

Histo-Pathological Effects of Raphanus Sativus Leaf Extract on Carbon Tetrachloride Induced Hepatotoxicity

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ABSTRACT

Background: Raphanus sativus is a cruciferous plant which possesses free radical scavenging properties that defend the body against the deleterious effects of reactive oxygen species. The study objective was to assess the protective effect of Raphanus sativus in carbon tetrachloride induced hepatotoxicity in albino Wistar rats.

Methodology: This animal based experimental research was conducted from September 2019 to March 2020 at the Postgraduate Research Laboratory, Isra University, Hyderabad. Non-random purposive sampling technique was used for selection of study animals. Rats were distributed evenly in three different groups: Group 1 (control group), Group 2 (CCI4 induced experimental group), and Group 3 (CCI4 plus Raphanus sativus treatment group). Data was analyzed using SPSS version 24.

Results: Liver weight and serum markers of hepatic function were high in group 2 as compared to group 1 and C rats ($p < 0.05$). Treatment with Raphanus Sativus significantly reduced serum levels of LFTs ($p < 0.05$). There was a significant decline in the plasma levels of oxidative markers in group 2 while marked histo-pathological changes like necrosis, sinusoidal dilatation and congestion observed among animals of group 2.

Conclusion: Raphanus Sativus wields anti-oxidative as well as hepato-protective effect against carbon tetrachloride induced hepatotoxicity and tissue damage.

Keywords: Carbon Tetrachloride, Raphanus, Oxidative Stress

Authors' Contribution:

¹Conception; Literature research; manuscript design and drafting; ² Critical analysis and manuscript review; ³ Data analysis; ⁴Manuscript Editing⁵.

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Introduction

Liver, with its crucial role in the conservation of homeostasis and detoxifying baneful drugs, is one of the most vital organs required for survival.⁽¹⁾ Globally, liver diseases account for more than 1.5 million deaths every year, half of which are owed to complications of liver disease such as cirrhosis and liver cancer. Altogether, liver diseases are responsible for more than 3% of overall mortality

around the world.⁽²⁾ Various elements recognized to adversely affect the structural/ and or functional properties of the hepatic tissue, leading to grave implications, have been regarded as hepato-toxic. Carbon tetrachloride (CCI4) being one of those elements, results in hepato-toxicity owing to its innate potential to undergo dehalogenation yielding trichloromethyl (CCl₃⁻), which in turn stimulates

oxidative stress eventually leading to cell-injury and cell death.⁽³⁾ In addition, actuation of Kupffer cells induces the onset of an inflammatory cascade, thereby increasing the serum levels of inflammatory markers.^(4, 5)

Due to reported side effects of certain drugs currently being used for the mitigation of hepatotoxicity, alternative plant based compounds have gained tremendous interest in recent years.⁽⁶⁾ Among such plants is *Raphanus sativus* (*R. Sativus*), a cruciferous plant also commonly called Radish, which has since long been used in traditional medicine.⁽⁷⁾ The chemicals found within the roots and leaves of *R. Sativus* include various nitrogenous compounds, alkaloids, and phenols, etc. which have shown to possess free radical scavenging properties that defend the body against the deleterious effects of reactive oxygen species.⁽⁸⁾ *R. Sativus* extract has also shown to augment the activity of various antioxidants within the body, such as catalase and glutathione peroxidase which consequently prevent lipid peroxidation.⁽⁹⁾

Pakistan, holding a liver disease related mortality rate of 23.24 per 100,000 deaths, bears a huge burden of liver disease.^(10, 11) To the best of our knowledge, no studies have been conducted in Pakistan, in recent years, on the possible hepatoprotective effect of the indigenous species of *R. Sativus* growing in the country, which itself poses a significant knowledge gap.

The aim of the current study was to explore the protective effects of *Raphanus sativus* on CCL4 induced hepatotoxicity by examining different makers of hepatic function as well as analysis of hepatic histological parameters.

