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The Protective Effects of Curcumin Oil and Flaxseed Oil on Cisplatin-Induced Nephrotoxicity and Hepatotoxicity in Male Albino Rats

Eman Gamal Eldin Helal [1*], Eman Refaat Younis [2], Aisha Emhammed Almokhtar Aljaghmani [3]

[1] Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt,

[2] Medical Biochemistry Department, National Research Centre, Egypt

***Corresponding Author:** Eman G.E. Helal, E-mail: emanhelal@hotmail.com,

Mobil: 00201001025364, **Orchid.org/0000-0003-0527-7028**

ABSTRACT

Background: Cisplatin, while potent against cancer, causes significant nephrotoxicity and hepatotoxicity.

Objective: To investigate the protective effects of curcumin and flaxseed oil against cisplatin-induced toxicity.

Materials and Methods: Animal models (rats) were divided into four groups: a control group, a cisplatin-alone group (single dose of 7.5 mg/kg i.p. on day six), a curcumin group, and a flaxseed oil group (0.1 ml/1 kg orally for ten days with a cisplatin dose on day six). Liver and kidney functions were assessed by measuring serum (alanine transaminase) ALT, aspartate aminotransferase (AST), urea, and creatinine levels. Protein metabolism was evaluated by measuring total protein, albumin, and globulin levels, and the albumin/globulin ratio, followed by histological examination of liver and kidney biopsies.

Results: Cisplatin significantly increased ALT, AST, urea, and creatinine levels indicating severe liver and kidney damage ($p < 0.001$). It also decreased total protein, albumin, and globulin levels, impairing protein metabolism ($p < 0.001$). Co-administration of curcumin or flaxseed with cisplatin significantly reduced ALT, AST, urea, and creatinine levels, while improving total protein, albumin, and globulin levels and improved histological results compared to the cisplatin-alone group.

Conclusion: Curcumin and flaxseed effectively mitigated cisplatin-induced hepatotoxicity, nephrotoxicity, and disturbances in protein metabolism. Thus, they could be potential adjuvant therapies in cisplatin chemotherapy to reduce its side effects.

Keywords: Cisplatin, Curcumin, Flaxseed, Hepatotoxicity, Nephrotoxicity, Chemotherapy.

INTRODUCTION

The high incidence of cancer and the significant adverse effects associated with conventional chemotherapy present substantial challenges for researchers seeking to develop new interventions aimed at reducing these toxicities [1].

Cisplatin, a potent chemotherapeutic agent, is known to cause severe toxicity in multiple organs, including the kidneys, liver, gastrointestinal tract, and nervous system. This nephrotoxicity and hepatotoxicity result from the inhibition of antioxidant enzymes and proteins [2].

Current research efforts are focused on mitigating the effects of cisplatin during chemotherapy. Recent studies indicate that combining cisplatin with plant extracts may enhance its antitumor efficacy while minimizing its side effects [3].

For instance, curcumin has been extensively utilized to combat cisplatin-resistant cancer cells and to alleviate its adverse effects, such as ototoxicity, nephrotoxicity, and neurotoxicity [4].

Flaxseed, a rich dietary source of omega-3 fatty acids among plant-based options, has gained recognition as an important alternative source of these essential nutrients. Supplementation with flaxseed oil (FXO) has been shown to influence fatty acid metabolism, thereby affecting the balance of proinflammatory mediators and atherogenic lipids. This holds significant potential for modulating inflammatory diseases [5].

The aim of this study was to investigate the protective effects of curcumin and flaxseed oil against cisplatin-induced toxicity.

MATERIALS AND METHODS

Materials

Cisplatin was purchased as vial contents (50 mg/50 ml) from the Hikma Pharmaceutical Company (Giza, Egypt). Curcumin oil and flaxseed oil were purchased from EL-Hawag for Natural Oils company, Egypt.

Animals

The experiments utilized male albino rats weighing between 160 to 200 grams, provided by the animal facility at NODCAR, Egypt. The animals were maintained in a controlled environment at a constant temperature of 22±1°C with a 12-hour light-dark cycle and had unrestricted access to food and tap water.

