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Original Article

Sophora pachycarpa Root Extract has Ameliorative Effects on Testicular Injury but is Hepatotoxic in Carbon Tetrachloride Intoxicated Rats

Seyed Mahdi Banan Khojasteh[1] and Reihaneh Javanmard Khameneh[2*]

[1]*Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran*
[2]*Department of Biology, Faculty of Sciences, University of Urmia, Urmia, Iran*

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***Corresponding author**
javanmardr96@gmail.com

ABSTRACT

Background: Carbon tetrachloride (CCl_4) causes damages to the testicles, liver, kidneys, lungs and brain. In the present study, we investigated protective effects of *Sophora pachycarpa* Schrenk ex C.A.Mey. root extract on testicular damage, liver histopathology and sex hormone levels in CCl_4 intoxicated male rats.

Methods: In present study the rats were divided into 6 experimental groups (n=6): 3 groups as pre-treatment groups that received doses of 50, 100 and 250 mg/kg/days of *S. pachycarpa* extract respectively by gavage for 21 days before intraperitoneal injection of 500µl/kg CCl_4. CCl_4 group received 500µl/kg CCl_4 on the day of 21. Post-treatment group received 100 mg/kg/day *S. pachycarpa* for 10 days and 12h after injection of 250 µl/kg CCl_4. Control group received 1ml distilled water for 21 days. Liver and testis tissues were sampled for microscopic examination and sex hormone levels in the serum were evaluated.

Results: Treatment of *S. pachycarpa* root extract (250 mg/kg) lead to a considerable improvement in the histopathological changes caused by CCl_4 in the testis. However, the extract (250 mg/kg) caused inflammation and adverse tissue effects in liver. Serum levels of hormones also increased significantly in pre-treatment groups.

Conclusion: Based on the results, the *S. pachycarpa* extract can improve the changes caused by CCl_4 in the testis and sex hormones levels. But its higher doses can cause adverse effects in liver tissue.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a clear, colorless, heavy, volatile and non-flammable liquid [1]. This chemical compound is an industrial solvent that is used for different purposes [1]. As an industrial material, CCl_4 is widely used in home cleaners, fire extinguishers, coolers, stimulants etc. Due to the harmful effects of carbon tetrachloride, it is important to control, prevent and eliminate its harmful effects [2,3]. Humans may be exposed to carbon tetrachloride through inhalation or oral [4].

CCl_4 causes damages to testes, kidneys, liver, brain and lungs through oxidative stress [5]. It induces oxidative stress by production of free radicals, trichloromethyl ($CCl_3^{\cdot}$), trichloromethyldioxy (CCl_3O_2) and other metabolites [6]. Oxidative stress means disturbance in the ratio of production of free radicals and oxidants of the body [7]. Free radicals are molecules, atoms or ions that contain one or more unpaired electrons in the outer orbit of the molecules [8]. Free radicals are active chemicals that can rapidly react with cell components [9].

The liver is more vulnerable to oxidative stress than other organs [10]. CCl_4 is known as a liver toxin that can have effects such as fatty degeneration, fibrosis, hepatocellular death and carcinogenesis. In fact, CCl_4 is activated by cytochrome P450 to form the trichloromethyl radical ($CCl_3^{\cdot}$). This radical can bind to cellular components (Nucleic acid, protein and lipid) [11].

Testicular toxicity had been known as the result of oxidative stress, caused by CCl_4 toxication. Testicular oxidative stress causes germ cells apoptosis, hormone regulation disorders and even male infertility [12]. Previous findings show that fruits and vegetables have a variety of nutrients and non-nutritive substances, which are called phytochemical compounds that can inhibit free

radicals, interception the fatty oxidation and breaking of DNA, support the immune system by their antimicrobial, antioxidant and anticancer effects [13,14].

The *Sophora* plant belongs to Fabaceae family. It has 52 species reported worldwide, that three species are found in Iran, including *S. molis*, *Sophora alopecuroides* and *S. pachycarpa* (*S. pachycarpa*). The leaves of this plant are comprised, periodic and pinnate with a length of 10-18 cm [15]. The calyx has flowers similar to the flowers of the Pea family, white to yellow and fruits are club-shaped [15]. In Iran, *Sophora* can be found countrywide, especially in arid and semi-arid regions [15].

Study of chemical composition of *S. pachycarpa* has showed presence of alkaloids, flavonoids and steroid glucosides [16] which has anti-cancer, antibacterial, anti-inflammatory and antiviral activity [17]. In present study, the protective effects of *S. pachycarpa* root extract (SpRE) has been investigated in CCl_4 induced toxicity in male rats.

