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Potential role of Moringa Oleifera in alleviating paracetamol-induced nephrotoxicity in rat

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SUMMARY

The kidney plays a vital role in eliminating the toxic substances and drug metabolites from blood. Therefore, it is prone to hazards in case of abuse or overdose of drugs. The aim of the work was to clarify the impact of Moringa oleifera (MO) against the nephrotoxic effect of paracetamol in adult male albino rats. Twenty-eight adult male albino rats were used in this study and divided equally into four groups (G); G1 (control G), G2 (paracetamol-treated animals), G3 (paracetamol- plus high MO-treated G), G4 (high MO-treated rats). Kidney specimens were harvested after anesthetizing the animals, and then processed for histological and DNA comet analysis. Biochemical blood investigations for kidney function were also performed through assessment of serum creatinine (Cr) and blood urea nitrogen (BUN). Administration of paracetamol resulted in genotoxicity and marked biochemical and renal histological changes. It caused a significant increase in Cr and BUN levels and degeneration in both proximal and distal convoluted tubules with glomerular changes. Administration of MO with paracetamol led to a noticeable amelioration of previous mentioned changes. Moringa oleifera is suggested to be an effective nephroprotection against paracetamol-induced damage. Further studies are recommended to confirm the protective role of MO using large numbers of different animal models before investigating it in humans.

Key words: Moringa oleifera – Paracetamol – Kidney – Nephroprotection – Apoptosis – Microanatomy

INTRODUCTION

The kidney is an important organ, formed of two main parts of different embryological origins. One excreting portion arises from the intermediate mesoderm and constitutes structural and functional units of the kidney called nephrons. The other portion develops from the ureteric bud and forms the collecting passages (Hegazy, 2014). The unit (nephron) of excretory portion that functions in eliminating waste products, medications metabolites and toxic substances such as creatinine and urea from blood consists of a glomerulus, the loop of Henle and proximal (PCT) and distal convoluted tubules (DCT) (Gounden and Jialal, 2019).

Paracetamol or acetaminophen (N-acetyl-p-aminophenol [APAP]), is an acylated aromatic amide, a metabolite of phenacetin. It is widely used in outpatient and inpatient settings in a widespread manner as a single-component medication, and also as a component of a plethora of combinations for treatment of mild pain and fever. Despite its

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safety when used properly, paracetamol poisoning is one of the common overdoses reported to poison centers. This is because paracetamol is readily available over-the-counter without physician's prescription in many countries; and so, overdose is a common means of self-poisoning worldwide (Ghaffar and Tadvi, 2014). The toxic dose of paracetamol is highly variable. The recommended maximum daily dose for healthy adults is 4 grams. Higher doses lead to increasing risk of toxicity. In adults, single doses above 10 grams or 200 mg/kg of body weight, or multiple smaller doses within 24 hours when they exceed these levels, have a reasonable likelihood of causing toxicity (Daly et al., 2008).

Potentially, an acute paracetamol overdose causes fatal liver damage; and this is even noticed with normal doses taken by rare individuals. In both the United Kingdom and the United States, it is the most common cause of acute liver failure. Although hepatotoxicity is more addressed than nephrotoxicity in paracetamol overdoses, Paracetamol-induced renal damages such as renal tubular damage and acute renal failure are usually life-threatening; and there is no specific treatment for them up till now (Khashab et al., 2007; Peng et al., 2010).

After oral administration, about 63% of paracetamol is metabolized via glucuronidation and 34% via sulphation primarily in the liver. The water-soluble metabolites consisting of these metabolic pathways are excreted via the kidney. N-Acetyl-p-benzoquinone (NAPQI) is a reactive intermediate that occurs when oxidization of 55% of paracetamol takes place by the microsomal P-450 enzyme system. NAPQI is detoxified by intracellular glutathione (GSH) in therapeutic doses. Accordingly, NAPQI has been implicated as a responsible metabolite of paracetamol toxicity. In paracetamol overdosing cases, glutathione stores are depleted; NAPQI are not eliminated from the body and their rapid increase in concentration causes necrosis. Lipid peroxidation is initiated in renal tubular cells, disturbing the body's natural antioxidants system and ultimately causing death of renal tubular cells. The reactive oxygen species (ROS) or free radicals are important mediators of paracetamol-induced nephrotoxicity. APAP (acetyl-para-aminophenol), a phenacetin metabolite, also makes nephritic syndrome and renal papillary necrosis possible (chronic analgesic nephropathy) (Ijaz et al., 2016).

Moringa oleifera (MO) is a tree that grows widely in many tropical and subtropical countries. It is known as the drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils. In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutrition content: they contain vitamins, minerals, amino acids, and fatty acids (Mbikay, 2012; Abdull Razis et al., 2014). The moringa plant is beginning to gain more popularity as a new superfood for its powerful tissue-protective properties, among many other health benefits. It has been used for generations in Eastern countries to treat and prevent diseases. The leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids. Various preparations of MO are used for their anti-inflammatory, antihypertensive, antimicrobial, diuretic, antidiabetic, antineoplastic and hepatoprotective activities, etc. No adverse effects have been reported in any of the human studies that have been conducted to date (Abdull Razis et al., 2014).

Therefore, the aim of this work was to evaluate the role of MO against paracetamol-induced nephrotoxicity in rats.

MATERIALS AND METHODS

The study was performed according to the Institutional Review Board of Zagazig University (ZU-IRB) instructions. Approval of the Animal Care and Use Committee of our Institution was received.

Materials

Paracetamol ($C_8H_9NO_2$) (N-acetyl-p-aminophenol [APAP]), in the form of Cetal 500 mg tablets manufactured by EIPICO Company.

Seeds of Moringa oleifera, purchased from (El-Kahera Company for chemical and medical trading, Zagazig, Egypt).

Experimental animals

The present study was carried out for 10 days on 28 adult healthy male albino rats, weighing from 150 to 200 gm according to IRB instructions. They were obtained from the animal house unit in Faculty of Medicine, Zagazig University. All animals were housed in environmentally controlled rooms, in wire mesh cages.

The animals were equally divided into 4 groups (G), 7 animals for each, as follows:

G1 (control group): The rats were only on balanced diet and tap water without any additions.

G2 (paracetamol-treated group): The rats received a daily dose of paracetamol 2g/kg in 5 ml distilled water (Canayakin et al., 2016).

G3 (paracetamol plus MO-treated group): The rats received a daily dose of paracetamol 2g/kg in 5 ml distilled water and 400 mg/kg MO seed powder orally (Ijaz et al., 2016).

G4 (MO-treated group): The animals were given a daily dose of 400 mg/kg oral MO seed powder diluted in 5 ml distilled water by gastric tube.

Methods

The initial body weight (BW) of rats in different groups was estimated at the first day of experi-

