocardial NP-SH concentration in CCl<sub>4</sub>- intoxicated rats.

All values represent mean ± SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with CCl<sub>4</sub> only group

**Effect of PSME on renal oxidative stress markers**

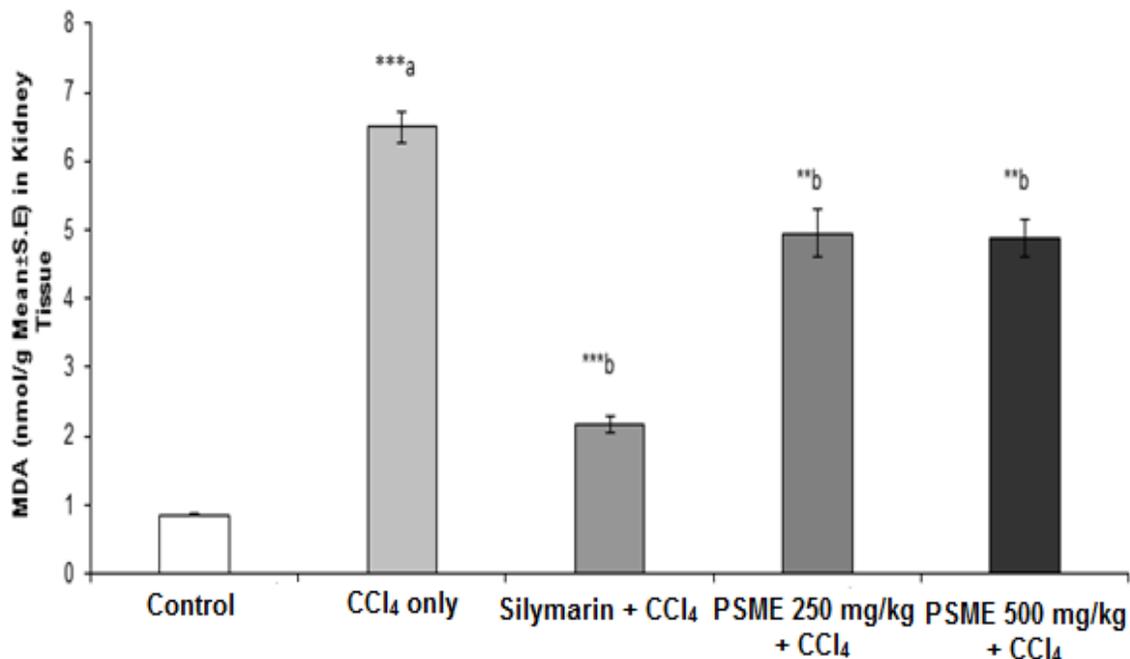
Total protein, MDA and NPSH (oxidative stress profile) of kidney tissues were shown in Figure 4-6. The level of total protein ( $\approx 49.5$ ) (Figure 4) in  $\text{CCl}_4$  intoxicated group was significantly decreased ( $p < 0.001$ ) when compare to the normal control group ( $\approx 145.3$ ). The level of total proteins in the PSME and Silymarin treated groups were showed the significant ( $p < 0.05-0.001$ ) protection. The MDA level ( $\approx 6.5$  nmol/g) was significantly ( $p < 0.001$ ) elevated in Whereas, treatment with PSME with higher dose and silymarin ( $\approx 4.55$  and  $\approx 4.74$  nmol/g, respectively) significantly ( $p < 0.001$ ) protected the kidney tissues.

$\text{CCl}_4$  intoxicated group when compared with normal control group ( $\approx 0.86$  nmol/g). The significant ( $p < 0.05-0.001$ ) protective level of MDA (Figure 5) in kidney tissues were showed in PSME (250 and 500 mg/kg) and silymarin ( $\approx 4.9$ ,  $\approx 4.88$  and  $\approx 2.17$  nmol/g respective) groups. NP-SH ( $\approx 3.23$  nmol/g) level (Figure 6) was significantly ( $p < 0.001$ ) decreased in  $\text{CCl}_4$  intoxication when compare to the normal control rats ( $\approx 5.52$  nmol/g).



