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RESEARCH ARTICLE
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Protective Impact of Flaxseed Oil against Acetaminophen-Induced Nephrotoxicity in Rats: Antioxidant and Anti-inflammatory Pathway

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ABSTRACT

Acetaminophen (AAP) is a commonly analgesic found in numerous non-prescription pharmaceuticals. High dose and chronic ingestion of AAP caused renal toxicity. This study is designed to assess the possible nephroprotective role of flaxseed oil (FSO) in male rats. Nephrotoxicity was induced in rats via ingested a single dose of AAP (3 g/kg). Five groups of rats were used; Control, AAP, FSO (1.5 ml/kg) + AAP, FSO (3 ml/kg) + AAP, and FSO (4.5 ml/kg) + AAP. Rats were received orally FSO for 30 days and at the 30th day received AAP 1 h before FSO. Serum renal function indices were determined. Also, antioxidants, oxidative stress, and pro-inflammatory cytokines indices were measured in serum. Ingestion of FSO (3 and 4.5 ml/kg) prior to AAP intoxication significantly decreased AAP-induced nephrotoxicity as evidenced by significant decrease in renal functions relative to the AAP group. Prevented the oxidative stress as evidenced by significant increases in SOD and GSH levels, concurrent with a significant decline in MDA level. Besides, there were significant decreases in IL-1α and TNF-α relative to the AAP group. FSO (3 and 4.5 ml/kg) preserved the renal parenchyma, glomerulus and tubules histological features induced by AAP. FSO (4.5 ml/kg) was markedly the most effective dose relative to the two other doses. In conclusion, FSO protects AAP-induced renal toxicity in a dose dependent manner via its potent antioxidant and anti-inflammatory activities.

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INTRODUCTION

One of the most frequent kidney issues is nephrotoxicity, which happens when the body is exposed to a toxin or drug).[1] Because there are more effective therapeutic medications available, a lot of them might negatively affect the kidney, causing chronic interstitial, chronic interstitial nephritis, acute renal failure and nephritic syndrome.[2] When used in therapeutic doses, acetaminophen is normally innocuous,[3] Adults should take immediate-release oral formulations in doses between 325 and 650 mg every four to six hours, or one gram every four to six hours as needed, but no more than 4 g per day. Children should take 10-15 mg/kg every 4-6 hours, up to a maximum of 50-75 mg/kg each day[4] However, it is known to cause toxicity when taken in a single or repeated large dose or after prolonged use[3]. Centrilobular hepatic necrosis, acute liver failure, hypoglycemia coma and renal tubular necrosis are all common adverse effects linked with acetaminophen consumption.[5-7]

The overproduction of reactive oxygen species (ROS) generated by the buildup of AAP in renal tubular cells has been attributed to AAP's nephrotoxic potential. Furthermore, antioxidant enzyme activity reduction causes morphological damage to intracellular organelles.[8] An important new insight into the mechanisms causing inflammation in AAP-induced acute renal damage.[9] An increasing body of evidence suggests that AAP causes significant NF-κB activation in the kidneys.[5]

The oil derived from flaxseeds, known as flaxseed oil (FSO), is frequently consumed as a dietary supplement worldwide.[10] Several biological activities of flaxseed oil are reported. It is known for its cardioprotective properties, it increases the high-density lipoproteins while it decreases the low-density lipoproteins if taken daily in routine diet.[11] The world health organization ranks the flaxseed oil quite high to lower down the risk of atherosclerosis.[12]

Flaxseed oil supplementation also showed improvement in antioxidant status of liver [13]. Additionally, it played a worthy role on kidney [14] (Ahmad et al., 2017).

In this research the effect of FSO on nephrotoxicity induced by AAP was evaluated.

KEYWORDS:

pelvic floor disorders, pelvic floor dysfunction, pelvic organ prolapse, vaginal wind, vaginal noise, vaginal gas, vaginal flatus

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MATERIAL AND METHODS

Plant, Drug, and Chemicals

Flaxseed oil (FSO) was bought from Abazeer organic food stories, Jeddah, SA. Acetaminophen (AAP) was obtained from Alnahdi Pharmacy. All chemicals and kits with high analytical grade were purchased from Sigma, USA.

Experiment Protocol

Fifty male Wister rats (170-190 g) were bought from the unit of rodent experimental, King Fahd Medical Research Center (KFMRS), KAU. The experiment, biochemical analysis and histopathological examination were conducted from January 2020 to December 2021.

