

Histopathological and Histochemical effects of Fresh Garlic Homogenate on reno-hepatic alterations in rats treated with Gentamicin, Cefotaxime and Metronidazole

Hosny Abd El Fadil* ; Abdel Alim F. A. * ; Raslan, Y. A. ** ; Amany M. El-Garhy. ** and Ahmady Y. Kamare**

* Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

** Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Abstract- Gentamicin is an aminoglycoside bactericidal antibiotic used in therapy mainly against Gram negative bacteria. Cefotaxime is a semisynthetic broad spectrum bactericidal cephalosporin antibiotic and used against Gram positive and Gram negative bacteria. Metronidazole is an antimicrobial drug used in treatment of protozoal and anaerobic bacterial infection which may be administered with Gentamicin and/or Cefotaxime in the treatment of mixed infections caused by anaerobic and aerobic organisms. Combination therapy has complementary mechanisms of action. The Present investigation aimed at evaluating the effect of fresh garlic homogenate (FGH) on histopathological and histochemical alterations of gentamicin, cefotaxime, metronidazole and their combinations-induced toxicity. For this purpose, eighty eight male albino rats were divided into eleven groups. (First): Served as control, (Second): Received FGH (500mg/kg.b.wt., p.o.), (Third): Received gentamicin (80 mg/ kg.b.wt., i.m), (Fourth): Received cefotaxime (540 mg/kg.b.wt., i.m), (Fifth): Received metronidazole (135 mg/kg.b.wt., p.o.), (Sixth): Received gentamicin with cefotaxime, (Seventh): Received gentamicin with cefotaxime and metronidazole, (Eighth): received FGH one hour prior gentamicin , (Ninth): Received FGH one hour prior cefotaxime, (Tenth): Received FGH one hour prior gentamicin and cefotaxime, (Eleventh): Received FGH one hour before gentamicin, cefotaxime and metronidazole for 14 successive days. Animals were sacrificed; kidney and liver were removed for homogenate measurements, histopathological & histochemical examination. Results indicated a significant ($p < 0.05$) decrease of TNF- α and elevation in GPx of liver and kidney homogenate. Our data indicated that FGH could protect the liver and kidney against the histopathological and histochemical alterations by blocking oxidative damages in addition to restoration of the antioxidant enzymatic profile.

Index Terms- Gentamicin, Cefotaxime, Metronidazole, Garlic, Histopathological, Histochemical.

I. INTRODUCTION

Aminoglycoside antibiotics have long been used in antibacterial therapy. Gentamicin is an aminoglycoside antibiotic derived from *Micromonospora purpurea*. It is effective against most of the life threatening Gram negative bacterial infections (1). Cefotaxime is a broad spectrum β -lactam

antibiotic. It is considered to be equivalent to ceftriaxone in terms of safety and efficacy (2). Metronidazole is a 5-nitroimidazole drug widely used in veterinary and human medicine for the treatment of *trichomoniasis*, *giardiasis*, *amebiasis* and *anaerobic* bacterial infections. In addition to its anti-protozoal and bactericidal properties (3).

Garlic (*Allium sativum*) FGH has been cultivated since ancient times and used as a spice and flavoring, and due to its potential benefits in preventive and curative medicine has been used in many cultures. It has antimicrobial, hypolipidemic, antihypertensive, hypoglycemic, anticoagulant and anti-atherosclerotic nephroprotective and hepatoprotective effects. Garlic is used as antidote for heavy metal poisoning (4).

The target of the present study was to investigate the possible ameliorative effect of garlic against histopathological and histochemical alterations induced by combination of gentamicin, cefotaxime and metronidazole in male albino rats.

Material and METHODS

1- Drugs

Gentamicin (80 mg / kg, I.M.) (5), Cefotaxime (540 mg / kg, I.M) (6), were obtained from Egyptian Int. Pharmaceutical Industrial Co. (10th of Ramadan, Egypt), Metronidazole (135 mg / kg, orally; Alex. Co. for pharmaceutical industries (Egypt), (7). Garlic (500 mg/kg, orally; local market, (8).

