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Abstract

The current study looked at the preventive effects of aqueous extracts of Psidium guajava leaves (EEPGL) and Artemisia annua on gentamicin-induced nephrotoxicity in rats. Thirty-six male albino rats were separated into two major groups as follows: (The first core group of six rats) This primary group was fed a basal diet and served as the control group. (The second major group (30 rats): This main group was fed a basal diet and administered gentamicin (GM) intraperitoneally at a dose rate of 80 mg/kg body weight of rat for 8 consecutive days to induce nephrotoxicity. The rats in the second major group (30 rats) were then divided into into five subgroups (each group had six rats) as follows:Group 1 was fed on B.D. and kept as the control (+ve) group.Group 2: fed on B.D. and treated with 250 mg/kg b.w. of guava leaf aqueous extract daily; Group 3: fed on B.D. and treated with 500mg/kg b.w. of guava leaf aqueous extract daily; Group 4: fed on B.D. and treated with Artemisia extract orally 100 mg/kg b.w. daily; and Group 5: fed on B.D. and treated with Artemisia extract orally 200 mg/kg b.w. daily. The current studies demonstrated that guava 500 mg/kg normalized AST, ALT, and ALP similarly to healthy control, while Artemisia 200 mg/kg exhibited comparable efficacy.Guava 500 mg/kg reduced serum urea, uric acid and creatinine, while Artemisia 200 mg/kg achieved near-normal levels of urea, uric acid and creatinine. Both *Psidium guajava* and *Artemisia annua* extracts demonstrated significant nephroprotective and hepatoprotective effects against gentamicin-induced toxicity. Higher doses (500 mg/kg guava, 200 mg/kg *Artemisia*) were most effective, restoring biochemical, histological, and antioxidant parameters to near-normal levels.

This study concluded that: Aqueous extracts of *Psidium guajava* and *Artemisia annua* leaves effectively mitigated gentamicin-induced nephrotoxicity in rats. Higher doses (500 mg/kg guava and 200 mg/kg Artemisia) showed the greatest protective effects, restoring kidney and liver function markers, as well as antioxidant and histological parameters, to near-normal levels.

***Keywords*:** *Psidium Guajava* ,*Artemisia annua,* ,Nephrotoxic.

ونبات الشيح ضد (Artemisia annua) أُجريت هذه الدراسة لبحث التأثيرات الوقائية للمستخلص المائى لأوراق الجوافةو السُمية الكلوية التي يسببها الجنتاميسين في الفئران. تم تقسيم ستة وثلاثين فأرًا ذكورًا من سلالة ألبينو إلى مجموعتين رئيسيتين على النحو التالي: **المجموعة الرئيسية الأولى (٦ فئران** تم تغذيتها على النظام الغذائي الأساسي واعتُبرت مجموعة تحكم سلبية . **المجموعة الرئيسية الثانية (٣٠ فأرًا** تم تغذيتها على النظام الغذائي الأساسي وحُقنت بالجنتاميسين.(BD) (−ve)

عن (GM)تم تغذيتها على النظام الغذائي الأساسي وحُقنت بالجنتاميسين (GM) بجرعة ٨٠ ملجم/كجم من وزن الجسم عن طريق الحقن البريتوني لمدة ٨ أيام متتالية لتحفيز السُمية الكلوية بعد ذلك، قُسمت فئران المجموعة الثانية إلى خمس مجموعات فرعية (٦ فئران لكل مجموعة **المجموعة الفرعية ١مجموعة تحكم ايجابية تغذية على النظام الاساسى. المجموعة الفرعية ٢ تغذية على الغذاء الاساسى مع** معاملة الفئران بمستخلص أوراق الجوافة المائي بجرعة ٢٥٠ ملجم/كجم يوميً المجموعة الفرعية ٣تغذية على النظام الاساسى مع معاملة الفئران بمستخلص أوراق الجوافة المائي بجرعة ٥٠٠ ملجم/كجم يوميًا: **المجموعة الفرعية٤ تغذية على النظام الاساسى**مع معاملة الفئران بمستخلص الشيح بجرعة ١٠٠ ملجم/كجم يوميًا عن طريق الفم. **المجموعة الفرعية٥ تغذية على النظام الاساسى** مع معاملة الفئران بمستخلص الشيح بجرعة ٢٠٠ ملجم/كجم يوميًا عن طريق الفم وكانت النتائج كالتالى:

أظهرت جرعة **الجوافة ٥٠٠ ملجم/كجم** قدرة على تطبيع مستويات إنزيمات الكبد الى مستويات قريبة من المجموعة السلبية. حققت جرعة **الشيح ٢٠٠ ملجم/كجم** فعالية مشابهة في تحسين وظائف الكبد. خفضت جرعة الجوافة ٥٠٠ ملجم/كجم مستويات اليوريا وحمض اليوريك والكرياتينين في الدم، بينما أوصلت جرعة الشنان ٢٠٠ ملجم/كجم هذه المؤشرات إلى مستويات شبه طبيعية. أظهر كل من مستخلصي الجوافة والشانان تأثيرات واقية للكلى والكبد ضد سُمية الجنتاميسين، مع تفوق الجرعات العالية (٥٠٠ ملجم/كجم للجوافة و٢٠٠ ملجم/كجم للشانان) في استعادة المؤشرات الكيميائية الحيوية والأنسجة ومستويات مضادات الأكسدة إلى الحالة الطبيعية تقريبًا.ومن ذلك يتضح ان تمتلك المستخلصات النباتية (الجوافة والشيح) فعالية كبيرة في الحماية من السُمية الكلوية والكبدية الناجمة عن الجنتاميسين، مع تفوق الجرعات الأعلى في تحقيق الاستعادة الشبه كاملة للوظائف الحيوية.

Introduction

The kidney is an essential organ in the body that plays an important part in execution. Drug-induced nephrotoxicity is a major cause of renal failure. Hydroxyl radicals contribute to the development of gentamicin nephrotoxicity. Gentamicin can reduce Na(+)-K(+)-ATPase activity and DNA synthesis in rats' proximal tubules, resulting in kidney dysfunction; this injury may be related to the reactive

oxygen metabolites produced by gentamicin. Renal cortical mitochondria produce reactive oxygen metabolites, which cause kidney damage (Nephrol Dial Transplant 1994)

` Guava leaves (Psidium guajava, L.) have long been used in traditional medicine to cure a variety of ailments. Díaz-de-Cerio et al. (2017) found that extracts from the leaves can treat many illnesses, both in vivo and in vitro. Guava (Psidium guajava, L.) is a nutrient-rich tree that contains alkaloids, carotenoids, anthocyanins, vitamin C, and triterpenes (Jayachandran et al. 2018). Recent studies have shown that an ethanolic extract of Psidium guajava Leaves has kidney-protective properties. (Mohan et al. 2014).

Artemisia is typically found in the northern hemisphere, at mid to high latitudes in arid and semi-arid regions. Central Asia is home to a number of Artemisia species (Hayat et al., 2009), 25 of which are found in Pakistan, with the majority of them in the northern regions, namely the Himalaya, Hindu Kush, and Karakorum mountain ranges in the Abbottabad, Chitral, Gilgit, Kashmir, Rawalpindi, Skardu, and Swat districts (Abdul et al., 2010). This plant, known locally as Afsantin or Afsantin jari, is used to treat malaria, with the leaves being used to treat coughs, common colds, diarrhea, and fevers (Hayat et al., 2009). The principal antioxidant phenolic chemicals discovered in Artemisia species include gallic acid, catechin, vanillic acid, caffeic acid, epicatechin, ferulic acid, sinapic acid, rutin, quercetin, luteolin, gentisic acid, chlorogenic acid, isoquercitrin, quercetol, kaempferol, and apigenin. (Odhav et al., 3007Phenolic compounds are widely distributed in plants and have been connected to the prevention of certain diseases in which oxidative stress is a prominent factor (Krishnan et al. 2009). Wormwood's antioxidant capacity correlates positively with its phenolic and flavonoid levels. (Jimoh et al., 2010).

The purpose of this study is to assess the potential effects of an aqueous extracts of Psidium guajava leaves (EEPGL) and Artemisia annua on nephrotoxicity in rats.

Materials And Methods

Material

The dried leaves of guava (Psidium guajava) and Artemisia annua were obtained from the Herbs and Spices Company in Obour City, El Kalubia Egypt. The kits utilized for analysis were obtained from Bio-diagnostic Co., Dokki, in Egypt. Gentamicin (GM) was purchased from the Memphis Company for Pharmaceutical and Chemical Industries in Cairo, Egypt. Casein (85% protein), cellulose, dextrose, choline chloride, DL-methionine, vitamins, and salt were obtained from Cairo Company Chemical Trading in Cairo, Egypt.Corn oil was purchased from a nearby grocery. Cornstarch was provided by Starch and Glucose Co. Helwan in Cairo, Egypt

Preparation of Artemisia and *guajava* Leaf Aqueous Extract

Artemisia leaves were ground, washed and put in boiling water (5g/l) for 15-20min. The aqueous extracts were filtrated using Whatmann filter paper and directly used for the experiment. The extract should be prepared daily **(Barkat et al., 2015).**The guava leaves were ground into powdered form using a laboratory blender and weighed 400g. The powdered fruit was dissolved in 4L of distilled water and kept for 48hours, after which it was filtered using filter paper **(Hyacinth Tochukwu Eze et al., 2024)**

Animals:

Thirty-six male albino rats of the Sprague Dawley strain (180 ± 10g) were collected from the animal colony at Helwan Farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt

Chemical Composition

Guava leaves were tested for moisture, ash, crude protein (Kieldhl, TN × 6.25), fat (Soxhlet using hexane). Carbohydrates were calculated using the following formula: % Carbohydrate = 100-(% Moisture+% Protein+% Fat+% Ash+% Fiber).

Determination of Total Phenolic Content

The total phenolic concentration in the extract was measured using the Folin-Ciocalteu reagent and external calibration with gallic acid, following the method of Chen et al. (2007). To proceed, shake 100 µl of diluted sample for 5 minutes with 750 µl of diluted Folin-Ciocalteu reagent (x10). Then, 750 µL of 20% Na_2CO_3 was added. The resulting mixture was agitated, then incubated at room temperature for 90 minutes. The absorbance was measured at 750 nm using a UV-VIS Bueco S-22 spectrophotometer. Gallic acid was used to create a standard curve (0.05-0.5 mg/ml, y=0.011x+0.04, R2=0.987, where y represents absorbance and x represents standard concentration). The phenolic content was calculated as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Determination of Total Flavonoids Content

The extract's total flavonoid content was determined using the method developed by Djeridane et al. (2006), which is based on the formation of a flavonoid-aluminum complex. A diluted sample of around 1 ml was combined with a methanolic solution containing 2% aluminum trichloride $AlCl_3$. After 15 minutes of incubation at room temperature, the absorbance of the reaction mixture at 430 nm was measured with a UV-VIS Bueco S-22 spectrophotometer. The standard curve was created with quercetin (0.05-0.5 mg/ml, y=0.036x+0.275, R2=0.97, where y is absorbance and x is standard concentration). The results were expressed as milligram quercetin equivalents (QE) per gram extract.

Experimental Design

Male albino rats of the Sprague Dawley strain weighing 180 ± 5 g were housed in clean metabolic cages. The rat's adaptation lasted one week before the start of

the experiment. According to Reeves et al. (1993), the rats were fed a basal diet (B. D.) and divided into two groups as follows: The first major group (6 rats). This major group was fed a basal diet and kept as a control (negative) group. The second main group (30 rats): This group was fed a Basal diet and administered gentamicin (GM) intraperitoneally at a dose rate of 80 mg/kg body weight of rat for 8 consecutive days to induce nephrotoxicity (Reddy et al., 2011). After that, the rats in the second major group were divided into five subgroups (each group comprised of six rats), as follows:

Group 1: Received the basal diet (B.D.) and served as the positive control group.

Group 2: Received the basal diet (B.D.) and also designated as a positive control group.

Group 3: Received the basal diet along with a daily oral dose of guava leaf aqueous extract at 250 mg/kg body weight.

Group 5: Received the basal diet along with a daily oral dose of guava leaf aqueous extract at 500 mg/kg body weight.

Group 6: Received the basal diet and was administered a daily oral dose of Artemisia extract at 100 mg/kg body weight.

Group 7: Received the basal diet and was administered a daily oral dose of Artemisia extract at 200 mg/kg body weight.

Blood sampling

At the end of the experiment, rats were fasted for 12 hours before being sacrificed under ether anesthesia. Blood samples were obtained from the aorta vein and placed in clean, dry centrifuge tubes. They were kept at room temperature for 15 minutes before being refrigerated for 2 hours and spun for 10 minutes at 3000 rpm to separate serum. Using a Pasteur pipette, serum was gently aspirated and transferred to dry, clean Wassermann tubes. The sample was kept at -20ºC until analysis.

Biological evaluation

The biological evaluation of the three diets was performed by calculating the body weight gain percentage (BWG%) and organ weight as a percentage of total body weight according to Chapman et al., (1959), using the following equations: - BWG% = [(final weight g - initial weight g) / initial weight g× 100]. To calculate relative organ weight (ROW%), divide the organ weight by the final weight and multiply by 100.

Determination of liver functions:

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured calorimetrically using a spectrophotometer (model DU 4700) at 505 nm using the Reitman and Frankel technique (1957). Doumas' (1971) approach was used to determine albumin levels. The total bilirubin level was measured at 535 nm using the method given by Walter and Gerade

(1970). The activity of alkaline phosphatase (ALP) was assessed calorimetrically at 510 nm using a spectrophotometer (model DU 4700) following the method of Belfield and Goldberg (1971).

Determination of the kidney functions:

Barham and Trinder (1972) measured serum uric acid with a spectrophotometer (model DU 4700) set to 510 nm. The serum urea nitrogen was measured using a spectrophotometer (model DU 4700) calibrated to 550 nm, as described by Batton and Crouch (1977). Tietz (1986) measured serum creatinine with a spectrophotometer (Model DU 4700) set to 510 nm.

Antioxidant enzymes

Glutathione peroxidase (GPx), serum catalase (CAT) activity, superoxide dismutase (SOD) activity, malondialdehyde (MDA), and total antioxidants were measured using the methods of Paglia and Valentine (1967), Aebi (1984), Nishikimi et al. (1972), Ohkawa et al. (1979), and Koracevic et al. (2001), respectively.

Henry (1974) methods were used to determine sodium (Na). Potassium (K) was Glutathione peroxidase (GPx), serum catalase (CAT) activity, superoxide dismutase (SOD) activity, malondialdehyde (MDA), and total antioxidants were determined using the methods of Paglia and Valentine (1967), Aebi (1984), Nishikimi et al. (1972), Ohkawa et al. (1979), and Koracevic et al. (2001), respectively.

Sodium (Na) was determined using the procedures developed by Henry (1974). Potassium (K) was determined using Trinder's (1951) techniques.

Histopathology investigation

Following removal from the animals, the rat kidney was immediately fixed in 10% buffered neutral formalin. Fixed tissues were then processed for histopathology examinations, as reported by **Bancroft** et al. (1996).

Statistical Analysis

Statistical analysis was performed using the one-way analysis of variance (ANOVA) test, followed by the Duncan test, using the statistical package for the social sciences (SPSS) version 16. The results were expressed as mean ± SD. Differences between means at $p < 0.05$ are deemed significant (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Table 1 displays chemical compositions for Artemisia annua and Guajava leaves. Moisture, crude protein, lipids, ash, crude fiber, and carbohydrates are calculated as percentages of dry weight. Results represent that the *Artemisia annua* per 100g contained(6.93^{a} ± 0.15) moisture, (25.43 ± 0.15) Crude protein, (6.67 ± 0.47) fats, (7.32 ± 0.25) ash,(9.5 ± 0.2) crude fiber and Carbohydrates (44.15 ± 0.48).. In the same table, Results represent that the guajava leaves per

100g contained (5.53±0.35) moisture, (3.13 ±0.35) Crude protein, (1.1^{b}±0.2) fats, (3.27^{b}±0.2) ash,(12.23±0.25) Crude fiber and Carbohydrates (74.73 ± 0.61). Guava leaves have a lower moisture content, indicating better shelf stability and resistance to microbial deterioration than Artemisia. Low moisture levels are beneficial for long-term storage of dried herbal items (Oyedeji et al., 2020). Artemisia leaves are extremely protein-rich, comparable to legumes such as soybeans (20-40% protein). Artemisia's high protein level makes it a viable alternative protein source for functional meals or animal feed. Guava, on the other hand, has a low protein content that is comparable to that of leafy greens, limiting its use as a protein supplement(Bvenura & Sivakumar, 2017). The higher lipid content in *Artemisia* may reflect the presence of essential oils and bioactive compounds (e.g., artemisinin), which are linked to antimicrobial and anti-inflammatory properties. Guava's low fat content is typical of non-oilseed plants, though its lipids may still contain antioxidants like carotenoids (Gutiérrez et al., 2021). Ash content correlates with mineral density. *Artemisia*'s higher ash suggests it is a richer source of minerals (e.g., calcium, magnesium), beneficial for bone health and metabolic functions. Guava's lower ash content may still contribute essential minerals like potassium, which supports cardiovascular health (Ullah et al., 2021).

Table (1): Proximate composition of Artemisia and *guajava* Leaf (g/100g) on the dry weight basis.

