ASSESSMENT OF ROLE OF EMPAGLIFLOZIN, ROYAL JELLY AND HYDROXYCHLOROQUINE IN PREVENTION OF ACUTE GENTAMYCIN INDUCED NEPHROTOXICITY IN ALBINO RATS

Ezzeldin Shalaby (1), Marwa Abdel Naser Mohammed (2), Karima Mokhtar Mohamed Ahmed (1), Nermin Nabil Fayed (1)

1-Department of Forensic medicine and clinical toxicology-Faculty of medicine-Cairo University.
2-Department of Pathology-Faculty of medicine-Cairo University.

Corresponding Author: Ezzeldin Shalaby. Email: ezz.shalaby@yahoo.com

Submit Date 2025-05-20
Revise Date 2025-07-08
Accept Date 2025-07-09

ABSTRACT

Background: Gentamycin is aminoglycoside antibiotic which has potent bactericidal activities, less bacterial resistance, post-antibiotic effects and low cost but its uses limited due to nephrotoxicity. The Reno-protective effects of new sodium-glucose cotransporter 2 inhibitor drug Empagliflozin has been studied in Acute Kidney Injury and in some drugs induced nephrotoxicity. Protective effect of royal jelly was studied in many drug nephrotoxicity. Hydroxychloroquine antimalarial drug has potential protective mechanisms in kidney especially from toxins.

Aim: we aimed in the present study to assess role of Empagliflozin, Royal jelly and Hydroxychloroquine in prevention of acute gentamycin induced nephrotoxicity in albino rats.

Methods: In the present study, 25 male Wistar albino rats used in our study divided into 5 groups each group 5 rats: Group A control group. Then the four other groups received gentamycin SC 100 mg/kg/day for seven consecutive days to induce Nephrotoxicity, Group B gentamycin alone, Group C received Gentamicin + oral Royal Jelly 100 mg/kg for 7 days. Group D received Gentamicin + oral Empagliflozin 30 mg/kg/day for 7 days and Group E received Gentamicin + Hydroxychloroquine 1mg /kg/day for 7 days, we drew blood sample from all rats for Urea and Creatinine, on day 8, in addition to kidneys histopathology of sacrificed rats.

Result: In the present study, Hydroxychloroquine followed by Empagliflozin show significant Reno-protection, but Royal Jelly show mild Reno-protection against Gentamycin induced acute nephrotoxicity in both laboratory and histopathological results.

Conclusion: Hydroxychloroquine and Empagliflozin protect against Gentamycin induced acute nephrotoxicity but Royal Jelly minimize but not prevent it.

Key words: Gentamycin, Empagliflozin, Royal jelly, Hydroxychloroquine, Nephrotoxicity

INTRODUCTION

Gentamycin antibiotic has powerful bactericidal action with low resistance, and cheap, but its uses is limited due to nephrotoxicity. (**Balakumar, et al., 2010)**

Gentamycin induced nephrotoxicity mainly due to production of Reactive Oxygen Species (ROS) in tubular, glomerular and vascular tissues. **(Zarei and Elyasi, 2022)**

ROS affects antioxidant defense mechanisms. **(Hoseinynejad, et al., 2021)**

Gentamycin impairs mitochondrial respiration as it release Acid hydrolases in kidney, which lead to Induction of acute tubular necrosis, apoptosis and intracellular edema. (**Balakumar, et al., 2010)**

Gentamycin nephrotoxicity also through elevation of endothelin I, basal membrane disruption, increment of monocyte/ macrophages infiltration and glomerular congestion. **(Alsharidah, et al., 2021)**

The EMPA-KIDNEY trial found that Empagliflozin; a sodium-glucose cotransporter 2 (SGLT2) inhibitor, effective in type 2 diabetes mellitus control has also positive cardio-renal protective effects. (**Davidson, 2024)**

Reduction of toxic albumin and modulation of autophagic processes mediate protective effect of Empagliflozin. **(Matsui , et al., 2025)**

Meta-analyses prove that Empagliflozin effective in protection of kidneys from acute insults. (**Baigent, et al., 2022)**

Empagliflozin has also Reno-protection action from toxins in Wister rats. (**Eslamlou , et al., 2024)**

Royal jelly (RJ), as a product from honeybees, has potential therapeutic intervention as it has antioxidant activates and minimize inflammation. **(Kumar, et al., 2024)**

Royal jelly minimize nephrotoxicity induced by fluoride in rats. **(Aslan, et al., 2022)**

Antimalarial drug - chloroquine and its derivate hydroxychloroquine increase nitric oxide synthase with increase in glomerular filtration rate and urine flow rate. **(Ahmed, et al., 2003)**

Hydroxychloroquine also protect kidney from nephrotoxic drugs through reactive oxygen species (ROS) modulation. **(Klouda and Stone., 2020)**

We aim to assess the role of Empagliflozin, Royal jelly and Hydroxychloroquine in prevention of acute nephrotoxic effect of gentamycin in albino rats.

Rational: Gentamycin is potent and cheap antibiotic, it has also broad spectrum antibacterial action with less drug resistance by comparison with most famous antibiotic, but Gentamycin can cause nephrotoxicity which limit its uses or complete the course.

Aim of the study:

We aim to assess the role of Empagliflozin, Royal jelly and Hydroxychloroquine in prevention of acute nephrotoxic effect of gentamycin in albino rats, so it allow its uses safely without nephrotoxicity.

MATERIALS AND METHODS

Type of study:

Experimental animal study.

Ethical consideration:

We did the study after approval of The Institutional Animal Care and Use Committee Cairo University number CU/III/F/3/25.

Chemical substances:

Gentamycin from Schering-Plough Company, Empagliflozin from Pharmaglob Company, Hydroxychloroquine from Sanofi Company **and** Royal jellyfrom Pharco Company, all medication was bringed from Ali and Ali pharmacy Kasr Alaini branch.

Experimental design:

-Rats involved in our study breaded in suitable laboratory environment in wire mesh cages with water and standard nourishment, Animals lived at room temperature 22-24 ^{o}C and light/ dark cycles (12:12 hours). **(Reeves et al., 1993).**

- In the present study, 25 Female albino rats, body weight 150–200 g from Kasr Al Aini Faculty of medicine animal house used in our study divided into 5 groups each group 5 rats:

Group A represented the control group.

Group B received Gentamicin at dose 100 mg/kg/day subcutaneous (SC) for seven consecutive days to induce Nephrotoxicity. (**Udupa and Prakash., 2019)**

Group C received Gentamicin at dose 100 mg/kg/day SC + Royal Jelly 100 mg/kg via oral route for 7 days. **(Aslan, et al., 2022)**

Group D received Gentamicin at dose 100 mg/kg/day SC + Empagliflozin 30 mg/kg/day via oral route for 7 days. **(Mishriki, et al., 2024)**

Group E received Gentamicin at dose 100 mg/kg/day SC—+ Hydroxychloroquine 1 mg/kg/day via oral route for 7 days. **(Brkić, et al., 2022)**

We drew blood sample from all rats participated in our study including serum Urea and Creatinine on day 8, in addition to histopathological microscopic studies of Sacrificed rats kidneys on day 8.

