Global Veterinaria 7 (4): 353-357, 2011
ISSN 1992-6197

Effects of *Salvia officinalis* Extract on Carbon Tetrachloride Induced Hepatotoxicity

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Abstract: The objective of this investigation is to evaluate the protective effects of *Salvia officinalis* extract against carbon tetrachloride-induced hepatotoxicity in rats through biochemical assays. Thirty male Wistar rats were randomly divided into five groups. Dried *Salvia officinalis* was slowly boiled in 100 ml of distilled water and heated for 30 minutes. The extracts were then filtered and administered orally to the animals. Group A served as normal control. Group B was a negative control and rats were treated intraperitoneally with a single dose of CCl4. The rats in Groups C, D and E were pretreated with *Salvia officinalis* at dose of 10, 15 and 20 mg/ml orally once daily for 14 days and then they were treated intraperitoneally with CCl4 as group B. On the 15th day, the rats were anesthetized using thiopental and blood was collected from abdominal artery. Biochemical factors, AST, ALT, GSH, SOD, CAT and MDA, were used as the biochemical markers of the hepatic damage. After treatment with CCl4, levels of serum ALT and AST was significantly ($P<0.05$) increased compared to the control. Pre-treatment with extract of three *Salvia officinalis* doses reduced the CCl4-induced elevation of serum ALT and AST activities. Hepatic MDA shows significant increase ($P<0.01$), post CCL4 administration. However pre-treatment with any of the three doses of OS reduced the elevation in hepatic MDA levels associated with AZP treatment alone. In CCL4-treated rats a significant decrease in hepatic SOD and CAT activity ($P < 0.01$) was observed post-treatment. This inhibition was significantly released with pre-treatment with SO. *Saliva officinalis* extract has been found to possess significant reactive oxygen species (ROS) scavenging activity and it seems that neutralizing such radicals could have a hepatoprotective effect.

Key words: *Salvia Officinalis* · Hepatotoxicity · Carbon Tetrachloride · Biochemical Factors

INTRODUCTION

Formation of reactive oxygen species (ROS) is an unavoidable consequence in aerobic organisms during respiration. It has been shown that overproduction of unstable ROS leads to unwanted reactions with other groups or substances in the body, resulting in cell or tissue injury. In addition, numerous studies have revealed that uncontrolled lipid peroxidation is involved in the occurrence of many diseases, including Parkinson's, arthritis, myocardial infarction, Alzheimer's, cancer, cardiovascular disease and liver damage [1]. Therefore, during the last few decades, human nutrition and biochemistry research focused on antioxidants derived from foods that could prevent or diminish ROS-induced damage. Liver damage is a widespread disease which can be caused by reactive oxygen species (ROS) and is characterized by a progression from steatosis to chronic hepatitis, cirrhosis and hepatocellular carcinoma [2]. Several compounds, such as carbon tetrachloride (CC14), acetaminophen, bromobenzene, ethanol and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases [3]. CCl4 is a classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis [4]. The mechanism of liver injury induced by CCl4 is thought to involve free radicals and lipid peroxidation. CCl4 requires bioactivation by phase I cytochrome P450 system in the liver to form reactive metabolic trichloromethyl radical (CCl3.) and proxy trichloromethyl radical (OOCCl3) [5]. Since free radicals are very unstable, they are immediately neutralized by antioxidants in the cell once they are generated in normal metabolism pathway, so increasing the antioxidant content in cells may play an important role against CCl4-induced liver injury. Due to the risks of synthetic antioxidants, there is a growing interest in the

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use of natural antioxidants to prevent oxidative stress-related liver pathologies [6]. A major defence mechanism involves antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GSH), which neutralize ROS in cells [7].

Salvia officinalis (SO), a plant endemic to the Mediterranean region is one of the most popular herbal remedy in the Middle East to treat common health complications such as cold and abdominal pain [8]. Salvia species (*Labiatae*) are generally known for their multiple pharmacological effects including their antibacterial [9], hypoglycemic [10], antianoxic [11], antitumor [12], antioxidative [13] and anticholestatic [14] effects. Phytochemically, the whole plant contains several antioxidants that prevent peroxidative damage to hepatocytes such as water-soluble compounds; salvianolic acid A, salvianolic acid B and rosmarinic acid [15], tanshinone IIA [16] and several phenolic glycosides [17]. The objective of this investigation is to evaluate the protective effects of *Salvia officinalis* against carbon tetrachloride-induced hepatotoxicity in rats through biochemical assays.

