



## Effects of *Salvia Miltiorrhiza* extract on the regulation of antioxidant enzyme activities in liver and kidney of rats exposed to TCA

Hanan S. Alnahdi<sup>1</sup>, Kholoud S. Ramadan<sup>1,2\*</sup>, Hoda E. A. Farid<sup>1,3</sup> and Najla O. Ayaz<sup>1</sup>

Department of Biochemistry,  
Faculty of Science-Al Faisaliah, King Abdulaziz University, Jeddah, Saudi Arabia.  
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### ABSTRACT

The present study aimed to investigate the protective role of *Salvia Miltiorrhiza* against Trichloroacetic acid (TCA) induced liver and kidney toxicity in rats. Twenty eight adults Wister albino male rats were divided into 4 equal groups. Group 1 served as control, while group 2 received SM extracts at 200 mg/kg body weight; group 3 was treated with 50 mg/kg/day TCA by gavage daily. Group 4 received the extract at 200 mg/kg body weight and 50 mg/kg/day TCA for two months. A significant increase was observed in liver function, kidney function and MDA (malondialdehyde) levels among the TCA administered animals compared to normal control. Daily oral administration of SM normalized most biochemical changes observed among the TCA treated animals. Histopathologically, higher amount of mononuclear cells infiltration, necrotic cells and few fibroblasts were observed in liver and kidney of TCA treated group. The administration of *Salvia Miltiorrhiza* extract regulates and decreases liver and kidney damage.

**Key words:** Antioxidant system, Liver enzymes, Kidney function, *Salvia Miltiorrhiza*, Trichloroacetic Acid.

### INTRODUCTION

*Salvia Miltiorrhiza* is widely used for circulatory and heart health; it appears to be somewhat effective at this claim and is one of the best selling Chinese Medicines for heart health. It works through a few primary ingredients, which all work synergistically on various parameters of heart and circulatory health. It can hunt the oxygen free radicals, originated from ischemia-reperfusion injury as efficiently as SOD in the myocardium, utilizing the low temperature technique of electron spin resonance. The extract can also be established to increase the antioxidants enzyme-activities of CAT and SOD, which is persistent with the originated outcomes with both *in vivo* and *in vitro* (Sun *et al.*, 2005).

Trichloroacetic acid (TCA) is a toxicologically important metabolite of industrial solvents and by-product of drinking water chlorination. Different reports suggested, that TCA induce oxidative stress and overproduction of free radicals because of it cause liver injury and nephrotoxicity. The genotoxicity is ambiguous and nongenotoxic mechanisms cause tumor development in liver (Komulainen, 2004). Chronic studies on TCA treated animals indicated that the induction of different biomarkers of oxidative stress and transition of liver, animal antioxidant enzyme activities were significantly stimulated earlier than induction of any hepatotoxic or hepatocarcinogenic effects leading to them (Hassoun *et al.*, 2010). The supplementation of food with different antioxidants became highly popular, because many

diseases have been linked with oxidative stress. Therefore, in the last years, the free radical scavengers have been fully concentrated, which can protect against the irregular effects of free radicals (Akpinar *et al.*, 2008).

In recent years, the search for natural plant antioxidants gain more significance. *Salvia Miltiorrhiza* Bunge (SM), known as Danshen in Chinese, is considered as traditional medicine for multiple therapeutic remedies. Danshen exhibits properties; such as, microcirculation enhancement, coronary vasodilatation; restrain the aggregation and adhesion of platelets etc. It is extensively used for treatment of coronary artery and different cardiovascular diseases (Shi *et al.*, 2014).

Seven phenolic compounds, isolated from *S. miltiorrhiza* as active components had been demonstrated for strong protective actions against oxidative damage for liver microsomes, hepatocytes and erythrocytes (Liu *et al.*, 1992). Scaduto *et al* (1988) studied the effects of *Salvia miltiorrhiza* ethanol extract on cellular bio-antioxidant, GSH, which is known to be depleted following an ischemic insult. The extract helped in replenishing the GSH pool (Scaduto *et al.*, 1988). Moreover, Chen *et al* (2012) reported that *Salvia miltiorrhiza* ethanol extract causes a significant increase in CAT, GSH-Px and SOD activities in comparison with renal ischemia and reperfusion group. It might have an antioxidant effect through the increase in SOD, GSH-Px and CAT enzyme activities.

