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Investigation of the Protective Effect of Sığla Oil Against Carbon Tetrachloride-Induced Toxication in Kidney

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Abstract

Objective: In this study, the intention was to investigate the relation between carbon tetrachloride (CCl_4)-induced renal damage and malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and nitric oxide (NO) enzyme activities; as well as the effect of sığla oil (storax, also commonly known as *Turkish sweetgum*) on the metabolism.

Materials and Methods: Rats were randomly divided into 5 groups as control (n=10), CCl_4 (n=10), CCl_4+sığla oil (50 mg/kg) (n=10), CCl_4+ sığla oil (100 mg/kg) (n=10), and CCl_4+ sığla oil (200 mg/kg) (n=10).

Results: In the group administered carbon tetrachloride, the CAT, GPx and GSH levels were observed to be decreased and MDA and NO levels were increased. In the treatment group (CCl_4+sığla oil), the MDA and NO levels decreased and the CAT, GPx and GSH levels increased close to that of the control group.

Conclusion: In this study, where the effect of sığla oil on CCl_4-induced renal oxidative damage was investigated, it was observed that the sığla oil reinforced the antioxidant system by suppressing the oxidative stress, and that the results obtained with the increased doses, in particular, were most effective.

Key Words: Carbon tetrachloride, kidney damage, sığla oil (Turkish *sweetgum*), antioxidant.

INTRODUCTION

If we consider the fact that biochemical reactions in living organisms occur continuously, we can say that an uninterrupted production of oxidizing agents is available in metabolism, as well. Therefore, the amount of oxidizing agents in the organism must be balanced, to a certain extent. A balance can be ensured only when the excess amount of oxidant is inactivated by cleaners called antioxidants. The balance between oxidants and antioxidants may be disrupted by cellular and biological factors [1 and 2]. Disruption of this balance leads to an increase in the amount of free radicals and to a cell damage [3]. Free radicals have been shown to damage proteins, lipids, carbohydrates, and DNA, both in vitro and in vivo [4 and 5].

Carbon tetrachloride is a hepatotoxic agent that has been investigated the most both biochemically and pathologically all over the world [6]. CCl_4 causes destruction of many organs such as liver, thymus, lymph nodes, spleen, kidney, brain and pancreas [7; 8 and 9]. CCl_4 turns into trichloromethyl (CCl_3) radical, and CCl_3 turns into trichlorometil peroksit (CCl_3O_2) radical. CCl_3O_2 is alleged to create alkylation reaction by directly inactivating the enzymes through membrane proteins and covalent bonds in particular, by means of the first mechanism; or to stimulate lipid peroxidation by affecting membrane fatty acids, by means of the second mechanism [10;11 and 12].

Because of their antioxidant and antiradical effects, plants have been studied extensively in recent years [13]. Storax is a balsam obtained from sığla (*Liquidambar orientalis*) tree. With the research studies carried out up to this time, sığla oil has been alleged to contain high-molecular compounds such as acid, alcohol, ester and phenol; as well as molecules such as cinnamic acid, styracine, styrol, styrene, storesinol and storegen [14].

The purpose of this study was to investigate the protective effect of sığla oil in kidney damage induced by CCl_4 in rats.

MATERIALS AND METHODS

The study was carried out with 3-4 month old male Sprague-Dawley rats. Rats were randomly divided into 5 groups as 1. control (n=10), 2. CCl_4 (n=10), 3. CCl_4+sığla oil (50 mg/kg) (n=10), 4. CCl_4+ sığla oil (100 mg/kg) (n=10), and 5. CCl_4+ sığla oil (200 mg/kg) (n=10). The rats were fed a diet consisting of standard rat chow and water for one week, under a 12-hour light/dark cycle, at AKU (Afyon Kocatepe University) Research and Application Centre for Laboratory Animals, were heating and ventilation were adjusted according to the laboratory methods and needs of the animals and were kept under observation.

0.8 ml/kg CCl_4 dissolved in olive oil (1/2) was administered intraperitoneally and sığla oil was administered—with gavage method—intragastrically to rats, on a daily basis. 24 hours after the last injection, animals were sacrificed under ketamine-xylazine anesthesia, and their renal tissues were removed. The removed tissue samples were homogenized with homogenizers in pH 7.4 phosphate buffer (1:5), in an icy environment. The homogenates were centrifuged at 20 000 rpm in a centrifuge cooled to + 4 °C. The supernatants were taken and stored at -80 °C in a freezer until the time of analysis.

