Original Research Article

Histological and Biochemical Effects of Lutein on the Kidneys of Adult Wistar Rats Following Paraquat-induced Toxicity.

ABSTRACT

Introduction: Paraquat (PQ) poisoning in human tends to result from the formation of reactive oxygen species. Till date, there is no proven antidotefollowing the poisoning, which is associated with significant kidney injury and high mortality globally.Free radical scavenger and antioxidant agent may attenuate 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: There wasmarked histological distortion with compromised dimensions of the bowman space and the kidney tubules in group B. However, the lutein-treated groups had dose-dependent similar features with the control. There was significant increase in the plasma creatinine (*P*=0.003) and urine protein (*P*=0.001)with reduced plasma protein (*P*=0.004) in group B when compared WITH the treated groups. Group Ehad significant improvement in histo-architectural and biochemical findings compared to control.

Conclusion: This study showed significant alteration in the histo-architecture and biochemical analyses of the renal profile following paraquat toxicity. Meanwhile, the lutein-treated groups showed some significant similar correlation with the control group.

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]. Its 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) in 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 an ethical approval. They were acclimatised 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 oral route by 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 histo-architecture 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 anucleated epithelial cells given 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 high-lutein-dosage groups 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 with the control group (figures 1 and 2). In the treated groups C, D, and E,there was no significant tubular difference when compared to the control.

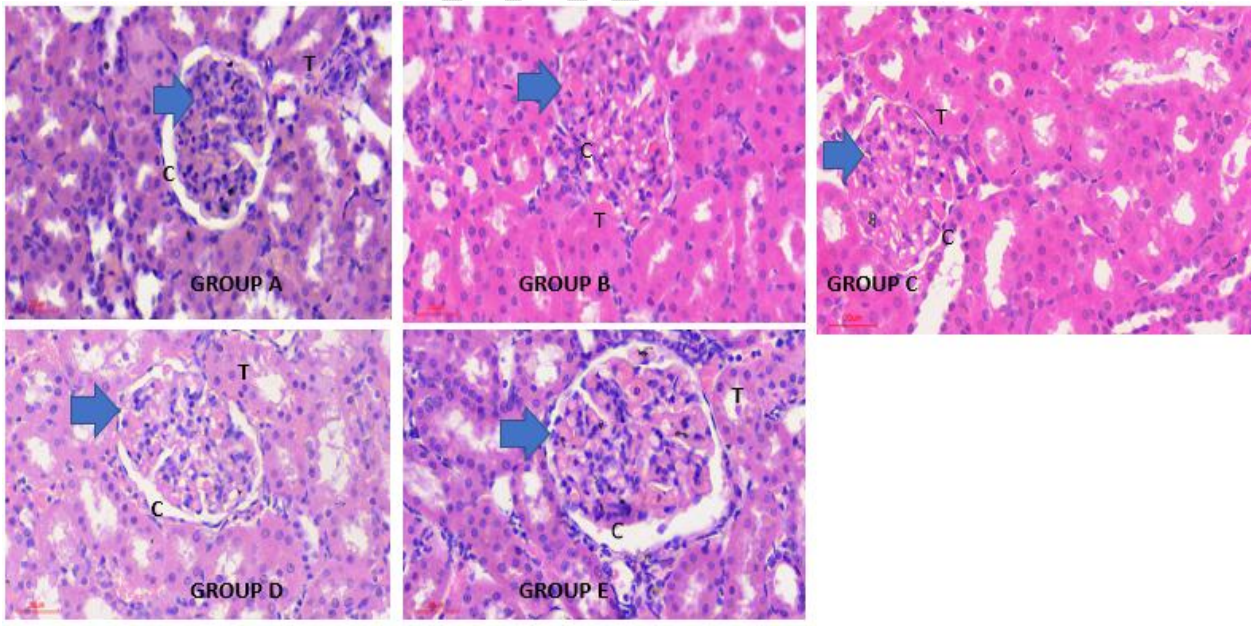


Plate 1: **Photomicrographs of kidney section (glomeruli with few tubules) using Motic scanner:** Group A (Control), B (Paraquat +Normal Saline), C (Paraquat+50mg/kg of Lutein),

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.

