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Original Article

Histological and biochemical effects of Lutein on the liver of adult Wistar rats following Paraquat-induced toxicity

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Abstract

BACKGROUND AND AIM: Paraquat is a common herbicide worldwide with potentials for human poisoning through the generation of reactive oxygen species. There is presently dearth of evidence-based cure for paraquat (PQ) poisoning, which is associated with significant hepatic injury and a high mortality globally. Lutein is a carotenoid with free radical-scavenging and antioxidant effects. The study is aimed at investigating the mitigating effect of lutein on paraquat-induced hepatic toxicity in Wistar rats.
MATERIALS AND METHODS: Forty male Wistar rats weighing 150-180 g were randomly grouped (A to E) for this study. Paraquat (PQ) toxicity was induced in groups B to E, at a dose of 5mg/kg through oral route. Lutein was administered at graded doses of 50 mg/kg, 100 mg/kg and 150 mg/kg to groups C to E through oral route for twenty-one days respectively. Group A (negative control) was given only normal saline, while group B (positive control) had only paraquat. Twenty-four hours after the last administration, blood samples were collected for the biochemical analyses of plasma aspartate and alanine transaminases; thereafter, the animals were sacrificed before the excision of the liver for histological examination.
RESULTS: There was significant increase in the plasma alanine transaminase (ALT) and aspartate transaminase (AST) (p=0.01) in group B when compared with the treated groups. The concentrations of catalase and glutathione in group B were significantly lower (p=0.01 and 0.009 respectively) relative to the negative control and lutein-treated groups, especially at higher doses. Malondialdehyde concentration was significantly higher in group B than others (p=0.043). There was marked histological distortion with a reduction in hepatocyte count through the use of image J software in group B, which was given PQ only. However, the lutein-treated groups had dose-dependent improvement in hepatocyte count similar to the control. Group E, which had the highest dose of lutein, had remarkable similarity in histo-architectural and biochemical findings when compared to the negative control.
CONCLUSION: This study showed significant alteration in the hepatic biochemical analyses and histo-architecture following paraquat toxicity. However, the groups treated with high doses of lutein showed remarkable similarity with the control group. Hence, study underscores the potentials of lutein to mitigate paraquat-induced toxicity in Wistar rats.

Keywords:
Paraquat; lutein; histological; liver

INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-dipyridylium) is one of the most commonly used herbicides worldwide (Gawarammana *et al*., 2011; Adejumo *et al*., 2016). The toxic phytochemical was first synthesized in 1882 as a redox indicator but its herbicidal property was recognized in the 1950s (Shashibhushan *et al*., 2015). Paraquat poisoning in humans has been widely reported globally. The most common route of of human exposure to paraquat poison is oral ingestion, which can occur after intake of contaminated foods or through deliberate self-harm (Eizadi-Mood *et al*., 2011; Adejumo *et al*., 2016). Its chemical poisoning is also possible after skin exposure especially in the presence of preexisting skin lesions (Zhou *et al*., 2013).

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) (Shashibhushan *et al*., 2015). Ingestion of a little quantity usually leads to toxicity in few target organs, while fulminant multi-organs failure may result from ingestion of large volume (Ito *et al*.,

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2019). The lungs, kidney and liver have been found to have the highest concentrations of paraquat following accidental ingestion in human and animals (Ujowundu *et al*., 2018). The toxic features tend to develop over days to weeks (Gawarammana and Buckley 2011, Wunnapuk *et al*., 2014).

Lutein is an active carotenoid and a natural source of antioxidant. It is widely distributed carotenoids in fruits and vegetables. The carotenoid has been found to have protective effects against oxidative damage (Severins and Mensink 2014). 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 compared to other carotenoids. It promotes the antioxidant enzyme system in blood and liver tissue. (Hirdyani *et al*., 2017). The carotenoid has numerous pharmacological and biological benefits which is not limited to hepato-protective (Li *et al*., 2015), nephron-protective (Bilgic *et al*., 2022), cardio-protective (Liu 2014; Ouyang *et al*., 2019), and anti-neoplastic effects (Zhang *et al*., 2018).

