PROTECTIVE EFFECTS OF TOMATO LYCOPENE AND ZINGIBER OFFICINALE EXTRACTS ON GENTAMICIN-INDUCED NEPHROTOXICITY IN ADULT ALBINO RATS (COMPARATIVE STUDY)

Ghadeer MM Abdelaal; Laila ME Sabik; Rasha L Etewa*and Wafaa I Soliman
*Departments of Forensic Medicine & Clinical Toxicology and Medical Biochemistry**
Faculty of Medicine, Zagazig University

ABSTRACT

Background: Gentamicin (GEN) is an aminoglycoside antibiotic that is widely used in clinical practice in treatment of severe gram negative bacterial infections. Tomato lycopene (LYC) and Zingiber officinaleethyl acetate extract (ZOEAE) are powerful antioxidants against free radicals and oxidative attacks. **Aim of the work**: the aim of this study was to investigate the protective effects of tomato LYC and ZOEAE against GEN induced oxidative stress and apoptosis on kidney of adult albino rats. **Material and methods**: the study was conducted for 10 days on 72 adult albino rats of both sexes, divided into six main groups; one control (subdivided into 4 subgroups) and five treated groups (8 rats in each subgroup & group). Apart from Group I(control groups) the treated groups are treated as follow: Group II (LYC) treated orally with LYC (200 mg /kg/day). Group III (ZOEAE) treated orally with ZOEAE (200 mg /kg/day). Group IV (GEN) treated with IP injection of GEN (67.4 mg/kg/day). Group V (GEN + LYC) treated with GEN (67.4 mg/kg/day) along with LYC (200mg/kg/day). Group VI (GEN + ZOEAE) treated with GEN (67.4 mg /kg/day) along with LYC (200 mg/kg/day). All animals were sacrificed 24 hours after the last dose, their blood and kidney tissues were subjected to biochemical analysis, while the remaining kidney tissues were stained for histopathological study. **Results**: GEN administration for 10 days significantly increased serum urea and creatinine concomitant with marked renal histopathological changes, suggesting nephtotoxicity. Also, it significantly increased renal malondialdehyde and decreased renal reduced glutathione and catalase activity, suggesting free radical formation. GEN also caused DNA fragmentation, suggesting apoptosis. Co-administration with either tomato LYC or ZOEAE produced approximate comprehensive improvements of all studied parameters with the superiority of ZOEAE as regard histopathology. **Recommendations**: Supplementation with antioxidants e.g. LYC and ZOEAE in GEN treated patients had beneficial impacts through alleviating the oxidative stress and improving renal functions
DNA fragmentation assay – DNA laddering **Keywords**: Gentamicin – Lycopene – Ginger-Nephtotoxicity – Apoptosis – Oxidative Stress.

INTRODUCTION

Gentamicin (GEN) is an aminoglycoside antibiotic, which is commonly applied, in human clinical practices for treatment of life-threatening gram-negative infections (**Atta et al., 2014**). Routine therapeutic uses of GEN for treatment of urinary tract infections may confront patients with toxic side effects of aminoglycosides known as nephrotoxicity **(Derakhshanfar et al., 2008).** Studies showed that, GEN not only induces lipid peroxidation, but also has a direct stimulatory action on the production of reactive oxygen species (ROS), which in turn augment oxidative damages to cell membrane transport and affects cell energy pathways **(Rodrigues et al .,2014**). The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of GEN nephrotoxicity (**Yanagida et al., 2004**).

Natural products and their active principles as sources for new drug discovery have attracted attention in recent years. Antioxidants have been shown to ameliorate signs of GEN-induced nephrotoxicity (**Raju et al, 2011**). Scavenging ROS is a popular approach in modulation of apoptosis, given their effect on lysosomal and mitochondrial pathways to apoptosis (**Servais et al., 2008**). Accordingly, the administration of several compounds with antioxidant and antiapoptotic

activities has been successfully used to prevent or ameliorate GEN-induced nephrotoxicity (**Ali, 2003 and Stojiljkovic et al., 2012)**.

Lycopene (LYC) is a natural carotenoid found in tomato, an essential component of the Mediterranean diet (**Palozza et al., 2010)**. It has many biochemical functions as an antioxidant scavenger, antihyperlipidemic agent and inhibitor of proinflammatory and prothrombotic factors (**Mordente et al., 2011).**

Zingiber officinale Roscoe, commonly known as ginger, is one of the frequently used spices in the world and medicinally in folk and home remedies to treat cold, fever, headache, nausea, digestive problems and arthritis (**Rasmussen, 2011**). Its root contain polyphenol compounds (6-gingerol and shogaol), which have a high antioxidant activity **(Stoilova et al., 2007).**

Aim of the work

The aim of this work was to evaluate and compare the protective effects of tomato lycopene (LYC) and Zingiber officinale ethyl acetate extract (ZOEAE) on gentamicin-induced nephrotoxicity in adult albino rats.

MATERIAL AND METHODS

A) Chemicals

Gentamicin sulphate: Used as Garamycin ampoules each contains 40 mg/mL GEN sulphate ; Schering-Plough, Weesp, Netherlands

Reagents and commercial kits: They were supplied from Sigma/Aldrich, USA and Biodiagnostic, Egypt Chemical Companies.

Gum acacia: obtained from Algomhoreya Company.

B) Preparation of fresh plant extracts

Tomatoes (Solanum lycopersicum L., Solanaceae) and fresh ginger Zingiber officinaleRoscoe, Zingiberacae) rhizomes were purchased from local commercial sources. The botanical authentication and extraction were done at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

1- Extraction of lycopene

Fresh and finely ground tomato peels (200 gm) were placed into a colored amber flask. Then 0.05% w/v butylated hydroxytoluene (BHT) in acetone (500 mL), ethanol (500 mL) and hexane (250 mL) were rapidly added. The flask was magnetically stirred for 15 min. De-ionized water (400 mL) was poured into the flask and shaked. The flask was stirred for 5 min and then the system was left at room temperature for further 5 min. The upper hexane layer was separated and evaporated to dryness under reduced pressure at 40°C using Heidolph rotatory evaporator (Hei-VAP Value Digital, Germany) **(Luo and Wu, 2011).** The process was repeated 5 times and the total extract was calculated as the sum of the values obtained in each extraction stage. The obtained LYC extract weighed 4 grams. This amount was prepared in 80 mL of corn oil (50 mg mg/mL) and preserved in a refrigerator at a refrigerator at 4 C in a dark container to avoid oxidation of LYC.

2- Ethyl Acetate extract of Fresh ginger rhizomes

Fresh ginger rhizomes (400 gm) were divided, macerated and saturated with cold methanol (2 L) for 3 days. The extract was filtered and subjected to drying. The residues were re-extracted for 3 times. The dried extract was dissolved in least amount of methanol (50 mL) and distilled H_2O was added, then fractionation was done by ethyl acetate using separating funnel at room temperature. Zingiber officinale ethyl acetate extract (ZOEAE) was evaporated to dryness under reduced pressure at 40°C using Heidolph rotatory evaporator (Hei-VAP Value Digital, Germany) **(Lakshmi and Sudhakar, 2010).** The obtained extract (ZOEAE) was oily semisolid mass weighed 4 grams. This amount was prepared in80 mL of 1% w/v gum acacia (50mg/mL) and preserved in a refrigerator at 4 C.

Experimental design

The study has been designed in the Faculty of Medicine; Zagazig University .The study was performed after approval from the Institutional Review Board of the Faculty of Medicine, Zagazig University, Egypt.

Animals C-

The study was carried out on 72 adult albino rats of both sexes weighing 120 –

150 gm. The period of study continued for 10 days.

All animals received human care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with "The Guide for the Care and Use of Laboratory Animals" (**Institute of Laboratory Animal Resources, 1996).** Rats were obtained from the animal house of Faculty of Medicine, Zagazig University. Before starting the experiment, all animals subjected to 14 days of passive preliminaries for house acclimatization, to ascertain their physical well-being and to exclude any diseased animal. They were kept in polypropylene cages at the temperature 24°C, 45% relative humidity, and 12-h light and dark cycles with free access to drinking water and food. After housing acclimatization, the rats were divided into six main groups; control (32 rats) and five treated groups (8 rats in each).

Groups:

Group I (control): It was further subdivided into 4 subgroups (8 rats each):

Ia (negative control group): Each rat received only regular diet and tap water to measure the basic parameters .

: *(***Ib (saline**): Each rat received a daily (IP) injection of 0.5 mL isotonic saline (solvent of GEN) for 10 days

Ic (corn oil): Each rat received a daily 0.5 mL corn oil for 10 days by oral gavage.

(**Id (gum acacia**

Each rat received a daily 0.5 mL gum acacia 1% aqueous solution for 10 days by oral gavage.

Group II (LYC)

Rats received a daily 200 mg/kg/day of LYC for 10 days by oral gavage **(Stacewicz-apuntzakis and Bowen, 2005)**

Group III (ZOEAE)

Rats received a daily 200 mg/kg/day of ZOEAE for 10days by oral gavage **(El-Sharaky et al., 2009)**

Group IV (GEN)

Gentamicin was injected to animals (IP) at the dose of 67.4 mg /kg/day for 10 days, which equals to 1/10 of LD50 of GEN sulphate **(Robbins et al, 1971)** GEN dosage range that is typically used in rodents to simulate clinical GEN toxicity is 40–120 mg/kg for 7–10 days **(Zager, 2007)**

Group V (GEN + LYC)

Rats received simultaneous 67.4 mg/kg/day (IP) GEN and 200 mg/ kg/ day LYC (oral gavage) for 10 successive days.

Group VI (GEN + ZOEAE)

Rats received simultaneous 67.4 mg/kg/day (IP) GEN and 200 mg/kg/day ZOEAE (oral gavage). For 10 successive days

After 24 hours from the last dose (on the 11th day), rats from each group were subjected to:

- Estimation **of** kidney function tests: serum urea and creatinine, MDA, catalase, GSH, DNA extraction and fragmentation assay.

Histopathological examination of kidney tissue by light microscope. -

II- Methods

A) Blood and kidney tissue samples collection

At the end of the experimental period, venous blood samples were collected from animals by means of micro–capillary glass tubes from the retro- orbital plexus under light ether anesthesia (**Johnson, 2007)**

One kidney was used freshly for the determination of oxidative stress markers. The other kidney was divided into 2 longitudinal halves ; one kept frozen at -80 °C until used for evaluation of DNA fragmentation and the other half kept in 10 % formalin for the histological assessment.