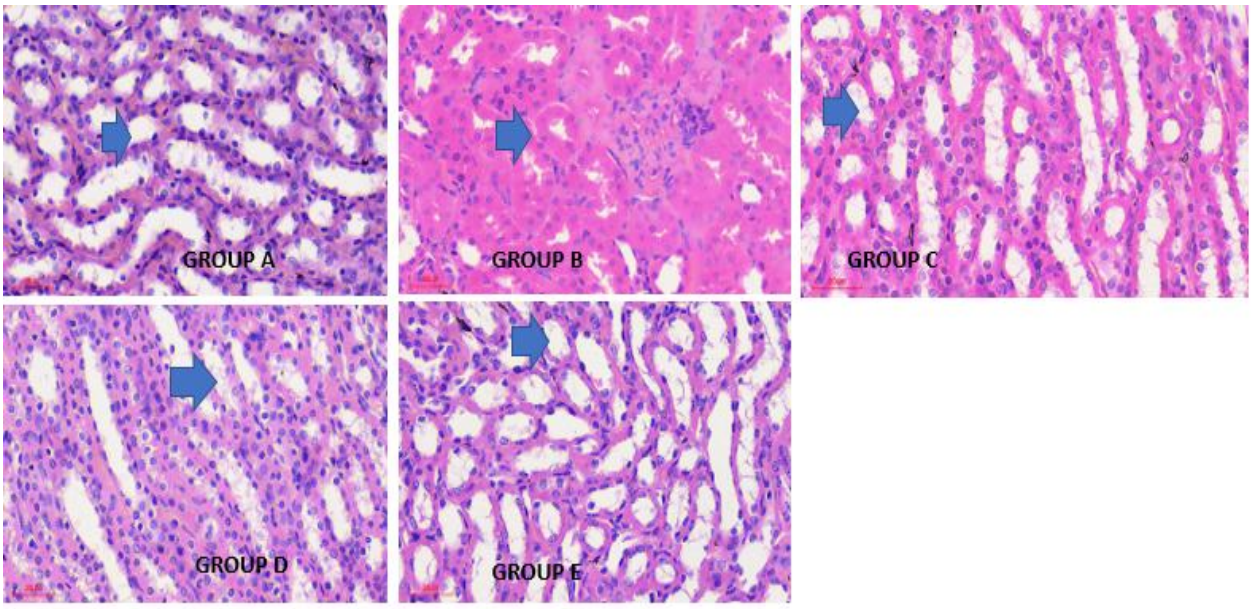


Plate 2: **Photomicrographs of kidney tubules using Motic scanner:** A Control, B (Paraquat+ normal saline), C (Paraquat+ 50mg/kg of Lutein), D (Paraquat+ 100mg/kg), E (Paraquat+ 150mg/kg), Blue arrow shows kidney tubules. H&E (X400)

