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(RESEARCH ARTICLE)

Olive Oil's protective potential against cyclophosphamide-induced nephrotoxicity in Swiss albino rats

Fatma Hassan Boshahma [1], Ali H. Mahmoud Masoud [2], Nisreen Hassan Alferjani [3], Zahra Mohammed Elfazani [3], Heba Salem Sassi [3], Salwa Momen Eldresi [3], Thanaa Ali Elharabi [3] and Abeer Hussein Amer [1, 4, *]

[1] *Department of Basic Medical Sciences, Faculty of Applied Medical Sciences, Libyan International Medical University, Benghazi, Libya.*
[2] *Department of Pathology, Faculty of Medicine, University of Benghazi, Benghazi, Libya.*
[3] *Department of Cytotechnology, Faculty of Biomedical Sciences, University of Benghazi, Libya.*
[4] *Department of Histology, Faculty of Medicine, University of Benghazi, Benghazi, Libya.*

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Abstract

Background: Cyclophosphamide is a widely used chemotherapy drug, known for its cytotoxic and mutagenic effects on mammalian tissues and cells. Olive oil, rich in antioxidants, has potential protective effects against drug-induced organ damage. This study investigates the protective role of olive oil against cyclophosphamide-induced toxicity in mice.

Objective: To evaluate the protective effects of olive oil against cyclophosphamide-induced kidney toxicity in rats.

Material and Method: Male albino mice (n=9) were divided into three groups: Control, Cyclophosphamide (150 mg/kg), and Cyclophosphamide (150 mg/kg) + Olive Oil (200 mg/kg). After one week of treatment, kidney tissues were collected and stained with hematoxylin and Eosin for histopathological analysis.

Results: The administration of olive oil led to a significant decrease in cellular damage of the kidney tissue. In this study the histopathological examination revealed marked improvement in kidney tissue architecture, with reduced cellular damage, inflammation, and fibrosis compared to the group treated with cyclophosphamide alone. These findings suggest that olive oil has a protective effect against cyclophosphamide-induced hepatic and renal toxicity in mice.

Conclusion: Cyclophosphamide caused significant histopathological damage to kidney tissues in mice. Co-administration of olive oil mitigated these adverse effects, likely due to its antioxidant properties. This suggests that olive oil may serve as a protective adjunct in cyclophosphamide chemotherapy.

Keywords: Cyclophosphomide toxicity; Kidney tissue; Histopathology; Olive oil

1. Introduction

Cyclophosphamide is a member of the oxazaphosphorine family of alkylating agent. It is widely used as a chemotherapy drug to treat various neoplastic diseases (1). It is frequently used in conjunction with other chemotherapy medications to treat a variety of malignancies, such as lung, ovarian, breast, endometrial, neuroblastoma, leukemia, and neuroblastoma. As an immunosuppressant, cyclophosphamide is usually taken to manage chronic autoimmune diseases like multiple sclerosis, rheumatoid arthritis, autoimmune skin diseases, systemic vasculitides, and systemic lupus erythematosus (2). The administration of cyclophosphamide is mostly intravenously, depending on the particular condition being treated. Cyclophosphamide works on DNA by introducing an alkyl group. It functions by joining an alkyl group to the DNA guanine base at the imidazole ring's seventh nitrogen atom. Consequently, at the G2 and S stages of

* Corresponding author: Abeer H. A. Amer

the cell cycle, this results in irreversible cross-linkages in the DNA strands, which cause cell death (3). Additionally, cyclophosphamide is inactivated by side-chain oxidation, which produces metabolites that are neurotoxic (4,5). In severe cases of aplastic anemia and other autoimmune conditions, high doses of cyclophosphamide have shown positive outcomes (6). However, Cyclophosphamide can cause a range of side effects due to its cytotoxic effects on rapidly dividing cells. Adverse effects of cyclophosphamide include hair loss, nausea, vomiting, low platelets count, mucosal ulcerations, dizziness, striations in the nails, increased skin pigmentation, pulmonary fibrosis, diarrhoea, hemorrhagic cystitis and petechial haemorrhage in lungs and small bowel (3).

