RESEARCH ARTICLE

The hepatoprotective potentials of Olea europaea L. leaves against carbon tetrachloride-induced hepatic injury in rats

Amber Ayaz Memon, Lubna Naz

Abstract

Objective: To examine the therapeutic effects of Olea europaea L. leaves extract on carbon tetrachloride-induced liver injury in rats.

Method: The experimental study was conducted at the Department of Physiology, University of Karachi, Karachi, in July 2021, and comprised Albino Wistar male rats weighing 180-220gm. The animals were divided into control group I, carbon tetrachloride group II, Olea europaea L. + carbon tetrachloride group III and Olea europaea L. group IV. In Vitro model of hepatic toxicity was developed by carbon tetrachloride. A daily dose of 50mg/kg of aqueous extract of olive leaves was administered orally and 0.8ml/kg of carbon tetrachloride was administered twice a week subcutaneously for 28 days. On the 29th day, the animals were sacrificed, and tested for hepatic enzymes, lipid peroxidation markers and histopathology. Data was analysed using SPSS 20.

Results: Of the 24 rats, 6(25%) were in each of the 4 groups. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin levels were significantly reduced ($p<0.05$) in group II whereas, 4-hydroxynonenal, isoprostane and malondialdehyde levels were significantly increased ($p<0.05$). However, total antioxidant level increased significantly ($p<0.05$) in group III compared to group II. Histopathology showed severe liver damage in group II and mild damage in group III.

Conclusion: Olea europaea L. leaves extract was found to have profound hepatoprotective effects.

Key Words: Antioxidants, Olea, carbon tetrachloride, alanine transaminase, alkaline phosphatase, liver injury, chronic, lipid peroxidation, bilirubin, aspartate aminotransferases, malondialdehyde, isoprostanes

(JPMA 74: S-63 (Supple-2); 2024) **DOI: https://doi.org/10.47391/JPMA-DUHS-S13**

Introduction

The liver carries out many important metabolic processes. It has the capacity to regenerate, but in case healing is not successful, serious liver dysfunction may result. Globally, liver illnesses have been on the rise, largely due to bad lifestyle choices. Hepatotoxicity indicates liver damage instigated by chemicals. The chemicals taken in overdoses and, occasionally, taken in therapeutic range may injure the organs. Other chemical agents, such as those used in laboratories, industries or naturally occurring chemicals, may cause hepatotoxicity. The chemicals that induce liver injury are known as hepatotoxins[1].

In the liver, carbon tetrachloride (CCl^4) induces oxidative stress (OS). The disproportion of oxidation and antioxidant systems causes liver injury owing to OS, resulting in the formation of excessive free radicals and a reduction in antioxidant capacity[2]. It is thought that CCl^4 invades hepatocytes and generates free radicals, causing peroxidation that causes liver structure to be disrupted and liver function to be damaged[3]. According to a different mechanism, CCl^4 damages argyrophilic nucleolar organiser regions (AgNORs), which disperse with irregularly shaped particles and cause genotoxicity by breaking deoxyribonucleic acid (DNA) strands[4]. It can cause hepatic injury. Hence, it is widely used to construct as toxic liver model in rats[5].

Defence against oxidative harm is evolved and essentially reliant on antioxidant enzymes, substrate supply, and injury repair. These defences strengthen in response to oxidants and other electrophiles, boosting the capacity to repair oxidative damage, detoxify oxidants, and electrophiles. The main strategies underpinning antioxidant therapy are agents that boost these defences. Extensive research on antioxidant enzyme induction has focussed on regulatory mechanisms, clinical consequences, and prospective inducers with therapeutic potential[6].

Olea europaea L., commonly known as olive, belongs to the Oleaceae family, and is grown all over the world, with the Mediterranean region being the primary location of agricultural output accounting for over 98% of global production[7]. The olive tree is widely grown outside of the Mediterranean region throughout Asia, India and the

Department of Physiology, University of Karachi, Karachi, Pakistan.