Methodology

This animal based, quasi-experimental study was conducted from September 2019 to March 2020. Total thirty male, healthy Wistar Albino rats of age from 8-10 weeks and having body weight 250-300 grams, were included in the study. The study animals were acquired from the Sindh

Agriculture University, Tando Jam, Sindh. While the experiment was conducted at the Postgraduate Research Laboratory, Isra University, Hyderabad. Selection of rats was done by non-random purposive sampling technique while the standard method of power analysis for animal studies was used for the sample size calculation.^(12, 13)

The procured Wistar rats were placed in plastic cages at postgraduate laboratory in Isra University, Hyderabad. The animals were kept in a for ten days acclimatization period at the optimal temperature of 24-26°C in a day-night (12/12) hours cycle. Each cage was having nozzles of stainless steel bedded with sawdust along with feed containers to avoid any harm to the study animals. While rats were provided with chow diet and clean water ad libitum during this period.

Fresh leaves of *Raphanus sativus* were procured, taxonomically identified and authenticated from the department of Horticulture, Sindh Agriculture University, Tando Jam. Fresh leaves were splashed thoroughly to remove dirt or any potential contaminants. The leaves were dried at room temperature for 10 days. After that all the leaves were further dried at the temperature of 60°C for six hours in a hot air oven. The dried leaves then grinded using an electric grinder and the powder obtained was then filtered through mesh sieve. Later the 80% ethanol was mixed with the filtered powder and then extracted with as well as filtered with filter paper (Whatman No. 2). The extracts were concentrated in a hot air oven at 37°C, lyophilized by freeze drying apparatus (Christ Germany model # Alpha 1-4LSC) and subsequently air tightly stored at -20°C.⁽¹⁴⁾ After the period of acclimatization, the rats were weighed individually before the experiment began.

Rats were divided into three groups, each group have same number (n=10) of rats. Group 1 was the control group, in which rats were given a normal chow diet and clean water ad libitum only,

Group 2 was the experimental group, in which rats were given (single dose of CCl₄, 1.2 mg/kg in 50 mM phosphate buffer solution, subcutaneously), and Group 3 (single dose of CCl₄, 1.2 mg/kg in 50 mM phosphate buffer solution, subcutaneously + 100 mg/Kg R. sativus extract). Treatment of R. Sativus extract orally was done by force feeding the animals for a span of 28 days through a stainless-steel feeding syringe. The level of the orally administered dosage of R.Sativus extract (100 mg/Kg) and the subcutaneous dose of CCl₄ (CCl₄ 1.2 mg/kg) was based on previous studies.^(15, 16)

On completion of experiment period of four weeks, the body weight of rats in all three groups were measured once again using an electronic precision balance. Later, all rats were sacrificed by cervical dislocation under anesthesia. Later, blood samples were collect from all rats by cardiac puncture for analysis of oxidative and liver function markers.

The viscera of animals were dissected out and preserved after weighing them on an electronic scale. The liver of all animals were used for further evaluation, 10% buffered formalin was used for fixing and then the tissues were lodged in paraffin wax after passing in xylene for clearing purpose. 4-µm thick slices of the liver tissues were then obtained via a rotary microtome 290. Hematoxylin and Eosin (H&E) was used for staining. Histo-pathological analysis of hepatic tissue was performed by; appraising the degree of infiltrated inflammatory cells, fibrosis, necrosis, sinusoidal dilatations, and congestion of portal vein. A grading scale was adopted for observing the alterations and extent of tissue damage. Scale was comprised of four categories (none, mild, moderate and severe) depends on the level of alterations and severity.⁽¹²⁾

The collected data was entered and analyzed in SPSS version 24.0. One-way ANOVA with Post hoc Tukey's analysis was applied. Significance level of P-value ≤ .05 was considered as significant.

Results

The mean pre-experiment body weight of group 1 was 213.2±3.82 grams, group 2 was 215.4±3.78 grams and group 3 was 218.6±3.84 grams. There was a significant difference in mean post experimental body weight in all three groups i.e., in group 1 there was a rise in body weight (226.3±3.76 grams), while in experimental groups 2 and 3 ,significant decline in mean body weight (189.4±2.77 grams and 213.2±3.54 grams) respectively. However, in group 3 the weight loss was not as much as seen in group 2. There was a statistically significant difference (p<0.05) between the experimental groups. The relative liver weight was significantly raised in Group 2 as compared with other experimental groups (p<0.05).