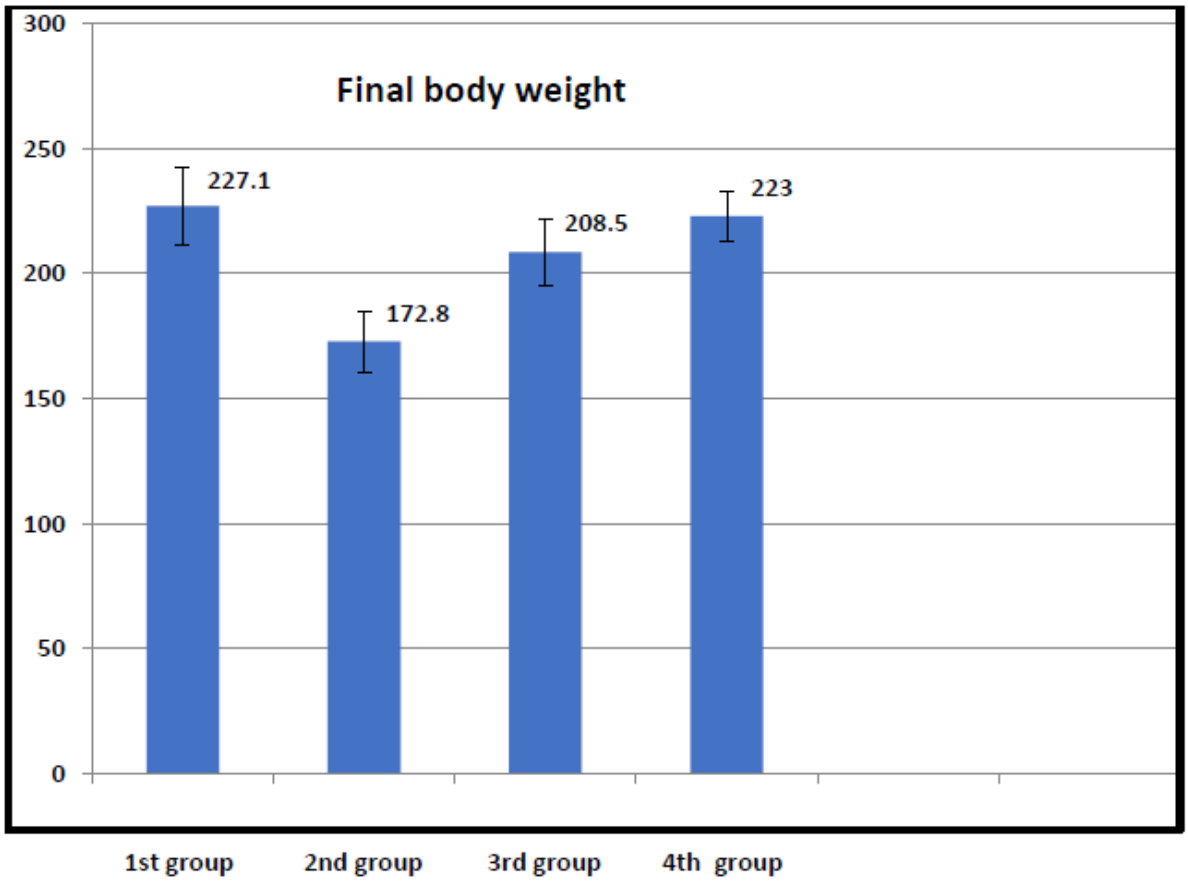

Fig 1. Columns showing the variations in means of final BW (in gm) of different groups.

ment before administration of food and drugs using a digital balance. The BW was again assessed at the end of the experiment using the same digital balance.

Venous blood samples were collected from animals by means of micro–capillary glass tubes from the retro–orbital plexus under light ether anaesthesia. The blood samples were used to investigate kidney function through assessment of serum creatinine (Cr) and blood urea nitrogen (BUN). The analysis was performed in the Clinical Pathology Department, Zagazig University Hospitals.

Then, the animals were slaughtered and their abdomens were opened. Kidneys were released from their fatty covering connective tissue, gently removed and weighed by digital balance.

Each kidney was cut into two halves across the renal pelvis along its longitudinal axis to expose cortex, medulla and papilla. The specimens were immediately immersed in 10% formal saline for 48 hours to be processed and embedded in paraffin; then slides' specimens were stained with haematoxylin and eosin (H&E) (Hegazy and Hegazy, 2015). The histological structure of specimens was investigated using light microscopy.

The quantitative and qualitative extent of DNA damage in the cells was assessed by measuring the length of DNA migration and the percentage of migrated DNA by Comet 5 image analysis software. A Comet 5 image analysis software developed by kinetic imaging, Ltd. (Liverpool, UK), linked to a CCD camera was used. The analysis was carried out at Animal Reproduction Research Institute (ARRI), Cairo.

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science), version 18.0. Quantitative data were expressed as mean ± SD (Standard deviation).

RESULTS

Weight

The initial BW showed a non-significant difference among different groups (Table 1), while final BW showed highly statistically significant difference among different groups. Concerning the com-

Table 1. Means of initial BW in the four different groups

Groups	Number of Rats (24)	Initial BW mean ±SD (Range)	F-Test	*P*-value
G1	7	158±7.6 gm (150-170)	2.3	0.07
G2	7	165.1±11.3 gm (150-180)		
G3	7	177.5±14.1 gm (155-192)		
G4	7	167.4±9.3 gm (153-180)		

Table 2. Multiple comparison analysis for comparing final BW within the four different groups

Groups	*Final body weight*	*P*-value
G1	*G2*	0.001**
	G3	0.006*
	G4	0.5
G2	*G3*	0.001**
	G4	0.001**
G3	*G4*	0.03*

* Statistically significant difference (*P* ≤ 0.05)
** Statistically highly significant difference (*P* ≤ 0.001)

parison of final BW of different groups, there were statistically significant differences (p-value=0.0001), with highest final body weight in G1 (mean: 227.1 ± 15.7 and ranged from 205 to 250 gm), followed by G4 (mean: 223 ± 9.4 gm and ranged from 213 to 240 gm). The G1 showed a statistically significant difference with all other groups except G4. Regarding the G2, there was a statistically significant difference with other groups, as BW showed a decrease in the paracetamol-treated animals (Fig. 1; Table 2).

Assessment of kidney weight (KW) showed that there was a statistically a significant difference between the G2 and other groups as there was statistical decrease in KW in the paracetamol-treated group. Regarding the G1, there was statistically significant difference in comparison with other groups except with G4 (Tables 3-4).

Table 3. Comparison between KW of the four different groups.

Groups	Number of rats (24)	KW mean ± SD (Range)	F-Test	*P*-value
G1	7	0.7±0.08 (0.58-0.8)	44.2	**0.001****
G2	7	0.39±0.05 (0.32-0.46)		
G3	7	0.56±0.05 (0.47-0.62)		
G4	7	0.83±0.04 (0.78-0.9)		

** Statistically highly significant difference (P ≤ 0.001)

Biochemical results

Administration of paracetamol at dose 2g/kg in 5 ml distilled water orally in G2 significantly elevated serum Cr (P ≤ 0.05) (Fig. 2; Tables 5, 6) and BUN levels (Fig. 3; Tables 7,8) as compared to their respective levels in G1. There were statistically significant differences between the four different groups in serum Cr level (P = 0.001), with highest creatinine level in the G2 with a mean of 0.37±0.05 (range: 0.3 to 0.44), followed by the G3 with a mean of 0.28±0.07) (range: 0.2 to 0.43) (Fig. 2). Regarding BUN, there were also statistically significant differences between the four different groups (P = 0.0001), with highest level in G2 (mean: 16.6 ± 0.9 mg/dl; range: 15.1 to 17.8), followed by G3 (mean: 12.7 ± 0.39 mg/dl; range: 12.1-13.4) (Fig. 3).

Contrary to noticed statistically significant posi-

Table 4. Multiple comparison analysis for comparing KW within the four different groups

Groups	*KW*	*P*-value
G1	*G2*	0.001**
	G3	0.001**
	G4	0. 001**
G2	*G3*	0.001**
	G4	0.001**
G3	*G4*	0.001**

* Statistically highly significant difference (P ≤ 0.001)

Table 5. Cr level of the four different groups

Groups	Number of rats (42)	Serum Cr mean ± SD (Range)	F-Test	*P*-value
G1	7	0.27±0.08 (0.21-0.42)	5.9	**0.001****
G2	7	0.37±0.05 (0.3-0.44)		
G3	7	0.28±0.07 (0.2-0.43)		
G4	7	0.15±0.01 (0.08-0.38)		

** Statistically highly significant difference (P ≤ 0.001)

Table 6. Multiple comparison analysis for comparing serum creatinine level within the four different groups

Groups	*Serum creatinine* mean ± SD (Range)	*P*-value
G1	*G2*	0.04*
	G3	0.7
	G4	0.01*
G2	G3	0.06
	G4	0.001**
G3	*G4*	0.005*

* Statistically significant difference (P ≤ 0.05)
** Statistically highly significant difference (P ≤ 0.001)

Table 7. BUN level of the four animal groups

Groups	Number of rats (28)	*BUN* mean ± SD (Range)	F-Test	*P*-value
G1	7	11.8±0.47 (11.1-12.2)	16.9	**0.0001****
G2	7	16.6±0.9 (15.1-17.8)		
G3	7	12.7±0.39 (12.1-13.4)		
G4	7	9.6±0.55 (9-10.5)		