2-Experimental animals

Eighty eight adult male albino rats (weighing 200 \pm 10 gm) were used in the present study. They were obtained from the Animal Breeding Unite, National Organization for drug control and research (Giza, Egypt). Animal groups were caged in separate cages, in controlled temperature (23–25 °C), humidity (60%), light and dark cycles of 12 hours each. The animals were fed on standard pelleted diet and water *ad libitum*. The experiment was conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Veterinary Medicine, Zagazig University, Egypt and comply with the Guide for the Care and Use of Laboratory Animals (9).

3-Animal grouping:

After two weeks of acclimatization, eighty eight adult male albino rats were divided equally into eleven groups (8 rats each).

Group 1: (Control)

Group2: animals received FGH for 14 successive days.

Group3: animals received gentamicin for 14 successive days.

Group4: animals received cefotaxime for 14 successive days.

Group5: animals received metronidazole for 14 successive days.

Group6: animals received gentamicin simultaneously with cefotaxime administration for 14 successive days.

Group7: animals received gentamicin simultaneously with cefotaxime and metronidazole administration for 14 successive days.

Group8: animals received garlic one hour prior gentamicin administration for 14 successive days.

Group9: animals received garlic one hour prior cefotaxime administration for 14 successive days.

Group10: animals received garlic one hour prior gentamicin and cefotaxime administration for 14 successive days.

Group11: animals received garlic one hour prior gentamicin, cefotaxime and metronidazole administration for 14 successive days.

Twenty four hours after last dose the animals were anaesthetized using Phenobarbital, kidney and liver were immediately removed and washed in ice saline, and divided into 2 pieces one kept in formalin for histopathology and histochemistry, the 2nd was homogenized in phosphate buffer (10), used for determination of Malondialdehyde, Glutathione peroxidase and Tumor necrosis factor alpha (TNF- α) (11). Kidney and liver were examined histopathologically and histochemically according to (12).

3-Statistical analysis:

Results were expressed as mean \pm standard errors of the means (S.E.M.). Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Tukey-Kramer's Multiple Comparison Test, where $P < 0.05$ was considered significant (13).

II. RESULTS

I-Effect of fresh garlic homogenate on MDA, GPx and TNF- α of kidney and liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole.

Tables (1) and (2) illustrated that, oral metronidazole treated group produced a non-significant difference in kidney MDA, GPx and TNF- α for the normal control group, but gentamicin or cefotaxime groups evoked a significant decrease in GPx and increase in MDA and TNF- α versus control group, whereas oral fresh garlic homogenate administration prior gentamicin or cefotaxime or gentamicin with cefotaxime or gentamicin, cefotaxime with metronidazole groups induced a significant increase in GPx and decrease in MDA and TNF- α as compared to gentamicin, cefotaxime, gentamicin and cefotaxime or gentamicin, cefotaxime with metronidazole groups respectively.

Table (1): Effect of fresh garlic homogenate on MDA and GPx of kidney and liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole. (Mean \pm SE) (n = 8).

Groups	Kidney		Liver	
	MDA(nmol/g)	GPx(U.g.t)	MDA(nmol/g)	GPx(U.g.t)
(1) Control(Cont)	30.47 \pm 0.477 ^e	36.96 \pm 1.944 ^a	22.24 \pm 0.425 ^d	55.94 \pm 2.438 ^a
(3) Gentamicin(Gen.)	86.80 \pm 4.371 ^a	4.610 \pm 0.407 ^c	78.68 \pm 5.612 ^a	7.360 \pm 0.785 ^c
(4) Cefotaxime(Cef.)	37.36 \pm 1.205 ^d	24.32 \pm 2.809 ^{ab}	31.16 \pm 0.344 ^c	36.47 \pm 2.430 ^{ab}
(5) Metronidazol(Met)	32.75 \pm 0.748 ^{de}	32.42 \pm 3.240 ^{ab}	25.96 \pm 0.926 ^d	36.47 \pm 3.033 ^{ab}
(6) Gen. + Cef.	74.49 \pm 0.595 ^b	21.40 \pm 1.946 ^b	66.08 \pm 1.566 ^b	31.61 \pm 2.430 ^b
(7) Gen+Cef+ Met	75.19 \pm 0.844 ^b	21.40 \pm 2.089 ^b	67.30 \pm 0.631 ^b	31.61 \pm 4.655 ^b
(8) Gar. + Gen.	48.81 \pm 1.548 ^c	26.75 \pm 4.656 ^{ab}	37.212 \pm 2.007 ^c	36.47 \pm 2.430 ^{ab}
(9) Gar. + Cef.	30.43 \pm 0.413 ^e	31.61 \pm 2.430 ^{ab}	22.23 \pm 0.542 ^d	34.04 \pm 8.424 ^b
(10) Gar + Gen +Cef	36.65 \pm 1.004 ^{de}	35.01 \pm 2.381 ^a	28.48 \pm 2.224 ^{cd}	53.50 \pm 2.809 ^a
(11) Gar+Gen+Cef+Met.	36.05 \pm 1.190 ^{de}	35.01 \pm 2.581 ^a	29.31 \pm 2.377 ^{cd}	53.50 \pm 4.865 ^a

