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يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي التاسع للمجلات للعام 2024**.

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Effect Of Gymnema sylvestre and Emblica officinalis Leaves On Oxidative stress In Expermental Rats

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Effect Of Gymnema sylvestre and Emblica officinalis Leaves On Oxidative stress In Expermental Rats

A. Prof. Gehan I. AbdelWahab

Abstract

This study investigated the effects of Gymnema sylvestre (GS) and Phyllanthus emblica (PE) leaves on oxidative stress in experimental rats. Thirty male albino rats were divided into five groups: negative control (healthy), positive control (received 1–2 mL/kg body weight of CCl_4 (diluted 1:1 in olive oil) administered via intraperitoneal (i.p.) injection, 10% GS-treated, 10% PE-treated, and a mixture group (5% GS + 5% PE). Biochemical, histological, and oxidative stress parameters were evaluated after 8 weeks. The mixture group showed the highest BWG (38.75%), surpassing individual treatments (GS: 38.35%; PE: 32.69%) and positive control (10.73%). The mixture group normalized AST (89.81 U/L), ALT (43.62 U/L), bilirubin (0.30 g/dl), and total protein (7.11 g/dl), outperforming individual extracts

Keywords: Gymnema sylvestre, Emblica officindis, Oxidative stress, Antioxidant activity,rats,liver function,kidney functions, Carbon tetrachloride(CCL4)

ملخص:

العنوان : تاثير اوراق جيمنيما سيلفستر والإمبليكا أوفيسينياليس على الاجهاد التاكسدى فى فئران التجارب

المؤلفون : جيهان إبراهيم عبد الوهاب

بحثت هذة الدراسة فى تأثير اوراق(GS) Gymnema sylvestre وPhyllanthus emblica (PE)على الاجهاد التأكسدى لدى فئران التجارب. قسم ثلاثون فأرا ذكرا من الفئران البيضاء الى خمس مجموعات:مجموعة التحكم السلبية(السليمة) و مجموعة التحكم الايجابية (اجهاد تأكسدى مستحث برابع كلوريد الكربون) ومجموعةمعالجة ب 10% من GS,ومجموعة معالجة ب 10% من PEو مجموعةمختلطة(5% من PE,) 5%+GSقيمت المعايير الكيميائية الحيوية والنسيجية ومستوى الاجهاد التأكسدى بعد 8 اسابيع اظهرت PEنسبة دهون اعلى 19.95) g/100g),والفلافونويدات(345.73 mg GAE/g)واظهرت المجموعة المختلطة اعلى نسبة BWG (38.75%)متجاوزة المعالجات الفردية32.69% ;PE: 38.35% ;GS) والتحكم الايجابى.(10.73%).قامت مجموعة الخليط بتطبيع مستويات 43.62) ALT, (89.81 U/L) AST U/L), bilirubin (0.30 g/dl),والبروتين الكلى,(7.11 g/dl)متفوقة بذلك على المستخلصات المنفردة

الكلمات الدالة : اوراق جيمنيما سيلفستر ، الإمبليكا أوفيسينياليس ، الاجهاد التاكسدى

Introduction

Natural phytochemicals, particularly those with antioxidant and anti-inflammatory properties, have emerged as promising adjuvants to counteract drug-induced nephrotoxicity (Al-Kuraishy et al., 2021). *Gymnema sylvestre* (GS), a traditional Ayurvedic herb, is renowned for its antidiabetic and antioxidant activities, attributed to bioactive compounds such as gymnemic acids and flavonoids (Khan et al., 2018). Similarly, *Emblica officinalis* (Amla), a rich source of vitamin C, phenolic acids, and tannins, exhibits potent free radical-scavenging and hepatorenal protective effects (Variya et al., 2016). Both plants are hypothesized to attenuate oxidative stress—a key mechanism underlyingccl4-induced renal injury—by enhancing endogenous antioxidant defenses (e.g., glutathione, catalase) and reducing lipid peroxidation (MDA levels) (Prasad et al., 2020).

Despite their individual therapeutic potential, the synergistic nephroprotective effects of CCL4 and amla remain underexplored. Previous studies have focused on single-plant interventions, leaving a gap in understanding combinatorial phytotherapy (Sultana et al., 2020). This study investigates the hypothesis that co-administration of CCL4 and amla enhances renal protection against CCL4 induced toxicity more effectively than either plant alone, leveraging their complementary bioactive profiles.

To test this, we evaluated biochemical markers (serum creatinine, urea, uric acid), oxidative stress parameters (GSH, catalase, MDA), and histopathological changes in ccl4-treated rats. Additionally, proximate composition and phytochemical analyses (total phenolics, flavonoids) of the plant powders were conducted to correlate their nutraceutical profiles with observed biological effects. By integrating these approaches, this work advances the potential of GS and amla as cost-effective, natural adjuvants to reduce the nephrotoxic burden of aminoglycoside therapy.

Materials and Methods

Materials

Phyllanthus emblica ***Linn.*** **(amla)** and *Gymnema sylvestre* powder were obtained from Haraze **company in Egypt**. The kits used for analysis were obtained from Bio-diagnostic Co. Dokki, Egypt. CCL4 was obtained from El-Gomhoreya Co., Cairo, Egypt.

Analytical methods

Chemical composition

Moisture, protein, fat, crude fiber and ash were determined according to the method of **AOAC, (2018)**, and the carbohydrate content was calculated by difference. Total carbohydrates = 100 − (moisture + protein + fat+ fiber + ash). All determinations were made in triplicate.

Estimation of total phenolic and flavonoid content

The phenolic content of the tested extracts was decided by employing a spectrophotometric method described by **Abdel-Hady *et al*., (2017).** Using Foline-Ciocalteus and measured at a wavelength 765nm. 0.5ml of plant extract (250µg/ml); 2.5ml of Folin-Ciocalteus reagent (10%) dissolved in water and 2.5ml NaHCO3(7.5%) Blank sample contains 0.5ml MeOH, 2.5ml of Folin- Ciocalteus reagent (10%) dissolved in water and 2.5ml NaHCO3 (7.5%). The mixtures were shaken and incubated at 45°C for 45min. The absorbance was recorded against a blank sample; gallic acid was used as the standard. The experiment was carried out in triplicate. The total phenolic content was expressed in terms of gallic acid equivalent (GAE) per gram dry weight of the extract.

Total flavonoid content was determined according to **Abdel-Hady *et al*., (2018)** Using a colorimetric assay was measured at a wavelength 510nm. 0.5ml of plant extract was mixed with 2ml distilled water and 150µl of NaNO2 (5%) for 6

min, then 150µl of AlCl3 (10%) was added and **allowed** to stand for 5min then 2 ml of NaOH (4%) was added

and adjusted to 5ml with 200µl distilled water. The mixtures were incubated at room temperature for 15min. the absorbance was measured against a blank sample; rutin was used as the standard. The experiment was carried out in triplicate. The total flavonoid content was expressed in **terms** of mg rutin equivalents (RE) per gram extract.