B) Biochemical studies:

I. Kidney function tests

Determination of serum creatinine and urea level (mg/dl):

Serum creatinine level was assayed colorimetrically according to the method proposed by **Schirmeister et al., (1964)** while Serum urea level was assayed colorimetrically according to the method proposed by **Patton and Crouch, (1977)**

II Oxidative stress parameters

Determination of MDA (nmol/ g tissue)

MDA was assayed colorimetrically according to the method proposed by **(Ohkawa et al., 1979).**

Determination of GSH (mmol/g tissue)

GSH was assayed colorimetrically according to the method proposed by **(Beutler et al., 1963)**

Determination of catalase activity (U/g)

Catalase activity was assayed colorimetrically according to the method proposed by **(Aebi, 1983**)

III Detection of DNA fragmentation

DNA laddering

It is a qualitative analysis of DNA fragmentation by agarose gel electrophoresis, the presence of DNA ladder was determined according to **Wlodek et al., (1991).**

DNA extraction and storage

Kidney samples were coded and analyzed in blind manner for genomic DNA extraction using the commercially available G-spin ™ Total DNA Extraction Kit

*(*iNtron bio-tehnology, Seongnam-Si, Gyeonggi-do, Korea *)*

Protocol used for DNA extraction from kidney tissues was followed according to **Buffone and Darlington, (1985)**

DNA was stored at -20°C until used for gel 32lectrophoresis

The DNA was visualized and photographed, under UV Trans-illuminator with 100 bp ladder (Pharmacia Biotech, USA).

NB: Wear eye and skin protection when UVR is on.

DNA fragmentation assay

Quantitative analysis of DNA fragmentation by diphenylamine (DPA), DNA fragmentation was measured by the diphenylaminespectro-photometric method according to **Perandones et al., (1993)** with some modifications from **Burton, (1956)**

C- Histopathological studies

Kidney tissues were fixed in 10% formalin and processed to get 5 u thickness paraffin sections which were stained with hematoxylin and Eosin then used for histopathological examination under light microscope **(Kiernan, 2001)**

D) Statistical analysis

Data were analyzed by Statistical Package of Social Science (SPSS), software version 16.0 (SP**SS Inc., 2007).** Quantitative data were summarized as mean ±standard deviation (SD). Comparison of several means were done by One Way Analysis of Variance (ANOVA), followed by Least Significance Difference test (LSD) for multiple comparisons between groups. Probability (P value < 0.05 means significant difference).

RESULTS

Biochemical results

Biochemical results of control, LYC and ZOEAE treated groups:

The biochemical findings among negative, saline , corn oil and gum acacia control groups and LYC & ZOEAE treated groups, were not statistically significant as regard all studied parameters **(Table 1**). So, the negative control group was used as a control group for comparison with other treated groups

Biochemical results of studied groups:

There was a highly significant increase of serum creatinine and urea (P<0.001) in GEN-treated group as compared with the control group. While there was a highly significant decrease (P<0.001) in GEN + LYC- and GEN + ZOEAE- treated groups when compared with the GEN-treated group. Moreover, by comparing GEN + LYC- and GEN + ZOEAE- treated groups there was no significant difference (P >0.05) as shown in **table (2).**

Oxidative Stress Markers:

There were significant differences of the mean values of kidney MDA, catalase activity and GSH among the studied groups (P <0.001). There was highly significant increase of kidney MDA, besides significant decrease of kidney GSH and catalase in the GEN-treated group when compared with the control group. While in GEN + LYC- and GEN + ZOEAE-treated groups, kidney MDA level showed a significant decrease , while kidney GSH level and catalase activity showed significant increase in GEN + LYC- treated group and in GEN +ZOEAE- treated group when compared to the GEN-treated group. Moreover, by comparing GEN + LYC- and GEN +ZOEAE- treated groups there was no significant difference regarding all oxidative stress markers as shown in **table 3.**

Table (1): Statistical comparison among negative (Neg.) control, saline, corn oil, gum acacia, lycopene (LYC) and Zingiber officinale ethyl acetate extract groups (ZOEAE) groups as regard mean values of kidney function tests, oxidative stress markers and Kidney fragmented DNA using ANOVA test

Groups / Biochemical parameters	Neg. control (Ia) N = 8 Mean ± SD	Saline (Ib) N = 8 Mean ± SD	Corn oil (Ic) N = 8 Mean ± SD	Gum acacia (Id) N = 8 Mean ± SD	LYC N = 8 Mean ± SD	ZOEAE N = 8 Mean ± SD	F value	P value
Serum creatinine (mg/dl)	0.47 ± 0.15	0.46 ± 0.14	0.51 ± 0.13	0.55 ± 0.15	0.50± 0.10	0.48 ± 0.10	0.77	0.58
Serum urea (mg/dl)	21.75± 3.92	22.73 ± 3.98	23.87 ± 4.08	23.38 ± 3.36	22.32 + 3.66	22.54 ±3.66	0.32	0.90
MDA (nmol/g tissue)	159.75 ± 50.70	160.02± 50.62	181.30 ± 56.30	177.71 ± 55.13	166.99 ± 54.95	165.54± 53.55	0.23	0.95
GSH (µmol/g tissue)	0.90 ± 0.29	0.89 ± 0.29	0.84 ± 0.26	0.85 ± 0.27	0.98 ± 0.30	1.02 ± 0.33	0.51	0.77
Catalase activity (U/g tissue)	5.18 ± 0.86	5.28 ± 0.86	4.99 ± 0.74	5.00 ± 0.65	5.13 ± 0.86	5.19 ± 0.48	0.17	0.97
Kidney fragmented DNA%	0.37 ± 0.10	0.38 ± 0.12	0.44 ± 0.10	0.42 ± 0.12	0.40 ± 0.14	0.40 ± 0.10	0.35	0.88

NB All values are expressed as Mean± SD N: Number of rats in each group=8 rats
SD: standard deviation P: Probability
P: >0.05 non significant P: < 0.05 significant

Table (2): Statistical comparison among negative (Neg.) control, gentamicin, gentamicin + lycopene (LYC) and gentamicin + *Zingiber officinale* ethyl acetate extract (ZOEAE) groups as regard mean values of kidney function tests (serum creatinine and urea levels) after 10 days of treatment, using ANOVA test

Groups / Parameters	Neg. control (Ia) N = 8 Mean ± SD	Gentamicin (IV) N = 8 Mean ± SD	Gentamicin + LYC (V) N = 8 Mean ± SD	Gentamicin + ZOEAE (VI) N = 8 Mean ± SD	F value	P value
Serum creatinine (mg/dl)	0.47 ± 0.15^{A}	2.43 ± 0.75^{B}	0.74 ± 0.17^{A}	0.62 ± 0.17^{A}	42.01	<0.001
Serum urea (mg/dl)	21.75 ± 3.92^{A}	65.88 ± 5.80^{B}	49.71 ± 7.26^{AB}	44.92 ± 8.52^{AB}	61.01	<0.001

NB All values are expressed as Mean± SD N: Number of rats in each group=8 rats
SD: standard deviation P: Probability
P: >0.05 non significant P: < 0.05 significant
^{A}: significant with gentamicin group. ^{B}: significant with negative control group

Table (3): Statistical comparison among negative (Neg.) control, gentamicin, gentamicin+ lycopene (LYC) and gentamicin + *Zingiber officinale* ethyl acetate extract (ZOEAE) groups as regard mean values of oxidative stress markers of kidney (malondialdhyde, glutathione reductase and catalase activity) after 10 days of treatment, using ANOVA test.

Groups Parameters	Neg. control (Ia)	Gentamicin (IV)	Gentamicin + LYC (V)	Gentamicin + ZOEAE (VI)	F value	P Value
	N = 8 Mean ± SD	N = 8 Mean ± SD	N = 8 Mean ± SD	N = 8 Mean ± SD		
MDA (nmol/g tissue)	159.75 ± 50.70^{A}	315.65 ± 94.48^{B}	210.18± 67.57^{A}	180.34± 55.60^{A}	8.02	< 0.001
GSH (µmol/g tissue)	0.90 ± 0.29^{A}	0.32 ± 0.10^{B}	0.73± 0.23^{A}	0.84 ± 0.21^{A}	11.56	<0.001
Catalase activity (U/g tissue)	5.18 ± 0.86^{A}	2.40 ± 0.71^{B}	3.41 ± 0.59^{AB}	3.94 ± 0.67^{AB}	21.19	<0.001

NB All values are expressed as Mean± SD N: Number of rats in each group=8 rats
SD: standard deviation P: Probability
P: >0.05 non significant

^{A}: significant with gentamicin group. ^{B}: significant with negative control group
P: < 0.05 significant
*: significant

Apoptotic Markers

Qualitative assessment of DNA fragmentation (DNA laddering)

DNA ladder assay showed no appreciable fragmentation of DNA in the kidney tissues of negative control rats (Fig. 1). DNA was observed in the kidneys of rats treated with GEN alone (Fig. 2). DNA shearing was mild in kidney samples of GEN + LYC- and GEN + ZOEAE-treated groups (Figs. 3 and 4).

Quantitative assessmentof DNA fragmentation (Kidney fragmented DNA %)

There was a highly significant difference of DNA fragmentation % among the studied groups (P<0.001) by ANOVA test. There was significant increase of DNA fragmentation % (P<0.001) in the GEN-treated group when compared to the control group. In the GEN + LYC and GEN + ZOEAE- treated groups, DNA fragmentation % showed a significant decrease when compared with the GEN-treated group. There was no significant difference between GEN + LYC- and GEN + ZOEAE- treated groups (**Table 4**).

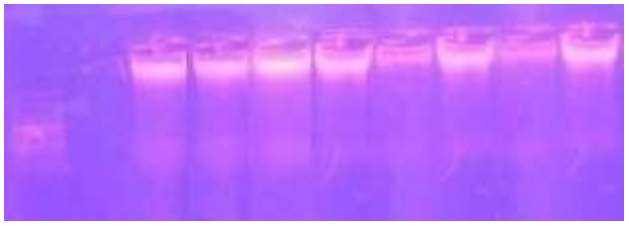


Fig. (1): Agarose gel electrophoresis of DNA isolated from adult albino rats kidney of negative control group after 10 days showing; Lane 1: DNA ladder, Lanes 2-9: normal DNA bands

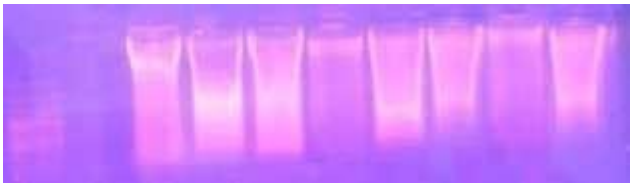


Fig. (2): Agarose gel electrophoresis of DNA isolated from adult albino rats kidney of gentamicin group after 10 days of treatment showing; Lane 1: DNA ladder, Lanes 2-9: advanced shearing of DNA.