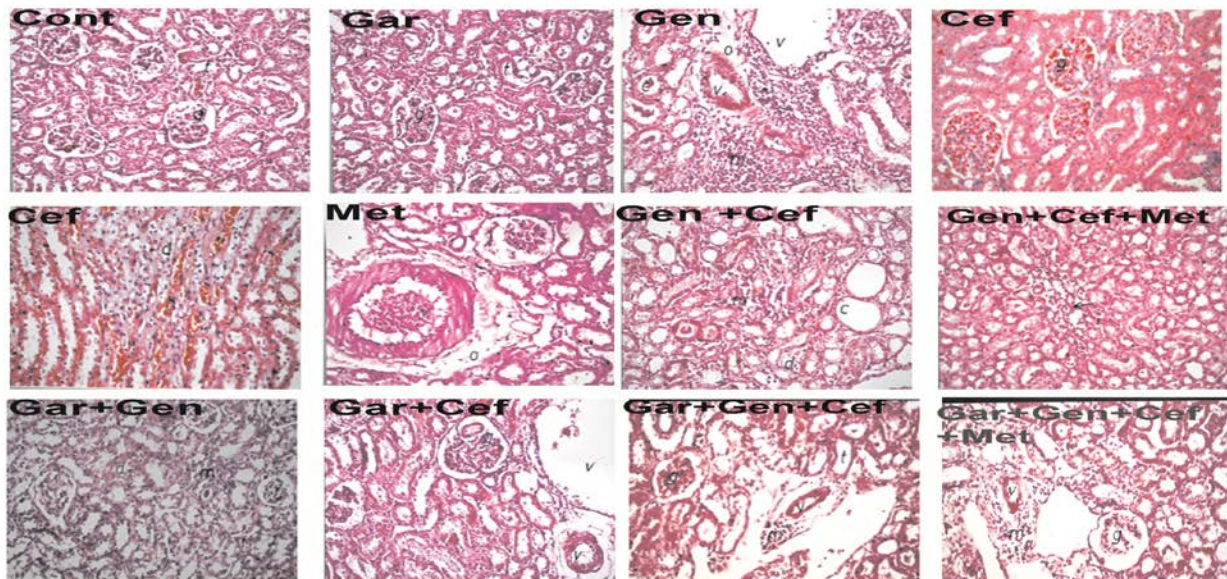
Means with different superscripts in the column are significant (p < 0.05)

Table(2): Effect of fresh garlic homogenate on TNF- α of kidney and liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole (Mean \pm SE) (n = 8).

Groups	Kidney TNF- α (Pg/g.t.)	Liver TNF- α (Pg/g.t.)
(1) Control(Cont)	46.6 \pm 2.02 ^d	46.6 \pm 2.02 ^d
(3) Gentamicin(Gen.)	228.7 \pm 10.04 ^a	228.7 \pm 10.04 ^a
(4) Cefotaxime(Cef.)	94 \pm 3.05 ^c	94 \pm 3.05 ^c
(5)Metronidazol(Met)	53 \pm 4.04 ^{cd}	53 \pm 4.04 ^{cd}
(6) Gen. + Cef.	156 \pm 5.13 ^b	156 \pm 5.13 ^b
(7)Gsen+Cef+ Met	165.3 \pm 3.75 ^b	165.3 \pm 3.75 ^b
(8) Gar. + Gen.	172 \pm 3.05 ^b	172 \pm 3.05 ^b
(9)Gar. + Cef.	52 \pm 5.77 ^d	52 \pm 5.77 ^d
(10)Gar + Gen +Cef	71.33 \pm 10.84 ^{cd}	71.33 \pm 10.84 ^{cd}
(11)Gar+Gen+Cef+Met.	64 \pm 12.22 ^{cd}	64 \pm 12.22 ^{cd}

Means with different superscripts in the column are significant (p < 0.05)

II-Effect of fresh garlic homogenate on histopathological picture of kidney in male albino rats treated with gentamicin, cefotaxime and/or metronidazole.