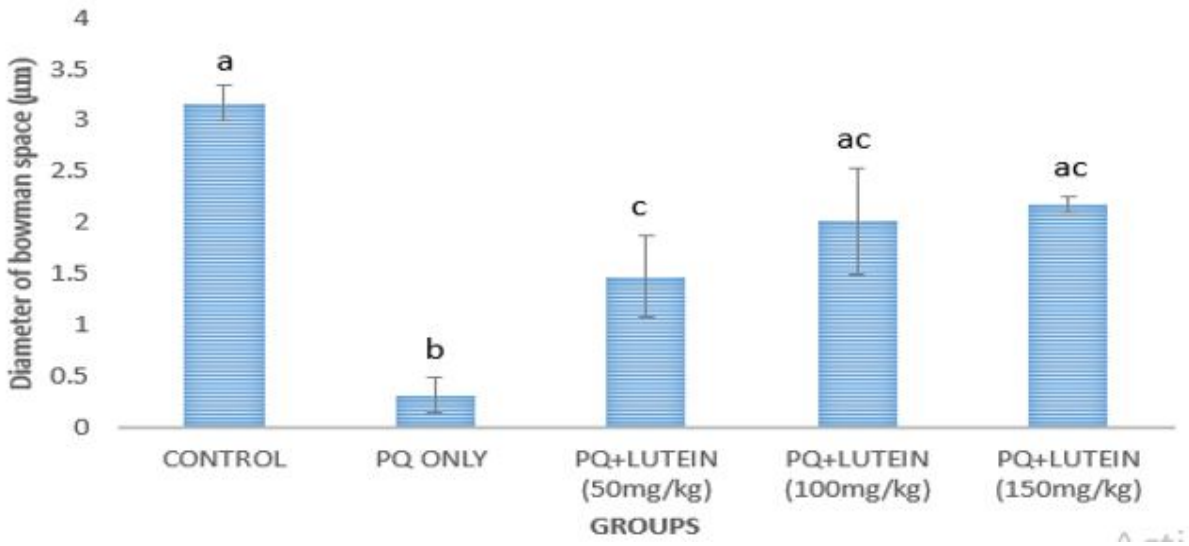


Figure 1: Shows diameter of the bowman space in the kidney using image J: values are given Mean ± SEM in each group. a, b, c within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05 . PQ=Paraquat.

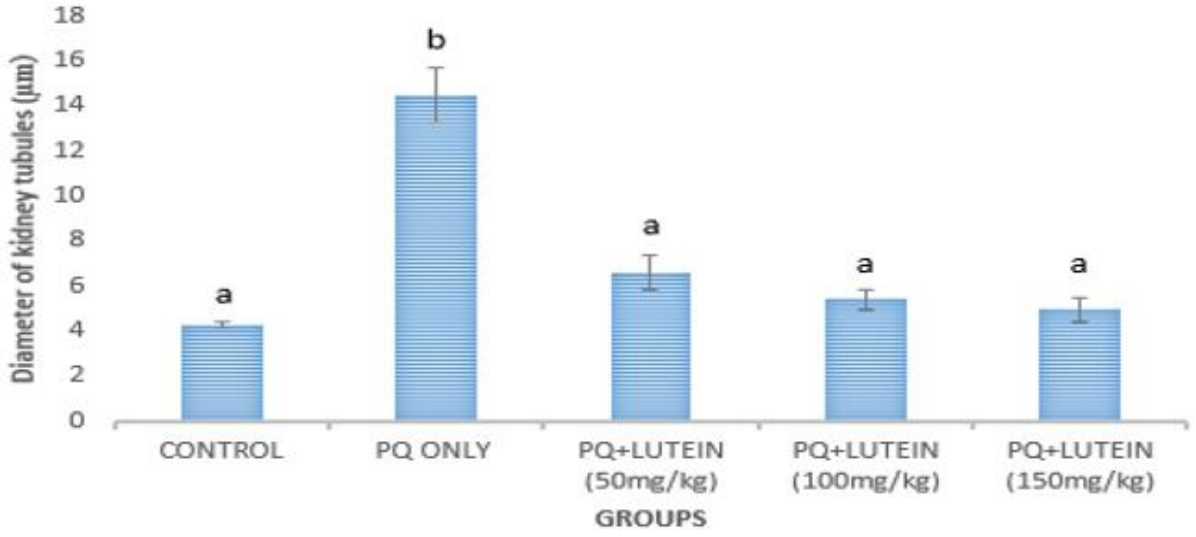


Figure 2: Shows transverse diameter of the kidney tubules using image J: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05 . PQ=Paraquat.

