Protective effect Investigation of "Sour Orange Peel Extract" on Liver Histopathological Changes, Caused by the use of CCl₄, in Female Wistar Rats

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ABSTRACT

Introduction and objective: Orange (Citrus Aurantium L.) is known as a medicinal plant used in traditional medicine. The protective effects of citrus fruits against the risk of some chronic diseases have been shown. The purpose of this study is to evaluate the antioxidant effects of ethanol extract of orange peel on liver tissue on the mice damaged by CCL₄. Materials and Methods: This experimental study was performed on 48 male Wistar rats, weighing 200-150 g. The mice were divided into 6 groups as follows: 1) Control group (mice were kept without reception of any materials), 2) The recipient group of CCL₄ that receive daily 5 gr per kilogram of body weight of carbon tetrachloride orally, 3) The receptor group of the extract at a dose of 100 ml per kg body weight, 4) The receptor group of the extract at a dose of 400 ml per kg body weight, 5) The receptor group of CCL₄ with the extract at a dose of 1 ml per kg body weight, 6) The receptor group of CCL₄ with the extract at a dose of 4 ml/kg. It should be noted that the method of treatment is orally. In the end, the mice in all groups were anatomized and then liver tissues were removed for histological studies. For this purpose, liver tissues injury with microscopic and histopathological examination were carried out and the results were reported as follows. Findings: In a study of the histopathologic changes of liver tissue in different groups, the vasculature in the liver sinusoids and portal liver damaged by carbon tetrachloride in comparison to the control group were observed. These changes reduced in the treated groups with extract of orange peel - and in carbon tetrachloride + 400 orange induced liver injury is far less than the other + carbon tetrachloride + 100 orange peel. Conclusion: The results of this research indicate that orange peel extract may have liver protective effects against carbon tetrachloride which may be related to the present flavonoids in this extract and can be seen more at higher doses effect.

INTRODUCTION

Today, with the return to nature and according to its blessings, once again the use of natural compounds found in medicinal plants is preferred to synthetic molecules, as the adverse effects thereof are unpredictably dangerous. With regard to plants, and due to the fact that humans have long enjoyed plants and as a food source and for medical needs, many manuscript are available, all of which illustrate the important role of plants in human life [1]. Citrus, an important fruit of trees in semi-tropical areas, is of considerable importance in terms of economics, employment, drug and vitamin supply. Among citrus, orange (Citrus Aurantium L.) is considered a medicinal plant of high-consumption and native to Iran. In Iranian traditional medicine, the flowers of this plant are used to treat nervous disorders such as hysteria, epilepsy, and neurasthenia. Additionally, this plant has been known as to relieve palpitations [2]. On the other hand, the antioxidants found in fruits and vegetables containing ascorbic acid, carotenoids, flavonoids and tannins, play an important role in preventing disease[3]. Incidentally, various species of citrus fruit are considered as flavonoid important resources [4]. Also, the phenolic compounds of citrus fruits are regarded as a great resources of antioxidant material[5].

Orange with the scientific name Citrus Aurantium L. of the family Rutaceae [Citrus] is known by the conventional names of Bitter Orange, Citrus vulgaris Orange, Citrus. Orange [Citrus Aurantium L.] is a shrub that reaches 6-4 meters in height, this tree like other citrus trees is an evergreen. Orange flowers in Iran are well known as orange blossoms which bloom in mid-spring and fall seasons [6].

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The orange medicine sectors including fresh and dried peel fruits, flowers, seeds and oil extracted from it [7]. The extracted essence of orange that contains 20 kinds of compound the most common of which are linalool [34%], Linalyl acetate [6-17%], Limonin [15%], pinene, geraniol, nerol, Methyl anthranilate, indole, Citral, Farneh soul and jasmon [8].

The essence compounds in the leaves of plant, flowers and peel of this fruit are significantly different, in particular, Linalyl acetate [Patigreen] with 50% in the leaf essence, and Linalool with 35% in essential oils of nerol flowers[9]. Limonin orange peel oil contains 90%, flavonoids, coumarin, vitamin C, carotene and pakten. Flavonoids have anti-inflammatory, antibacterial and antifungal properties[8]. The bitterness associated with Citrus Naringin and Limonin is due to being oxidized in air. Naringin is one of the flavonoids that is found in orange and its structural and chemical formula is as follows [8]. Orange [Citrus Aurantium L.] is used to treat throat inflammation, chest pain, cough, headache, seizures and hiccup. Orange peel is a laxative and is suggested for stomach that has a lot of acerb[acid] is digestive of food[7]. Research has stated that carbon tetrachloride, with the chemical formula of C-CL4 and molecular weight of 82/153 isfrom halogenated aliphatic hydrocarbons families, saturated halogenated hydrocarbons, halogenated alkanes, haloalkane, Tetra Halo alkanes, chloroethyl alkanes, chlorine methane. Carbon tetrachloride is a colorless liquid, with a pleasant odor, like chloroform. This liquid is non-polar and insoluble in water and has no specific PH, but in non-polar solvents such as ethanol, benzene, chloroform, diethyl ether, carbon disulfide, petroleum ether and acetone have good solubility. This chemical combination is also known by other names such as: Carbona, carbon chloride, carbon Tett, methane tetrachloride, Prochloroethyl methane, methane chloroethyl Tetra, Tetra form, tetrachloroethyl carbon dioxide.

