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# Protective Effect of Flax Seed Oil on acetaminophen-induced toxicity in the renal cortex of albino rats

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## ABSTRACT

**Introduction:** Acetaminophen is known for its analgesic and antipyretic properties. Its use in therapeutic doses is safe but overdosing or misuse makes it harmful. It has toxic effects on the cell of liver and kidneys. Scientific evidence highlights the antioxidant effect of flax seed oil in preventing the damage caused by acetaminophen on the kidneys.
**Aims and Objectives:** To observe the nephrotoxic effect of acetaminophen and see the protective effects of flax seed oil on nephrotoxicity induced by acetaminophen.
**Place and Duration of study:** The study was carried out in the research lab of University of Health Sciences, Lahore with ERB number No. UHS/ANAT/6827. The duration of the study was three months.
**Material and Methods:** Twenty-four male Albino rats were divided randomly into three groups, consisting of 8 animals each. The rats were 6-8 weeks old and weighed between 175-225 gm. Animals of group A served as control and were given 5ml/kg normal saline (NS) each, intraperitoneally on day1. Animals of group B were given 1000 mg/kg acetaminophen (AAP) intraperitoneally dissolved in 5ml of NS on day1. Group C animals were given flax seed oil (FSO), 2 ml at a dose of 1.86gm/kg orally, once daily. On the 14th day, a single intraperitoneal dose of acetaminophen, 1000 mg/kg dissolved in 5ml/kg normal saline was given, one hour after giving flax seed oil. The animals of group A were sacrificed on the 15th day, whereas animals of group B and C were sacrificed 48 hours after giving acetaminophen. Kidneys were removed and excised after necropsy and prepared for histological examination.
**Results:** The cortical region in the kidneys of group A showed normal histological finding. All the AAP treated animals showed evidence of renal damage. In Group C, FSO pretreatment significantly reduced AAP induced changes in kidney as shown by restoration of its histological structure.
**Conclusion:** AAP is nephrotoxic and FSO ameliorates its effect in rats.


**Keywords:** Acetaminophen (AAP), nephrotoxicity, flax seed oil (FSO).

## INTRODUCTION

AAP is one of the most popular drugs used for pyrexia and analgesia[1]. It is considered as a safe drug for those in whom nonsteroidal anti-inflammatory drugs (NSAIDS) are contraindicated[2]. AAP (paracetamol) in therapeutic doses is a drug of choice in children and adolescents[3]. It can be used alone or combined with other medications in conditions like cough, flu and fever[4]. Although it is a safe drug but its misuse results in renal damage, leading to its failure.

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However, the relative risk of renal failure is much lower with AAP than other NSAIDs[5]. When taken orally, the digestion of AAP starts in the proximal part of small intestine[6], with peak plasma concentrations reaching 30–60 min after ingestion[7]. In therapeutic doses, it is primarily metabolized in the liver by enzymes, glucuronyl transferases (50–60%) and sulfotransferases (25–30%); The remaining AAP is metabolized by oxidation with cytochrome P450 (<10%) resulting in the formation of a small amounts of N- acetyl-p- benzoquinone imine (NAPQI), a toxic metabolite; NAPQI is reduced and detoxified by glutathione and excreted by kidneys as benign compounds, mercapturic acid cysteine[7]. In acute AAP over dosage, glucoronidation and sulphation pathways get saturated, diverting the excessive amount of AAP to be metabolized by cytochrome P-450 system. Glutathione, which detoxifies NAPQI in liver, gets depleted, resulting in the formation of increased levels of un-conjugated metabolite, NAPQI; which binds covalently with cellular proteins, producing their oxidation and starting lipid peroxidation

resulting in oxidative stress resulting in injury to liver and kidney cells[8].

