

Original Article

Ameliorating Effect of Gamma Irradiated Chicory against Carbon Tetra-Chloride Induced Kidney and Testis Damage in RatFereshteh Ezzati Ghadi¹ Abdollah Ramzani Ghara^{1*} Zahra Rodbari²

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Abstract

Background and purpose: Gamma radiation can affect the content of phytochemicals. The aim of this study was to investigate the ability of gamma irradiated chicorium intybus L. root extract to protect against carbon tetra-chloride (CCl₄) induced kidneys and testes injury.

Materials and Methods: The rats were divided into six groups according to treatment: I) control, II) CCl₄ (1ml/kg body weight by intraperitoneal injection (IP)), III) gamma irradiated chicory root extract (500 mg/kg body weight) + CCl₄, IV) non-irradiated chicory root extract (500 mg/kg body weight) + CCl₄, V) gamma irradiated chicory root extract, and VI) non-irradiated chicory root extract. The level of BUN and creatinine, and also histological study of kidney and testis tissues were estimated twenty-four hours after the last treatment at the end of four weeks.

Results: Gamma irradiated chicory root extract significantly decreased elevated level of BUN and creatinine in the serum of CCl₄ treated rats. Histological evaluation revealed that gamma and non-irradiated chicory root extract treatment to CCl₄ rats demonstrated reduce Bowman's space and basement thickening of kidney. Moreover, the results showed the normalization of testes of CCl₄ treated animals in group III and IV.

Conclusion: The gamma irradiated chicory root showed kidney and testis protection against CCl₄ in rats. Based on the results of the present study, it can be concluded that chicory has a potent protective effect more than non-irradiated chicory root extract due to containing phenolic content.

Key words: Gamma Ray; Chicory root; CCl₄; Kidney; Testis

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1. Introduction

Carbon tetra-chloride (CCl₄) is known to be hepatotoxic as well as nephrotoxic leading to testis damage to humans and experimental animals (1). The initial step in the tissue injury induced by CCl₄ is its cytochrome P450 mediated formation of trichloromethyl radical (CCl₃) and trichloromethylperoxyl (CCl₃OO) free radicals which can react with sulfhydryl groups (glutathione and protein thiols) in addition to antioxidant enzymes, such as catalase and superoxide dismutase. Over the production of trichloromethyl free radicals a membrane lipid peroxidation is initiated, eventually leading to various pathological changes.

Male sexual derangement is caused by various agents, such as alcoholism, drug abuse, smoking, some drugs and chemicals (2). Testicular dysfunction is the main underlying cause of male developmental and sexual dysfunctions. Spermatozoa are very sensitive to ROS, as their plasma membrane is comprised of polyunsaturated fatty acids (PUFA), which can be easily oxidized, and they also lack cytoplasm to produce a robust preventive and repair mechanism against free radicals (3). However, this makes spermatozoa particularly vulnerable to attack by reactive oxygen species (ROS) (4). Administration of CCl₄ causes an increase in lipid peroxidation products and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney(5). The CCl₃ and CCl₃OO radicals are reported to enhance lipid peroxidation and protein oxidation, resulting in widespread membrane damage and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney (6). Chicory is an indigenous vegetable widely cultivated in Europe, America, and Asia. In ancient times, the leaves, flowers,

seeds, and roots were used as a wealth of health benefits including its tonic effects, the ability to ease digestive problems, and to detoxify liver (7). Several authors have reported the medicinal importance of chicory due to the presence of a number of compounds, such as inulin, sesquiterpene lactones, alkaloids, coumarins, chlorophyll pigments, flavonoids unsaturated sterols, vitamins saponins, and tannins (8).

Radiation processing is well established as a physical, non-thermal method to preserve various food products, and it involves the exposure of food products (raw or processed) to ionizing or non-ionizing radiation (9). Irradiation of food products causes a minimal modification in the flavor, color, nutrients, taste, and other quality attributes of food (10). It has been reported that gamma irradiation could increase phenolic content in almond skin, and cinnamon (11). However, little information is still available regarding the changes on chemical or bioactive properties, as well as effect on the different diseases of processed medicinal plants by gamma ray. Thus, the current study was conducted to investigate whether gamma irradiated chicory has any ameliorating effect on histopathological lesions associated with the oxidative stress.

