Original Article

# Potential Protective Effect of Pumpkin Seed Oil against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats



**Abdul Majid[1], Asim Mehmood[3], Rasheed Ahmed Soomro[4], Samreen Pandhiani[5], Aftab Abbasi[2], Hina Mawani[6]**

## ABSTRACT

**Objective:** To determine the potential protective effect of pumpkin seed oil (PSO) against Carbon tetrachloride Induced Hepatotoxicity in Albino Rats.

**Study Design:** Experimental study

**Place and Duration of Study:** This study was conducted at the Department of Anatomy, Pharmacology and Pathology, SRMC, T. Adam from March 2019 to January 2020.

**Materials and Methods:** 100 rats were divided equally into five groups. Group A negative control and Group B (positive control) ($CCl_4$ (0.2 mL/kg), Group C – $CCl_4$ (0.2 mL/kg) + PSO (1 mL/Kg bwt), Group D –$CCl_4$ (0.2 mL/kg+ PSO (2 mL/Kg) and Group E– $CCl_4$ (0.2 mL/kg)+(PSO (3 mL/Kg). Carbon tetrachloride was administered intraperitoneal at a dose of 0.2 mL/kg twice a week and PSO for 8 weeks. Blood samples were collected from the retro – orbital capillary plexus after anesthesia. Sera were used for the biochemical analysis; liver function tests (spectrophotometric), superoxide dismutase, glutathione peroxidase and catalase were detected by ELISA assay. Data was analyzed on statistical SPSS package (ver. 21.0) at 95% Confidence interval.

**Results:** We observed carbon tetrachloride ($CCl_4$) induced hepatotoxicity was improved significantly after 8 weeks pumpkin seed oil (PSO). Serum bilirubin, PT, ALT, AST and GGT were mitigated significantly ($P\leq 0.0005$). Pumpkin seed oil (PSO) augmented the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) activity after 8 weeks therapy ($P\leq 0.0005$).

**Conclusion:** The present study reports hepatoprotective potential of pumpkin seed oil against carbon tetrachloride induced hepatotoxicity in male Wistar albino rats.

**Key Words:** Pumpkin Seed Oil, Carbon tetrachloride, Hepatotoxicity, Rats

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## INTRODUCTION

Liver is largest gland of body that detoxifies the digested food particles and portal vein blood. Detoxification is major function of liver besides others. Disturbance in the liver functions manifests clinically as derangement of physiological functions throughout the body. Liver injury also occurs as its detoxification function declines resulting in fatty liver, chronic hepatitis, fibrosis, etc.[1] Organisms are taking thousands of xenobiotic in food as contaminants that, if not detoxified by liver produced adverse toxic effects. Liver dysfunction caused by xenobiotics is known as hepatotoxicity. Xenobiotics that selectively damage liver are called the hepatotoxin.[2,3] One of the hepatotoxin, the carbon tetrachloride ($CCl_4$) is used for experimental injury in animal models to observe the hepatoprotective effects of herbs and drugs.[4] Carbon tetrachloride ($CCl_4$) is used for scientific experimental studies in laboratory animals producing chemical liver injury.[4,5] Carbon tetrachloride ($CCl_4$) is biotransformed into free radicals, the trichloromethyl and proxy chloromethyl by the liver microsomal CYP P450 system. Trichloromethyl and proxy chloromethyl react with various cell components such as the amino acids, nucleic acids, phospholipids, proteins, fatty acids, etc.[3,5-7] Nowadays, many natural herbs have gained

[1.] Department of Pathology / Anatomy[2], Isra University, Hyderabad, Sindh.
[3.] Department of Anatomy / Pathology[4] / Pharmacology[5], Suleman Roshan Medical College, Tando Adam, Sindh.
[6.] Department of Anatomy, Indus Medical College, Tando Muhammad Khan, Sindh.

Correspondence: Dr. Asim Mehmood, Assistant Professor of Anatomy, Suleman Roshan Medical College, Tando Adam, Sindh.
Contact No: 0300 3046690
Email: mailboxKxm@gmail.com

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attention for the chemical induced liver injury. One such herbal compound is the pumpkin seed oil (PSO), this has been used by previous studies.[8] PSO contains minerals, proteins, poly unsaturated fatty acids (PUFA) and phyto – sterols. Investigators have reported anti – bacterial, anti – fungal, anti – nematocidial, anti – carcinogenic, anti – helminthic and anti – oxidant, and hepatoprotective potential of PSO. Investigators have observed the PSO ameliorates tissue architecture in liver toxicity animal models.[9,10] However, limited literature is available on the effects of PSO on biochemical markers of chemical induced hepatotoxicity.[11-13] We planned an experimental rat study to analyze the effects of PSO on biochemical markers of chemical induced hepatotoxicity. The present rat model experimental study was conducted to determine the potential protective effect of pumpkin seed oil (PSO) against Carbon tetrachloride -Induced Hepatotoxicity in Albino Rats.