Experimental Design: The rats were randomly assigned into four groups, each comprising six rats:

- **Group 1 (Control Group):** Consisted of normal, untreated rats.
- **Group 2:** Administered a single intraperitoneal dose of cisplatin (7.5 mg/kg body weight) on the sixth day of the experiment.

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- **Group 3:** Received oral curcumin oil (0.1 ml/kg body weight) daily for ten days, with an intraperitoneal injection of cisplatin (6 mg/kg body weight) on the sixth day.
- **Group 4:** Received oral flaxseed oil (0.1 ml/kg body weight) daily for ten days, with an intraperitoneal injection of cisplatin (6 mg/kg body weight) on the sixth day.

On day 11, blood was collected from all animals, which were then sacrificed and decapitated. The collected blood was used to determine serum concentrations of ALT, AST, urea, and creatinine, as well as plasma levels of total protein (TP), albumin, globulin, and the albumin/globulin ratio. Blood samples were obtained from the retro-orbital sinuses of the rats. After separation, serum and plasma samples were frozen until analysis.

a. Determination of liver function biomarkers:

Colorimetric assay kits (BioMerieux, France) were employed to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

b. Determination of renal function:

Renal function biomarkers were evaluated by measuring serum creatinine and urea concentrations using BioMerieux kits (France).

c. Determination of protein biomarkers:

Total protein and albumin levels were measured using colorimetric assay kits (BioMerieux, France). Globulin levels and the albumin/globulin (A/G) ratio were then calculated as follows:

- Globulin (g/dl) = Total protein (g/dl) - Albumin (g/dl)
-Albumin/globulin ratio (A/G ratio) = Albumin/Globulin

Histopathological examination:

Autopsy samples were collected from the kidneys of rats in the various experimental groups and were fixed in 10% formal saline for twenty-four hours. After washing in tap water, the samples underwent dehydration through a series of alcohol solutions (methyl, ethyl, and absolute ethyl). The specimens were then cleared in xylene and embedded in paraffin wax to create tissue blocks. These blocks were sectioned at a thickness of 4 microns using a rotary LEITZ microtome. The tissue sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin stain [6] for examination under a light microscope.

Ethical consideration:

The experiment received approval from the Ethics Committee of the Faculty of Science at Al-Azhar University. The study adhered to the ethical guidelines and procedures established by the Animal Care and Use Committee of the Faculty of Science, Al-Azhar University, Cairo, Egypt.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test. The results were analyzed using SPSS software (version 27), with a significance threshold set at $p<0.05$.

RESULTS

Table 1 compares the four experimental groups regarding ALT and AST activities. The findings indicate that cisplatin treatment significantly increased ALT and AST activities relative to the control group. Conversely, curcumin and flaxseed treatments resulted in significantly lower activities of these enzymes compared to cisplatin.

Table (1): Comparison between the studied four groups regarding ALT levels and AST levels (liver enzymes) of the studied cases

Groups	Group 1 (Control) No. = 6	Group 2 (Cisplatin alone) No. = 6	Group 3 (Cisplatin + Curcumin) No. = 6	Group 4 (Cisplatin + Flaxseed) No. = 6	P-value
ALT (mg/dl)					
Mean ± SD	16.17 ± 1.60	73.50 ± 7.66[a]	25.33 ± 5.72 [a, A]	39.83 ± 6.11 [a, A]	<0.001
% of change Vs Control group	–	354.55	56.65	146.32	
% of change Vs Cisplatin	–	–	-65.53	-45.81	
AST (mg/dl)					
Mean ± SD	24.00 ± 3.46	61.83 ± 4.88[a]	26.33 ± 5.72[A]	33.83 ± 4.79 [a, A]	<0.001
% of change Vs Control group	–	157.63	9.71	40.96	
% of change Vs Cisplatin	–	–	-57.42	-45.29	

a: Significant difference from negative control; A: Significant difference from positive control (Cisplatin group).

Table 2 compares the four groups regarding urea and creatinine levels. The results show that cisplatin treatment significantly elevated urea and creatinine levels compared to the control group. However, treatments with curcumin and flaxseed significantly lowered these levels compared to the cisplatin group.