MATERIALS AND METHODS

Male Wistar rats (150-200g) were maintained on standard pellet diet and tap water *ad libitum*. The animals were kept in plastic cages under a 12 hr light/dark cycle and room temperature 22-24°C. They were acclimatized to the environment for two weeks prior to experimental use. All the animals were acclimatized for at least one week prior to experiment and then randomly divided into five groups (6 rats per group). Ten gm of dried plant was slowly boiled in 100 ml of distilled water and heated for 30 minutes. The extracts were then filtered and administered orally to the animals at a volume of 10 ml of extract/kg body weight.

Group A served as normal control. Group B was a negative control and rats were treated intraperitoneally with a single dose of CCl4 (2 mL/kg of body weight). The rats in Groups C, D and E were pretreated with SO at dose of 10, 15 and 20 mg/ml orally once daily for 14 days, then they were treated intraperitoneally with CCl4 as group B. On the 15th day, the rats were anesthetized using thiopental (40 mg/kg) and blood was collected from abdominal artery and kept at 37°C in the incubator for 30 minutes. Afterwards, it was cold centrifuged at 2000 rpm for 15 min to isolate the supernatant serum, which was used for the biochemical estimations.

Hepatic enzymes, asparate aminotransferase (AST) and alanine aminotransferase (ALT), were used as the biochemical markers of the hepatic damage. The serum activities of AST and ALT were assayed using commercial kits. The enzyme activities of AST and ALT were expressed as U/L. Liver tissue homogenate was used for the following assay of antioxidant enzyme activity. The activities of GSH, SOD and CAT in the liver were evaluated using commercial kits and a spectrophotometer. The specific enzyme activities of SOD, GSH and CAT were expressed as U/mg protein. Lipid peroxidation (LPO) in the liver tissue homogenate was measured by estimating the formation of MDA using the TBARS assay. The protein concentration of liver homogenate was determined by the method of Lowry *et al.* [18] using bovine serum albumin as a standard. The results were expressed as the amount of MDA formed per mg protein.

Statistical Analysis: Results are expressed as mean ± SD Multiple comparisons were performed by ANOVA and followed by the Tukey honestly significant difference (HSD) test. In all analyses, the level of significance was set to ($P<0.05$ or 0.01).

RESULTS

The damage to the structural integrity of liver is commonly assessed by the determination of serum aminotransferases (ALT and AST) activities. After treatment with CCl4, levels of serum ALT and AST was significantly ($P<0.05$) increased compared to the control (Table 1). In contrast, pre-treatment with water extract of any of the three OS doses used in this study (10, 15 or 20 mg/ml) reduced the CCl4-induced elevation of serum ALT and AST activities. The highest ALT and AST reduction was observed in group E with highest dose of OS. The effects of OS extract on the CCL4-induced LP were examined through monitoring the levels of MDA. Hepatic MDA shows significant increase ($P<0.01$), post CCL4 administration. However pre-treatment with any of the three doses of OS reduced the elevation in hepatic MDA levels associated with AZP treatment alone (Table 1). The highest MDA reduction was observed in group E with highest dose of OS. In CCL4-treated rats a significant decrease in hepatic SOD and CAT activity ($P < 0.001$) was observed post-treatment (Table 1). This inhibition was significantly released with pre-treatment with SO. Best results were observed in group E. Findlay, none of the factors, did not return to normal values, by any of OS treatment groups.

Table 1: Effects of different doses of *Salvia officinalis* on biochemical parameters, following CCl4-induced hepatotoxicity

	Groups				
Factor	A	B	C	D	E
ALT (U/L)	51.6±2.4^{e}	839±49.4^{a}	783±35.5^{b}	545±24.5^{c}	231±12.6^{d}
AST (U/L)	55.8±3.5^{e}	1512±56.5^{a}	1366±62.2^{b}	853±68.3^{c}	182±21.6^{d}
MDA (nmol/mg)	209±12.3^{e}	486±42.2^{a}	452±33.3^{ab}	389±32.5^{c}	312±23.8^{d}
GSH (U/mg)	61±3.5^{a}	24±2.1^{e}	36±3.3^{d}	48±4.1^{c}	52±3.5^{b}
CAT (U/mg)	79±5.3^{a}	31±2.1^{e}	44±3.3^{d}	62±4.6^{bc}	70±5.2^{ab}
SOD (U/mg)	8.1±0.03^{a}	4.2±0.05^{e}	4.9±0.04^{d}	6.6±0.05^{c}	7.3±0.06^{b}

AST = aspartate aminotransferase; ALT = alanine aminotransferase. MDA = malondialdehyde; GSH= glutathione peroxidase; SOD= superoxide dismutase; CAT= catalase

Data are expressed as mean ± SD in each group (n = 6). Data with different letters (a-e) in the same row are significantly different at $P < 0.01$

DISCUSSION

The attention to hepatoprotective effects of medicinal plants against drug models of hepatotoxicity is rising. Liver injury induced by CCl4 is the most characterized system of drug-induced hepatotoxicity and is considered a new used model for the study of plant potential hepatoprotective activities.

