Histological and Biochemical Effects of Lutein on Paraquat-Induced Renal Toxicity in Wistar Rats

ABSTRACT

Introduction: Paraquat (PQ) poisoning in humans predominantly results from the generation of reactive oxygen species (ROS). To date, no proven antidote exists for PQ poisoning, which is characterized by severe kidney injury and high mortality rates globally. However, free radical scavengers and antioxidant agents have shown potential in mitigating PQ toxicity.

Aim: The study is aimed at investigating lutein, an antioxidant, for possible mitigation of paraquat-induced renal toxicity.

Study design: Preclinical experimental study.

Place and Duration of Study: Department of Anatomy, Obafemi Awolowo University (OAU), Nigeria, between 2022 and 2023.

Methods: Fortywistar rats weighing 150-180 grammes were randomly grouped (A to E) for this study. Paraquat (PQ) toxicity was induced in groups B to E. Lutein was administered at graded doses of 50 mg/kg, 100 mg/kg and 150 mg/kg to groups C to E for twenty-one days respectively. Group A (positive control) was given only normal saline, while group B had paraquat only. Twenty-four hours after the last administration, urine and blood samples were collected and the animals were sacrificed before the excision of the kidneys.

Results: Marked histological distortion, which was characterized by compromised Bowman's space and renal tubule dimensions, was observed in Group B. In contrast, the lutein-treated groups exhibited dose-dependent improvements, with outcomes comparable to the control group. Notably, Group B showed significant elevations in plasma creatinine (P = 0.003) and urine protein (P = 0.001), accompanied by reduced plasma protein (P = 0.004), relative to the treated

groups. However, Group E, which received the highest lutein dose, demonstrated substantial improvement and histoarchitectural and biochemical findings similar to the control group."

Conclusion: This study demonstrated significant alterations in the histoarchitecture and biochemical profiles of renal tissue following paraquat exposure. Notably, the lutein-treated groups exhibited substantial improvement, with results comparable to those of the control group. These findings suggest that lutein may possess potential therapeutic properties to mitigate paraquat-induced renal toxicity.

Keywords: Paraquat; lutein; histological; biochemical; kidneys

INTRODUCTION

Paraquat (1, 1′-dimethyl-4,4′-dipyridylium) is one of the most widely used herbicides worldwide [1,2]. The toxic phytochemical was first produced in 1882 as a redox indicator, but its herbicidal property was recognised in the 1950s [3]. Paraquat poisoning in humans has been reported globally. The most common route of human exposure to paraquat poison is oral ingestion, which can occur after intake of contaminated foods or through deliberate self-harm [1,4].The chemical poisoning is also possible after skin exposure, especially in the presence of preexisting skin lesions [5].

Paraquat-induced toxicity results from its ability to generate reactive oxygen species (ROS) in many organs. The lethal dose (LD50) of the poisoningin humans is approximately 35 mg/kg (10-15 mL of a 20% v/v solution) [3]. Ingestion of a little quantity usually leads to toxicity in few target organs, while fulminant multi-organ failure may result from ingestion of large volume [6]. The lungs, kidneys and liver have been found to have the highest concentrations of paraquat following accidental ingestion in humans and animals [7]. The toxic features tend to develop over days to weeks [1,8].

Lutein is an active carotenoid and a natural source of antioxidants. It is widely distributed in carotenoids in fruits and vegetables. The carotenoid has been found to have protective effects against oxidative damage [9]. Lutein has a free radical scavenging ability as a result of its polarity, conjugated double bond, and the two hydroxyl groups on both ends, making it stronger antioxidant as compared to other carotenoids. It promotes significantly the antioxidant enzyme system in blood and liver tissue [10]. The carotenoid has numerous pharmacological and biological benefits, which are not limited to hepatoprotective[11], nephroprotective[12], cardioprotective[13, 14] and anti-neoplastic effects [15].

Even in the best of intensive care units, the probability of death from PQ poisoning exceeds 50% [16]. Till date, there is no proven antidote nor widely accepted guidelines for treatment of affected patients [1,2]; hence, there is a need to investigate a probable treatment using an antioxidant agent, lutein, for possible mitigation of paraquat-induced renal toxicity. This is aimed at providing an effective interventional strategy towards reducing the burden of the poisoning in terms of morbidity and mortality to humans.

MATERIALS AND METHODS

Forty male Wistar rats weighing between 150-180 grams were used for this study after obtainingan ethical approval. They were acclimatized for two weeks and fed with standard laboratory rat pellets with access to clean water ad libitum**.** The rats were randomly assigned into five groups of eight rats per group (Groups A, B, C, D, and E). Group A served as the positive control (which was given normal saline), group B was the negative control (had only paraquat), and groups C, D and E were the treated groups. Administrations of all drugs and other substances were given through the mouthby oral cannula.

Paraquat toxicity was induced in groups B, C, D, and E by administration of 5 mg/kg of paraquat for three days. At the same time, group A was given an equivalent volume of normal saline. Twenty-four hours after the last dose, groups C, D, and E were given lutein at graded dosages of 50, 100, and 150 mg/kg once daily, respectively, for twenty-one days, after the dissolution of the compound in normal saline.