FSO obtained after extraction from flax seeds is famous for its nutritional and pharmaceutical values [9]. Nutrients present in flaxseeds and their oil are known for their antioxidant and anti-inflammatory properties. [10]. Flax seeds contain dietary fiber, alpha-linolenic acid and lignans. Together alpha-linolenic acid and lignans have antioxidant and anti-inflammatory properties[11]. FSO, lignans and fiber are very beneficial for health and bring improvement in diabetes, heart diseases, atherosclerosis, arthritis, osteoporosis, autoimmune disorders.[12]and blood cholesterol[13]. Its anticarcinogenic[14], hepatoprotective[15] and nephroprotective[16, 17] effects have also been studied. Since nephroprotective effect of FSO has never been explored in AAP induced nephrotoxicity, this study, therefore, was designed to observe AAP induced histological changes in the kidneys of rats and the effect of FSO in restoring the histological parameters of the kidneys.

## MATERIAL AND METHODS

The study was carried out in the research lab of the University of Health Sciences, Lahore with ERB number No. UHS/ANAT/6827. The duration of the study was three months. It was an experimental study. Twenty-four healthy male Albino Wistar rats, weighing between 175-225 gm and aged 6-8 weeks were obtained from the animal house of University of Health Sciences (UHS), Lahore, Pakistan. The sample size was calculated using open epi software at 95% confidence level, power of study at 80% and anticipated factor as mean difference in superoxide ????? dismutase levels (SOD, an antioxidant) among AAP group alone and AAP plus FSO group. In AAP group, SOD level was 50.36±2.63, whereas in AAP plus FSO group, mean SOD levels were 53.23±1.99[18]. The calculated sample size is 22. We have recruited 24 rats for this study.

Random balloting was done, and the animals were divided into three groups A, B, and C having eight rats each; the groups were individually housed in stainless steel cages. The animals were fed on standard rat diet and water ad libitum and were kept at controlled room temperature (23±2°C), humidity (50±5%) and light and dark cycles of 12 hours each. The study was carried out in the research laboratory of UHS Lahore. The experiment started one week after acclimatization of the animals. Intra peritoneal route was opted for the experiment [19]. Group A was the control group and received intraperitoneal injection of NS, 5ml/kg on the 1st day. Group B received intraperitoneal injection of AAP, 1000 mg/kg dissolved in NS, 5ml/kg on 1st day. Group C was given FSO, 2ml orally, once daily for 14 days, followed by a single intraperitoneal injection of AAP, 1000 mg/ kg dissolved in 5ml/kg of NS. The animals of group A were sacrificed on day 15, whereas animals of groups B and C were sacrificed 48 hours after administration of AAP(Table1). The animals were then sacrificed painlessly, and kidneys were removed; from the tissue pieces of kidney 5µ thick sections were prepared for histological examination after H&E staining. The proximal and distal convoluted tubules were selected and examined for epithelial necrosis, vacuoles and protein casts as histological parameters; PCTs were also examined for brush border. Changes in the histological parameters of tubules were noted and were considered as normal (N), mild (+), moderate (++) and severe (+++). The damage was marked as mild (+), when it involved less than 25% of the total number of tubules, moderate (++), when involved 26-50% of the tubules and severe (+++) when more than half of the tubules were involved and showed necrosis (>50%). Luminal casts were also similarly graded.

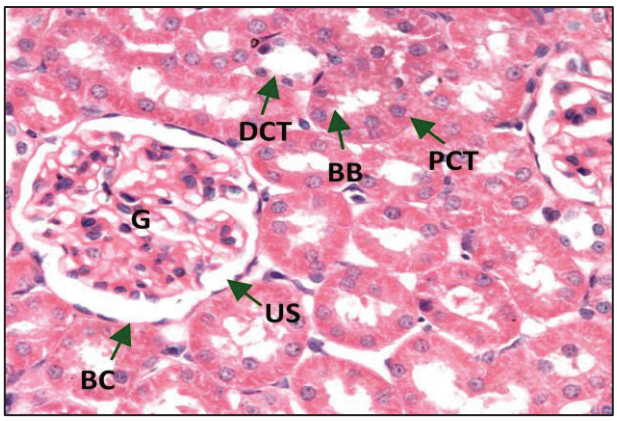
**Data Analysis**

Data analysis was done using version 20 of software SPSS; frequency and percentages were given for qualitative parameters. Comparison of morphological features among groups was made by applying Fisher Exact / Chi-square tests. The level was considered statistically significant with p-value ≤0.05.