MATERIALS AND METHODS

Extract Preparation

S. pachycarpa Schrenk ex C.A.Mey. plant was prepared from herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. The roots of *S. pachycarpa* were placed in methanol at room temperature after drying. In order to prepare the crude methanolic extract, the extract filtered and solvent was evaporated at 40-45 ◦C. Then, for providing of required dosages, crude methanolic extract was dissolved in distilled water and KCl.

Animals

Male albino rats of Wistar strain (150-200g) were taken from Tabriz University of Medical Sciences. The rats were kept in polypropylene cages. The room temperature was kept at 20 ± 22 ◦C, with 40% - 60% relative humidity. Animals received standard food (pellets) and water. Rats were divided into 6 experimental groups, including 6 animals in each group.

Experimental Plan

Groups I, II and III as pre-treatment groups, received doses of 50, 100 and 250 mg/kg SpRE, respectively for 21 days by gavage. All three pre-treatment groups also received CCl_4 (500 µl/kg) intraperitoneally (ip) on the day of 21, 3 – 4 hours after the last dose administration of the extract [18].

Group IV was considered as control group that received 1 ml distilled water for 21 days.

Group V was considered as CCl_4 group that received CCl_4 (500 µl/kg) on the day of 21.

Group VI considered as post-treatment group that received 250 µl/kg CCl_4 after 21 days and subsequently received 100 mg/kg SpRE for 10 days.

Histological Preparations

After the end of treatment period, animals were dissected. Testis and liver tissue samples were fixed in fixative (formalin 10%) for 48 h and then tissue slides were prepared by routine histotechnique method in order to light microscopic examination. Also, blood samples were taken for measuring serum levels of sex hormones by radio-immunoassay method.

Statistical Analysis

SPSS software (version 20) was used to analyze of the data. All results were analyzed using One-Way Variance test and Tukey's test ($P< 0.05$) and calculated as mean ± standard error.

RESULTS

Hormonal Analysis

The data shown in table1 illustrate that follicle stimulating hormone (FSH) levels were markedly ($P< 0.05$) higher in pre-treatment III (8.86 ± 0.06) and post-treatment groups (6.64 ± 0.41) in comparison to CCl_4- group (5.54 ± 0.5) (Fig. 1).

Also, the mean concentration of luteinizing hormone (LH) was significantly increased ($P< 0.05$) in pre-treatment I (28.38 ± 0.57), II (29.15 ± 0.45), III (31.76 ± 0.44) groups and post- treatment group (24.32± 0.33) in comparison with CCl_4- group (16.98 ± 0.22) (Fig.1).

Testosterone levels in pre-treatment I (25.44 ± 0.14), pre-treatment II (26.88 ± 0.28) and pre-treatment III (40.28 ± 0.23) groups has increased significantly ($P< 0.05$) compared to CCl_4- group (22.74 ± 0.4) (Fig. 1).

Histopathological Studies

According to histologic examination, spermatogenic cells are limited to the lumen of the seminiferous tubular basement membrane in CCl_4- group (Fig. 2.A).