Carbon tetrachloride has the highest toxicity on the liver and because it quickly turns into vapor form, and through inhalation rapidly enters into the nasal passages, it is very dangerous and harmful. This substance affects on the central nervous system and its symptoms can be noted by headache, nausea, dizziness and drowsiness, vomiting, drunkenness and lack of coordination of body movements[10, 11]. In another study performed by Mishra and Roa in 1998, hepatic protective effects of methanol extract of parviflora have been demonstrated over the toxicity caused by carbon tetrachloride. In other researches, an important compound of the plant called Muvmtyl fumarate, which is known as an anti-fever substance [12]. Moreover, in another study, the protective effects of alcoholic extract of pistachio blue-gum on the liver against toxicity induced by carbon tetrachloride in rats was investigated. Hepatic activities were measured by biochemical variables including glutamic pyruvate transaminase and indicated that these variables were significantly increased [13]. According to the mentioned subjects and the destructive effects of carbon tetrachloride on the liver tissue, the most important aim of the present study is to determine the suitable antioxidants in order to reduce the effects of carbon tetrachloride. As no research related to antioxidant effect of orange plant peel on the carbon tetrachloride on liver texture has been performed to date, the main aim of this study is to investigate this issue.

The work method:

This study was conducted in vitro and completely random. All of the work ethic principles have been followed with regard to the experimental animals of this research. The number of 48 adult female Wistar rats aged 2-3 months and weighing 200 to 150 gr were prepared at the Animal House of Jahrom Medical Sciences University. These rats were kept for 2 weeks in in vitro conditions, including the temperature of 2 ± 21°C and 12 hours light/12 hours dark cycle.

Rats were kept in metal cages with mesh door and were fed the standard food. Water was also provided from special glass bottles. Their cages were disinfected three times a week with 70% alcohol. In this study, carbon tetrachloride, in the amount of 5 mg / kg in corn oil [0.2-0.5 ml]: PH in time of before use was solved and in terms of single dose and orally by gavage or by injection about 2 ml was received. Moreover, orange [Citrus Aurantium L.] peel extract as well as the two minimum dose [100 ml/ kg of body weight] and maximum dose [400 ml/ kg of body weight] was given orally. In the present study these rats were divided into groups of eight as follows:

1- Control Group: In this group no treatment was performed and only the standard food and water were provided during the 14-day trial period for the mice.
2- Carbon tetrachloride Group: This group received carbon tetrachloride dissolved in corn oil for every 5g/kg body weight at the beginning of the research.
3- Carbon tetrachloride Group+ Minimum dose: This group received carbon tetrachloride for every 5 g/kg body weight at the beginning of the research and then were treated with 1 ml of orange peel extract for 14 days.
4- Carbon tetrachloride Group+ Maximum dose: This group received carbon tetrachloride for every 5 g/kg body weight at the beginning of the research and then were treated with 4 ml of orange peel extract for 14 days. It should be noted that the treatment time of the mice with the orange peel extract was 14 days after carbon tetrachloride gavage was performed.
5- Citrus[Orange] peel extract group + Minimum dose: This treated group was treated with 1 ml of orange peel extract per 100 g body weight for 14 days during the trial period.
6- Citrus[Orange] peel extract group + Maximum dose: This group was treated with 4 ml of orange peel extract per 100 g body weight for 14 days during the trial period.

After completion of the research, all groups of mice after anesthesia were anatomized and liver tissue after rinsing with saline and placed in Formalin solution 10% and for the preparation of the next steps, the slides of tissue sections and hematoxylin eosin staining were sent to the laboratory. The related tissues that were prepared from different sections of liver, in terms of tissue cell structure and all its components, including Hepatocytes, platter space, sinusoids as well as other survey factors of apoptosis amount in these sections were examined by light microscopy and the number of necrotic cells was reported in each liver.

The prepared slices and slides of liver, in terms of morphological were examined and thus the cell death by observing the color change of hepatocytes compared to control slides and the lack of organizing of these cells was detected.