Constituents (%)	Artemisia	guajava
Moisture	6.93^{a} ± 0.15	5.53^{b}±0.35
Crude Protein	25.43^{a} ± 0.15	3.13^{b} ±0.35
Fats	6.67^{a}± 0.47	1.1^{b}±0.2
Ash	7.32^{a}± 0.25	3.27^{b}±0.2
Crude fiber	9.5^{b} ± 0.2	12.23^{a}±0.25
Carbohydrates	44.15^{b} ± 0.48	74.73^{a} ± 0.61

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

Table shows the total phenolic and total flavonoid content in Artemisia and *guajava* leaf aqueous extract

Results represent that the *Artemisia annua* contained(160.61±0.36) **Total Phenolic** (mg gallic acid), **while *guajava*** leaf contained **(**650.81±1.14) **Total Phenolic (**mg gallic acid**)** Phenolic compounds have well-known antioxidant capabilities; they are effective chelators of redox-active metal ions and can inactivate free radical chain events by inhibiting the conversion of hydroperoxides to reactive oxyradicals (Sahreen et al., 2009). To combat chronic diseases, phenolic chemicals modulate a variety of physiological processes such as cell proliferation, enzymatic activity, cellular redox potential, and signal transduction pathways (Luca et al., 2018)... On the other hand, results in table (2) showed that *Artemisia*

annua contained(45.81± 0.3) total flavonoids **(mg quercetin equivalents /g),** Guajava leaf co contained (56.7 ±0.45) total flavonoids (mg quercetin equivalents per gram). Flavonoids' free radical scavenging ability is well established through complex formation with metal ions (Adrian et al., 2015).

Table (2) Total phenolic and total flavonoid contents in Artemisia and *guajava* Leaf Aqueous Extract

Constituents	Artemisia	guajava
Total Phenolic (mg Gallic acid)	160.61^{b}±0.36	650.81^{a}±1.14
Total flavonoids (mg quercetin equivalents /g)	45.81^{b}± 0.3	56.7^{a}±0.45

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

The results in Table (3) indicate that the highest body weight gain (49.09%) corresponds with typical growth patterns in healthy rats under standard diets((G1). (Smith et al., 2021 It was also observed that group G4 exhibited the highest body weight gain (BWG) among all groups. This significant increase in weight for G4 (500 mg/kg guava) may be attributed to its potential in improving gut health and reducing inflammation.

Guava extracts are known to modulate gut microbiota, improving nutrient absorption and energy metabolism (Rahim et al., 2023). Conversely, G6 (200 mg/kg Artemisia) showed an improved weight gain of 48.43%, approaching the levels observed in G1, which indicates a dose-dependent effect. . *Artemisia*'s bioactive compounds (e.g., artemisinin, flavonoids) may enhance anti-inflammatory and antioxidant pathways, indirectly supporting weight gain (Mouffouk *et al*., 2021).

Table (3): Mean body weight gain (%) of experimental rats which treated with Artemisia and *guajava*

Groups	Initial weight IBW	Final weigh FBW	Body gain BWG/wk %
G 1 Control (ve-)	182.66^{d}±2.88	271.33^{c}±7.37	49.09^{b}±3.42
G 2 Control (ve+)	185.33^{b}±1.53	199.67^{d}±1.53	8.54^{f}±2.38
G3 guajava (250mg/kg)	186.00^{a}±1.00	272.00^{c}±4.58	47.67^{d}±4.13
G4 guajava (500mg/kg)	185.00^{b}±3.00	282.00^{a}±7.94	55.26^{a}±1.82
G5 Artemisia (100 mg/ kg)	184.67^{c}±1.53	271.67^{c}±4.93	46.65^{e}±2.97
G6 Artemisia (200 mg/ kg)	185.33^{b}±1.53	279.00^{b}±3.00	48.43^{c}±2.09

Data are presented as means ± SEM (*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

Table (4): Mean organ weight/body weight (%) of experimental rats which treated with Artemisia and *guajava*

Groups	Organ's weight (%)	
	liver	kidney
G 1 Control (ve-)	2.45^{f}±0.06	0.53^{e}±0.005
G 2 Control (ve+)	5.42^{a}±0.08	0.92^{a}±0.04
G3 guajava (250mg/kg)	3.39^{b}±0.2	0.73^{b}±0.05
G4 guajava (500mg/kg)	2.95^{d}±0.11	0.66^{c}±0.03
G5 Artemisia (100 mg/ kg)	3.15^{c}±0.11	0.63^{d}±0.05
G6 Artemisia (200 mg/ kg)	2.89^{e}±0.1	0.56^{e}±0.05

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

Results in Table (٤) represent that the **highest** liver weight **(**5.42±0.08) **in positive control (G2)** comparable to negative control (G1) (2.45±0.06). This likely reflects hepatotoxicity or metabolic stress, as increased liver weight is often associated with inflammation, fatty infiltration, or hypertrophy.(Anonymous Authors. 2022).

On **the other hand** It was also, noticed that Group G3 (guava at 250 mg/kg) caused the highest increase in liver weight (3.39b±0.2) compared to groups G4 (500 mg/kg guava), G5 (100 mg/kg Artemisia), and G6 (200 mg/kg Artemisia). While G4 guajava **500 mg/kg decrees** liver weight to (2.95% ± 0.11). This aligns with studies showing guava's hepatoprotective properties, attributed to polyphenols (e.g., quercetin) and polysaccharides that mitigate oxidative stress and inflammation. Higher doses may enhance antioxidant enzyme activity, counteracting toxin-induced damage. **(Adeyemi and Pintel 2014) .** In the same table result represent that G6 Artemisia (200 mg/ kg)) caused the lowest decrease in liver weight(2.89±0.1) compared to G 2 Control (ve+) (5.42±0.08). This dose-dependent efficacy mirrors findings where *Artemisia* extracts reduced hepatic damage via anti-inflammatory and antioxidant mechanisms, such as scavenging reactive oxygen species (ROS).(Lee.,2012). in the same table result represent that the **highest** kidney weight **(**0.92^{a}±0.04) **in positive control (G2)** comparable to negative control (G1) (0.53^{e}±0.005). suggests renal hypertrophy or injury, potentially due to oxidative stress or nephrotoxic agents. .(**Adeyemi and Pintel 2014).** In the same table result represent that G6 Artemisia (200 mg/ kg) caused the lowest decrease in kidney weight (0.56±0.05) compared to G 2 Control (ve+) (0.92±0.04). This is consistent with previous studies demonstrating that *Artemisia annua* extracts mitigate diclofenac-induced renal damage by decreasing lipid peroxidation and increasing glutathione levels. This is consistent with previous studies demonstrating that *Artemisia annua* extracts mitigate diclofenac-induced renal damage by decreasing lipid peroxidation and increasing glutathione levels. (Anonymous Authors. 2022).

4.3.4. Biochemical analysis

Table (5): Liver enzyme levels in all experimental rats treated with *guajava* and Artemisia extracts.

Groups	Parameters (U/L)			
	AST	ALT	ALP	T. Bil
G 1 Control (ve-)	39.96^{e}±0.63	60.63^{e}± 1.28	63.87^{e}± 0.45	0.46^{e}± 0.03
G 2 Control (ve+)	72.28^{a}±1.4	109.53^{a}± 1.89	128.45^{a}±1.18	1.003^{a}± 0.05
G3 guajava (250mg/kg)	53.95^{b}± 0.58	93.48^{b}± 1.32	100.8^{b}±0.4	0.61^{b}± 0.005
G4 guajava (500mg/kg)	41.29^{d}± 1.29	62.106^{d} ± 1.25	65.99^{d}± 0.38	0.53^{c}±0.01
G5 Artemisia (100 mg/ kg)	46.61^{c}± 0.88	77.27^{c}±1.45	91.88^{c}± 1.78	0.49^{d}±0.02
G6 Artemisia (200 mg/ kg)	38.37^{f}±1.87	60.88^{e}±0.46	61.23^{f}±1.23	0.48^{d}±0.02

Data are presented as means ± SDM (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; ALP: alkaline phosphatase; T. Bil: Total Bilburine

Results in Table (5) show that the highest enzyme levels (AST,ALT,ALP, and total bilirubin (T. Bil)) **in positive control (G2)** comparable to negative control (G1). indicating induced liver injury, likely mimicking a pathological state such as oxidative stress or cholestasis (Kwo et al., 2017). Elevated AST and ALT are hallmarks of hepatocellular damage, while increased ALP and bilirubin suggest cholestatic injury (Thapa & Walia, 2007). On the other hand, G3 guajava (250mg/kg) reduced AST, ALT, ALP, and bilirubin compared to G2, though levels remained elevated relative to the healthy control (G1). In the same table Results represent that G4 (guajava 500mg/kg) restored enzyme levels nearly to G 1 Control (ve-) , indicating a **dose-dependent hepatoprotective effect**. This aligns with studies showing guajava's phenolic compounds (e.g., quercetin, gallic acid) mitigate oxidative stress and inhibit lipid peroxidation in liver tissue (Naseer et al., 2018). For example, guajava leaf extract has been shown to reduce CCl4-induced hepatotoxicity in rats by enhancing antioxidant enzyme activity (Naseer et al., 2018). In the same table results represent that G5 Artemisia (100 mg/ kg) decrease enzyme levels (AST,ALT,ALP, and total bilirubin (T. Bil) comparable to G 2 Control (ve+),on the other hand G6 Artemisia (200 mg/ kg) caused the lowest decrease in enzyme levels (AST,ALT,ALP, and total bilirubin (T. Bil) comparable G 2 Control (ve+). This suggests *Artemisia*'s efficacy at higher doses, likely due to sesquiterpene lactones (e.g., artemisinin) and flavonoids with anti-inflammatory and antioxidant characteristics
(Ekiert et al., 2020).

Table (6): Kidney function (mg/dl) in experimental rats treated with guava and Artemisia extracts.

Groups	Parameters(mg/dl)			
	Urea	Uric Acid	Creatinine	Albumins
G 1 Control (ve-)	48.50^{e}±1.03	1.67^{d}±0.07	0.67^{e} ± 0.04	3.5^{f}±0.2
G 2 Control (ve+)	139.52^{a}±1.23	4.58^{a}±0.45	5.46^{a}± 0.46	6.76^{a}±0.50
G3 guajava (250mg/kg)	77.81^{b}±0.78	2.43^{b}±0.28	1.49^{b}±0.06	4.6^{b}±0.103
G4 guajava (500mg/kg)	49.81^{d}±0.22	2.1^{c}±0.08	0.8^{c}±0.02	3.9^{c}±0.13
G5 Artemisia (100 mg/ kg)	72.29^{c}±0.34	1.64^{e}±0.09	1.54^{b}±0.04	3.84^{d}±0.205
G6 Artemisia (200 mg/ kg)	48.11e±0.59	1.63^{e}±0.02	0.75^{d}±0.05	3.65^{e}±0.11

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

Table (6) shows that the positive control (G2) had higher Levels of urea, uric acid, creatinine, and albumins than the negative control (G1) suggest induced kidney injury, likely mimicking oxidative stress or toxin-mediated nephropathy (e.g., cisplatin or gentamicin models) (Peres et al., 2019). On the other hand G3 guajava (250mg/kg) reduced urea (77.81 mg/dl), uric acid (2.43 mg/dl), and creatinine (1.49 mg/dl) compared to G 2 control (ve+), but levels remained higher than G 1 Control (ve-) . This supports partial nephroprotection, most likely caused by guajava's flavonoids (quercetin, rutin) and tannins, which scavenge free radicals and prevent lipid peroxidation (Naseer et al., ٢٠١٨). In the same table results represent that G4 (guajava 500mg/kg) restore urea (49.81 mg/dl), uric acid (2.1 mg/dl), creatinine (0.8 mg/dl) **and Albumins (**3.9±0.13) to near Control (ve-) (G1), showing dose-dependent effectiveness. Guajava's high polyphenol content can boost antioxidant enzymes (SOD CAT) and lower inflammatory cytokines (TNF-α, IL-6) in renal tissue. (Alam et al., 2021). In the same table results represent that G5 Artemisia (100 mg/ kg) decrease levels of urea (72.29±0.34),uric Acid (1.64±0.09), **creatinine (**1.54±0.04) and a**lbumins** (3.84±0.205) comparable to G 2 Control (ve+),on the other hand G6 artemisia (200 mg/ kg) caused the lowest decrease in urea(48.11e±0.59) , uric acid (1.63^{e}±0.02); creatinine (0.75±0.05)albumins(3.65±0.11) comparable G 2 Control (ve+) . This is consistent with artemisia's sesquiterpene lactones (such as artemisinin) and phenolic acids, which reduce oxidative stress and apoptosis in renal tubular cells. (Ekiert et al., 2020).

Table (7): Mineral content (Potassium and Sodium) in experimental rats treated with *guajava* and Artemisia extracts.

Groups	Parameters(mg/dl)	
	Potassium	**Sodium**
G 1 Control (ve-)	4.55^{d}±0.4	140.66^{a}± 0.57
G 2 Control (ve+)	8.4^{a}± 0.79	114.33^{e}± 2.3
G3 guajava (250mg/kg)	6.62^{b}±0.25	132.66^{d}±2.3
G4 guajava (500mg/kg)	5.21^{c}±0.32	138.33^{c}±2.08
G5 Artemisia (100 mg/ kg)	6.09^{c}±0.75	138.83^{b}±0.75
G6 Artemisia (200 mg/ kg)	5.12^{c}±0.05	138.33^{c}±0.57

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

Table (7) indicates that the positive control group (G2) had considerably higher potassium levels (8.4±0.79) than the negative control group (G1). (4.55±0.4)." Elevated potassium (8.4± 0.79) suggests impaired renal excretion likely owing to acute kidney injury (AKI) or decreased glomerular filtration rate (GFR) (Kovesdy, 2022). In the same table, the positive control group (G2) revealed reduced sodium levels (114.33± 2.3) compared to the negative control group (G1) (140.66± 0.57), **which may indicate** tubular dysfunction or syndrome of inappropriate antidiuretic hormone secretion (SIADH) secondary to renal stress (Hoorn & Zietse, ٢٠١٥).On the other hand G3 guajava (250mg/kg) partially corrected potassium

(6.62±0.25) and sodium (132.66±2.3), indicating moderate renal functional recovery .In the same table G4 guajava (500mg/kg)Near-normalization of potassium (5.21±0.32) and sodium (138.33±2.08), suggesting **dose-dependent restoration of electrolyte homeostasis**. Guajava's flavonoids (e.g., quercetin) may enhance renal tubular Na^{+}/K^{+}-ATPase activity and reduce oxidative damage to nephrons (Alam et al., 2021).). In the same table results represent that G5 Artemisia (100 mg/ kg) showed higher potassium (6.09±0.75) levels compared to the negative control group (G1) (4.55±0.4)." while sodium (138.83±0.75) approached baseline, indicating selective efficacy in Na^{+} regulation. On the other hand**G6 (200 mg/kg)**: Achieved near-normal potassium (5.12 mg/dl) and sodium (5.12^{c}±0.05), outperforming guajava's 250 mg/kg dose. This aligns with *Artemisia*'s sesquiterpene lactones (e.g., artemisinin), which improve renal blood flow and tubular integrity (Ekiert et al., 2020).

Table (8): Serum levels of catalase, glutathione reductase, superoxide dismutase, malondialdehyde, and total antioxidants in experimental rats treated with *guajava* and Artemisia extracts.

Groups	Parameters				
	CAT catalase **(µ/L)**	**Glutathione reductase (µ /dl)**	**SOD (µ /dl)**: Superoxidedism utase	**MDA (ng/ml)** malondialdehyde	**Total antioxidants**
G 1 Control (ve-)	52.54c±0.27	49.87^{b}±0.08	21.16^{b}±0.66	4.96^{d}±0.16	1.34^{d}±0.02
G 2 Control (ve+)	35.8^{f}±0.26	30.25^{e}±0.45	15.05^{e}±0.47	23.27^{a}±0.65	0.41^{e}±0.01
G3 guajava (250mg/kg)	48.34^{e}±0.53	45.56^{d}±0.07	19.11^{d}±0.61	7.74^{b}±1.01	1.62^{c}±0.03
G4 guajava (500mg/kg)	53.69^{b}±0.42	50.34^{a}±0.39	20.88^{c}±0.74	6.22^{c}±0.53	1.81^{b}±0.02
G5 Artemisia (100 mg/ kg)	51.02^{d}±0.77	45.54^{d}±0.52	19.15^{d}±0.202	4.13^{e}±0.38	1.91^{a}±0.02
G6 Artemisia (200 mg/ kg)	54.11^{a}±0.79	49.02^{c}±0.03	21.58^{a}±0.19	3.5^{f}±0.43	1.89^{a}±0.01

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

Results in Table (٨) represent that **Positive Control (G2, ve+)**: Marked oxidative stress is evident, with **elevated MDA (23.27 ng/ml)** and suppressed antioxidant enzymes (CAT: 35.8 µ/L; SOD: 15.05 µ/dl). This aligns with studies where disease models (e.g., chemically induced oxidative stress) show increased lipid peroxidation (MDA) and depleted antioxidant defenses due to excessive ROS generation (Halliwell & Gutteridge, 2015).On the other hand G3 guajava (250mg/kg) Partial restoration of CAT (48.34±0.53) and SOD (19.11^{d}±0.61) activities, along with reduced MDA (7.74±1.01) compared to G2. This suggests guajava's polyphenols (e.g., quercetin, gallic acid) mitigate ROS via free radical scavenging (Rahim, ٢٠٢٣).In the same table G4 guajava (500mg/kg)Near-normalization of CAT (53.69±0.42) and SOD (20.88±0.74), with MDA (6.22±0.53) approaching G1 levels. The higher dose's efficacy correlates with guajava's **dose-dependent antioxidant activity**, attributed to its vitamin C and flavonoid content, which enhance glutathione reductase and SOD synthesis (Dos Santos et al., 2022).On the other hand **Artemisia 100 mg/kg (G5)** Significantly reduced MDA (4.13^{e}±0.38) and elevated total antioxidants (1.91^{a}±0.02) compared to G2. This aligns with studies showing *Artemisia*'s sesquiterpene lactones (e.g., artemisinin) inhibit lipid peroxidation (Abdelgadir et al., 2021).On the other hand **Artemisia 200 mg/kg (G6)** Achieved the **highest SOD (21.58 µ/dl)** and **lowest**

MDA (3.5 ng/ml), surpassing even G1. This superior efficacy may stem from *Artemisia*'s synergistic phytochemicals (e.g., flavonoids, coumarins) that activate Nrf2 pathways, boosting endogenous antioxidant enzymes (Kshirsagar et al., 2023).