Laboratory examination:

Serum Urea and serum creatinine were analyzed by chromatography technique through spectrophotometer in Al Borg central lab. (**Krishnegowda, et al., 2017)**

The rat sacrifice method:

Cervical dislocation (CD) under tranquilization Ketamine: 75 mg/kg + Xylasine: 16 mg/kg IP (in same syringe) **(Richardson., 2016)**

Histopathological examination:

After rats scarification, kidneys was removed, embedded in 10 % formalin solution for 24 hours, then we cut 4µm thickness Sections, fixed at slide then hematoxylin and eosin-stained. Sections then were coded then examined by Leica DM500 light microscope to which ICC 50 camera was attached. **(Bancroft and Gamble., 2008).**

We **divided Histopathological changes,** which have been seen by the light microscope**, into mild , moderate and severe according to certain % of changes** in each used parameter Glomerular congestion, tubular injury, mesangial

hypercellularity, interstitial inflammation, glomerular inflammation and glomerular edema By using Histopathological scoring:

All the microscopic lesions of the kidney for each group were presented in tables to demonstrate the type of lesion and its severity according to (**Chen et al., 2018**) as follow: Kidney lesions ranged from 0 to 4. Histopathological score is (0 = no lesions), (1= mild), (2= moderate), (3= severe) and (4= very severe lesions).

The percentage of histological changes in the cortex and medulla were scored using a semiquantitative scale designed to evaluate the degree of necrosis, cell loss, and necrotic casts on a five-point scale based on extent of involvement as follows: 0, normal kidney; 0.5, < 10 %; 1, 10–25 %; 2, 25–50 %; 3, 50–75 %; and 4, 75–100 % (**Ascon et al., 2009**).

Statistical analysis:

The statistical package for social sciences, version 26.0 (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. Qualitative variables analyzed as number and percentages, regarding quantitative data analyzed as mean± standard deviation and ranges when their distribution was parametric (normal) while non-normally distributed variables (non-parametric data) were presented as median with inter-quartile range (IQR).

We used also in our study A one-way analysis of variance (ANOVA), Chi-square test, Fisher's exact test, Probability (P-value) and Post Hoc test for Multiple comparison between groups **(Bursac et al., 2008).**

RESULTS

A-Laboratory results:-

As shown in table (1), and figures (1-2)

Multiple comparison between groups through Post Hoc test showed that there was a highly statistically significant highest mean value of serum urea (mg/dL) in Group B was 65.40±6.69, followed by Group C was 51.60±2.51, then the Group D was 49.40±3.21, followed by Group A was 24.00±4.06, then the Group E was 24.80±3.49, with p-value (p<0.001)

Also, there was a highly statistically significant highest mean value of serum creatinine mg/dL in Group B was 1.22±0.19, followed by Group C was 0.93±0.12, then the Group D was 0.66±0.10, followed by Group A was 0.41±0.11 and then the Group E was 0.37±0.13.

For Group E there was no significant difference i.e the levels were the same as Group A meaning that levels return to normal**.**

Table (1): Comparison between groups according to laboratory data.

Laboratory data		Group A	Group B	Group C	Group D	Group E	Test value	p-value
Serum Urea mg/dL	Mean±SD	24.00¨	65.40#	51.60▲	49.40▲	24.80¨	90.796	<0.001**
		4.06	6.69	2.51	3.21	3.49		
	Range	18	56	50	45	20		
		28	73	56	53	29		
Serum creatinine mg/dL	Mean±SD	0.41§	1.22#	0.93▲	0.66¨	0.37§	36.675	<0.001**
		0.11	0.19	0.12	0.1	0.13		
	Range	0.23	1	0.8	0.55	0.23		
		0.5	1.5	1.1	0.8	0.5		

Using: One way Analysis of Variance test was performed for Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test

Different capital letters indicate significant difference at (p<0.05) among means in the same row

*p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant*

Group A Control group; Group B Gentamycin group

Group C Gentamycin group+Royal Jelly Group

Group D Gentamycin group+Empagliflozin Group

Group E Gentamycin group+Hydroxychloroquine Group

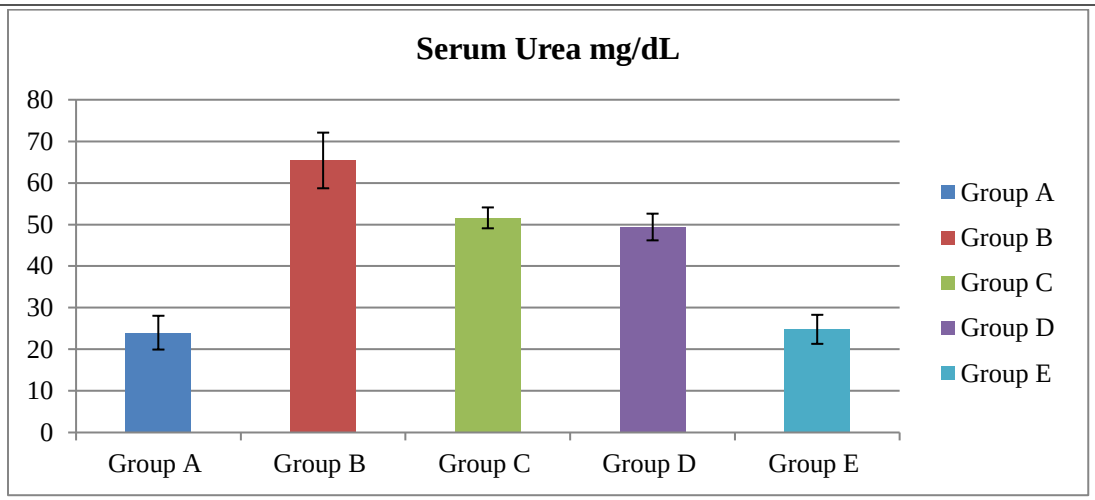

Fig. (1): Comparison between groups according to Serum Urea mg/dL.

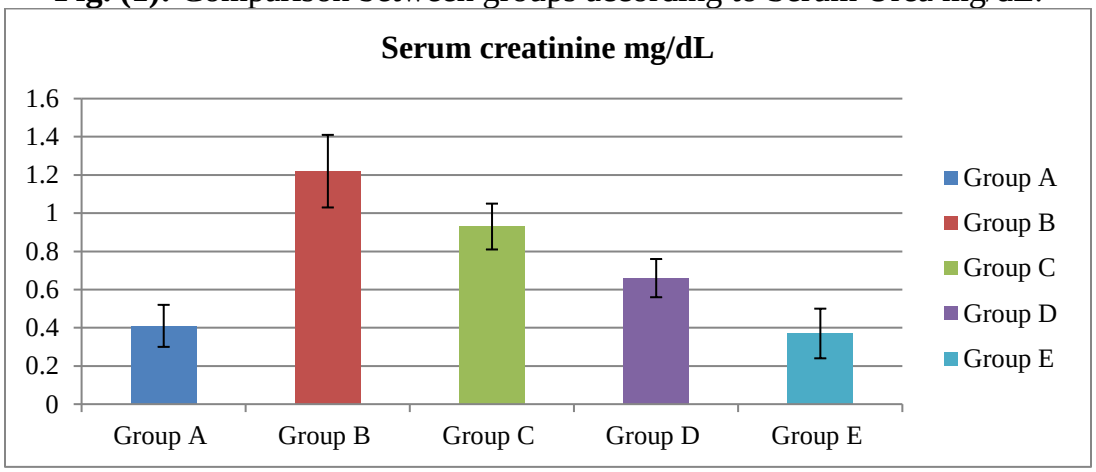

Fig. (2): Comparison between groups according to Serum creatinine mg/dL.

B-Histopathological results:-

As shown in table (2); and Figures (3-9)

Table (2): Comparison between groups according to pathology results.