Table (2): Comparison between the studied four groups regarding urea levels and creatinine levels of the studied cases

Groups	Group 1 (Control) No. = 6	Group 2 (Cisplatin alone) No. = 6	Group 3 (Cisplatin + Curcumin) No. = 6	Group 4 (Cisplatin + Flaxseed) No. = 6	P-value
Urea (mg/dl)					
Mean ± SD	44.88 ± 8.91	109.23 ± 7.46[a]	101.50 ± 7.02 [a]	77.53 ± 14.53 [a, A]	<0.001
% of change Vs Control group	–	143.38	126.16	72.75	
% of change Vs Cisplatin	–	–	-7.08	-29.02	
Creatinine (mg/dl)					
Mean ± SD	0.18 ± 0.07	1.62 ± 0.39[a]	0.94 ± 0.12 [a, A]	0.53 ± 0.13 [a, A]	<0.001
% of change Vs Control group	–	800	422.22	194.44	
% of change Vs Cisplatin	–	–	-41.98	-67.28	

a: Significant difference from negative control; A: Significant difference from positive control (Cisplatin group)

Table 3 examines the four groups regarding total protein, albumin, globulin, and the albumin/globulin ratio in the studied cases. Cisplatin treatment significantly decreased total protein and albumin levels while altering the albumin/globulin ratio. Curcumin and flaxseed treatments showed significant improvements in these parameters compared to cisplatin. The percentage change calculations further highlight the degree of impact, each treatment had relative to the control groups.

Table (3): Comparison between the studied four groups regarding total protein and albumin and globulin and albumin /globulin ratio (g/dl) of the studied cases

Groups	Group 1 (Control) No. = 6	Group 2 (Cisplatin alone) No. = 6	Group 3 (Cisplatin + Curcumin) No. = 6	Group 4 (Cisplatin + Flaxseed) No. = 6	P-value
Total protein (gm/dl)					
Mean ± SD	8.27 ± 0.41	3.03 ± 0.37 [a]	6.73 ± 1.21 [a, A]	6.23 ± 1.34 [a, A]	<0.001
% of change Vs Control group	–	-63.36	-18.62	-24.3	
% of change Vs Cisplatin	–	–	122.11	105.61	
Albumin (gm/dl)					
Mean ± SD	5.15 ± 0.29 [a, A]	1.80 ± 0.40 [a, A]	4.52 ± 0.54 [a, A]	3.88 ± 0.83 [a, A]	<0.001
% of change Vs Control group	–	-65.05	-12.23	-24.66	
% of change Vs Cisplatin	–	–	151.11	115.56	
Globulin (g/dl)					
Mean ± SD	3.12 ± 0.38	1.23 ± 0.26[a]	2.22 ± 1.08	2.82 ± 1.47 [A]	<0.001
% of change Vs Control group	–	-60.58	-28.85	-9.62	
% of change Vs Cisplatin	–	–	80.49	129.27	
Albumin/globulin ratio (g/dl)					
Mean ± SD	1.65 ± 0.23	1.46 ± 0.45	2.04 ± 0.34	1.38 ± 0.36	<0.001
% of change Vs Control group	–	-11.52	23.64	-16.36	
% of change Vs Cisplatin	–	–	39.73	-5.48	

a: Significant difference from negative control; A: Significant difference from positive control (Cisplatin group)

Histopathological Findings of Liver

1- Control Group: No histopathological changes were observed, and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma was maintained (Figure 1).

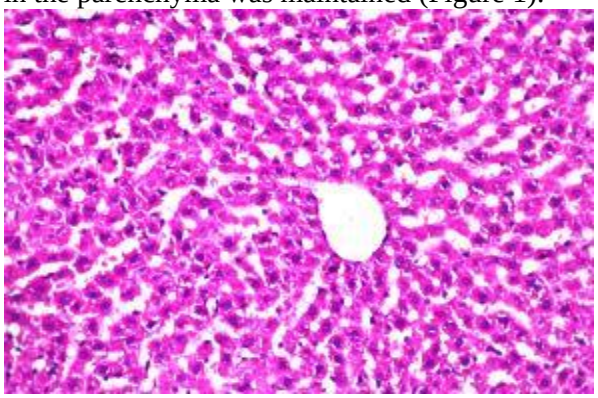

Figure (1): Depicts the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (H&E stain, X40).