Following PQ poisoning, the risk of death is over 50% even in the best of intensive care facilities (Goudarzi *et al*., 2014). Till date, there is no proven antidote nor widely accepted guidelines for treatment of affected patients (Gawarammana *et al*., 2011, Adejumo *et al*., 2016).

The study was aimed at investigating an antioxidant agent, lutein, for possible mitigation of paraquat-induced hepatic toxicity. This is geared towards providing effective interventional strategy to reduce the burden of the poisoning in term of morbidity and mortality to humans.

MATERIALS AND METHODS

Forty male Wistar rats weighing between 150-180 grams were used for this study after obtaining an ethical approval (Number: 071/10/2024) from the Ethical Research Committee of Ladoke Akintola University Technology, Ogbomosho. They were acclimatized for two weeks and fed with standard rat pellets with free access to clean water. 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 negative control (which was given only normal saline), group B was the positive 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 body weight (Shalaby *et al*., 2020) of PQ for three days. At the same time, group A was given equivalent volume of normal saline. Twenty-four hours after the last dose, groups C, D, E were given lutein at graded dosages of 50, 100, and 150 mg/kg body weight once daily respectively for twenty-one days after the dissolving the compound in normal saline. Lutein dosage was determined after the determination of the LD_{50}.

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. The quantitative concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using Randox kit. Malondialdehyde (MDA) product was estimated by the thiobarbituric acid (TBA) method. Catalase activity was determined in erythrocyte lysate using method. Glutathione (GSH) was measured by Beutler and Kelly method.

Animal sacrifice, histological Preparation: Twenty-four hour after the last administration, the animals were euthanized through cervical dislocation. A mid-line incision was made along the anterior abdominal wall and the liver was exercised. The excised liver 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 X-100 and X-400 magnifications. Image analysis with processing for Java (image J) and public domain software were used for assessment of the hepatocytes count in line with standard protocol.

RESULTS

Hepatic Alanine Transaminase and Aspartate Transaminase

The ALT and AST results showed significant increase (P=0.01) in group B as shown in figure 2 and 3 when compared with group A (Figures 1 and 2). There was a significant increase (p= 0.01, F= 4.18) in the activity of alanine transaminase in group B when compared to the treated groups D and E except group C. There was no significant difference when groups C, D, and E were compared with one another.

Haematoxylin and Eosin (H &E) Staining and Image J section of the liver

The histological photomicrograph of the liver is demonstrated in plates 1 (X-100) and plate 2 (X-400). The H and E staining result showed a normal histo-architecture characterized by the central vein and the portal tract in the control group (group A). In plate 2, the paraqaut-treated group demonstrated partial occlusion of the central vein with necrosis of the surrounding hepatocyte; numerous fatty changes with infiltration of inflammatory cells were also observed in group B. Group C (which paraquat with 50mg/kg of lutein) was characterized by some fatty changes with necrosis, but group D (paraquat with 100mg/kg of lutein) and E (paraquat with 150mg/kg of lutein) showed unremarkable liver tissue with normal portal tract and hepatocytes.

The hepatocytes count in group B (paraquat-only group) was significantly lower relative to group **A** at X-400 magnification using Motic software. Comparing group B with the treated groups C, D and E, there was significant reduction in the hepatocyte count except in group C (which had the lowest dose of lutein) using image J as shown in figure 3.

Antioxidants (Catalase and Glutathione) and lipid peroxidation product (Malondialdehyde)

The concentration of catalase in group B was significantly lower (p= 0.01) relative to group A. With the exception of group C, the other treated groups (D and E) showed a significant increase in catalase in a dose dependent manner relative to group B. Similarly, the level of GSH in group B was significantly lower (p= 0.009) compared to groups A and group E, but there was no significant difference when compared to groups C and D. Malondialdehyde concentration was significantly higher (p= 0.043) in group B relative to group A and the treated groups (C, D and E) in a dose dependent fashion as shown in table 1