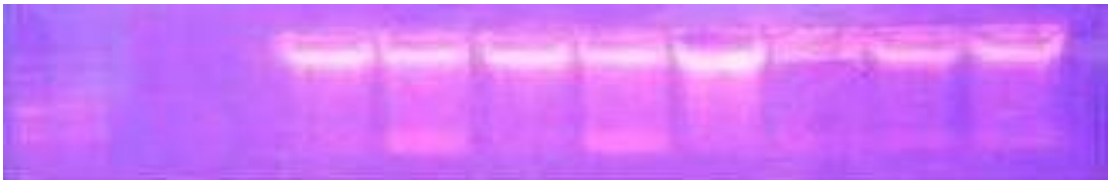


Fig. (3): Agarose gel electrophoresis of DNA isolated from adult albino rats kidney of gentamicin + lycopene group after 10 days of treatment showing; Lane 1: DNA ladder, Lanes 2-9: mild DNA shearing

Fig. (4): Agarose gel electrophoresis of DNA isolated from adult albino rats kidney of gentamicin + *Zingiber officinale* ethyl acetate extract group after 10 days of treatment showing; Lane 1: DNA ladder, Lanes 2-9: mild DNA shearing

Table (4): Statistical comparison among negative (Neg.) control, gentamicin, gentamicin+ lycopene (LYC) and gentamicin + *Zingiber officinale* ethyl acetate extract (ZOEAE) groups as regard mean values of kidney DNA fragmentation % after 10 days of treatment, using ANOVA test

Groups / Parameter	Neg. control (Ia)	Gentamicin (IV)	Gentamicin + LYC (V)	Gentamicin + ZOEAE (VI)	F value	P value
	N = 8 Mean ± SD	N = 8 Mean ± SD	N = 8 Mean ± SD	N = 8 Mean ± SD		
Kidney fragmented DNA%	0.37 ± 0.10^{A}	3.21 ± 0.34^{B}	0.97 ± 0.32^{AB}	0.81 ± 0.27^{AB}	171.22	<0.001

NB All values are expressed as Mean± SD N: Number of rats in each group=8 rats
SD: standard deviation P: Probability
P: >0.05 non significant P: < 0.05 significant
[A]: significant with gentamicin group. [B]: significant with negative control group

Histopathological Results

Control, LYC and ZOEAE treated groups

light microscopical examination of hematoxylin and eosin (H&E) stained sections from the kidney of negative control, saline, corn oil, gum acacia, LYC, and ZOEAE groups, showed normal renal tissue formed of renal glomeruli surrounded by closely packed renal tubules (Figs. 5, 6 and 7)

As regard other treated groups

Gentamicin treated group

The light microscopic examination of H&E stained sections of the kidney of rats treated with GEN alone for 10 days showed marked proximal tubular necrosis, apoptotic tubular cells (pyknotic nuclei), hyaline casts in tubular lumen, desquamation of the tubular epithelial cellsand interstitial nephritis with dilated congested vascular spaces throughout the cortex (Figs. 8, 9, 10, 11 and 12).

Gentamicin + lycopene treated group

The light microscopic examination of H&E stained sections of the kidney revealed mild affection in 4 rats (50% of cases) in the form of mild aggregation of inflammatory cells with few and small tubular casts, there was no tubular necrosis (Fig. 13)

The rest of the group showed normal renal tissue with absence of necrosis, casts and inflammatory aggregates (Fig. 14).

Gentamicin + Zingiber officinale ethyl acetate extract treated group

light microscopic examination of H&E stained sections of the kidney of rats revealed normal renal tissue in all rats (100% of cases) (Figs. 15 and fig.16).

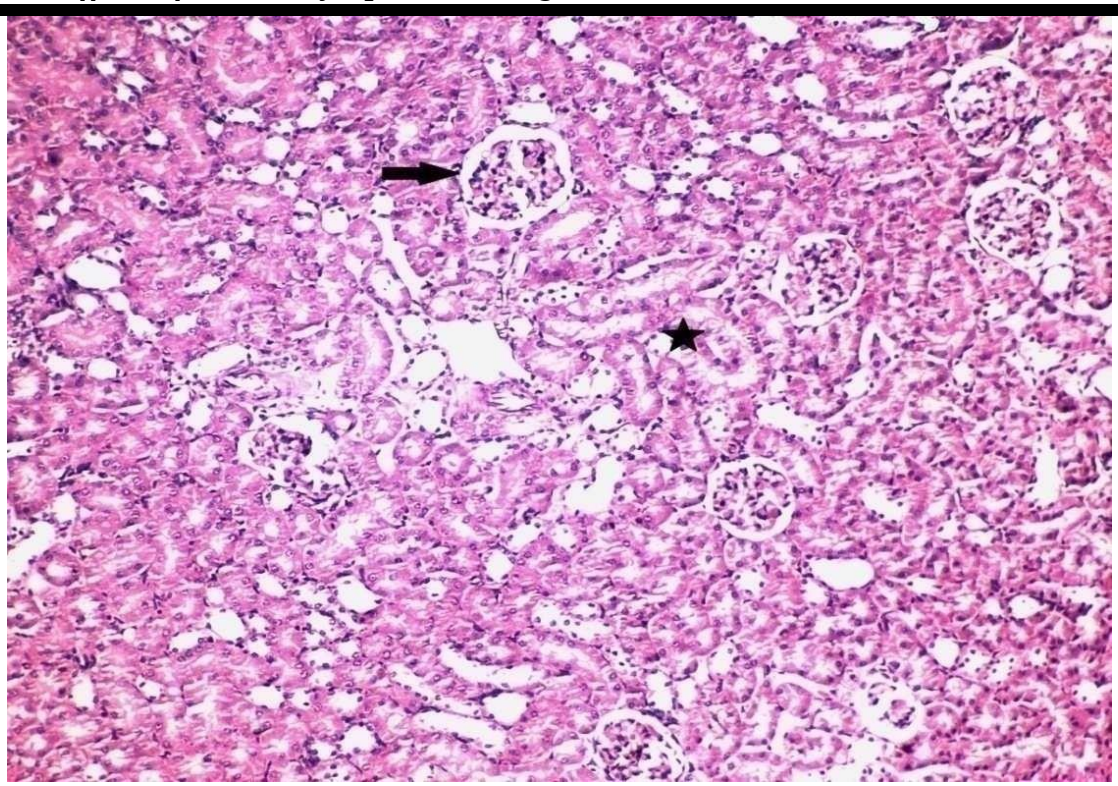


Fig. (5): A photomicrograph of renal tissue of adult albino rat of saline control group, injected intraperitoneally by 0.5 ml saline for 10 days, showing normal renal glomeruli (arrow) surrounded by closely packed renal tubules (black filled star) (H & E × 200).

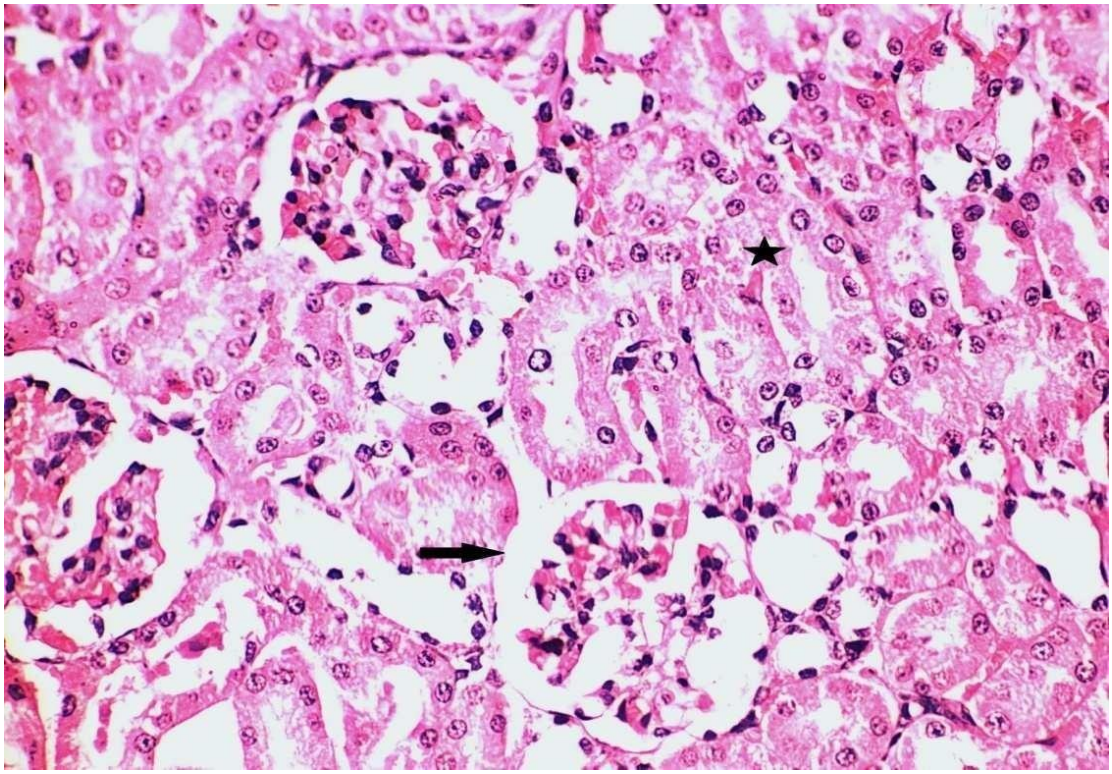


Fig. (6): A photomicrograph of renal tissue of adult albino rat, gavaged with 200mg/kg/day lycopene for 10 days, showing normal renal glomeruli (arrow) surrounded by closely packed renal tubules (black filled star) (H & E × 400)