## RESULTS

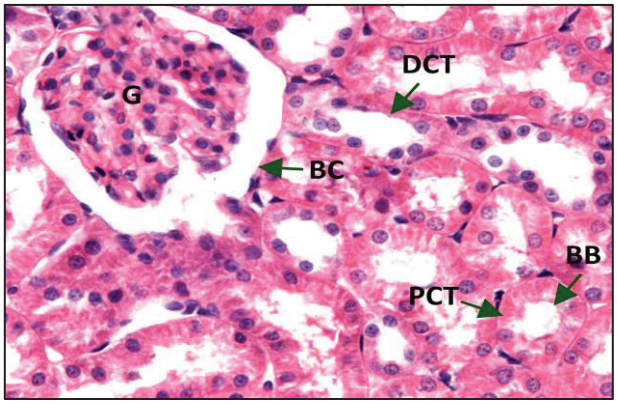
The histology of kidney cortex comprises of renal parenchyma showing renal corpuscles having a tuft of capillaries, surrounded by Bowman's capsule, lined by squamous epithelial cells and cortical tubules consisting of proximal and distal convoluted parts. PCTs were lined by simple cuboidal epithelium with prominent brush border and narrow lumen; DCTs also lined by simple cuboidal epithelium with rounded nuclei and wide lumen (fig. 1).

**Fig.1: (Below) Light photomicrograph of cortex of kidney sections from control group, A, showing normal structure of kidney cortex comprising of glomerulus (G) surrounded by urinary space (US) of Bowman's capsule (BC); PCTs are lined by simple cuboidal cells with prominent brush border (BB) and DCTs are lined by simple cuboidal epithelium. H&E stain X400.**



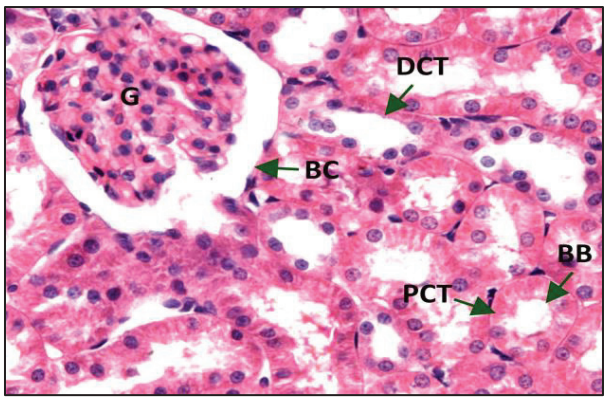

The AAP treated group resulted in marked degenerative changes both in PCTs and DCTs which showed marked necrosis and vacuoles with loss of brush border in PCTs. Luminal casts were seen in both types of convoluted tubules (fig. 2).

**Fig.2: Light photomicrograph of cortex from the kidney sections of experimental group B showing glomerulus (G), enclosed in Bowman's capsule (BC); PCT with loss of brush border (BB); degenerative necrotic tubules (NT) with smudge like appearance. The lumen of DCT, cast (C) and desquamating cell (DC) are seen. Cytoplasmic vacuoles (V) are seen both in PCTs and DCTs. H&E stain. X400.**




Flax seed oil pretreatment in group C prevented the degenerative changes and the tubules appeared nearly normal, resembling those of group A; PCTs showed mild loss of brush border and vacuolation was discernibly reduced in both tubules (fig. 3).