2- Cisplatin Group: The portal area exhibited fibrosis accompanied by infiltration of inflammatory cells (Figure 2).

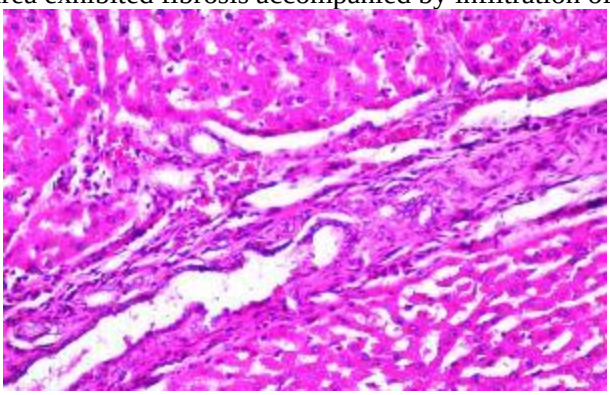

Figure (2): Shows the fibrosis and inflammatory cell infiltration in the portal area (H&E stain, X40).

3- Curcumin Group: The histopathological examination revealed normal findings (Figure 3).

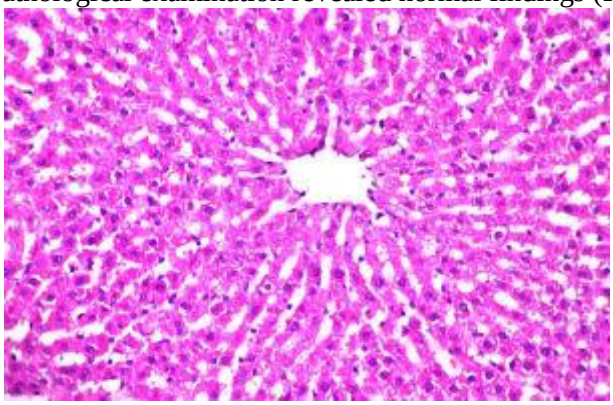

Figure (3): Illustrates the normal histological structure (H&E stain, X40).

4- Flaxseed Group: Inflammatory cell infiltration was observed in the portal area (Figure 4).

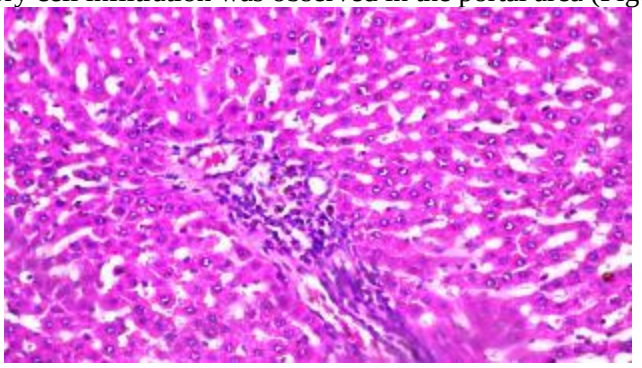

Figure (4): Depicts inflammatory cell infiltration in the portal area (H&E stain, X40).

Histopathological Findings of Kidney

1- Control Group: No histopathological alterations were observed, and the normal histological structure of the glomeruli and tubules in the cortex was maintained (Figure 5).