2. Materials and Methods

2.1. Radiation facility

Chicory roots were collected from Jiroft, Iran. The roots were washed with distilled water and were subjected to 8 kGy of gamma radiation using ⁶⁰Co source (Gamma cell 220) at room temperature in Atomic Energy Organization of Iran.

2.2. Preparation of Plant extract

The irradiated and non-irradiated chicory roots were dried in shade and made into a

powder. Then 1 g of each sample was mixed with 20 ml of 95% ethanol and stirred for 2 hours using magnetic stirrer, and then held for 48-72 hours. The resulting extracts were filtered through filter paper number 1, and dried using rotary evaporator, and then stored at 4°C until use.

2.3. Experimental design

Twenty four male rats (180-200 g) were assigned to six groups: I) animals served as normal control and were given water and diet *ad libitum*, II) rats were given IP injections of CCl₄ (1ml/kg b.w) suspended in olive oil at a ratio of 2:3 by volume twice a week for four weeks (12), III) animals were given irradiated chicory root extract (500 mg/kg b.w by oral gavage) to CCl₄ treated rats three times a week (13), IV) Animals were treated with non-irradiated chicory root extract as group III ,V) The normal control were given irradiated chicory root extract, and VI) animals received only non-irradiated chicory root with dose of 500 mg/kg b.w three times a week.

At the end of four weeks, all animals were sacrificed, and blood was collected from all treatment groups. The serums were collected and kept at -20°C for biochemical estimations.

2.5. Relative weight

The relative kidney weight was calculated using the formula as follows (14):

$$\begin{aligned} & \text{Relative kidney weight (\%)} \\ &= \frac{\text{kidney weight (g)}}{\text{Body weight (g)}} \\ & \times 100\% \end{aligned}$$

2.6. BUN and creatinine estimation

Creatinine and BUN concentration was estimated using spectrophotometric assay (Pars Azmoon Inc, Tehran, Iran).

2.7. Histopathological study

The histopathological study was conducted according to the method of Humanson(15).

2.8. Statistical analysis

The collected data were analyzed statistically, using SPSS Version 20. One-way analysis of variance (ANOVA) was performed to compare the different groups and to determine the overall effect of each treatment, followed by a multiple post hoc test, while the least significant difference of 5% ($p < 0.05$) was considered as significant. The results are represented as mean \pm standard deviation.

3. Results

3.1. Serum biochemical estimation

The CCl₄ treated rats demonstrated a significant increase in the level of creatinine ($p < 0.001$) and BUN ($p < 0.001$) in serum as compared to the normal control group (Table. 1). Gamma irradiated chicory root extract also significantly decreased creatinine ($p < 0.05$) and BUN ($p < 0.01$) content in serum in group III. Moreover, the elevated level of BUN was decreased in the non-irradiated chicory root extract in group IV.

Table 1. The effects of gamma irradiated chicory root extract on creatinine and BUN of CCl₄ treated rats

Serum biochemical estimation		
Groups	Creatinine (mg/dl)	BUN (mg/dl)
I	0.487± 0.05	49.25± 3.86
II	0.685 ± 0.11 ^c	62.00± 3.91 ^c
III	0.572± 0.057 ^x	54.00± 5.77 ^y
IV	0.630 ± 0.04 ^b	55.50± 3.69 ^{a,x}
V	0.477 ± 0.05	49.25± 1.25
VI	0.492 ± 0.03	43.75± 2.21

Results are presented as mean ± S.D. N=4. ^a*p*<0.05, ^b*p*< 0.01 and ^c*p*< 0.001 represented the statistical significant between group I animals and all treated groups. ^x*p*< 0.05 and ^y*p*< 0.01 also showed the significant difference between group II and groups III and IV.