** Statistically highly significant difference (P ≤ 0.001)

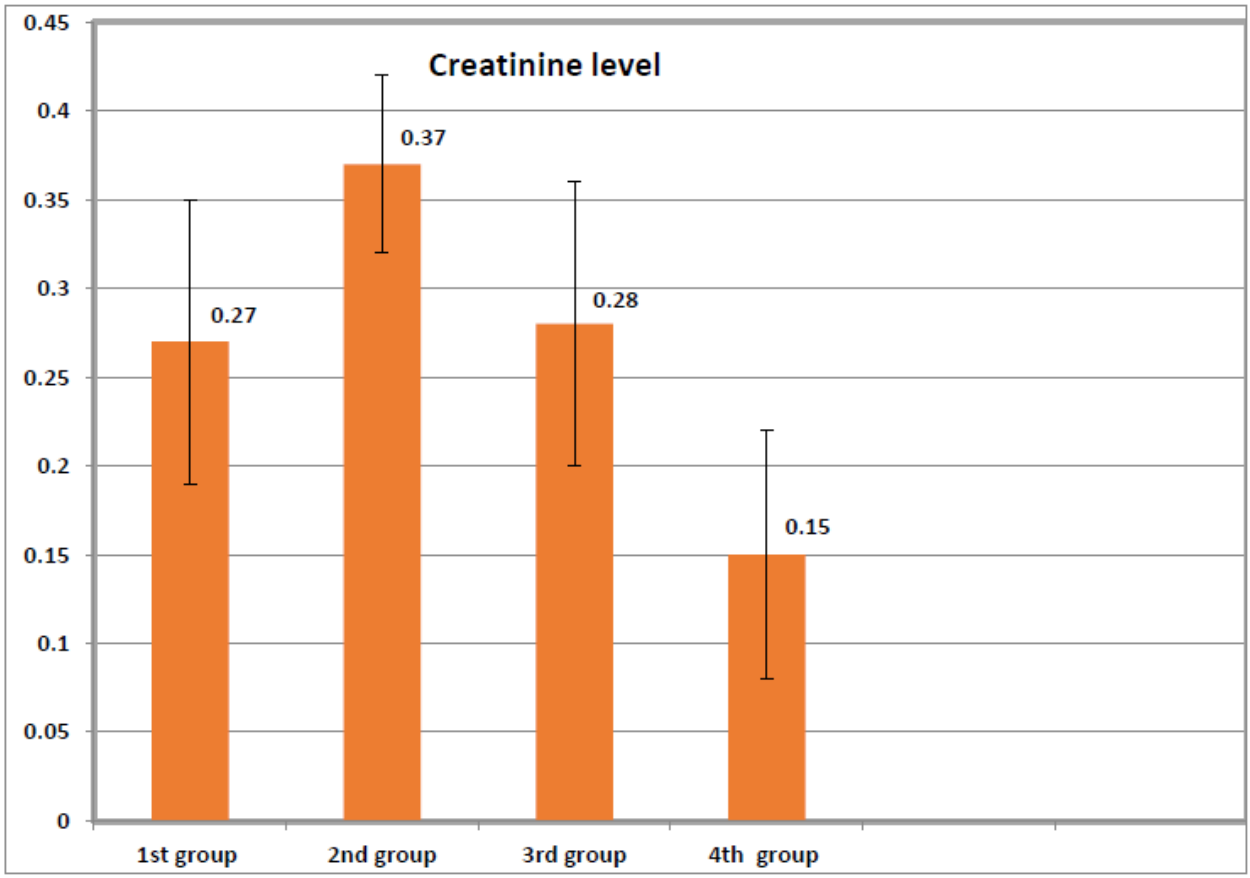

Fig 2. Bar chart showing means of Cr level (mg/dl) in the studied groups.

tive correlation with initial body weight and kidney weight, there was statistically significant negative correlation between final BW with serum Cr and BUN levels (Fig. 4; Table 9).

Histological features

Regarding the microscopic tissue sections of the kidneys of control animals (G1), the glomeruli were surrounded by visceral and parietal layers of Bowman's capsule and separated by Bowman's space. Proximal and distal convoluted tubules with normal lumen and intact lining epithelium were observed (Fig. 5). Normal collecting tubules and normal loop of Henle with flat capillaries were obvious (Fig. 6).

Meanwhile, in G2 animals there were changes in renal glomeruli, represented by shrinkage and atrophy in some glomeruli, and hypertrophy and hypercellularity with lobulations of others. There were also widening or obliteration of Bowman's space, congestion of glomerular capillaries, dark stained nuclei of parietal cell layer and Peri-glomerular inflammatory cell infiltrations (Figs. 7-9).

As regard to the renal tubules, there were dilatation, tubular cell necrosis, sloughing of necrotic tubular epithelial cells into the lumens and dark stained nuclei of distal convoluted tubules, marked swelling and cytoplasmic vacuolization of proximal tubular cells (Figs. 9-10). Deposition of acidophilic hyaline material or cellular debris containing pyknotic nuclei in tubular lumen were noticed (Fig. 8). Moreover, there were loss of architecture of the renal interstitial tissues, interstitial hemorrhage, mono-nuclear inflammatory cell infiltration (in the

Table 8. Multiple comparison analysis for comparing BUN level within the four different groups

Groups	*BUN*	*P*-value
G1	*G2*	0.001**
	G3	0.004*
	G4	0.001**
G2	*G3*	0.004*
	G4	0.001**
G3	*G4*	0.001**

* Statistically significant difference (P ≤ 0.05)
** Statistically highly significant difference (P ≤ 0.001)

Table 9. Correlation between final BW with initial BW, BUN, serum Cr and KW in the studied group

Variable	*Final BW* r ^	*P*	SIG
Initial BW	0.4	> 0.03*	S
BUN	-0.8	0.001**	HS
Serum Cr	-0.8	0.001**	HS
KW	0.7	0.001**	HS

* Statistically significant difference (P ≤ 0.05)
** Statistically highly significant difference (P ≤ 0.001)