\*Corresponding author's e-mail: ramadankholoud@yahoo.com <sup>1</sup>Department of Biochemistry, Faculty of Science-Al Faisaliah Campus, King Abdulaziz University, Jeddah, Saudi Arabia. <sup>2</sup>Biochemistry Department, Faculty of Science, Ain Shams University, Egypt. <sup>3</sup>Biochemistry Department, Faculty of Agriculture, Minufiya University, Egypt.

## MATERIALS AND METHODS

**Chemicals:** The chemicals and reagents used in the study have been obtained from Sigma-Aldrich Chemical Company (StLouis, MO, USA) and were of the highest available grade. The plant was identified by the Herpal Museum, Department of pharmacology, Faculty of Science, King Abdulaziz University of Medical Sciences. There is no deposition number. *Salvia Miltiorrhiza* was taken from the department of pharmacology, King Abdulaziz University of Medical Sciences, Saudi Arabia.

**Preparation of SM extract:** The plant extraction was prepared by soaking 10 gm of the powdered SM root (Haraz store for medicinal plant, Egypt) in 199 ml of distilled water in a conical flask leaving at room temperature for 72 hrs., allowing an extraction of active components in water. Thereafter, the mixture of extract was filtered through Whatman No. 1 filter paper (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and dried using a rotary vacuum evaporator.

**Animals and experimental design:** Twenty-eight Wister albino adult male rats (weighting 170 - 200 g) were obtained from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The local committee approved the design of experiments and protocols that have been carried out, according to the guidelines of institute. All rats have been housed into the cages made up of stainless steel. All cages then placed in a well-ventilated rat house, maintained for two weeks as acclimatization period under the standard laboratory conditions on free supply of food and water ad libitum, and subjected to 12 hour natural light and dark cycles.

**Blood collection and tissue preparation:** At the end of experiment, the 8 hrs fasted rats were anesthetized and sacrificed by cervical dislocation. Blood samples were collected; the clear non hemolyzed serum was collected and stored at -20°C till used in biochemical tests. The liver and kidney were removed immediately and washed with chilled saline solution.

**Biochemical analysis:** The serum samples were analyzed for various activities, such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Gamma Glutamyl Transferase (GGT) (Quimica Clinica Aplicada S.A. kits. Aspartado, Amposta, Spain). The serum levels of conjugated bilirubin

and total protein were determined using the kits from Sentinel Ch. (via principle Eugenio 5-20155 Milan, Italy) and albumin, urea, creatinine and uric acid were measured spectrophotometrically with the help of manual commercial reagent kits. The levels of malondialdehyde (MDA) and reduced glutathione (GSH) were measured spectrophotometrically in liver and kidney tissues with the help of manual commercial reagent kits. Superoxide Dismutase (SOD), Glutathione peroxidase (GPx), and catalase (CAT) activities will be measured in liver and kidney tissues by standard enzyme colorimetric methods using commercial kits.

**Histopathological examinations:** The liver and kidney tissues were cut into small pieces and immersed in a neutral buffered formaline for 24 hours. The fixed tissues were routinely processed, embedded in paraffin, sectioned, deparaffinized, and rehydrated by using standard techniques. The extent of TCA-induced necrosis has been evaluated by assessing the morphological changes in liver and kidney sections stained with Hematoxylin and Eosin (H and E), using some standardized techniques through the light microscope.

**Statistical analysis:** The results have been expressed as mean  $\pm$  Standard Deviation (SD) by using quantitative research design. One-way Analyses of Variance (ANOVA) and student's t-test has been applied using SPSS 15.0 program. A p-value  $\leq$  0.05 is considered to be significant.

**Ethical consideration:** All experimental procedures, described in this study, were approved by King Abdulaziz University ethical committee.

## RESULTS AND DISCUSSION

**Effects of TCA on biochemical liver markers:** It has been revealed through outcomes that the treatment with TCA at 50mg/kg BW induced a significant elevation in serum enzyme activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP), and Gamma-glutamyl transpeptidase (GGT) and level of conjugated bilirubin while the levels of total protein and albumin were decreased compared to control rats, as shown in Table 1. A treatment with SM allowed these parameters to decrease and come near the control group values. *Salvia Miltiorrhiza* has revealed its ability to restore the normal functional status of intoxicated liver and also to protect against the subsequent TCA hepatotoxicity.