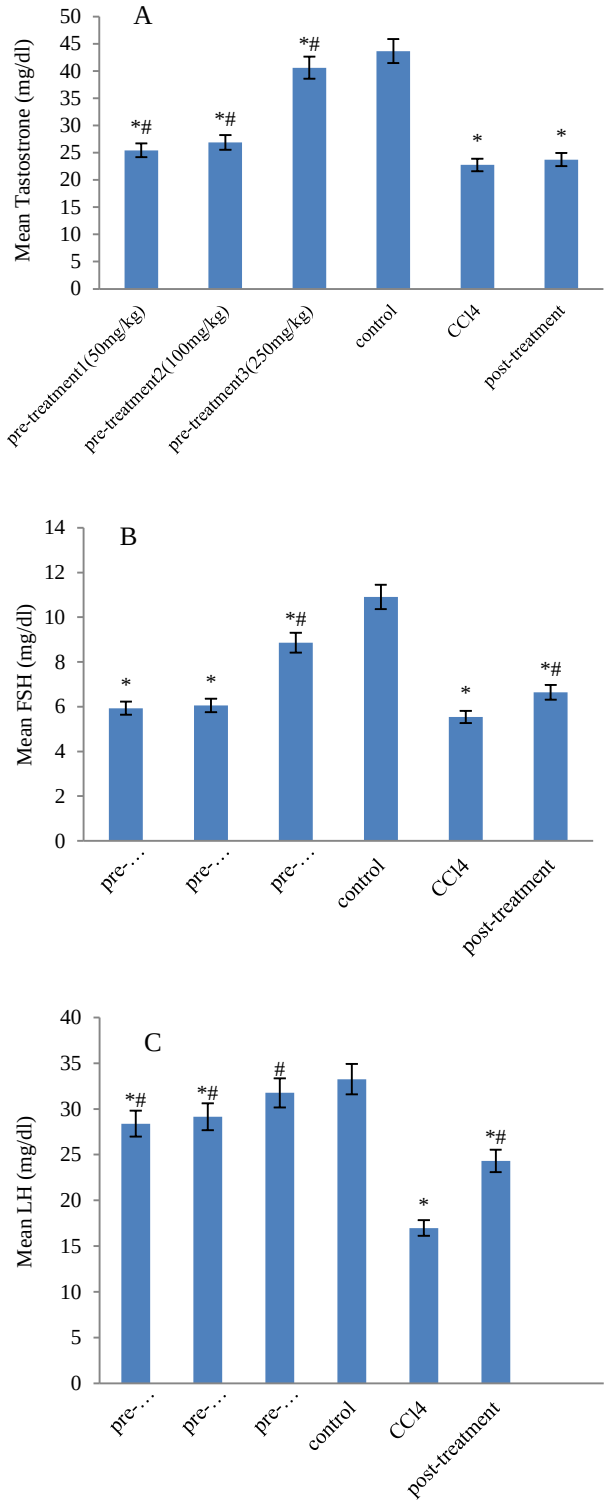

Fig.1 Comparison of the mean serum concentrations of LH, FSH and testosterone among the studied groups. The significant difference ($P< 0.05$) of the experimental groups compared to the control group is shown with * and the significant differences (P<0.05) of the treatment groups compared to CCl_4 group is shown with #.

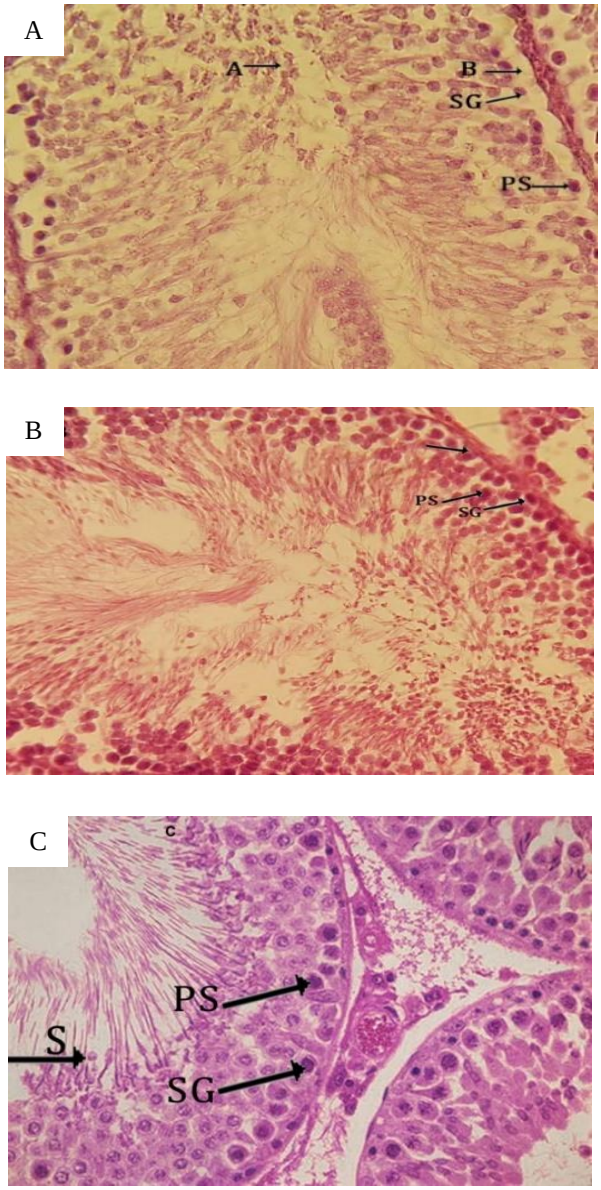

Fig. 2 A. Section from seminiferous tubules of CCl4 group. A: loss of spermatogenic cells inside the lumen; B: development of space between the basement membrane and spermatogenic cells (H&E stain, ×400). B. Seminiferous tubular section of SpRE group 250 mg/kg + CCl4 (H&E stain, ×400). C. Section from seminiferous tubule of control group. S, Spermatid; PS: primary spermatocyte; SG: spermatogonia (H&E stain, ×400).