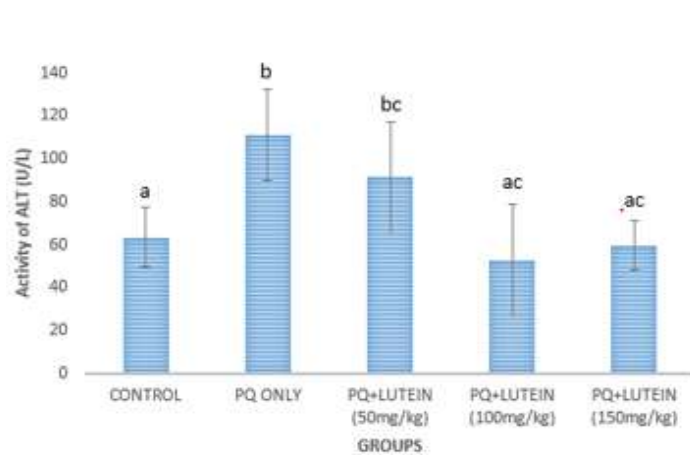

Figure 1: Shows activity of blood alanine transaminase: values are given Mean ±SEM in each group. a, b, c, within column signifies that mean with different letters differs significantly at p=0.01. PQ=paraquat

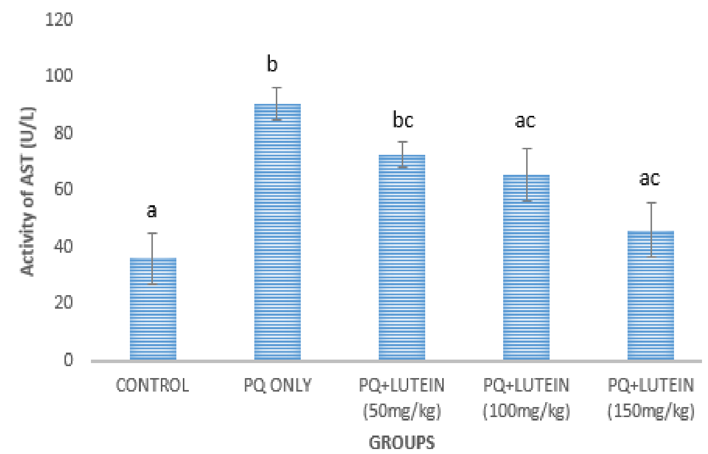

Figure 2: Shows activity of blood aspartate transaminase: values are given Mean ±SEM in each group. a, b, c, within coloumn signifies that mean with different letters differs significantly at p=0.01. PQ=paraquat.

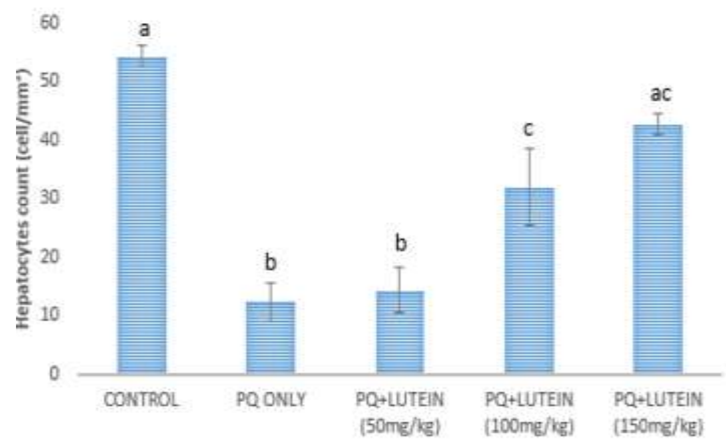

Figure 3: Shows hepatocytes count in liver using image J: values are given Mean ± SEM in each group. a, b, c, within column signifies that mean with different letters differs significantly at p=0.00 while mean with the same letter does not differ significantly at p= 0.00. PQ=Paraquat.