# MATERIALS AND METHODS

The present rat animal experimental study was conducted at the Department of Anatomy, Pharmacology and Pathology, SRMC, T. Adam. Approval for conducting experimental study was taken in writing in collaboration with Animal house, Sindh Agriculture University. Animals were housed at the animal house of Sindh Agriculture University; Tando Jam. Experimental study duration was from March 2019 to January 2020.

**Animal Housing:** One hundred male Wistar albino rats were purchased from the animal house SAUTA. Inclusion criteria were male gender, body weight 180 – 200 grams, moving and feeding actively. Female rats were excluded. Animals were housed in accordance to the Guidelines of Animal Experimentation of Institute. Animal house is equipped with all facilities. Five rats were put per cage under appropriate conditions. Room temperature was maintained at (21 ± 2°C), ventilation (12 rpm), 12 – 12 hours light/dark cycle and humidity (50 ± 5%). Feeding and water were available ad – libitum.

**Dose calculation of $CCl_4$ and PSO:** $CCl_4$ was administered at dose of 0.2 mL/kg as 1:1 mixture in corn oil, twice a week for 8 weeks as cited.[3] This dose is well documented to cause hepatotoxicity in rats.[3] PSO dose was given 1, 2 and 3 mL/kg/bwt as cited[14] given for total 8 weeks duration.

**Experimental Grouping:** One hundred rats were divided into five groups. Group A (negative control) – received 0.9% NaCl orally as placebo therapy. Group B (negative control) – received $CCl_4$ (0.2 mL/kg as 1:1 mixture in corn oil, twice a week for 8 weeks). It was left untreated. Group C – received $CCl_4$ (0.2 mL/kg as 1:1 mixture in corn oil, twice a week for 8 weeks) + treated with PSO (1 mL/Kg bwt orally for 8 weeks). Group D – received $CCl_4$ (0.2 mL/kg as 1:1 mixture in corn oil, twice a week for 8 weeks) + treated with PSO (2 mL/Kg bwt orally for 8 weeks), and Group E– received $CCl_4$ (0.2 mL/kg as 1:1 mixture in corn oil, twice a week for 8 weeks) + treated with PSO (3 mL/Kg bwt orally for 8 weeks). Carbon tetrachloride was administered intraperitoneal at a dose of 0.2 mL/kg as 1:1 mixture with corn oil, twice a week for 8 weeks.

**Blood sampling:** After the experiment duration was over, the rats were anesthetized by ketamine (50 mg/kg intramuscularly) and xylazine (10 mg/kg intramuscularly) injection. Blood samples were collected from the retro – orbital capillary plexus by putting a capillary tube below and behind the eyeball.[15] Blood samples were centrifuged (x1300g rpm) for 15 minutes. Sera were separated and stored at temperature -20°C in refrigerator for biochemical analysis later on.

**Biochemical analysis**: Sera were used for the biochemical analysis. Liver function tests (7 parameters) were analyzed and estimated (spectrophotometric).

Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) were detected by ELISA assay kit (Fortress Diagnostics) on Roche Cobas Biochemistry Analyzer

**Statistical Analysis:** Research variable results were saved in Microsoft Excel Sheet. Values were copied and pasted on SPSS package (ver. 21.0, IBM, incorporation, USA) for data analysis. One – way analysis variance (1- ANOVA) post – Hoc Benforinni test analyzed the results of different groups with descriptive analysis. Level of statistical significance was taken at Confidence interval 95% ($p \leq 0.05$).

# RESULTS

We observed carbon tetrachloride ($CCl_4$) induced hepatotoxicity was improved significantly after 8 weeks pumpkin seed oil (PSO) in experimental groups C-E.

**Table No.1. Liver function tests after 8 weeks therapy**

| | Group A | Group B | Group C | Group D | Group E | P |
|---|---|---|---|---|---|---|
| Bilirubin | 0.57±0.03 | 2.7±0.32 | 1.5±0.64 | 1.1±0.27 | 1.01±0.07 | 0.0001 |
| PT (sec) | 7.1±1.54 | 13.6±1.7 | 11.5±3.15 | 9.1±2.43 | 9.1±1.12 | 0.0003 |
| ALT | 32.7±6.39 | 69.8±13.19 | 57.5±8.21 | 53.1±7.63 | 52.1±7.1 | 0.0001 |
| AST | 31.6±6.51 | 42.9±19.2 | 36.5±10.71 | 35.5±8.3 | 33.1±3.1 | 0.0004 |
| ALP | 79.5±17.3 | 139.5±32.3 | 115.3±41.5 | 97.3±31.1 | 91.3±31.2 | 0.0001 |
| LDH | 109.1±16.5 | 161.3±31.1 | 139.5±31.1 | 130.1±19.1 | 119.3±10.3 | 0.0005 |
| GGT | 35.1±4.8 | 71.1±17.3 | 57.3±18.7 | 47.3±19.1 | 41.1±11.0 | 0.0001 |