Figure(1) showed that, normal histological structure of kidney section of normal control and garlic groups, severe inflammatory cells infiltration (m) with dilatation of blood vessels (v) and perivascular oedema (o) as well as severe tubular degeneration with eosinophilic casts formation (c) in renal tubules of gentamicin group, severe congestion in glomerular tuft (g) at the cortex and severe haemorrhage (h) in between the degenerated tubules (d) at the corticomedullary portion of cefotaxime group, dilatation and congestion in sclerotic blood vessels (v) with perivascular oedema in metronidazole treated rats, gentamicin and cefotaxime group, showed Focal

inflammatory cells infiltration (m) was detected in between the degenerated (d) and cystically (c) dilated ducts at the corticomedullary portion, Rats were treated with gentamicin, cefotaxime and metronidazole group, showed inflammatory cells infiltration between the degenerated tubules (arrow) at corticomedullary portion, focal inflammatory cells infiltration (m) in between mild degenerated renal tubules (d) at cortex of garlic and gentamicin rats, garlic and cefotaxime group, dilatation in the blood vessels as well as normal histological structure of the glomeruli (g) and tubules. Kidney section of rat treated with garlic, gentamicin and cefotaxime group, showed

perivascular few inflammatory cells infiltration (m) with normal glomeruli(g) and renal tubules(t) and rat treated with garlic, gentamicin, cefotaxime and metronidazole group, showed

normal glomeruli(g) with few inflammatory cells infiltration (m) and perivascular oedema in the cortical portion.(H&E x40).

III-Effect of fresh garlic homogenate on histopathological picture of liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole.