Pathology results			Group A	Group B	Group C	Group D	Group E	Test value	p-value
Glomerular congestion	Absent	No.	5	0	1	2	5	32.404	<0.001**
		%	100.00%	0.00%	20.00%	40.00%	100.00%		
	Present	No.	0▲	5#	4#	3#	0▲		
		%	0.00%	100.00%	80.00%	60.00%	0.00%		
	Mild	No.	0	1	4	3	0		
		%	0.00%	20.00%	80.00%	60.00%	0.00%		
	Moderate	No.	0	4	0	0	0		
		%	0.00%	80.00%	0.00%	0.00%	0.00%		
Tubular injury	Absent	No.	5	0	0	2	5	43.75	<0.001**
		%	100.00%	0.00%	0.00%	40.00%	100.00%		
	Present	No.	0▲	5#	5#	3#	0▲		
		%	0.00%	100.00%	100.00%	60.00%	0.00%		
	Mild	No.	0	0	5	3	0		
		%	0.00%	0.00%	100.00%	60.00%	0.00%		
	Moderate	No.	0	4	0	0	0		

		%	0.00%	80.00%	0.00%	0.00%	0.00%		
	Severe	No.	0	1	0	0	0		
		%	0.00%	20.00%	0.00%	0.00%	0.00%		
Tubular casts	Absent	No.	5	2	2	3	5	8.456	0.076
		%	100.00%	40.00%	40.00%	60.00%	100.00%		
	Present	No.	0▲	3#	3#	2#	0▲		
		%	0.00%	60.00%	60.00%	40.00%	0.00%		
	Mild	No.	0	3	3	2	0		
		%	0.00%	60.00%	60.00%	40.00%	0.00%		
Mesangial hypercellularity	Absent	No.	5	1	3	3	5	10.294	0.036*
		%	100.00%	20.00%	60.00%	60.00%	100.00%		
	Present	No.	0▲	4#	2#	2#	0▲		
		%	0.00%	80.00%	40.00%	40.00%	0.00%		
	Mild	No.	0	4	2	2	0		
		%	0.00%	80.00%	40.00%	40.00%	0.00%		
Interstitial inflammation	Absent	No.	5	0	2	5	4	27.875	<0.001**
		%	100.00%	0.00%	40.00%	100.00%	80.00%		
	Present	No.	0▲	5#	3#	0▲	1▲		
		%	0.00%	100.00%	60.00%	0.00%	20.00%		
	Mild	No.	0	1	3	0	1		
		%	0.00%	20.00%	60.00%	0.00%	20.00%		
	Moderate	No.	0	4	0	0	0		
		%	0.00%	80.00%	0.00%	0.00%	0.00%		
Glomerular inflammation	Absent	No.	5	0	3	4	5	15.809	0.003*
		%	100.00%	0.00%	60.00%	80.00%	100.00%		
	Present	No.	0¨	5#	2▲	1▲	0¨		
		%	0.00%	100.00%	40.00%	20.00%	0.00%		
	Mild	No.	0	5	2	1	0		
		%	0.00%	100.00%	40.00%	20.00%	0.00%		
Glomerular edema	Absent	No.	5	1	4	5	5	15	0.005*
		%	100.00%	20.00%	80.00%	100.00%	100.00%		
	Present	No.	0▲	4#	1▲	0▲	0▲		
		%	0.00%	80.00%	20.00%	0.00%	0.00%		
	Mild	No.	0	4	1	0	0		
		%	0.00%	80.00%	20.00%	0.00%	0.00%		

Using: x2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
Different symbols indicate significant difference at (p<0.05) among means in the same row
*p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant*
Group A Control group; Group B Gentamycin group
Group C Gentamycin group+Royal Jelly Group
Group D Gentamycin group+Empagliflozin Group
Group E Gentamycin group+Hydroxychloroquine Group

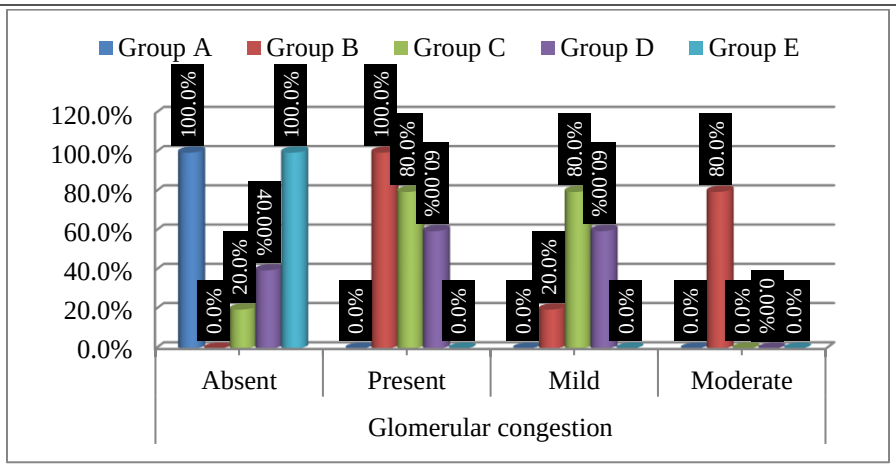

Fig. (3): Comparison between groups according to Glomerular congestion.

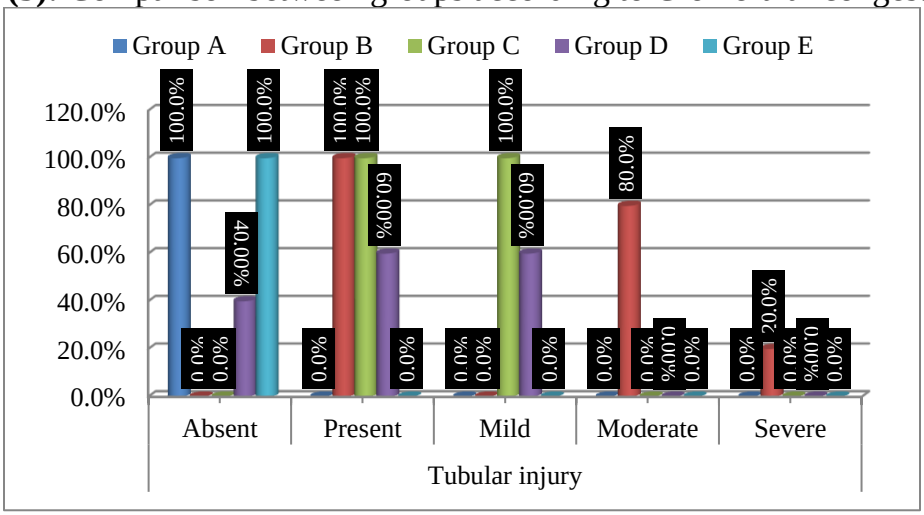

Fig. (4): Comparison between groups according to Tubular injury.

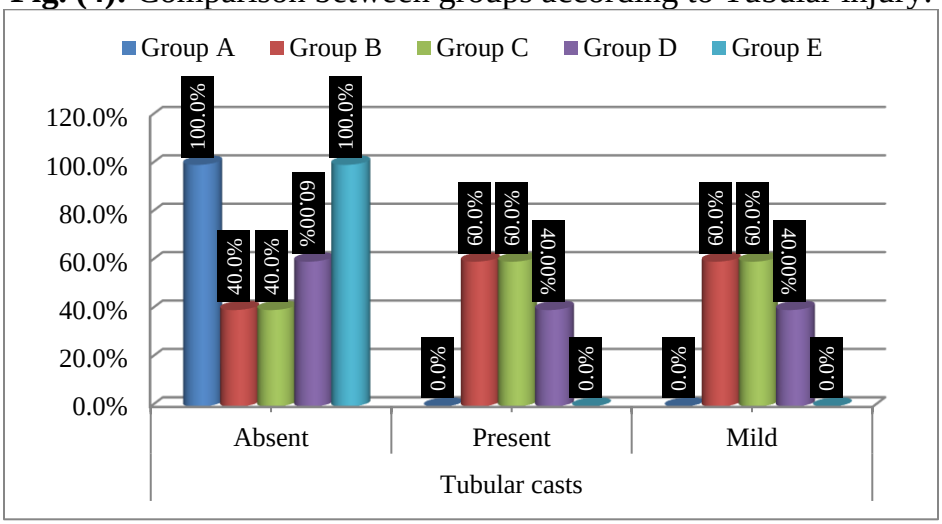

Fig. (5): Comparison between groups according to Tubular casts.