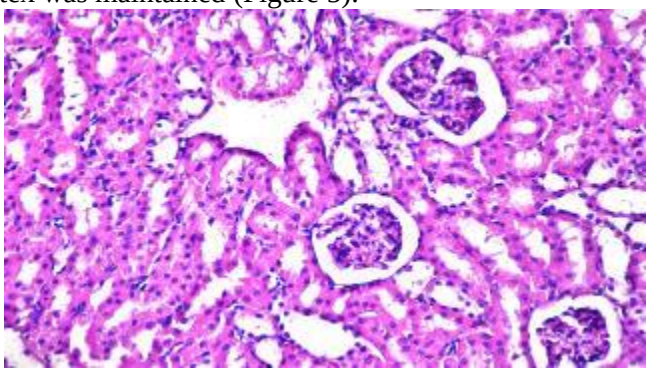

Figure (5): Shows the normal histological structure of the glomeruli and tubules in the cortex (H&E stain, X40).

2- Cisplatin Group: Degenerative changes, necrosis, and desquamation were detected in the lining tubular epithelium at the cortex and corticomedullary junction (Figures 6).

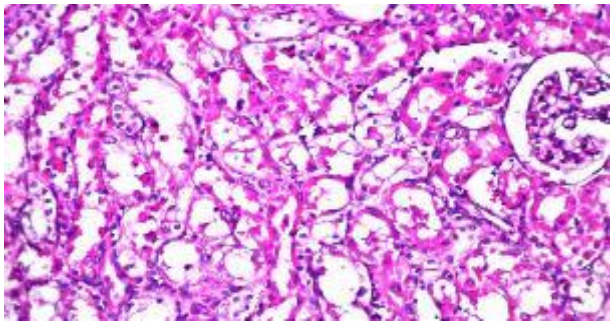

Figure (6): Shows degeneration, necrosis, and desquamation of the tubular lining epithelium in the cortex.

3- Curcumin Group: Degenerative changes were observed in the tubular lining epithelium of the cortex (Figure 7A). The corticomedullary portion showed degeneration and necrosis with a few eosinophilic casts in the tubular lumen (Figure 7B). Focal inflammatory cell infiltration was also detected between the degenerated and necrotic tubules in the corticomedullary portion (Figure 7C).