Table 1: shows the liver concentrations of catalase, glutathione and Malondialdehyde (Mean ± SE)

Group	Catalase (µmol/mL)	GSH (µmol/mL)	MDA (µmol/mL)
A	1.00 ± 0.001[a]	2.23 ± 0.400[a]	1.20 ± 0.040[a]
B	0.20 ± 0.060[b]	0.83 ± 0.100[b]	5.50 ± 2.200[b]
C	0.43 ± 0.030[b]	1.23 ± 0.200[ab]	2.60 ± 0.970[a]
D	0.83 ± 0.090[c]	1.33 ± 0.100[ab]	1.40 ± 0.100[a]
E	0.97 ± 0.030[ac]	2.20 ± 0.600[a]	1.20 ± 0.100[a]
	p- value= 0.01	*p-value = 0.009*	*p-value= 0.043*

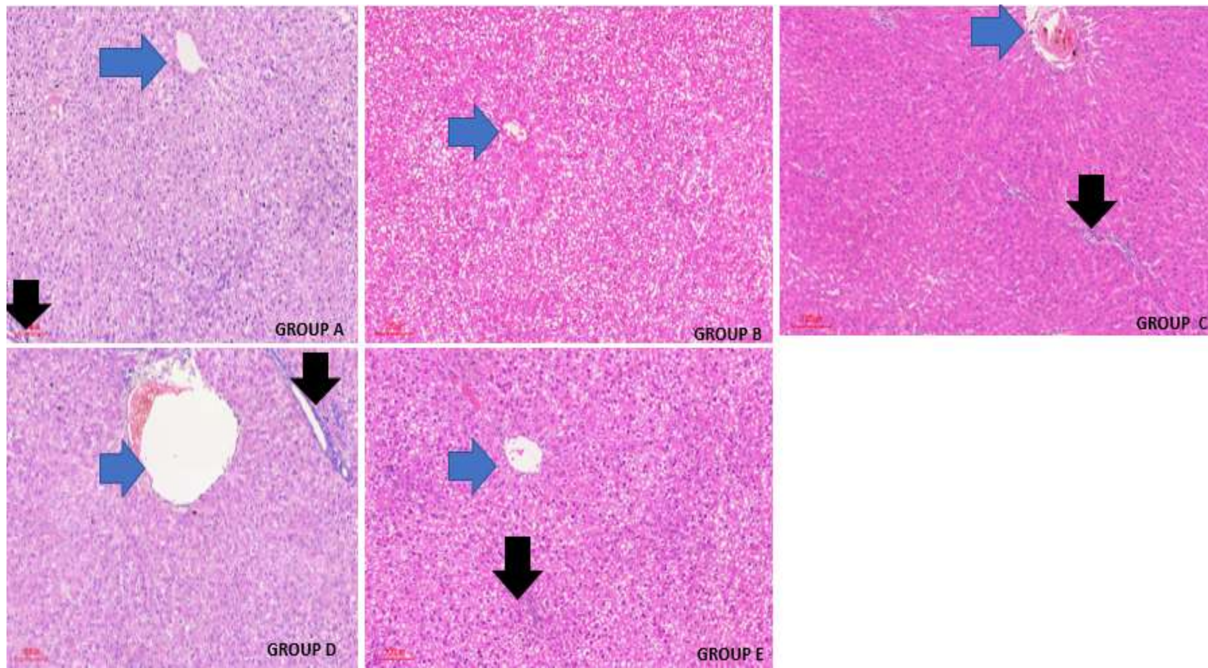

Plate 1: Photomicrograph of liver section using Motic scanner: Group A (Control), B (Paraquat +Normal Saline), C (Paraquat+50mg/kg of Lutein), D (Paraquat+100mg/kg of lutein), E (Paraquat +150mg/kg of Lutein) using (H & E X100). Blue arrow= central vein, Black arrow = portal tract