Animal sacrifice, histological Preparation: Twenty-four hour after the last administration, the animals were euthanized. A mid-line incision was made along the anterior abdominal wall and the kidneys were exercised.The excised organ was fixed in 10% formol saline and processed using paraffin wax embedding method. The sectioning was done at 5µm thickness using a rotary microtome and stained with haematoxylin and eosin for general histoarchitecture. The tissue was processed using the recommended procedure by Bancroft and Gamble (2002).

Photomicrography, Processing and Biochemical Assay: The stained section was examined under ‘Motic Scanner’ and photomicrograph was taken at various magnifications. Image analysis

and processing for Java (image J) and public domain software were used for the measurement of the kidney tubules and bowman space.

Blood samples were collected via ocular puncture in heparinized tubes and centrifuged at 2500 revolution per minutes (rpm) for 15minutes after which the plasma was separated and stored at – 20^0C for analysis. Blood protein was assayed as described by Holme and poeck, (1998). Electrolytes were also measured by Henry 1974 method.

RESULT

Heamatoxylin and Eosin staining with Renal Dimensional Evaluation

Plates 1 and 2 show normal histoarchitecture of the kidney tissue in the control group A, characterised by the uniform tubules with viable epithelial cells. However, group B showed increased cytoplasmic eosinophilia, with some non-nucleated epithelial cells,giving a ghost town appearance, which is a characteristic of acute tubular necrosis. There was also evidence of glomerular sclerosis; its glassy nature is a sign of kidney disease. In the groups with high dosages of lutein (D and E), there were well-arranged tubular epithelial cells with near-normal glomeruli. There was also a significant reduction in the Bowman space of the glomerulus (P = 0.000, F = 2.30) and a significant increase in the tubules (P = 0.000, F = 12.05) of the animals in the PQ only (group B) when compared to the control group (Figures 1 and 2). In the treated groups C, D, and E,there was no significant tubular difference whencomparedto the control.

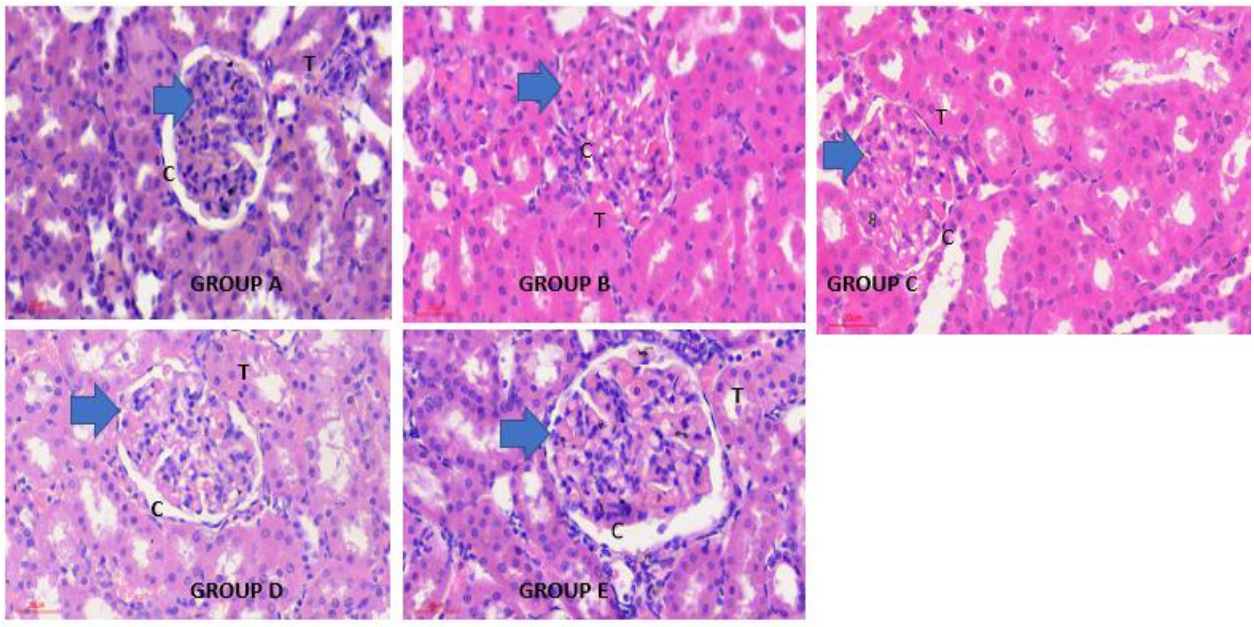


Plate 1: **Photomicrographs of kidney section (glomeruli with few tubules) using Motic scanner:** Group A (Control), B (Paraquat +Normal Saline), C (Paraquat+50mg/kg of Lutein), D (Paraquat+100mg/kg of Lutein) and E (Paraquat +150mg/kg of Lutein). Blue arrow shows the glomerulus,**T** shows tubules and **C** is bowman space H & E× 400.