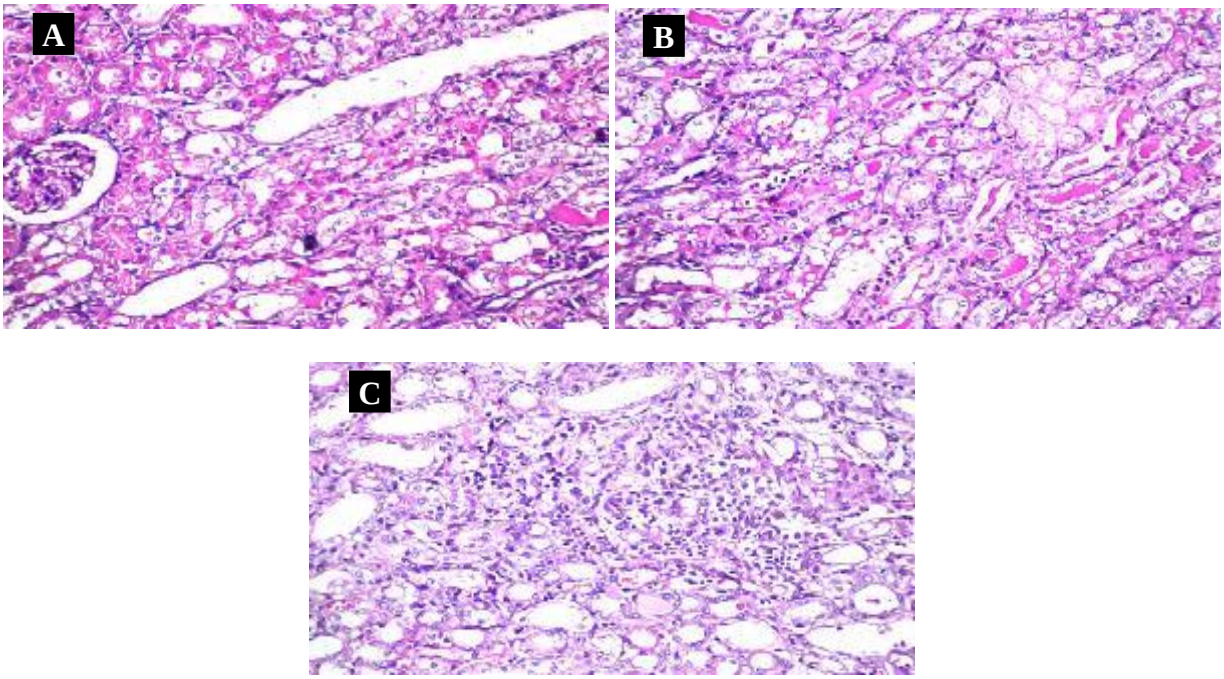

Figure (7): A) Shows degeneration in the tubular lining epithelium at the cortex. B) Depicts degeneration and necrosis with a few eosinophilic cast formations in the tubules of the corticomedullary portion. C) Illustrates focal inflammatory cell infiltration between the degenerated and necrotic tubules in the corticomedullary portion (H&E stain, X40).

4- Flaxseed Group: Degenerative changes were detected in the tubular lining epithelium at the cortex (Figure 8A). The corticomedullary portion exhibited eosinophilic cast formation in the tubular lumen (Figure 8B), as well as tubular cystic dilation with flattened lining epithelium (Figure 8C).

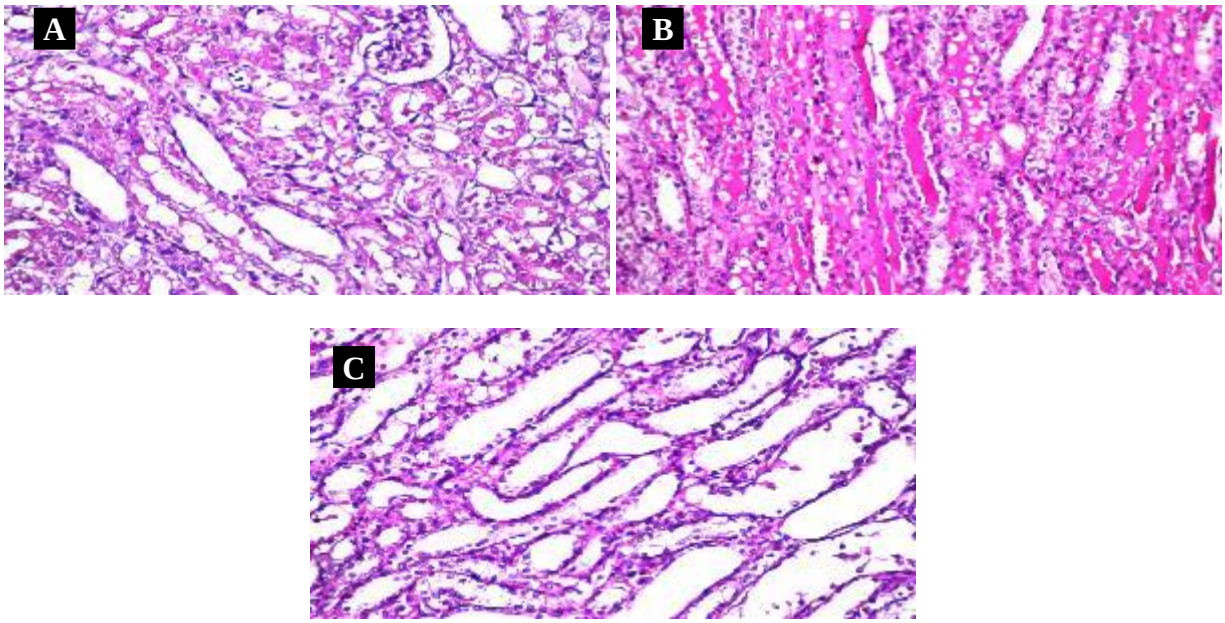

Figure (8): A) Shows degeneration in the lining tubular epithelium at the cortex. B) Depicts eosinophilic cast formation in the lumen of the tubules in the corticomedullary portion. C) Illustrates tubular cystic dilation with flattened lining epithelium in the tubules of the corticomedullary portion (H&E stain, X40).