Many adverse drug reactions are possible with cyclophosphamide. Also, a small proportion of people experience severe responses, which can be lethal or result in birth malformations, congenital anomalies, or illnesses necessitating long-term hospitalization (7). Effective prevention of drug-induced nephrotoxicity requires knowledge of patient-related risk factors, pathogenic processes of renal injury, and drug-related risk factors. Drug-induced nephrotoxicity is common in specific clinical circumstances. Acrolein and phosphoramide mustard are the two main active metabolites that cause renal cell damage as a result. Although phosphoramide mustard has anticancer properties, acrolein produces free radicals that interact with the body's antioxidant defenses, particularly glutathione, to cause oxidative damage (8,9). Rat kidneys treated with cyclophosphamide exhibit cortical tubular vacuolization, interstitial edema, and glomerulonephritis, according to histological analysis (10). Renal injury is a result of both increased protein concentration and decreased lysosomal enzyme activity. Hemorrhagic cystitis is caused by inflammatory reactions in the bladder that are characterized by changed cholinergic activity and nitric oxide release from muscarinic receptors, especially sixty hours after treatment (11). Olive oil components have been proved to have anticancer activity through reducing DNA oxidation, arresting the cell cycle, and inducing apoptosis in tumor cells (12). Consumption of olive oil in the diet has been suggested to be responsible for protection of DNA and the reduction in cancer incidence. The aim from this research is to evaluate the protective effects of olive oil against cyclophosphamide-induced kidney toxicity in rats.

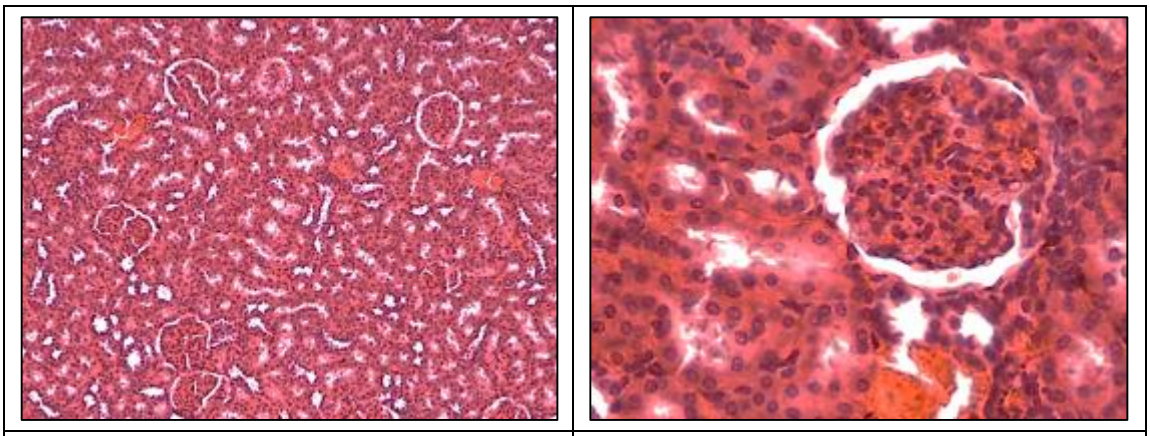
2. Materials and Methods

Experimental Animals: The study was conducted on 9 healthy male albino mice. The average weight of the mice was deemed appropriate for the study. The temperature was 24°C, with Light/Dark Cycle of 15 /9 hours. Throughout the duration of the experiment, food and water were provided *ad libitum*. Body weights were recorded before the onset of the experiment and again prior to the sacrifice of the animals. The mice were divided into three groups, with 3 mice in each group: Control Group: Received 1 ml of normal saline. Cyclophosphamide treated Group: Treated with 1 ml of a mixture containing cyclophosphamide (150 mg/kg body weight) diluted with 50 ml of normal saline solution. Cyclophosphamide and Olive Oil Group: Treated with 1 ml of a mixture containing cyclophosphamide (150 mg/kg body weight) diluted with 50 ml of normal saline solution and an oral dose of 200 mg/kg olive oil. Sacrifice was done humanely and the samples were collected rapidly. The tissues were preserved in formalin for histopathological analysis. The sections were stained with Hematoxylin and Eosin (H&E).