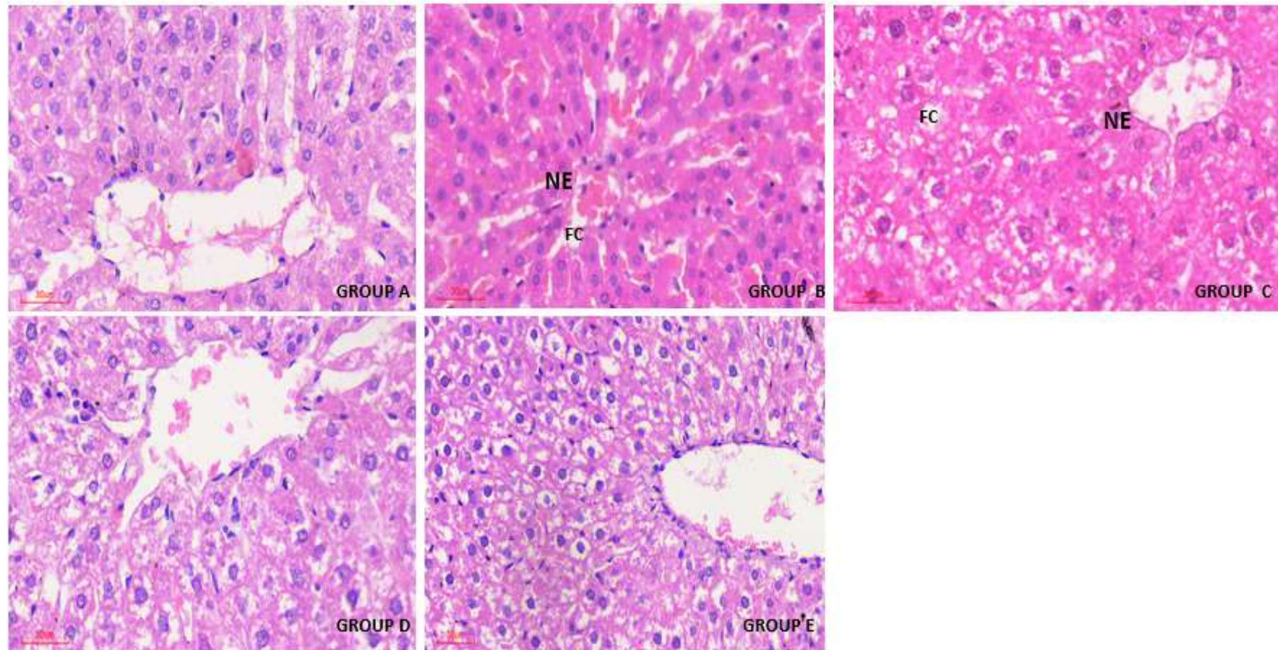

Plate 2: Photomicrograph of liver section using Motic scanner: Group A (Control), B (Paraquat +Normal Saline), C (Paraquat+50mg/kg of Lutein), D (Paraquat+100mg/kg of lutein), E (Paraquat +150mg/kg of Lutein) using (H & E X400). FC: Fatty changes; NE: Necrosis.

DISCUSSION

The study showed that paraquat exposure caused severe histological changes in the liver especially in paraquat-only group (Group B) which demonstrated necrosis of the hepatocytes with venular occlusion as a result of thrombosis. This is consistent with previous studies that showed alteration of hepatic cells around the central vein, necrosis, narrowing of sinusoids, and central

venous congestion also (Han *et al*., 2014, Kheiripour *et al*., 2021). Numerous fatty changes were also seen in the hepatocytes distal to the central vein with inflammatory cells; these findings are supported by Ujowundu *et al* (2018) who observed hepatic fatty changes indicative of failure in the oxidation of fatty acid. Studies have shown that exposure to paraquat causes derangement in fatty acid metabolism leading to accumulation of fatty acids in hepatocytes (Chochan *et al*., 2010, Atashpour *et al*., 2017).

However, the study demonstrated significant improvement in the lutein-treated groups in a dose dependent manner in line with the findings by Li *et al* (2015) and Zhao *et al* (2023) who observed that lutein alleviated the pathological alteration following liver toxicity. This improvement in the histo-architecture of the liver may be linked to the anti-inflammatory and anti- oxidative properties of lutein (Du *et al*., 2015).