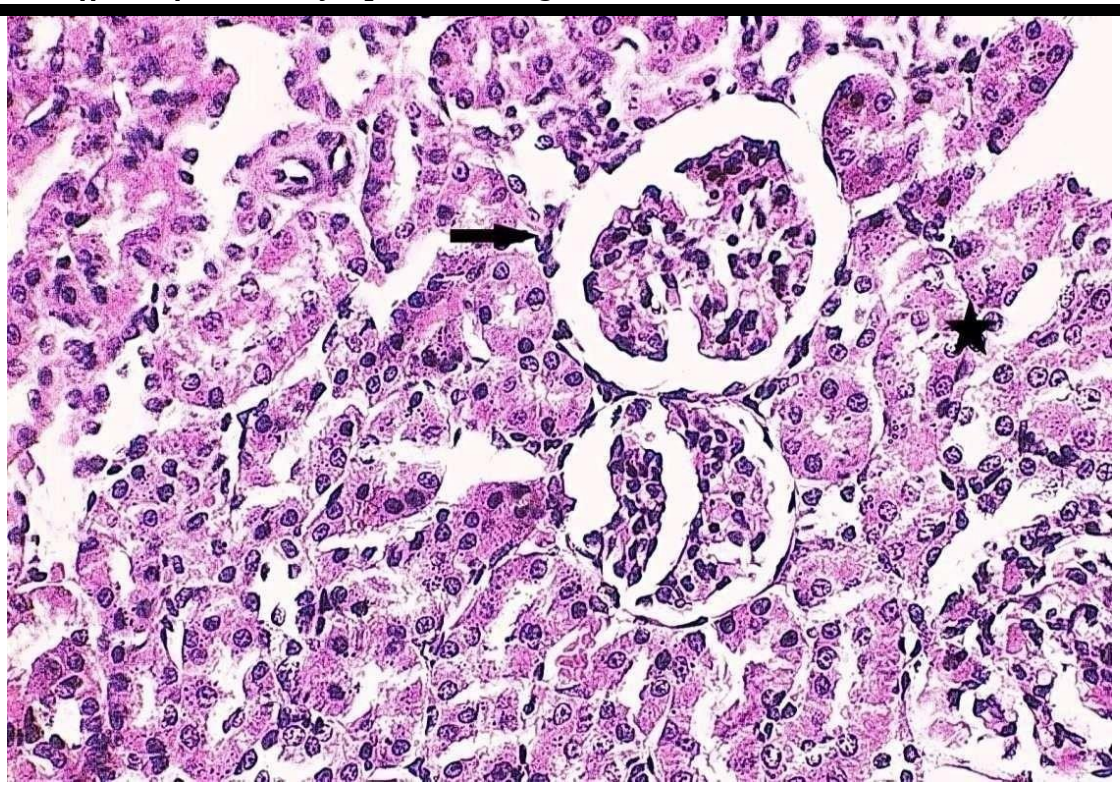


Fig. (7): A photomicrograph of renal tissue of adult albino rat, gavaged with 200mg/kg/day *Zingiber officinale* ethyl acetate extract for 10 days, showing normal renal glomeruli (arrow) surrounded by closely packed renal tubules (black filled star) (H & E × 400)

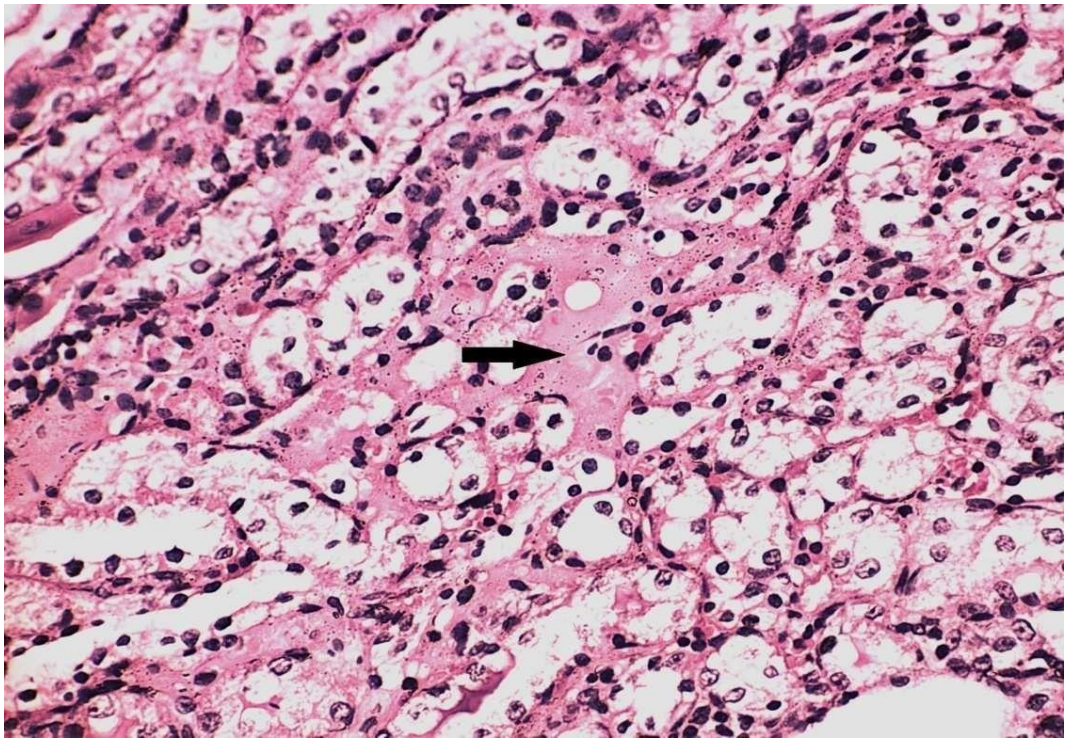


Fig. (8): A photomicrograph of renal tissue of adult albino rat after daily intaperitoneal injection of 67.4 mg/kg gentamicin for 10 days showing large area of interstitial edema (arrow) (H & E × 400)

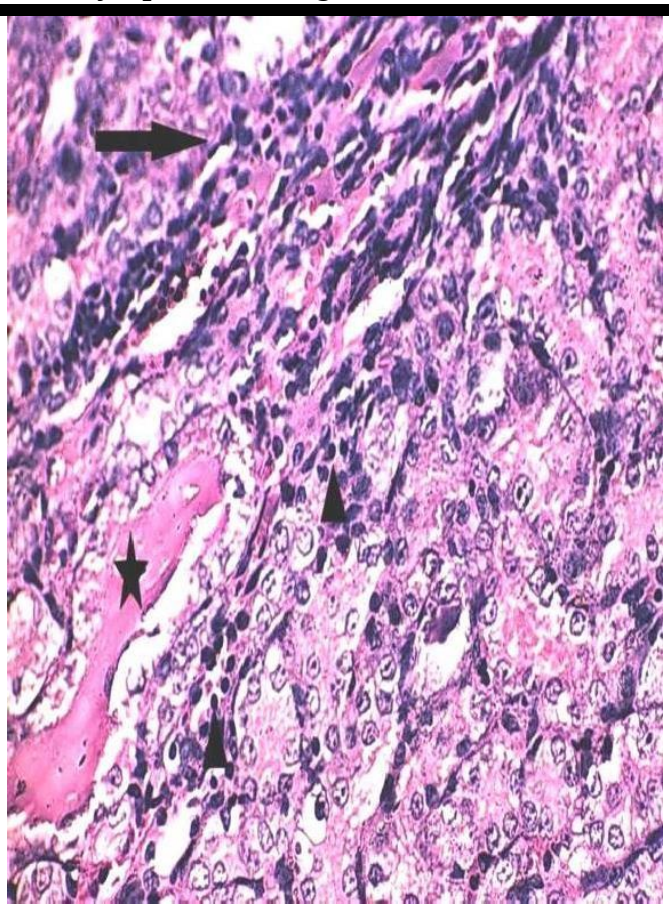


Fig. (9): A photomicrograph of renal tissue of adult albino rat after daily intaperitoneal injection of 67.4 mg/kg gentamicin for 10 days showing tubular cast (black filled star) with aggregates of inflammatory cells (arrow) and scattered apoptotic cells (arrow head) (H & E × 400)

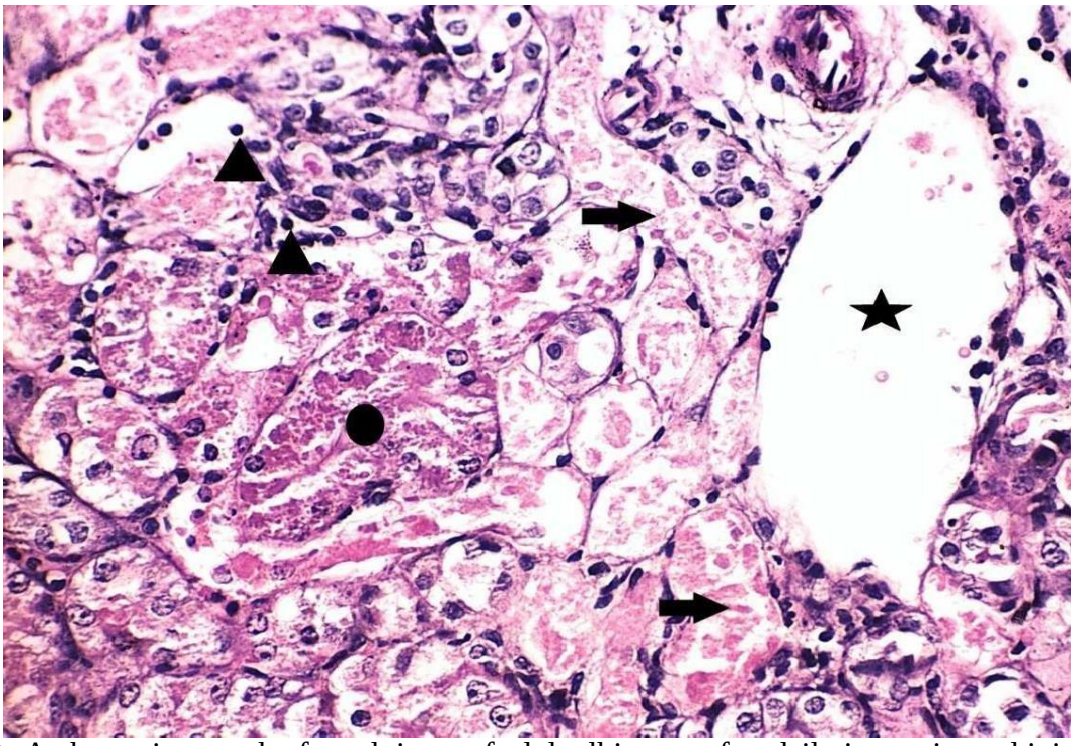


Fig. (10): A photomicrograph of renal tissue of adult albino rat after daily intaperitoneal injection of 67.4 mg/kg gentamicin for 10 days showing moderate area of necrosis (arrow) with apoptotic cells (arrow head), desquamted cells(black filled circle) and dilated vascular space (black filled star) (H & E × 400)