Induction of Nephrotoxicity and Experimental Design

All rats were allowed to one-week adjustment, they fed a nutritionally balanced diet and drinking water freely in a standard environment conditions before being used for this study. After adjustment period, rats were divided into 5 groups (10 rats each). Control: rats were ingested orally saline; AAP: rats were ingested a single dose of AAP (3 g/kg) at day 30,[15] FSO (1.5 ml/kg) + AAP: rats were ingested orally FSO for 30 days and at the 30th day received AAP 1 h before FSO. FSO (3 ml/kg) + AAP: rats were ingested orally FSO for 30 days and at the 30th day received AAP 1 h before FSO,[16] and FSO (4.5 ml/kg) + AAP: rats were ingested FSO for 30 days and at the 30th day received APAP 1 h before FSO. After thirty days, blood samples were taken under light ether anaesthesia from the inner canthus of the eye using capillary tubes, and centrifuged for 15 min at 3000 rpm. Separated serum samples were frozen at -20°C until used for the biochemical analysis. Renal samples were collected for histopathological examination.

Estimation of Renal Function Indices

Renal function biomarkers were assessed through determination of serum creatinine, uric acid, and urea levels by using BioMerieux kits, France.

Estimation of Antioxidants/oxidative Stress Indices

Serum was used to measure an enzymatic antioxidant (superoxide dismutase (SOD)), non-enzymatic antioxidant (reduced glutathione (GSH)) and an oxidative stress marker (malondialdehyde (MDA)). These markers were determined using Elisa kits Cayman's, according to the manufacturer's instructions.

Estimation of Serum Inflammatory Cytokines

Serum was used to measure interleukin 1 alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) using Sandwich-ELISA kits.

Histopathological Examination of Renal Tissue

Renal Specimens after being stained were examined under the light microscope to detect the histopathological changes in different groups.

Statistical Analysis

The obtained data were analyzed using (SPSS version 27). All data represented as mean with their standard deviation. One-way analysis of variance (ANOVA) was used to determine the differences between experimental groups. Results considered as significant if $p \leq 0.05$.

RESULTS

Impact of Flaxseed oil (FSO) on renal function biomarkers

As shown in Figure 1, injection of acetaminophen (AAP) significantly induced elevated in renal functions relative to the control rats. Ingestion of FSO (300 and 450 mg/kg) inhibited the elevation of renal function markers relative to the AAP group. FSO (450 mg/kg) restored renal function markers to the normal value. Interestingly, ingestion of FSO (450 mg/kg) showed significant changes in these parameters relative to the other two doses (150 and 300 mg/kg).

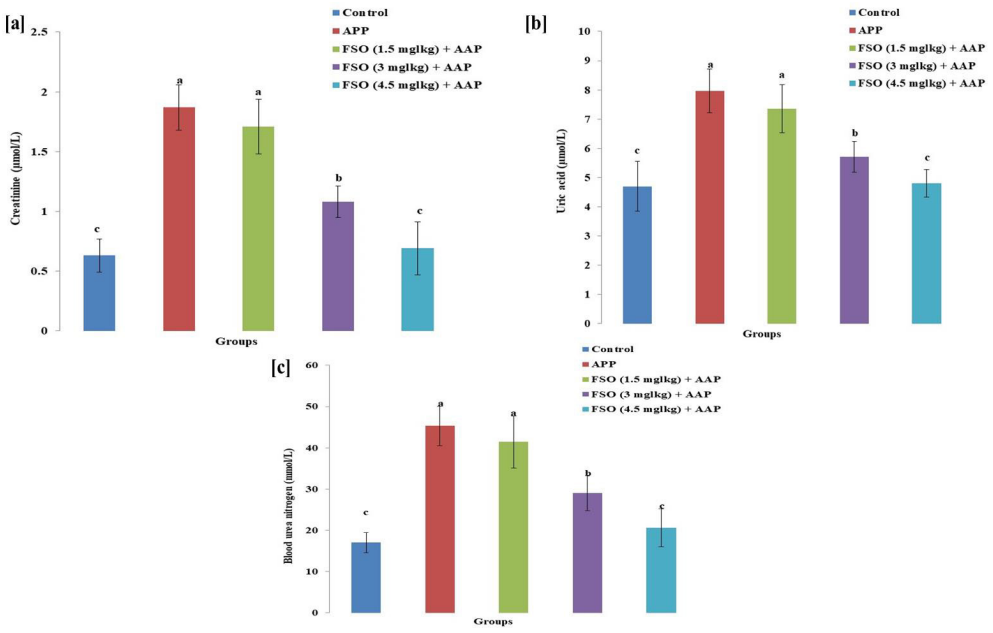

Fig. 1: Impact of FSO on creatinine [a], uric acid [b], and blood urea nitrogen [c] measured in AAP-induced nephrotoxicity in male rats. Data were tabulated as means ± standard deviation, (n = 10). Data with changed superscripts within the column are significantly different ($p \leq 0.05$).