Correspondence: Amber Ayaz Memon. Email: amberayaz96@gmail.com

ORCID ID: 0009-0009-1903-4589

Arabian Peninsula[8]. The main product extracted from the olive tree is olive oil that has been extensively studied for its antioxidant effects. However, olive leaves contain significant quantity of phenolic compounds that have intrigued interest of scientists around the world. Many investigations have been conducted for its beneficial effect on health, including their antioxidant and anti-inflammatory properties as well as the potential to treat a variety of diseases[9]. Oleuropein, a substance found in olive leaves, is regarded to be the main factor behind many of these effects. Oleuropein is a phenolic secoiridoid glycoside made up of a glucose molecule, hydroxytyrosol and olenolic acid. This is the most abundant bioactive substance found in olive leaves[10].

The current study was designed to investigate hepatoprotective potential of Olea europaea L. leaves extract (OLE) against CCl^4-induced hepatic injury in rats.

Materials and Methods

The experimental study was conducted at the Department of Physiology, University of Karachi, Karachi, in July 2021, and comprised Albino Wistar male rats weighing 180-220gm. Female rats and animals weighing <180gm were excluded. The animals were acclimatised in a well-ventilated and temperature-controlled animal house. The study was approved by the institutional ethics review board. Dried olive leaves were obtained from the local market and extracts were obtained as per Chaker et al.[11]

The animals were divided into control group I, CCl^4 group II, OLE. + CCl^4 group III and OLE group IV. Group I comprised healthy rats, group II received 0.8ml/kg CCl4 subcutaneously along with olive oil in a 1:1 ratio twice a week, group III received 0.8ml/kg CCl^4 subcutaneously along with olive oil in a 1:1 ratio together with OLE 50mg/kg through gavage per day. Group 1V was treated with OLE 50mg/kg through gavage per day. The intervention continued for 28 days. On the 29th day, the animals were sacrificed, and blood and tissue samples were collected.

Hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated by using commercial kits (Randox Laboratories Ltd., United Kingdom, and Amitech Diagnostics Inc., Canada). Total bilirubin levels were also assessed. Lipid peroxidation markers 4-hydroxynonenal (4-HNE and isoprostane as well as total antioxidant capacity (TAC) levels were assayed using enzyme-linked immunosorbent assay (ELISA) kits (Glory Sciences Co. Ltd., United States). For histopathological investigations, the liver tissue samples were embedded in paraffin blocks after being fixed in formalin, sectioned at 4mm and stained with haematotoxylin and eosin (H&E) for microscopic examination. Histological sections were graded and scored to determine the extent of hepatic damage. Histological sections were used to determine the severity of liver injury using numerical scores.[12]

Data was analysed using SPSS 20. Post-hoc Tukey's multiple comparison test was conducted. $P<0.05$ was considered significant.

Results

Of the 24 rats, 6(25%) were in each of the 4 groups. ALT, ALP, AST and total bilirubin levels ($p<0.05$) were raised in group II compared to group I and decreased ($p<0.05$) in group III compared to group II (Table 1).

Table-1: Intergroup comparison of serum markers of hepatic injury.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)
Control (n=6)	15.5 ± 1.7	12.2 ± 0.8	122 ± 4.6	0.44 ± 0.04
Toxic Group (CCl4) (n=6)	24.2 ± 2.0 α *	19.5 ± 1.1 α *	163.7 ± 6.5 α *	0.66 ± 0.04 α *
CCl4 + OLE Group (n=6)	17.2 ± 1.7 β *	14.2 ± 1.1 β *	132.5 ± 6.1 β *	0.50 ± 0.01 β *
OLE Group (n=6)	9.7 ± 0.8 π *	8.5 ± 1.0 π *	108 ± 1.9 π *	0.29 ± 0.03 π *

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, A:P: Alkaline phosphatase, CCl4: Carbon tetrachloride, OLE: Olive leaves extract, *: Statistical significance ($p<0.05$), α: Comparison of control group and Toxic group, β: Comparison of Toxic group and CCL4+ OLE group π: Comparison of Control group and OLE group.
The values are mean ± standard error of the mean (SEM).

Table-2: Intergroup comparison of lipid peroxidation markers and total antioxidant levels.