3.2. Histopathology of kidney and testis

Kidney of control rats showed normal structure. In CCl₄ treated animals, kidney sections demonstrated atrophy of glomerular capillaries with increased Bowman's space (Figure 1b). In group III, Bowman's space, glomerular capillaries were improved towards normal conditions (Figure c and d, Table 2). There was no significant difference between control and gamma treated and non-irradiated chicory in glomerular structure, as shown in Table

2, and Figure 1(e, f). Seminiferous tubules were completely healthy in terms of appearance, number of spermatogonia cells, and thickness of the basement membrane (Figure 2 (a), Table 2). CCl₄ treated group, on the other hand, showed a reduction in spermatogonia cells and seminiferous tubules diameter, in addition to an increase in the thickness of basement membrane (Figure 2 (b), Table 2). Groups III and IV also showed that changes were improved towards normal.

Table 2. The effects of gamma irradiated chicory root extract on kidneys and testis of CCl₄ treated rats

Groups	Kidney			Testis	
	Diameter of glomerular capillaries (µm)	Diameter of Bowman's space (µm)	Relative weight of kidney (%)	Number of spermatogonia	Epithelium (µm)
I	342.33 ± 11.23	38.33 ± 3.05	0.73 ± 0.05	51.33 ± 15.05	377.41 ± 29.32
II	200.00 ± 17.32 ^c	294.00 ± 5.02 ^a	0.65 ± 0.06 ^a	28.00 ± 2.00 ^b	239.83 ± 21.84 ^b
III	277.66 ± 26.57 ^{c,z}	78.66 ± 5.50 ^{a,z}	0.74± 0.05 ^x	43.66 ± 4.16 ^x	406.75 ± 62.08 ^z
IV	318.66 ± 2.30 ^z	86.66 ± 5.13 ^{b,z}	0.75± 0.04 ^y	43.33 ± 1.52 ^x	448.41 ± 63.46 ^z
V	355.66 ± 2.51	46.00 ± 6.08	0.71± 0.02	51.66 ± 6.11	456.41 ± 20.38
VI	333.66 ± 20.20	44.00 ± 7.54	0.72± 0.02	41.33 ± 4.16	384.28 ± 51.51

Results are presented as mean ± S.D. ^a*p*< 0.05 and ^b*p*< 0.01 represented the statistical significant between group I animals and all treated groups. ^x*p*< 0.05, ^y*p*< 0.01 and ^z*p*< 0.001 also showed the significant difference between group II and groups III and IV.