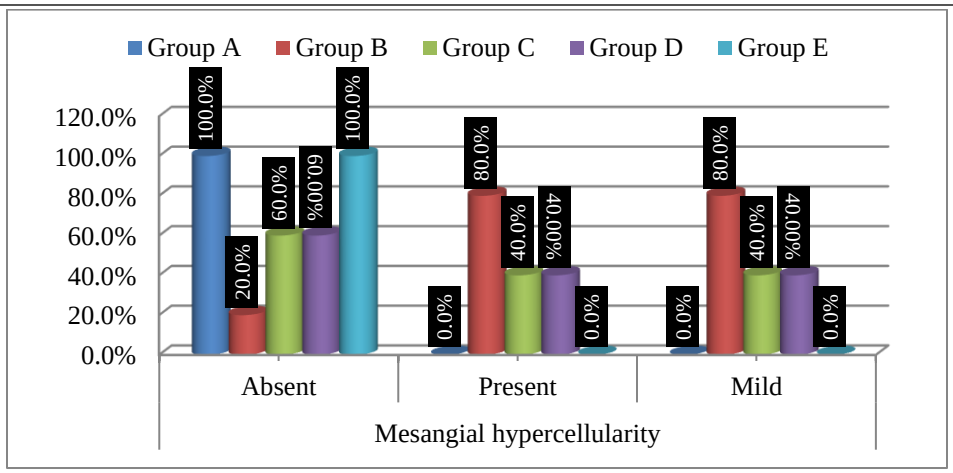

Fig. (6): Comparison between groups according to mesangial hypercellularity.

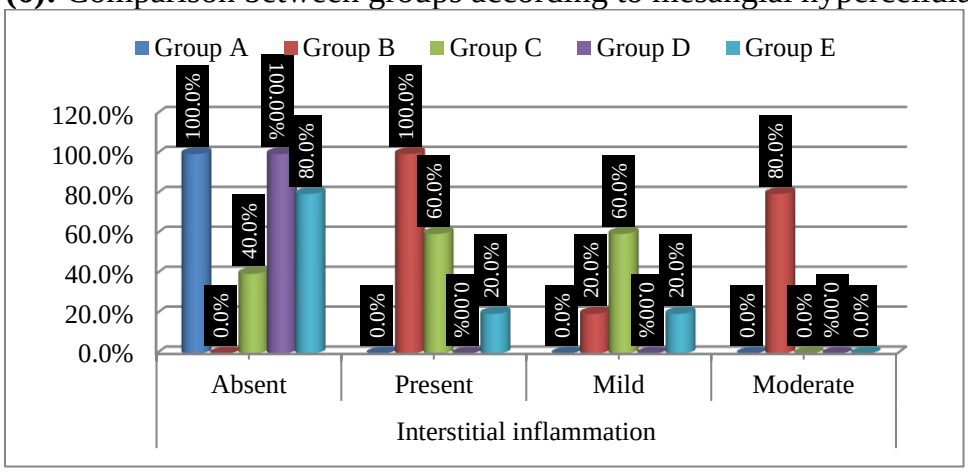

Fig. (7): Comparison between groups according to interstitial inflammation.

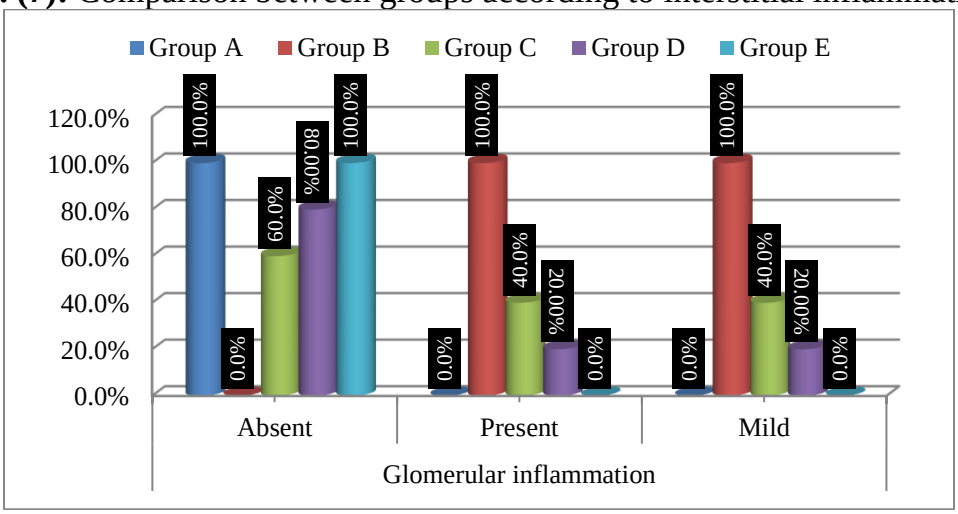

Fig. (8): Comparison between groups according to Glomerular inflammation.