**Fig.3: Light photomicrograph of cortex from the kidney sections of group C showing glomerulus (G), surrounded by Bowman's capsule (BC). PCT is seen with partial loss of brush border (BB); this was comparable with group A in which there was no tubular vacuolation or loss of brush border in proximal convoluted tubules; DCTs are lined with simple cuboidal epithelium. Epithelial vacuolation is apparent and reduced. H&E stain. X400.**




A notable difference was observed in percentages of tubular necrosis ($p < 0.001$), tubular cast ($p < 0.001$), tubular vacuolation ($p < 0.001$), and loss of brush border ($p < 0.001$) among the groups (Table 2).

**Table 1: Showing experimental grouping of animals and intervention.**

| Groups | Intervention and Dosage | Route of Administration | Duration of Administration | Day of Sacrifice |
|---|---|---|---|---|
| Group A | NS 5ml/kg | Intraperitoneal | Day 1 | sacrificed on 15th day |
| Group B | AAP 1000mg/kg dissolved in 5 ml of NS | Intraperitoneal | Day 1 | sacrificed after 48 hours |
| Group C | FSO, 2 ml at a dose of 1.86 g/kg | Orally | Day 1 to day 14 | sacrificed after 48 hours of giving acetaminophen |
| | AAP 1000mg/kg dissolved in 5 ml of NS | Intraperitoneal | Day 14 | |

**Table 2: Shows comparison of histological parameters among groups A, B and C**

| Parameters | | Group A n = 8 | Group B n =8 | Group C n = 8 | p-value |
|---|---|---|---|---|---|
| Epithelial Necrosis | Absent (-) | 8(100 %) | 1(12.5 %) | 5(62.5 %) | <0.001 |
| | Mild (+)<25% | 0(0.0%) | 2(25.0 %) | 3(37.5 %) | |

| | | | | | |
|---|---|---|---|---|---|
| | Moderate (++)26-50% | 0(0.0%) | 3(37.5%) | 0(0.0%) | |
| | Severe (+++)>50% | 0(0.0%) | 2(25.0%) | 0(0.0%) | |
| Loss of brush border | Absent (-) | 8(100%) | 0(0.0%) | 6(75.0%) | <0.001 |
| | Mild (+)<25% | 0(0.0%) | 2(25.0%) | 2(25.0%) | |
| | Moderate (++)26-50% | 0(0.0%) | 4(50.0%) | 0(0.0%) | |
| | Severe (+++)>50% | 0(0.0%) | 2(25.0%) | 0(0.0%) | |
| Absence of vacuolation | Absent (-) | 8(100%) | 0(0.0%) | 6(75.0%) | <0.001 |
| | Mild (+)<25% | 0(0.0%) | 1(12.5%) | 2(25.0%) | |
| | Moderate (++)26-50% | 0(0.0%) | 4(50.0%) | 0(0.0%) | |
| | Severe (+++)>50% | 0(0.0%) | 3(37.5%) | 0(0.0%) | |
| Protein casts | Absent (-) | 8(100%) | 1(12.5%) | 8(100%) | <0.001 |
| | Mild (+)<25% | 0(0.0%) | 2(25.0%) | 0(0.0%) | |
| | Moderate (++)26-50% | 0(0.0%) | 4(50.0%) | 0(0.0%) | |
| | Severe (+++)>50% | 0(0.0%) | 1(12.5%) | 0(0.0%) | |

## DISCUSSION

Nephrotoxicity induced by AAP was evidenced by a variety of histopathological changes which were observed earlier in other studies[20.21.22].