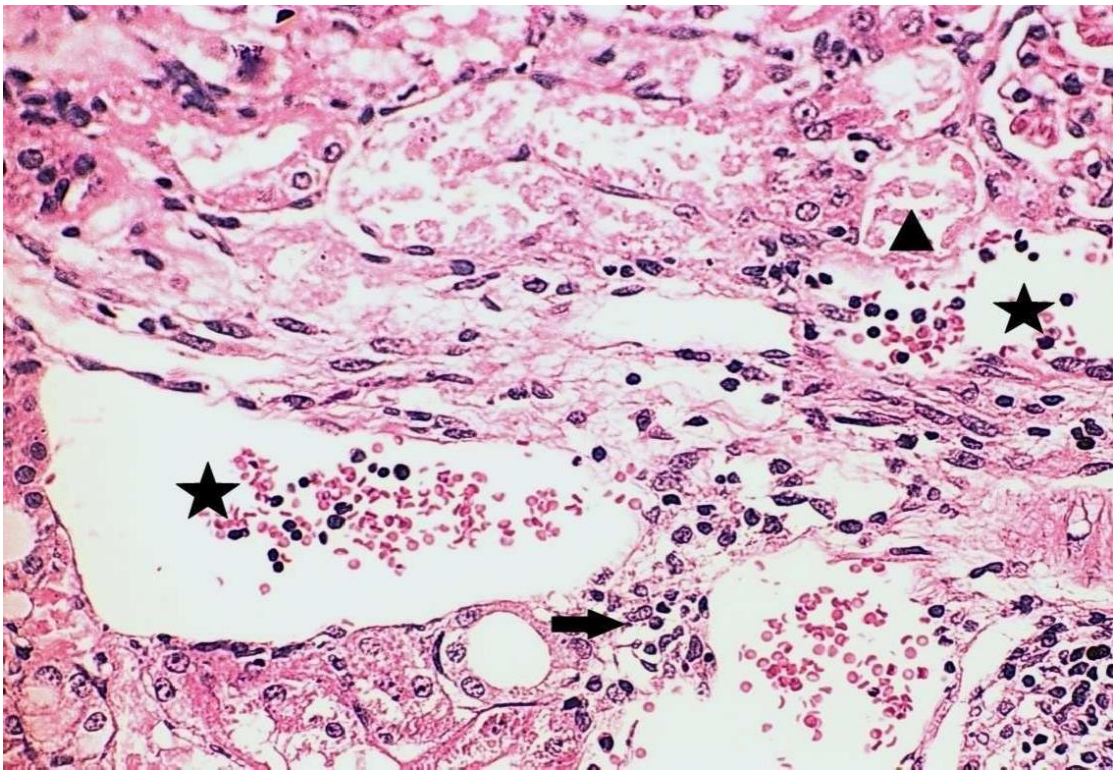
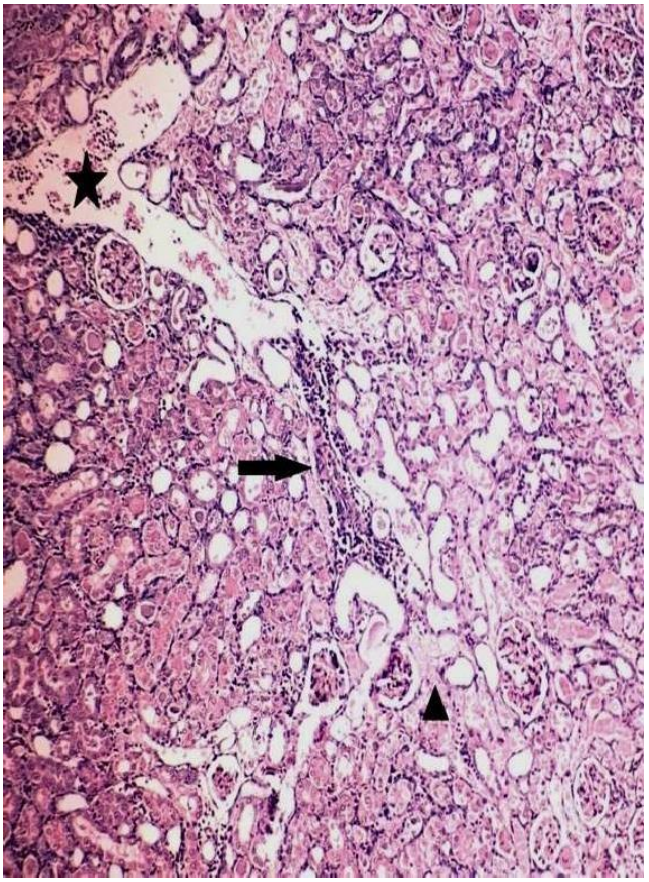


Fig. (11): A photomicrograph of renal tissue of adult albino rat after daily intaperitoneal injection of 67.4 mg/kg gentamicin for 10 days showing dilated congested vascular spaces (black filled star) with areas of desaquamation (arrow head) and aggregates of inflammatory cells (arrow) (H & E × 400)



Fig(12): A photomicrograph of renal tissue of adult albino rat daily Intaperitoneal injection of 67.4 mg/kg gentamicin for 10 day showing aggregates of inflammatory cells (arrow) with dilated congested vascular spaces (black filled star) and areas of necrosis (arrow head) (H&E × 200)

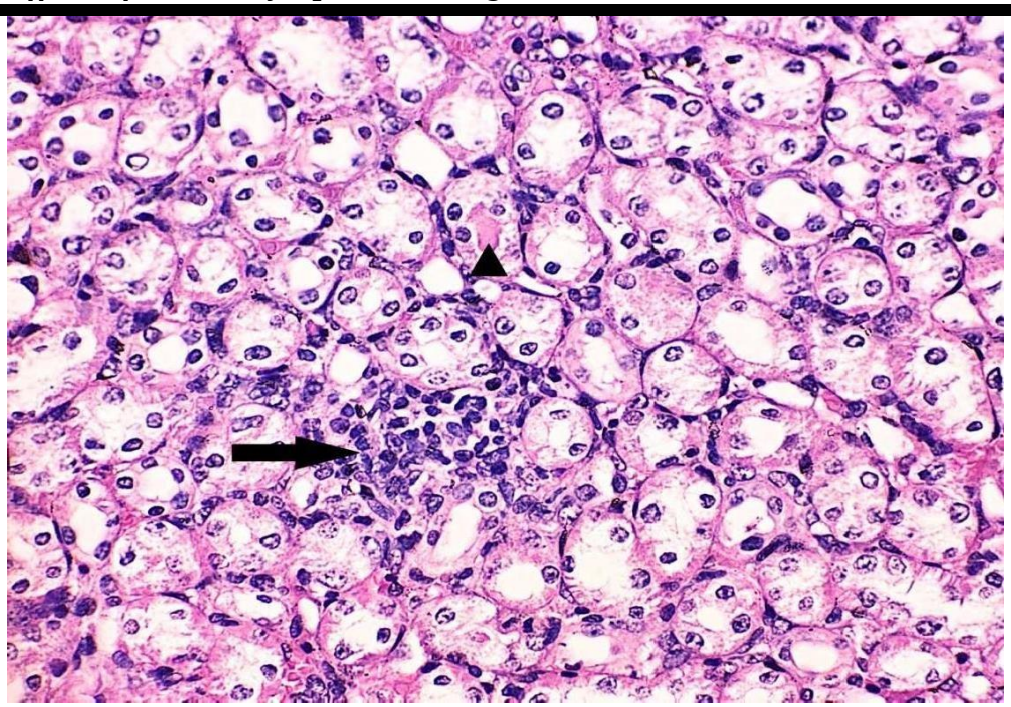


Fig. (13): A photomicrograph of renal tissue of adult albino rat after daily intraperitoneal injection of gentamicin (67.4 mg/kg) oral gavage with lycopene (200mg/kg/day) for 10 days showing mild aggregation of inflammatory cells (arrow) with small tubular cast (arrow head) (H & E × 400)

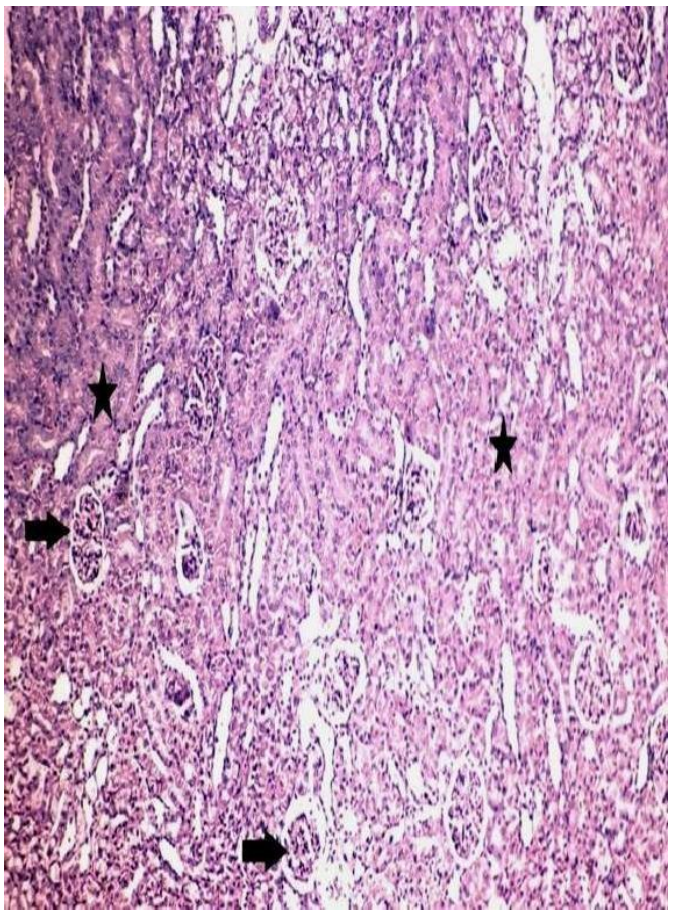


Fig. (14): A photomicrograph of normal renal tissue of adult albino rat afterdaily intraperitoneal injection of gentamicin (67.4 mg/kg) + oral gavage withlycopene (200mg/kg/day) for 10 days showing normal renal glomeruli (arrow)surrounded by closely packed renal tubules (black filled star) (H & E × 200).