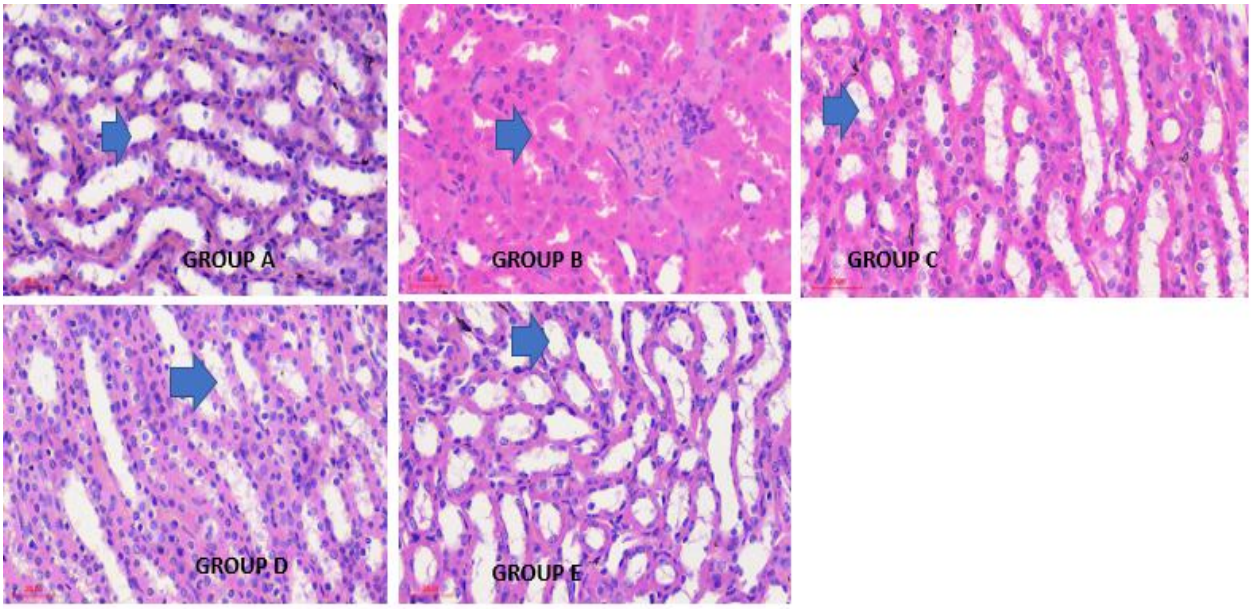


Plate 2: **Photomicrographs of kidney tubules using Motic scanner:** A Control, B (Paraquat+ normal saline), C (Paraquat+ 50mg/kg of Lutein), D (Paraquat+ 100mg/kg), E (Paraquat+ 150mg/kg), Blue arrow shows kidney tubules. H&E (X400)

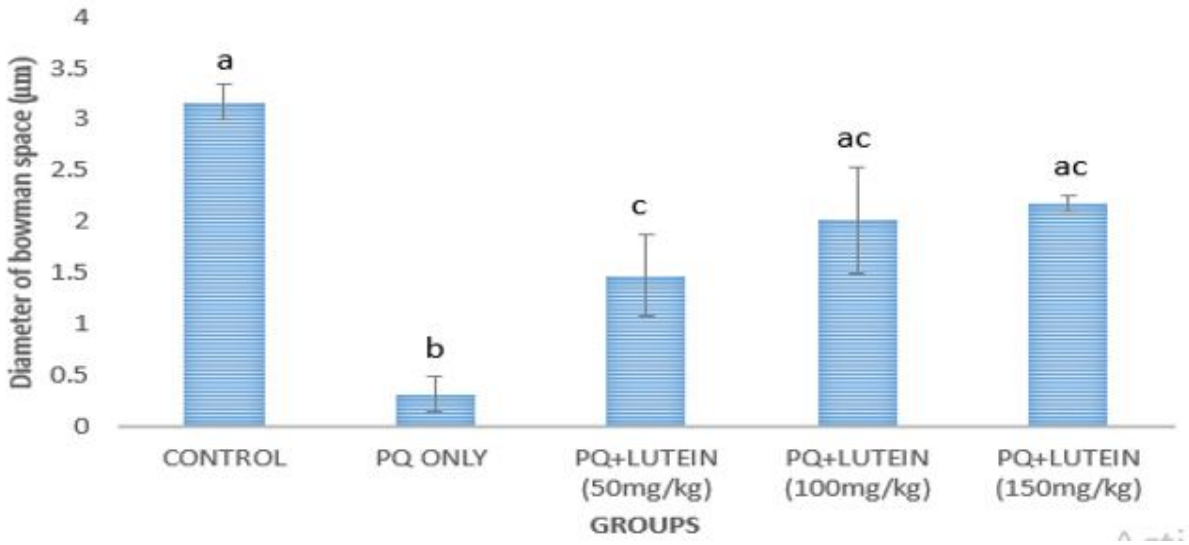


Figure 1: Shows diameter of the bowman space in the kidney using image J: values are given Mean ± SEM in each group. a, b, c within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05 . PQ=Paraquat.