MDA measurement was made according to the method of Jain et al. [15], NO measurement was made according to the method used by Miranda et al. [16], GSH measurement was made according to the method of Buetler et al. [17], CAT measurement was made according to the method used by Aebi [18], and GPx measurement was made with Elisa kit (Cayman, 8543).

Statistical calculations of the obtained findings were made by use of SPSS 18.0 software package, and the data obtained from the study were refer to as "mean ± standard deviation" (X ± SD). The groups were first subjected to normality test, and all the data were observed to be normally distributed. In this context, the statistical relationship was determined by applying analysis of variance (ANOVA) and DUNCAN test—among the parametric tests—to the data ascertained to be normally distributed. p was considered to be < 0.05 for the statistical significance.

RESULTS

A statistically significant difference was observed in MDA levels of the control and CCl_4 groups. The statistical difference between CCl_4 and treatment groups (3rd, 4th and 5th groups) was found to be significant. Statistical proximity to the control group was observed in the 5th group (Table 1).

The difference between NO level in CCl_4 group and that of the control group was found to be statistically significant. Statistical difference was observed between CCl_4 and treatment groups (3rd, 4th and 5th groups). Statistical difference was not observed between 4rd and 5th groups but was observed between 3rd and 4th groups (Table 1).

The difference between CAT level in CCl_4 group and that of the control group was found to be statistically significant. Any statistical difference between CCl_4 and 3rd and 4th groups did not occur. The treatment groups showed proximity to the control group, especially 5th group(Table 2).

A statistically significant difference was found between GPx levels of CCl_4 and control groups. A statistical difference was observed between CCl_4 group and 5th group (Table 2).

A statistically significant difference was found between GSH levels of CCl_4 and control groups. The difference between 3rd and 4th groups, and 4th and 5th groups were found to be statistically significant. A statistical difference was not observed between CCl_4 group and treatment groups (3rd and 5th groups) but 4th group showed proximity to the control group (Table 2).

DISCUSSION AND CONCLUSION

In CCl_4-mediated studies, CCl_4 was ascertained to cause destruction of many organs such as liver, thymus, lymph nodes, spleen, kidney, brain and pancreas [7; 8 and 9]. In their study, Jayakumar et al. [19] have determined that CCl_4 causes toxicity in the heart, brain, and kidney. In their study, Tulin et al. [20] have showed the changes—at fine structure level— in renal tissues of rats subjected to low-dose CCl_4. Dogukan et al. [21] have applied 0.15 ml/kg dose of CCl_4 for 7 weeks, and found interstitial fibrosis and inflammation, as a result of the evaluation of light microscopy. Özturk et al. [22] have applied 1 ml/kg CCl_4 for a period of 11 days and observed an intense cortical damage and focal glomerulosclerosis in their study, where they have investigated the tissue protective effect of betaine in CCl_4-induced renal damage. In a study conducted on rats, Kim et al. [23] have showed that the renal toxicity of CCl_4 varies depending on gender difference. Male rats have been found to be more sensitive to CCl_4 nephrotoxicity, when compared to female rats. As in conformity with the literature, renal toxicity formation was determined in our study, as well.

Table 1. Levels of MDA and NO in kidney tissues

Gruplar	n	MDA (µmol/mg protein)	NO (µmol/mg protein)
1.Control	10	6.44 ± 0.9^{a}	7.86 ± 0.95^{a}
2.CCl_4	10	11.46 ± 1.44^{d}	20.19 ± 1.63^{d}
3.CCl_4+50 mg/kg *sığla oil*	10	8.95 ± 0.77^{c}	13.07 ± 1.69^{c}
4.CCl_4+100 mg/kg *sığla oil*	10	8.09 ± 0.58^{b}	10.58 ± 1.14^{b}
5.CCl_4+200 mg/kg *sığla oil*	10	7.35 ± 0.66^{b}	9.93 ± 1.07^{b}

a,b,c,d: Values in a column followed by different letters are significantly different ($p<0.05$).