Impact of FSO on Renal Histology (H&E).

The control photo showed normal kidney structure. AAP group photo showed marked interstitial nephritis and congestion of renal blood vessels. FSO (1.5 ml/kg) + AAP group photo showed dilatation of Bowman's space and focal interstitial nephritis. FSO (3 ml/kg) + AAP group photo showed slightly congestion of glomerular tuft and inflammatory cells infiltration. FSO (4.5 ml/kg) + AAP group photo showed apparently no histopathological alterations Figure 2.

Impact of FSO on Antioxidants / Oxidative Stress Markers

Injection of AAP significantly induced oxidative stress as evidenced by significant reduction in serum SOD and GSH levels, concurrent with a significant elevation in serum MDA relative to the control rats. Ingestion of FSO (300 and 450 mg/kg) exhibited antioxidant effects as evidenced by significant increases in serum SOD and GSH levels, concurrent with a significant decline in MDA level relative to the AAP group. FSO (450 mg/kg) restored normalized SOD, GSH, and MDA levels in serum. Interestingly, ingestion of FSO (450 mg/kg) showed significant changes in these antioxidants and oxidative stress markers relative to the other two doses of FSO (150 and 300 mg/kg) Table 1.

Impact of FSO on Pro-inflammatory Cytokines

As shown in Figure 3, injection of AAP induced inflammation as evidenced by significantly elevated in serum IL-1α and TNF-α levels relative to the control rats. Ingestion of FSO (300 and 450 mg/kg) significantly decreased the IL-1α and TNF-α relative to the AAP group. FSO (450 mg/kg) restored IL-1α and TNF-α to the normal value. Ingestion of FSO (450 mg/kg) showed significant changes in these pro-inflammatory parameters relative to the two other doses (150 and 300 mg/kg).