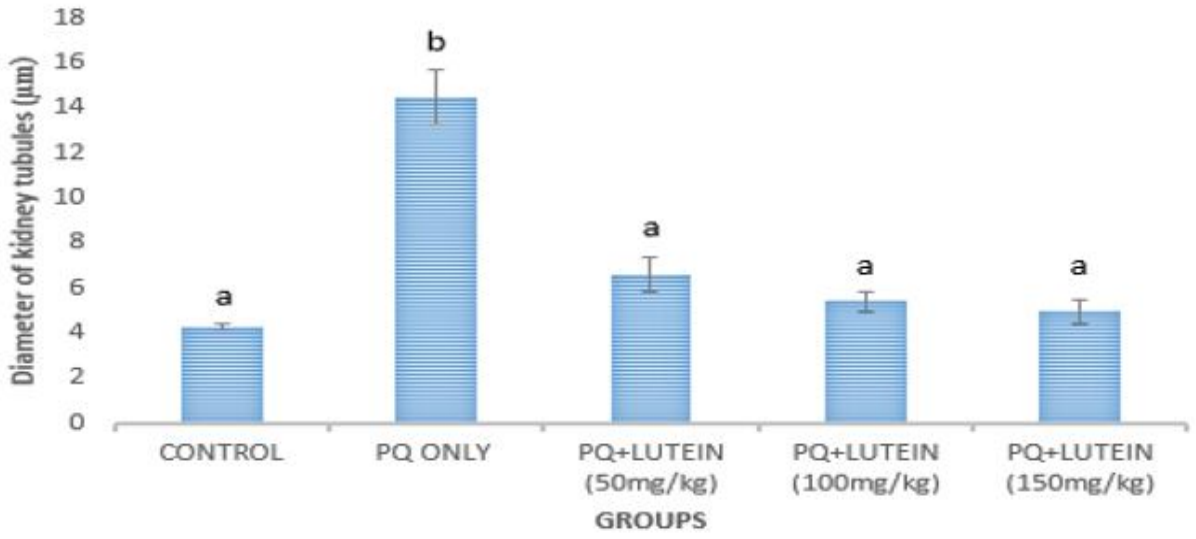


Figure 2: Shows transverse diameter of the kidney tubules using image J: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05 . PQ=Paraquat.

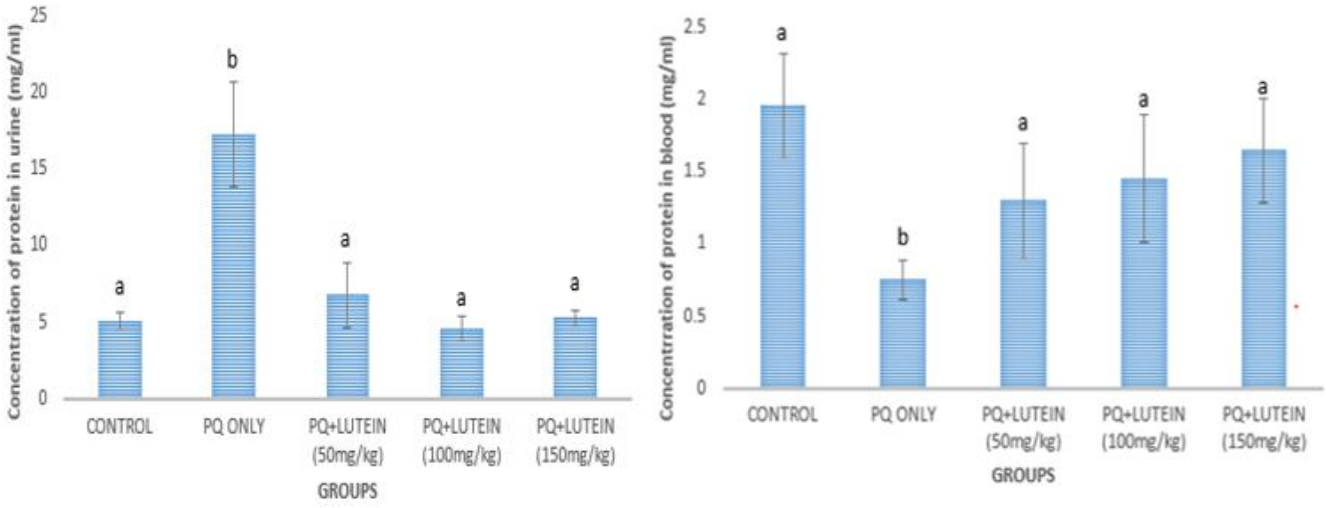
Biochemical Parameters of the Kidneys

The concentration of urine protein in group B was significantly higher (*P* = 0.001, F = 8.23) when compared to the treated groups (C, D, and E) and the control group A. Meanwhile, there was no significant difference between the treated groups and the positive control. Regarding plasma concentration of protein, there was a significant decrease (*P* = 0.004, F = 1.04) in group B when compared to groups C, D, E, and group A. However, there was no significant difference in control group A when compared with the treated groups C, D, and E (Figure 3).