Administration of CCl_4 caused destruction in germinative epithelium of seminiferous tubules, loss of germ cells and falling of cells into the lumen. Also, nuclei of spermatogenic cells were crumpled and the cells were out of order. There was loss of intercellular connections. Also results showed that at pre-treatment groups SpRE moderated the effects of CCl_4, especially at higher doses (250 mg/kg). Liver histopathological examination of normal group revealed normal distinct cells, portal spaces and hepatocytes. In comparison, pre- treatment group I (50 mg/kg) showed tissue damage. There

was cell swelling and karyomegaly in pre-treatment group II (100mg/kg). As well as inflammation, cell swelling, and cell death were illustrated in pre-treatment group III (Fig. 3).

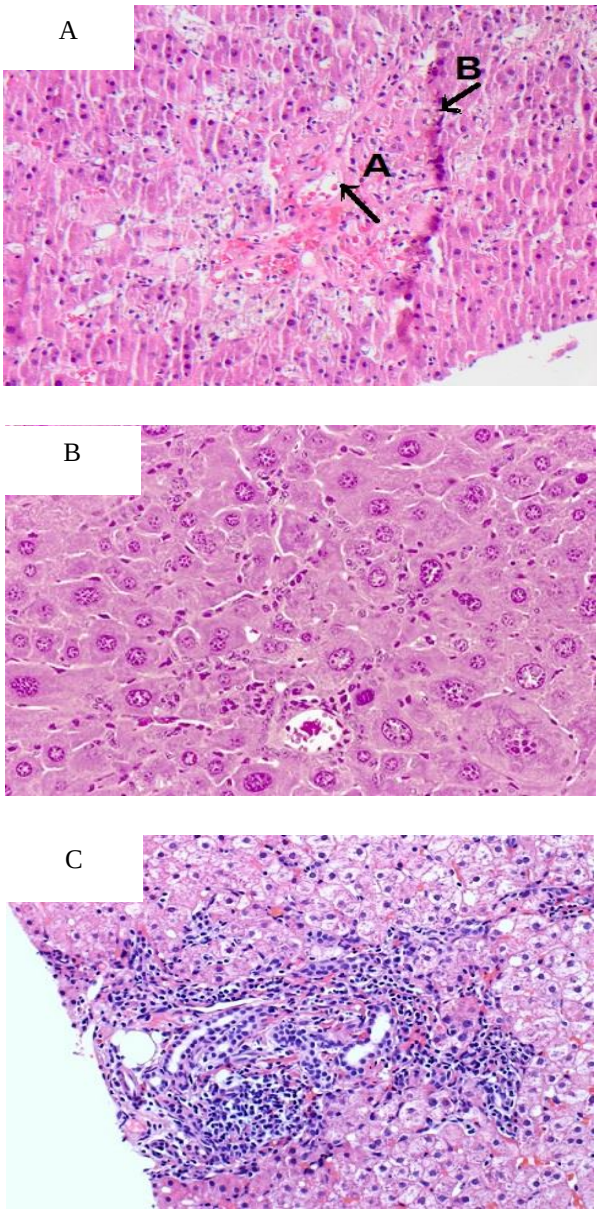

Fig. 3 Liver Section. A. Necrosis in pre-treatment group I. A: expansion of sinusoids, B: Apoptotic bodies. B. Karyomegaly of hepatocytes in pre-treatment II. C. Hepatitis in pre-treatment group III. (H&E stain, × 400).

DISCUSSION

Chloride hydrocarbons are industrial or organic solvents. These hydrocarbons are used in chemical industry, medical and other fields [19]. Carbon tetrachloride that penetrates the body through the skin and inhalation cause damage to organs including testis and liver [2,3].

The testis is one of the organs that carbon tetrachloride causes damage through oxidative stress [12]. Oxidative stress leads to germ cells apoptosis; subsequently causes hypo-spermatogenesis and endocrine signaling. Therefore, oxidative stress has been reported as one of the factors of male infertility [20].

The regulation of hepatocytes by thyroid hormones has indicated that there is a clear correlation between the liver and thyroid hormones [21]. Hyperthyroidism significantly alters the gonadotropin and prolactin axis and severely affecting sperm function [22].

The liver is known as one of the organs affected by carbon tetrachloride. In fact, carbon tetrachloride is known as one of the reasons of hepatic-cirrhosis as a result of reducing the levels of antioxidant enzyme activities such as catalase, peroxidase, superoxide dismutase and etc. [23].