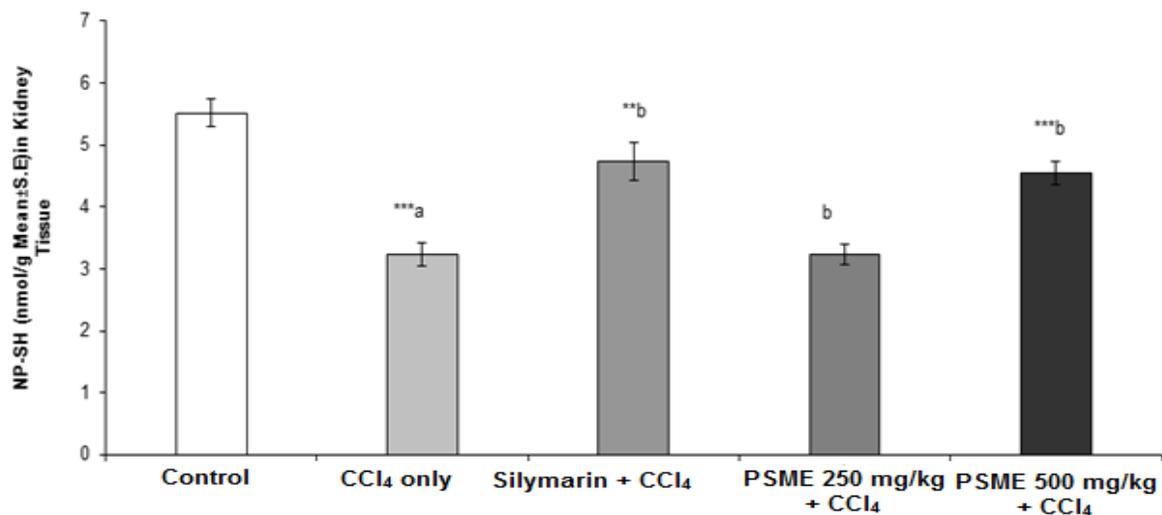
**Figure 4:** Effect of PSME on renal total protein concentration in  $\text{CCl}_4$ - intoxicated rats.

All values represent mean  $\pm$  SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with  $\text{CCl}_4$  only group