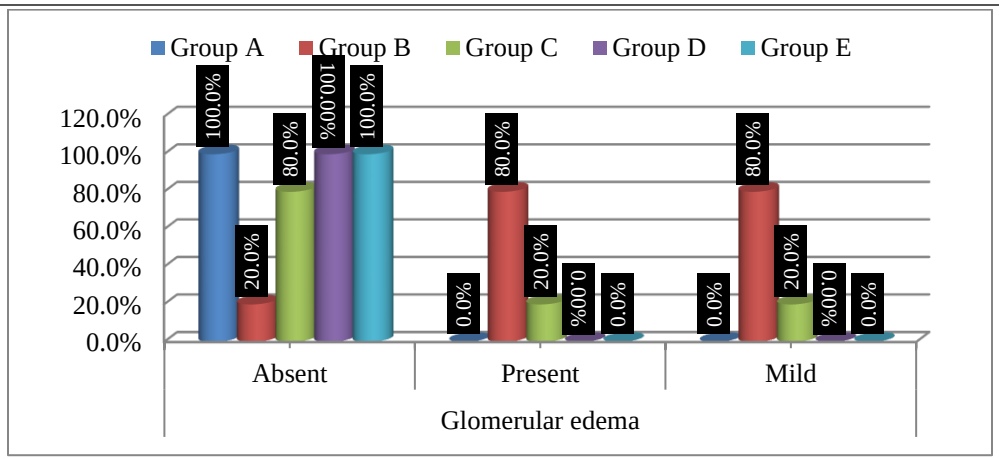

Fig. (9): Comparison between groups according to Glomerular edema

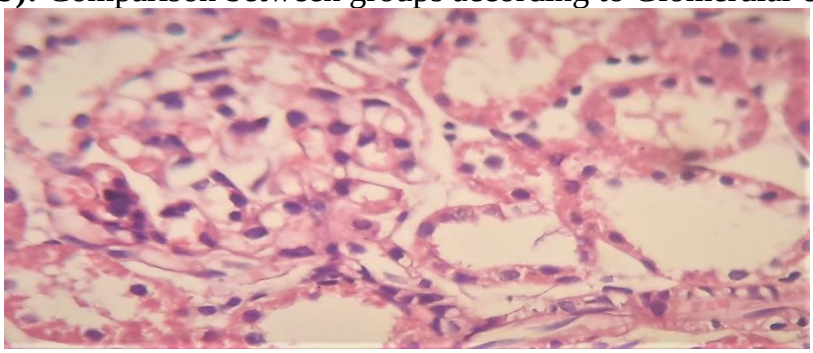

Figure (10) Normal Glomerulus in kidney cortex (H&E 200X) in control Group A

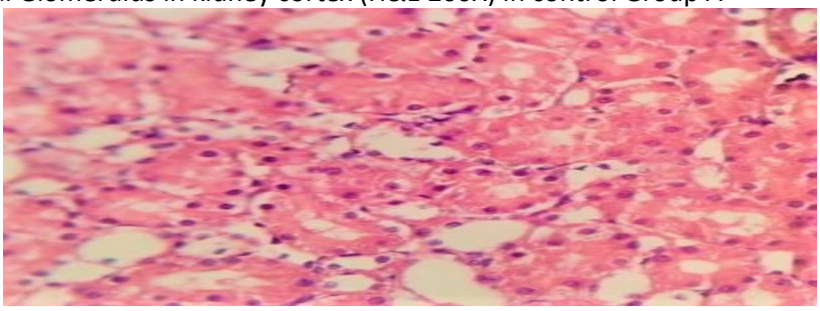

Figure (11) Normal proximal convoluted tubules in kidney cortex (H&E 200X) in control Group A

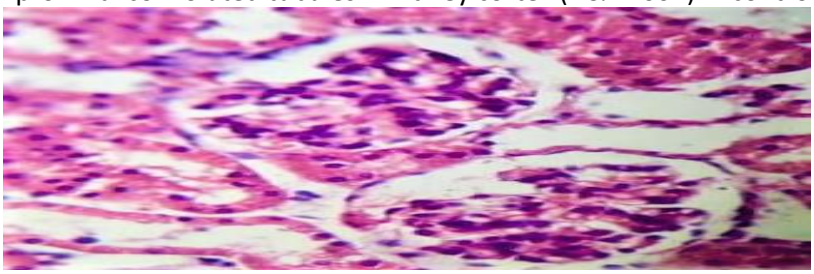

Figure (12) Two Glomeruli show lobulations with marked mesangial hypercellularity (H&E 200X) in Group B

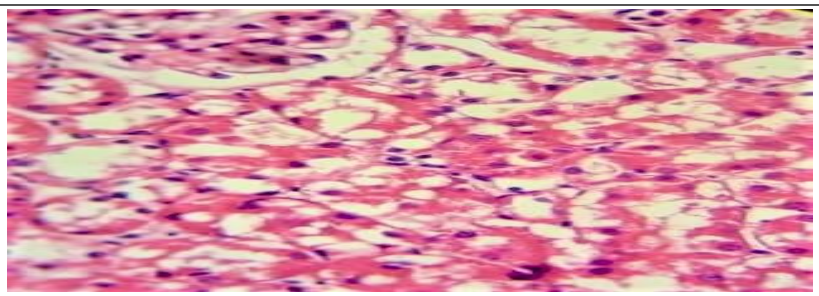

Figure (13) Marked Tubular Injury with cytoplasmic vacuolations of proximal convoluted tubules (H&E 200X) in Group B

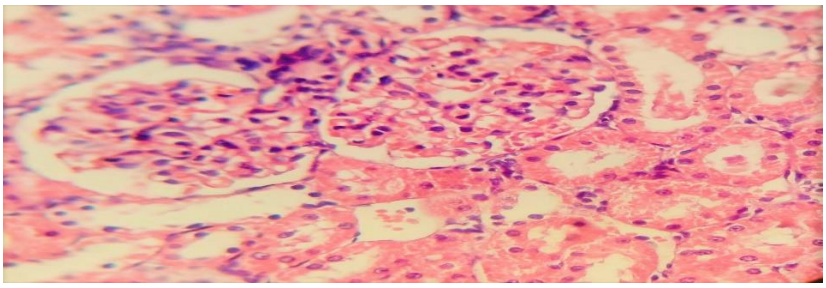

Figure (14) Two Glomeruli show variable degrees of Congestion and mild mesangial hypercellularity (H&E 200X) in Group C

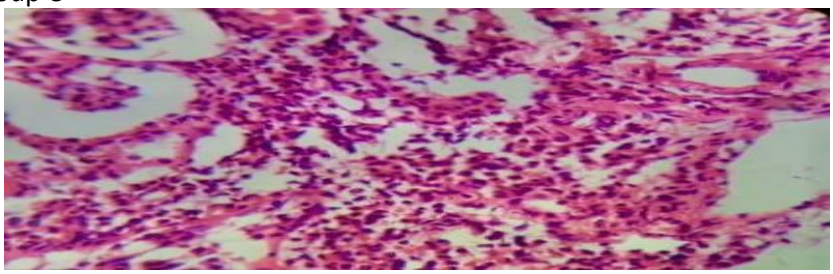

Figure (15) Dense mixed inflammatory infiltration in the interstitium (H&E 200X) in Group C

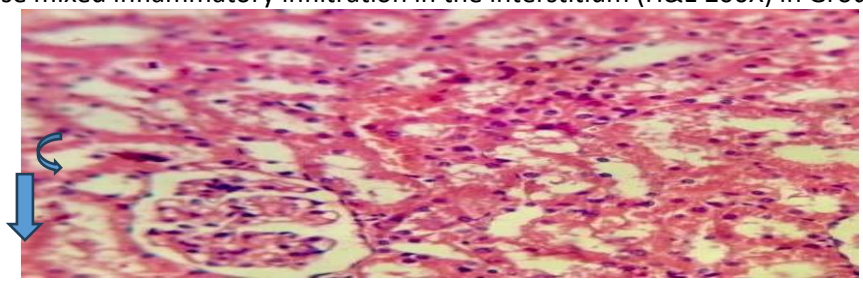

Figure (16) The glomerulus show mesangial hypercellularity (arrow) and vascular congestion (curve arrow) of peritubular capillaries (H&E 200X) in Group D

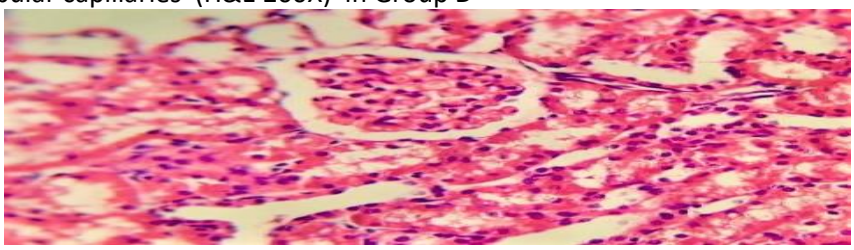

Figure (17) The glomerulus shows mild congestion, normal cellularity and mild tubular injury (H&E 200X) in Group D

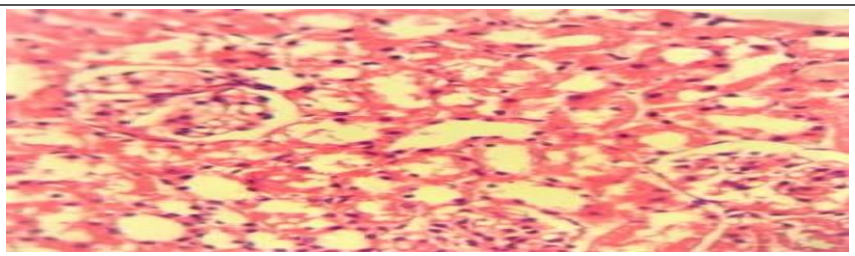

Figure (18) The glomeruli show normal cellularity and mild tubular injury (H&E 200X) in Group E

There was a statistically significant difference between groups according to pathology results about glomerular congestion, tubular injury, mesangial hypercellularity, interstitial inflammation, glomerular inflammation and glomerular edema, with p-value ($p<0.05$) as follow:-

In group A (control group): normal histopathological result.