Many chemical reagents such as CCl4 could significantly increase the serum AST and ALT levels and cause serious injury to the liver. Sturgill and Lambert [19] noted that once the liver was exposed to CCl4, AST and ALT were released from the liver into blood and caused the level of these serum maker enzymes to increase significantly. Valcheva-Kuzmanova *et al*. [20] reported that natural fruit juice from *Aronia melanocarpa* could decrease the serum AST and ALT level of rats. Augusti *et al*. [21] noted that the liver damage by CCl4 was satisfactorily prevented by garlic oil as effectively as vitamin E. The results proved that bioflavonoids and allylic sulphide could give protection from toxins like CCl4. Srivastava and Shivanandappa [2] also reported that aqueous extract of the roots of *Decalepis hamiltonii* which exhibited strong free radicals scavenging activity could effectively protect against CCl4-induced oxidative stress and liver injury in rats. The hepatotoxicity induced by CCl4 was due to its metabolite CCl3, a free radical that alkylates cellular proteins as well as other macromolecules and simultaneously attacks polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides, leading to liver damage [22]. To counteract ROS and to prevent their possible damage to biological molecules, all oxygen-consuming organisms are endowed with well-integrated antioxidant systems, which include enzymes such as GSH, SOD and CAT. And these antioxidant enzymes are the first line of defences against free radical induced oxidative stress [23]. Glutathione dependent defence against xenobiotic toxicity is a multifaceted phenomenon that has been well characterized in animals. GSH is an enzyme which prevents the generation of hydrogen peroxide and alkyl hydroperoxides in association with GSH and GSH-reductase, as well as the generation of more harmful metabolites such as the hydroxyl radical [24]. SOD is an exceedingly effective defense enzyme that converts superoxide anions into hydrogen peroxide (H_2O_2) [25] and CAT is a haemeprotein in all aerobic cells that metabolize H_2O_2 to oxygen and water. These antioxidant enzymes are inactivated by lipid peroxides or ROS. Under normal conditions, excess free radicals are neutralized immediately by enzymatic scavengers such as SOD and GSH, which contribute to the maintenance of a normal oxidation-reduction balance. Jayakumar *et al*. [26] noted that pretreatment with ethanol extract of oyster mushroom could significantly increase SOD, CAT and GSH activities in the liver of CCl4-administered rats compared to the negative group. Guo *et al*. [27] revealed that corn peptides could significantly decrease the levels of SOD, GSH and GSH ($P < 0.01$) in immunological liver injury of mice. In present study, the level of GSH, SOD and CAT activities in liver were significantly elevated by administration of CCl4 to rats, suggesting that it has the ability to restore these enzymes' activities in CCl4-damaged livers and this observation may be due to the reducing power of the peptides. The similar results in another study confirmed that porcine plasma albumin hydrolysate could increase the activities of SOD, CAT and GSH in 4-nitroquiunoline-1-oxide-4-NQO) induced liver damage of mice [28]. The protective effects of SO extract on liver result from its antioxidant activity, including stabilization in the intracellular defence systems and reductions in the lipid peroxidation products.

Treatment with CCl4 promoted lipid peroxidation, the extent of which was specified by the level of MDA. Pre-treatment with SO extract markedly reduced the formation of MDA, indicating that the administration of SO effectively inhibited lipid peroxidation as induced by Ccl4.

Lipid peroxidation is one of the major characteristics that can be included as an oxidative damage marker. Furthermore, tissues and cells are subjected to oxidative injury when there is overproduction of ROS or when antioxidant systems fail to function effectively. When the liver is damaged by some chemical toxin, hepatocytes generate a large number of free radicals, causing lipid peroxidation of the cytomembrane to produce MDA. MDA levels indirectly reflect the extent of cellular damage by free radicals and are widely used as an index of free radical mediated lipid peroxidation [29]. The increase in MDA and hydroperoxide levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals [30]. Opoku *et al*. [31] reported that aqueous extract of Zulu medicinal plant significantly decreased MDA levels in CCl4-induced acute liver injury in rats.

In conclusion, Saliva officinalis extract has been found to possess significant reactive oxygen species (ROS) scavenging activity and it seems that neutralizing such radicals could have a hepatoprotective effect.

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