Histopathological examination of kidneys:

Microscopically, kidney slices from rats in group 1 (Control ve-) showed normal renal parenchymal structure (Figs. 1, 2, and 3). In contrast, the kidneys of rats in group 2 (Control ve+) revealed histological abnormalities defined by cytoplasmic vacuolization of epithelial lining renal tubules (Figs. 4 & 5), renal blood vessel congestion (Fig. 5), and epithelial lining renal tubule necrobiosis (Fig. 6). Meanwhile, the kidneys of rats in group 3 (guajava 250mg/kg) showed no histological alterations (Fig. 7), except for cytoplasmic vacuolization of the epithelial lining of some renal tubules (Figs. 8 and 9)..The near-normal renal morphology in this group, except for mild vacuolization, suggests that guava extract may attenuate renal injury. Polyphenols in guava (*Psidium guajava*)—such as quercetin and gallic acid—are known to scavenge free radicals, reduce lipid peroxidation, and inhibit pro-inflammatory pathways. (Flores,2020). Similar results were reported by (Ojewole 2020), where guava leaf extract preserved renal tubules in diabetic rats(. The residual vacuolization could reflect transient stress, possibly due to incomplete suppression of oxidative damage. On the other hand, the kidneys of rats in group 4 displayed eosinophilic proteinaceous casts in the lumen of some renal tubules (Figs. 10 & 11), as well as pyknosis of the nuclei of epithelium lining some renal tubules (Fig. 12). Otherwise, the kidneys of rats in group 5 (Artemisia 100 mg/kg) showed cytoplasmic vacuolization of epithelium lining some renal tubules (Figs. 13 & 14) and pyknosis of the nuclei of epithelial lining some renal tubules (Fig. 15). Furthermore, the kidneys of rats from group 6 showed no histological abnormalities (Figs. 16 and 17) except congestion of glomerular tufts (Fig. 18) was noticed in some sections. The vacuolization and pyknosis here resemble early-stage tubular damage. While *Artemisia* species possess anti-inflammatory properties (Ekiert et al.,,2021), certain sesquiterpene lactones in these plants may induce cytotoxicity at higher doses(Efferth 2021). This dual effect aligns with studies showing dose-dependent nephroprotection or toxicity in *Artemisia* extracts(Al-Megrin et al., ٢٠٢٠).

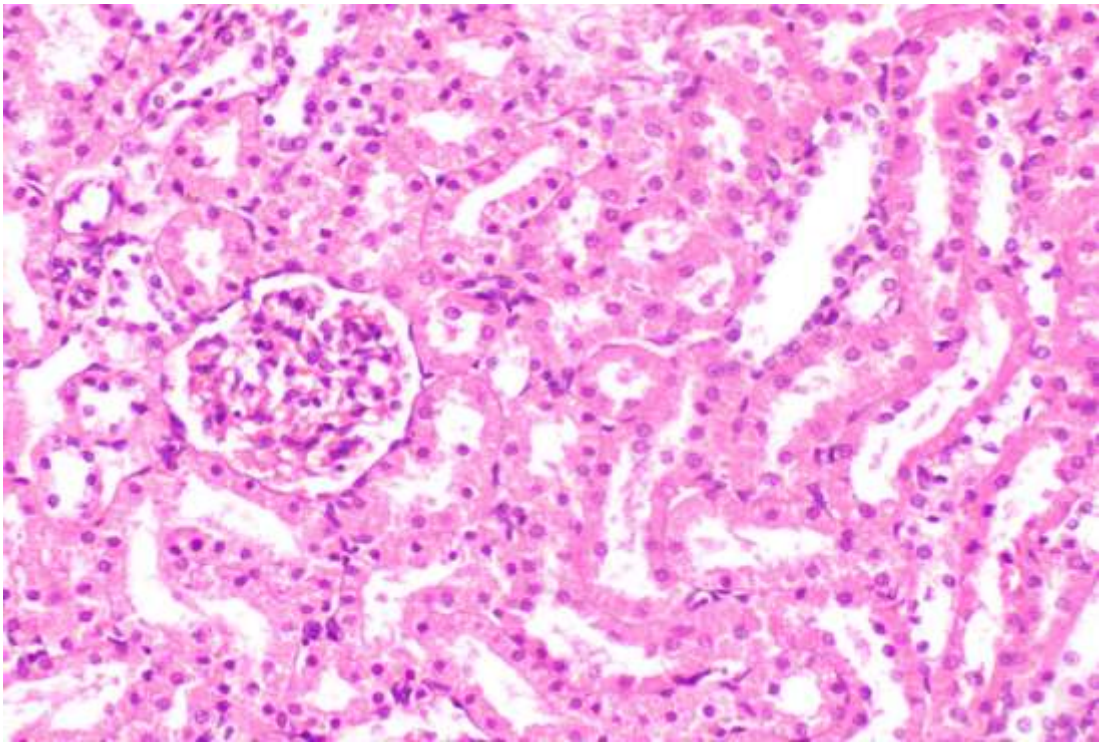


Fig. (1): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H & E X 400).

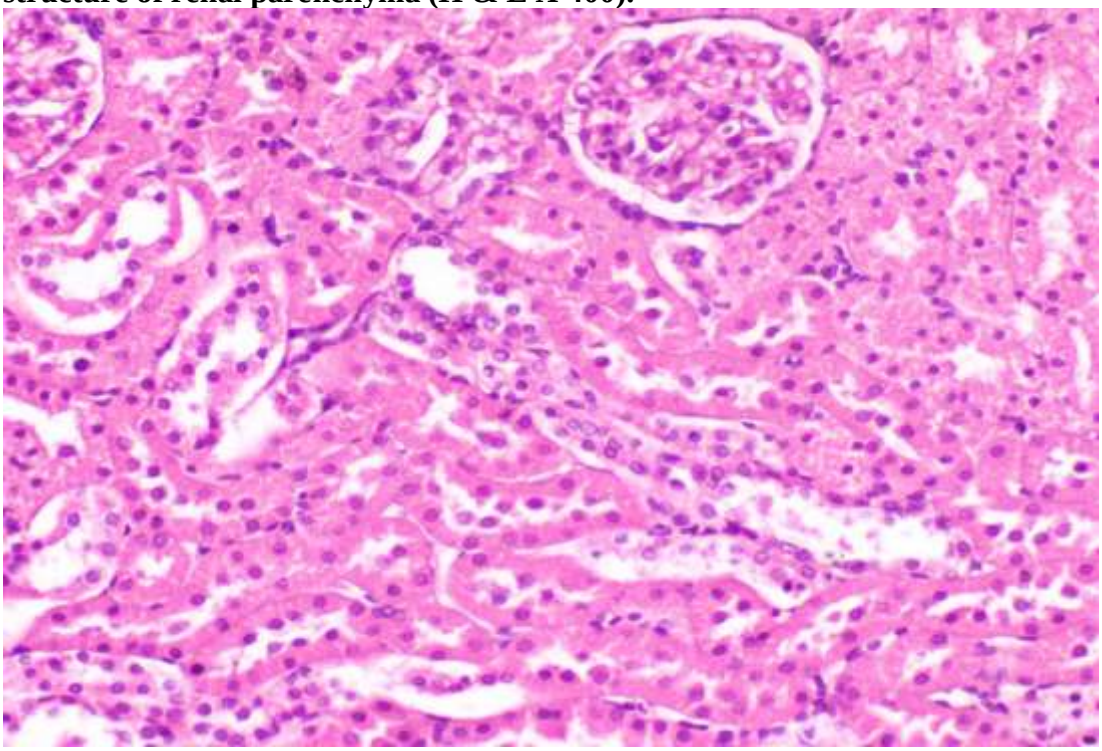


Fig. (2): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H & E X 400).

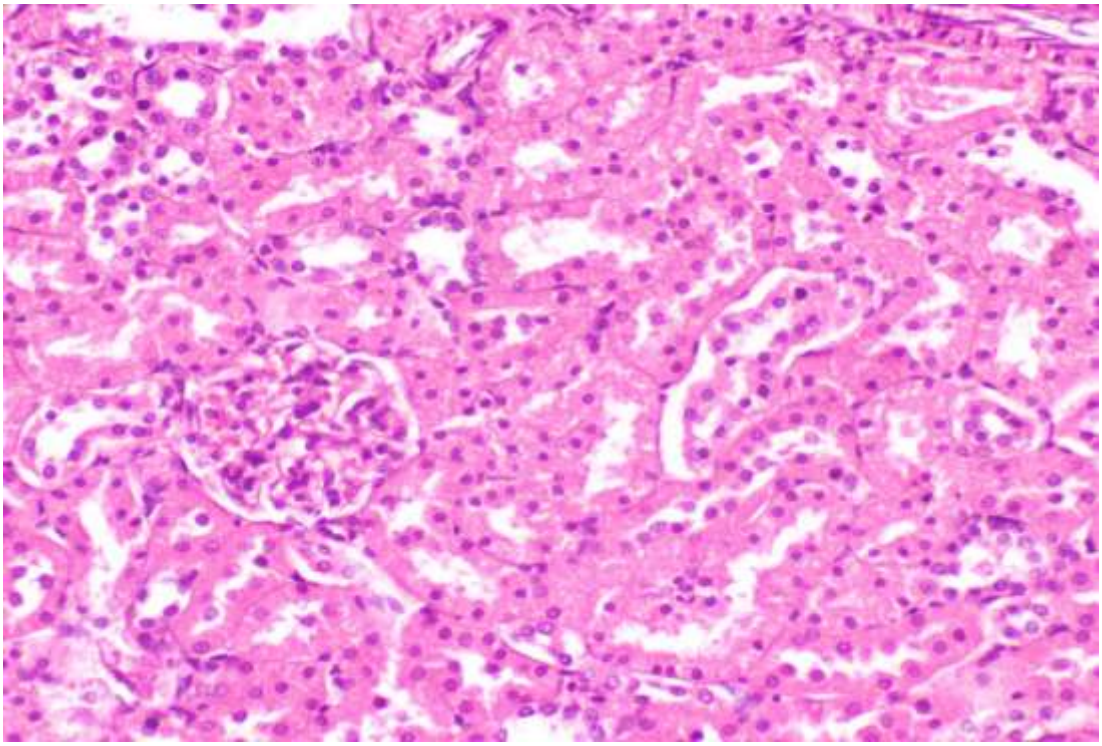


Fig. (3): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H & E X 400).

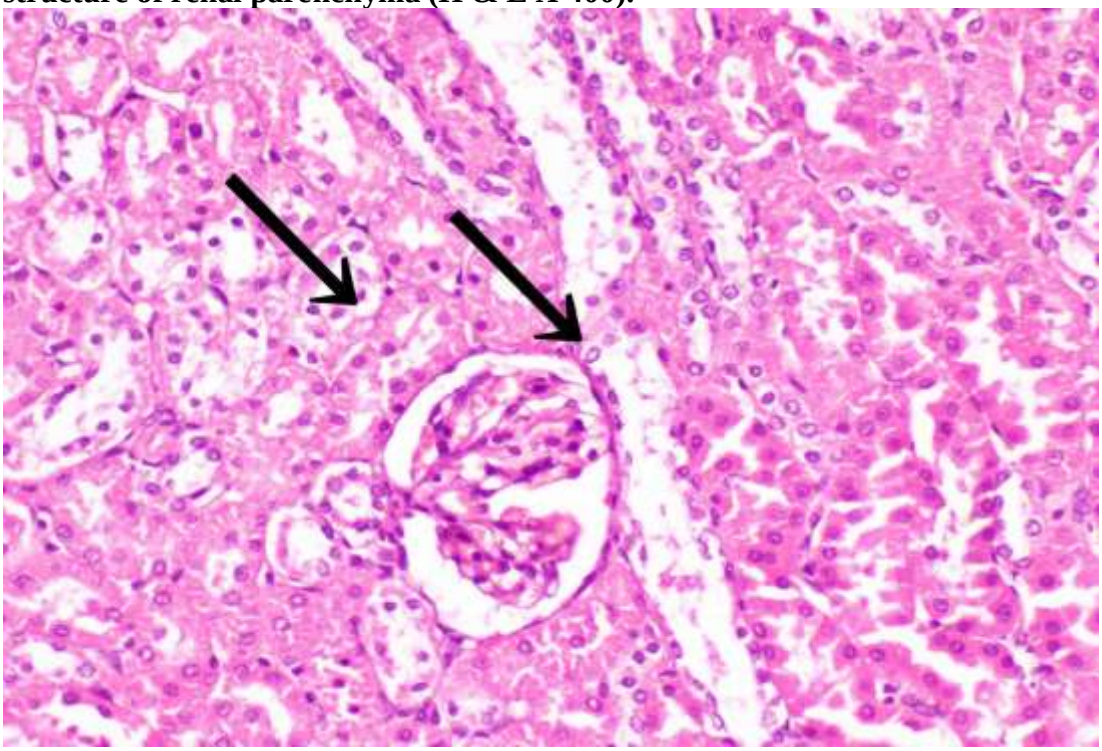


Fig. (4): Photomicrograph of kidney of rat from group 2 showing cytoplasmic vacuolization of epithelial lining renal tubules (black arrow) (H & E X 400).

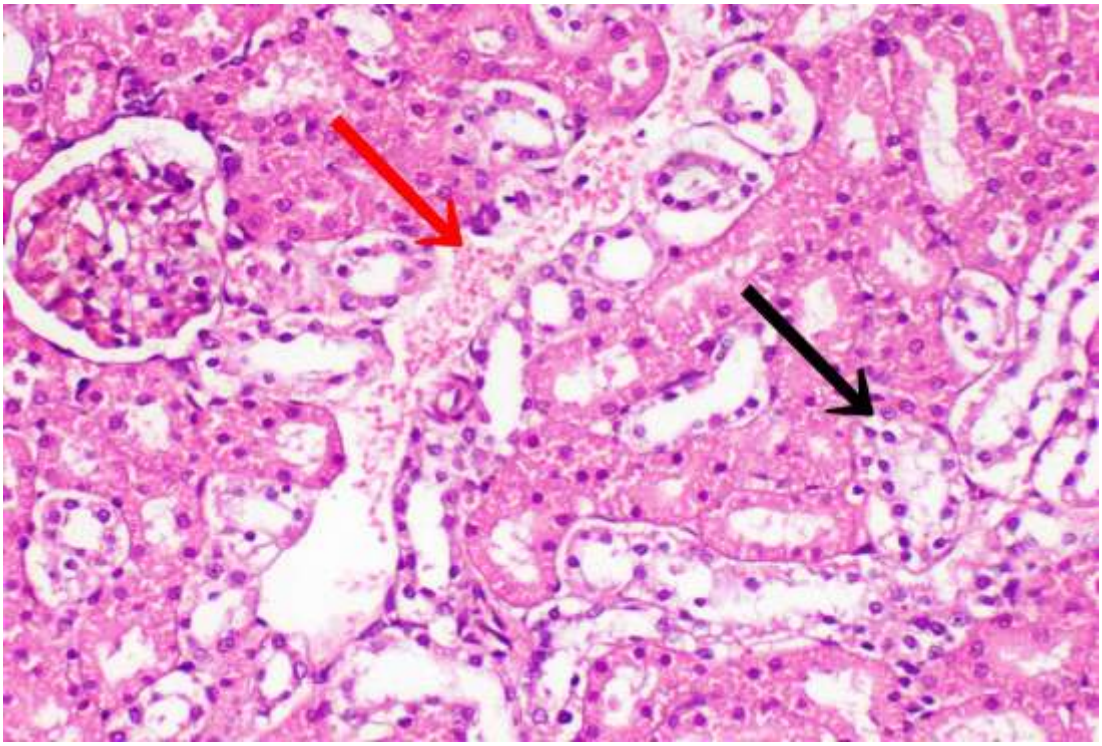


Fig. (5): Photomicrograph of kidney of rat from group 2 showing cytoplasmic vacuolization of epithelial lining renal tubules (black arrow) and congestion of renal blood vessel (red arrow) (H & E X 400).