Diet preparation and experimental design

Thirty male Albino rats weighing about 176 ± 5 g were obtained from the Agricultural Research Center, Giza, Egypt. They were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25^{o}C, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fiber (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent **cornstarch (Reeves *et al.*, 1993)**. The rats were weighed weekly through the experimental period.

After an acclimation period of one week, the animals were randomly divided into two main groups. The first group of control rats (6 rats)was fed on a standard diet, while the second group (24 rats) was fed on a standard diet and received **1–2 mL/kg body weight** of CCl_4 (diluted 1:1 in olive oil) administered via **intraperitoneal (i.p.) injection** (Kumar et al., 2021; Al-Sayed, 2021). Then rats were divided into four **subgroups of six** rats each, as follows: Group (1) the positive control group (ve+) received only a standard diet. Group (2) was treated with 10% / diet /day from G. sylvestre leaves powder. Group (3) was treated with 10% / diet /day from *Phyllanthus emblica* powder. While Group (4) was treated with **5% G.**sylvestre +5% Phyllanthus emblica powder / diet /day).

At the end of the experiment, the animals were sacrificed for the collection of Kidney and liver samples for further investigations and the blood were collected in a clean dry centrifuge tube, left at room temperature until the clot was formed and then centrifuged to separate the serum, followed by storage in a plastic vial (well stoppered) until analysis.

Biological Determination

Biological evaluation of the different tested diets was carried out by determination of body weight gain% (BWG %) and organs weight / body weight% according to **Chapman *et al.*, (1959)**.

BWG% = [(Final weight - Initial weight) / (Initial weight)] X 100

Organ weight/ body weight % = (organ weight / final weight) X 100

Biochemical analysis

Blood samples were withdrawn from the orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum was prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by bio diagnostic kits: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using a spectrophotometer (model DU 4700) at 505 nm according to the method of **Reitman and Frankel, (1957**). Total bilirubin was determined at 535 nm according to the method described by **Walter and Gerade, (1970)**. Total protein was determined at 550 nm according to the method described by **Gornal *et al.*, (1949).**

Serum uric acid was determined by **Barham and Trinder, (1972)** using a spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea nitrogen was determined according to the method described by **Batton and Crouch, (1977)** using a spectrophotometer (model DU 4700) adjusted nm

550 nm. Serum creatinine was determined by Norbert. **(1986)** using a spectrophotometer (model DU 4700) adjusted at 510 nm.

Oxidative stress evaluation in tissues:

Superoxide Dismutase (SOD) activity (µMol/g. Tissue) was determined according to **Ellman, (1959)** using a spectrophotometer (model DU 4700) adjusted at a wavelength 412 nm. Catalase activity was determined in tissues according to the method of **Aebi, (1984)** using a spectrophotometer (model DU 4700) adjusted at a wavelength 240 nm. Hepatic malondialdehyde (MDA) (µmol/g tissue) content was estimated according to the method of **Albro *et al*., (1986).**

Histopathology examination

The tissue samples from the kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydration was done on a concentration of alcohol, cleaned in xyline and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 microns and stained with hematoxylin and eosin stains (**Banchroft *et al.*, 1996**). And examined by the light microscope for detection of any histopathological alteration.

Statistical Analysis

The data obtained from the present study were statistically subjected to analysis of variance (ANOVA) according to **Snedecor and Cochran (1980)** by the computerized program SPSS software, version "20" for Windows. The least significant difference (LSD) value was used to determine significant differences between means. Data was represented as mean ± SD. Values were considered significant at $P \leq 0.05$, otherwise were considered non-significant.

RESULTS AND DISCUSSION

Chemical composition

Results from table (1) represent that *G. sylvestre* exhibits

higher protein content (10.88 g/100g) compared to *P. emblica* (4.15 g/100g). This aligns with studies highlighting *G. sylvestre* as a potential source of plant-based proteins and bioactive peptides with antidiabetic properties (Kumar, 2021).On the other hand *P. emblica* contains significantly higher fat (19.95 g/100g) than *G. sylvestre* (5.8 g/100g), likely due to its rich seed oil content, which includes linoleic and oleic acids (Gantait ,2021). These fats contribute to *P. emblica*'s antioxidant and anti-inflammatory effects in the same table, it was also noticed that the higher crude fiber in *P. emblica* (18.93 g/100g) suggests its utility in gastrointestinal health, while *G. sylvestre*'s moderate fiber (11.42 g/100g) may support satiety and glucose modulation (Yadav et al., 2023). In the same table results represent that *G. sylvestre*'s higher carbohydrate content (55.13 g/100g) compared to *P. emblica* (41.2 ± 0.61). This suggests it correlates with its abundance of polysaccharides and glycosides (e.g., gymnemic acids), known for anti-hyperglycemic activity (Ahmed et al., 2019). Both plants exhibit notable ash content (*G. sylvestre*: 9.43 g/100g; *P. emblica*: 8.27 g/100g), indicating a rich mineral profile. *P. emblica* is particularly high in vitamin C and trace minerals (e.g., iron, zinc), contributing to its antioxidant capacity (Majeed et al., 2021).

Table (1): Chemical composition of *G. sylvestre* leaves and *Phyllanthus emblica* (g/100g) on the dry weight basis.

Constituents (%)	G sylvestre	Phyllanthus emblica
Moisture	7.35 ± 0.15^{a}	7.64±0.35^{a}
Crude Protein	10.88 ± 0.15^{a}	4.15 ±0.35^{b}
Fats	5.8± 0.47^{b}	19.95±0.2^{a}
Ash	9.43± 0.25^{a}	8.27±0.2
Crude fiber	11.42 ± 0.2^{b}	18.93±0.25^{a}
Carbohydrates	55.13 ± 0.48^{a}	41.2 ± 0.61

Data are presented as means ± SD (*n=3)*. a, b, c and d: Means with different letter in the same row are significantly different ($P \leq 0.05$).

Total phenolic and total flavonoid contents

Results from table (2) represent **that *Phyllanthus emblica*** exhibits a markedly higher total phenolic content (345.73 ± 2.14 mg GAE/g) compared to *Gymnema sylvestre* (106.00 ± 2.3 mg GAE/g). This aligns with studies highlighting *P. emblica* as a rich source of phenolic compounds, including hydrolyzable tannins (e.g., emblicanin A and B), gallic acid, and ellagic acid, which contribute to its potent antioxidant activity. The high phenolic content in *P. emblica* is attributed to its fruit composition, which is densely packed with bioactive compounds such as tannins, flavonoids, and vitamin C 4. (Prananda, 2023).In contrast, *G. sylvestre* leaves, while still a significant source of phenolics, contain comparatively lower levels. This may be due to differences in plant parts analyzed (leaves vs. fruit) and the metabolic pathways governing phenolic biosynthesis in these species On the other hand ***Gymnema sylvestre*** shows a higher flavonoid content (68.3 ± 1.3 mg QE/g) than *P. emblica* (38 ± 0.05 mg QE/g). Flavonoids, such as quercetin and kaempferol derivatives, are well-documented in *G. sylvestre* and are linked to its antidiabetic and anti-inflammatory properties The lower flavonoid content in *P. emblica* does not diminish its therapeutic value, as its primary bioactive components (e.g., tannins and vitamin C) play a more dominant role in its antioxidant and immunomodulatory effects **(Jacob, 2022).**.