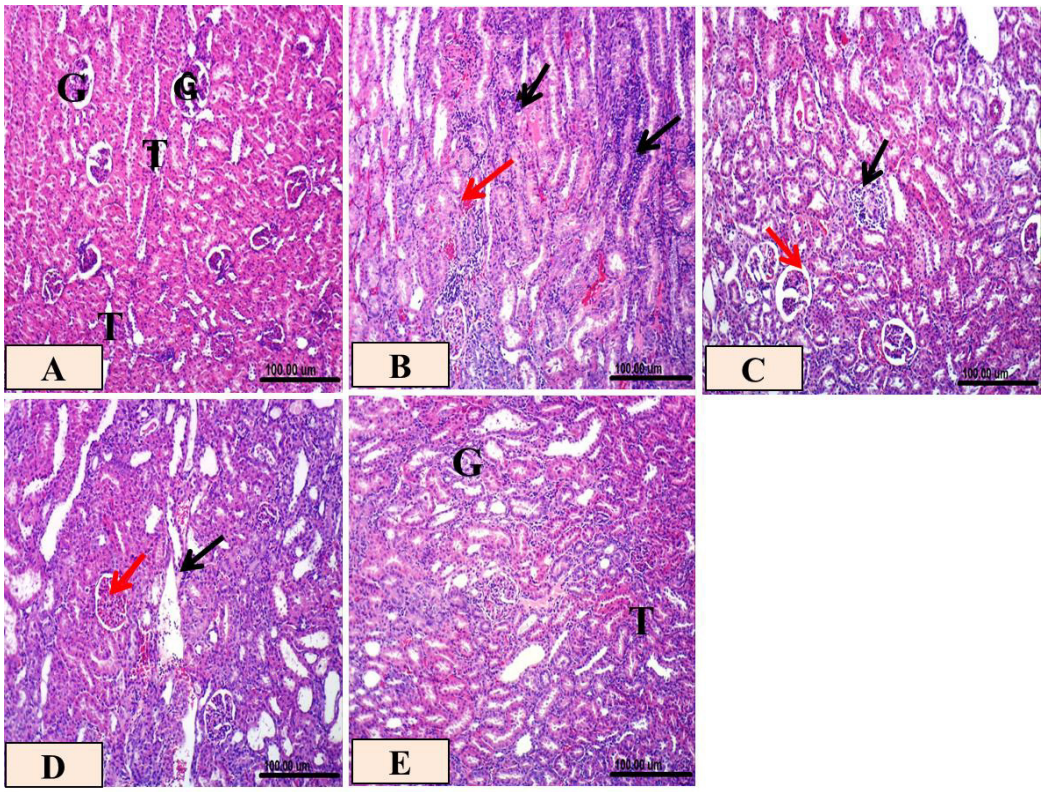

Fig. 2: Impact of FSO on renal histology in AAP-induced nephrotoxicity in male rats. Sections stained with haematoxylin and eosin. Photo [A] control showed normal structure of glomeruli (G), parenchyma, and tubules (T). Photo [B] AAP group showed interstitial nephritis (black arrow) and congestion of renal blood vessels (red arrow). Photo [C] FSO (1.5 ml/kg) + AAP group showed slight dilatation of Bowman's space (red arrow) and focal interstitial nephritis (black arrow). Photo [D] FSO (3 ml/kg) + AAP group showed few inflammatory cells infiltration (black arrow) and congestion of glomerular tuft (red arrow). Photo [E] FSO (4.5 ml/kg) + AAP group showed apparently no histopathological alterations.

Table 1: Effect of FSO on serum SOD, GSH, and MDA measured in AAP-induced nephrotoxicity in male rats.

Groups	*SOD (U/mg protein)*	*GSH (µg/mg protein)*	*MDA (nmol/g protein)*
Control	65.72 ± 2.55 a	7.83 ± 0.23 a	3.72 ± 0.04 c
AAP (3 g/kg)	50.36 ± 2.63 c	4.12 ±0.16 c	12.03 ± 1.03 a
FSO 1.5 ml/kg + AAP	53.23 ± 1.99 c	4.87 ± 0.09 c	10.63 ± 0.99 a
FSO 3 ml/kg + AAP	60.88 ± 2.74 b	6.52 ± 0.27 b	4.89 ± 0.02 b
FSO 4.5 ml/kg + AAP	64.95 ± 3.42 a	7.36 ± 0.64 a	3.63 ± 0.25 c

Data were tabulated as means ± standard deviation, (n = 10). Data with changed superscripts within the column are significantly different ($p \leq 0.05$).

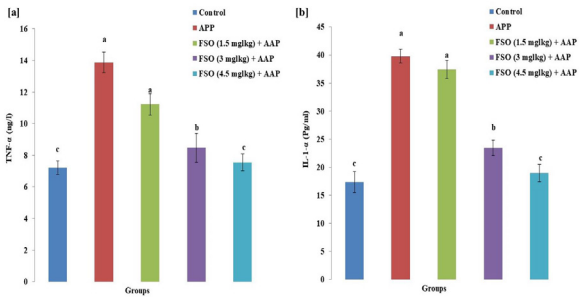

Figure 3: Effect of FSO on TNF- α [a] and IL-1α [b] measured in AAP-induced nephrotoxicity in male rats.
Data were tabulated as means ± standard deviation, (n = 10). Data with changed superscripts within the column are significantly different (p ≤ 0.05).

DISCUSSION

The kidney eliminates metabolic wastes such creatinine, urea, and uric acid. When there is renal disease or injury, the quantities of the metabolites in the blood rise [17]. This could be because of lipid peroxidation, elevated levels of triacylglycerol and cholesterol, and, high xanthine oxidase activity [18]. The most popular antipyretic and pain reliever is acetaminophen, also known as N-acetyl p-aminophenol and paracetamol. It has been sold over the counter either alone or in conjunction with other medications [19] Although AAP use has been associated with a variety of adverse effects since its approval in the 1950s [20]. While acetaminophen is thought to be largely safe when used in therapeutic doses, it is well recognized to be toxic when administered in high dosages, either once or repeatedly, or when used for a prolonged period (Siemionow et al., 2016). In this research, the protective effects of FSO against AAP-induced nephrotoxicity were investigated. The results showed that oral FSO consumption decreased the nephrotoxicity of AAP. The blood's concentrations of a few important biochemical markers were employed to diagnose kidney injury.

In treated groups as compared to the positive control rats, FSO at various dosage levels caused a substantial (P≤0.05) decrease in creatinine, uric acid, and blood urea nitrogen. These results were supported by EL-Sahar and Abed EL-Rahman [21], who reported that flaxseed oil (20, 30 and 40 g/kg diet) improved kidney functions in rats. Flaxseed (3, 5 and 7 g/100g diet) reduced urea and uric acid values in rats suffer from nephropathy [22]. Al-Bishri [23] reported that flaxseed supplemented diet significantly lowered the plasma level of kidney functional markers including urea, uric acid, and creatinine in hypertensive rats.