DISCUSSION

Cisplatin is a potent chemotherapy agent widely used for treating various cancers due to its efficacy in inducing apoptosis in cancer cells [7]. However, its clinical utility is often limited by severe side effects, notably nephrotoxicity and hepatotoxicity [8]. This study explores the protective effects of curcumin and flaxseed against cisplatin-induced toxicity, focusing on liver and kidney function markers, as well as protein metabolism.

Cisplatin alone significantly increased both ALT and AST activities, indicating severe hepatotoxicity ($p < 0.001$). This finding is consistent with previous research showing that cisplatin induces oxidative stress and inflammation in the liver, leading to elevated transaminase levels [9]. Co-administration of curcumin or flaxseed significantly reduced these enzyme levels and improve histological results compared to cisplatin alone, suggesting a hepatoprotective effect. Curcumin, a polyphenol from turmeric, is known to inhibit NF-κB signaling and reduce inflammatory cytokines [10]. Flaxseed, rich in alpha-linolenic acid and lignans, exerts hepatoprotective effects through its antioxidant activity and modulation of lipid metabolism [11].

Cisplatin treatment significantly increased urea and creatinine, indicating nephrotoxicity ($p < 0.001$), consistent with its known mechanisms of renal damage through reactive oxygen species (ROS) generation and apoptosis [12]. Both curcumin and flaxseed significantly lowered urea and creatinine levels and improve histological results compared to cisplatin alone. Curcumin's nephroprotective effects are attributed to its ability to scavenge ROS, upregulate antioxidant enzymes like SOD and catalase, and inhibit inflammatory pathways [13]. Flaxseed oil has been shown to protect against nephrotoxicity by enhancing glutathione peroxidase and reducing lipid peroxidation [14].

Cisplatin administration led to significant decreases in total protein and albumin levels ($p < 0.001$), reflecting impaired protein synthesis and liver function [15]. Curcumin and flaxseed significantly improved these levels compared to cisplatin alone. Curcumin enhances protein synthesis by modulating signaling pathways such as PI3K/Akt, while flaxseed's beneficial effects are due to its high content of omega-3 fatty acids and lignans that modulate lipid and protein metabolism [16].

Curcumin is the primary active ingredient in turmeric (Curcuma longa), a spice commonly used in traditional medicine. Curcumin's protective effects against cisplatin-induced toxicity are largely due to its potent anti-inflammatory and antioxidant properties. Curcumin inhibits the NF-κB signaling pathway, which reduces the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-110. It also enhances the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase, which help neutralize reactive oxygen species (ROS) and reduce oxidative stress-induced damage [13]. Additionally, curcumin modulates key signaling pathways involved in cell survival and apoptosis, including the PI3K/Akt and MAPK pathways, thereby promoting cell survival and reducing cisplatin-induced cytotoxicity [17].

Flaxseed is rich in alpha-linolenic acid (ALA), an omega-3 fatty acid, and lignans, which are phytoestrogens with antioxidant properties. The nephroprotective effects of flaxseed are primarily attributed to its high ALA content, which has been shown to reduce inflammation and oxidative stress in renal tissues. ALA is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body, which are known to have anti-inflammatory effects. Flaxseed also enhances the activity of antioxidant enzymes like glutathione peroxidase, which helps mitigate oxidative stress and protect against lipid peroxidation [14]. Lignans in flaxseed contribute to its antioxidant activity by scavenging free radicals and protecting cellular membranes from oxidative damage [18]. These combined effects help maintain renal function and prevent the deterioration of kidney tissues induced by cisplatin.

These findings are supported by existing literature on the protective effects of curcumin and flaxseed against cisplatin-induced toxicity. Curcumin's anti-inflammatory and antioxidant properties have been extensively documented [17], and flaxseed's ability to protect against oxidative stress and improve metabolic profiles is well-established [18].

CONCLUSION

This study demonstrates that curcumin and flaxseed provide significant hepatoprotective, nephroprotective, and protein homeostasis-maintaining effects against cisplatin-induced toxicity. Flaxseeds has more potent ameliorative effect than curcumin.

- **Conflict of Interest:** None to declare.
- **Funding Source:** None.

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