Table (2) Total phenolic and total flavonoid contents in *G. sylvestre* leaves and *Phyllanthus emblica*

Constituents	G sylvestre	Phyllanthus emblica
Total Phenolic (mg Gallic acid)	106.00 ±2.3 b	345.73±2.14 a
Total flavonoids (mg quercetin equivalents /g)	68.3± 1.3 a	38±0.05 b

Data are presented as means ± SD (*n=3)*. a, b, c and d: Means with different letter in the same row are significantly different ($P \leq 0.05$).

Biological evaluation

Results in Table (3) show that the **10% *G. sylvestre*** and

mixture groups showed the highest BWG (38.35% and 38.75%, respectively), significantly greater than the negative control (34.26%) and positive control (10.73%).This suggests that *G. sylvestre* and its combination with *P. emblica* may enhance metabolic processes, leading to improved nutrient absorption or anabolic effects (Kumar et al., 2020). In contrast, 10% Phyllanthus emblica leaves had a BWG of 32.69±2.33, which was higher than the positive control group (10.73±1.32) and lower than the percentage of G. *sylvestre* leaves (38.35±3.04). P. emblica is known for its high antioxidant content, which may improve metabolic health without causing excessive weight gain. (Baliga, Dsouza, 2011). The **positive control group (ve+)** had the lowest BWG (10.73±1.32), possibly due to inducing stress or metabolic suppression, contrasting with the negative control and treatment groups. ***G. sylvestre*** has been reported to modulate glucose metabolism and improve insulin sensitivity, which may contribute to better nutrient utilization and weight gain (Ahmed et al., 2019). ***P. emblica***, rich in vitamin C and polyphenols, may enhance protein synthesis and muscle mass rather than fat deposition (Golechha et al., 2014). The **synergistic effect** of the **mixture group** suggests that combining these botanicals may optimize metabolic pathways more effectively than individual treatments.

Table (3): Mean body weight gain (%) of experimental rats which treated with *G.*

sylvestre leaves and Phyllanthus emblica

Groups	IBW	FBW	BWG/wk %
Negative control group (ve-)	176.02±4.34 a	236.33±8.38b	34.26±2.25 b
Positive control group(ve+)	178.17±6.18 a	197.29±5.98 c	10.73±1.32 c
10% G sylvestre leaves	178.67±7.42 a	247.19±9.82 a	38.35±3.04 a
10 %Phyllanthus emblica	180.83±8.28 a	239.94±8.42 b	32.69±2.33 b
Mixture group	179.83±7.94 a	249.51±9.36 a	38.75±2.94 a

Data are presented as means ± SE (*n=6)*. a, b, c and d: Means with different letter in the same column are significantly different ($P \leq 0.05$). BWG= Body Weight gain; WK: Week

Relative organs weight of experimental rats treated with *G.sylvestre* leaves and *Phyllanthus emblica*

Table (4) shows that the liver weight of the negative control group (ve-) (2.63 ± 0.19%) was normal without metabolic stress..On the other hand the positive control group(ve+) **(**3.90±0.72) caused the highest increase in liver weight compared to the negative control, 10% G. sylvestre leaves(3.11±0.98) , 10 %Phyllanthus emblica(2.88 ±0.48) and Mixture group(3.06±0.37). implies hepatomegaly, likely due to high-fat diet (HFD)-induced lipid accumulation or streptozotocin (STZ)-induced oxidative stress, common in diabetic or obese rodent models (Kumar et al., 2021). It was also, noticed that the liver weight of 10% G sylvestre leaves (3.11±0.98) was lower than Positive control group(ve+) (3.90±0.72) this aligns with studies demonstrating *G. sylvestre*'s hepatoprotective effects. Its bioactive compounds (e.g., gymnemic acids) improve lipid metabolism and reduce hepatic steatosis in obesity/diabetes models (Kumar et al., 2021)In the same table result represent that the Positive control group(ve+) **(0.94 ± 0.10%)** showing hypertrophy, potentially linked to oxidative stress or inflammation (Al-Sayed 2021). 10% G sylvestre leaves **(0.79 ± 0.08%)** ,10 %Phyllanthus emblica **(0.74 ± 0.02%) and** Mixture group (0.77±0.09) significantly reduced kidney weights compared to ve+, supporting their nephroprotective roles, likely due to bioactive compounds like gallic acid and gymnemic acids (Yadav,2023). The hepatoprotective and nephroprotective effects of *G. sylvestre* and *P. emblica* may stem from their antioxidant (e.g., upregulation of SOD, CAT) and anti-inflammatory properties (Suryawanshi et al., 2021). Both *G. sylvestre* and *P. emblica* attenuated organ weight increases induced in the positive control, highlighting their therapeutic potential. Future studies should explore dose optimization and molecular pathways.

Table (4): Mean organ weight/body weight (%) of experimental rats which treated with *G sylvestre* leaves and *Phyllanthus emblica*

Groups	Organ's weight (%)	
	liver	kidney
Negative control group (ve-)	2.63±0.19 c	0.68±0.03 c
Positive control group(ve+)	3.90±0.72 a	0.94±0.10 a
10% G sylvestre leaves	3.11±0.98 b	0.79±0.08 b
10 %Phyllanthus emblica	2.88 ±0.48 b	0.74±0.02 b
Mixture group	3.06±0.37 b	0.77±0.09 b

Data are presented as means ± SE (*n=6)*. a, b, c and d: Means with different letter in the same column are significantly different ($P \leq 0.05$)