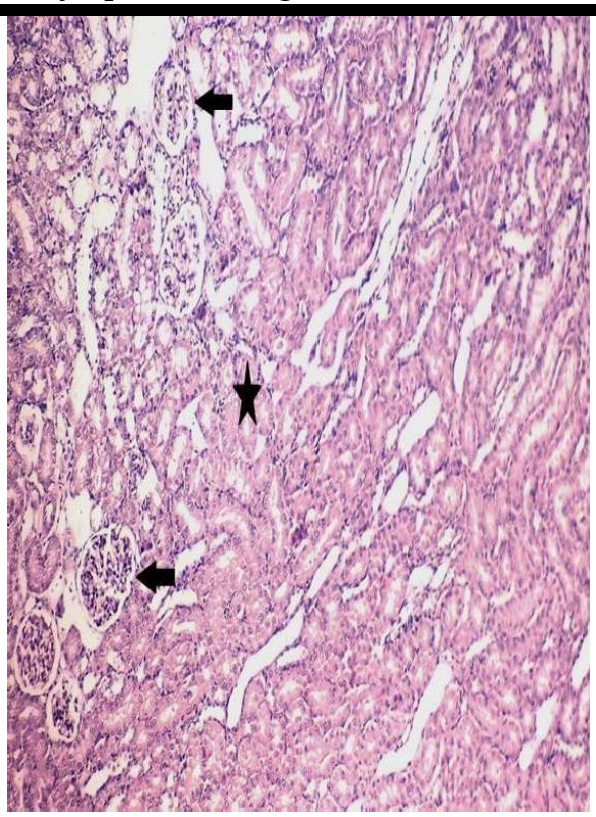


Fig. (15): A photomicrograph of normal renal tissue after treatment with intraperitoneal injection of gentamicin (67.4 mg/kg) + oral gavage with *Zingiber officinale* ethyl acetate extract (200mg/kg/day) for 10 days showing normal renal glomeruli (arrow) surrounded by closely packed renal tubules (black filled star) (H & E × 200).

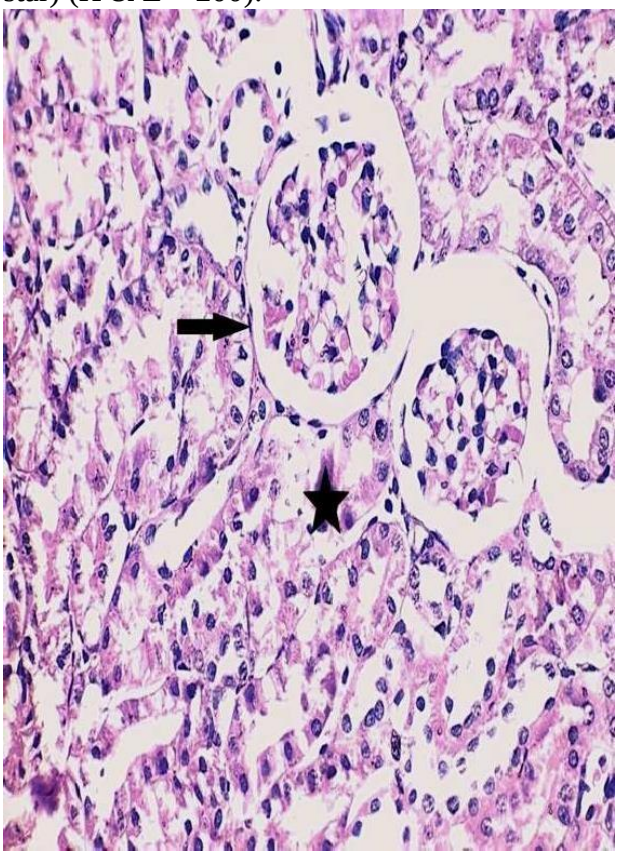


Fig. (16): A photomicrograph of normal renal tissue of the same previous group with higher power (H & E × 400).

DISCUSSION

Aminoglycosides have been successfully used for decades in the treatment of gram-negative bacterial infections, careful serum monitoring is essential to prevent their nephrotoxicity especially in populations at risk **(Denamur et al., 2011).** Gentamicin (GEN), a typical aminoglycoside, is widely used in the treatment of severe gram-negative bacterial infections. Possessing a potent bactericidal activity and less bacterial resistance than other antibiotics, favor its usage in the clinical practice despite its nephrotoxicity. Therefore, reducing or preventing the development of GEN induced nephrotoxicity has attracted considerable efforts **(Balakumar et al., 2010).**

Herbal medicine plays an essential role in health care; about 80% of the world's population relies on natural remedies for their primary health care **(Hasan and Rahman, 2011).** Lycopene (LYC) is a major carotenoid, available primarily from tomato, which is an integral part of the ordinary Egyptian diet. Of all carotenoids, LYC has been shown to exhibit the highest quenching ability of ROS. Moreover, it proved to have antiapoptotic properties **(Palabiyik et al., 2013).** Ginger is one of the safe herbal medicines. It is available in Egypt as a spice and widely used as a home remedy. It is a powerful antioxidant and may either mitigate or prevent generation of free radicals **(Zahedi et al., 2012).**

Given the current misuse of antibiotics among Egyptian population**(Sabry et al., 2014), as** well as, the high prevalence of chronic liver disease in Egypt **(Wantuck et al., 2014)**with its known potentiating effect on aminoglycosides-induced nephrotoxicity, make the proposed point of research an interesting and a potentially rewarding one. The present study has been conducted to evaluate and compare the protective effects of tomato LYC and Zingiber officinale ethyl acetate extract (ZOEAE) on GEN-induced nephrotoxicity in adult albino rats.

In the present study, rats treated with GEN alone revealed highly significant increase of serum creatinine and urea when compared with the control group. These alterations in biochemical parameters were well correlated with the renal histopathological findings that revealed marked proximal tubular necrosis, apoptotic cells with pyknotic nuclei, hyalinecasts, desquamation of the tubular epithelial cells and interstitial nephritis with dilated congested vascular spaces throughout the cortex. Both biochemical and histopathological changes recorded in the present study indicated nephrotoxicity.

Hosten, (1990)and **Yaman and Balikci, (2010)** reported that creatinine level is much more reliable indicator than urea in the first phases of kidney disease because urea is far more likely to be affected by dietary and physiologic conditions not related to renal function. Urea level begins to increase only after renal parenchymal injury

Histopathological results induced by GEN in the current work could be related to its excessive accumulation in cells of renal proximal tubules as reported by (**Rodrigues et al., 2014).** The increased urea and creatinine levels besides the histopathological changes recorded in the present study were coincided with previous studies stated by **Atessahin et al., (2003)**; **Randjelovic et al., (2012) and Tavafi et al., (2012**) who reported that treatment with GEN causes significant reduction in renal functions, in addition to marked proximal tubular damage that indicated nephrotoxicity.

In addition, the present study revealed that GEN induced apoptosis in renal proximal tubules, which was demonstrated by light microscopical examination of renal tissues of rats treated with GEN alone. Confirmation was done qualitatively by gel electrophoresis of DNA extracted from kidneys of the GEN treated group, which exhibited acontinuous banding pattern of fragmented DNA, and quantitatively by spectro-photometric method, which revealed a significant increase of DNA fragmentation % in the GEN-treated group when compared with the control group. GEN induced apoptosis in renal proximal tubules in the present work was reported in previous studies (**Servais et al., 2006** and **Denamur et al., 2011**). DNA fragmentation of the current work coincided with results of **Khan et al., (2011)** who reported a significant

increase in DNA fragmentation in liver and renal tissue samples of Sprague-Dawley male rats treated with GEN 100 mg/kg IP for ten days. Moreover, DNA ladder assay showed a continuous banding pattern of fragmented DNA induced with GEN in both organs, liver and kidneys.

Gross et al., (1999) attributed apoptotic activity of rat kidney induced by GEN treatment to over expression of Bax protein. The Bax protein is known as pro-apoptotic protein and over expression of Bax has been shown to upset the anti-apoptotic effects of Bcl-2 and Bcl- xL. **Kalkan et al., (2012**) added that GEN decreased the Bcl-2 expression and increased the Bax protein activation in kidney of adult male Sprague Dawley rats. They explained apoptosis induced by GEN by its ability to modulate membrane enzyme activities and changes pericellular and mitochondrial membranes permeability, so increasing cytosolic calcium concentration. These changes can cause the apoptotic activation in kidney.

GEN developed a significant increase of kidney MDA, while renal GSH level and catalase activity were diminished as compared to control group. These results were parallel to those of **Parlakpinar et al., (2005**) who reported that GEN induced nephrotoxicity was associated with significant increase in kidney MDA and low levels of GSH and catalase in the renal cortex. They suggested that these changes indicated not only consumption of antioxidants but also aggravation of the oxidative damage.

Safa et al., (2010) attributed the pathogenesis of GEN induced renal dysfunction to iron that capable of catalyzing free-radical formation GEN acts as an iron chelator and that have been shown to cause release of iron from renal cortical mitochondria. Iron-GEN complex is a potent catalyst of free-radical formation and enhance the generation of ROS.

Another proposed mechanism of GEN-induced oxidative stress was mentioned by **Tavafi et al., (2012**) who stated that ROS formation is the central key in the mechanisms that lead to tubular necrosis and decrease of glomerular filtration rate. They added that ROS activates nuclear factor kappa B that plays a key role in the inception of inflammatory process. They suggested that the central role of GEN- induced nephrotoxicity is oxidative stress and inflammation.

Moreover, **Servais et al., (2006**) **and Hsu et al., (2014)** have proved that the stimulation of apoptosis is a significant cytotoxic mechanism of GEN in renal proximal tubular cells and mesangial cells.

In another study, **Atessahin et al., (2003)** reported that GEN caused depletion in kidney GSH level; however, glutathione peroxidase (GSH-Px) and catalase were unaffected. Also, **Karahan et al., (2005)** found an increase in MDA levels, decreases in catalase activity, but no alteration in GSH levels. The short duration (6 days) used in both studies, in addition to different rodent species (Male Sprague-Dawley rats) used in the later study may the cause of controversy with the findings of the present study.

The results of the present work regarding co-administration of **LYC with GEN treatment** for 10 days caused significant decrease in serum creatinine and urea when compared with the GEN-treated group ($P<0.001$ each). This appeared also in the histopathological results that showed complete recovery in sections of 50% of rats treated with LYC+ GEN, the other half showed a marked histological amelioration with only mild inflammatory cells and occasionally small tubular casts.

The improvement in urea and creatinine levels, besides the histopathological amelioration recorded in the present study by using LYC were coincided with those obtained **byKarahan et al., (2005).**

Atessahin et al., (2005); Gado et al., (2013)andPalabiyik et al., (2013) found the same protective effects of LYC on nephrotoxicity induced by different nephrotoxic agents; cisplatin,cyclosporine and ochratoxin A respectively.

DNA ladder assay in this study denoted that DNA damage was comprehensively eliminated by LYC when co-administered with GEN as compared with the GEN-treated group. With respect to DNA fragmentation assay, GEN + LYC treated group showed

significant decrease of DNA fragmentation % when compared with the GEN-treated group.

The anti-apoptotic effect of LYC in this study was recorded in a previous work studied on a different organ (heart) reported by **Upaganlawar et al., (2012**)who observed that tomato LYC attenuates myocardial infarction induced by isoproterenol. Pre/co treatment of LYC with isoproterenol significantly prevented the isoproterenol DNA fragmentation and apoptotic changes of the myocardium. Gel electrophoresis revealed a significant decrease in DNA fragmentation, along with a significant reduction in caspase-3 protease activity.