The concentration of creatinine in plasma in group B was significantly elevated (*P* = 0.003, F = 2.52) when compared to the treated groups C, D, E, and the control. However, there was no significant difference noticed between the control groups A and the treated groups, as shown in figure 4a. There was a decrease in the concentration of creatinine in urine in group B but no

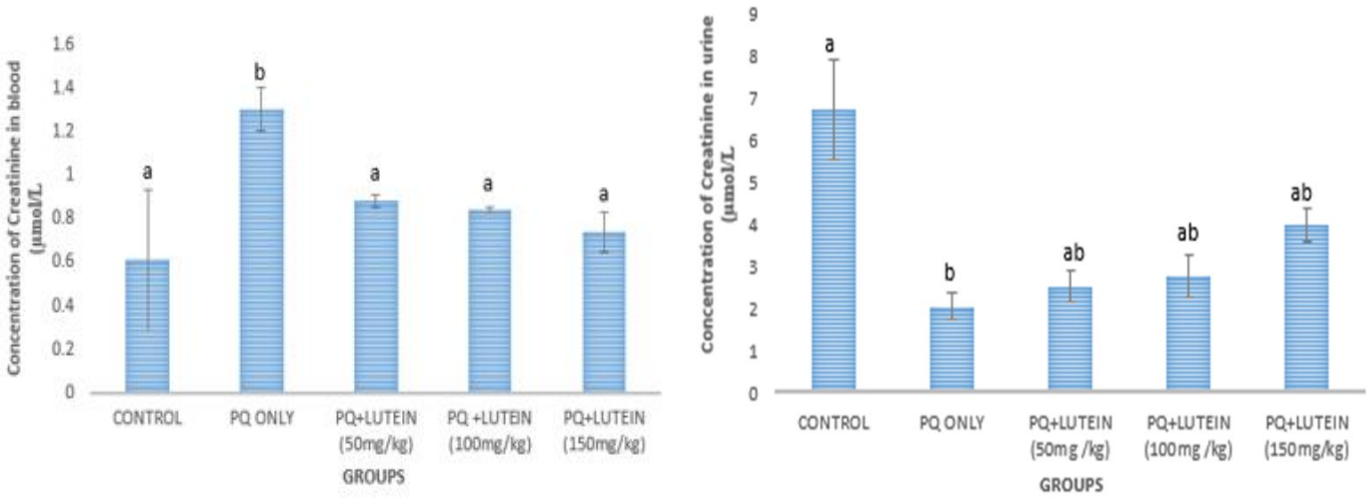
significant difference when compared to the treated groups C, D, and E. Meanwhile, group A showed a significant increase in creatinine concentrationwhen compared to group B (P = 0.03). Comparing the treated groups with the control, there was no significant difference (Figure 4b).

The plasma concentrations of potassium in both groups B (paraquat only) and C (which had the lowest dose of lutein) were significantly lower (P = 0.003, F = 6.01) when compared to the other treated groups (D, E) and the control (Figure 5). Similarly, the concentration of plasma sodium in group B was significantly lower (P = 0.001, F = 7.49) when compared to all the treated groups C, D, E, and the control. However, there was no significant difference between the control (group A) and the treated groups, as shown in figure 6.



a **b**

Figure 3: Shows Concentration of protein inurine (a) and blood (b): values are given Mean ±SEM in each group. a, b, within coloumn signifies that mean with different letters differs significantly at P=0.05. PQ=paraquat.



(a) **(b)**

Figure 4: Shows Concentration of creatinine in (a) blood and (b) urine: values are given Mean ±SEM in each group. a, b, within coloumn signifies that mean with different letters differs significantly at *P*=0.05. PQ=paraquat.

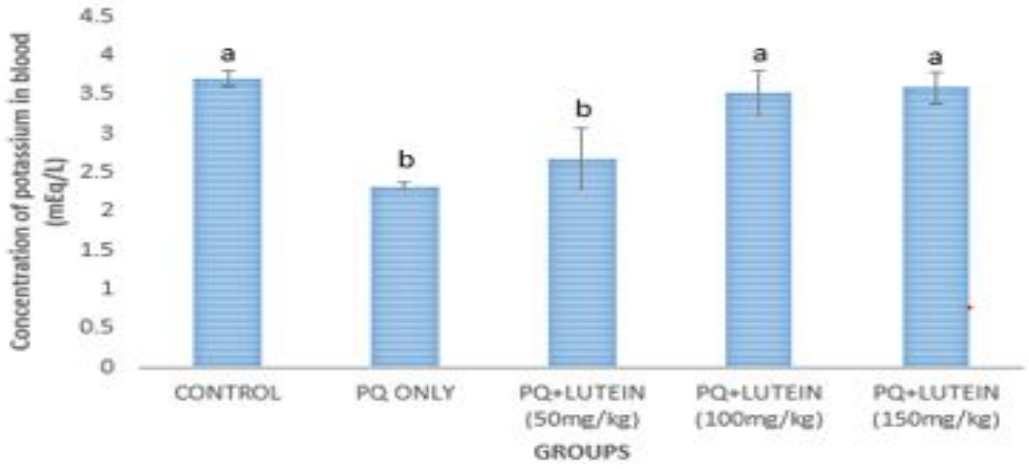


Figure 5: Shows Concentration of Potassium in the blood: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05. PQ=Paraquat.