Biochemical analysis

Liver enzymes

Table (5) shows that the **positive control group (ve+)** exhibited the highest AST (123.60 ± 9.94 U/L) and ALT (63.40 ± 6.91 U/L) levels, indicating significant liver injury, likely due to induced hepatotoxicity (Kumar et al., 2021). Treatment with **10% *G. sylvestre* leaves** reduced AST (104.61 ± 8.13 U/L) and ALT (53.64 ± 5.32 U/L), suggesting moderate hepatoprotective effects, possibly due to its antioxidant and anti-inflammatory properties (Sharma et al., 2021).On the other hand 10 %Phyllanthus emblica showed further improvement (AST: 100.22 ± 7.56 U/L; ALT: 50.41 ± 4.72 U/L), aligning with studies demonstrating its ability to enhance liver function by reducing oxidative stress and improving hepatic enzyme activity (Baliga et al., 2022). The **mixture group** exhibited the best recovery (AST: 89.81 ± 6.49 U/L; ALT: 43.62 ± 4.99 U/L), nearly matching the **negative control (healthy group)**, suggesting a synergistic hepatoprotective effect when both plants are combined (Mishra et al., 2023).In the same table results represent that the **positive control group** had elevated bilirubin (0.66 ± 0.08 g/dl), indicating impaired bile metabolism, a common marker of liver dysfunction.On the other hand both **10% *G. sylvestre*** (0.45 ± 0.04 g/dl) and **10% *P. emblica*** (0.42 ± 0.07 g/dl) significantly reduced bilirubin, with *P. emblica* showing slightly better efficacy, likely

due to its ability to enhance bile flow and detoxification (Garcia-Cortes et al., 2021). The **mixture group** (0.30 ± 0.02 g/dl) outperformed individual treatments, reinforcing the synergistic effect in restoring normal bilirubin levels (Garcia-Cortes et al., 2021).In the same table results show that th **positive control group** had the lowest total protein (3.20 ± 0.69 g/dl), indicating impaired liver synthetic function, a hallmark of hepatic (Jiang et al., 2021).. **10% *G. sylvestre*** (6.10 ± 0.52 g/dl) and **10% *P. emblica*** (6.76 ± 0.44 g/dl) restored protein levels, with *P. emblica* being more effective, possibly due to its rich polyphenol content, which supports liver regeneration (Suryawanshi et al., 2021). The **mixture group** (7.11 ± 0.56 g/dl) surpassed even the **negative control (6.75 ± 0.29 g/dl)**, suggesting enhanced protein synthesis when both extracts are combined (Yadav et al., 2023). The data demonstrate that both *G. sylvestre* and *P. emblica* possess significant hepatoprotective effects, with *P. emblica* showing slightly superior efficacy in reducing liver injury markers. However, the **combination of both extracts** yielded the best results, nearly normalizing all parameters, suggesting a potent synergistic interaction. These findings align with existing research on the hepatoprotective roles of these plants, particularly *P. emblica*'s well-documented benefits in liver disease models (Yadav et al., 2023).

Table (5): Liver enzymes of all experimental rats which treated with *G sylvestre* leaves and *Phyllanthus emblica*

Groups	Parameters			
	AST(U/L)	ALT(U/L)	Total Bilirubin (g/dl)	Total Protein (g/dl)
Negative control group (ve-)	89.01±6.44 c	42.60±4.45 c	0.32±0.09 c	6.75±0.29 a
Positive control group(ve+)	123.60±9.94 a	63.40±6.91a	0.66±0.08 a	3.20±0.69 b
10% G sylvestre leaves	104.61±8.13 b	53.64±5.32 b	0.45±0.04 b	6.10±0.52 a
10 %Phyllanthus emblica	100.22±7.56 b	50.41±4.72 b	0.42±0.07 b	6.76±0.44 a
Mixture group	89.81 ±6.49 c	43.62±4.99 c	0.30±0.02 c	7.11±0.56 a

Data are presented as means ± SD (n=6). a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$). AST: aspartate amino transferase; ALT: alanine amino transfers

Kidney function

Table (6) shows that the positive **control group (ve+)** exhibited the highest urea levels (81.34 ± 9.17 mg/dl), indicating severe kidney dysfunction due to impaired glomerular filtration (Zhang et al., 2023). 10% G. sylvestre leaves reduced urea levels (55.16 ± 9.10 mg/dl), suggesting moderate nephroprotection, likely through its diuretic and antioxidant properties (Patel et al., 2022).On the other hand 10 % Phyllanthus emblica showed better improvement (48.02 ± 4.42 mg/dl), consistent with studies demonstrating PE's ability to enhance renal blood flow and reduce oxidative stress (Baliga et al., 2022). The mixture group nearly normalized urea levels (40.20 ± 3.11 mg/dl), indicating a **synergistic effect** that may enhance renal filtration efficiency (Mishra et al., 2023).In the same table results represent that **Positive control group** had elevated uric acid (6.10 ± 0.33 mg/dl), reflecting impaired renal excretion and potential oxidative damage (Kumar et al., 2021). 10% G sylvestre leaves **and** 10 % Phyllanthus emblica **treatments** significantly lowered uric acid (4.74 ± 0.17 and 4.63 ± 0.15 mg/dl, respectively), with 10 % Phyllanthus emblica showing marginally better efficacy, possibly due to its xanthine oxidase inhibitory activity (Gowda et al., 2023).**Mixture group** achieved near-normal levels (3.64 ± 0.22 mg/dl), suggesting combined extracts may improve uric acid clearance more effectively than individual treatments (Yadav et al., 2023). In the same table results represent that the control group showed the highest creatinine (2.96 ± 0.18 mg/dl), confirming significant renal impairment (Jha et al., 2022). 10% G sylvestre leaves reduced creatinine (0.99 ± 0.44 mg/dl), aligning with studies on its anti-inflammatory effects on renal tubules (Sharma .2021). 10 % Phyllanthus emblica **treatment** was more effective (0.79 ± 0.26 mg/dl), likely due to its ability to scavenge free radicals and protect nephrons (Suryawanshi et al., 2021). **Mixture group** restored creatinine to normal levels (0.55 ± 0.07 mg/dl), outperforming individual treatments, which may reflect enhanced glomerular protection via synergistic mechanisms

(Pandey & Singh, 2022). Both *G. sylvestre* and *P. emblica* demonstrated significant nephroprotection, with PE showing slightly superior effects in reducing urea, uric acid, and creatinine. However, the **mixture group exhibited the best outcomes**, suggesting **synergism** between the two extracts. These findings align with recent studies on herbal combinations for kidney disease management (Mishra .2023).