**Table 1:** S.M. Aqueous Extract on serum Marker Enzymes Activities, total protein, albumin, and conjugated bilirubin levels.

Parameters	Control	Extract	TCA	Extract + TCA
ALT (U/L)	18 $\pm$ 3 <sup>a</sup>	20 $\pm$ 2 <sup>a</sup>	41 $\pm$ 6 <sup>b</sup>	26 $\pm$ 3 <sup>c</sup>
AST (U/L)	35 $\pm$ 5 <sup>a</sup>	38 $\pm$ 4 <sup>a</sup>	54 $\pm$ 4 <sup>b</sup>	42 $\pm$ 5 <sup>c</sup>
ALP (U/L)	74 $\pm$ 7 <sup>a</sup>	70 $\pm$ 5 <sup>a</sup>	126 $\pm$ 6 <sup>b</sup>	110 $\pm$ 8 <sup>c</sup>
GGT (U/L)	25.1 $\pm$ 0.3 <sup>a</sup>	25.5 $\pm$ 0.34 <sup>a</sup>	53.4 $\pm$ 0.02 <sup>b</sup>	25.8 $\pm$ 0.4 <sup>a</sup>
TP( g/dl)	6.1 $\pm$ 0.14 <sup>a</sup>	6 $\pm$ 0.67 <sup>a</sup>	4.01 $\pm$ 0.48 <sup>b</sup>	6.08 $\pm$ 0.6 <sup>a</sup>
Albumin (g/dl)	4.12 $\pm$ 0.13 <sup>a</sup>	4 $\pm$ 0.23 <sup>a</sup>	2.98 $\pm$ 0.19 <sup>b</sup>	4.01 $\pm$ 0.61 <sup>a</sup>
Conjugated Bilirubin (mg/dl)	0.23 $\pm$ 0.02 <sup>a</sup>	0.24 $\pm$ 0.022 <sup>a</sup>	0.45 $\pm$ 0.016 <sup>b</sup>	0.28 $\pm$ 0.02 <sup>a</sup>

**Table 2:** S.M. Aqueous extract on serum Urea, Uric Acid, and Creatinine Levels Treated with TCA

Parameters	Control	Extract	TCA	Extract + TCA
Creatinine (mg/dl)	0.3 ± 0.08a	0.33 ± 0.06a	0.59 ± 0.03 b	0.34 ± 0.06 a
Uric acid (mg/dl)	0.5 ± 0.01a	0.45 ± 0.012a	0.7 ± 0.014b	0.42 ± 0.019a
Urea (mg/dl)	30 ± 0.03a	33 ± 0.02a	50 ± 0.05b	36 ± 0.06a