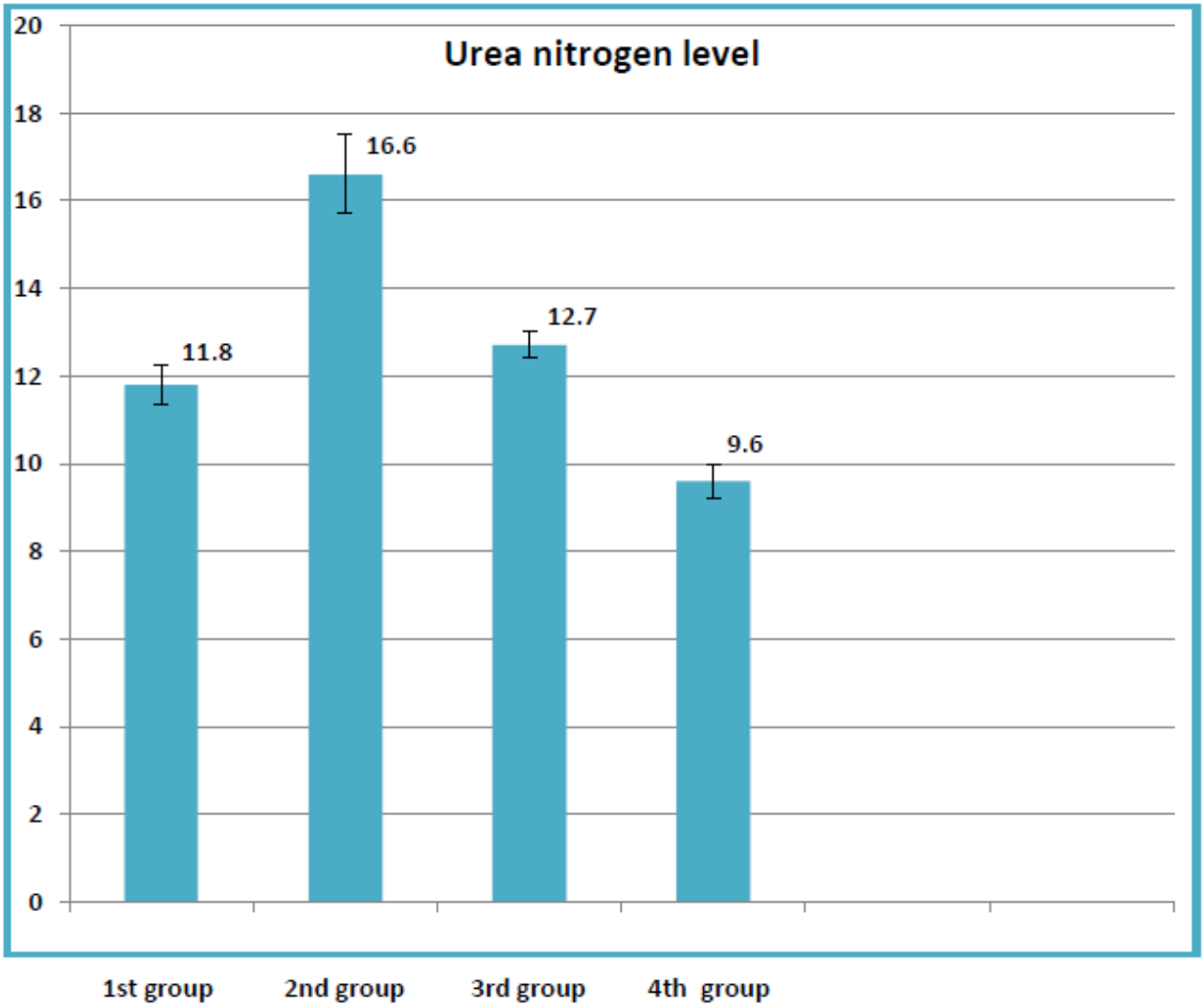

Fig 3. Bar chart showing means of BUN level (mg/dl) in the studied groups.

form of many small focal or massive areas between the renal tubules) and congestion and thickening of wall of blood vessels (Figs. 7-9).

Co-administration of MO in G3 showed that the renal glomeruli and tubules appeared almost normal (Fig. 11). However, dispersed areas of lobulated glomeruli, congestion and few periglomerular inflammatory cells were still noticed (Fig. 12). Few areas of almost normal collecting ducts with exfoliated nuclei and vacuolated cells were also seen (Fig. 13).

Comparing the microscopic tissue sections of MO-treated animals (G4) with other groups, G4 showed almost the same normal structure of renal cortex and medulla found in G1 (Figs. 5,6,14,15).

Morphometry

On comparing the measurements of the capillary

Table 10. Cortical Morphometric measures among the different groups

Parameter *Group*	Capillary tuft area (μm^2) Mean ± SD	Bowman space (µm) Mean ± SD	PCT diameter (µm) Mean ± SD	DCT diameter (µm) Mean ± SD
G1	1109.60±198.1	5.174±1.691	27.78±3.604	47.83±5.923
G2	641.3±102.1 aaa	9.785±2.265 aaa	36.88±3.494 aaa	67.32±7.703 aaa
G3	986.1±74.71 b	7.633±1.894 b	29.89±4.070 bb	52.76±9.838 bb
G4	1095±157.6	5.360±1.912	26.20±3.859	48.53±4.788
F	6.799	20.81	9.009	9.672
P	0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***

No. of measurements in each group = 8. F: ANOVA test. ***: Highly significant (P <0.001); aaa: High significant change at P <0.001 in comparison with Control; b: Mild significant change at P <0.05 in comparison with Paracetamol; bb: Moderate significant change at P <0.01 in comparison with Paracetamol.

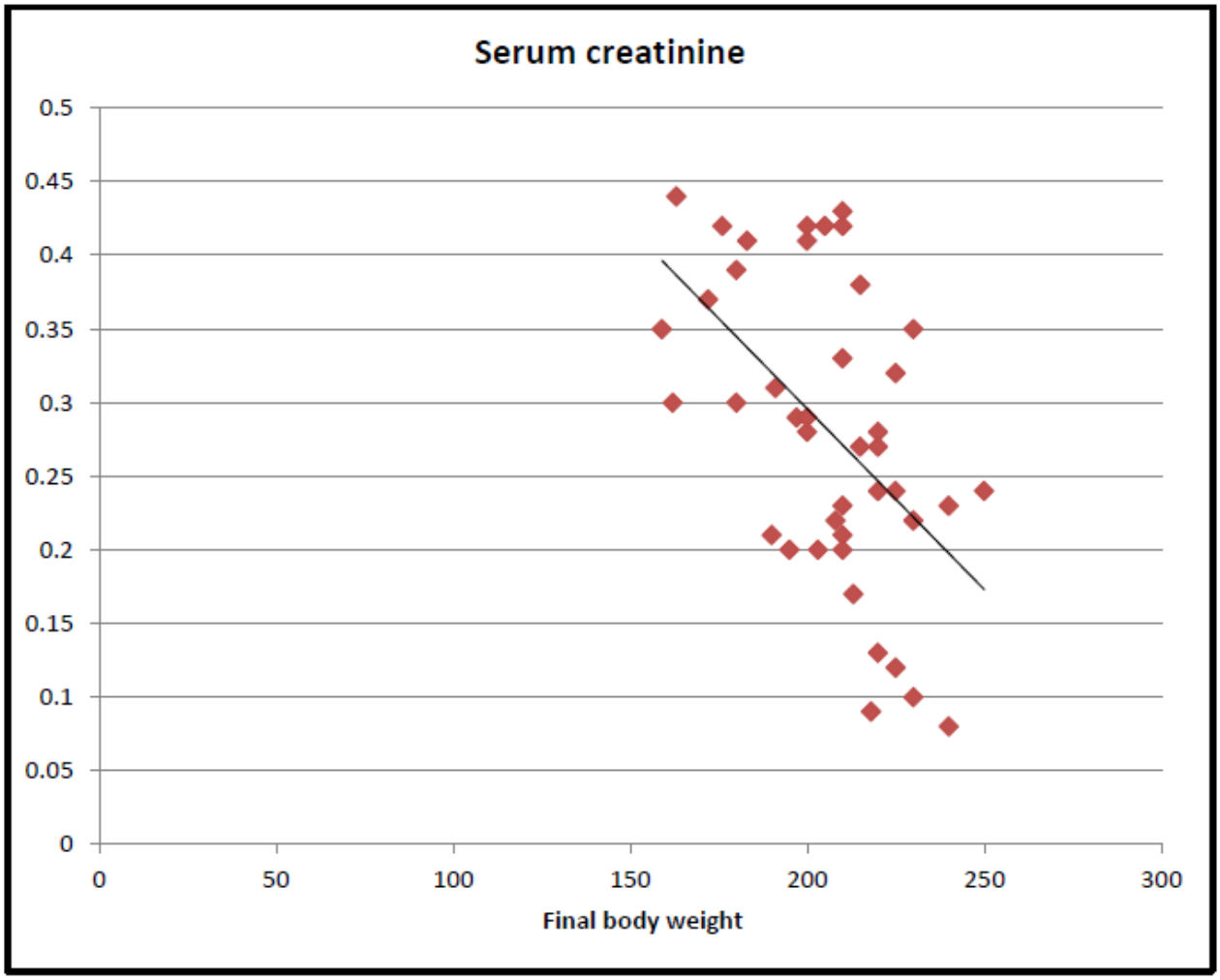

Fig 4. Scatter plot with line chart for negative correlation between final weight and serum creatinine in the studied groups.

tuft area, Bowman's space, proximal convoluted tubule (PCT) diameter and distal convoluted tubule (DCT) diameter among the various groups, they revealed highly significant changes of the second group with respect to other groups (Table 10). There was some improvement in G3 animals co-administered with MO. At the same time, animals of G4 receiving only MO showed measures approximately similar to that of G1.