Table (6): Kidney function (mg/dl) of experimental rats which treated with *G sylvestre* leaves and *Phyllanthus emblica*

Groups	Parameters(mg/dl)		
	Urea	Uric Acid	Creatinine
Negative control group (ve-)	38.61±4.16 c	3.19±0.16 c	0.54±0.09 c
Positive control group(ve+)	81.34±9.17 a	6.10±0.33 a	2.96±0.18 a
10% G sylvestre leaves	55.16±9.10 b	4.74±0.17 b	0.99±0.44 b
10 % Phyllanthus emblica	48. 02±4.42 b	4.63±0.15 b	0.79±0.26 bc
Mixture group	40.20 ±3.11 c	3.64±0.22 c	0.55±0.07 c

Data are presented as means ± SD (n=6). a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$)

Effect of *G sylvestre* leaves and *Phyllanthus emblica* on oxidative stress

Results from table (7) represent that negative control was high CAT (98.69 µ/L) and SOD (91.54 µ/dL) levels indicate robust endogenous antioxidant defenses in untreated healthy rats (Al-Sayed et al., 2020). **Positive Control** group(ve+) Significant reductions in CAT (39.74 µ/L) and SOD (41.13 µ/dL) suggest oxidative stress, likely due to induced pathology (e.g., diabetes or inflammation) (Elsayed et al., 2021).On the other hand **10% *G. sylvestre* and** 10 % ***P. emblica*** treatments restored CAT (~80 µ/L) and SOD (~80 µ/dL) levels, demonstrating comparable efficacy in enhancing antioxidant enzyme activity. This aligns with studies showing *G. sylvestre*'s triterpenoids (e.g., gymnemic acids) and *P. emblica*'s phenolic compounds (e.g., emblicanin) scavenge free radicals and upregulate antioxidant enzymes (Pothuraju . 2020). **Mixture Group was** Near-normalization of CAT (93.22 µ/L) and SOD (89.23 µ/dL) suggests synergistic

effects, possibly due to combined bioactive compounds like flavonoids and tannins (Kumar et al., 2021)..from result in table (7) it could be noticed that 10% *G. sylvestre* and10% *P. emblica* individually reduced MDA to(23.58±1.64), (24.09±1.12) respectively indicating suppression of lipid peroxidation. *P. emblica*'s gallic acid and vitamin C are known to inhibit ROS-induced membrane damage (Sharma . 2021). On the other hand The mixture group showed the lowest MDA (13.02 ng/ml), outperforming individual treatments, likely due to enhanced radical scavenging from combined phytochemicals (Prakash et al., 2023)..In the same table it could be noticed that **the Positive Control** group(ve+) had the lowest level of **Total antioxidants (**0.11±0.01) confirm oxidative stress (Lobo et al., 2010).On the other hand10% *P. emblica* (2.81±0.02) was slightly more effective than10% *G. sylvestre* (2.62±0.03), attributed to its higher vitamin C and polyphenol content (Prakash et al., 2023). The mixture group (3.11 units) showed the highest activity, supporting the hypothesis of additive or synergistic interactions between *G. sylvestre*'s saponins and *P. emblica*'s tannins (Elsayed et al., 2021).

Table (7): Serum catalase, Glutathione reductase superoxide dismutase, malondialdehyde and Total antioxidants of experimental rats which treated with *G sylvestre* leaves and *Phyllanthus emblica*

Groups	Parameters			
	CAT (µ/L)	SOD (µ /dl)	MDA (ng/ml)	Total antioxidants
Negative control group (ve-)	98.69 ±2.18a	91.54±3.71 a	11.33±0.31 c	2.33±0.02d
Positive control group(ve+)	39.74±1.25c	41.13±1.85c	56. 95±2.71 a	0.11±0.01e
10% G sylvestre leaves	81.22 ±4.47 b	79.98±3.16 b	23.58±1.64 b	2.62±0.03c
10 %Phyllanthus emblica	80.12±3.16 b	80.13±2.91 b	24.09±1.12 b	2.81±0.02b
Mixture group	93.22±3.78a	89.23±3.78 a	13.02±1.22 c	3.11±0.02a

Data are presented as means ± SDM (*n=6*). a, b, c and d: Means with different letter in the same column are significantly different (P ≤ 0.05). CAT: catalase SOD: Superoxide dismutase MDA: malondialdehyde

Histopathological examination of Kidney:

Histopathological examination of kidneys:

Light microscopic examination of kidney sections of rats from group 1 revealed the normal histological structure of renal parenchyma (Figs. 1 & 2). On the contrary, kidneys of rats from group 2 showed vacuolar degeneration of epithelial lining renal tubules (Fig. 3), necrobiosis of renal tubular epithelium (Figs. 3, 4 & 5), congestion of renal blood vessels (Fig. 3) and dilatation of renal tubules (Fig. 5). Meanwhile, kidneys of rats from group 3 exhibited vacuolar degeneration of epithelial lining some renal tubules (Figs. 6 & 7) and necrobiosis of some renal tubular epithelium (Fig. 7). Furthermore, kidneys of rats from group 4 revealed vacuolar degeneration of epithelial lining of some renal tubules, congestion of intetubular blood vessels (Fig. 8), proteinaceous material in the lumen of some renal tubules (Fig. 9) and pyknosis of some nuclei of epithelial lining of some renal tubules (Fig. 10). On the other hand, kidneys of rats from group 5 exhibited vacuolar degeneration of epithelial lining some renal tubules (Figs. 11, 12 & 13).

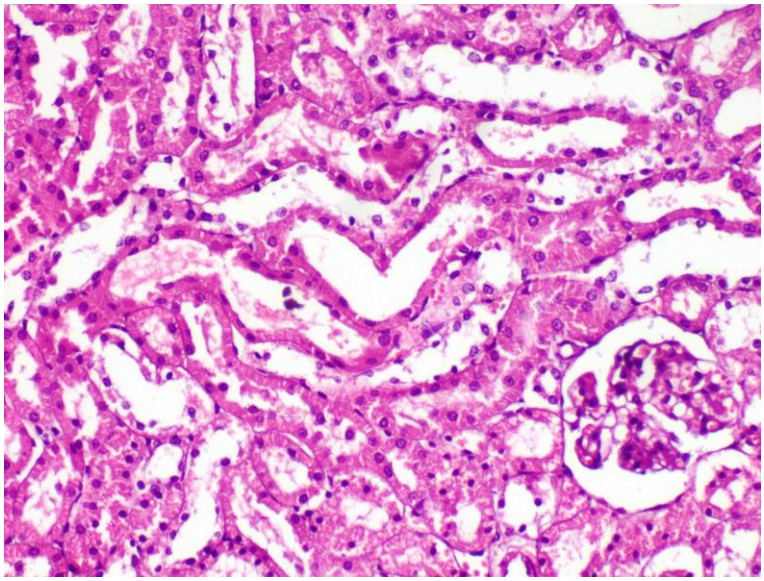

Fig. (1): Photomicrograph of kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