**Effect of S.M. Aqueous Extract Pre-treatment on Liver and Renal SOD, CAT and GPx Activities**

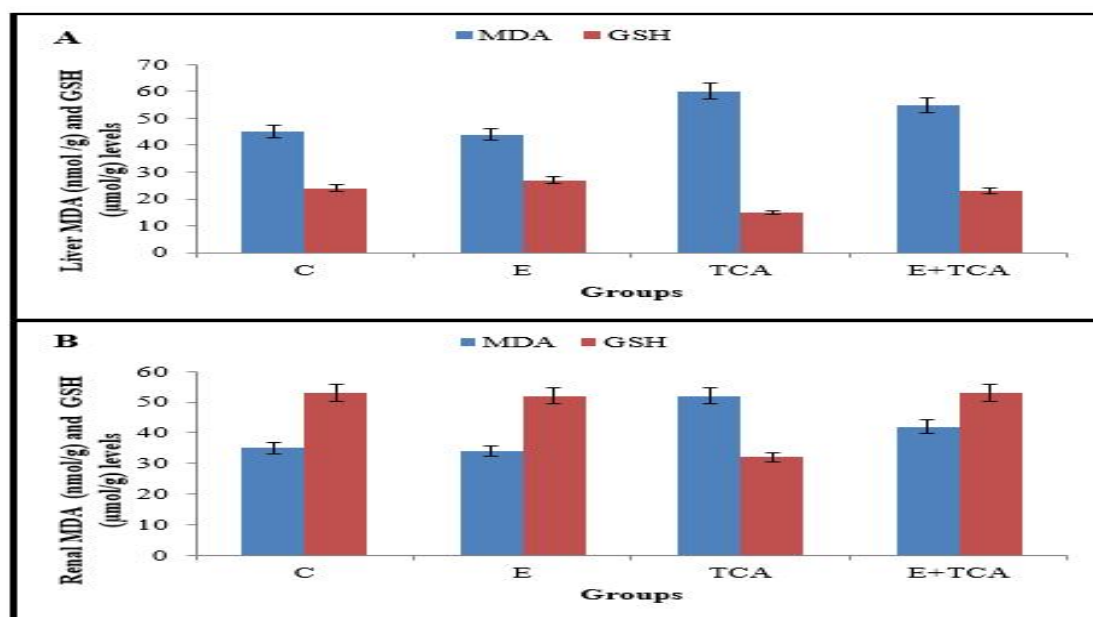
Parameters	Control	Extract	TCA	E + TCA
<i>Liver</i>				
SOD(U/mg protein)	20 ± 1 <sup>a</sup>	22 ± 1.5 <sup>a</sup>	12 ± 2.3 <sup>b</sup>	20 ± 2.4 <sup>a</sup>
CAT(U/mg protein)	25 ± 1 <sup>a</sup>	23 ± 3 <sup>a</sup>	15 ± 2 <sup>b</sup>	25 ± 2.4 <sup>a</sup>
GPx(U/mg protein)	40 ± 1.5 <sup>a</sup>	41 ± 1.1 <sup>a</sup>	29 ± 3.2 <sup>b</sup>	38 ± 3 <sup>a</sup>
<i>Kidney</i>				
SOD(U/mg protein)	95 ± 1 <sup>a</sup>	94 ± 1.5 <sup>a</sup>	44 ± 3.2 <sup>b</sup>	94 ± 4.2 <sup>a</sup>
CAT(U/mg protein)	82 ± 3.5 <sup>a</sup>	83 ± 4.4 <sup>a</sup>	25 ± 1.8 <sup>b</sup>	90 ± 4.8 <sup>a</sup>
GPx(U/ mg protein)	37 ± 4.3 <sup>a</sup>	38 ± 2 <sup>a</sup>	29 ± 4 <sup>b</sup>	39 ± 3.1 <sup>a</sup>

Data are expressed as means ± SD (n=7 rats per group). Comparison between groups was made using Duncan test. Values in the same columns not sharing a common letter (a–b) differ significantly at  $p < 0.05$ .

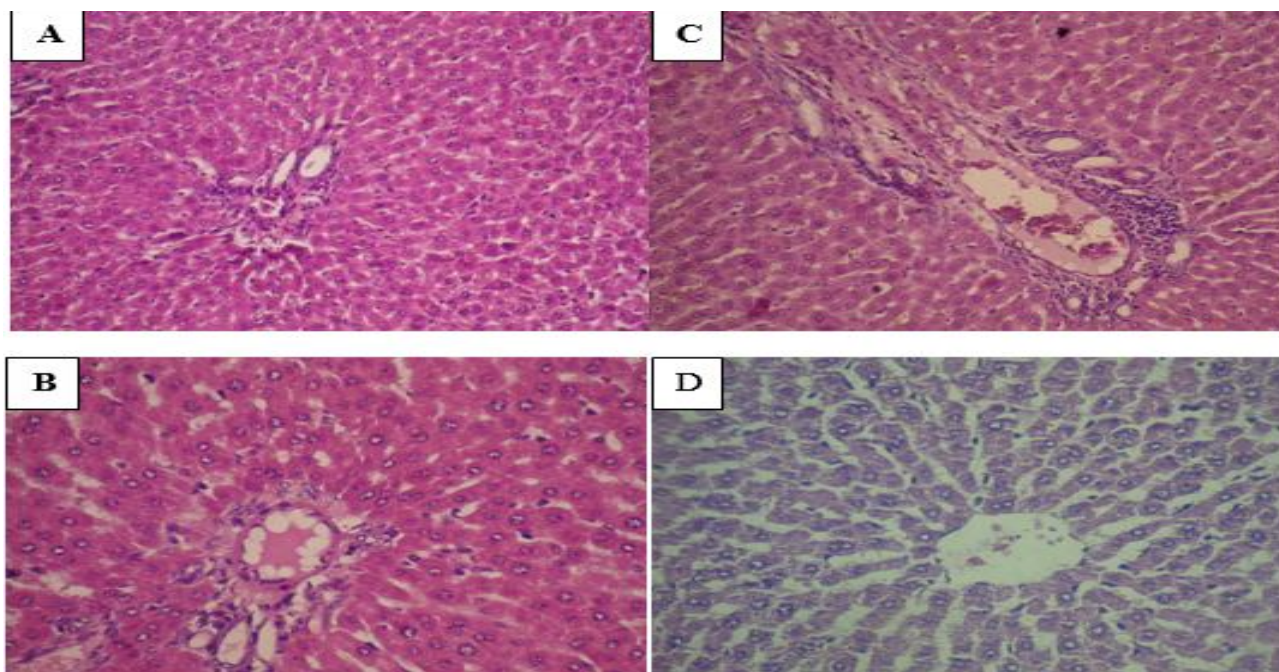
Data are expressed as means ± SD (n=7 rats per group). Comparison between groups was made using Duncan test. Values in the same columns not sharing a common letter (a–b) differ significantly at  $p < 0.05$ .