3. Results

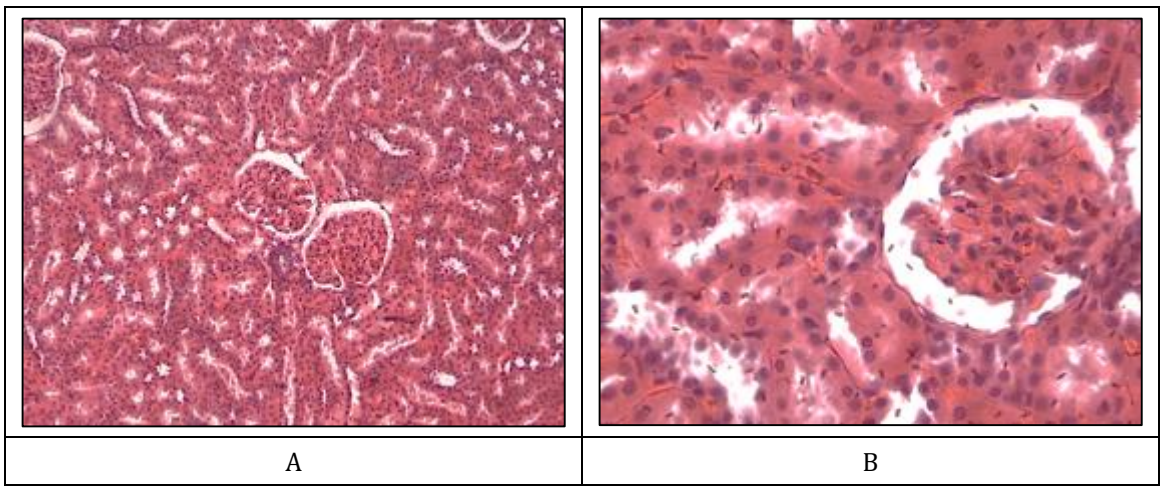
Histological sections from kidneys of the control group showed normal view of tubules and glomeruli of the cortex. Figure 1.A. Shows healthy rounded glomeruli, the proximal convoluted tubules with a narrow lumen and rounded vesicular nuclei. The distal convoluted tubules are with wide lumen and lined by simple cuboidal cells with rounded nuclei. Each glomerulus was surrounded by a narrow capsular space lined by flat squamous cells of the parietal layer of Bowman's capsule shown clearly in figure 1.B. On the other hand, in the toxic group (dosed with 150 mg/kg body weight cyclophosphamide), the changes were more severe compared to the control group, with increase in the tubular necrosis and damage to renal glomeruli, also the tissue was characterized by cellular swelling and vacuolization in the kidney cortical tubules as in figure 2.A. Furthermore, the toxic group treated with 150 mg/kg cyclophosphamide showed a decrease in glomerulus size with increase in Bowman's space with vacuolar changes with casts intraluminally seen to surrounded tubules, Casts are cylindrical structures composed mainly of mucoprotein (the Tamm-Horsfall mucoprotein) which is secreted by epithelial cells lining the loops of Henle, the distal tubules and the collecting ducts. Renal tubular epithelial cell casts reflect damage to tubule cells in the kidney (Figure 2.B.). In figure 2.C. the section shows cellular vacuolization of collecting tubules of the medulla. The epithelial cells lining both the proximal and distal convoluted tubules are swollen and inflamed, indicating cell stress and potential necrosis. The vital brush border, essential for reabsorption, is absent, further confirming impaired tubular function. Numerous pyknotic nuclei within the epithelial cells are evident, a hallmark of cell death. Exfoliation of epithelial cell fragments into the tubular lumen reinforces the extent of cell loss and disruption of the tubular lining (Figure 2.A. and B). The third group was the group from the rats treated with cyclophosphamide and olive oil Group: the group was treated with cyclophosphamide (150 mg/kg body weight) and an oral dose of 200 mg/kg olive oil showed mild congestion in the glomerular capillaries, while