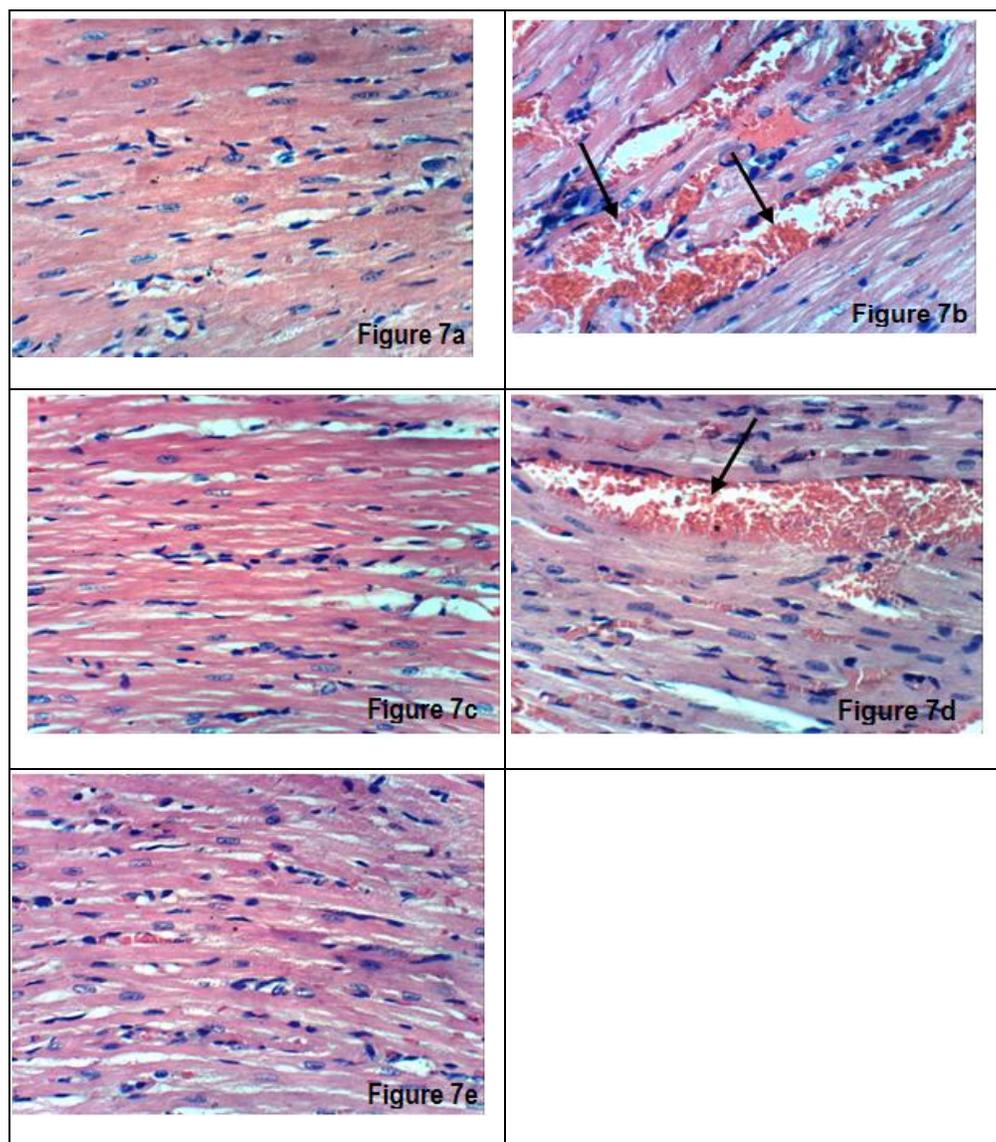
**Figure 5:** Effect of PSME on renal MDA concentration in CCl<sub>4</sub>- intoxicated rats.

All values represent mean  $\pm$  SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with CCl<sub>4</sub> only group