**Table No.2: SOD, GPX and CAT after 8 weeks therapy**

| | Group A | Group B | Group C | Group D | Group E | P |
|---|---|---|---|---|---|---|
| SOD **(U/ml)** | 136.0±33.8 | 75.3±15.3 | 112.7±19.2 | 121.5±13.2 | 131.1±11.31 | 0.0001 |
| GPX **(nM/mL)** | 137.1±31.2 | 87.1±21.3 | 117.1±15.7 | 121.3±11.0 | 129.5±13.1 | 0.0005 |
| CAT**(nM/mL)** | 307.3±30.3 | 133.6±52.3 | 201.3±71.1 | 276.1±61.3 | 187.7±77.6 | 0.0001 |

SOD- superoxide dismutase, GPX- glutathione peroxidase, CAT- catalase

Differences in serum bilirubin, PT, ALT, AST and GGT were found statistically significant among groups as shown in table –1 (P≤ 0.0005). Pumpkin seed oil (PSO) therapy shows rise in the natural anti – oxidant enzymes the; superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) after 8 weeks therapy as shown in table – 2 (P≤ 0.0005).

# DISCUSSION

During the modern era, the human beings are constantly exposed to chemical toxins in form of pollutants, agriculture pesticides, drugs, etc. Most of the chemical toxins are seriously injurious to the liver producing acute chronic liver toxicity. Various agents have been experimented against the chemical induced liver injuries.[10-12] Present report is the first experimental research conducted to observe the PSO against the $CCl_4$ induced hepatotoxicity rat model. We report the PSO shows excellent anti – toxic and hepatoprotective effects against $CCl_4$ induced hepatotoxicity. Liver is major site of biotransformation of drugs, toxins and chemical pollutants. Hepatocyte enzymes leak out of cell as the cell injury occurs and are best biomarkers of hepatotoxicity.[2,16] Serum ALT, AST, ALP, LDH and GGT are best predictors of liver toxicity in experimental rat models. ALT is the first to rise and identified by standard laboratory investigations.[2] Raised serum ALT is an indicator of disruption of functional integrity of hepatocyte cell membrane and its waning levels indicates healing of liver.[16,17] Hepatotoxicity causes leakage of liver enzymes into the circulating blood.[17] In present study, it was observed carbon tetrachloride ($CCl_4$) induced hepatotoxicity was improved significantly after 8 weeks pumpkin seed oil (PSO) in experimental groups C-E. Differences in serum bilirubin, PT, ALT, AST and GGT were found statistically significant among groups as shown in table –1 (P≤ 0.0005). The findigns of present study are in agreement with previous studies.[10,12,16,17] Pumpkin seed oil (PSO) increased the natural anti – oxidant enzymes status in present study that is in agreement with previous studies.[16,17] In present study, the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were found increased in experimental groups C to E after 8 weeks therapy (table – 2) (P≤ 0.0005). It has been reported the serum ALT raises at the earliest indicating most vital parameter of liver function test,[4,18,19] that has been confirmed in the present study. In present study, the liver functions derangement was too much in positive control B that was ameliorated by eight weeks PSO therapy in experimental groups C to E. The findigns are in keeping line with previous studies.[2,17,20] In present study, the $CCl_4$ at dose of 0.2 mL/kg as 1:1 mixture in corn oil for 8 weeks was sufficient to produce hepatotoxicity rat model. This is in agreement with previous studies.[3,17] In present study the dose of PSO used was in range of 1-3 mL/kg that proved of therapeutic effects ameliorating the liver function tests and anti – oxidant enzyme status of experimental rat groups. Finding is supported by a previous study.[14,17] A previous study[21] reported the PSO augments Islet β- cell functions of pancreas, resulted in increased insulin secretion sufficient to normalize the blood glucose levels. Evidence based of present study proves the biological potential of PSO against the $CCl_4$ induced hepatotoxicity in rats. Hepatoprotective effects of PSO may be attributed to its rich polyphenol and polyunsaturated fatty acids contents. Hence PSO may be used as hepatoprotective agents for human beings however; this needs clinical trials in patients with hepatic affections.

# CONCLUSION

The present study reports potential protective effect of pumpkin seed oil (PSO) against carbon tetrachloride ($CCl_4$) induced hepatotoxicity in male Wistar albino rats. PSO in doses of 1, 2 and 3 ml/Kg body weight was significantly improved and mitigated the carbon tetrachloride induced liver injury. PSO improved the liver functioning enzymes, ALT, AST, ALP, LDH and GGT along with boosting of superoxide dismutase, glutathione peroxidase and catalase. Future animal studies are recommended and clinical trials too to make this remedy available for liver affections in clinical practice to better patient management with cost effective herbal therapy.

**Author's Contribution:**

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|---|---|
| Concept & Design of Study: | Abdul Majid |
| Drafting: | Asim Mehmood, Rasheed Ahmed Soomro |
| Data Analysis: | Samreen Pandhiani, Aftab Abbasi, Hina Mawani |
| Revisiting Critically: | Abdul Majid, Asim Mehmood |
| Final Approval of version: | Abdul Majid |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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