In group B Significant histopathological changes in the form of Glomerular congestion 100% divided into 20% mild and 80% moderate, Tubular injury 100% divided into 80% moderate and 20% severe, Tubular cast 60% which is mild, Mesangial hypercellularity 80% which is mild, Interstitial inflammation 100% which is mild in 20% and moderate in 80%, Glomerular inflammation in 100% which is mild and Glomerular edema in 80% which is mild.

In group C histopathological results in the form of mild Glomerular congestion 80%, mild Tubular injury 100%, mild Tubular cast 60%, mild Mesangial hypercellularity 40%, minimal Glomerular inflammation 40%, mild Interstitial inflammation 60% and mild Glomerular edema 20%.

In group D histopathological results in the form of mild Glomerular congestion 60% , mild Tubular injury 60%, mild Tubular cast 40% , mild Mesangial hypercellularity 40%, minimal Glomerular inflammation 20% and no Interstitial inflammation and no Glomerular edema.

In group E normal histopathological results except 20% interstitial inflammation.

DISCUSSION

Gentamycin antibiotic has powerful bactericidal action with low resistance, and cheap, but its uses is limited due to nephrotoxicity. (**Balakumar, et al., 2010)**

The present study confirmed that injection of gentamicin 100 mg/kg/day subcutaneous for 7 days lead to nephrotoxicity in rats with raising of serum urea with mean value 65.40±6.69 and serum creatinine mean value 1.22±0.19.

Our study is in harmony with studies of **Abd-Elhamid, et.al (2018); Babaeenezhad, et al., (2021; Medić, et al (2019)** which prove that injection of gentamicin 100 mg/kg/day subcutaneous for 7 days lead to nephrotoxicity in rats and raise serum urea and creatinine.

the present study proved that gentamycin induced kidney histopathological changes in the form of Glomerular congestion 100% divided into 20% mild and 80% moderate, Tubular injury 100% divided into 80% moderate and 20% severe, Tubular cast 60% which is mild, Mesangial hypercellularity 80% which is mild, Interstitial inflammation 100% which is mild in 20% and moderate in 80%, Glomerular inflammation in 100% which is mild and Glomerular edema in 80% which is mild.

Our study is in harmony with the study of **Udupa and Prakash (2019)** which found significant histopathological changes due to gentamycin toxicity increase with increase dose and duration in the form of tubular damage, tubular necrosis, tubular cast, interstitial inflammation.

In addition, our study is in harmony with a study of **Alarifi, et al (2011)** which declare that gentamycin induce tubular necrosis, degenerative changes and glomerular inflammation and edema.

In the present study, Hydroxychloroquine at a dose of 1mg/Kg oral showed significant Reno-protection against Gentamycin induced acute nephrotoxicity in laboratory with urea mean value 24.80±3.49, and creatinine mean 0.37±0.13 which is nearly equal to control group, with normal histopathological results except 20% Interstitial inflammation.

Our study is in harmony with the study of **Brkić, et al (2022),** which declare that Hydroxychloroquine at low doses (0.3 mg/kg and

1 mg/kg) decrease urea and creatinine level due to antioxidant action but higher in group received higher dose 3 mg/Kg.

In addition, our study is in harmony with the study of **Idris and Olufunke (2024),** which approve potential of chloroquine in the management of Acrylamide-induced nephropathy, which mediated via anti-inflammatory, anti-apoptotic and immune mediated mechanisms.

However, our study is against the study of **Helal, et al (2023),** which declared that chloroquine treatment with dose of 970 mg/kg body weight resulted in a state of peroxidation of membrane lipids and oxidative stress-mediated kidney tissue injury and raising of serum urea and creatinine, which is improving with ginkgo biloba extract.

In addition, **Pari and murugan, (2006),** reported that chloroquine-treated rats showed numerous hemorrhagic and necrotic areas, and cloudy swelling of renal tubules that happen as result of chloroquine -induced oxidative damage also at chloroquine dose 970 mg/kg body weight.

Explanation: Chloroquine effect on rats kidneys is dose dependent, at low dose as we use in our study 1 mg/kg and similar studies has Reno-protective effect as it has antioxidant effect and Attenuates Oxidative Stress, but in higher dose 3 mg/kg has mild toxic effect on kidneys and at toxic higher dose 970 mg/kg has nephrotoxic effect result of chloroquine -induced oxidative damage **(Idris and Olufunke ,2024).**

In the present study, Empagliflozin at a dose of 30 mg/Kg orally for 7 days showed significant Reno protection against Gentamycin induced acute nephrotoxicity with laboratory result show urea mean value 49.40±3.21, and creatinine mean 0.66±0.10, with histopathological results in the form of mild Glomerular congestion 60% , mild Tubular injury 60%, mild Tubular cast 40% , mild Mesangial hypercellularity 40%, minimal Glomerular inflammation 20% and no Interstitial inflammation and no Glomerular edema.

The present study is in harmony with **Botros, et al (2022),** who proved that empagliflozin orally at dose 10 and 20 mg/kg for 7 days has a protective effect against gentamicin-induced nephrotoxicity due to its antioxidant and anti-apoptotic actions, with more protective effect at the higher dose.

In addition, the present study is in harmony with **Mosalam, et al (2025**),

Who proved that empagliflozin at 10 mg/kg and 20 mg/kg for one week confers significant Reno-protective especially with higher dose against paracetamol-induced kidney injury mainly due to its antioxidant, anti-inflammatory, anti-apoptotic, and various metabolic regulatory properties.

In addition, the present study is in harmony with the study of **Mishriki, et al (2024**) ,which proved that Empagliflozin at dose of 30 mg / Kg for one week prevent Nephrotoxic effect due to methotrexate in rats.

In addition, the present study is in harmony with the study of **Matsui, et al (2025)** who proved that empagliflozin 10 mg/ kg for two weeks lead to reduction of toxic albumin and modulation of autophagic processes mediate its protective effect.

Unfortunately, our study is against the study of **Cha, et al (2024)** who declared that Dapagliflozin (SGLT2 inhibitors) had no protective effect in Adriamycin-induced kidney injury.

Explanation: New SGLT2 inhibitors Empagliflozin has proven Reno protection effect against nephrotoxic drugs include gentamycin in many studies as mentioned previously but nephroprotection effect not proven significant with others SGLT2 inhibitors (**Matsui, et al ,2025)**

We recommend also more studies.

In the present study, Royal Jelly at a dose of 100 mg/Kg orally for 7 days show mild Reno protection against Gentamycin induced acute nephrotoxicity with laboratory result show urea mean value 51.60±2.51, and creatinine mean 0.93±0.12, with histopathological results in the form of mild Glomerular congestion 80% , mild Tubular injury 100%, mild Tubular cast 60% , mild Mesangial hypercellularity 40%, minimal Glomerular inflammation 40%, mild Interstitial inflammation 60% and mild Glomerular edema 20%.

Our study is in harmony with **Aslan, et al, (2022),** who use RJ [100 mg/kg] with fluoride toxic dose for 8 weeks RJ reducing kidney damage through increased tumor necrosis factor alpha level and decreased caspases levels in fluoride-treated rats.

The present study is in harmony with **Hassan, et al (2017)** who proved that Gentamycin 100 mg/ kg SC + Royal Jelly orally 50 mg/ kg body weight for 10 days ameliorate gentamycin toxicity but not prevent it in rats.

However, the present study is against **Alaraj (2020)** who prove that Aliskiren decrease gentamycin toxicity in rats but Royal Jelly 150 mg/ Kg for 10 days decrease nephroprotective effect of Aliskiren.