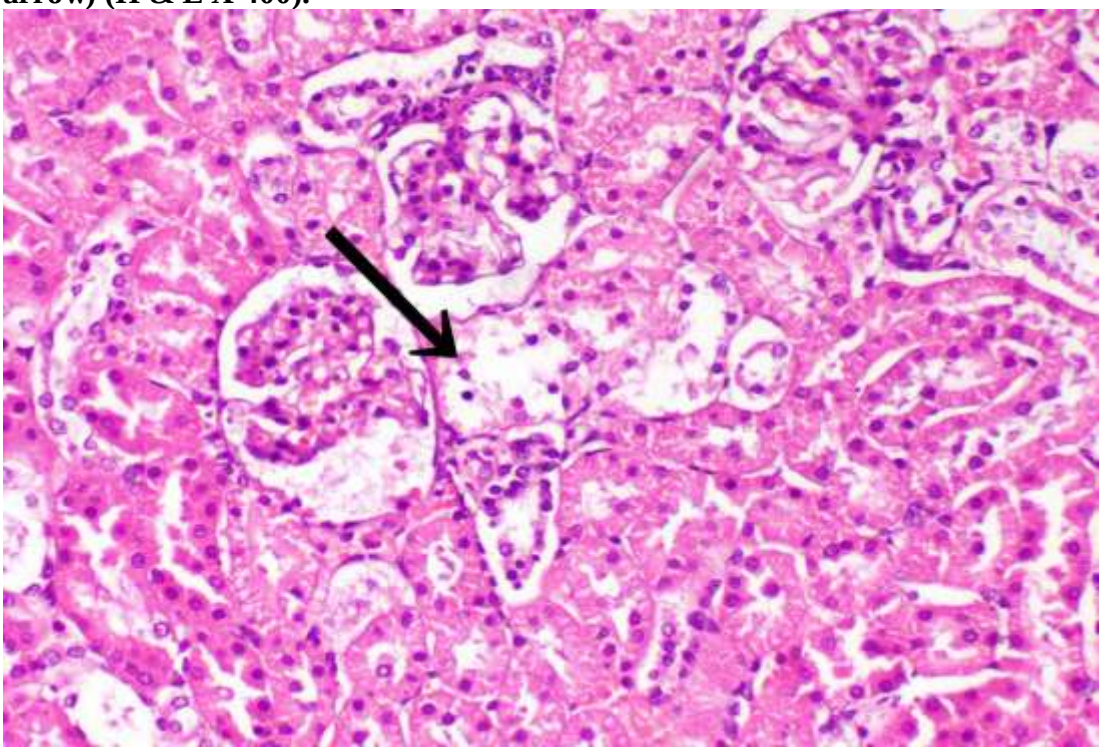
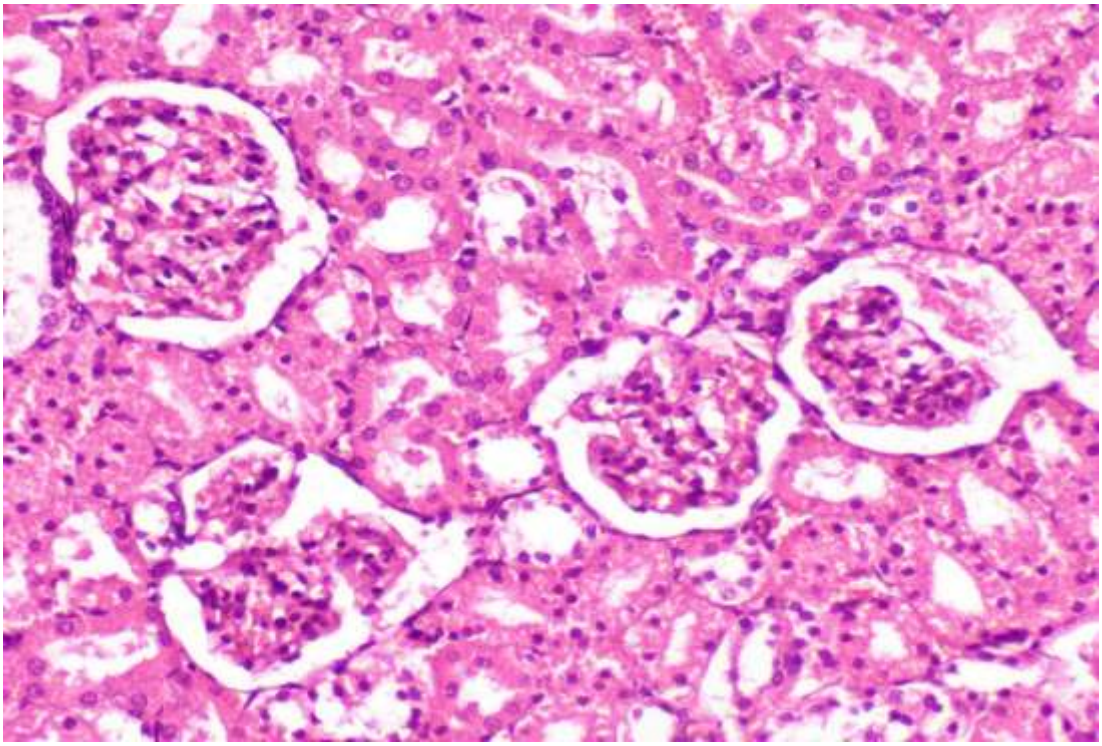


Fig. (6): Photomicrograph of kidney of rat from group 2 showing necrobiosis of epithelial lining renal tubules (black arrow) (H & E X 400).



(7): Photomicrograph of kidney of rat from group 3 showing no histopathological changes (H & E X 400).

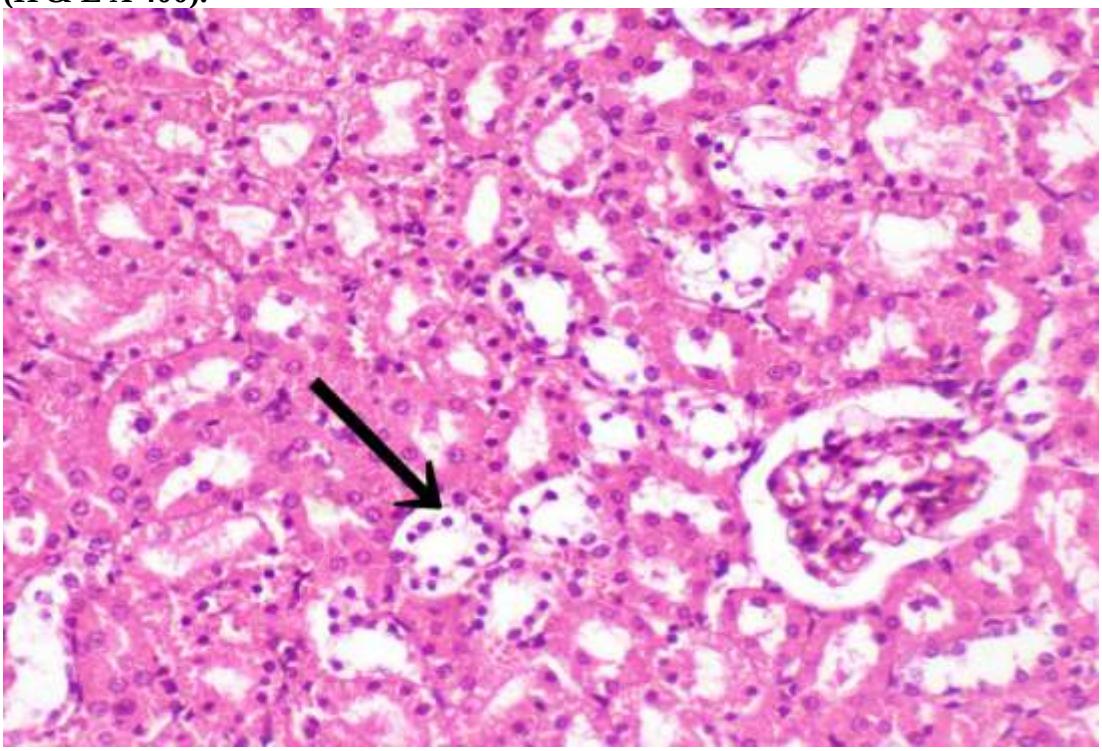


Fig. (8): Photomicrograph of kidney of rat from group 3 showing cytoplasmic vacuolization of epithelial lining some renal tubules (black arrow) (H & E X 400).

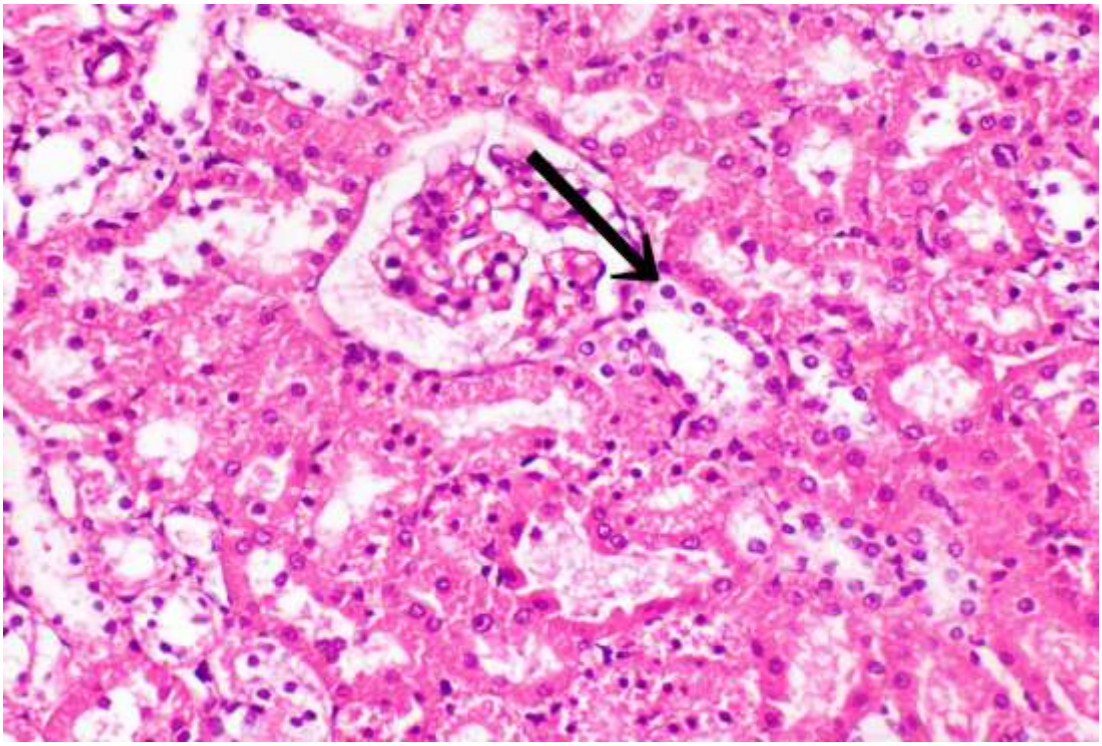


Fig. (9): Photomicrograph of kidney of rat from group 3 showing cytoplasmic vacuolization of epithelial lining some renal tubules (black arrow) (H & E X 400).

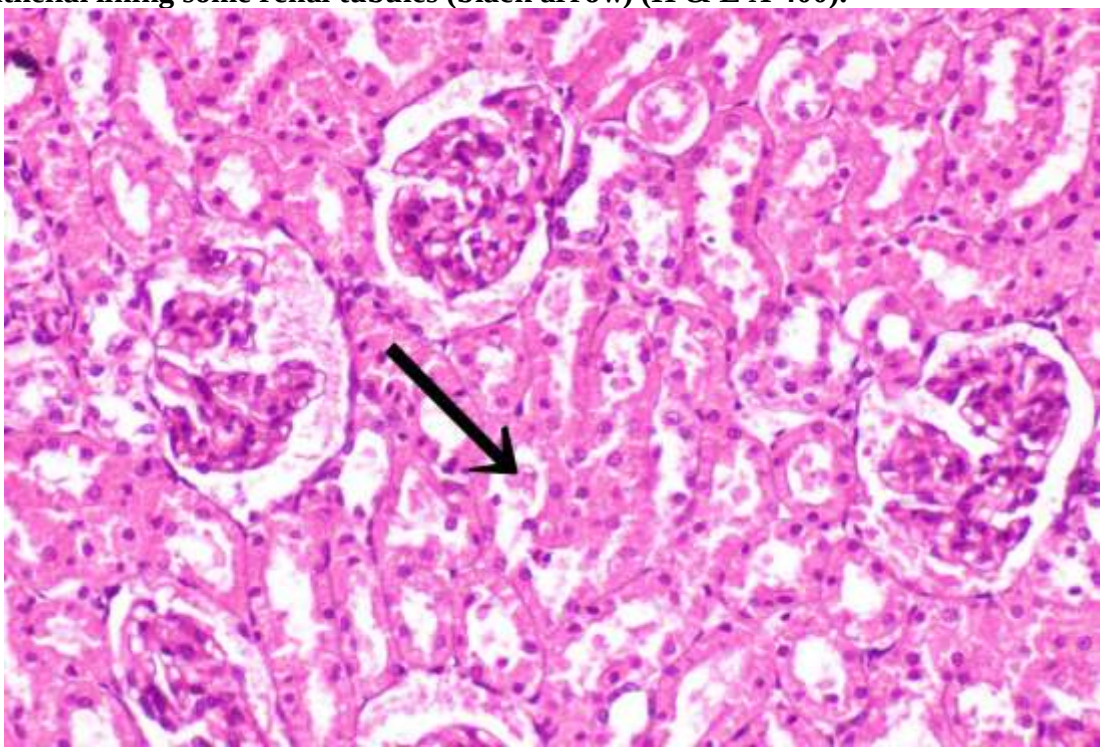


Fig. (10): Photomicrograph of kidney of rat from group 4 showing eosinophilic proteinaceous casts in the lumen of some renal tubules (black arrow) (H & E X 400).

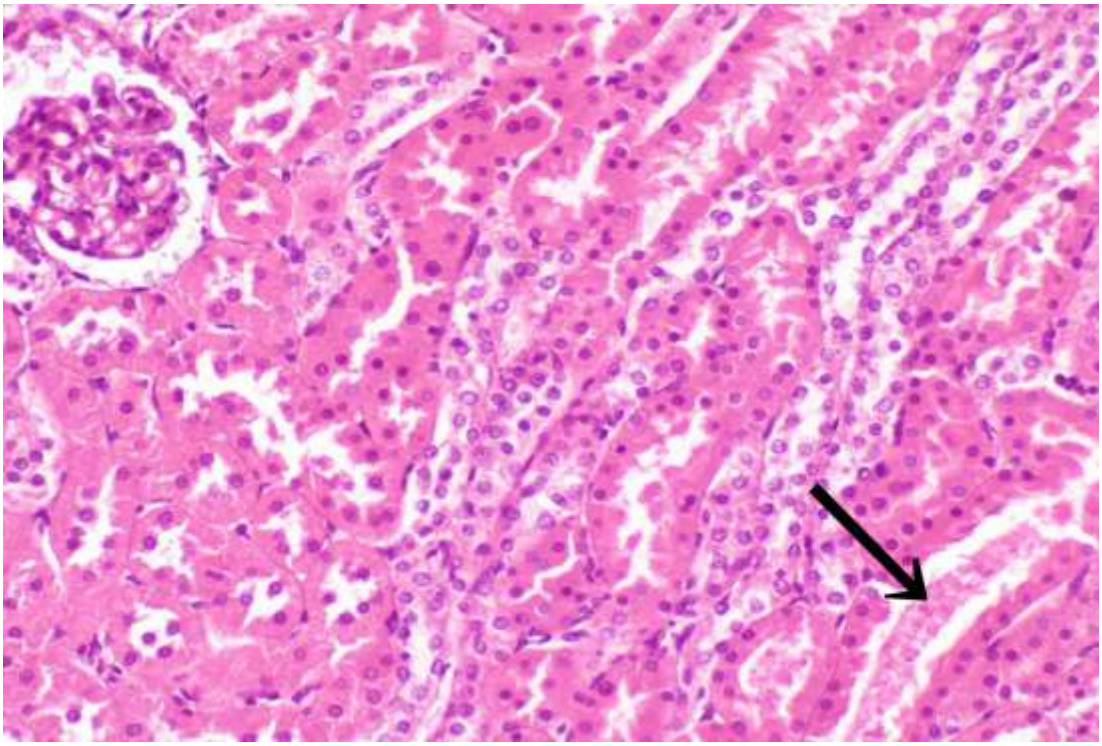


Fig. (11): Photomicrograph of kidney of rat from group 4 showing eosinophilic proteinaceous casts in the lumen of some renal tubules (black arrow) (H & E X 400).