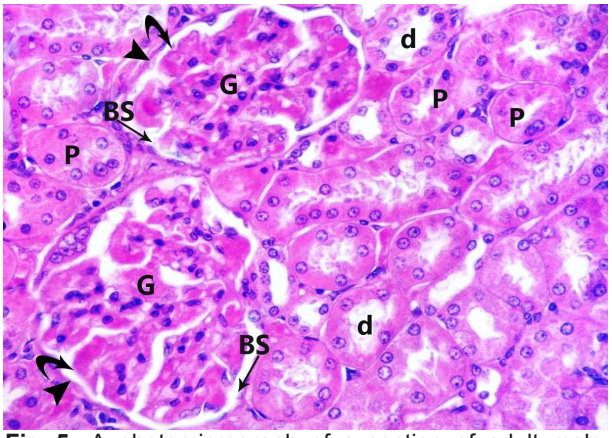

Fig 5. A photomicrograph of a section of adult male albino rat kidney of G1 showing the glomeruli (**G**). The glomeruli are surrounded by visceral (**curved arrow**) and parietal (**arrow head**) layers of Bowman's capsule and separated by Bowman's space (**BS**). PCTs (**p**) with narrow lumen and DCTs (**d**) with wide lumen are also seen. (H&E, x400).

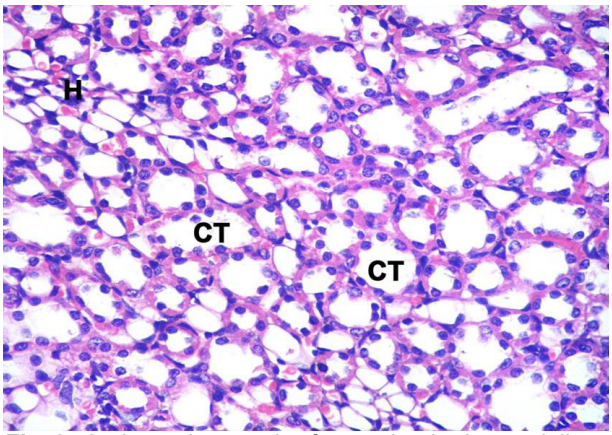

Fig 6. A photomicrograph of a section in the medullary tissue of a rat's kidney of G1 showing normal collecting tubules (**CT**) and normal loop of Henle (**H**) with flat capillaries. (H&E, x400).

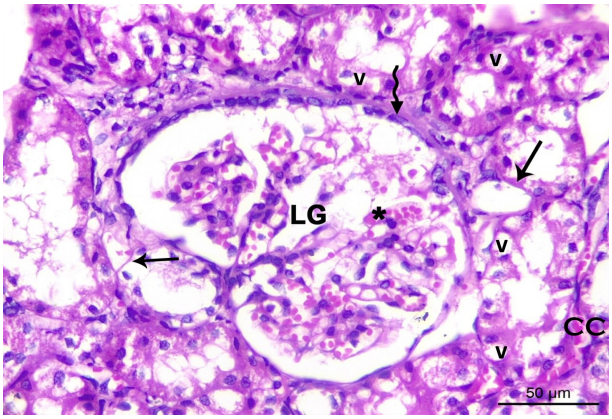

Fig 7. A photomicrograph of a section of adult male albino rat renal cortex of G2 showing lobulated hypertrophied glomerulus (**LG**) with congested glomerular capillaries (*) and hypercellularity. The glomerulus shows thickening in lining epithelium of parietal layer (**zigzag arrow**). The convoluted tubules display cytoplasmic vacuolation (**V**) and loss in the lining epithelium (**arrow**). Capillary congestion is also observed. (H&E, x400).

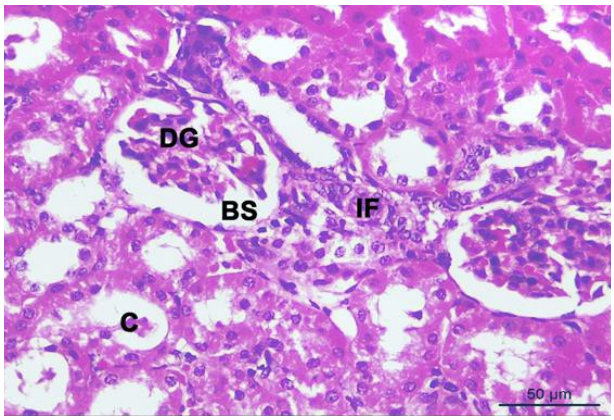

Fig 8. A photomicrograph of a section of adult male albino rat renal cortex of **G2** showing shrunken degenerated glomerulus (**DG**) with dilated Bowman's space (**BS**). Peri glomerular inflammatory cell infiltrations (**IF**) can be noticed. There are hyaline casts in the tubules (**C**).

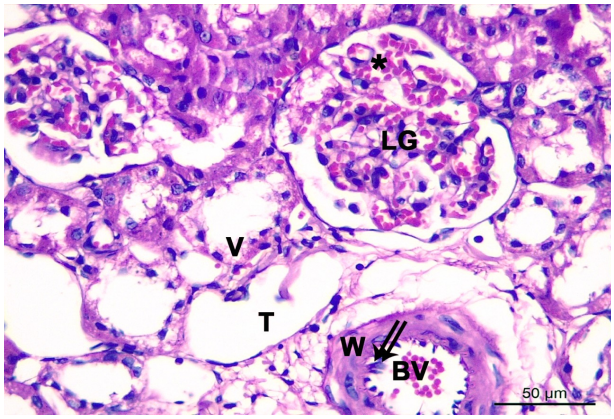

Fig 9. A photomicrograph of a section of adult male albino rat renal cortex showing lobulated glomerulus (**LG**) with congested glomerular capillaries (*), congested blood vessel (**BV**) with markedly thick wall (**W**) and altered dimension of endothelial lining (double arrows). Disorganized dilated tubules (**T**) with darkly stained nuclei and vacuolated cells (**V**) can be noticed. (H&E, x400).

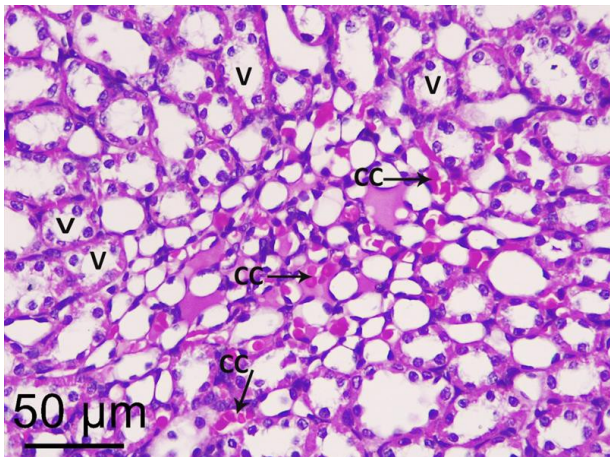

Fig 10. A photomicrograph of a section in the medulla of a rat kidney of **G2** showing congested capillaries (**CC**) and vacuolated cells (**V**). (H&E, x400).

DNA Comet

Regarding percent of tail DNA, G2 had the highest percent of damage (2.99%) denoted by increased migration length of stained DNA. This was then followed by G3 (2.1%); and the least percent was in G4 (Figs. 16-17).

DISCUSSION

The kidney plays a vital role in eliminating the toxic substances and drug metabolites (Gounden and Jialal, 2019). Therefore, it is prone to hazards in case of abuse or overdose of drugs. In this study, we tried to induce nephrotoxicity by administration of high doses of paracetamol suggested by Canayakin et al. (2016) in order to investigate the MO role in alleviating such kidney damage. We did the experiment on male rats other than females to exclude effects of hormonal changes of estrous cycles in female animals (Hegazy et al., 2018).

In the current work, there was statistically significant decrease in the BW of paracetamol-treated animals when compared with the control group. This result is in agreement with Pareta et al. (2011), who reported that the percentage of the increase in BW of paracetamol-treated rats was dramatically lesser than the other groups due to the toxicity of paracetamol, gastrointestinal toxicity, reduced feed and water intake. Also, according to European Agency for the Evaluation of Medicinal Products (1999), there is a decrease in BW gain in rats at daily doses of paracetamol higher than 800 mg/kg BW. However, Oyedeji et al. (2013) found non-significant changes in rat BW relative to the

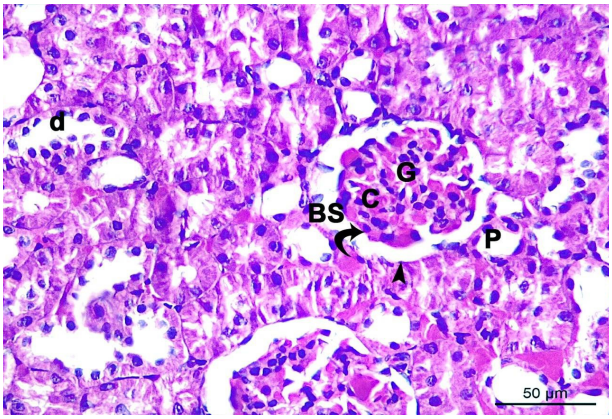

Fig 11. A photomicrograph of a section of adult male rat renal cortex of **G3** showing almost normal glomerulus (**G**) formed of a tuft of glomerular capillaries (**C**). It is surrounded by visceral (**curved arrow**) and parietal (**arrow head**) layers of Bowman's capsule. Both layers are separated by Bowman's space (**BS**). PCTs (**p**) with narrow lumen and DCTs (**d**) with wide lumen are also seen. (H&E, x400).