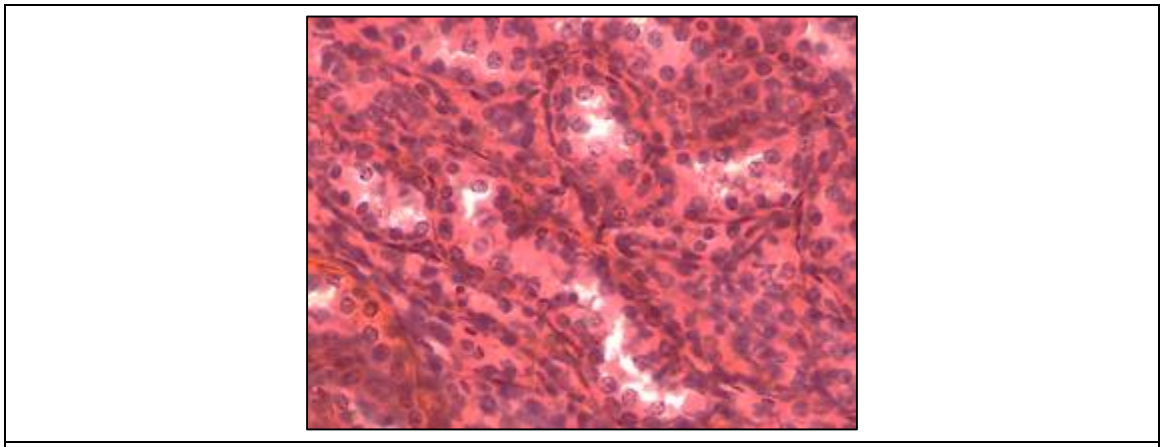
the epithelial cells in both the proximal and distal convoluted tubules were swelled. Furthermore, swollen cells of tubules and some pyknotic nuclei could be seen in some areas. Also, the medulla of the kidney revealed rarefaction of the cytoplasm found in the renal tubules. Furthermore, some pyknotic nucleus were found in some sections of the medulla tissue. Figure 3.A., showed a micrograph section of the cortex of the kidney showing a decrease in the glomerulus size with marked increase space of Bowman, sclerotic changes of glomerulus, scattered pyknotic nuclei. In Figure 3.B., the section illustrations marked intraluminal casts like structures in the lumen of the cortical tubules. While Figure 3.C., displayed a part of glomerulus lower right with normal configuration, whereas surrounded casts seen intraluminal of tubules, gives picture of thyroidization of glomerulonephritis. This is a bit less than in the tissues treated only with cyclophosphamide but with no olive oil. Figure 3.D., demonstrates necrotic changes in the epithelial lining of the collecting tubules of the medulla. The main histopathological features found in all the slides taken from all the groups of the animals were scored and recorded in table 1.



A. Shows healthy rounded glomeruli, the proximal convoluted tubules with a narrow lumen and rounded vesicular nuclei. Magnification 100X. **B.** Shows glomerulus was surrounded by a narrow capsular space lined by flat squamous cells of the parietal layer of Bowman's capsule. Magnification 400X.