Moreover,**Aydin et al., (2013)** and **Palabiyik et al., (2013)** observed that LYC supplementation with Ochratoxin A provided a significant protection against apoptosis when compared to ochratoxin A treated group by using two different assays (alkaline comet assay and TUNEL assay respectively).

The results of the present study can be explained by those of **Upaganlawar et al., (2012**) who stated that the protective effect of LYC against DNA fragmentation and apoptosis is attributed to its potent antioxidant activity, which scavenges the highly toxic free radicals and prevents the DNA from damage, and thereby inhibiting caspase-3activity.

In addition, **Tang et al., (2009)** attributed the antiapoptotic effect of LYC against injury of endothelial cells induced by H2O2 to attenuation of p53 and caspase-3 mRNA expressions in injured cells. They stated that active caspase-3 may promote apoptosis by degrading key structural and regulatory proteins within the cell.

The studied oxidative stress markers in this work revealed that; co-administration of LYC with GEN caused a significant decrease of kidney MDA, while renal GSH and catalase activity showed a significant increase in GEN + LYC- treated group when compared with GEN-treated group. These results pass parallel with those of **Karahan et al., (2005)** who demonstrated that simultaneous treatment of LYC with GEN normalized kidney MDA levels, besides significant increases in kidney GSH and catalase activity when compared to GEN treated group. In addition**, Dogukan et al., (2011); Meydan et al., (2011) and Gado et al., (2013**) demonstrated the ameliorative effect of LYC dietary supplementation against oxidative stress induced by cisplatin on kidney, radiation on liver and cyclosporine on kidney of rats respectively. Their results revealed a significant reduction in MDA levels (decreased lipid peroxidation), in addition the later two studies revealed rise in GSH-Px and SOD activities.

Tapiero et al., (2004) explained the antioxidant effect of LYC by its highly efficient scavenger activity of singlet oxygen (O•) and other excited species. They stated that during O• quenching, energy is transferred from O• to the LYC molecule. In addition, trapping of other ROS, like OH•, NO2 or peroxynitrite. Thus, LYC may protect in vivo lipids, proteins, and DNA against oxidation. Also, **El-Gerbed, (2012)** added to the antioxidant activity of LYC its ability to inhibit interleukin-6, thus improves the inflammatory process induced by GEN.

Co-administration of ZOEAE with GEN treatment for 10 days in the present work caused significant decrease in serum creatinine and urea when compared with the GEN-treated group. This appeared also in the histopathological results that showed complete recovery in 100% of sections of rats treated with ZOEAE + GEN. These results were in line with the same results obtained **by Lakshmi and Sudhakar, (2010)**. Also**, Rodrigues et al., (2014**) found that oral treatment with an enriched solution of gingerols in GEN treated rats promoted amelioration in renal function parameters,as well as, the histopathological findings.

Ajith et al., (2008); Hamed et al., (2012) and Krim et al., (2013) recorded the same protective effects of ginger on nephrotoxicity induced by different nephrotoxic agents; doxorubicin, Carbon Tetrachloride and chromate respectively.

DNA ladder assay in this study denoted that DNA damage was comprehensively eliminated by ZOEAE when co-administered with GEN as compared with the GEN-treated group. With respect to DNA fragmentation assay, GEN + ZOEAE treated group showed a significant decrease of DNA fragmentation

% when compared with the GEN-treated group.

The anti-apoptotic effect of ZOEAE in this work was recorded by **Zahedi et al., (2012),** who stated that when GEN was administrated with ginger rhizome, TUNEL assay revealed that apoptotic cells percent was significantly decreased indicating anti-apoptotic property of ginger. Other investigators have proved the anti-apoptotic effect of ginger against apoptosis induced by bromobenzene, lead acetate and metiram respectively **(El-Sharaky et al., 2009;Khaki and khaki, 2010and Sakr and Badawy, 2011).**

El-Sharaky et al., (2009) explained the inhibitory effect of ginger extract on the apoptotic cell death by its potent ameliorating effects on oxidative stress and local inflammation.

The studied oxidative stress markers in our work revealed that; co-administration of ZOEAE with GEN caused a significant decrease of kidney MDA, while renal GSH and catalase activity showed a significant increase in GEN + ZOEAE- treated group when compared with GEN-treated group. These results pass parallel with those of **Ademiluyi et al., (2013**) who demonstrated that pretreatment with ginger in GEN treated rats significantly protected the kidney and attenuated oxidative stress by modulating renal damage and antioxidant indices MDA and GSH contents as well as renal antioxidant enzymes (catalase, glutathione-S-transferase, GSH-Px and SOD).

In addition, **Rodrigues et al., (2014**) reported that oral treatment with an enriched solution of gingerols in GEN treated rats reduced lipid peroxidation and nitrosative stress, besides increasing the level of GSH and SOD activity.

The results of current study pass in accordance with those of **Ajith et al., (2008) and Krim et al., (2013)**, who found the same protective effects of ginger against oxidative stress of kidney induced by different nephrotoxic agents, doxorubicin and chromate respectively. Co-administration of ginger significantly reduced MDA levels, and restored renal GSH concentration and catalase activity to normal.

Sakr and Badawy, (2011) and Nasri et al., (2013) attributed the antioxidant activity of ginger to its major ingredients namely gingerols, shogoals, gingerdiol, zingerone and zingiberene. In addition,**Lakshmi and Sudhakar, (2010)** reported that, the ability of ginger to enhance renal mitochondrial antioxidant system is likely related to the nephron protection against GEN toxicity.

Moreover, **Siddaraju and Dharmesh, (2007**) stated that, ginger-free phenolic and hydrolysed phenolic fractions of ginger exhibited free radical scavenging, DNA protection, inhibited lipid peroxidation and reduced power abilities, indicating strong antioxidant properties.

In comparison between GEN + LYC and GEN + ZOEAE treated groups, it was found that, all the studied biochemical parameters (serum creatinine & urea, kidney MDA, GSH & catalase activity), showed no significant difference between GEN + LYC- and GEN+ZOEAE- treated groups. Consequently, both LYC and ginger extracts had approximate potency that could overcome oxidative stress induced by GEN. Also, histopathological results of kidney sections in the present study revealed better results with ZOEAE as complete recovery 100% was observed compared to 50% of rats treated with LYC +GEN. The other half of LYC + GEN treated group showed a marked amelioration but not complete resolution. In addition, DNA ladder assay in our study denoted that DNA damage was comprehensively eliminated by LYC and ginger when co-administered with GEN. With respect to DNA fragmentation assay, both extracts showed significant decrease of DNA fragmentation %. So, both LYC and ginger extracts could overcome DNA damage.

In conclusion, GEN has an oxidative stress on renal tissue and damaging effect on DNA. Lycopene as well as ZOEAE have antiapoptotic and antioxidant effects and their administration along with GEN led to marked functional and structural improvement of the nephrotoxicity as they improved all studied parameters while the histopathological findings revealed superiority of ginger over LYC.

RECOMMENDATIONS

It is recommended that, all patients treated with GEN should undergo periodic assessment of kidney functions. Suitable supplementary doses of LYC and/ or fresh vegetables or fruits containing LYC, as well as ginger and its extracts, can be added to the diet of GEN-treated patients.

REFERENCES

Ademiluyi AO; Oboh G; Ogunsuyi OB and Akinyemi AJ (2013): Attenuation of gentamicin-induced nephrotoxicity inrats by dietary inclusion of ginger (Zingiber officinale) and turmeric (Curcuma longa) rhizomes. Nutr Health. [Epub ahead of print].

Aebi H (1983): Catalase. In: Methods in enzymatic analysis, Bergmeyer HU (ed.), volume 3. Academic Press, New York, PP. 276–286.

Ajith TA; Aswathy MS and Hema U (2008): Protective effect of Zingiber officinale roscoe against anticancer drug doxorubicin-induced acute nephrotoxicity. Food Chem Toxicol; 46: 3178–3181

Ali BH (2003): Agents ameliorating or augmenting experimental gentamicinnephrotoxicity: some recent research. Food Chem Toxicol; 41: 1447–145.

Atessahin A; Karahan I; Yilmaz S; Ceribasi AO and Princci I (2003): The effect of manganese chloride on gentamicine-inducednephrotoxicity in rats. Pharmacol Res; 48: 637–642.

Atessahin A; Yilmaz S; Karahan I; Ceribasi AO and Karaoglu A (2005): Effects of lycopene against cisplatin-induce nephrotoxicity and oxidative stress in rats. Toxicol; 212: 116–123.

Atta AH; Nasr SM; Mouneir SM; Abdel-Aziem SH and Nassar SA (2014): Egyptian Propolis alleviates gentamicin-induced nephrotoxicity in rats. J advances chem; 6: 1109-1119.

Aydin S; Palabiyik ŞS; Erkekoglu P; Sahin G; Başaran N and Giray BK (2013): The carotenoid lycopene protects rats against DNA damage induced by Ochratoxin A. Toxicon; 73: 96-103.

Balakumar P; Rohilla A and Thangathirupathi A (2010): Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Pharmacol Res; 62:179–186.

Beutler E; Duron O and Kelly MB (1963): Improved method for the determination of blood glutathione. J Lab Clin Med; 61: 882-888.

Buffone GJ and Darlington GJ (1985): Isolation of DNA from biological specimens without extraction with phenol. Clin chem; 31: 164-165.

Burton K (1956): A study of the conditions and mechanism of thediphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J; 62: 315.

Denamur S; Tyteca D; Marchand-Brynaert J; Van Bambeke F; Tulkens PM; Courtoy PJ and Mingeot-Leclercq MP (2011): Role of oxidative stress in lysosomal membrane permeabilization and apoptosis induced by gentamicin; an aminoglycoside antibiotic. Free Radic Biol Med; 51: 1656–1665.

Derakhshanfar A; Bidadkosh A and Yazdi A (2008): Dopamine protects gentamicin early induced nephrotoxicity in Sprague–Dawley rats. Comp Clin Pathol; 17: 99–104.

Dogukan A; Tuzcu M; Agca CA; Gencoglu H; Sahin N; Onderci M; Ozercan IH; Ilhan N; Kucuk O and Sahin K (2011): A tomato lycopene complex protects the kidney from cisplatin-induced injury via affectingoxidative stress as well as Bax, Bcl-2, and HSPs expression. Nutr Cancer; 63: 427–434.

El-Gerbed MSA (2012): Protective effect of lycopene on deltamethrin-induced histological and ultrastructural changes in kidney tissue of rats. Toxicol Ind Health; 28: 1–14.

El-Sharaky AS; Newairy AA; Kamel MA and Eweda SM (2009): Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. Food Chem Toxicol; 47: 1584-1590.