A statistically significant rise in serum markers of hepatic function (LFT) was observed after CCl₄ administration in Group 2. Treatment with Raphanus Sativus administration significantly reduced serum levels of LFTs (p<0.05). (Table I)

Table I. Comparative Analysis of Liver Function Markers among Animal Groups.

	Group 1	Group 2	Group 3	P-Value
ALT (IU/L)	43.21±8.63	210.4±17.73 ^a	72.11±9.63 ^{a,b}	<0.05
AST (IU/L)	45.81±7.4	165.7±12.03 ^a	71.56±9.02 ^{a,b}	<0.05
ALP (IU/L)	81.6±15.12	261.9±6.73 ^a	120.3±6.04 ^{a,b}	<0.05
Total Bilirubin (mg/dL)	0.26±0.07	1.39.3±0.08 ^a	0.51±0.08 ^{a,b}	<0.05
Direct Bilirubin (mg/dL)	0.19±0.03	1.12.2±0.06 ^a	0.28±0.03 ^{a,b}	<0.05

^a p value < 0.05 as compared with Group 1

^b p value < 0.05 as compared with Group 2

^c p value <0.05 as compared Group 3

Statistically significant difference (p<0.05) in markers of oxidative stress was observed in all three groups i.e., in experimental group 2, there was a decline in the plasma levels of oxidative markers

while in group 3 the decline on oxidative markers was not as much as seen in group 2. (Table. II)

Table II. Distribution of markers of oxidative stress among animal groups.

	Group 1	Group 2	Group 3	P-Value
MDA (nmol/mg)	2.19 ± 1.57 ^b	4.6 ± 0.73 ^{c,a}	2.9 ± 0.08 ^b	<0.05
CAT (U/mg)	24.31 ± 0.68 ^{b,c}	18.11 ± 0.62 ^{a,c}	21.14 ± 0.61 ^{a,b}	<0.05
GPX (ng/dl)	1.39 ± 0.17 ^b	0.94 ± 0.09 ^{a,c}	1.28 ± 0.11 ^b	<0.05

^a p value < 0.05 as compared with Group 1

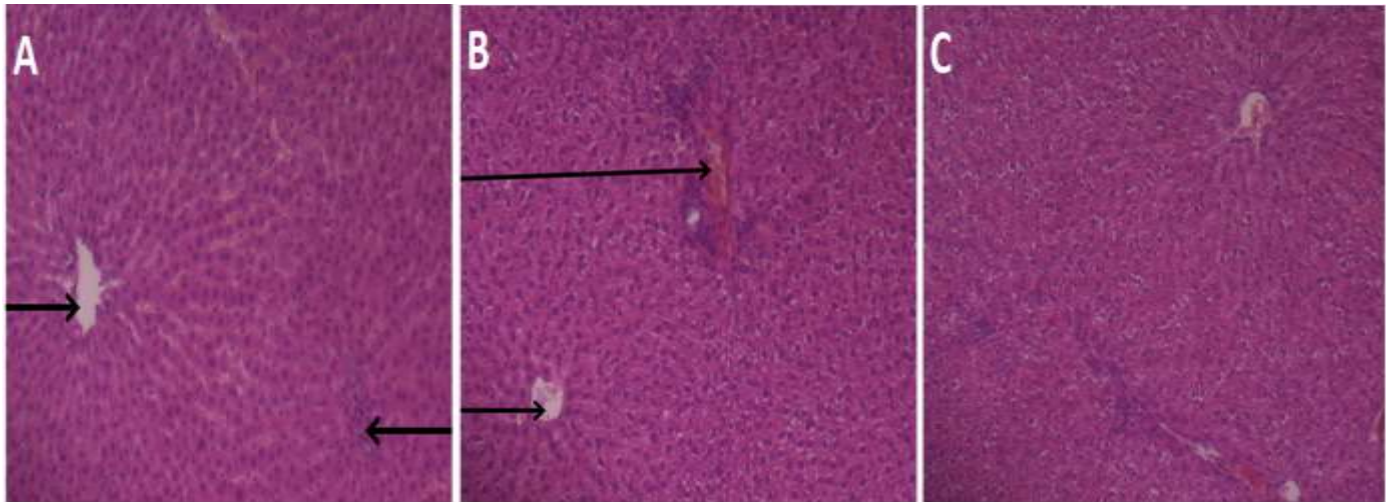
^b p value < 0.05 as compared with Group 2