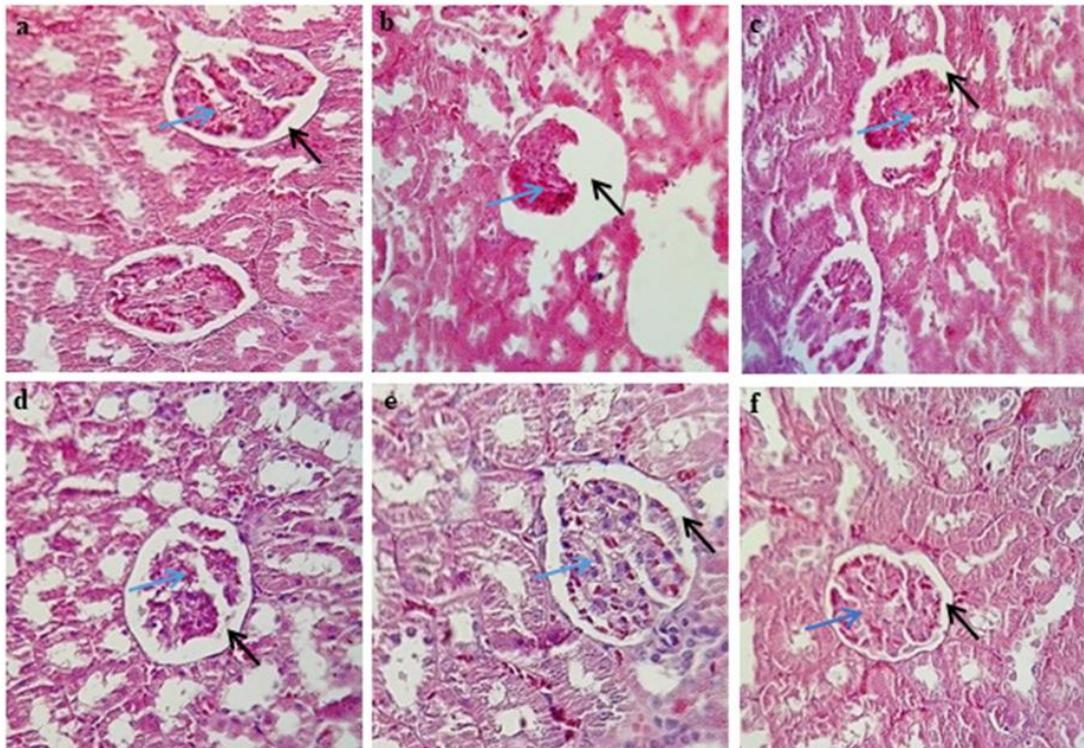


Figure 1. Histopathological evaluation of kidney sections. (a) Control, (b) CCl₄, (c) CCl₄ + gamma irradiated chicory root, (d) CCl₄ + non-irradiated chicory root, (e) Gamma irradiated chicory, and (f) Non-irradiated chicory root treated animals. Bowman's space (black arrow) and glomerular capillaries (blue arrow). (H&E stained and $\times 400$ magnification).

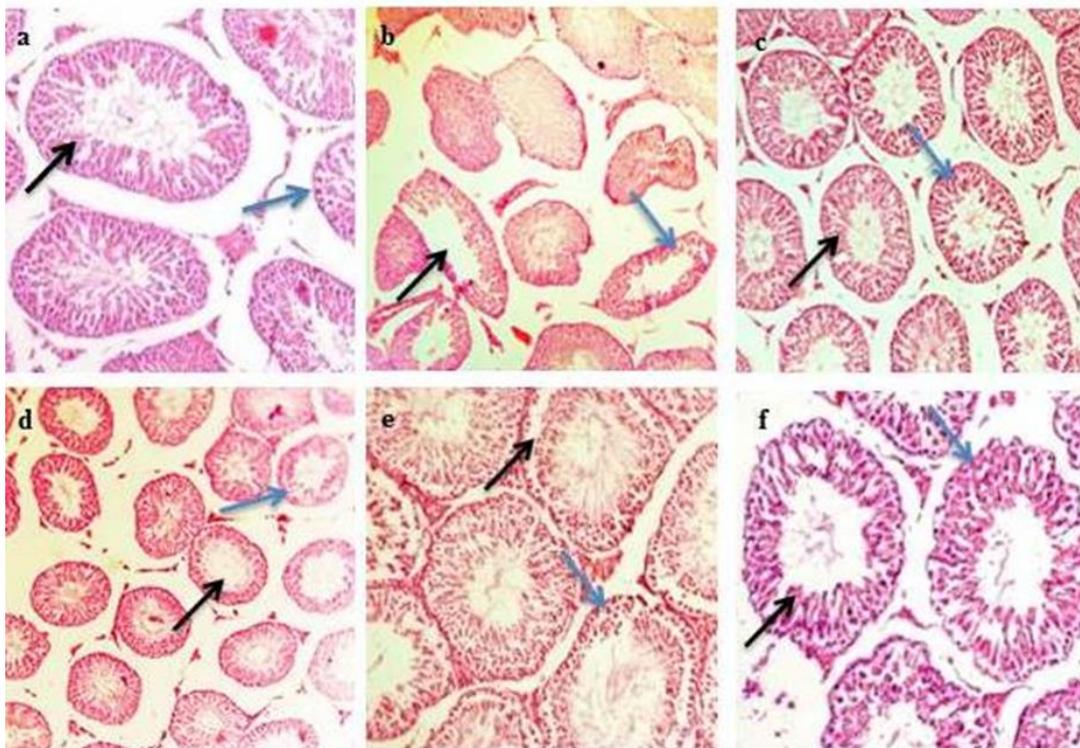


Figure 2. Histopathological evaluation of testes sections. (a) Control, (b) CCl₄, (c) CCl₄ + gamma irradiated chicory root, (d) CCl₄ + non-irradiated chicory root, (e) Gamma irradiated chicory, and (f) Non-irradiated chicory root. Seminiferous tubules (black arrow), and Spermatogonia (blue arrow). (H&E stained and $\times 100$ magnification).