Medicinal plants have been used for treatment of many diseases in the recent decades, due to cheapness, availability and to be effective [24]. Plants are known as a rich source of antioxidant compounds such as flavonoids. Studies have shown that the use of plants containing antioxidants has a significant relationship with the reduction of human diseases related to oxidative stress [25].

In this study, CCl_4 reduced levels of the sex hormones in comparison with control group. Therefore, it can be said that CCl_4 can cause infertility in men. However, *S. pachycarpa* root extract as a medicinal plant led to increase the sex hormones levels contrasted to CCl_4 group, especially in pre-treatment group III. So this medicinal plant can be effective in preventing infertility caused by CCl_4 [26].

In a similar study, moderating effects of various fractions of *Rumex hastatus* roots in CCl_4- induced testicular and hepatic damage has been shown [26].

Histopathological examination in present study was indicated that administration of SpRE before exposure to CCl_4 (especially in 250 mg/kg) improves changes caused by CCl_4 in testis. However, SpRE aggravated the toxic effects of CCl_4 in the liver of male rats. Therefore, SpRE may has anti-cancer properties so that lead to hepatotoxicity. It seems that apoptosis caused by dichloromethane and *Sophora* flavonoids G are involved in this toxicity; therefore, caution is required in its use [27].

It was demonstrated that *Rumex hastatus* root extract can improve liver disorders induced by CCl_4 such as necrosis, inflammatory cells and ballooning

of cells. This extract improved the disorders induced by CCl_4 in the testis and liver [26].

The study of Xiaohe and *et al*. showed that administration of Byakangelicine (100 mg/kg) after four weeks through oral gavage until the end of eight weeks modified disorders such as fibrosis, hepatocyte apoptosis and stellate cell inhibition caused by carbon tetrachloride in liver [28].

In other study, it has showed that after treatment with Propolis (200 mg/kg), there were improvement in hormone levels and spermatogenesis. This may be due to the antioxidant properties of the extract [5].

Aramjoo and *et al*, stated that *S. pachycarpa* seed (300mg/kg) has flavonoids such as polyohenols, that lead to improvements of renal toxicity and reproductive disorders [29]. They showed that *S. pachycarpa* seed extract at dose of 300 mg/kg can improve centrilobular necrosis, fatty change and infiltration of inflammatory cells in CCl_4 intoxicated rats that is contrary to the results of the present study [29].

A similar study on carbon tetrachloride-induced hepatotoxicity and hypogonadism in rats indicated moderating effects of Rutin (vitamin P) on toxicity. Rutin contains quercein flavonoids and disaccharide rutinose [30]. It has been found that Rutin prevents liver damage caused by methotrexate in rats [31]. Also, Rutin has a promising efficacy on infertility induced by oxidative stress in males [32]. In study by Elsawy H and *et al*, in CCl_4 group showed intense centrilobular necrosis, hepatocyte degeneration, vacuolar fatty alteration and slight mononuclear cell infiltration [33]. carbon tetrachloride affected spermatogenesis. In this way, the serum levels of testosterone, LH and FSH were significantly reduced compared to the control group. Carbon tetrachloride decreased the secretion of hormones in rats directly through the central nervous system and Leydig cells or indirectly through the hypothalamic-pituitary estrogen receptor axis [34].

Elsawy and *et al*.'s research showed that high levels of serum cholesterol are related to testicular disorders and decreased sperm quality. Therefore A diet high in antioxidants and low in cholesterol improves spermatogenesis [35].

In another study by Badr and Colleagues was indicated that carbon tetrachloride (3ml/kg) poisoning caused hypospermatogenesis in irregular seminiferous tubules with primary edema in the intestitial spaces in male rats. But the administration of Parsley Leaf (*Petroselinum crispum*) in a dose of 0.5 ml/kg moderated these changes. Also, Parsley Leaf treatment induced Significant increase in serum levels of Testosterone, LH and FSH. This improvement was due to the presence of natural antioxidants in the extract [12].

CONCLUSION

This study determined that exposure to CCl_4 in male rats damages the liver and testis. Due to presence of various flavonoids, the root extract of *S. pachycarpa* can improve the harmful effects of the carbon tetrachloride in testicular tissue and the levels of sex hormones. Therefore, it can be said that SpRE is useful in preventing infertility caused by CCl_4. However, SpRE led to cytotoxicity in intact hepatic cells.

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Conflict of Interest

The authors declare no conflict of interest.

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