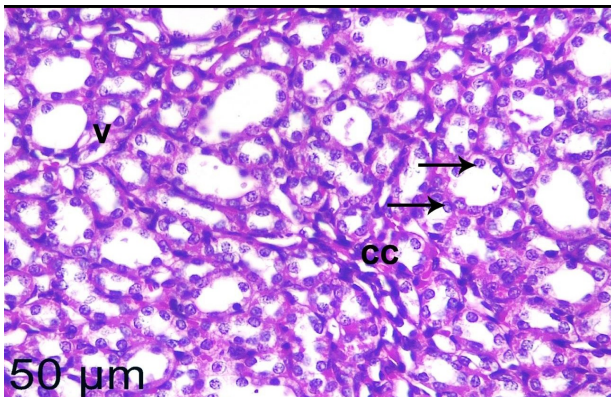

Fig 13. A photomicrograph of a section in the medulla of a rat kidney of **G3** showing almost normal collecting ducts with exfoliated nuclei (**arrow**) and vacuolated cells (**V**). Small areas of congested capillaries (**CC**) are observed. (H&E, x400).

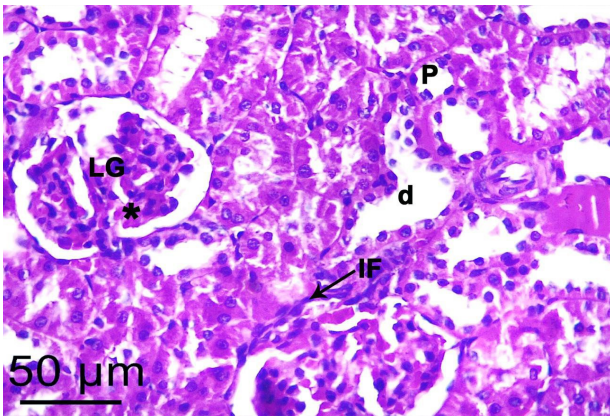

Fig 12. A photomicrograph of a section of adult male albino rat renal cortex of **G3** showing a lobulated glomerulus (**LG**) with congested glomerular capillaries **(*).** Few peri glomerular inflammatory cell infiltrations (**IF**) can be noticed. PCTs (**p**) with narrow lumen and DCTs (**d**) with wide lumen are also seen. (H&E, x400).

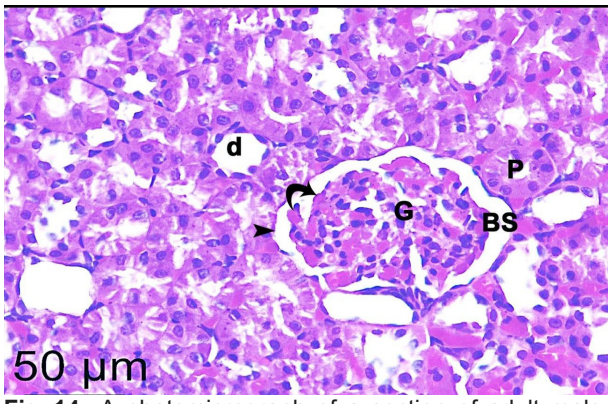

Fig 14. A photomicrograph of a section of adult male albino rat renal cortex of **G4** showing a totally normal section: the glomeruli (**G**). The glomeruli are surrounded by visceral (**curved arrow**) and parietal (**arrow head**) layers of Bowman's capsule and separated by Bowman's space (**BS**). PCTs (**p**) with narrow lumen and DCTs (**d**) with wide lumen are also seen. (H&E, x400).

control ones after their treatment for 42 days with 7.5 mg/kg BW of paracetamol. On the other hand, the MO-treated animals (G4) showed a significant increase in the BW in comparison to paracetamol-treated group. The animals' BW gain may be due to an increase of their appetite as well as the nutritious effect of MO as detected with Mbikay (2012) and Abdull Razis et al. (2014).

Regarding KW, there was a statistical decrease in paracetamol-treated animals (G2) in comparison with other groups. It might be a part of total decrease of BW. However, this result differs from findings of Pareta et al. (2011) that stated a treatment-related increase in KW. On the other hand, the MO-treated group (G4) showed a KW increase in comparison to the other groups. This contradicts the findings of Asogwa et al. (2017).

Retro-orbital plexus route for getting blood samples from small animals such as rats is the commonly used method in our Animal House. It might be performed to get a large volume of blood to be tested. It was done following light anesthesia to alleviate the stress that could result from blood sampling (Mahl et al., 2000). The serum Cr and BUN are indicators of renal functions; and their levels are increased in case of kidney tissues' damage (Ndukaku et al., 2014). In the current study, administration of paracetamol at dose 2g/kg in 5 ml distilled water orally (G2) significantly ($P \leq 0.05$) elevated serum Cr and BUN levels as compared to their respective levels in control group. These results are in agreement with Ramadan and Schaalan (2011), who noticed their elevation in rats after paracetamol administration at a dose of

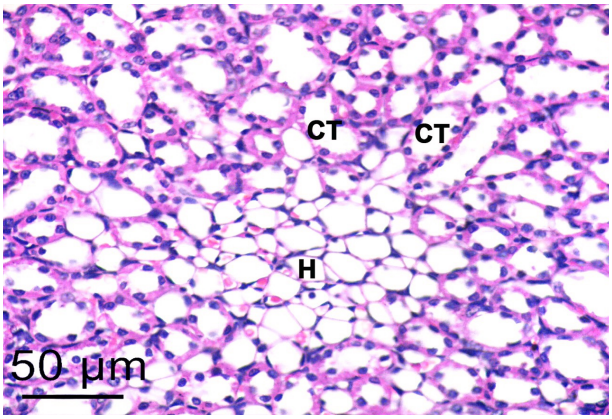

Fig 15. A photomicrograph of a section in the medulla of a rat kidney of **G4** showing normal collecting tubules (**CT**) and normal loop of Henle (**H**) with flat capillaries. (H&E, x400).

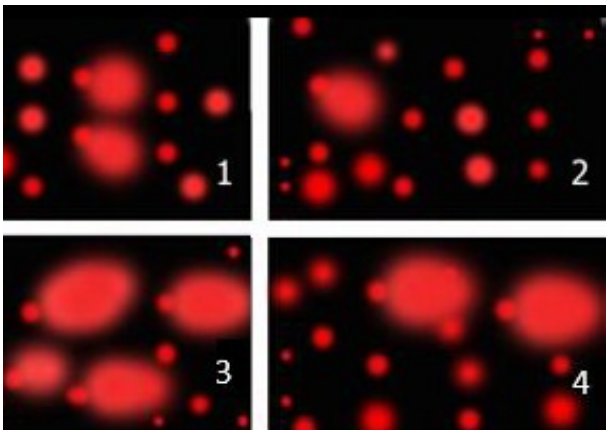

Fig 16. Photomicrographs showing the length of DNA migration and damage (DNA comet) in different groups; **G1** (1), **G2** (3), **G3** (4) and **G4** (2).

only 1 g/kg BW. However, the normal values in control animals differ from the previous studies. This might be due to the subtype of the rats used and/or the methods adopted in the different studies. Moreover, Satirapoj et al. (2009) added that even therapeutic dose of paracetamol could result in elevations in BUN and Cr especially in case of acute febrile illness.