In the present study, the histopathological features of nephrotoxicity produced by AAP were characterized by significant degenerative changes as evidenced by marked necrosis and vacuolization of tubular epithelial cells in animals of group B. These changes were seen in proximal as well as distal convoluted tubules, which showed that toxic dose of AAP was responsible for tubular damage after 48 hours. Similar changes like tubular necrosis and degeneration, with hyper cellularity in the glomerulus have been reported earlier[23]. These changes are also evidenced by another study in which degenerative changes were observed in the PCTs. The tubules showed necrotic epithelial lining with cytoplasmic vacuolation. Disorganization of tubules and desquamation of epithelial cells was also seen[24]. In the current study, loss of brush border was seen in the animals of group B. These results are comparable to those reported earlier in which there was loss of brush border in PCTs when AAP was given in toxic doses[24]. In our experiment, pretreatment with FSO largely prevented tubular epithelial necrosis, vacuolation and brush border loss. Our results are supported by a previous study in which administration of lead acetate caused degeneration, vacuolation and dilatation of kidney tubules which contained many apoptotic cells. Treatment with FSO largely prevented lead acetate induced histopathological changes in kidney[25]. It has been reported that when AAP is given in toxic doses, glutathione gets depleted; so an intermediate toxic metabolite, NAPQI is produced in large amount which forms covalent binding with cellular proteins causing disruption of homeostasis and leading to tissue injury and dysfunction of kidneys[23]. Depletion of glutathione in the kidney causes mitochondrial oxidative stress[21]. The current investigations reported that FSO significantly restored nephrotoxic changes such as necrosis and vacuolization of epithelial cells in proximal and distal convoluted tubules and loss of brush border in PCTs which were evident in group C, pretreated with FSO for 14 days. These features were comparable to those of control group A and are attributed to the antioxidant properties of FSO due to the presence of alpha linolenic acid[10].

**Future considerations and Limitations:**

The study would have been more meaningful if histopathological findings and biochemical testing had been combined to assess and correlate the nephrotoxic effects of AAP.

## CONCLUSION

FXO prevents occurrence of renal damage induced by AAP in Albino rats.