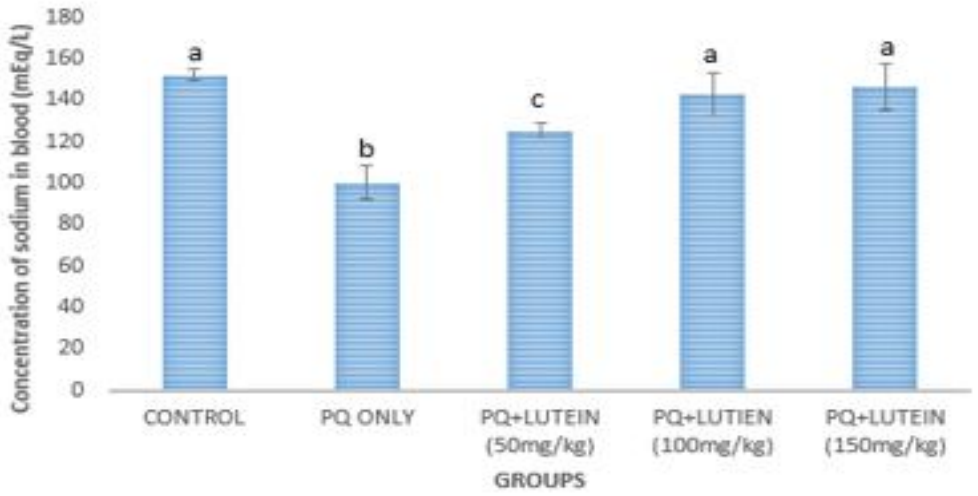


Figure 6:Shows Concentration of Sodium in the blood: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05. PQ=Paraquat.

DISCUSSION

The study showed that paraquat exposure caused severe histopathological changes in the kidneys, especially in the paraquat-only group (Group B). The finding was similar to the report by Jia*et al,*[17] who observed degeneration in the glomeruli and tubules of the kidneys with evidence of necrosis. In the groups treated with lutein, the histomorphology of the glomeruli and tubules showed near-normal histoarchitecture in a dose-dependent manner. This finding may be associated with the antioxidant, anti-inflammatory, and anti-apoptotic properties of lutein, in line with Gundogdu*et al*[18] and Gad El-Karim *et al,*[19] who reported that lutein successfully mitigated renal toxicity.

Singh *et al*[20]explain that the structural base of the antioxidative effect of lutein is believed to contribute to the delocalization of unpaired electrons by its conjugated double-bonded structure. This allows lutein to effectively scavenge free radicals. There was also a reduction in the Bowman space of Group-B relative to the control and lutein-treated groups, which may be linked to severe fibrosis associated with paraquat toxicity. Bowman capsules are lined by podocytes, which play a role in the restriction of plasma protein in the urine [21].

The study shows a significant increase in the serum creatinine level in PQ-only group in line with other findings [7,17,22]. This probably reflects increased generation of creatine and creatinine to meet energy demand following significant oxidative stress [23]. Direct oxidative damage to the renal tubules by PQ can induce elevated blood creatinine [25]. Studies by Roberts *et al*[26]and Mohamed *et al*[23] also found decrease in glomerular filtration capacity with elevated blood creatinine concentration, which is closely related to acute kidney injury and direct reflection of progressive kidney damage. Significant decrease in the serum creatinine of the rats treated with lutein may be due to the reno-protective anti-inflammatory and anti-oxidant

properties of the agent [26,27]. This is due to the free radical scavenging ability of its polarity, conjugated double bond, and the two hydroxyl groups [10, 28].

There was an increase in urine protein (proteinuria) in this study, which may be as a result of the glomerular and tubular injury; the injury is evident from the histological findings and corroborated by other studies [29, 30]. It has been reported that albuminuria can result from paraquat-induced glomerular damage with an associated increase in filtration of albumin or from tubular injury that impairs reabsorption [1].

In this study, there was a significant decrease in the sodium and potassium levels of the rats treated with paraquat only. This finding is supported by other works [31], which observed that increased production of reactive oxygen and lipid peroxidation in paraquat-poisoning tends to provoke inhibition of the medullary ($Na,^{+}$ K+) ATPase. [32] also explained that ROS-mediated alterations in the renal renin angiotensin-aldosterone system (RAAS) expression and active Na+ transport machinery could lead to fluid wasting and electrolyte depletion in herbicide-associated acute kidney injury.