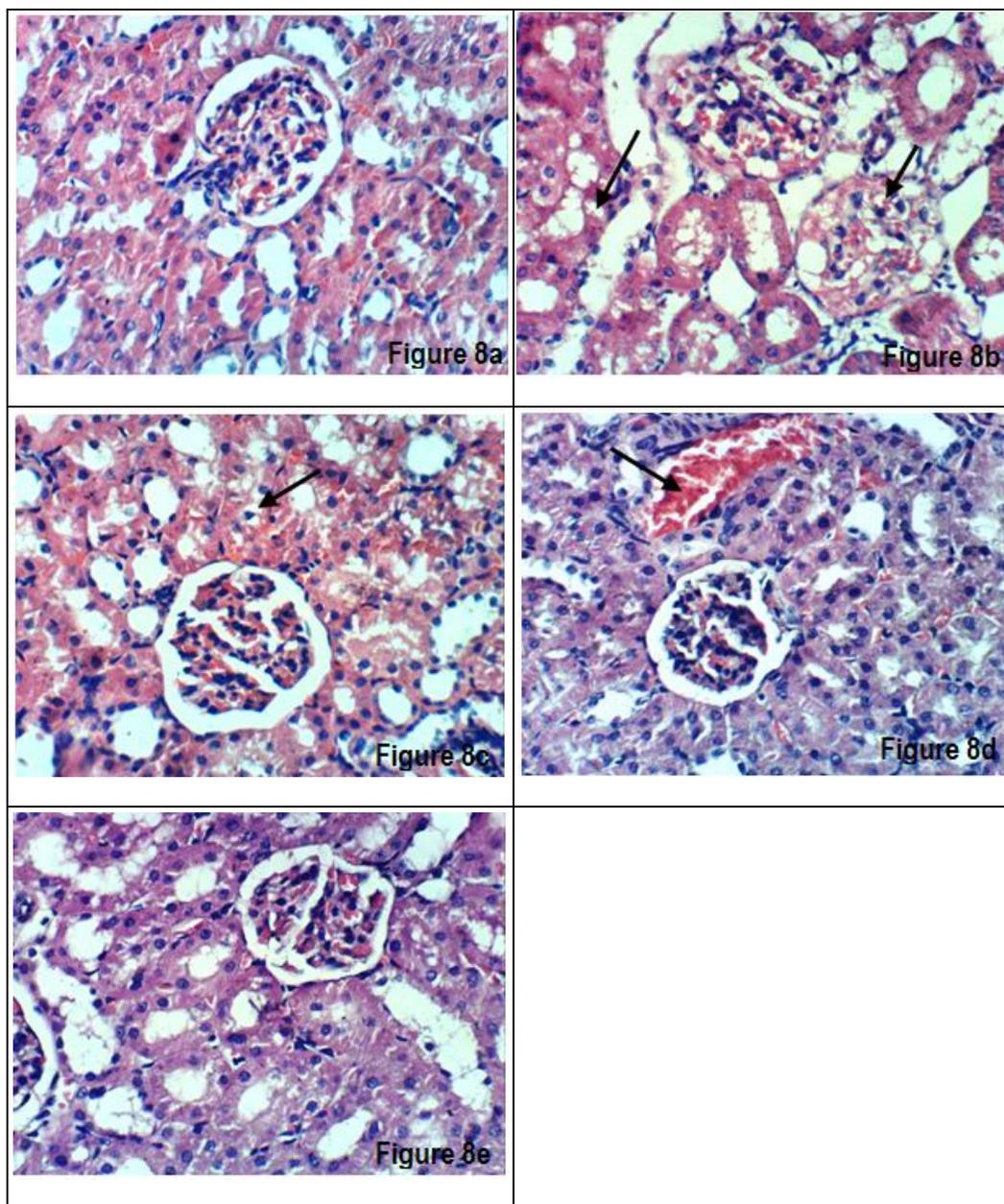


**Figure 6:** Effect of PSME on renal NP-SH concentration in CCl<sub>4</sub>- intoxicated rats.

All values represent mean  $\pm$  SEM (n=6), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Where <sup>a</sup> as compared with Control group and <sup>b</sup> as compared with CCl<sub>4</sub> only group



**Figure 7:** Histopathological section of myocardial tissues (H and E  $\times$  400), (Figure 7a) Section of normal control rat (Group-I), showing normal architecture of cardiac tissue, (Figure 7b) Section of  $\text{CCl}_4$  intoxicated rat (Group-II) showing dilatation and congestion of myocardial blood vessels, (Figure 7c) Section of Silymarin treated rat (Group-III) showing normal architecture with no histopathological changes, (Figure 7d) PSME (250 mg/kg b. wt.) treated rat (Group-IV) showing dilatation and congestion of myocardial blood vessels and (Figure 7e) PSME (500 mg/kg) treated rat (Group-V) showing no histopathological changes.



**Figure 8:** Histopathological section of Kidney tissues (H and E  $\times$  400), (Figure 8a) Section of normal control rat (Group-1) showed normal histological structure of renal parenchyma, (Figure 8b) Section of  $\text{CCl}_4$  intoxicated rat (Group-II) showing vacuolation and congestion of glomerular tuft (small arrow) as well as vacuolation of renal tubular epithelium, (Figure 8c) Section of Silymarin treated rat (Group-III) showing slight vacuolation of renal tubular epithelium, (Figure 8d) PSME (250 mg/kg) treated rat (Group-IV) showing congestion of renal blood vessel, (Figure 8e) PSME (500 mg/kg) treated rat (Group-V) showing no histopathological changes.

#### Effect of PSME on histopathology

The brief histopathological assessment of heart and kidney tissues were given in Fig. 7 and 8, respectively. The photomicrograph of normal rats showed normal cardiac architecture with no histopathological changes (Figure 7a), whereas, the  $\text{CCl}_4$  intoxicated group showed dilatation and congestion of myocardial blood vessels (Figure 7b).