Kidney functions of normal rats and rats fed different levels of flaxseed oil were shown in Figure (1). creatinine, uric acid, and blood urea nitrogen values of rats under this study were within the normal and safe range with the exception of positive control rats which were higher than the normal and save range. Positive control rats had higher (P≤0.05) creatinine, uric acid, and blood urea nitrogen than negative control rats and rats orally given different dosage levels of flaxseed oil. The results were in the same line with Barakat and Mahmoud[24] (2011) who stated that giving rats a diet high in cholesterol led to a considerable rise in serum urea compared to rats the positive control rats, flaxseed oil at various replacement doses significantly (P≤0.05) decreased blood urea nitrogen, uric acid, and creatinine. These results were supported by EL-Sahar and Abed EL- Rahman [21], who reported that flaxseed oil (20, 30 and 40 g/kg diet) improved kidney functions in rats. Flaxseed (3, 5 and 7 g/100g diet) reduced urea and uric acid values in rats suffer from nephropathy [22] (El-Sayed *et al*, 2014). Hypercholesterolemic rats fed different replacement levels of flaxseed oil had lower (P≤0.05) urea values than negative control rats. In contrary to, they had higher (P≤0.05) uric acid values than negative control rats. Data indicated that oral administration of FSO with different replacement levels to rats returned the kidney function values to the normal and save range value. The preventive effect was confirmed by the fact that a histological study of kidney tissues demonstrated less damage.

The mechanism underlying the higher values of the biochemical parameters may be the AAP-induced changes in the permeability of the cell membrane and decreased structural integrity that allow the enzymes to permeate into the circulation [6] (De-Giorgio et al. 2013). An examination of the kidney tissue's histopathology backed up these findings. The findings of the current study are consistent with the study by Olaleye *et al*.[25]

In the current research AAP causes a significant increase in renal lipid peroxidation (MDA), which in turn causes an unbalanced situation between oxidants and antioxidants that is manifested by a decrease in the kidneys' ability to protect themselves from oxidative damage by using antioxidants like SOD and GSH these results were in the line with Jaeschke et al.[26] (2011). According to the histological examination of the renal tissues, oxidative stress results in multiple pathological destructions. These results were consistent with the results attained by Hegazy *et al*.[27] (2021). In contrast to the AAP-injected group, pretreatment with FSO in the current investigation led to a considerable drop in renal MDA levels and an increase in the activities of renal SOD and GSH enzymes. The most notable effect was seen at dosages of FSO of 4.5 ml/kg. The antioxidant capabilities of FSO, this might be due to that FSO is a rich source of omega-3 fatty acids which serve to scavenge free radicals and prevent DNA damage, which in turn could explain the enhanced activity of the GSH and SOD enzymes.[28]

The study's findings showed a high prevalence of renal inflammation, which was supported by the considerable increases in serum TNF- and IL-1 levels in the AAP group compared to the control negative group. The FSO groups showed anti-inflammatory characteristics by significantly reducing levels of pro-inflammatory cytokines, Treatment with FSO induced decrease in TNF-α and IL-1α which explained by Farrah *et al*. [29], who reported that the anti-inflammatory effects of n-3 PUFA, which are present in flaxseed oil, are mediated by the generation of anti-inflammatory eicosanoids. Histopathological analysis of a kidney segment was used to confirm the biochemical findings of the current experiment. The AAP group had interstitial nephritis and cystic tubule dilatation. According to histological investigations, the rats' histological structure was improved by the FSO pretreatment. The interstitial nephritis and congestion of renal blood vessels in the renal portion of the AAP rats treated with FSO were all mildly altered, and the Bowman's space and parietal layer of the Bowman's capsule were both somewhat dilated.

The normal histological features of the kidney parenchyma, normal glomeruli, and normal tubules were recovered by the FSO pretreatment (4.5 ml/kg). The current findings are in line with earlier research that found that FSO's efforts to scavenge free radicals appear to lessen histopathological changes related to oxidative damage and inflammation in nephrotoxicity.[30,31]

CONCLUSION

According to the research exists, flaxseed oil at various replacement levels significantly improved the kidney function, antioxidants and oxidative stress markers and pro-inflammatory parameters. No conflicts of interest exist, according to the authors, with the publishing of this paper.

Ethical Approval: Ethical approval was cleared by Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, Saudi Arabia (Ethical approve No. 91060231).

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