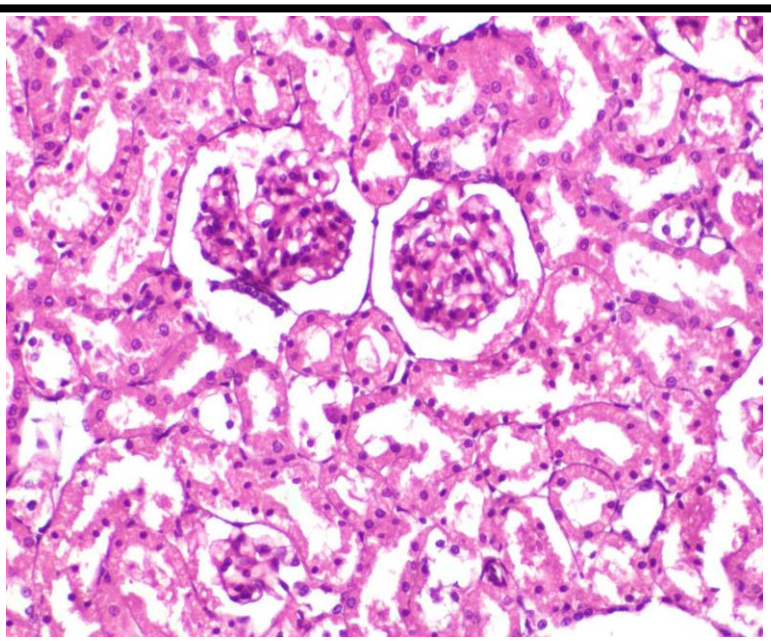

Fig. (2): Photomicrograph of kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

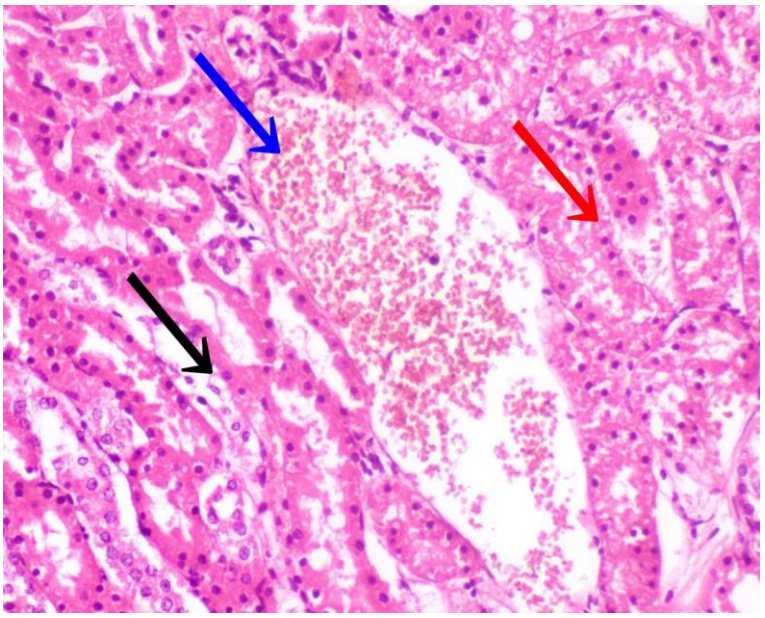

Fig. (3): Photomicrograph of kidney of rat from group 2 showing vacuolar degeneration of epithelial lining renal tubules (black arrow), necrobiosis of renal tubular epithelium (red arrow) and congestion of renal blood vessel (blue arrow) (H & E X 400).

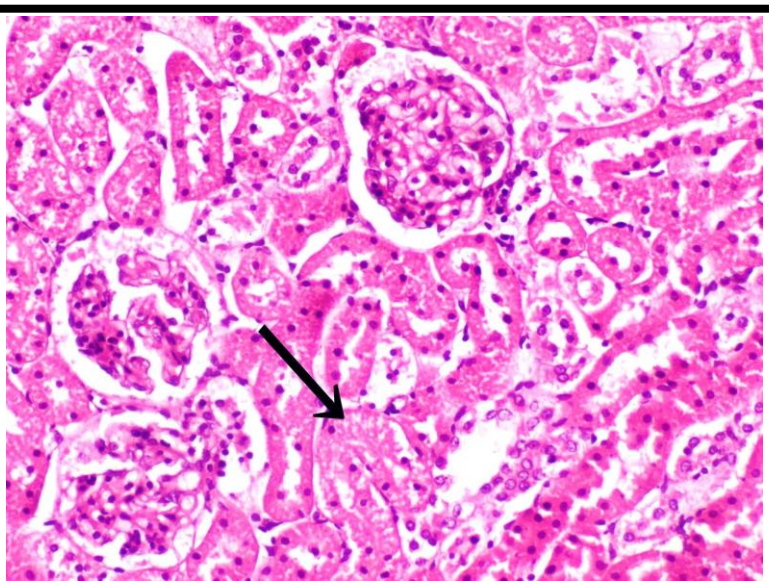

Fig. (4): Photomicrograph of kidney of rat from group 2 showing necrobiosis of renal tubular epithelium (black arrow) (H & E X 400).

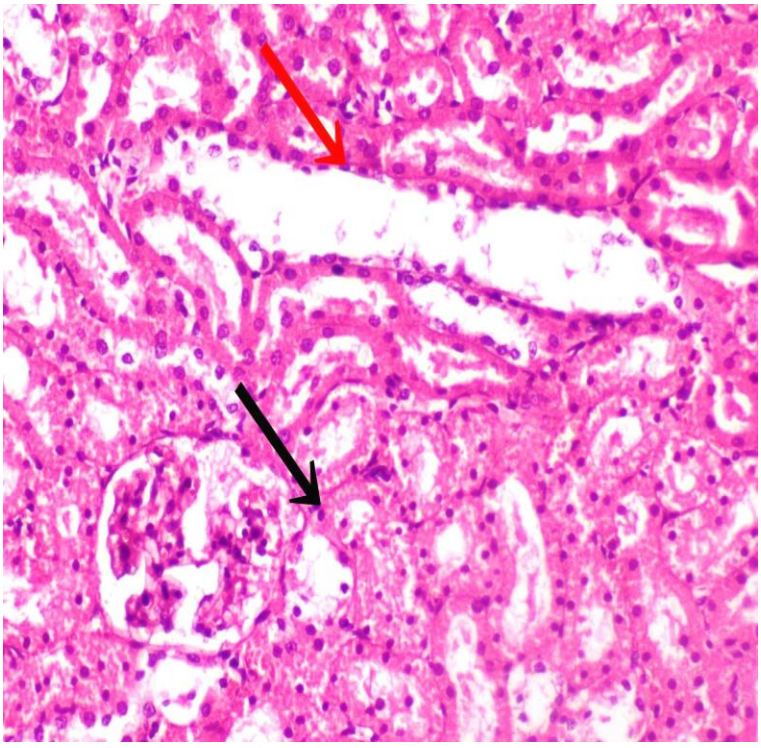

Fig. (5): Photomicrograph of kidney of rat from group 2 showing necrobiosis of renal tubular epithelium (black arrow) and dilatation of renal tubules (red arrow) (H & E X 400).