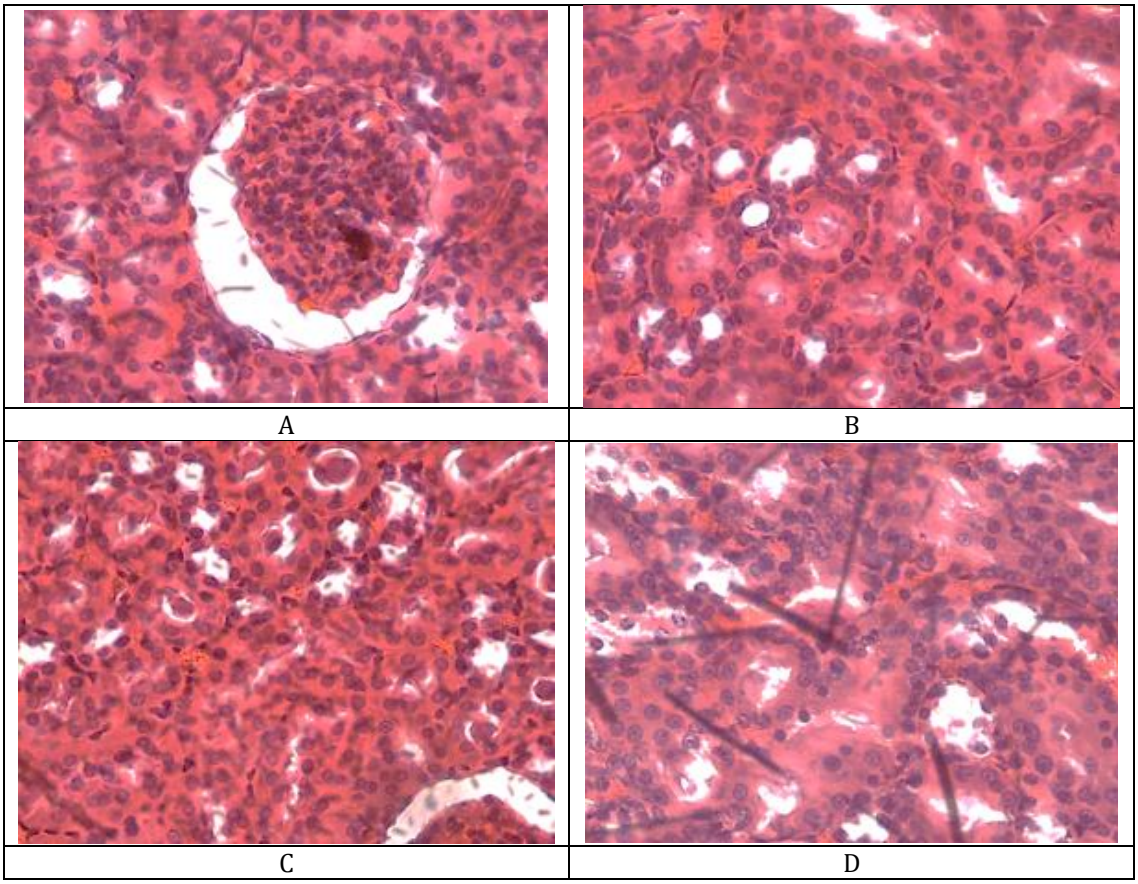
Figure 1 Micrograph of the tissue of the cortex of the rat kidney from the control group treated with normal saline. Stained with Hematoxylin and Eosin





A. The tissue was characterized by cellular swelling and vacuolization in the kidney cortical tubules. Magnification 200X**. B.** Showed a decrease in glomerulus size with increase in Bowman's space with vacuolar changes with casts intraluminally seen to surrounded tubules. 400X. **C.** Shows cellular vacuolization of collecting tubules of the medulla. Magnification 400X.

Figure 2 Micrograph of the tissue of the cortex of the rat kidney from the Treated group treated with 150 mg/kg body weight cyclophosphamide. Stained with Hematoxylin and Eosin



A. Showed a micrograph section of the cortex of the kidney showing a decrease in the glomerulus size with marked increase space of Bowman, sclerotic changes of glomerulus, scattered pyknotic nuclei. **B.** Illustrations marked intraluminal casts like structures in the lumen of the cortical tubules. **C.** displayed a part of glomerulus lower right with normal configuration, whereas surrounded casts seen intraluminal of tubules, gives picture of thyroidization of glomerulonephritis. **D.** Demonstrates necrotic changes in the epithelial lining of the collecting tubules of the medulla.

Figure 3 Micrograph of the tissue of the cortex of the rat kidney from the Cyclophosphamide and Olive Oil Group: Treated with cyclophosphamide (150 mg/kg body weight) and an oral dose of 200 mg/kg olive oil, Stained with Hematoxylin and Eosin. Magnification 400X

Table 1 Comparing the Histopathological features shown in each of the groups

	Cortex						Medulla			
	Glomerulus			Tubules			Tubules			
	Pyknotic nuclei	Swelling Change - Vacuolar	Necrotic Changes	Lymphocytic infiltration	Swelling Cellular Changes - Vacuolar	Necrotic Changes	Swelling Change - Vacuolar	Nuclear Changes Pyknotic	Necrotic Changes	Lymphocytic infiltration
Con.	-	-	-	-	-	-	-	-	-	-
CPA	+	+	-	-	+	+	+	+	-	-
CPA and Olive Oil	+	-	-	-	-	-	+	-	-	-

Con. = Control group; CPA = Cyclophosphamide Group; CPA and Olive Oil = = Cyclophosphamide Group and olive oil