The anti-apoptotic and aforementioned properties of lutein may be responsible for the therapeutic benefits in line with Gundogdu *et al* (2022) and also Gad El-Karim *et al* (2023) who reported that lutein successfully mitigated hepato-renal toxicity. Singh *et al*., 2006, explained that the structural base of the anti-oxidative 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 significant decrease in a dose-dependent fashion in the blood aspartate and alanine amino-transaminase levels of the groups treated with lutein. This may be as a result of the hepato-protective activities of lutein (Li *et al*., 2015, Zhao *et al*., 2023); the authors reported that lutein reduces oxidative damage following arsenic-induced toxicity through the process of activating Nrf2 pathway. Increase in the hepatocytes count of the groups treated with lutein may be as a result of the protective antioxidant effect of lutein on hepatocytes (Sindhu *et al*., 2010, Ouyang *et al*., 2019).

The present study showed a significant increase in the MDA activity in rats that had paraquat only. This finding is in line with Onur *et al* (2022) as well as El-Boghdady *et al* (2017); Ray *et al* (2007), who worked on paraquat toxicity in liver and kidney cells. However, in the treated groups, there was significant reduction in the MDA activity and this may be linked to the potential of lutein to scavenge superoxide radicals, hydroxyl radicals, and inhibit lipid peroxidation (Sindhu *et al*., 2010). In addition, treatment with lutein increased the antioxidant enzyme activity and attenuated the rise in reactive oxygen species (ROS) and MDA as reported by Faud *et al* (2020) who worked on arsenic-induced toxicity. Malondialdehyde functions as an indicator of lipid peroxidation and oxidative stress assessment (Del Roi *et al*., 2005); paraquat hepatotoxicity is linked to lipid peroxidation as a result of free radical generation including reactive oxygen species.

The significant decrease in the hepatic GSH concentrations of paraquat-only group relative to the treated groups can be linked to the effect of oxidative stress induced by paraquat on the organ. Glutathione (GSH) is an antioxidant that is capable of preventing oxidative damage to cellular components (Lu 2013; Pompella *et al*., 2003). Any reduction in the level of GSH can lead to compromise in its function in the organ. (Mirzaee *et al*., 2019). This result is in line with the finding by Cai *et al* (2024) who worked on the Silybin-alleviated hepatic injury following paraquat-induced oxidative stress.

There was significant an increase in GSH concentration in a dose dependent manner in the lutein groups similar to the control groups. This finding is corroborated by Faud *et al* (2020) who observed that lutein was able to increase radical absorbance potential of glutathione. Also, Du *et al* (2015) reported that lutein activities occur by inhibition of lipid peroxidation, TNF-α, IL-1β, and nuclear factor kappa B (NF-κβ) production and by preventing the reduction of GSH levels. These effects can be attributed to anti- inflammatory and antioxidative effect of lutein (Johnson 2002, Vijayapadma *et al*., 2014, Mammadov *et al*., 2019). This is due to the free radical scavenging ability of its polarity, conjugated double bond and the two hydroxyl groups (Li *et al*., 2013, Hirdyani *et al*., 2017, Fuad *et al*., 2020).

This present study also showed a significant decrease in the concentration of catalase in liver tissue of rats treated with paraquat only; however, there was a significant increase in a dose dependent fashion in the level of catalase concentration in the treated groups. Studies by Vijayapadma *et al* (2014) and Mammadov *et al* (2019) attributed this similar finding to the antioxidant properties of lutein, which is also supported by other authors (Hayes *et al.,* 2009). This increment in catalase concentration is probably one of the targeted preservative potentials of lutein against tissue oxidative stress. Serpeloni *et al* (2014) reported that supplementation of the diets of rats with lutein under severe oxidative stress significantly improved the antioxidant defense system by directly scavenging ROS.

In conclusion, the study showed significant alteration in the hepatic biochemical analyses and histo-architecture following paraquat toxicity. However, the groups treated with a high doses of lutein showed remarkable similarity with the control group. Hence, study underscores the potentials of lutein to mitigate paraquat-induced toxicity in Wistar rats.

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