Groups	Isoprostane (ng/dl)	4-HNE (Pg/ml)	MDA levels in liver (umol/g)	TAC (U/ml)
Control (n=6)	9.38± 1.76	6.20±0.91	0.51 ± 0.05	0.44 ± 0.03
Toxic Group (CCl_4) (n=6)	17.65±1.75 α*	14.52±0.96 α*	1.05 ± 0.09 α*	0.29 ± 0.03 α
CCl_4 + OLE Group (n=6)	12.72±0.72 β*	10.18±0.79 β *	0.77 ± 0.07 β*	0.47 ± 0.02 β
OLE Group (n=6)	5.7±0.60 π	8.24±0.76 π	0.53 ± 0.05 π	0.41 ± 0.01 π

4-HNE: 4-hydroxynonenal, MDA: Malondialdehyde, TAC: Total antioxidant capacity, CCl4: Carbon tetrachloride, OLE: Olive leaves extract, *: Statistical significance ($p<0.05$), α: Comparison of control group and Toxic group, β: Comparison of Toxic group and CCL4 + OLE group, π: Comparison of Control group and OLE group.
The values are mean ± standard error of the mean (SEM).

There was a significant increase in serum 4-HNE, isoprostane and MDA levels ($p<0.05$) in group II compared to group I and decreased ($p<0.05$) in group III

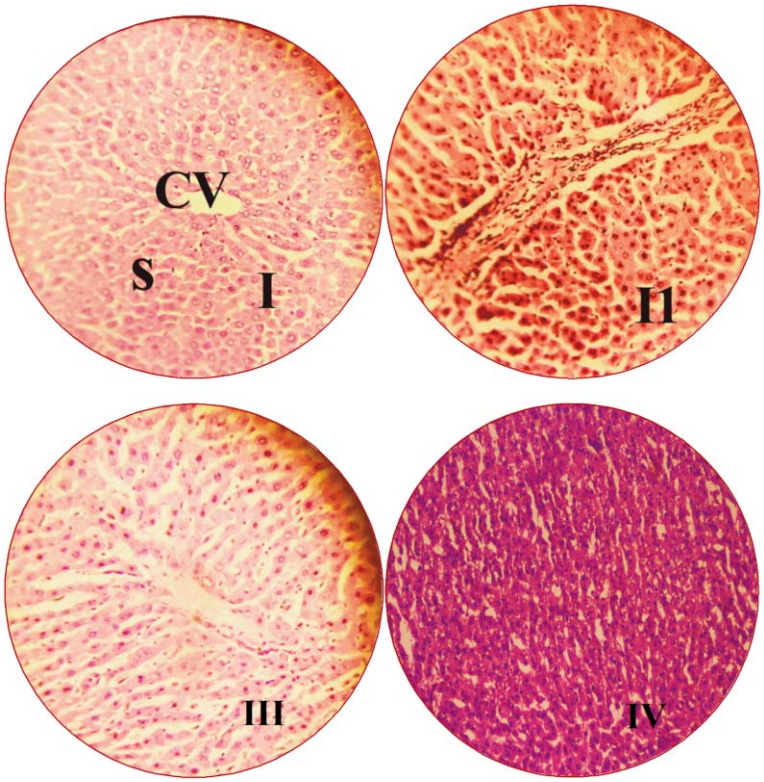

Figure: Histopathological features of rat liver.
I: Control group, II: CCl4 group, III: CCl4 + OLE group, IV: OLE group
CV: Central vein, S: Sinusoids.
CCl4: Carbon tetrachloride, OLE: Olive leaves extract.

compared to group II. TAC levels were significantly higher ($p>0.05$) in group III compared to group II, and lower ($p>0.05$) in group II compared to group I (Table 2).

Histopathological Findings showed enlargement, fatty changes, paleness and mild portal inflammation in groups II and III (Figure).

Discussion

Liver has an amazing regeneration capability. In animal models in which hepatocytes are damaged directly and necrosis occurs, cytokine-mediated pathways and similar growth-factor are triggered, as they are in partial hepatectomy. Hepatocyte proliferation is crucial for liver regeneration following hepatocyte necrosis or apoptosis driven by hepatic toxins, such as CCl^4. There are considerable changes in the architecture of the liver both after partial hepatectomy and after hepatic necrosis. Basement membrane proteins, such as fibronectin, and additional forms of cell adhesion proteins are developed. During regeneration, changes in intracellular and intercellular connections are transitory, and normal liver architecture is only restored until the original liver mass has been restored[13].