4. Discussion

This study showed a high toxicity of the cyclophosphamide on the kidney tissue of rats treated with a high dose, these results are similar to the results of other studies (10, 13, 14). This study, in contrast to some others, also found that cyclophosphamide caused pathological alterations in the kidneys, such as glomerular shrinkage, tubular cell edema, and inflammation. The research emphasizes the significance of utilizing renoprotective medicines in conjunction with cyclophosphamide to minimize harm to organs and suggests routine evaluation of renal and liver functioning while undergoing chemotherapy (14). In 2022 Elrashdy tested the effects of cyclophosphamide on Golden Hamsters with similar toxic results of the liver tissue. Her results showed considerable hepatotoxicity even one week after starting the treatment. (15,16). In Poland, at a single dosage of 150 mg/kg, cyclophosphamide significantly reduced urine pH, produced substantial proteinuria, and caused polyuria. Rats also showed higher levels of blood urea and BUN, decreased urine uric acid and creatinine, and increased urinary NGAL-1 excretion; nevertheless, their 24-hour excretion was comparable to that of control animals. Histopathology showed that the kidneys were largely normal, with the exception of mild congestion, and that there were inflammatory lesions in the bladders, this is consistent with a study on Golden Hamsters by Benomran (10). Other studies; at the same dosage, ifosfamide (IF) produced diuresis similar to controls, but it also significantly increased proteinuria and lowered the pH of the urine. In addition to increased blood urea and BUN and increased urine NGAL-1 excretion, conventional kidney function tests did not reveal any evidence of substantial renal impairment. Increased urine NGAL-1 excretion following cyclophosphamide and IF treatment suggests a possible renal tubulopathy most likely caused by tubular dysfunction. This is corroborated by the presence of aberrant urine pH, proteinuria, and polyuria, even in the absence of severe histological kidney damage. NGAL-1 may function as a prodromal marker of renal injury, possibly predating observable clinical alterations. (17) It is limited in its capacity to fully clarify whether the increased urine NGAL-1 is caused by generalized inflammation or by localized kidney injury because the study did not measure plasma NGAL-1 levels. (17). Mice administered with cyclophosphamide showed hazy swelling, vacuolar degeneration, renal tubule epithelial necrosis, and glomerular tuft atrophy in their kidneys. In addition, there was extensive bleeding, total renal tissue loss, and perivascular lymphocytic cuffing causing blood vessel obstruction (18). According to biochemical analysis, renal dysfunction—likely caused by damage from oxidative stress—was suggested by the large increases in kidney weight, serum urea, and creatinine levels that followed CPA treatment. Hepatic damage was indicated by decreased albumin and higher levels of AST, ALT, and total bilirubin in liver function tests (18). These results are similar to with other research that suggests the toxic metabolites of cyclophosphamide harm the liver and produce renal failure; these pathways may involve oxidative stress and altered excretory functioning (18). The group of rats treated with olive oil and cyclophosphamide showed lest effect of the nephrotoxic effects on the kidney tissue suggesting the soothing or protective effects of the live oil on the renal tissue. This is similar to researches conducted earlier on female Sprague-Dawley rats supposing that oleuropein which is a major component of the olive oil could reduce the levels of DNA damage and serum pro-inflammatory cytokines. It improved some of the deteriorated hemogram and biochemical parameters, because of antineoplastic drugs. Oleuropein increases the amount of antioxidant parameters in the tissues (19). These results suggested that olive oil might have protective effects against the toxicity induced by the cyclophosphamide.

5. Conclusion

The findings of this study demonstrate that cyclophosphamide induces significant histopathological damage to both the kidneys and liver of albino mice. However, the co-administration of olive oil appears to offer some protection against this damage, likely due to its antioxidant properties. These results suggest that olive oil could be a beneficial adjunct therapy to reduce the toxic side effects of cyclophosphamide. Further research, including molecular studies and clinical trials, is warranted to explore the potential of olive oil as a protective agent in cyclophosphamide chemotherapy.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared no conflict of interest.

Statement of ethical approval

All steps were in compliance with Ethics Committee of Faculty of Biomedical Sciences, University of Benghazi Institutional Review Board.

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