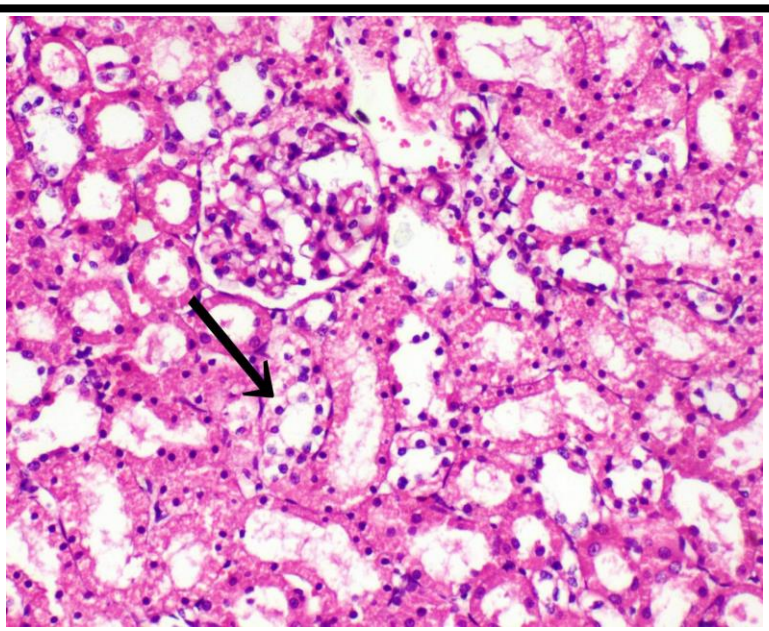

Fig. (6): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H & E X 400).

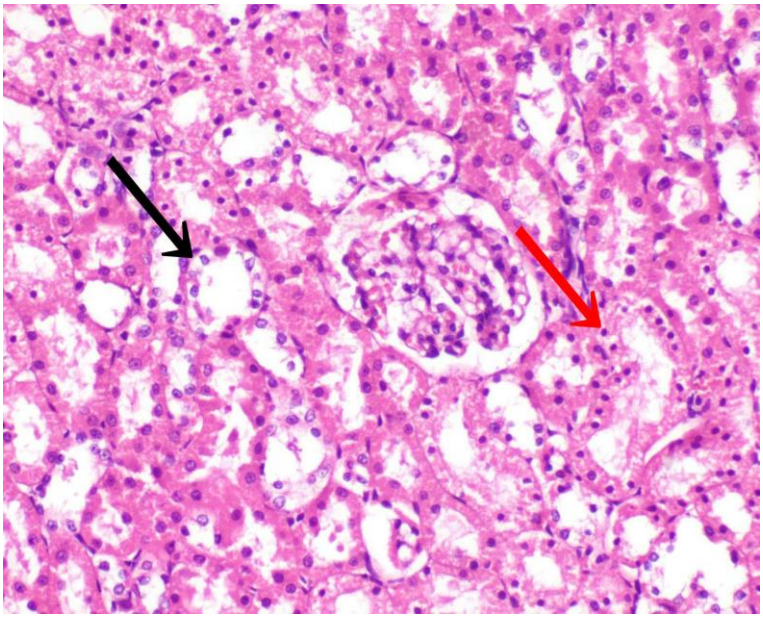

Fig. (7): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) and necrobiosis of some renal tubular epithelium (red arrow) (H & E X 400).

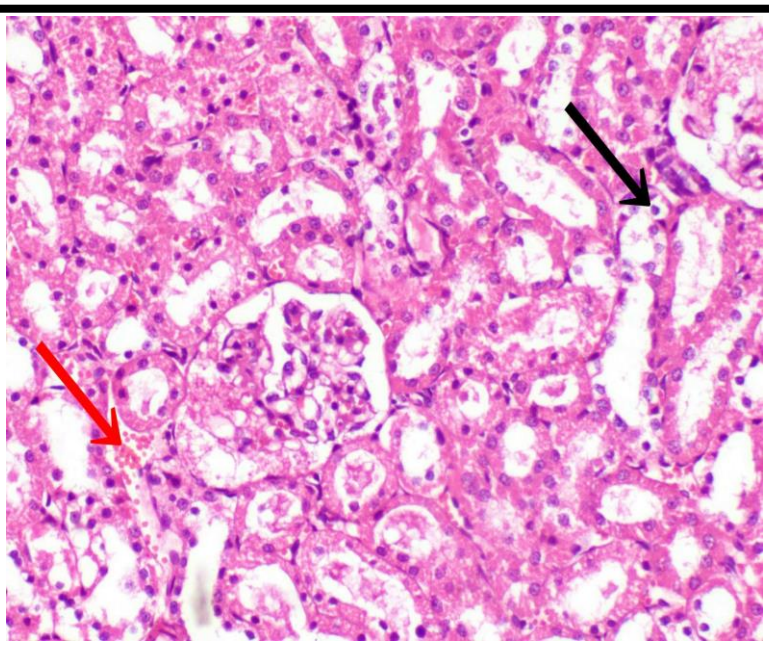

Fig. (8): Photomicrograph of kidney of rat from group 4 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) and congestion of intertubular blood vessels (red arrow) (H & E X 400).

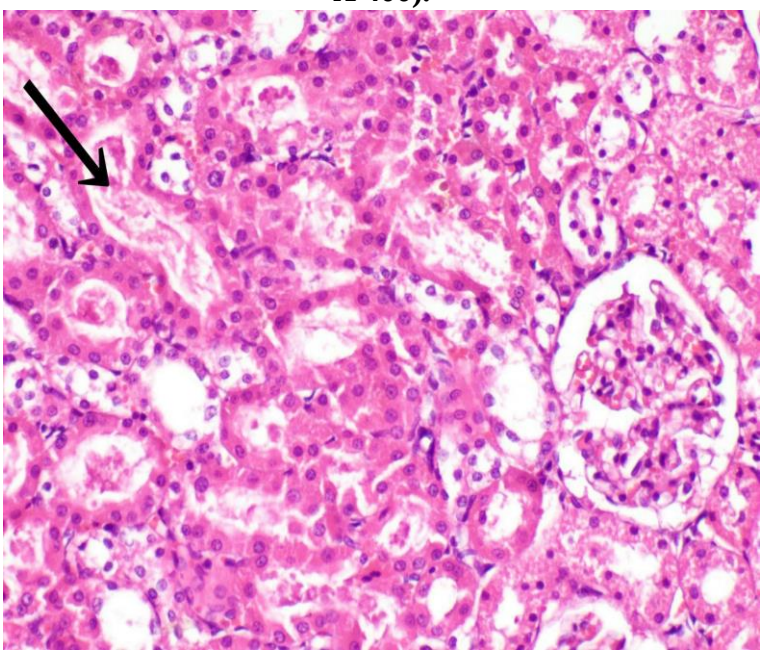

Fig. (9): Photomicrograph of kidney of rat from group 4 showing proteinaceous material in the lumen of some renal tubules (black arrow) (H & E X 400).