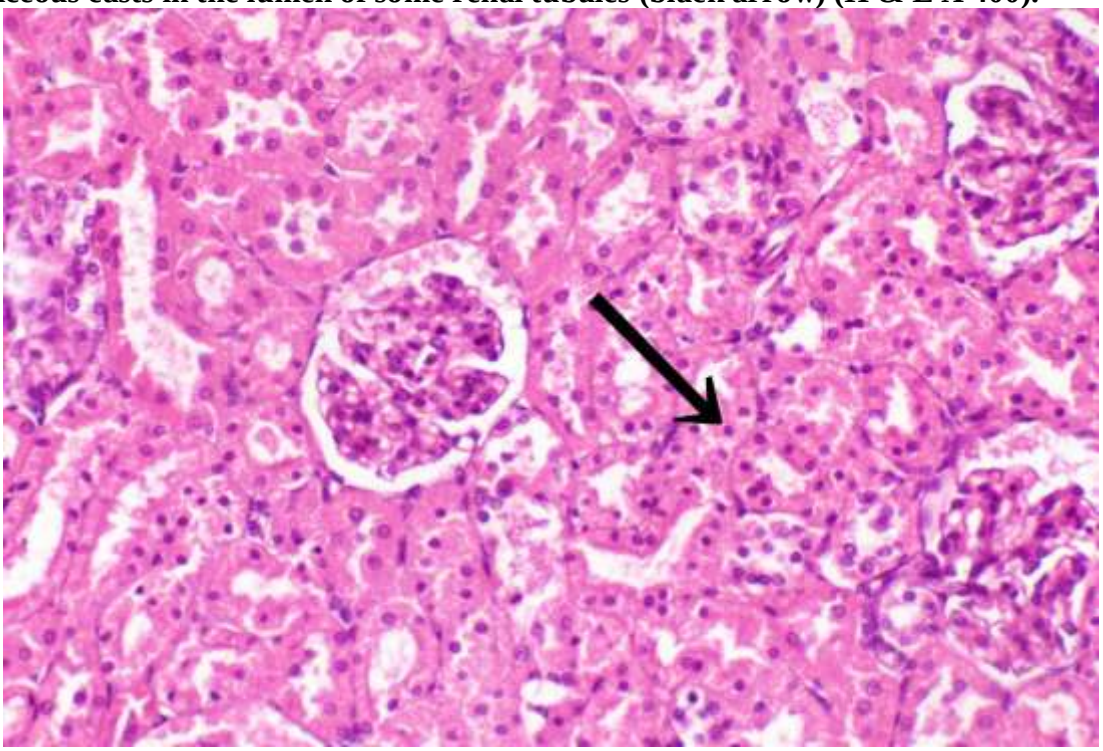


Fig. (12): Photomicrograph of kidney of rat from group 4 showing pyknosis of the nuclei of epithelial lining some renal tubules (black arrow) (H & E X 400).

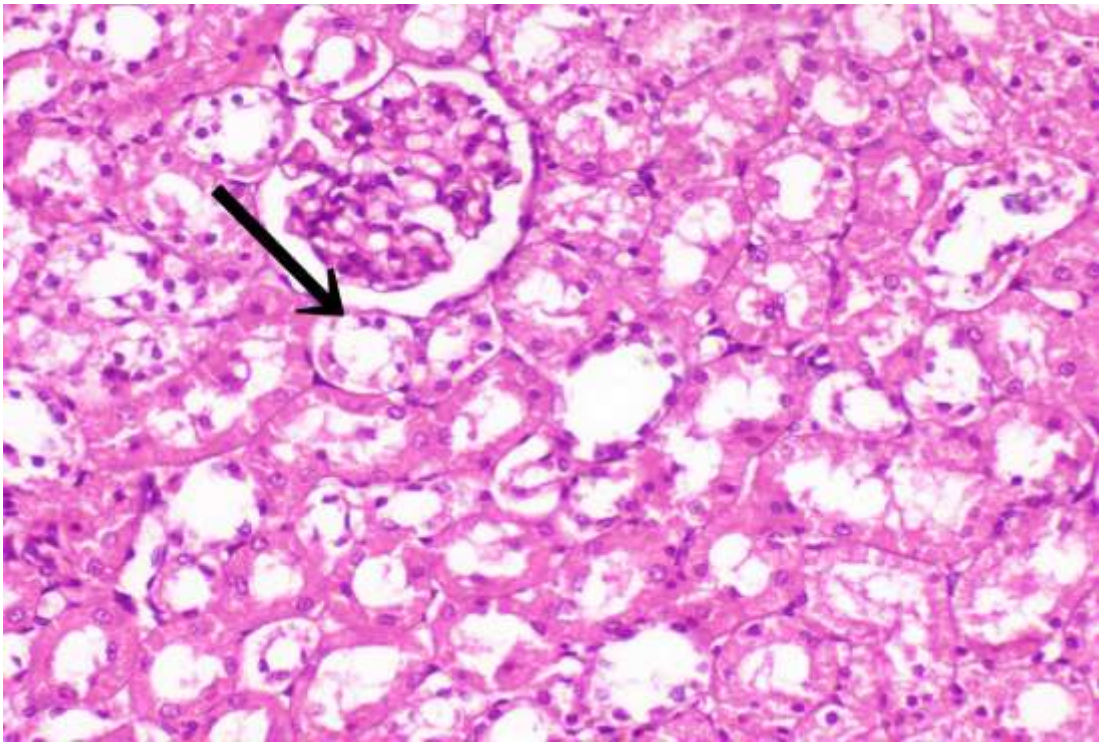


Fig. (13): Photomicrograph of kidney of rat from group 5 showing cytoplasmic vacuolization of epithelial lining some renal tubules (black arrow) (H & E X 400).

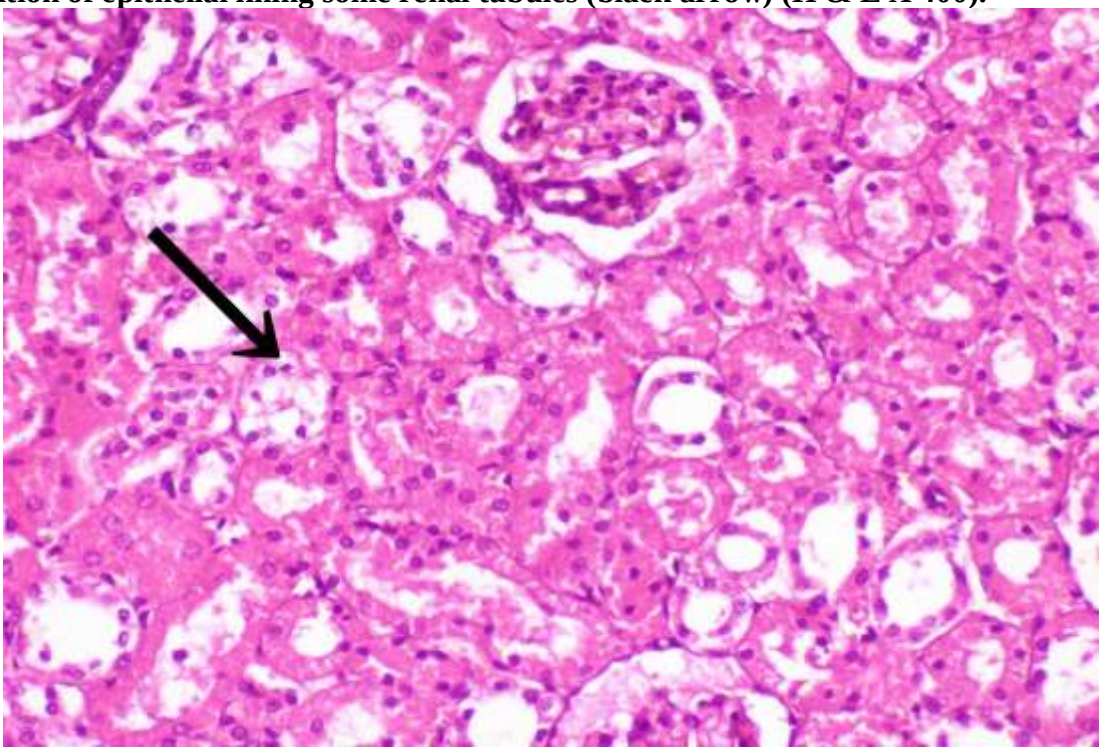


Fig. (14): Photomicrograph of kidney of rat from group 5 showing cytoplasmic vacuolization of epithelial lining some renal tubules (black arrow) (H & E X 400).

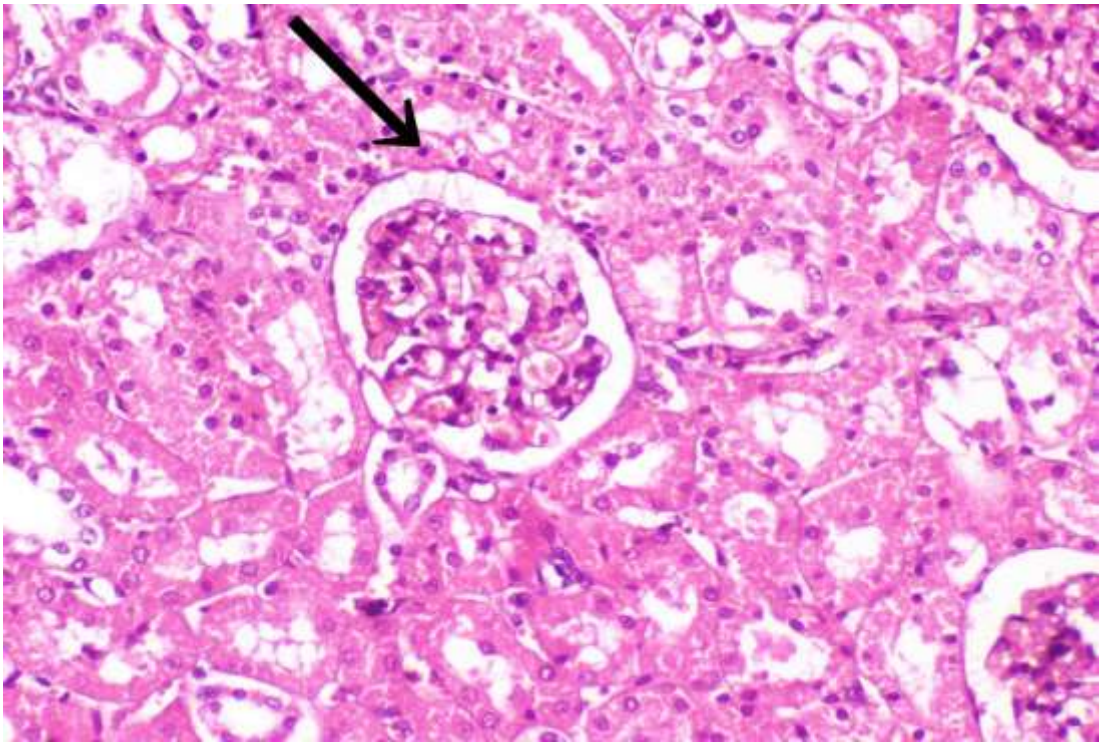


Fig. (15): Photomicrograph of kidney of rat from group 5 showing pyknosis of the nuclei of epithelial lining some renal tubules (black arrow) (H & E X 400).

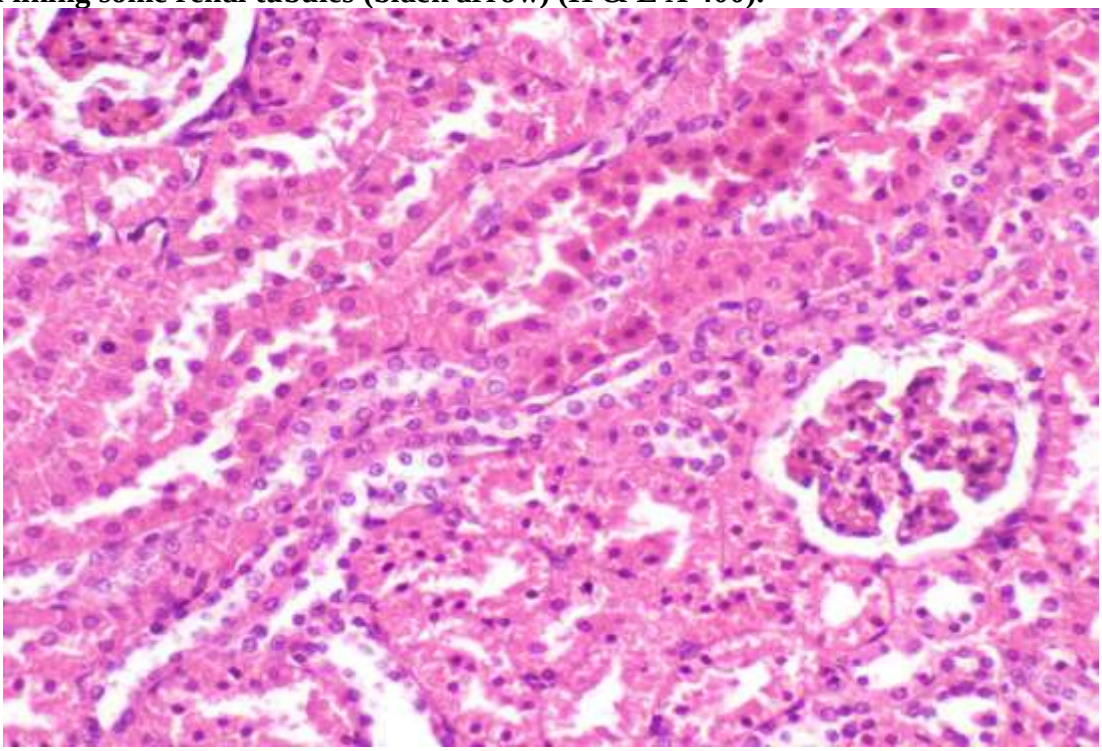


Fig. (16): Photomicrograph of kidney of rat from group 6 showing no histopathological lesions (H & E X 400).

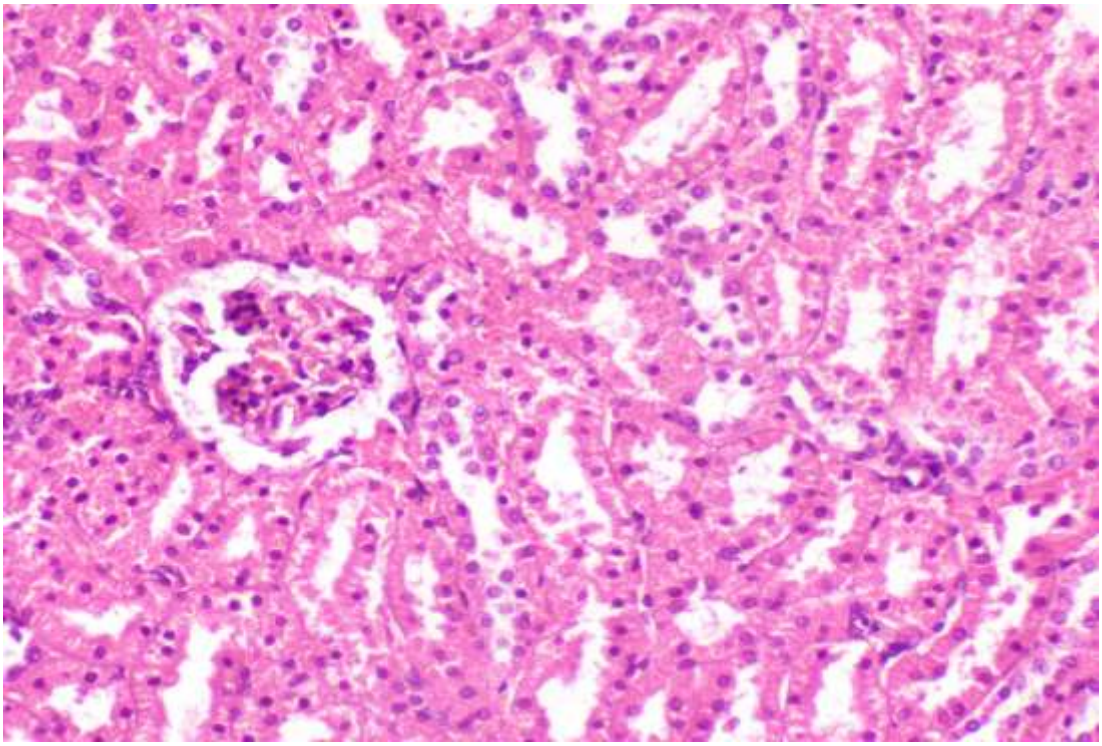


Fig. (17): Photomicrograph of kidney of rat from group 6 showing no histopathological lesions (H & E X 400).

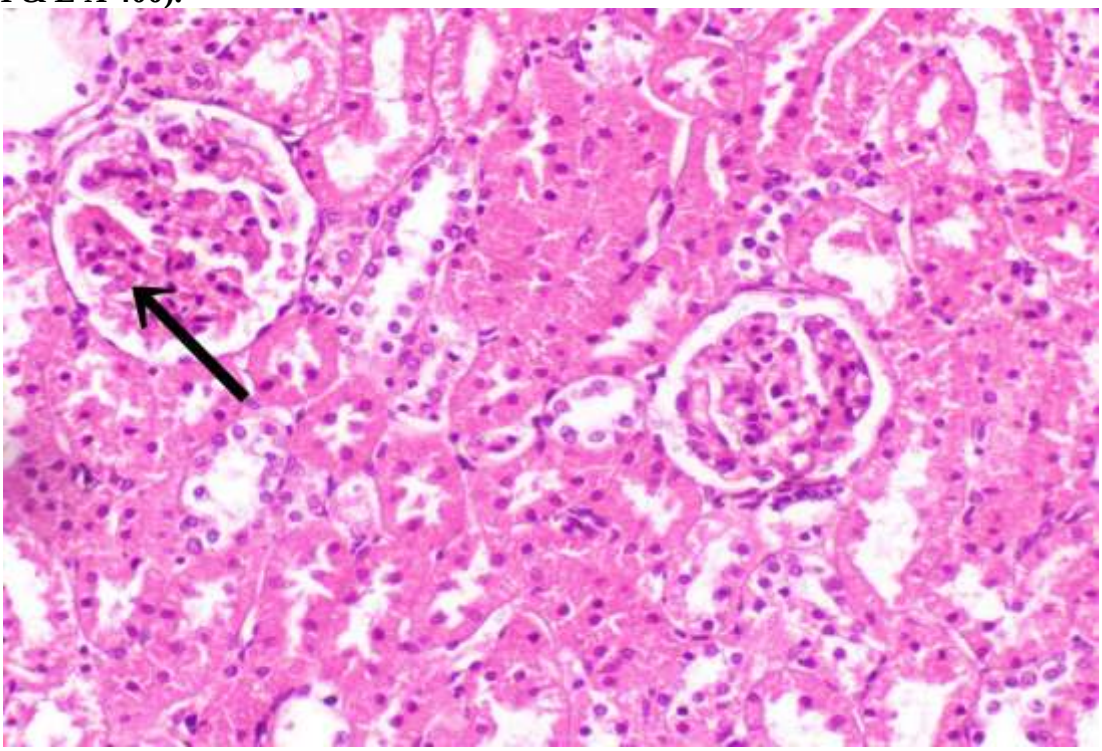


Fig. (18): Photomicrograph of kidney of rat from group 6 showing congestion of glomerular tuft (H & E X 400).