4. Discussion

CCl₄ is well-known as typical toxic agent which causes tissue damage in liver, kidney, lung, brain, and testis (1). It has been reported that CCl₄ administrated to rats is distributed at higher concentrations in the kidney than in the liver (16,17). CCl₄ causes renal damage through generation of free radicals. At the same time, kidney tissue has great affinity for CCl₄ due to the predominant presence of cytochrome P450 in the renal cortex. It has also been reported that free radicals of CCl₄ bind with polyunsaturated fatty acid of sperm membrane produce alkoxy and peroxy radicals that, in turn, generate lipid peroxides, and finally induce injury or necrosis. Plasma BUN and creatinine are known to be significantly elevated in certain forms of infection (18) and in chemical toxicity sufficient to cause a compromise in renal function (19). Chemical insults capable of causing substantial damage to the glomerulus are known to cause elevations in plasma urea and creatinine (20). The researchers also observed a significant increase in creatinine and BUN of CCl₄ group, which was in agreement with previous reports (21). Decrease in serum creatinine, BUN levels of groups that were treated with gamma irradiated and non-irradiated chicory confirmed a contributory mechanism of reduced oxidative stress. Previous studies have also revealed similar conclusions against CCl₄-induced oxidative stress in kidney while dealing with different plant extract/fractions or biflavonoids (22, 23). Changes in Bowman's space, atrophy of glomerular capillaries, relative weight of kidneys, and thickness of basement membrane were histopathologically investigated in CCl₄ treated rats, which

agreed with (24). Several investigations have also documented the different plant extracts significantly recovered biochemical marker fluctuations induced by CCl₄ intoxication (25). According to latter findings, plant extract and its fractions comprehensively ameliorate the injuries induced through CCl₄ intoxication, and affect the activity of biochemical enzymes, DNA strand breakage, and increase the activity of telomerase cancer marker enzyme of kidney tissue (26). It might be related to the antioxidant properties of these plant extracts which seem to be related to their molecular structure, presence of hydroxyl groups, double bond conjugation, resonance effects of substituents in the phenyl ring, and the position of its nitrogen atom relative to the N-H bond, as was previously reported (27). The findings of the current study also revealed that sections of testes from CCl₄ rats showed the disorganization of seminiferous tubule, and destruction of walls of seminiferous tubules, which was in accordance with Hanafi and Khan (28,29). These effects were found to be due to the production of oxygen radicals in excess of the stressed tissue (30). In the current study, use of gamma irradiated and non-irradiated chicory root extract was found to cause mild changes in kidney and testis when compared with the changes in the CCl₄ group.

Chicory contained high phenolic contents, especially in leave and roots (31). Our previous research work revealed, gamma irradiation at dose levels of 4, 6 and 8 kGy, caused significant elevation in the phenolic contents (32). Phenylalanine ammonia-lyase (PAL) was documented to be a key enzyme in the phenylpropanoid pathway

responsible for the synthesis of several plant phenolics. It was also reported in many instances that PAL activity may increase in response to various biotic and abiotic stress, such as wounding, drought, irradiation, and insect attacks (33). The above increase in phenolic contents could be due to the ability of gamma irradiation to stimulate activity of PAL. Moreover, this increase in phenolic content might be owing to the release of phenolic compounds from glycosidic components and degradation of larger phenolic compound into smaller ones by gamma irradiation. Finally, it can be concluded that the gamma irradiated chicory root extract has protective effect against renal and testis structural changes induced by CCl₄ in rats.

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Conflicts of Interest

The authors claim that there is no conflict of interest.

Author's contribution:

Ramzani Ghara and Ezzati Ghadi have made a substantial contribution to conception design and interpretation of data, drafting and revising the manuscript for intellectual content. Also, Rodbari made a significant contribution to analysis of data. All authors read and approved the final manuscript.

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