^c p value < 0.05 as compared Group 3

Table III: Histo-pathological grading comparison of hepatic tissues in study groups rats

	Fibrosis	Necrosis	Inflammatory cell infiltration	Sinusoidal dilatations	Congested portal vein
Group 1	-	-	-	-	-
Group 2	+++	+++	+++	+++	+++
Group 3	+	++	++	+	++

Grading score: none (-), mild (+), moderate (++) and severe (+++)



On histological examination, fibrotic changes were significantly higher among experimental groups as compared to control group. Necrotic changes, hepatic inflammatory changes, sinusoidal dilatation and congestion due to inflammatory changes present in intra-lobular area, were found markedly higher among animals of group 2. Histomorphological changes in different groups of rats is presented in Table III.

Figure 1. Photomicrograph showing histological section of liver of control and experimental rats. (H&E) X 400. (A) – Control group rat with normal hepatic histological architecture without any infiltration. (B) – Experimental group 2 rat with areas of lymphocytic infiltration, marked congestion and fibrosis. (C) – Experimental group 3 rat with marked reduction in inflammation, necrosis and fibrosis.

Discussion

The aim of the current study was to explore the protective effects of *Raphanus sativus* on

hepatotoxicity produced by CCl₄ administration. This was achieved by examining different makers of hepatic function as well as analysis of hepatic histological parameters.

CCl₄ administration was associated with marked alteration of hepatic histological architecture as well as disruption of the normal liver function and lowered body weight. However, these toxic changes were significantly lower in the experimental animals receiving concomitant *Raphanus Sativus* therapy. This shows that *Raphanus sativus* exerts an ameliorative effect on the toxicity induced by CCl₄.

A commonly used solvent for various dry-cleaning agents as well as certain refrigerants, CCl₄ exerts both nephrotoxic and hepatotoxic effects.⁽¹⁷⁾ In the current study, CCl₄ administration led to a decline in the body weight of experimental animals. On the other hand, the experimental animals receiving concomitant *raphanus sativus* therapy showed a far less decline in their body weight. This is consistent with the results reported by Anwar et al. who also reported that *Raphanus Sativus* therapy prevents the weight loss in experimental animals which occurs secondary to liver toxicity.⁽¹⁶⁾

In the present study, a marked increase in the serum LFTs levels was noted in the animals receiving CCl₄ therapy as compared with the animals of the control group. These findings are in accordance with the results reported by Jeongtae et al.⁽¹⁸⁾ However, the serum LFT levels in the experimental animals receiving concomitant *Raphanus* therapy were less than those receiving sole CCl₄ therapy. These findings are also in accordance with the results reported by Jeongtae et al. and Rahman et al.^{(18) (15)} CCl₄ administration also led to oxidative stress, which was evident from the elevated levels of Malondialdehyde (MDA) and decreased serum levels of Glutathione peroxidase (GP_x) and Catalase (CAT).

However, these changes were not as exaggerated in the experimental animals receiving adjunct *Raphanus Sativus* therapy. These findings are consistent with those reported by Rahman et al.,

Meejung et al., and Shariq et al. who reported that *Raphanus sativus* therapy ameliorates oxidative stress.^(7, 15, 19)

In the current study, CCl₄ administration also caused marked alteration of hepatic histological architecture with significant inflammatory cells infiltration, necrosis, fibrosis and congestion, which is in accordance with previous literature.⁽²⁰⁾ However, these changes were not as markedly evident in the experimental animals receiving adjunct therapy of *Raphanus Sativus*. These results are in accordance with those reported in previous studies by Shariq et al. and Rahman et al., who also observed that the histological anomalies were less pronounced in the experimental animals that were receiving adjunct *Raphanus Sativus* therapy.^(15, 19)

Owing to limited availability of time and monetary resources, other parameters such as inflammatory markers could not be explored. Therefore, further studies are recommended to investigate the effects of *Raphanus Sativus*, both individually as well as in combination with other antioxidants.

Conclusion

Raphanus Sativus exerts a protective effect on hepatic tissue against CCl₄ induced hepatotoxicity and oxidative stress.

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