Histopathological examination of liver:

Light microscopic examination of liver sections of rats from group 1 revealed the normal histological architecture of hepatic lobule (Figs. 1, 2 & 3). In contrast,

liver of rats from group 2(G 2 Control ve+) showed histopathological damage characterized by vacuolar degeneration of hepatocytes, fibroplasia in the portal triad (Fig. 4), sinusoidal leukocytosis (Fig. 5) and focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (Fig. 6). The severe histopathological alterations in G2, including **vacuolar degeneration, portal fibroplasia, sinusoidal leukocytosis, and necrosis/apoptosis**, are consistent with acute hepatic injury. Such findings are hallmark features of toxin-induced liver damage (e.g., paracetamol, CCl_4, or alcohol models) (Kumar, 2021). Meanwhile, hepatic tissue of rats from group 3(G3 guajava (250mg/kg) exhibited slight activation of Kupffer cells (Fig. 7), slight vacuolization of hepatocytes (Figs. 7 & 8) and small focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (Fig. 9). Otherwise, liver of rats from group 4 (G4 guajava (500mg/kg) exhibited no histopathological alterations (Figs. 10 & 11) except slight activation of Kupffer cells (Fig. 12. On the other hand, liver of rats from group 5 (G5 Artemisia (100 mg/ kg)showed slight vacuolization of hepatocytes (Figs. 13 & 14), slight activation of Kupffer cells (Fig. 14) and focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (Fig. 15). Otherwise, liver of rats from group 6(G6 Artemisia (200 mg/ kg) exhibited no histopathological lesions (Figs. 16 & 17) except slight activation of Kupffer cells (Fig. 180 in some sections. *Artemisia* spp. contain sesquiterpene lactones (e.g., artemisinin) and phenolic acids that mitigate oxidative stress via Nrf2 pathway activation and inhibit apoptosis by suppressing caspase-3 .

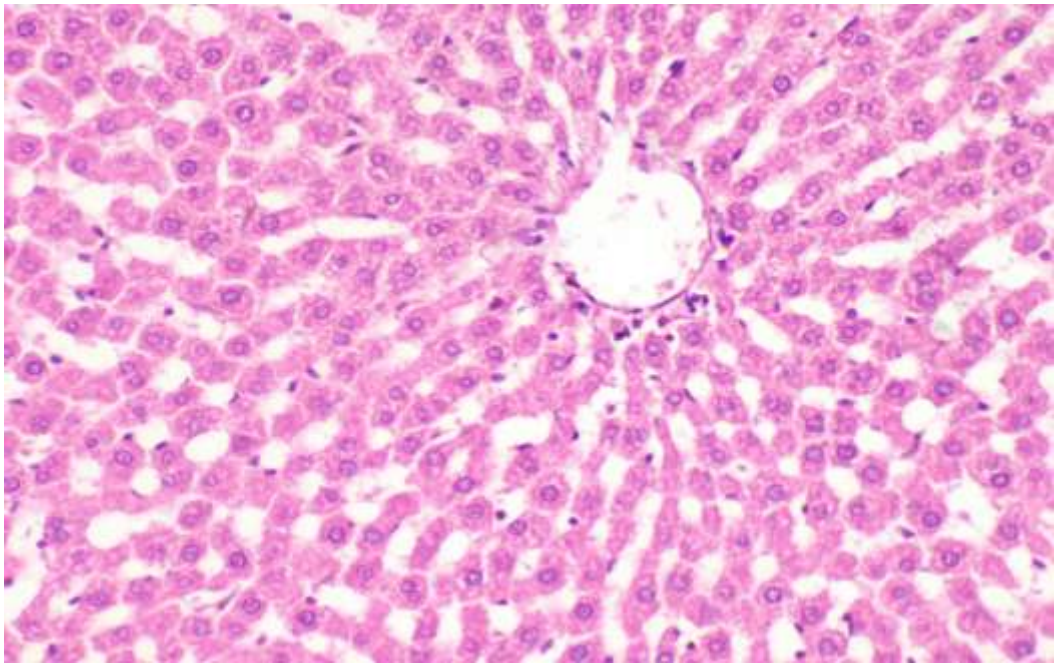


Fig. (1): Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X 400).

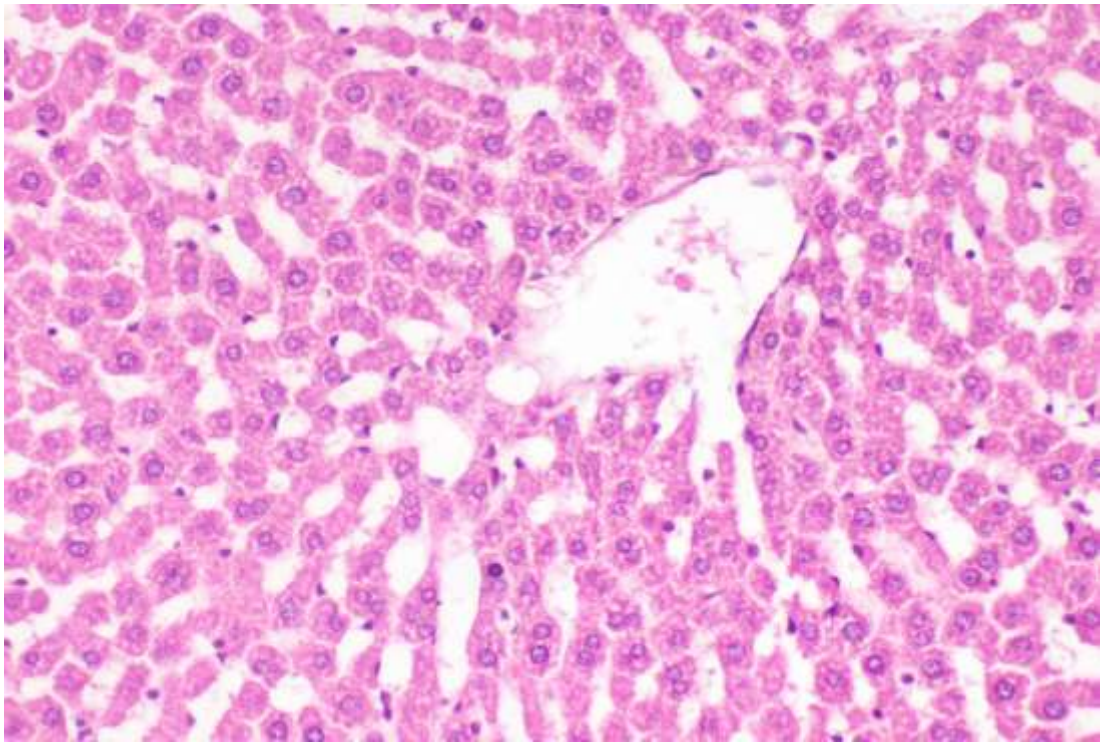


Fig. (2): Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X 400).

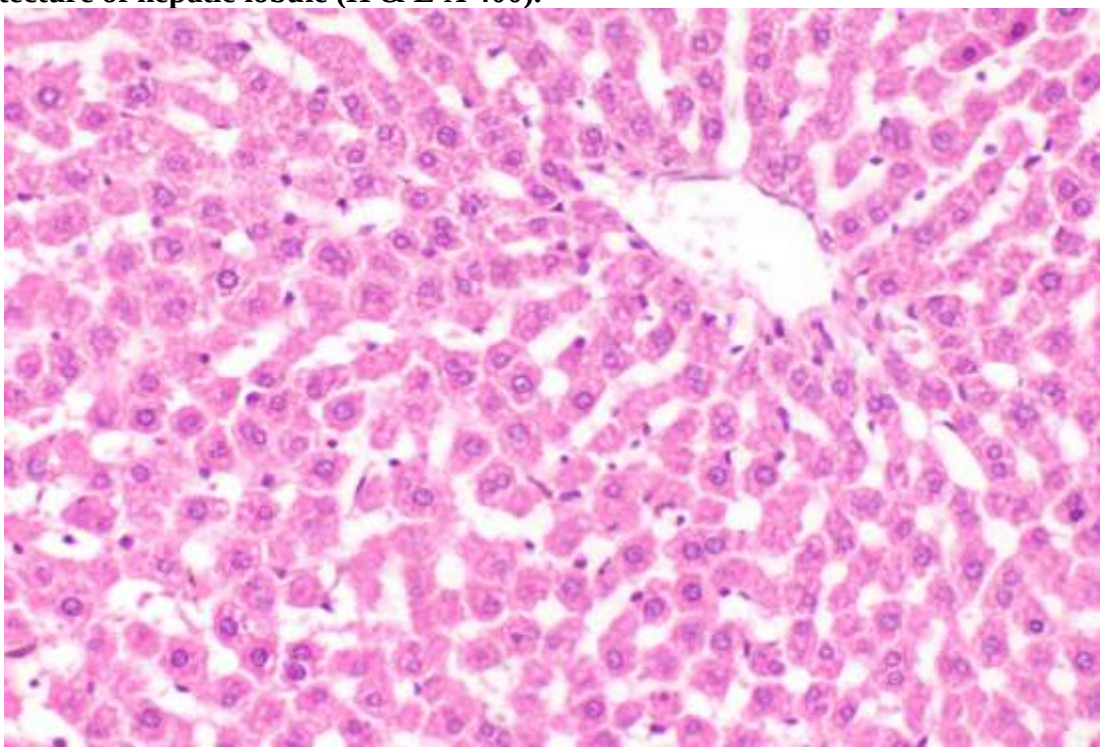


Fig. (3): Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X 400).

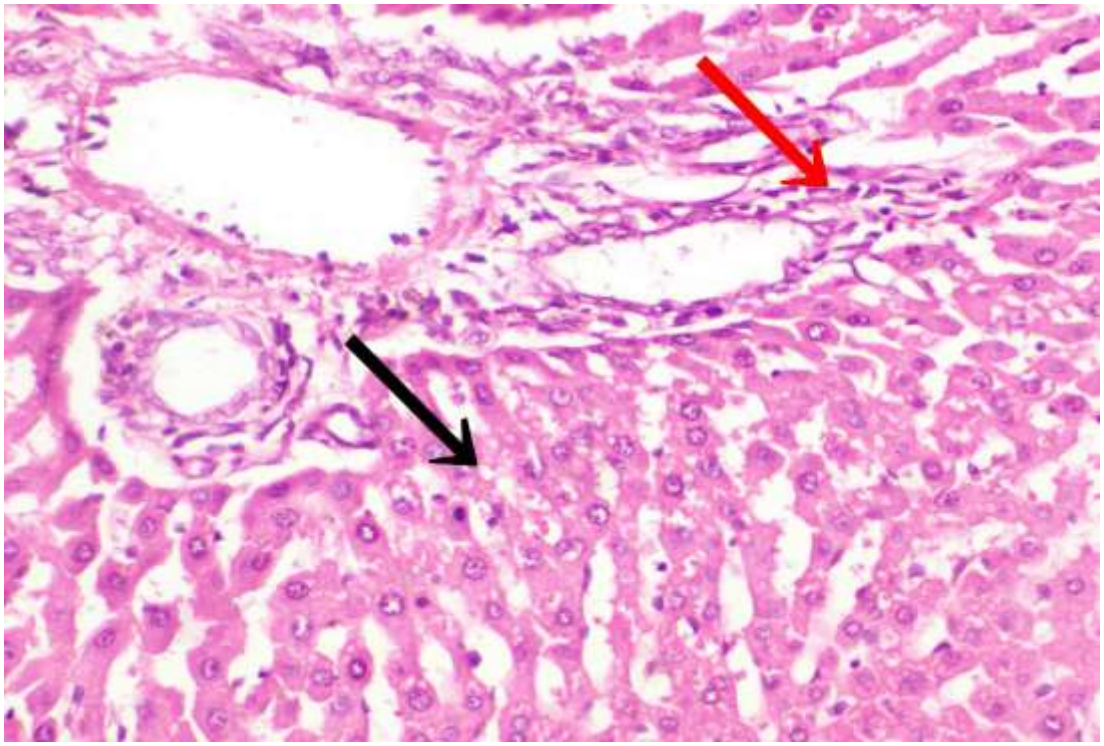


Fig. (4): Photomicrograph of liver of rat from group 2 showing vacuolar degeneration of hepatocytes (black arrow) and fibroplasia in the portal triad (red arrow) (H & E X 400).

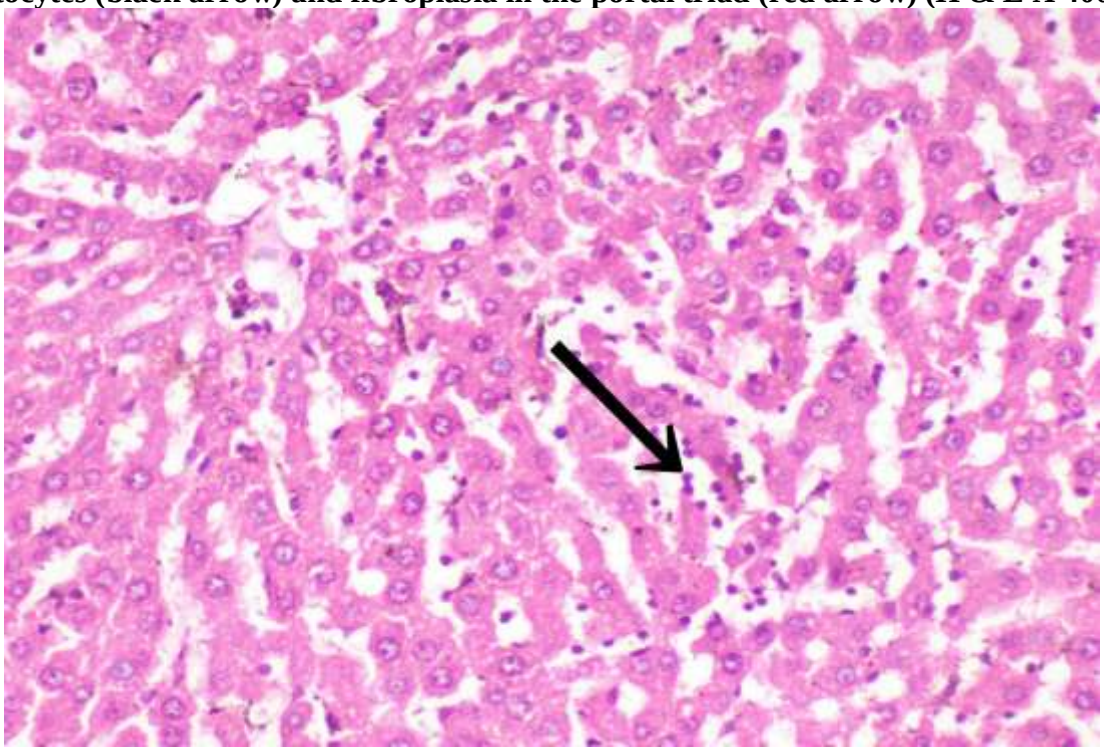


Fig. (5): Photomicrograph of liver of rat from group 2 showing sinusoidal leukocytosis (black arrow) (H & E X 400).