**Effects of TCA on biochemical kidney markers:** The results obtained in this study revealed that the sub-chronic treatment with TCA at 50mg/kg BW for 2 months caused a highly significant elevation (i.e.  $p$ -value  $< 0.05$ ) in the level of serum creatinine (96.7%), urea (66.7 %), and uric acid (40 %) compared to those of the control animals. The treatment with SM led to a decrease in these parameters to the nearest values of control group (Table 2).

**Enzymatic antioxidant status, MDA and GSH level in liver and kidney:** The effects of TCA administration on liver tissue damage index have been evaluated with reference to the levels of MDA, GSH, and antioxidative defense systems, which include Catalase (CAT), GPx, and SOD in the liver (Fig 1a and Table 2). It was observed that TCA resulted in significant decrease in the levels of SOD, CAT and GPx activities ( $p$ -value  $\leq 0.05$ ), and the content of GSH as compared to control rats. TCA also induced a significant ( $p < 0.05$ ) elevation in MDA concentrations as compared to the control animals (Fig 1a). The effects of TCA



**Fig-1:** Effects of *Salvia miltiorrhiza* extract on the level of MDA and GSH in rat; (A) liver (B) and kidney tissues after treatment of TCA at 50 mg/kg bw. Data are reported as the mean ± SD of seven animals in each group



**Fig 2:** Histopathological changes in liver of control and experimental rats; (A) Group I: Control rats showed central veins surrounded by columns of normal hepatocytes having abundant eosinophilic cytoplasm, central rounded nuclei separated by blood sinusoids; (B) Group II: animals treated with SM only showed normal histological structure; (C) Group III: a liver section of TCA-treated rats showing hydropic degeneration & inflammatory cell infiltration in portal tracts & dilated congested blood vessels; (D) Group IV: a liver section of SM + TCA-treated rats showing mild tubular necrosis with regenerated tubular cells (HE x 40)

administration on tissue damages index with reference to levels of MDA, GSH, and antioxidative defense systems such as CAT, GPx, and SOD in the kidney is presented in Figure 1b and Table 2).

**Histopathological changes:** Microscopic investigation of liver (Fig 2a and b) shows the normal histology structures in control and SM treated groups. There are hepatic lobules, which showed the central veins surrounded by the columns of normal hepatocytes, having abundant eosinophilic cytoplasm, central rounded nuclei separated by the blood sinusoids. It has been revealed through histological examination of TCA-induced rats that the liver with hepatocytes shows hydropic degeneration and infiltration of inflammatory cells in portal tracts and dilated congested blood vessels (Fig 2c). However, SM administration (Fig 2d) ameliorates these alterations showing preserved architecture, dilated central veins with mild degenerated hepatocytes, which are identical with control group.