Gado AM; Adam ANI and Aldahmash BA (2013): Protective effect of lycopene against nephrotoxicity induced by cyclosporine in rats. Life Sci J; 10: 1850-1856.

Gross A; Mc Donnell JM and Korsmeyer SJ (1999): Bcl-2 family members and the mitochondria in apoptosis. Genes Dev; 13: 1899–1911.

Hamed MA; Ali SA and Saba El-Rigal N (2012): Therapeutic potential of ginger against renal injury induced by carbon tetrachloride in rats. Sci World J; 2012: Article ID 840421, 12 pages.

Hasan MF and Rahman MM (2011): Screening of antibacterial, antifungal and cytotoxic activities of Polygonum hydropiper L.stem extracts. Int J Biosci; 1: 47-53.

Hosten AO (1990): BUN and Creatinine. In: Clinical Methods: The History, Physical, and Laboratory Examinations, Walker HK, Hall WD and Hurst JW (eds.), Chapter 193, 3rd edition. Butterworths, Boston, PP. 1234-1238. http://www.ncbi.nlm.nih.gov/books/NBK305/

Hsu YH; Chen TH; Wu MY; Lin YF; Chen WL; Cheng TH and Chen CH (2014): Protective effects of Zhibai Dihuang Wan on renal

tubular cells affected with gentamicin-induced apoptosis. J Ethnopharmacol; 151: 635-642. http://www.unimunester.de/imperia/md/content/ziv/service/software/spss/handb

Institute of Laboratory Animal Resources,Commissionon Life Sciences, National Research Council (1996): Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington DC, PP. 21-55. http://www.nap.edu/openbook.php

Johnson MD (2007): The Rats. In: Animal models of toxicology, Gad SC (ed.), Chapter 3, 2nd edition. CRC Press, Taylor & FrancisGroup, LLC, Boca, Raton, London, New York, PP. 187–188

Kalkan Y; Kapakin KAT; Kara A; Atabay T; Karadeniz A; Simsek N and Karakus E (2012):Protective effect of Panax ginseng against serum biochemical changes and apoptosis in kidney of rats treated with gentamicin sulphate. J Mol Hist; 43: 603–613

Karahan I; Atessahin A; Yılmaz S; Ceribasi AO and Sakin F (2005): Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicol; 215: 198–204.

Khaki AA and Khaki A (2010): Antioxidant effect of ginger to prevents lead induced liver tissue apoptosis in rat. J Med Plant Res; 4: 1492–1495.

Khan MR; Badar I and Siddiquah A (2011): Prevention of hepatorenal toxicity with Sonchus asper in gentamicin treated rats. BMC Complement Altern Med; 11: 113.

Kiernan J (2001): Histological and histochemical methods: theory and practice 3rd edition. MacMillan, Oxford, Boston, Johannsbury, New Delhi, PP. 111–113, 150–153.

Krim M; Messaadia A; Maidi I; Aouacheri O and Saka S (2013): Protective effect of ginger against toxicity induced by chromate in rats. Ann Biol Clin; 71:165-173.

Lakshmi BVS and Sudhakar M (2010): Protective Effect of Zingiber officinale on Gentamicin-Induced Nephrotoxicity in Rats. Internation J Pharmacol; 6: 58-62.

Luo C and Wu X-G (2011): Lycopene enhances antioxidant enzyme activities and immunity function in N-Methyl-N′ -nitro-N-nitrosoguanidine–inducedgastric cancer rats. Int J Mol Sci; 12: 3340–3351.

Meydan D; Gursel B; Bilgici B; Can B and Ozbek N (2011): Protective effect of lycopene against radiation-induced hepatic toxicity in rats. J Int Med Res; 39; 1239–1252.

Mordente A; Guantario B; Meucci E; Silvestrini A; Lombardi E; Martorana GE; Giardina B and Bohm V (2011): Lycopene and cardiovascular diseases: An uptade. Curr Med Chem; 18: 1146-116.

Nasri H; Nematbakhsh M; Ghobadi S; Ansari R; Shahinfard N and Rafieian-Kopaei M (2013): Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. Int J Prev Med; 4: 316–321

Ohkawa H; Ohishi N and Yagi K (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95: 351-358.

Palabiyik SS; Erkekoglu P; Zeybek ND; Kizilgun M; Baydar DE; Sahin G and Giray BK (2013): Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosis in rats. Exp Toxicol Pathol; 65: 853-861.

Palozza P; Parrone N; Simone RE and Catalano A (2010): Lycopene in atherosclerosis prevention: An integrated scheme of the potential mechanisms of action from cell culture studies. Arch Biochem Biophys; 504: 26–33.

Parlakpinar H; Tasdemir S; Polat A; Bay-Karabulut A; Vardi N; Ucar M and Acet A (2005): Protective role of caffeic acid phenethyl ester (CAPE) on gentamicin-induced acute renal toxicity in rats. Toxicol; 207: 169–177.

Patton C and Crouch SC (1977):Spectrophometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem; 49: 464-469.

Perandones CE; Illera VA; Peckham D; Stunz LL and Ashman RF (1993): Regulation of apoptosis in vitro in mature murine spleen T cells. J Immunol; 151: 3521-3529

Raju S; Kavimani S; Maheshwara Rao UV; Reddy SK andKumar VG (2011): Floral extract of Tecoma stans: A potent inhibitorof gentamicin-induced nephrotoxicity in vivo. Asian Pac J Trop Med; 4: 680-685.

Randjelovic P; Veljkovic S; Stojiljkovic N; Jankovic-Velickovic L; Sokolovic D; Stoiljkovic M and Ilic I (2012): Salicylic Acid Attenuates Gentamicin-InducedNephrotoxicity in Rats. The Sci World J; 2012: Article ID 390613, 6 pages.

Rasmussen P (2011): Ginger Zingiber officinale Roscoe; Zingiberaceae. J Prim Health Care; 3: 235–236.

Robbins GR; Castellano RF; Torning FE; Fabry A and Fielder FG (1971): Acute toxicity in rats (intramuscular, intravenous and intraperitoneal) and mice (intramuscular, intravenous, intraperitoneal and subcutaneous). Unpublished Report, P. 3967. Submitted to WHO

by Inveresk Research International, Trenant, Scotland, on behalf of Schering Plough Animal Health.

Rodrigues FA; Prata MM; Oliveira IC; Alves NT; Freitas RE; MonteiroHS; Silva JA; Vieira PC; Viana DA; Libório AB and Havt A (2014): Gingerol fraction from Zingiber officinale protects against gentamicin-induced nephrotoxicity. Antimicrob Agents Chemother. [Epub ahead of print].

Sabry NA; Farid SF and Dawoud DM (2014): Antibiotic dispensing in Egyptian community pharmacies: An observational study. Res Social Adm Pharm; 10: 168-184.

Safa J; Argani H; Bastani B; Nezami N; Ardebili BR;Ghorbanihaghjo A; Kalagheichi H; Amirfirouzi A; Mesgari M and Rad JR(2010): Protective effect of grape seed extract on gentamicin- inducedacute kidney injury. Iran J Kidney Dis; 4: 285-291.

Sakr SA and Badawy GM (2011): Effect of ginger (Zingiber officinale R.) onmetiram-inhibited spermatogenesis and induced apoptosis in albino mice. J Appl Pharmaceut Sci; 1: 131-136

Schirmeister J; Willmann H and Kiefer H (1964):Plasma creatinine concentration as a rough indicator of renal function. Dtsch Med Wschr; 89 : 1018.

Servais H; Jossin Y; Van Bambeke F; Tulkens PM and Mingeot- Leclercq MP (2006): Gentamicin causes apoptosis at low concentrations in renal LLC- PK1 cells subjected to electroporation. Antimicrob Agents Chemother; 50: 1213–1221.

Servais H; Ortiz A; Devuyst O; Denamur S; Tulkens PM and Mingeot-Leclercq MP (2008):Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. Apoptosis; 13: 11–32.

Siddaraju MN and Dharmesh SM (2007): Inhibition of gastric H (+), K (+) - ATPase and Helicobacter pylori growth by phenolic antioxidants of Zingiber officinale. Mol Nutr Food Res; 51: 324–332.

SPSS Inc (2007):SPSS for windows, version 16.0. Chicago, SPSS Inc.

Stacewicz-Sapuntzakis M and Bowen PE (2005):Role of lycopene and tomato products in prostate health. Biochim BiophysActa; 1740: 202-205

Stoilova I; Krastanov A; Stoyanova A; Denev P and Gargova S (2007): Antioxidant activity of a ginger extract (Zingiber officinale). Food Chem; 102: 764-770

Stojiljkovic N; Stoiljkovic M; Randjelovic P; Veljkovic S and Mihailovic D (2012): Cytoprotective effect of vitamin C against gentamicin-induced acutekidney injury in rats. Exp Toxicol Pathol; 64: 69–74.

Tang XY; Yang XD; Peng YF and Lin JH (2009): Protective effects of lycopene against H_2O_2 induced oxidative injury and apoptosis in human endothelial cells. Cardiovasc Drugs Ther; 23: 439-448.

Tapiero H; Townsend DM and Tew KD (2004): The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother; 58: 100–110.

Tavafi M; Ahmadvand H and Toolabi P (2012): Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis; 6: 25-32.

uecher/englisch/spss_brief_guide_16.0.pdf

Upaganlawar A; Patel V and Balaraman R (2012): Tomato lycopene attenuates myocardial infarction induced by isoproterenol: Electrocardiographic biochemical and anti–apoptotic study. Asian Pac J Trop Biomed; 2: 345–351.

Wantuck JM; Ahmed A and Nguyen MH (2014): Review article: the epidemiology and therapy of chronic hepatitis C genotypes 4, 5 and 6. Aliment Pharmacol Ther; 39: 137–147.

Wlodek D; Banath J and Olive PL (1991): Comparison between pulsed-fieldand constant-field gel electrophoresis for measurement of DNA double-strandbreaks in irradiated Chinese hamster ovary cells. Int J Radiat Biol; 60: 779-790.

Yaman I and Balikci E (2010): Protective effects of nigella sativa against gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol; 62: 183–190.

Yanagida C; Ito K; Komiya I and Horie T (2004):Protective effect of fosfomycin on gentamicin-induced lipid peroxidationof rat renal tissue. Chem Biol Interact; 148: 139–147

Zager RA (2007): Subclinical gentamicin nephrotoxicity: a potential risk factor for exaggerated endotoxin-driven TNF- production. Am J Physiol Renal Physiol; 293: 43–49.

Zahedi A; Fathiazad F; Khaki A and Ahmadnejad B (2012): Protective Effect of ginger on gentamicin-induced apoptosis intestis of rats. Adv Pharm Bull; 2: 197-200**.**