Many diseases are caused by OS triggered by liver-toxic substances, environmental contaminants, ionising radiations and drugs that cause hepatotoxicity. In the industrial domain, CCl^4 is a widely used chemical solvent. It is a well-known hepatotoxin, and has the best-studied hepatotoxicity caused by xenobiotic-induced free radicals in animal models. Hepatic damage is caused by CCl^4 in different ways. One of the processes contributing to hepatotoxicity is enhanced lipid peroxidation due to amplified free radical formation from CCl^4. The influx of inflammatory cells to the injured area by CCl^4 also activates the immune system[14]. Herbal treatment has been utilised to treat hepatic diseases for millennia. It has been explored that certain herbs and herbal extracts can shield the liver and kidney against CCl^4 intoxication[15]. The leaves of Olea europaea have been used for medicinal purposes since ancient times.[16]

Hepatic cytosolic enzymes can escape from membrane blebs or mitochondrial damage, and enter the circulation when membrane permeability is disturbed. The damage that occurs is brought on by metabolism or hepatocyte injury[17]. For assessing the severity of liver injury, ALT and AST levels were monitored in the current study. Elevated enzyme levels appear in the blood as the hepatocytes are damaged [18]. Bile leaks into the circulation as a result of hepatic damage. Increased uptake of free bilirubin or hepatic beta-glucuronidase is a result of hepatic insufficiency[19].

In the present study, ALT, ALP, AST and total bilirubin levels ($p<0.05$) were increased in group II, which was in line with Shahat et al.[20]. The current study found that OLE reduced the levels of ALT, AST, ALP and total bilirubin ($p<0.05$) in group III, indicating impediment of liver injury by CCl^4 (Table 1). Moreover, histopathological results depicted liver enlargement, fatty change paleness, mild portal inflammation groups II and III (Figure).

Additionally, the rats treated with CCl^4 showed elevated levels of lipid peroxidation marker MDA, which is the final product of lipid peroxidation, and its concentration was significantly raised ($p<0.05$). The results are consistent with those of another study[21]. In contrast, OLE treatment significantly reduced MDA production, reducing OS and lipid peroxidation in the current study.

Besides, 4-HNE is an extensive and commonly used indicator of the presence or amount of lipid peroxidation

formed by damage to arachidonic acid and linoleic acid. Because CCl^4 is an eminent compound in vivo activator of lipid peroxidation, it should mediate the generation of 4-HNE[22]. The current study found an increase in 4-HNE levels in the animals treated with CCl^4 ($p<0.05$), but were reduced in samples from animals given OLE and CCl^4, implying that these components reduced OS.

Isoprostanes are Prostaglandin isomers generated by the peroxidation of arachidonic acid catalysed by free radicals. As such, they have potential to be lipid peroxidation biomarkers, and can be easily tested in plasma and urine[23]. Isoprostane levels were elevated ($p<0.05$) in CCl^4 group compared to the control group, and declined ($p<0.05$) in OLE + CCl4 group (Table 2).

Increased OS is virtually always present in chronic liver illnesses, regardless of the cause of the liver disease. TAC levels were increased in the treated group compared to the toxic group in the current study.

The current study has limitations, as it did not comprise molecular investigation of its protective effect, analysis of specific doses, and isolation of natural ingredient in the olive leaf.

Conclusion

Olive leaves were found to have preventive qualities, and may include polyphenols that reduced OS and guarded against liver damage. When compared to the toxicant group, the parameters in the group treated with OLE revealed a clear difference.