The animals of G3 showed a significant lowering in serum Cr and BUN level in comparison to G2. This is because MO seed powder at oral doses of 400 mg/kg might protect the kidney against elevation of Cr and BUN. These results are in agreement with Ijaz et al. (2016) who reported that paracetamol-induced renal damage by significantly increasing levels of serum Cr and BUN ($P < 0.05$). However, MO seed powder at oral doses of 200 mg/kg, 400 mg/kg or 600 mg/kg exerted its nephroprotective and antioxidant role, and significantly lowered the acute elevation in the serum Cr and BUN ($P < 0.05$).

The present study showed that paracetamol induced different histopathological changes in the renal cortex and medulla. Paracetamol-treated animals revealed massive destructive damage of the renal cortex and medulla and congestion of glomerular capillaries. This could be explained, as mentioned by Ahmed et al. (2015), due to an in-

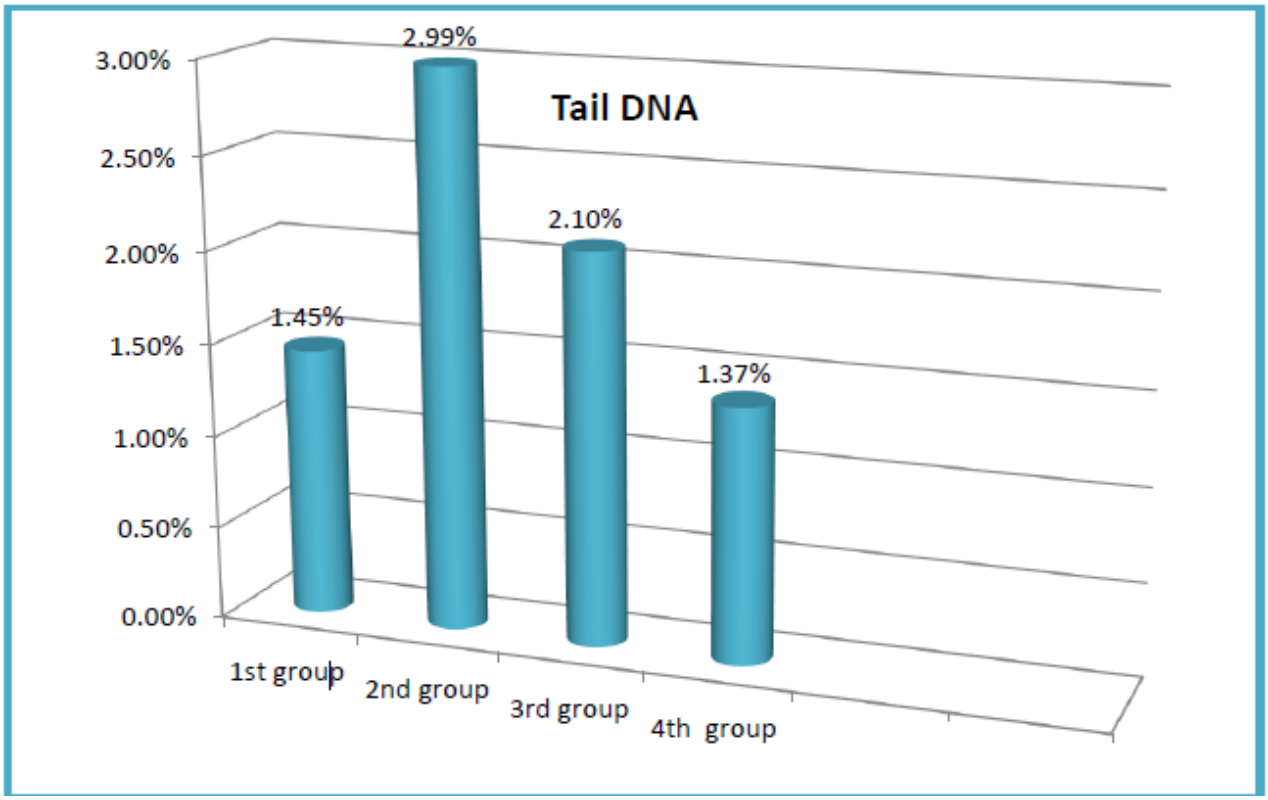

Fig 17. Bar chart for percent of tail DNA in the studied groups.

crease in renal blood vessel permeability caused by a high dose of paracetamol. On other side, some renal glomeruli in this study showed shrinkage and atrophy, which may be attributed to a decrease in the glomerular filtration of the drug as a result of capillary constriction.

With regard to the renal tubules, there was tubular dilatation, dark stained nuclei and tubular cell necrosis, sloughing of necrotic tubular epithelial cells into lumens of tubules, marked cytoplasmic vacuolization and swelling of tubular cells. These results are in accordance with Kirbas et al. (2015), who observed an intensive deformation of epithelial cell structures of both proximal and distal tubules in paracetamol-treated rats. This finding has been explained by Morsy et al. (2013), who reported the same results. They stated that exposure of epithelial cells to oxidant stress leads to an elevation in nitric oxide release and nitrite production, and decrease in cell viability.

This work demonstrated cytoplasmic vacuolization of tubular cells in the renal cortex of rats treated with paracetamol. These data could be explained by Suriyakumari et al. (2016), who demonstrated that cytoplasmic vacuolization occurs as one of primary responses to all forms of cell injury. They stated that increased permeability of cell membranes leads to an increase of intracellular water. As water sufficiently accumulates within the cell, it produces cytoplasmic vacuolization.

In the present work, examination of kidney sections treated with paracetamol revealed also inflammatory cell infiltration. These results are in agreement with Kumar et al. (2010) and Pareta et al. (2011), who stated that the paracetamol-treated group showed severe tubular necrosis, permeation of inflammatory cells, tubular deterioration, hemorrhage, distension of tubules and vacuolization. Kandemir et al. (2017) added that the kidney tissue showed increased pro-inflammatory cytokines, which could be another explanation of renal injury-induced by paracetamol.

In the current work, light microscopic examination of the kidney tissue of rats treated with paracetamol and MO (G3) revealed that renal glomeruli and tubules appeared almost normal when compared with the paracetamol-treated group. However, few atrophic renal glomeruli, some vacuolation of cells and mild dilation of some tubules were still evident, while other PCT and DCT began to retain their normal histological architecture. These findings are in consistence with those of Ijaz et al. (2016), who reported that MO significantly reduced the histopathological changes observed in the paracetamol-treated group. Animals administered with MO seed powder at doses of 200 & 400 mg/kg showed tubular epithelial cells with mild condensed nuclei. Ndhlala et al. (2014) proposed that MO reduces nephrotoxicity by its antioxidant properties. On the other hand, light microscopic examination of the kidney tissue of rats treated with MO only (G4) revealed normal architecture of kidney. These results are in accordance with Uma et al. (2010), who stated no toxic effect on rats with administration of 800 mg/kg leaves extract of MO for 15 consecutive days. Therefore, the plant is suggested to be safe for human consumption without any undesirable effects.

In morphometric measurements, the G2 showed increase in the diameter of PCT, DCT and Bowman's space in comparison to the other groups. Regarding G3, the diameters were less dilated than those in the G2 due to the nephroprotective effect of MO in these groups, which decreased the toxic effect of paracetamol on kidney. The diameters of PCT, DCT and Bowman's space in the G1 and G4 were in the normal range; this suggested the nephroprotective effect of MO in the G4. The capillary tuft area was the smallest in the G2, while the G3 showed larger capillary areas due to the protective effect of MO. Regarding the G1 and G4, their capillary tuft areas were nearly equal. These results are in accordance with those of Canayakin et al. (2016), who reported that the histopathology of the kidneys of the paracetamol-treated group showed severe proximal tubular distortion with enlarged lumina. This was found also by Pareta et al. (2011), who reported that the histopathological studies for Paracetamol treated group showed severe tubular necrosis, tubular deterioration, distension of tubules and vacuolization.