Results:
In a study of histopathological changes of liver tissue in different groups, the vasculature in the liver sinusoids and portal areas in the liver damaged group was observed by carbon tetrachloride compared to the control group [Fig. 1 and 2]. Also in this category, cell death and liver cells atresia and hepatocytes were observed [Fig. 2]. On the other hand, in study of the prepared slides from the livers of other groups, these changes in the treated groups with the orange peel extract decreased and in carbon tetrachloride + orange 400 induced, the liver injury alternatively was less than that of carbon tetrachloride + orange peel 100 [Fig. 3 and 4]. The histopathological changes in the liver tissue in Fig. 2 clearly indicate liver intoxication by carbon tetrachloride. The cell death, by observing liver cells fibrous and dense cores in the margin with H & E staining was detected. However, the damaged liver tissue with nuclei around the port space, reduction of sinusoids and the lack of cell organizing of this area and shrinkage nucleus was detected [Fig. 2]. Although these symptoms in liver tissues of mice of carbon tetrachloride group were observed, in samples of carbon tetrachloride + orange peel 100 and carbon tetrachloride + orange peel 400 it was rarely observed. In addition, in the receptor of the extract with minimum and maximum doses no changes were observed in liver tissue [Fig. 5 and 6].

![Image](image.png)

Fig.1: The optical photomicrograph of control group of healthy liver which shows sinusoids space in the above image with magnification ×10 and Hepatocytes core in the image below with magnification ×40.

Discussion:
Today, liver disease is one of the major problems facing society worldwide. Liver is one of the most important body organs and is responsible for important and different functions, such as body metabolism and is constantly faced with toxins and metabolites and many threats of the drugs, materials and microorganisms [14]. Carbon tetrachloride is a known potent toxin against liver which is widely used in laboratories and on human and animal models [15]. Severe liver damage is caused by carbon tetrachloride on the liver, including liver cell damage, inflammation, necrosis or severe tissue necrosis and the increase of oxidative stress in liver cells [15]. In recent years, scholars have argued that liver cell damage is caused not only by spontaneous destruction of its liver cell to exposure of carbon tetrachloride, but also might due to the sudden attack of an excess of oxidative stress in liver cells [15]. Inflammatory reaction of liver cells are mainly caused by alpha necrosis factor [TNF-α], interleukin B1 [IL-B1] and kinase in the region, and form tumors in which these factors are parts of the indirect cells inflammatory agents. In severe tissue inflammation interleukin 6 [IL-6] is also present [15]. In our study, the slides prepared from liver tissue were carefully examined under an optical microscope and showed that in the receptor group of Carbon tetrachloride a percentage of cell death was clearly visible and these damages in Carbon tetrachloride were treated with orange juice, but the amount of cell death in liver tissue, which is far lower than carbon tetrachloride was visible. In other words, carbon tetrachloride was able to penetrate into the liver cells. In a study conducted by Aktaly et al., orange had anti-lipid peroxidation effects, so liver protective effect was demonstrated and liver necrosis was prevented. Our results are in agreement with these experiments, but in the group treated with orange peel extract 100, these positive effects were observed less and orange peel extract at this dose could not reduce the serious liver wound from carbon tetrachloride to treated group with...
orange extract 400. In a study by Aktaly et al in 2000 on the plants that have similar compounds with orange peel extract, it was stated that the plants have anti-lipid peroxidation effects, so the liver protective effect was demonstrated and liver necrosis was prevented. However, severe hepatic lesions induced by carbon tetrachloride were not reduced [16]. Therefore, the probability exists in the present study that the adverse effects of low doses of carbon tetrachloride on liver tissue completely heal, so the use of higher doses of the extract is recommended.

Fig. 2: The optical photomicrograph of a damaged liver in the recipient group of carbon tetrachloride, which shows dense cores and shrinking small arrow] and congestion[large arrow] have been shown in this figure. H & E polychromy with magnification ×10.

In a study of Brown Tan on liver cells freshly isolated from the liver, the results indicate that herbal compounds similar to orange peel extract against hepatotoxicity induced by carbon tetrachloride which have sweeper properties of free radicals, represent the effects of antioxidant extracts [17]. In the present study, the receiver groups of orange peel extract in the improvement of liver tissue in toxicity with tetrachloride was observed.

Fig. 3: The optical photomicrograph of the liver in the recipient group of carbon tetrachloride and the minimum dose of the extract, indicating the destruction of sinusoids with H & E polychromy with magnification ×10.
Fig. 4: The optical photomicrograph of the liver in the recipient group of carbon tetrachloride and the maximum dose of the orange peel extract, which indicates congestion and hyperemia [arrows] with H & E polychromy with magnification ×10.

Fig. 5: The optical photomicrograph of the liver in the recipient group of carbon tetrachloride and the minimum dose of the orange peel extract, which indicates sinusoids space with H & E polychromy with magnification ×10.

Fig. 6: The optical photomicrograph of the liver in the recipient group of carbon tetrachloride and the maximum dose of the orange peel extract, which shows sinusoids space with H & E polychromy with magnification ×40.

Conclusions:
Accordingly, it can be said that the toxicity caused by carbon tetrachloride is related to the chlorine-carbon bond being broken and the formation of a free radical named proxy chloromethyl. Because the mechanism of toxicity caused by CCL4 is the production of free radicals, any substances that have antioxidant effects and are considered as the sweeper of free radicals in cells can reduce the toxicity. The results of this research show that orange peel extract may have liver protective effects against carbon tetrachloride, which may be related to the present flavonoids in this extract which can be seen more at higher doses.

REFERENCES