Low serum potassium is common among subjects with paraquat poisoning, as reported by various authors [33, 34,35]. The mechanism of PQ-induced hypokalaemia may also be multifactorial; this includes renal tubular necrosis leading to alteration in potassium re-absorption in the renal tubules [33]. Polyuric renal injury may also cause wastage of sodium and potassium, leading to hyponatraemia and hypokalaemia. Gastrointestinal ulceration with mucosal excoriation tends to occur in PQ-poisoning, and this may cause hypokalaemia and loss of other electrolytes [34,35]

Following PQ-induced oxidative stress, there may be an increase in the secretion of catecholamines and glucocorticoids with enhanced activity of sodium-potassium pump entry;

this tends to promote the transfer and entry of potassium from the extracellular compartment into the cells, ultimately potentiating hypokalaemia. Significant loss of potassium with accompanying hypokalaemia may also result from the use of diuretic agents, which have the capacity to promote PQ excretion after poisoning [35]. Hypokalaemia may be a poor prognostic marker and determinant of mortality following PQ poisoning [35].

Conclusion:This study demonstrated significant alterations in the histoarchitecture and biochemical profiles of renal tissue following paraquat exposure. Notably, the lutein-treated groups exhibited substantial improvement, with results comparable to those of the control group. These findings suggest that lutein may possess potential therapeutic properties to mitigate paraquat-induced renal toxicity.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

Disclaimer (Artificial intelligence):

The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

1. Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. British journal of clinical pharmacology. 2011;72(5):745-57.
2. Adejumo OA, Akinbodewa AA, Olafisoye OJ, Afolabi ON. Acute kidney injury following paraquat poisoning: An uncommon case of acute toxic nephropathy in Nigeria. Journal of Medicine in the Tropics. 2016;18(1):51-3.
3. Shashibhushan J, Venugopal K, Lingaraja M, Patanjali CP, Suresh C, Huggi V. Paraquat: A fatal poison. Medical Journal of Dr. DY Patil University. 2015 May 1;8(3):370-4.
4. Eizadi-Mood N, Sabzghabaee AM, Yaraghi A, Montazeri K, Golabi M, Sharifian A, Badri S. Effect of Antioxidants on the Outcome of Therapy in Paraquat-intoxicated Patients. Tropical Journal of Pharmaceutical Research. 2011;10(1).
5. Zhou Q, Kan B, Jian X, Zhang W, Liu H, Zhang Z. Paraquat poisoning by skin absorption: Two case reports and a literature review. Experimental and therapeutic medicine. 2013 Dec 1;6(6):1504-6.
6. Ito H. Silent acute renal impairment after low-dose paraquat ingestion. Case Reports in Acute Medicine. 2019;2(2):31-4.
7. Ujowundu CO, Nwaogu LA, Ujowundu FN, Oparaeche NN, Oyarebu AO. Hepatotoxicity of paraquat dichloride and ameliorative effect of nutritional supplements. Biochem. Mol. Biol. J. 2018;4:21.
8. Wunnapuk K, Mohammed F, Gawarammana I, Liu X, Verbeeck RK, Buckley NA, Roberts MS, Musuamba FT. Prediction of paraquat exposure and toxicity in clinically ill poisoned patients: a model based approach. British Journal of Clinical Pharmacology. 2014 Oct;78(4):855-66.

9. Severins N, Mensink RP, Plat J. Effects of lutein-enriched egg yolk in buttermilk or skimmed milk on serum lipids & lipoproteins of mildly hypercholesterolemic subjects. Nutrition, Metabolism and Cardiovascular Diseases. 2015;25(2):210-7.
10. Hirdyani H, Sheth M. Lutein—The less explored carotenoid. World Journal of Pharmaceutical Research. 2017; 6(6):528-53
11. 11. Li S, Ding Y, Niu Q, Xu S, Pang L, Ma R, Jing M, Feng G, Tang JX, Zhang Q, Ma X. Lutein has a protective effect on hepatotoxicity induced by arsenic via Nrf2 signaling. BioMed Research International. 2015;2015(1):315205..
12. Bilgiç S, Gür FM, Aktaş İ. Biochemical and Histopathological Investigation of the Protective Effect of Lutein in Rat Kidney Exposed to Cisplatin. Medical Records. 2022;4(3):433-8.
13. Liu XH, Yu RB, Liu R, Hao ZX, Han CC, Zhu ZH, Ma L. Association between lutein and zeaxanthin status and the risk of cataract: a meta-analysis. Nutrients. 2014; 6(1):452-65.
14. Ouyang B, Li Z, Ji X, Huang J, Zhang H, Jiang C. The protective role of lutein on isoproterenol-induced cardiac failure rat model through improving cardiac morphology, antioxidant status via positively regulating Nrf2/HO-1 signalling pathway. Pharmaceutical Biology. 2019;57(1):529-35.
15. Zhang WL, Zhao YN, Shi ZZ, Cong D, Bai YS. Lutein inhibits cell growth and activates apoptosis via the PI3K/AKT/mTORsignaling pathway in A549 human non-small-cell lung cancer cells. Journal of Environmental Pathology, Toxicology and Oncology. 2018;37(4).
16. Goudarzi F, Armandeh J, Jamali K, Rahmati H, Meisami A, Abbasi H. Mortality analysis of patients with paraquat poisoning treated at two university hospitals in Shiraz, Iran. 2014; 141-145
17. Jia C, Zhang Z, Wang J, Nie Z. Silymarin protects the rats against paraquat-induced acute kidney injury via Nrf2. Human & Experimental Toxicology. 2022; 41:09603271221074334.
18. Gündoğdu B, Taş HA, Süleyman B, Mamedov R, Yüce N, Kuyrukluyildiz U, Süleyman H. Effect of lutein on oxidants and proinflammatory cytokine-related liver ischemia-reperfusion injury. ActaPoloniaePharmaceutica-Drug Research. 2022;79(1).
19. Gad El-Karim DR, Lebda MA, Alotaibi BS, El-Kott AF, Ghamry HI, Shukry M. Lutein modulates oxidative stress, inflammatory and apoptotic biomarkers related to di-(2-ethylhexyl) phthalate (dehp) hepato-nephrotoxicity in male rats: role of nuclear factor kappa b. Toxics. 2023;11(9):742.