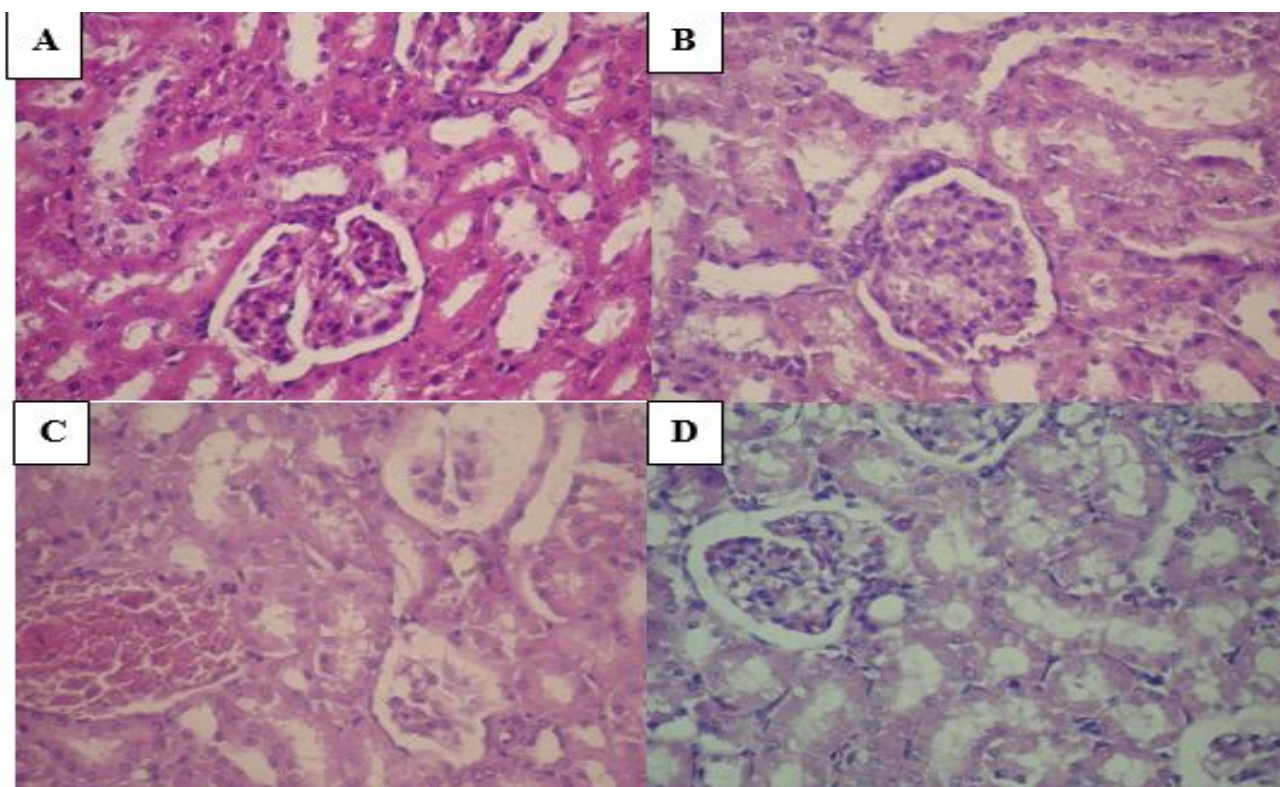
Histological investigation outcomes of kidney (Fig 3a and b) demonstrate normal structures in the control and SM treated groups. There are normal appearances of glomerulus, surrounded by a double-walled epithelial capsule. There is a urinary or Bowman's space between two layers of capsule with normal arrangement of renal tubules. Numerous alterations in medulla and cortical regions have been revealed in histological examination of TCA-induced kidney rats (Fig 3c), along with severe tubular necrosis,

degeneration and vacuolation. However, SM administration (Fig 3d) ameliorates these alterations showing mild tubular necrosis with regenerated tubular cells and normal appearance of glomerulus and renal tubules similar to control group.

The preventive and curative hepatoprotective SM effects against TCA-induced hepatotoxicity in rats have been evaluated. By treating with TCA, significant increases have been observed in serum liver enzyme activities (ALT, AST, ALP and GGT) and CB levels with concomitant significant decreases in TP, ALB levels.