Explanation: the present study proved that Royal Jelly decrease gentamycin nephrotoxicity but not prevent it, this matched with previously mentioned studies but regard Royal Jelly decrease Aliskiren nephroprotection against gentamycin is due to drug to drug interaction as both Royal Jelly and Aliskiren inhibits Renin Angiotensin-Aldosterone System (RAAS) more efficiently, thus leading to a marked decreased in the renal perfusion pressure, consequential failure of the glomerular filtration, and aggravated Gentamycin-induced nephrotoxicity, **Aslan, et al (2022).**

Conclusion:

The present study confirmed that the administration of gentamicin at the dose of 100 mg/kg/day subcutaneous for 7 days induced nephrotoxicity both laboratory and histopathological in rats.

Hydroxychloroquine and Empagliflozin protect against Gentamycin induced acute nephrotoxicity but Royal Jelly minimize but not prevent it.

Recommendation:

We recommend further prospective studies on humans for confirmation of the role of Hydroxychloroquine and Empagliflozin in protection against Gentamycin induced nephrotoxicity in human.

In addition, we recommend Hydroxychloroquine and Empagliflozin intake with gentamycin especially when medically indicated, also its protective role to be evaluated in more studies in other drugs induced nephrotoxicity.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

FUNDING:

No funding

REFERENCES

Abd-Elhamid T, Elgamal D, Ali S. (2018): Reno-protective effects of ursodeoxycholic acid against gentamicin-induced nephrotoxicity through modulation of NF-κB, eNOS and caspase-3 expressions. Cell Tissue Res. 374(2):367-387.

Ahmed MH, Ashton N and Balment RJ. (2003): Renal function in a rat model of analgesic nephropathy: effect of chloroquine. J Pharmacol Exp Ther, 305 (2):123–130.

Alaraj M (2020): Royal Jelly and Aliskiren mutually annul their protective effects against gentamicin-induced nephrotoxicity in rats. Veterinary World J 13(12): 2658-2662.

Alarifi S, Al-Doaiss A, Alkahtani S, et al (2011): Blood chemical changes and renal histological alterations induced by gentamicin in rats**.** Saudi J Biol Sci, 19 (1):103–110.

Alsharidah M, Abdel-Moneim A-MH, Alsharidah AS, et al. (2021): Thymoquinone, but Not Metformin, Protects against Gentamicin Induced Nephrotoxicity and Renal Dysfunction in Rats. Appl Sci J, 11(9):3981.

Ascon M, Ascon DB, Liu M, et al. (2009): Renal ischemia reperfusion leads to long-term infiltration of activated and effector memory T lymphocytes. Kidney Int J, 75(5): 526-535.

Aslan A, Beyaz S, Gok O, et al.(2022): Protective effect of royal jelly on fluoride-induced nephrotoxicity in rats via the some protein biomarkers signaling pathways: a new approach for kidney damage. Biomarkers J, 27(7): 637-647.

Babaeenezhad E, Hadipour Moradi F, Rahimi Monfared S, et al. (2021): D-Limonene Alleviates Acute Kidney Injury Following Gentamicin Administration in Rats: Role of NF-κB Pathway, Mitochondrial Apoptosis, Oxidative Stress, and PCNA. Oxid Med Cell Longev. 21(2):667-669.

Baigent C, Emberson J, Haynes R, et al. (2022): Impact of diabetes on the effects of sodium glucose co-transporter-2 inhibitors on kidney outcomes: collaborative meta-analysis of large placebo-controlled trials. Lancet J, 400(10365):1788–1801.

Balakumar P, Rohilla A, and Thangathirupathi A. (2010): Gentamicin-

induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Pharmacological Research J**,** 62(3) 3:179-186.

Bancroft, J.D. and Gamble, M., (2008): Theory and practice of histological techniques. 6 th edition. Churchill Living stone: Elsevier Health Science, Philadelphia USA: 121 – 134

Botros SR, Matouk AI, Anter A, et al (2022): Protective effect of empagliflozin on gentamicin-induced acute renal injury via regulation of SIRT1/NF-κB signaling pathway. Environmental Toxicology and Pharmacology**,** 94 (3): 10390-7.

Brkić B M, Rovčanin B, Stojanović M, et.al. (2022): Chloroquine Attenuates Oxidative Stress in Gentamicin-Induced Nephrotoxicity in Rats. Dose Response J, 20(3):155-163.

Bursac, Z., Gauss, C. H., Williams, D. K. and Hosmer, D. W. (2008): Purposeful selection of variables in logistic regression. Source code for biology and medicine, 8 (3): 15-17.

Cha JJ, Park HI, Yoo AJ, et al (2024): Dapagliflozin Does Not Protect against Adriamycin-Induced Kidney Injury in Mice. Kidney Blood Press Res J, 49 (1): 81–90.

Chen J, Ren J, Loo W, et al (2018): Lysyl oxidases expression and histopathological changes of the diabetic rat nephron. Molecular Medicine Reports J, 17(2):2431-2441.

Davidson R, 2024. US Pharmacist Journal. 3: 50-54

Empagliflozin mitigates methotrexate-induced nephrotoxicity in male albino rats: insights on the crosstalk of AMPK/Nrf2 signaling pathway. Future Journal of Pharmaceutical Sciences.10 (95):577 -580

Eslamlou NF, Momtaz S, Niknejad A, et al (2024): Empagliflozin protective effects against cisplatin-induced acute nephrotoxicity by interfering with oxidative stress and inflammation in Wistar rats. Naunyn Schmiedebergs Arch Pharmacol J. 397(9):7061-7070.

Hassan AB , Suliman MA , Bashir AH, et al (2017): Effect of royal jelly on gentamicin-induced nephrotoxicity in rats Biochemical and Cellular Archives 17(2):761-767.

Helal NE, Ibrahim FM, Abo safia HS, et al (2023): THE NEPHROTOXIC EFFECT OF CHLOROQUINE, THE OFF-LABEL ANTI COVID 19 AND POSSIBLE PROTECTIVE ROLE OF GINKGO BILOBA EXTRACT IN MALE ALBINO RATS. Egypt J. Forensic Sci. Appli. Toxicol. 23 (1): 49-59.

Hoseinynejad K, Mard SA and Dianat M. (2021): An Overview on Antioxidant and Antiinflammatory Properties of Ellagic Acid in Renal Dysfunction. Jundishapur J physiol, 2(1):16-21.

Idris AO and Olufunke O (2024): Chloroquine Attenuates Acrylamide-Induced Nephropathy in Male Wistar Rats. J Clin Nephrol Res, 11(2): 11-21.

Klouda CB and Stone WL. (2020): Oxidative Stress, Proton Fluxes, and Chloroquine/Hydroxychloroquine Treatment for COVID-19. Antioxidants J, 21(9):894.

Krishnegowda PA, Padmarajaiah N, Anantharaman S, et al (2017): Spectrophotometric assay of creatinine in human serum sample. Arabian Journal of Chemistry, 10(2):18-24

Kumar PR, Thakur A, Kumar S, et al. (2024): Royal jelly a promising therapeutic intervention and functional food supplement: A systematic review. Heliyon J, 10 (17): 371-378.

Matsui S, Yamamoto T, Takabatake Y, et al (2025): Empagliflozin protects the kidney by reducing toxic ALB (albumin) exposure and preventing autophagic stagnation in proximal tubules. Autophagy J, 21(3): 583-597

Medić B, Stojanović M, Rovčanin B, et al. (2019): Pioglitazone attenuates kidney injury in an experimental model of gentamicin-induced nephrotoxicity in rats. Sci Rep. 9(1):13689

Mishriki A A, Khalifa A K, Ibrahim D A, et al. (2024):

Mosalam EM, AboShabaan HS, Mahfouz MM, et al (2025): Protective effect of empagliflozin against paracetamol-induced acute kidney injury through modulation of AMPK/SIRT1/PGC-1α pathway in experimental mice. Toxicology and Applied Pharmacology 500 (2):117-122.