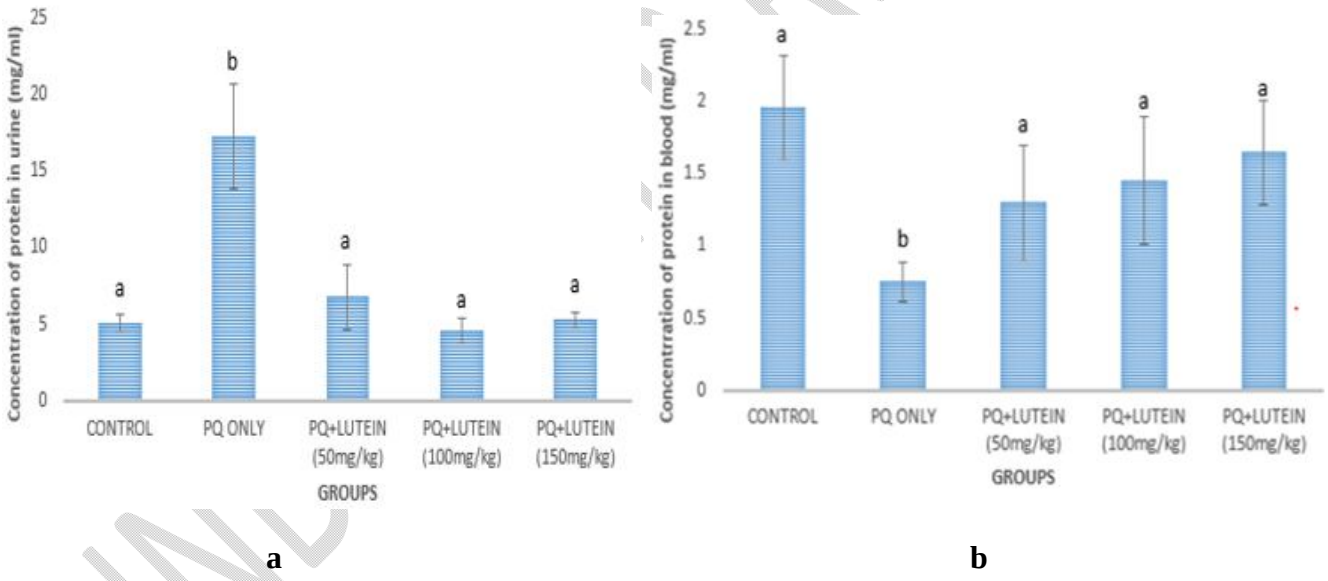
Biochemical Parameters of the Kidneys

The concentration of urine protein in group B was significantly increased (*P* = 0.001, F = 8.23) when compared with 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 with 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 increased (*P* = 0.003, F = 2.52) when compared with 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

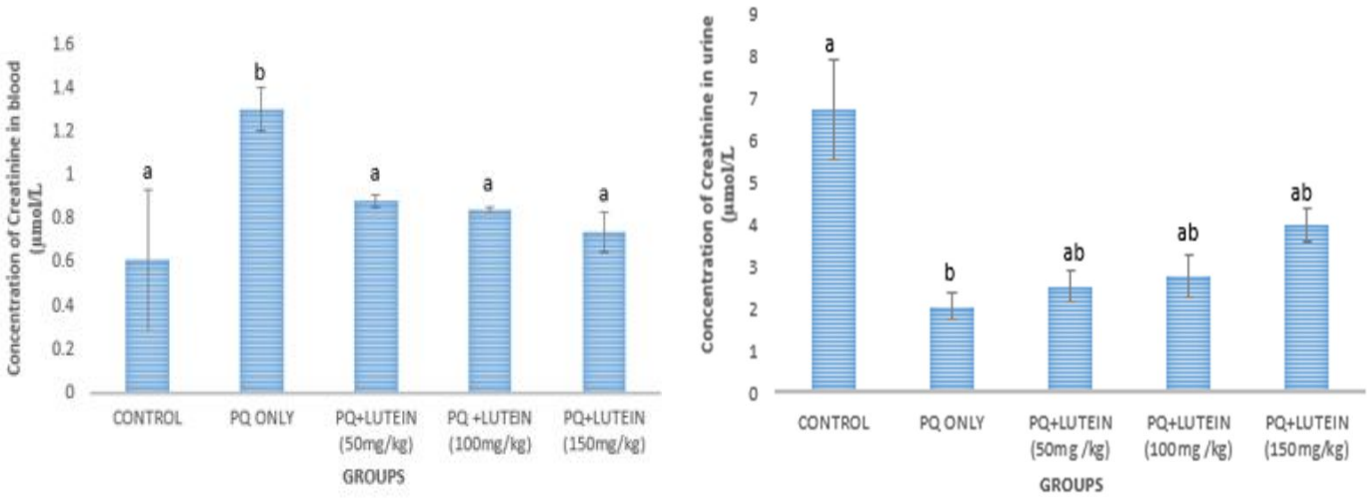
significant difference when compared with the treated groups C, D, and E. Meanwhile, group A showed a significant increase when compared with group B (P = 0.03). Comparing the treated groups with the control, there was no significant difference (Figure 4b).