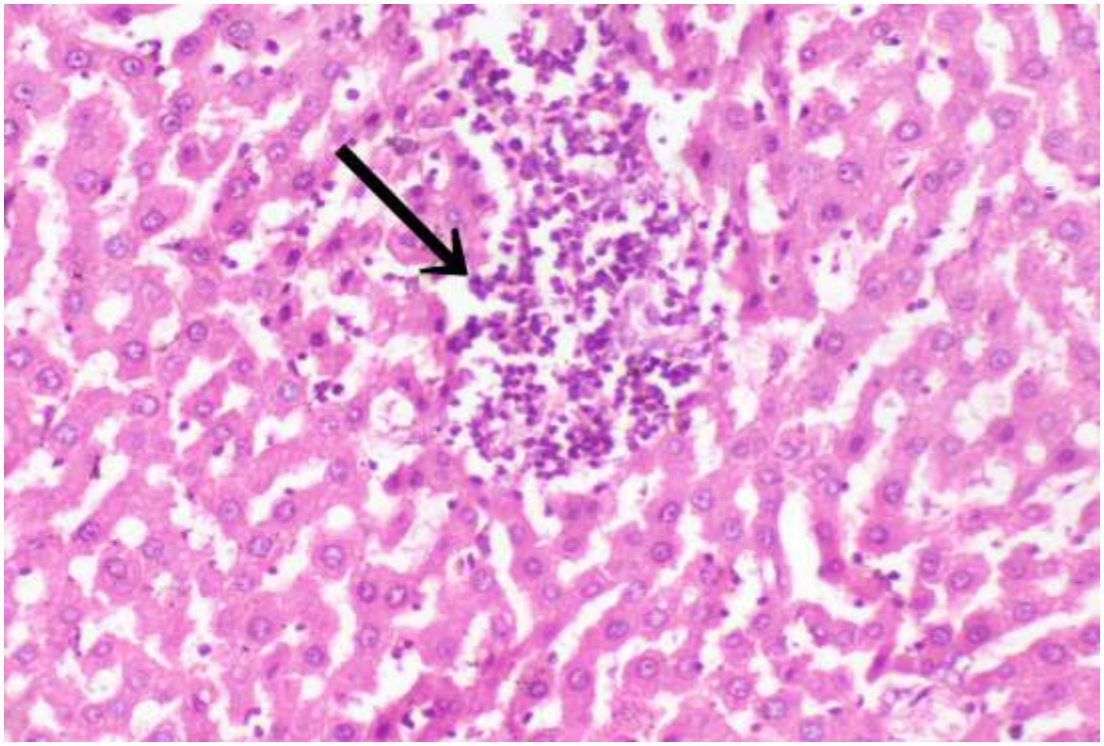


Fig. (6): Photomicrograph of liver of rat from group 2 showing focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (black arrow) (H & E X 400).

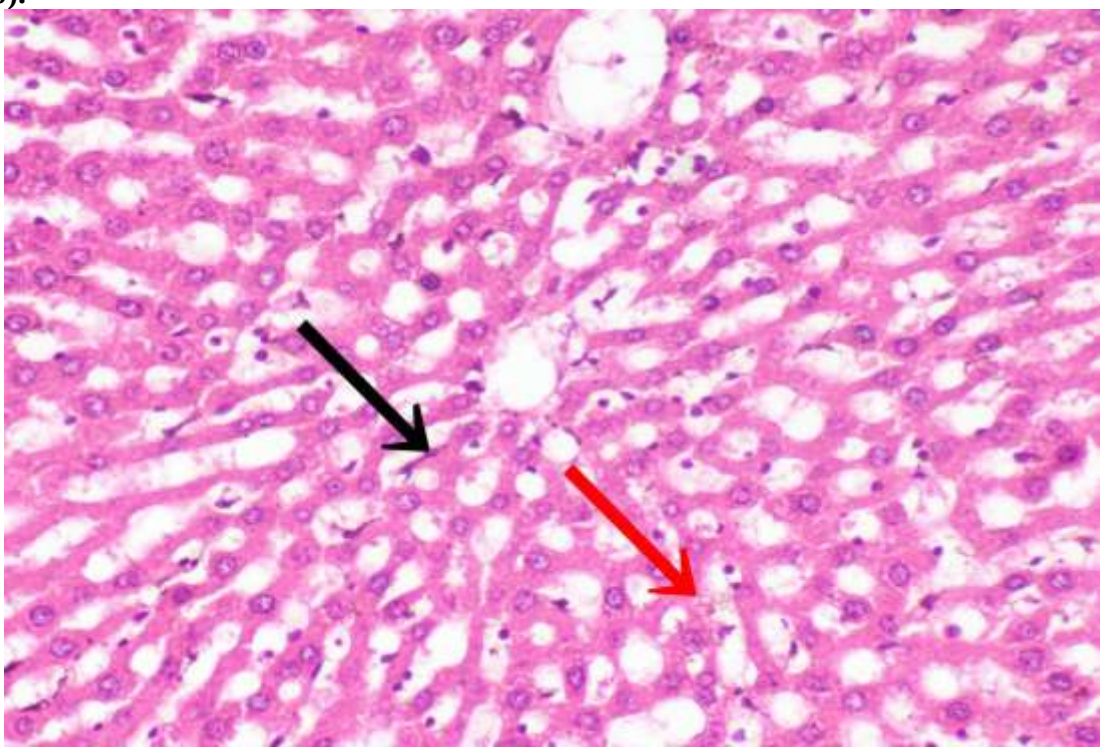


Fig. (7): Photomicrograph of liver of rat from group 3 showing slight activation of Kupffer cells (black arrow) and slight vacuolization of hepatocytes (red arrow) (H & E X 400).

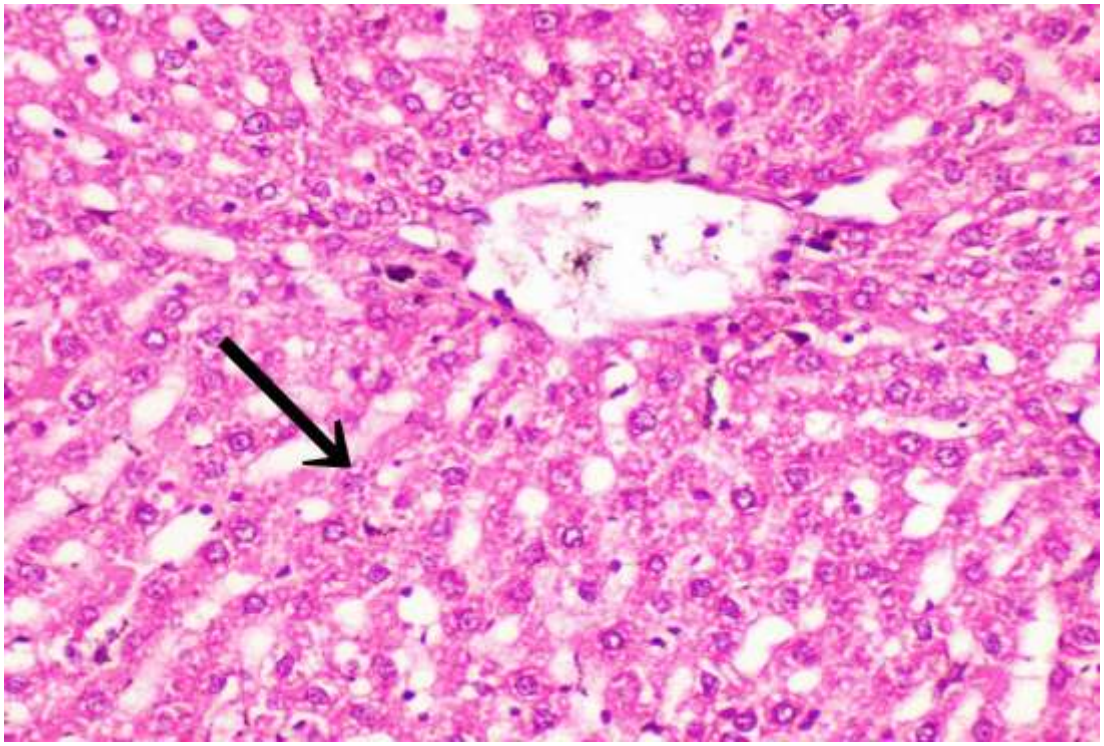


Fig. (8): Photomicrograph of liver of rat from group 3 showing slight vacuolization of hepatocytes (black arrow) (H & E X 400).

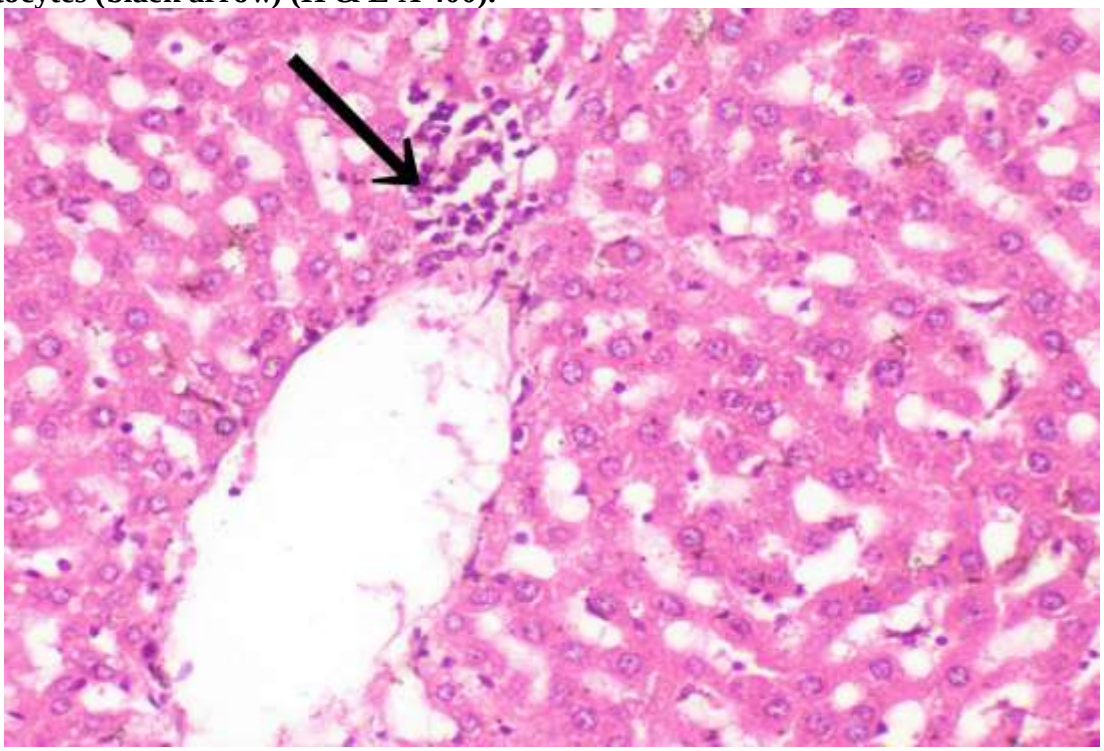


Fig. (9): Photomicrograph of liver of rat from group 3 showing small focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (black arrow) (H & E X 400).

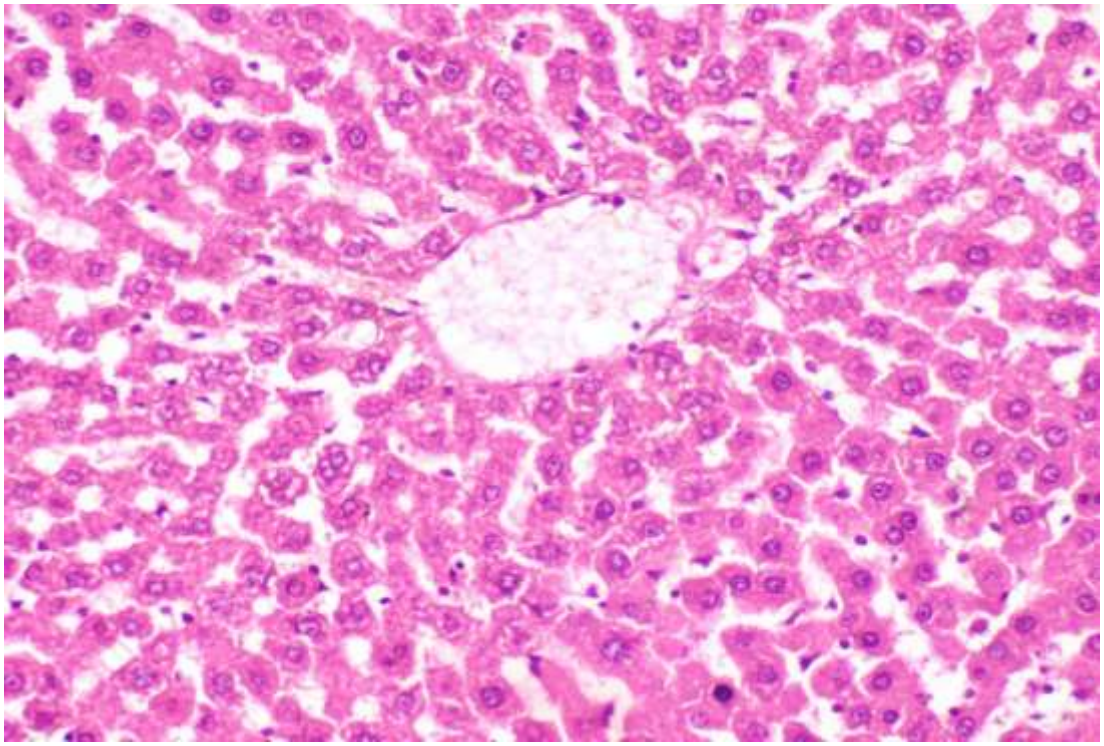


Fig. (10): Photomicrograph of liver of rat from group 4 showing no histopathological alterations (H & E X 400).

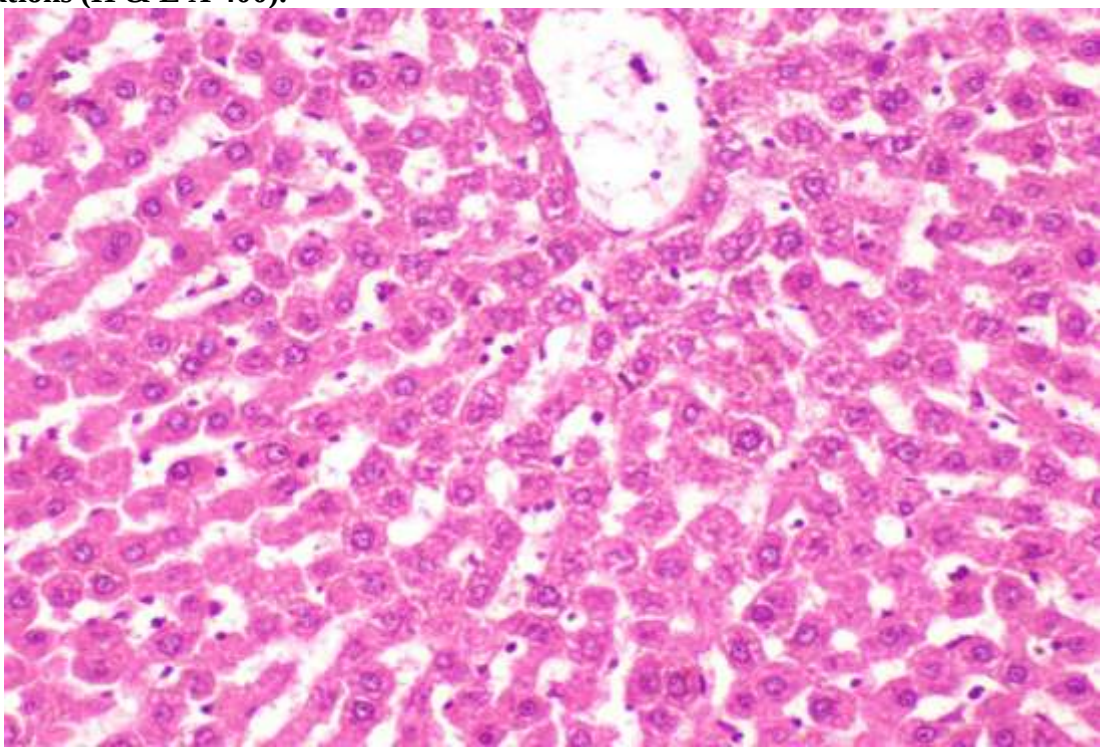


Fig. (11): Photomicrograph of liver of rat from group 4 showing no histopathological alterations (H & E X 400).

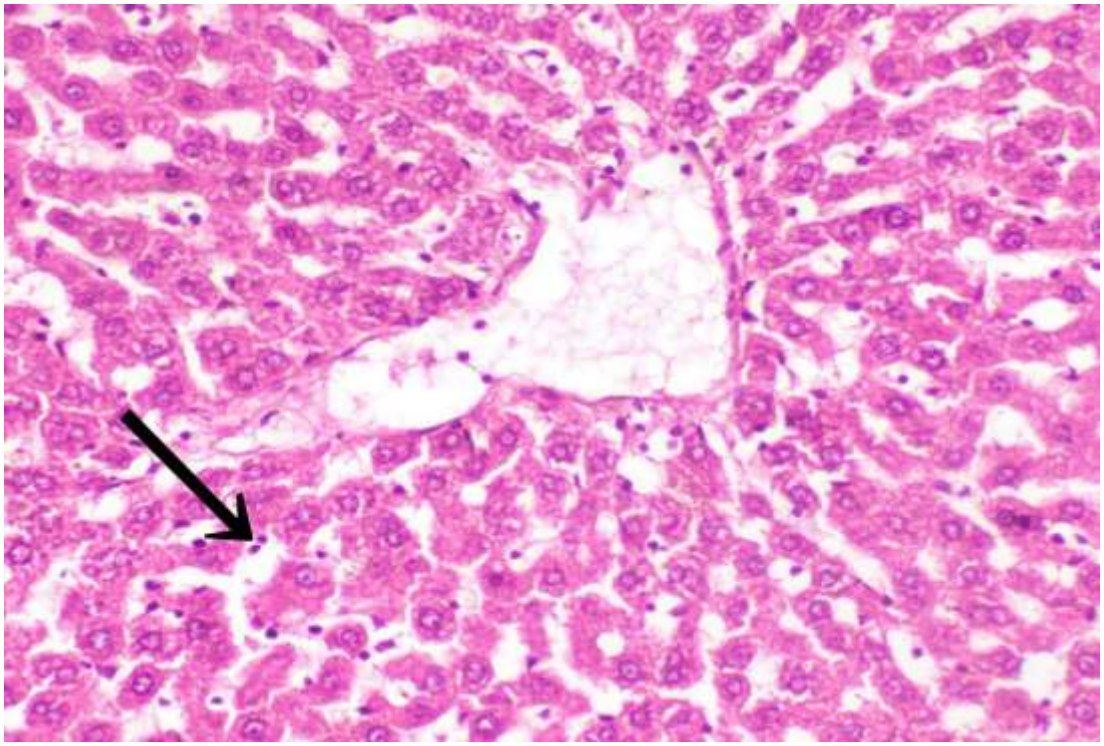


Fig. (12): Photomicrograph of liver of rat from group 4 showing slight activation of Kupffer cells (black arrow) (H & E X 400).