The role of oxidative stress in etiopathogenesis of hepatic disorders is well-documented (Basu, 2003). The free radicals generated as a result of reductive halogenation of TCA in presence of oxygen have led to an autoxidation of free fatty acids. Moreover, it also cause functional and morphological alterations in hepatocyte cell membrane after binding covalently to membrane lipids and proteins, or abstracting a hydrogen atom from unsaturated lipids and initiates the lipid peroxidation (Pramyothin *et al.*, 2004). Thus, prevention and inhibition of free radical generation and promotion of antioxidant activity constitutes an important defense system against TCA-induced hepatic injury (Murugesan *et al.*, 2007).

Study evaluates the effect of SM on TCA-induced oxidative stress, the hepatic tissue levels of GSH, MDA and activities of antioxidant enzymes CAT, SOD, and GPx have



**Fig 3:** Histopathological changes in kidney of control and experimental rats; (A) Group I: Control rats showed normal kidney, central glomeruli surrounded by Bowman's space, normal tubules lined by cuboidal epithelium; (B) Group II: animals treated with SM only showed normal histological structure; (C) Group III: a kidney section of TCA-treated rats showing severe tubular necrosis, degeneration & vacuolation; (D) Group IV: a kidney section of SM + TCA-treated rats showing mild tubular necrosis with regenerated tubular cells (HE x 40)

been measured. The antioxidant and antilipo oxidative properties of SME could be attributed to its constituent flavonoids and other polyphenolics, as these phyto-components have been widely reported to possess antioxidant and antilipo peroxidative activities (Yang *et al.*, 2010). The outcomes demonstrated that the MDA concentrations increased in liver and kidneys of TCA treated rats as compared to the control group. This enhancement in the content of MDA might have resulted from an increase in reactive oxygen species, as a result of stress in rats exposed to TCA toxication. Also, an elevation of MDA results by superoxide overproduction, after which dismutation produces single oxygen and  $H_2O_2$ , easily converted later into the reactive OH, which starts free-radical chain reactions of lipid peroxidation (Salah *et al.*, 2012). Furthermore, it has been examined that petroleum diethyl ether extracts of *Sideritis* sp. have antioxidative properties in vitro (Tunalier *et al.*, 2004).

The depletion of GSH also seems to be a major factor that permits lipid peroxidation in TCA-treated animals. The decreased level of renal GSH in TCA-treated rats can markedly increase TCA toxicity (Tunalier *et al.*, 2004). The antioxidant enzymes (SOD, CAT, and GPx) limit the effects of oxidant molecules on tissues. Due to the presence of free radical scavengers these enzymes are active in defending

oxidative cell injury. SOD and CAT are the primary antioxidant enzymes in line of defense against the deleterious effects of oxygen radicals in cells, which scavenge reactive oxygen species by catalyzing the dismutation of superoxide to  $H_2O_2$ . Hence, it has been evaluated that TCA administration induced depletion in the antioxidant activities of SOD, CAT and GPx in renal tissues. TCA administration might probably indicate a decreased efficiency in scavenging superoxide radicals and hydrogen peroxide.

There are several mechanisms of antioxidants in the literature, concerning polyphenolic compounds (Halake *et al.*, 2016). The initial one, polyphenolic compounds, which was bonded with the iron to manufacture catalytic activity center. It played an important role in antioxidant reaction. Next step is the polyphenolic compounds, which are easily oxidized to ketones or quinines substances. It combines DPPH to change the OD value. Polyphenolic compounds can inhabit some antioxidant enzyme and lessen the enzymatic activity.

## CONCLUSION

The outcomes of the present investigation demonstrate a high efficacy of aqueous *S.miltiorrhiza* extract in free radical scavenging by inhibiting the reactive oxygen species and lipid per oxidation produced by TCA. It may be

partially explained due to the presence of important antioxidative factors. The efficiency of methods varied with specific component to be extracted. The purpose, efficiency and economy must be taken into consideration while choosing appropriate method of extraction. *S. miltiorrhiza* showed a variety of bioactivities and many effects on health, such as the effect on cardiovascular diseases, and antioxidant effects. It has been recommended that future researchers should emphasize on anti-inflammation and antibacterial effects of *S. miltiorrhiza*. Besides this, the underlying

mechanisms of bioactivities should require detailed scientific explanation.

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#### Conflict of Interest

The authors declare no conflict of interest.

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