The plasma concentration of potassium in both groups B (paraquat only) and C (which had the lowest dose of lutein) was significantly lower (P = 0.003, F = 6.01) when compared with 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 with 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.

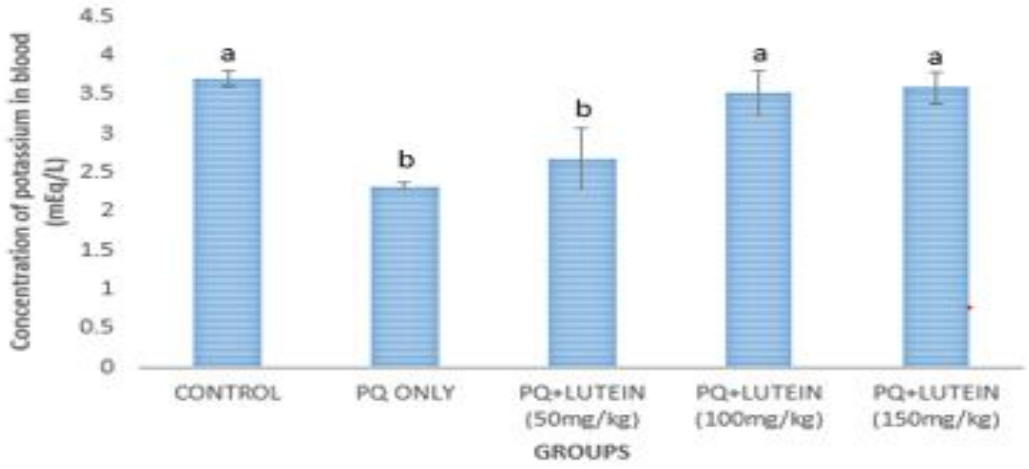


Figure 5: Shows Concentration of Potassium in the blood: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05. PQ=Paraquat.

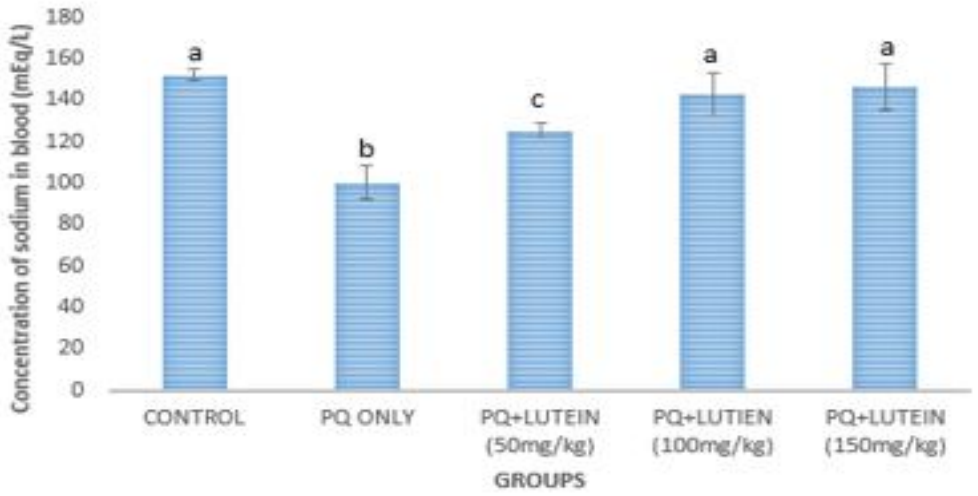


Figure 6:Shows Concentration of Sodium in the blood: values are given Mean ± SEM in each group. a, b, within column signifies that mean with different letters differs significantly at *P*= 0.05 while mean with the same letter does not differ significantly at *P*= 0.05. PQ=Paraquat.

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 architecture 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 delocalisation 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 hyponatreamia 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: our study found significant changes in the histo-architecture and biochemical assessments of the renal profile following paraquat exposure. Meanwhile, the lutein-treated groups shared some notable similarities with the control group. Many studies have shown that lutein is a more powerful antioxidant molecule than lycopene and other carotenoids.

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