20. Singh J, Upadhyay AK, Bahadur A, Singh B, Singh KP, Rai M. Antioxidant phytochemicals in cabbage (Brassica oleracea L. var. capitata). ScientiaHorticulturae. 2006 May 8;108(3):233-7.
21. Bronstein R, Pace J, Gowthaman Y, Salant DJ, Mallipattu SK. Podocyte-parietal epithelial cell interdependence in glomerular development and disease. Journal of the American Society of Nephrology. 2023;34(5):737-50.
22. Huang W, Zhang Z, Lu YQ. Serum creatinine in predicting mortality after paraquat poisoning: A systematic review and meta-analysis. Plos one. 2023 Feb 22;18(2):e0281897.
23. .Mohamed, F., Endre, Z., Jayamanne, S., Pianta, T., Peake, P., Palangasinghe, C., ...& Buckley, N. (2015). Mechanisms underlying early rapid increases in creatinine in paraquat poisoning. *PLoS One*, *10*(3), e0122357.
24. Kim SJ, Gil HW, Yang JO, Lee EY, Hong SY. The clinical features of acute kidney injury in patients with acute paraquat intoxication. Nephrology Dialysis Transplantation. 2009;24(4):1226-32.
25. Roberts DM, Wilks MF, Roberts MS, Swaminathan R, Mohamed F, Dawson AH, Buckley NA. Changes in the concentrations of creatinine, cystatin C and NGAL in patients with acute paraquat self-poisoning. Toxicology letters. 2011 Apr 10;202(1):69-74.
26. VijayaPadma V, Ramyaa P, Pavithra D, Krishnasamy R. Protective effect of lutein against benzo (a) pyrene-induced oxidative stress in human erythrocytes. Toxicology and industrial health. 2014;30(3):284-93.
27. Mammadov R, Suleyman B, Akturan S, Cimen FK, Kurt N, Suleyman Z, Malkoc İ. Effect of lutein on methotrexate-induced oxidative lung damage in rats: a biochemical and histopathological assessment. The Korean journal of internal medicine. 2019; 34(6):1279-1286
28. Fuad NI, Sekar M, Gan SH, Lum PT, Vaijanathappa J, Ravi S. Lutein: A comprehensive review on its chemical, biological activities and therapeutic potentials.Pharmacognosy Journal. 2020; 12. 1769-1778.
29. Williams JH, Whitehead Z, Van Wilpe E. Paraquat intoxication and associated pathological findings in three dogs in South Africa. Journal of the South African Veterinary Association. 2016;87(1):1-9.

30. Asaduzzaman M, Roy S, Pew ND, Roy AD, Kibria S, Roy RK, Alam MJ, Chakraborty SR. Paraquat induced acute kidney and lung injury with a dramatic response to methylprednisolone: A case report. Toxicology Reports. 2023; 11:350-4.
31. Cirilo MA, Santos VB, Lima NK, Muzi-Filho H, Paixão AD, Vieyra A, Vieira LD. Reactive oxygen species impair Na+ transport and renal components of the renin-angiotensin-aldosterone system after paraquat poisoning. Anais da Academia Brasileira de Ciências. 2024 Apr 5;96(1):e20230971.
32. Dedeke GA, Owagboriaye FO, Ademolu KO, Olujimi OO, Aladesida AA. Comparative assessment on mechanism underlying renal toxicity of commercial formulation of roundup herbicide and glyphosate alone in male albino rat. International journal of toxicology. 2018 Jul;37(4):285-95.
33. Wunnapuk K, Liu X, Peake P, Gobe G, Endre Z, Grice JE, Roberts MS, Buckley NA. Renal biomarkers predict nephrotoxicity after paraquat. Toxicology letters. 2013 Oct 9;222(3):280-8.
34. Biswas S, Barua P, Maruf-ul-Quader M, Chowdhury D, Biswas S, Das A. Paraquat Poisoning and AKI–A Rare Pediatric Case Report in a Tertiary Care Hospital. Journal of Pediatric Nephrology. 2021;9(2).
35. Yu J, Zhang L, Li X, Lv K, Sun S, Wu W, Ping L, Guo G, Tan W, Guo S, Wang K. Comparison of biochemical parameters between mouse model and human after Paraquat Poisoning. BioMed Research International. 2022;2022(1):1254824.