In addition to histological and biochemical evaluation, the damage of the kidney could be also assessed by comet assay (Abo-EL-Sooud et al., 2018). Duez et al. (2003) concluded that such method is an efficient way to demonstrate genotoxicity induced by physical or chemical agents. In the present study, the DNA COMET analysis results are in consistence with the findings of the European Agency for the Evaluation of Medicinal Products (1999), which stated the presence of evidences of chromosomal damage in high paracetamol doses in its report. De Boeck et al. (2000) also stated that increased DNA migration indicates the induction of DNA strand breaks. The percentage of DNA in the tail is the most appropriate parameter to analyze induced DNA damage. Also, Abo-EL-Sooud et al. (2018) stated that DNA damages are considered to be strongly related to cell death, because it can lead directly to chromosome aberrations and the loss of genetic material.

In conclusion, paracetamol administration could produce renal tissue damage predicted by noticed degenerative changes in the histological structure and DNA comet, and confirmed by disturbed kidney function tests. Co-administration of MO with paracetamol improves such deleterious changes. Moreover, MO has not only no bad sequelae if it is given alone, but also might be of beneficial effect indicated by least genotoxicity observed in G4. Accordingly, MO is suggested to have a protective

role against paracetamol-induced renal damage. Further studies to investigate MO on other animals with large numbers are recommended.

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REFERENCES

ABDULL RAZIS AF, IBRAHIM MD, KNTAYYA SB (2014) Health benefits of Moringa oleifera. Asian Pac J Cancer Prev, 15(20): 8571-8576.

ABO-EL-SOOUD K, HASHEM MM, BADR YA, ELEIWA MM, GAB-ALLAHA AQ, ABD-ELHAKIM YM, BAHY-EL-DIEN A (2018) Assessment of hepato-renal damage and genotoxicity induced by long-term exposure to five permitted food additives in rats. Environ Sci Pollut Res, 25(26): 26341-26350.

AHMED W, ZAKI A, NABIL T (2015) Prevention of methotrexate-induced nephrotoxicity by concomitant administration of garlic aqueous extract in rat. Turkish J Med Sci, 45(3): b507-516.

ASOGWA IS, ANI JC, OMAH EC (2017) Effect of moringa leaf powder supplementation on some biochemical indices of rats. IJRSB, 5(9): 48-58.

CANAYAKIN D, BAYIR Y, KILIC BAYGUTALP N, SEZEN KARAOGLAN E, ATMACA HT, KOCAK OZGERIS FB, KELES MS, HALICI Z (2016) Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharm Biol, 54 (10): 2082-2091.

DALY FF, FOUNTAIN JS, MURRAY L, GRAUDINS A, BUCKLEY NA (2008) Guidelines for the management of paracetamol poisoning in Australia and New Zealand explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. Med J Aust, 188 (5): 296-301.

DE BOECK M, TOUIL N, DE VISSCHER G, VANDE PA, KIRSCH-VOLDERS M (2000) Validation and implementation of an internal standard in comet assay analysis. Mutat Res Genet Toxicol Environ Mutagen, 469: 181-197.

DUEZ P, DEHON G, KUMPS A, DUBOIS J (2003) Statistics of the comet assay: a key to discriminate between genotoxic effects. Mutagenesis, 18: 159-166.

GHAFFAR UB, TADVI NA (2014) Paracetamol toxicity: a review. J Cont Med A Dent, 2(3): 12-15.

GOUNDEN V, JIALAL I (2019) Renal function tests. StatPearls [Internet]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK507821/

HEGAZY A (2014) Clinical Embryology for medical students and postgraduate doctors. Lambert Academic Publishing, Berlin.

HEGAZY R, HEGAZY A (2015) Hegazy' simplified method of tissue processing (consuming less time and chemicals). Ann Int Med Dental Res, 1(2): 57-61.

HEGAZY AA, AHMED MM, SHEHATA MA, ABDELFATTAH MM (2018) Changes in rats' liver structure induced by zinc oxide nanoparticles and the possible protective role of vitamin E. Int J Hum Anat, 1(3): 1-16.

IJAZ A, JAVED I, ASLAM B, KHAN JA, KHALIQ T, KHAN M., IQBAL Z, NAEEM MA, ASHRAF MM (2016) Nephroprotective and antioxidant effects of moringa oleifera (sohanjna) in paracetamol induced nephrotoxic albino rabbits. Pakistan Vet J, 36(3): 292-296. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/3841304

KANDEMIR FM, KUCUKLER S, CAGLAYAN C, GUR C, BATIL AA, GÜLÇIN İ (2017) Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: Biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. J Food Biochem, 41(5): 1-8.

KHASHAB M, TECTOR AJ, KWO PY (2007) Epidemiology of acute liver failure. Curr Gastroenterol Rep, 9(1): 66-73.

KIRBAS A, CURE MC, KALKAN Y, CURE E, TUMKAYA L, SAHIN OZ, PERGEL A (2015) Effect of infliximab on renal injury due to methotrexate in rat. Iran J Kidney Dis, 9(3): 221-229. http://www.ijkd.org/index.php/ijkd/article/view/1776/774

KUMAR G, HOTA D, SAIKIA UN, PANDHI P (2010) Evaluation of analgesic efficacy, gastrotoxicity and nephrotoxicity of fixed-dose combinations of nonselective, preferential and selective cyclooxgenase inhibitors with paracetamol in rats. Exp Toxicol Pathol, 62: 653-662.

MAHL A, HEINING P, ULRICH P, JAKUBOWSKI J, BOBADILLA M, ZELLER W, BERGMANN R, SINGER T, MEISTER L (2000) Comparison of clinical pathology parameters with two different blood sampling techniques in rats: retrobulbar plexus versus sublingual vein. Lab Anim, 34(4): 351-361.

MBIKAY M (2012) Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Front Pharmacol, 3: 24.

MORSY MA, IBRAHIM SA, AMIN EF, KAMEL MY, RIFAAI RA, HASSAN MK (2013) Curcumin ameliorates methotrexate-induced nephrotoxicity in rats. Adv Pharmacol Sci, 2013: 387071.

NDHLALA AR, MULAUUDZI R, NCUBE B, ABDELGADIR HA, DU PLOOY CP, VAN STADEN J (2014) Antioxidant, antimicrobial and phytochemical variations in thirteen Moringa oleifera Lam. Cultivars. Molecules, 19 (7):10480-10494.

NDUKAKU OY, OMOSUN G, PERPETUAL AI, EMMANUEL EU (2014) Some kidney function parameters of wistar albino rat fed Pentaclethra macrophylla seeds meal. Eur J Biotechnol Biosci, 2(3): 17-20.

OYEDEJI KO, BOLARINWA AF, OJENIRAN SS (2013) Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats. Res J Pharmacol, 7: 21-25.

PARETA SK, PATRA KC, HARWANSH R, KUMAR M, MEENA KP (2011) Protective effects of Boerhaavia diffusa against acetaminophen induced nephrotoxicity in rats. Pharmacol Online, 2: 698-706.

PENG HC, WANG YH, WEN CC, WANG WH, CHENG CC, CHEN YH (2010) Nephrotoxicity assessments of acetaminophen during zebrafish embryogenesis. Comp Biochem Physiol C Toxicol Pharmacol, 151(4): 480-486.

RAMADAN BK, SCHAALAN MF (2011) The renoprotective effect of honey on paracetamol-induced nephrotoxicity in adult male albino rats. Life Sci J, 8(3): 589-596.

SATIRAPOJ B, LOHACIT P, RUAMVANG T (2009) Therapeutic dose of acetaminophen with fatal hepatic necrosis and acute renal failure. J Med Assoc Thai, 90 (6): 1244-1247.

SURIYAKUMARI KVP, UDAYAKUMAR R, RUBA T (2016) Histological investigations on kidney of sildenafil citrate (edegra) treated albino mice. Int J Anat Res, 4(1): 1977-1980.

THE EUROPEAN AGENCY FOR THE EVALUATION OF MEDICINAL PRODUCTS (1999) Committee for veterinary medicinal products, Paracetamol, summary report. EMEA/MRL/551/99-FINAL. Available at: https://www.ema.europa.eu/en/documents/mrl-report/paracetamol-summary-report-committee-veterinary-medicinal-products_en.pdf 22-10-2019.

UMA N JR, FAKURAZI S, HAIRUSZAH I (2010) Moringa oleifera enhances liver antioxidant status via elevation of antioxidant enzymes activity and counteracts paracetamol-induced hepatotoxicity. Malays J Nutr, 16: 293-307