## REFERENCES


1. Ishitsuka Y, Kondo Y, Kadowaki D. Toxicological property of acetaminophen: the dark side of a safe antipyretic/analgesic drug. Biol. Pharm. Bull. 2020 Feb 1;43(2):195-206.
2. Ohashi N, Kohno T. Analgesic effect of acetaminophen: a review of known and novel mechanisms of action. Front. pharmacol. 2020 Nov 30;11:580289.
3. Shekunov J, Lewis CP, Vande Voort JL, Bostwick JM, Romanowicz M. Clinical characteristics, outcomes, disposition, and acute care of children and adolescents treated for acetaminophen toxicity. Psychiatric Services. 2021 Jul 1;72(7):758-65.
4. Toplis E, Mortimore G. Acute liver failure in paracetamol overdose: management, transplantation and best practice. Gastrointestinal Nursing. 2020 Jul 1;18(Sup6):S34-9.
5. Tomșa AM, Răchișan AL, Pandrea SL, Benea A, Uifălean A, Toma C, Popa R, Pârvu AE, Junie LM. Curcumin and vitamin C attenuate gentamicin-induced nephrotoxicity by modulating distinctive reactive species. Metabolites. 2022 Dec 28;13(1):49.
6. Mészáros P, Kovács S, Kulcsár G, Páskuj M, Almási A. Investigation of intestinal absorption and excretion of paracetamol in streptozotocin-induced hyperglycemia. Int. J. Mol. Sci. 2022 Oct 7;23(19):11913..
7. Freo U, Ruocco C, Valerio A, Scagnol I, Nisoli E. Paracetamol: a review of guideline recommendations. J. Clin. Med. 2021 Jul 31;10(15):3420.
8. Mitra M, Bandyopadhyay A, Datta G, Nandi DK. Effective Dose of Herbal Gold Nanoparticles for Protection of Acetaminophen-Induced Hepatotoxicity in Male Albino Rats. J. Bionanosci.2020 Dec;10:1094-106.
9. Abbasnezhad A, Salami F, Mohebbati R. A review: Systematic research approach on toxicity model of liver and kidney in laboratory animals. Animal models and experimental medicine. 2022 Oct;5(5):436-44.
10. Tomaszewska-Gras J, Islam M, Grzeca L, Kaczmarek A, Fornal E. Comprehensive thermal characteristics of different cultivars of flaxseed oil (Linum usittatissimum L.). Molecules. 2021 Mar 31;26(7):1958.
11. Chera EI, Pop TI, Pop RM, Pârvu M, Uifălean A, Cătoi FA, Cecan AD, Mîrza CM, Achimaș-Cadariu P, Pârvu AE. Flaxseed ethanol extract effect in acute experimental inflammation. Medicina. 2022 Apr 24;58(5):582.
12. Al-Madhagy S, Ashmawy NS, Mamdouh A, Eldahshan OA, Farag MA. A comprehensive review of the health benefits of flaxseed oil in relation to its chemical composition and comparison with other omega-3-rich oils. Eur. J. Med. Res. 2023 Jul 18;28(1):240
13. Kouamé KJ, Bora AF, Li X, Sun Y, Liu L. Novel trends and opportunities for microencapsulation of flaxseed oil in foods: A review. JFF.2021 Dec 1;87:104812.
14. Yakdhane A, Labidi S, Chaabane D, Tolnay A, Nath A, Koris A, Vatai G. Microencapsulation of flaxseed oil—State of art. Processes. 2021 Feb 3;9(2):295.
15. Gurumallu SC, Aqeel T, Bhaskar A, Chandramohan K, Javaraiah R. Synergistic hepatoprotective effects of ω-3 and ω-6 fatty acids from Indian flax and sesame seed oils against CCl4-induced oxidative stress-mediated liver damage in rats. Drug Chem. Toxicol. 2022 Sep 3;45(5):2221-32.
16. Ahmed SM, Abo El-Naga N I, Hussein M M, Bedir A M. Biological Study to Evaluate the Effect of Intake Flaxseed Oil on Kidney Failure Rats. ASEJ. 2022 Mar 1;43(2):9-19.
17. Shahper L, Farzana F, Muneer MQ, Hassan S, Hashim R, Ilyas M. Effects of whole-ground flax seeds versus flax oil for pesticide residues-induced nephrotoxicity in male Wistar rats. KMUJ. 2020 Jun 30;12(2):86-94.
18. Tashkandi B, Baghdadi GM, Baghdadi AM. Protective Impact of Flaxseed Oil against Acetaminophen-Induced Nephrotoxicity in Rats: Antioxidant and Anti-inflammatory Pathway. J. Complement. Med. Res.2023 Jan: 9;14(1):56-60
19. Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal route of drug administration: should it be used in experimental animal studies. Pharm Res. 2020 Jan;37(1):12.
20. Yaseen AH, Asker SA, Omar N, Abd El Fattah AA. N-Acetylcysteine mitigates histopathological and ultrastructural alterations induced by chronic usage of acetaminophen in the rat renal cortex. EJH. 2019 Mar 1;42(1):162-77.
21. Al-Doaiss AA. Hepatotoxicity-Induced by the therapeutic dose of acetaminophen and the ameliorative effect of oral co-administration of selenium/Tribulus terrestris extract in rats. Int. j. morphol. 2020 Oct 1;38(5):1444-54.
22. Akgun E, Boyacioglu M, Kum S. The potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats. Exp. Anim. 2021;70(1):54-62.
23. Reshi MS, Yadav D, Uthra C, Shrivastava S, Shukla S. Acetaminophen-induced renal toxicity: preventive effect of silver nanoparticles. Toxicol. Res. 2020 Jul;9(4):406-12.
24. Hegazy A, Abd Al Hameed EA, El-Wafaey D, Khorshed O. Effect of Paracetamol administration on the Rat kidney structure: A Morphological Study. ZUMJ. 2021 Jul 1;27(4):567-76.
25. Gaber DA, Badawy WA. Role of flaxseed oil and silymarin in amelioration of lead-induced kidney injury. Kasr Al Ainy Med. J.2019 Jan 1;25(1).

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**Authorship:**

**MS:** Conception, Interpretation of data**,** Research supervision,
**YB :** Data Collection
**SM:** Statistical Analysis
**FI :** Drafting