Pari L and Murugan P (2006): Tetrahydrocurcumin: Effect on Chloroquine Mediated Oxidative Damage in Rat Kidney.

Basic & Clinical Pharmacology & Toxicology, 99(5): 329–334.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C., (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. Journal of Nutrition, 123(11):1939-1951.

Richardson S (2016): Rat and mouse anesthesia and analgesia formulary and general drug information British Coumbia J. 3:2-3**.** http://ors.ubc.ca/contents/animal-care-sops-guidelines

Udupa V and Prakash V. (2019): Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol Rep J, 30(6):91–99.

Zarei B and Elyasi S. (2022): Saffron nephroprotective effects against medications and toxins: A review of preclinical data. Iran J Basic Med Sci, 25(4):419-34.

تقييم الدور الوقائي لعقار الايمباجلفلوزين، غذاء ملكات النحل والهيدروكسى كلوروكين على التأثير الكلوى السام لعقار الجنتاميسين في أناث الجرذان البيضاء

عزالدين شلبي 1، مروة عبد الناصر محمد 2، كريمة مختار محمد أحمد 1، نرمين نبيل فايد1
1قسم الطب الشرعي والسموم الإكلينيكية، كلية الطب، جامعة القاهرة
2قسم الباثولوجي، كلية الطب، جامعة القاهرة

مقدمة: إن عقار الجنتاميسين المنتمي لعائلة الأمينوجليكوزيد له تأثير قوي في القضاء على البكتيريا وتقليل مقاومتها واستمرارية تأثيره عليها رغم انخفاض سعره ولكن نظرا لأثره السام على الكلى لم يعد شائع الاستخدام.

إن عقار الايمباجلفلوزين المنتمي لعائلة مثبطات قنوات النقل المشارك صوديوم/جلوكوز 2 بالإضافة إلى أنه علاج فعال لمرض السكري ثبت حديثا أنه له أثر وقائي فعال في الفشل الكلوي الحاد وفي تقليل الأثر السمي لبعض العقاقير السامة للكلى.

ثبت أن غذاء ملكات النحل له دور فعال في الوقاية وتقليل الأثر السمي لبعض العقاقير على الكلى.

أن علاج الهيدروكسي كلوروكين المشتق من الكلوروكين والمستخدم في علاج الملاريا أثبتت بعض الدراسات دوره الفعال في حماية الكلى خاصه من العقاقير السامة للكلى.

الهدف من البحث: تقييم الدور الوقائي المحتمل لعقار الايمباجلفلوزين، غذاء ملكات النحل والهيدروكسى كلوروكين على التأثير الكلوي السام لعقار الجنتاميسين في أناث الجرذان البيضاء

طريقة البحث: بعد الحصول على موافقة لجنة اخلاقيات البحث العلمي لحيوانات التجارب بجامعة القاهرة تمت الدراسة على خمسة وعشرين فأرا بالغاً من الأناث لتصنيفها إلى خمس مجموعات، خمسة في كل مجموعة وتنقسم المجموعات كالاتي:- المجموعة (أ) الضابطة –وأعطيت المجموعات الأربعة الأخرى من (ب الى هـ) عقار الجينتاميسين بجرعة 100مجم/كجم تحت الجلد لمدة سبعة أيام لإحداث الأثر السمي على كلى الفئران حيث أن المجموعة (ب) تلقت عقار الجينتاميسين فقط أما المجموعة (ج) فتلقت عقار الجينتاميسين بالإضافة إلى غذاء ملكات النحل بجرعة 100 مجم/كجم لمدة سبعة أيام عن طريق الفم أما المجموعة (د) فتلقت عقار الجينتاميسين بالإضافة إلى عقار الايمباجلفلوزين بجرعة 30 مجم/كجم لمدة 7 أيام عن طريق الفم أما المجموعة (هـ) فتلقت عقار الجينتاميسين بالإضافة إلى عقارالهيدروكسي كلوروكين بجرعة 1مجم/كجم لمدة 7 أيام عن طريق الفم.

ثم في اليوم الثامن من بدء التجربة تم تخدير الحيوانات في المجموعات قيد الدراسه وذبحها. تم أخذ عينات الدم حتى يتم استخدامها للقياسات البيوكيميائية اليوريا والكرياتينين كما تم أخذ عينات من كلى الفئران في جميع المجموعات التجريبية حيث تم فحص الأنسجة الكلوية بعد تثبيتها في شمع البارافين وصبغها بصبغات الهيماتوكسيلين وإليوسين وتم فحص المقاطع وتصويرها باستخدام عدسات المجهر الضوئي

النتائج: بخصوص الفحص المعملى لعينات الدم في اليوم الثامن من التجربة لوحظ ارتفاع ذو دلالة إحصائية في مستويات وظائف الكلى اليوريا والكرياتين في مجموعة الجنتاميسين (ب) بمقارنة المجموعات الأخرى والمجموعة الضابطة حيث وجد ارتفاع في مستوى اليوريا والكارتين والذي انخفض بشكل ملحوظ بعد إعطاء عقار الهيدروكسى كلوروكين المجموعة (هـ) والذي يطابق في الدلالات الإحصائية المجموعة الضابطة ويليه في الانخفاض المجموعة (د) التي تلقت عقار الجينتاميسين بالإضافة إلى عقار الايمباجلفلوزين ثم يليه انخفاض بسيط في المجموعة (ج) التي تلقت عقار الجينتاميسين بالإضافة إلى غذاء ملكات النحل.

وأما نتائج فحص العينات الهستوباثولوجي لعينات النسيج الكلوي فأثبتت الأثر السمي والذى ارتفع بدلالة إحصائية في مجموعة الجنتاميسين (ب) بمقارنة المجموعات الأخرى والمجموعة الضابطة حيث حدث احتقان والتهاب كبيبي، إصابات بأنابيب الكلى، صب بأنابيب الكلى، زيادة مفرطة في خلايا مسراق الكبيبة والتهاب بالخلالي الكلوي والذى انخفض بشكل ملحوظ بعد إعطاء عقار الهيدروكسي كلوروكين المجموعة (هـ) والذي يطابق في الدلالات الإحصائية المجموعة الضابطة ويليه في الانخفاض المجموعة (د) التي تلقت عقار الجينتاميسين بالإضافة الى عقار الايمباجلفلوزين ثم يليه انخفاض بسيط في المجموعة (ج) التي تلقت عقار الجينتاميسين بالإضافة إلى غذاء ملكات النحل.

الاستنتاج: أثبتت الدراسة الحالية أن عقار الجنتاميسين بجرعة 100 مجم/كجم تحت الجلد لمدة سبعة أيام له أثر سمي ملحوظ على كلى فئران التجارب طبقا للتحاليل المختبرية ونتائج فحص العينات الهستوباثولوجية لعينات النسيج الكلوي. وأن عقار الهيدروكسى كلوروكين يليه عقار الايمباجلفلوزين له دور ملحوظ في الوقاية من الأثر السمي لعقار الجنتاميسين أما غذاء ملكات النحل يقلل فقط الأثر السمي له.

التوصيات: نوصي بعمل دراسات على الإنسان لتحديد الأثر الوقائي لعقار الهيدروكسي كلوروكين وعقار الايمباجلفلوزين من الأثر السمي لعقار الجنتاميسين على الكلى وكذلك دراسة دور عقار الهيدروكسي كلوروكين وعقار الايمباجلفلوزين في الوقاية من السموم الأخرى المؤثرة على الكلى.