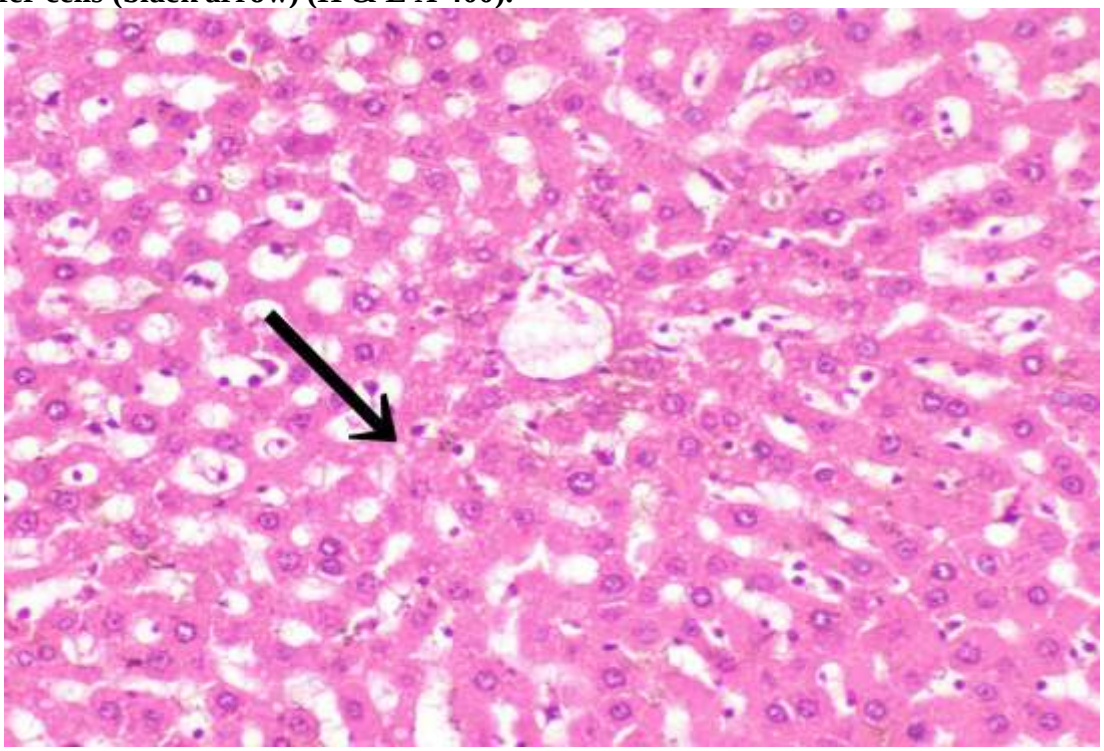


Fig. (13): Photomicrograph of liver of rat from group 5 showing slight vacuolization of hepatocytes (black arrow) (H & E X 400).

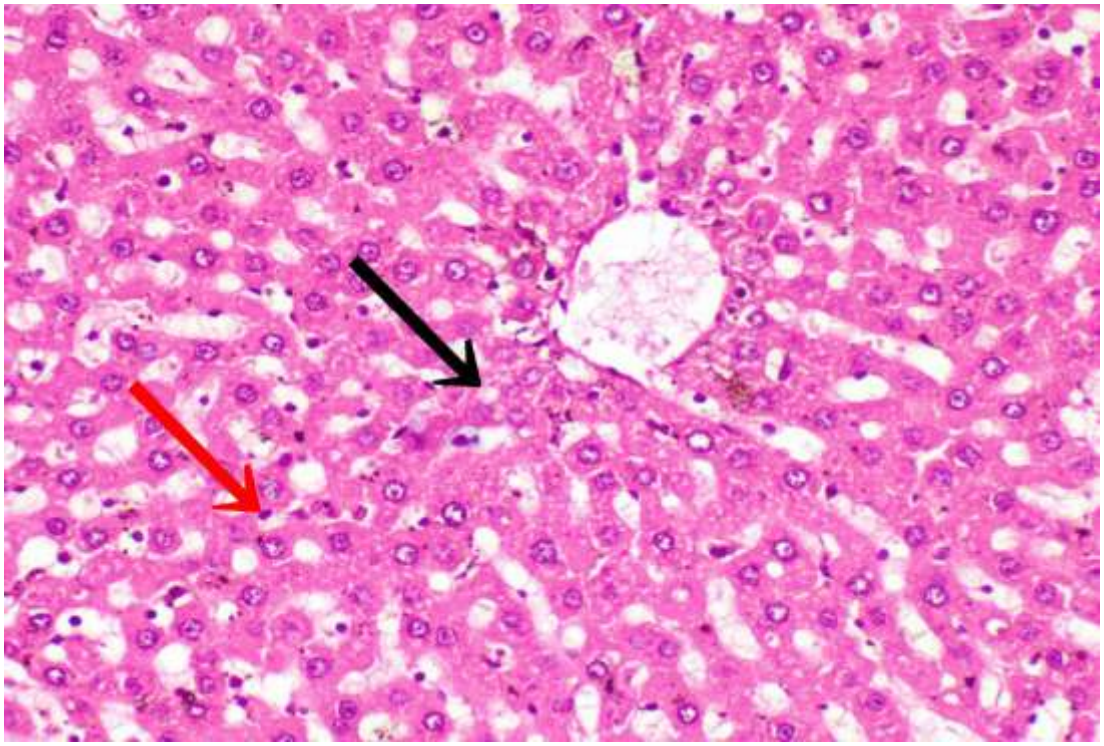


Fig. (14): Photomicrograph of liver of rat from group 5 showing slight vacuolization of hepatocytes (black arrow) and slight activation of Kupffer cells (red arrow) (H & E X 400).

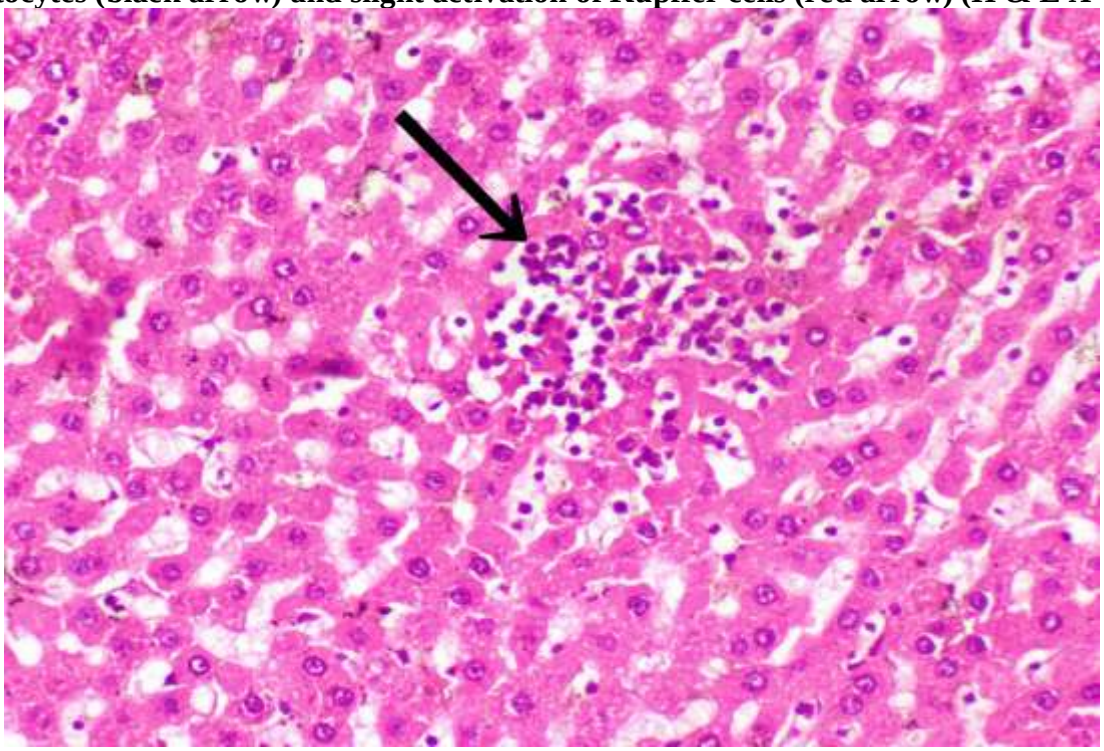


Fig. (15): Photomicrograph of liver of rat from group 5 showing focal hepatocellular necrosis and apoptosis associated with inflammatory cells infiltration (black arrow) (H & E X 400).

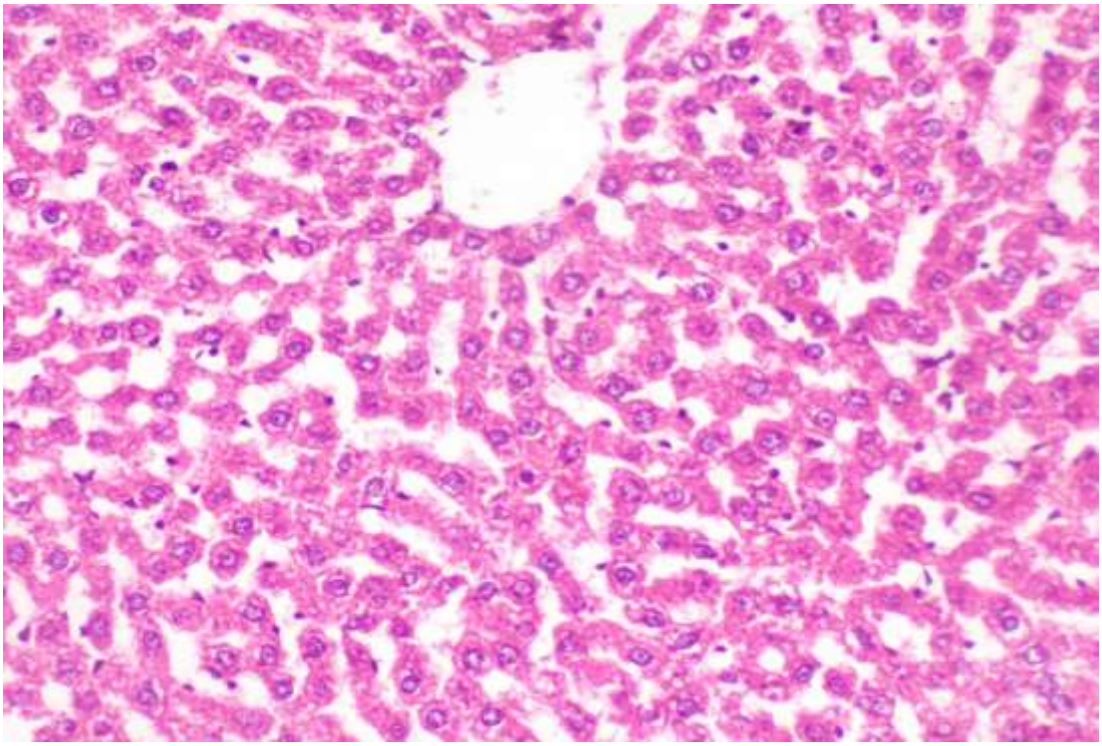


Fig. (16): Photomicrograph of liver of rat from group 6 showing no histopathological lesions (H & E X 400).

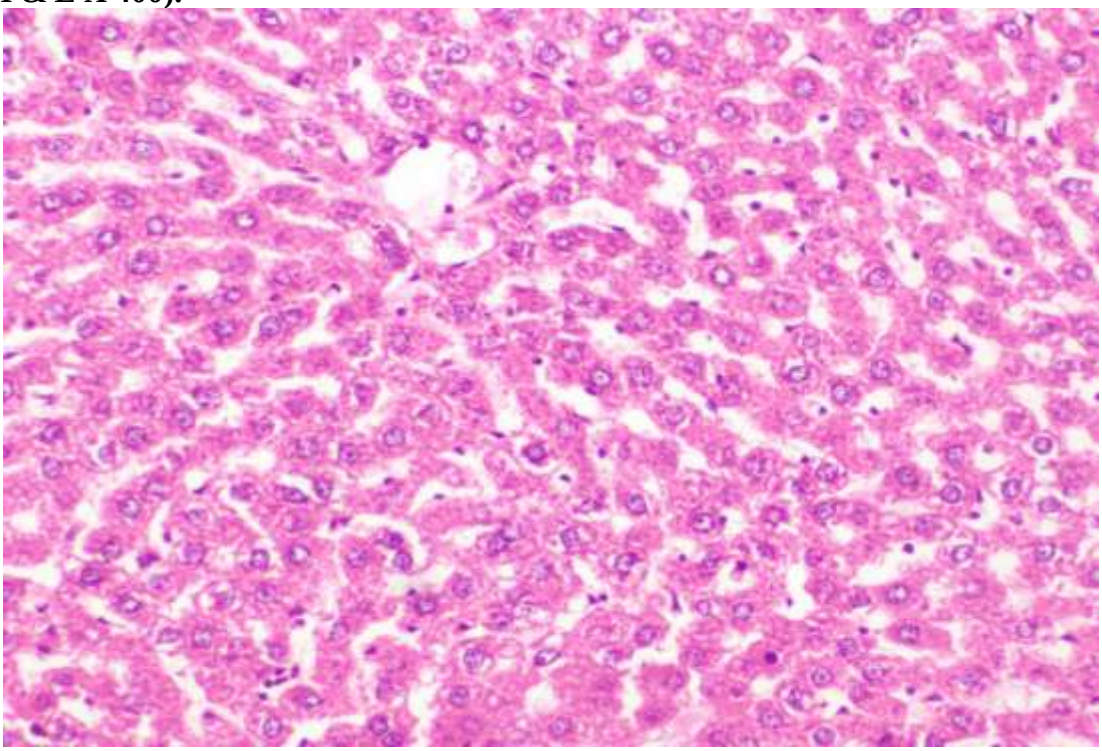


Fig. (17): Photomicrograph of liver of rat from group 6 showing no histopathological lesions (H & E X 400).

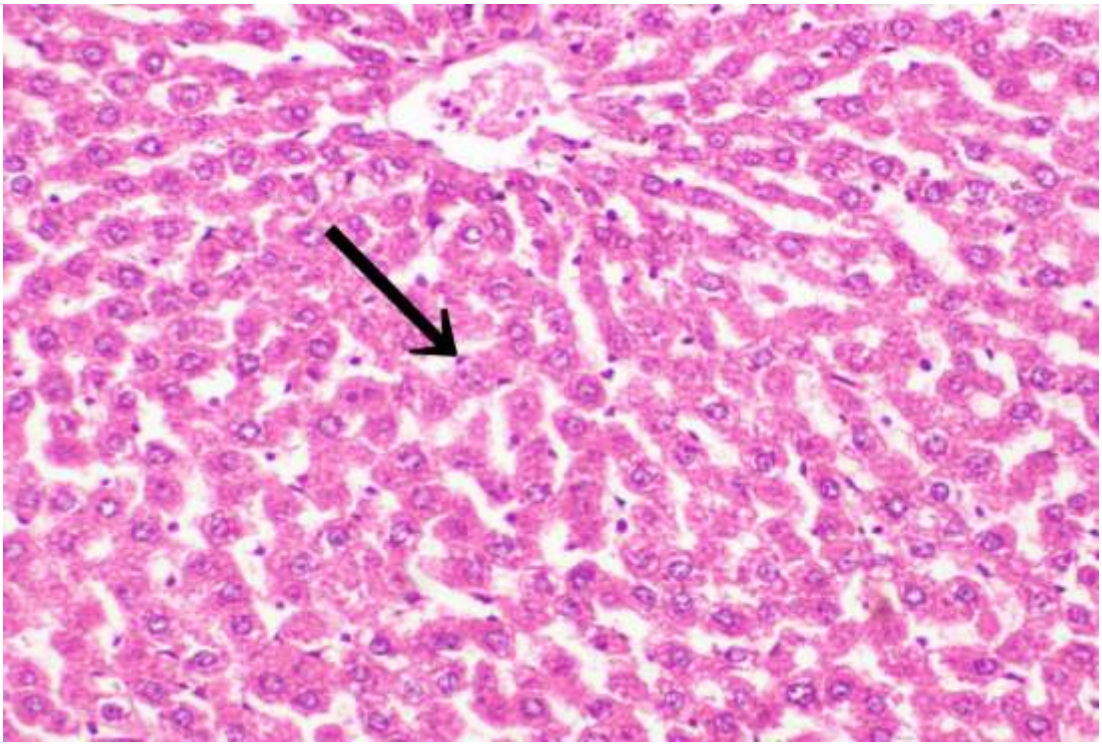


Fig. (18): Photomicrograph of liver of rat from group 6 showing slight activation of Kupffer cells (black arrow) (H & E X 400).

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