Table 2. Levels of CAT, GPx and GSH in kidney tissues

Gruplar	n	CAT (µmol/mg protein)	GPx (µmol/mg protein)	GSH (µmol/mg protein)
1.Control	10	1.99 ± 1.33^{b}	$0.027 \pm 0.002^{a,b}$	9.99 ± 1.67^{c}
2.CCl_4	10	0.75 ± 0.16^{a}	0.009 ± 0.001^{a}	3.21 ± 1.23^{a}
3.CCl_4+50 mg/kg *sığla oil*	10	1.0 ± 0.43^{a}	$0.019 \pm 0.001^{a,b}$	3.71 ± 1.16^{a}
4.CCl_4+100 mg/kg *sığla oil*	10	$1.33 \pm 0.47^{a,b}$	$0.027 \pm 0.017^{a,b}$	6.06 ± 1.14^{b}
5.CCl_4+200 mg/kg *sığla oil*	10	1.86 ± 0.68^{b}	0.036 ± 0.038^{b}	3.36 ± 1.14^{a}

a,b,c: Values in a column followed by different letters are significantly different ($p<0.05$).

Free radicals cause various damages in the body, when they overcome the defense capacity of antioxidant. Lipid peroxidation is a reaction initiated by free radicals that causes oxidation of polyunsaturated fatty acids in the cell membrane. MDA formed as a result of peroxidation of fatty acids is one of the most important indicators of lipid peroxidation. In their study [10 and 24] conducted with CCl_4, they observed CCl_4 to increase MDA level in the brain and kidney tissues. In presented study similarly, MDA levels increased in CCl_4-induced renal damage. The decreased levels of MDA in the groups applied sığla oil show that sığla oil effects lipid peroxidation positively, in a dose-dependent manner.

NO is a very important parameter for antioxidant defense in the organism. NO is a free radical containing unpaired (lone) electron. It is stable in the presence—but is unstable in the absence—of oxygen. In physiological intensities, it is released from endothelial cells through the agency of nitric oxide synthase (iNOS), and is normally non-toxic. NO creates peroxynitrite that is extremely harmful to tissues, by reacting with superoxide anion radical. This consisted substance causes cell damage, by initiating lipid peroxidation in cell membrane [25 and 26]. In our study, the significant increase observed in NO levels of the tissues after administration of CCl_4 was evaluated as an indicator of the fact that the consisted free radicals led cell lipid membranes to peroxidation. With studies carried out similarly, NO levels of the groups administered CCl_4 were observed to be increased [27; 28 and 29].

Catalase (CAT) is an enzyme involved in the degradation of H_2O_2 in high concentrations [30]. In their study, Hsiao et al. [31] have stated that catalase enables hydrogen peroxide in cells to turn into water and molecular oxygen; and that this enzyme plays an important role in the elimination of reactive oxygen species produced in tissues during redox process of xenobiotics. In studies conducted on the effect of carbon tetrachloride on antioxidant enzymes in the kidney, CCl_4 administered to rats has been reported to cause a significant decrease in the CAT activity in renal tissue, as consonant with our study [32; 33 and 34].

Glutathione is a strong antioxidant involved in the removal of resultant products from the environment, which are harmful to metabolism, by reacting easily with such products that come into existence depending on the increase in the amount of free radicals and lipid peroxidation caused by it. In consequence of its reaction with lipid peroxidation products, turns into oxidized glutathione. GSH and antioxidant enzymes metabolizing it provide a great defense against cellular damages caused by reactive oxygen derivatives [35 and 36]. In their study, where erythrocyte antioxidant systems in some mammalian species were compared, Kurata et al. [37] pointed out the depletion of GSH induced by antioxidant protection. In the study, the antioxidant defense in sığla oil was ascertained to be effective on preventing any decrease in the level of GSH and strengthening it.

GPx catalyses the reduction of hydrogen peroxide and lipid peroxides. It is considered to be an enzyme that provides an effective protection against lipid peroxidation [38]. In the study, the treatment groups were observed to be increased, compared to CCl_4 group; and on the other hand, remained low, compared to the control group, assumedly due to that GPx increased at the first stage with intent to neutralize such harmful effects, and then remained low and got closer to the control group because of its positive contribution to the repair. The extent of change in the 4th and 5th groups, which is greater than that in CCl_4 group, indicates the possibility of therapeutic effect of storax. With studies carried out similarly, GPx levels of the groups administered CCl_4 were observed to be decreased [39 and 40].

As a result, it was proven that higher doses of sığla oil are effective to reduce renal damage and oxidative stress caused by damaging carbon tetrachloride. Sığla oil is considered to be useful for humans when used with intent to cure renal damages or protect the kidney against damages; as a substance that can be utilized as a supporting element in clinical treatment of renal diseases; and can be one of ancillary therapies.

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