Acknowledgement: We are grateful to the administration of the Department of Physiology, University of Karachi, for facilitating the study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: a review. J Appl Pharm Sci 2012;2:233-43. DOI: 10.7324/JAPS.2012.2541
2. Lee HY, Lee GH, Yoon Y, Chae HJ. R. verniciflua and E. ulmoides Extract (ILF-RE) Protects against Chronic CCl_4-Induced Liver Damage by Enhancing Antioxidation. Nutrients 2019;11:382. doi: 10.3390/nu11020382.
3. Xu P, Yao J, Ji J, Shi H, Jiao Y, Hao S, et al. Deficiency of apoptosis-stimulating protein 2 of p53 protects mice from acute hepatic injury induced by CCl4 via autophagy. Toxicol Lett 2019;316:85-93. doi: 10.1016/j.toxlet.2019.09.006.
4. Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: protective role of Digera muricata. J Ethnopharmacol 2009;122:91-9. doi: 10.1016/j.jep.2008.12.006.
5. Memon AA, Naz L, Shabbir S, Khan Z. The Hepato-Renal Protective Effect of Nelumbo nucifera Gaertn Seeds against Carbon Tetra Chloride Toxicity in Rats. Int J Pure App Biosci 2019;7:15-24. DOI: 10.18782/2320-7051.7464
6. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021;20:689-709. doi: 10.1038/s41573-021-00233-1.
7. Ryan D, Robards K. Critical Review: Phenolic compounds in olives. Analyst 1998;123:31R-44R.
8. Somova LI, Shode FO, Ramnanan P, Nadar A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies africana leaves. J Ethnopharmacol 2003;84:299-305. doi: 10.1016/s0378-8741(02)00332-x.
9. Talhaoui N. Analytical, agronomic, and biological evaluation of phenolic compounds in olea europaea products and by-products. [Online] 2016 [Cited 2024 January 06]. Available form URL: https://digibug.ugr.es/bitstream/handle/10481/43641/26118956.pdf?sequence=6&isAllowed=y
10. Topuz S, Bayram M. Oleuropein extraction from leaves of three olive varieties (Olea europaea L.): Antioxidant and antimicrobial properties of purified oleuropein and oleuropein extracts. J Food Process Preserv 2022;46: e15697. Doi: 10.1111/jfpp.15697
11. Chaker R, Mansouri O, Hamamdia Z, Abdennour C. Olive leaves' extract may attenuate cadmium-induced liver damage in Wistar rat. J Stress Physiol Biochem 2020;16:14-25.
12. French SW. Intragastric ethanol infusion model for cellular and molecular studies of alcoholic liver disease. J Biomed Sci 2001;8:20-7. doi: 10.1007/BF02255967.
13. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004;5:836-47. doi: 10.1038/nrm1489.
14. Al-Harbi NO, Imam F, Nadeem A, Al-Harbi MM, Iqbal M, Ahmad SF. Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin. Int Immunopharmacol 2014;21:383-8. doi: 10.1016/j.intimp.2014.05.014.
15. Bellassoued K, Ben Hsouna A, Athmouni K, van Pelt J, Makni Ayadi F, Rebai T, et al. Protective effects of Mentha piperita L. leaf essential oil against CCl4 induced hepatic oxidative damage and renal failure in rats. Lipids Health Dis 2018;17:9. doi: 10.1186/s12944-017-0645-9.
16. Aliabadi MA, Darsanaki RK, Rokhi ML, Nourbakhsh M, Raeisi G. Antimicrobial activity of olive leaf aqueous extract. Ann Biol Res 2012;3:4189-91.
17. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 2007;45:1551-7. doi: 10.1016/j.fct.2007.06.007.
18. Huang HL, Wang YJ, Zhang QY, Liu B, Wang FY, Li JJ, et al. Hepatoprotective effects of baicalein against CCl_4-induced acute liver injury in mice. World J Gastroenterol 2012;18:6605-13. doi: 10.3748/wjg.v18.i45.6605.
19. Abdel-Aziz AD, Sayed AA, Ahmed AH, Dalia MU. The ameliorative effect of L-carnitine on experimentally induced liver cirrhosis in male albino rats. J Am Sci 2014;10:8-18.
20. Shahat AA, Ullah R, Alqahtani AS, Alsaid MS, Husseiny HA, Al Meanazel OTR. Hepatoprotective Effect of Eriobotrya japonica Leaf Extract and Its Various Fractions against Carbon Tetra Chloride Induced Hepatotoxicity in Rats. Evid Based Complement Alternat Med 2018;2018:e3782768. doi: 10.1155/2018/3782768.
21. Ullah H, Khan A, Baig MW, Ullah N, Ahmed N, Tipu MK, et al. Poncirin attenuates CCL4-induced liver injury through inhibition of oxidative stress and inflammatory cytokines in mice. BMC Complement Med Ther 2020;20:115. doi: 10.1186/s12906-020-02906-7.
22. Coballase-Urrutia E, Pedraza-Chaverri J, Cárdenas-Rodríguez N,

Huerta-Gertrudis B, García-Cruz ME, Ramírez-Morales A, et al. Hepatoprotective effect of acetonic and methanolic extracts of Heterotheca inuloides against CCl(4)-induced toxicity in rats. Exp Toxicol Pathol 2011;63:363-70. doi: 10.1016/j.etp.2010.02.012.

23. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, et al. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005;38:698-710. doi: 10.1016/j.freeradbiomed.2004.09.017.