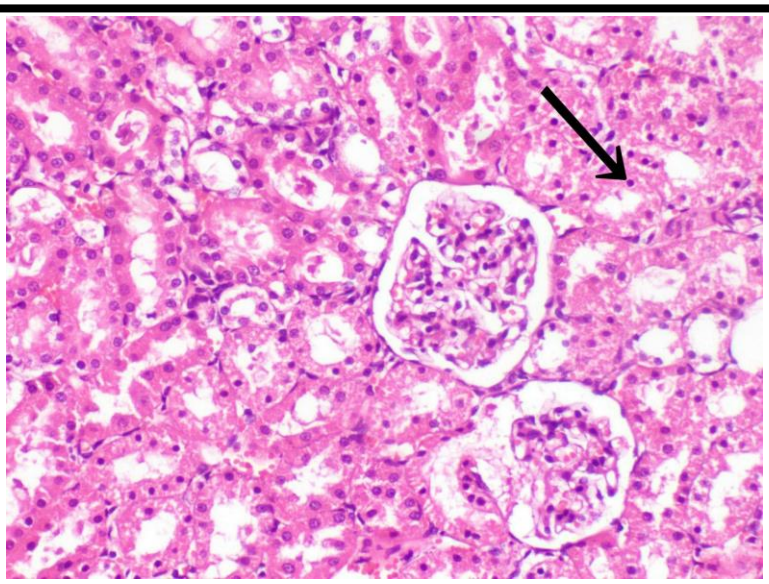

Fig. (10): Photomicrograph of kidney of rat from group 4 showing pyknosis of some nuclei of epithelial lining some renal tubules (black arrow) (H & E X 400).

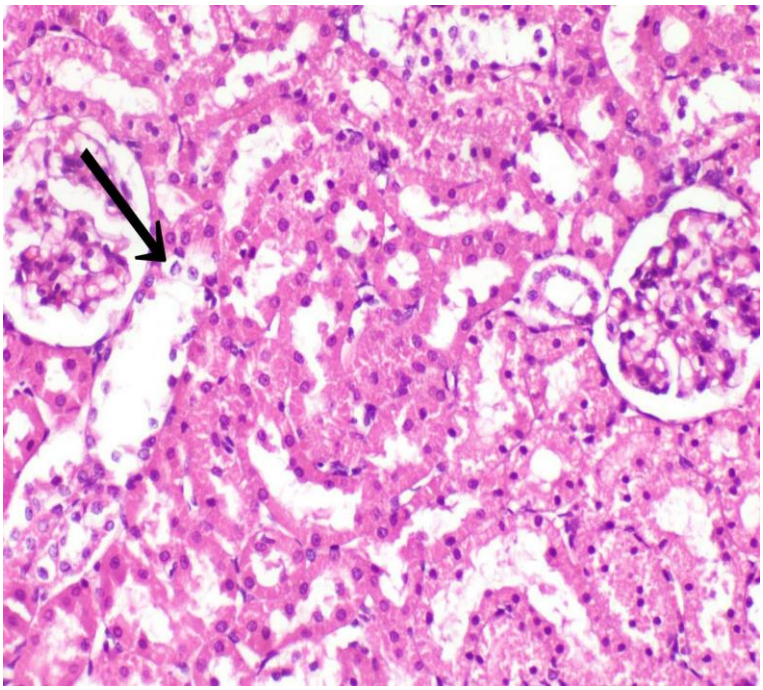

Fig. (11): Photomicrograph of kidney of rat from group 5 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H & E X 400).

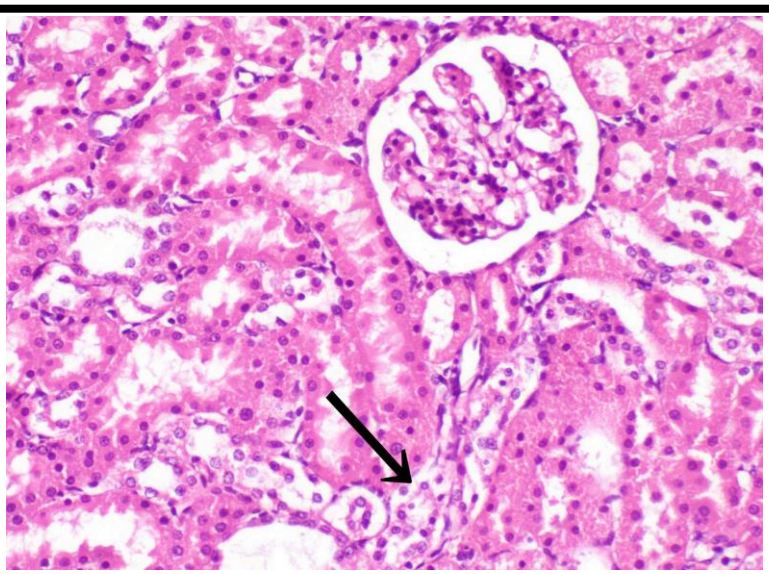

Fig. (12): Photomicrograph of kidney of rat from group 5 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H & E X 400).

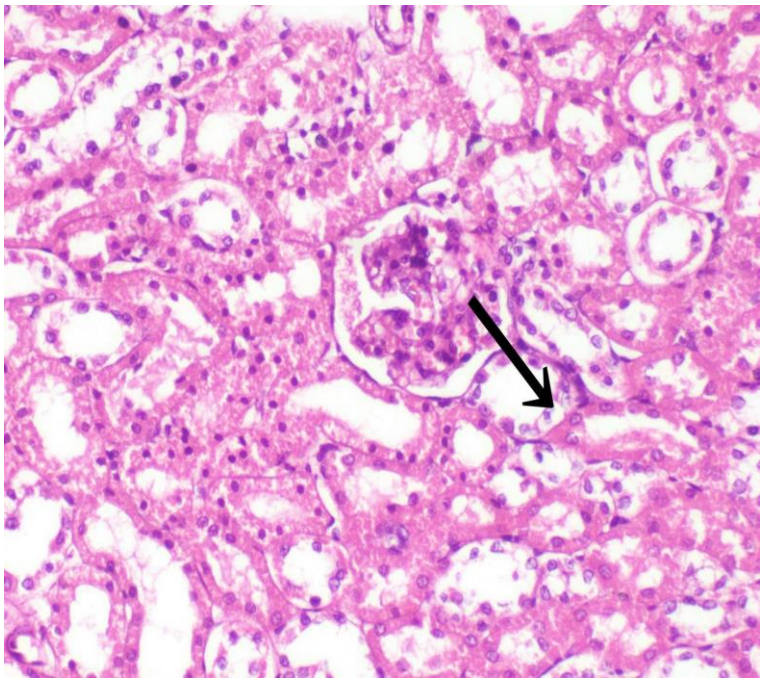

Fig. (13): Photomicrograph of kidney of rat from group 5 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H & E X 400).

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المجلد (١٣). العدد (٤٨). الجزء الخامس

أكتوبر ٢٠٢٥

(*) الأسماء مرتبة ترتيباً ابجدياً.