Treatment with Silymarin (Figure 7c), PSME at doses of 250 mg/kg (Figure 7d) and 500 mg/kg (Figure 7e) showed the reversal of myocardial changes. Similarly, renal photomicrograph of normal rats showed normal structure of renal parenchyma (Figure 8a), whereas, the  $\text{CCl}_4$ -intoxicated renal tissue showed vacuolation and congestion of glomerular tuft as well as vacuolation of renal tubular

epithelium (Figure 8b). Treatment with silymarin (Figure 8c), PSME at doses of 250 mg/kg (Figure 8d) and 500 mg/kg (Fig. 8e) exhibited prevention of renal tissue against CCl<sub>4</sub>-intoxicated changes.

### DISCUSSION:

Free radicals increased oxidative stress and decreased in antioxidant defenses is well recognized in several models of cardiotoxicity. [20,21] Carbon tetrachloride (CCl<sub>4</sub>), a typical toxic agent, produces the toxic effects by the production of free radicals. CCl<sub>4</sub> creates methyl trichloride radicals (CCl<sub>3</sub>\*) by the activation of liver cytochromes P450 enzymes, which immediately respond with cell membrane. [22] While liver is considered to be the primary target of CCl<sub>4</sub> toxicity, it also causes free radical generation in heart and kidneys. [23-25] The free radical produced by CCl<sub>4</sub> forms covalent bonds with unsaturated fatty acids and causes the production of chloroform and lipid radicals. [26] The biological membrane dramatically changed by the peroxidation of lipids, ensuing in severe cell damage and therefore, causes a significant role in the different ailments. [27-28] The findings of present study showed that injection of CCl<sub>4</sub> to rats induced oxidative heart damage which is proved by an increase in the LDH and creatine kinase (ATP restoration enzyme) and decreased albumin levels. The findings of present study are in corroborate to the previous recognition of cardiac damage, which involves the measurements of the several cardiac marker enzymes including creatine kinase. [29] Due to change of peroxidation of membrane by oxygen-derived free radicals, cardiac cell membrane gets disturbed, causing leakage of enzymes. [30] Hence, decreased activities of enzymes in heart tissue and increasing concentration in the serum as an indicator of heart injury. [31] The present finding also showed the decrease in total protein, NP-SH and increased in malondialdehyde (MDA) level in hearts of CCl<sub>4</sub>-treated rats when compared with normal rats, representing that the heart is one of the target organs affected by CCl<sub>4</sub>-toxicity. These findings are in agreement with earlier experiments that CCl<sub>4</sub> can cause oxidative damage and produced reactive oxygen species (ROS) in different organs including heart. [32-34] Similarly, increased oxidative stress and decrease in antioxidant defenses by oxygen free radicals is also well recognized in several models of nephrotoxicity. CCl<sub>4</sub> is well established model to induce hepatotoxicity and can also be applies for nephrotoxicity by the production of free radicals. [8,16,35] In present findings, administration of CCl<sub>4</sub> to rats induced oxidative kidney damage, confirmed by elevation in serum level of creatinine, urea, uric acid, Na, K and Ca. According to previous findings, these

pathological changes revealed potential damage to kidney structural integrity. [36] The previous data also showed the similar nephrotoxicity effect with CCl<sub>4</sub> intoxication so our findings are in agreement with previous studies. [23,37] The present finding also showed the decrease in total protein, NP-SH and increased in malondialdehyde (MDA) level in kidney tissues of CCl<sub>4</sub>-treated rats, when compared with normal rats. These findings are in agreement with earlier experiments showing oxidative damage and ROS produced by CCl<sub>4</sub> are responsible for nephrotoxicity. [38,39] The defensive effects of PSME may be due to protective effects against CCl<sub>4</sub> causing oxidative stress. [4] The microscopic structural changes in the cardiac and renal tissues of CCl<sub>4</sub> intoxicated rats were prevented by co-treatment with PSME in the experimental groups. The significant prevention in the structural alterations indicated that PSME scavenged the free radicals to reduce cellular damages.

### CONCLUSION:

To provide protective environment on PSME administration was probably due to protection of biomarkers and histological texture of both heart and kidney tissues against CCl<sub>4</sub> intoxication. Current findings suggested that PSME can be used in various heart and kidney diseases. Additional pharmacological studies are required before using the PSME in different ailments.

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