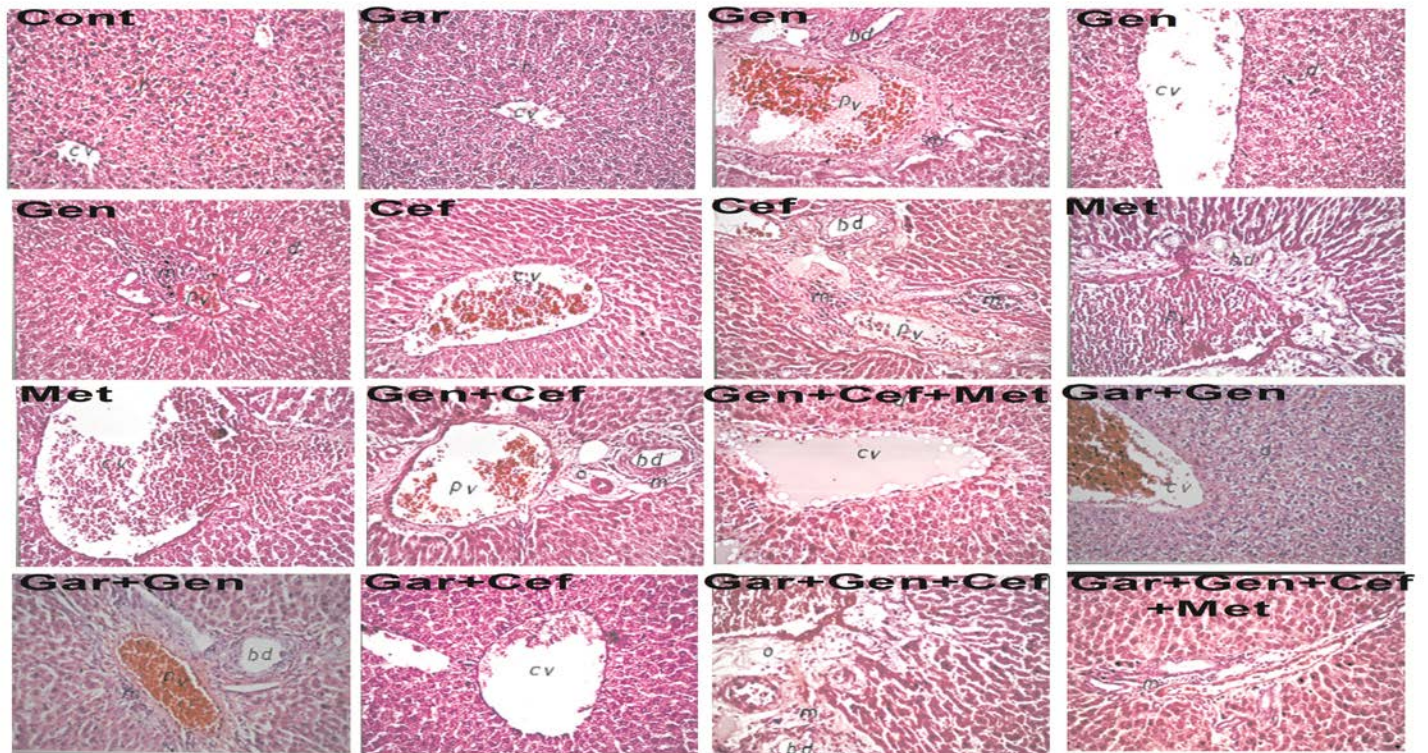
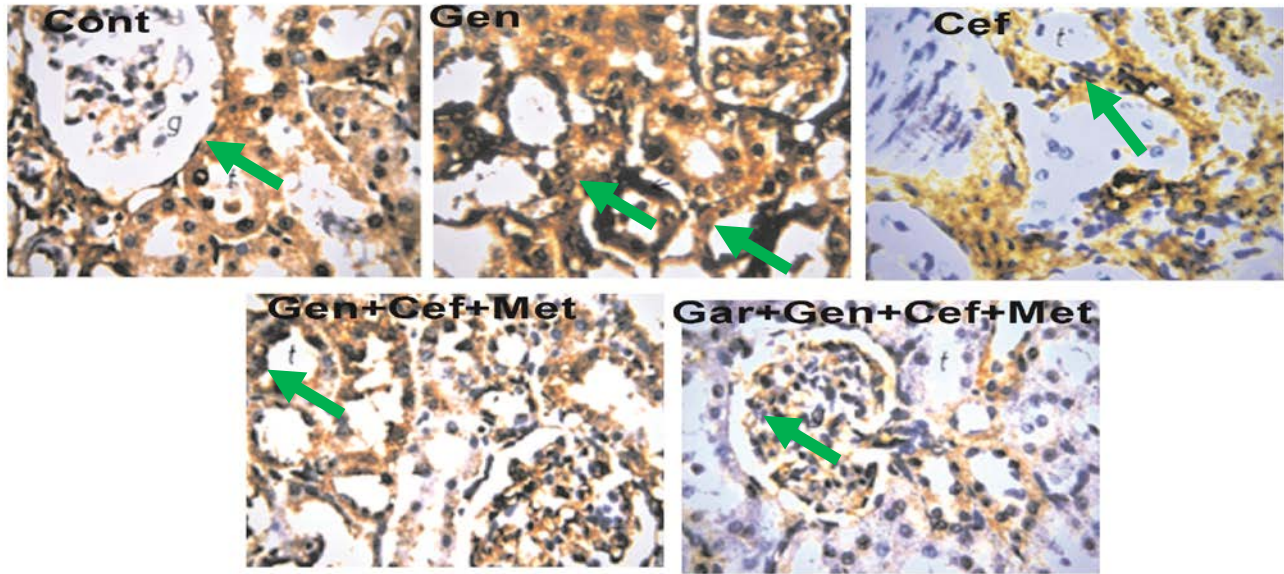


Figure (2) Liver section of normal control, garlic groups, there were no histopathological alteration and showed normal histological structure of the central vein (cv) and surrounding hepatocytes, rat treated with gentamicin group, showed severe dilatation in central vein (cv) with degeneration in the hepatocytes (d) and severe dilatation and congestion in portal vein (pv) with inflammatory cells infiltration (m) as well as dilatation in the bile ducts (bd) severe congestion in portal vein (pv) as well as severe inflammatory cells infiltration (m) in portal area and degeneration in the hepatocytes (h). Liver section of rat treated with cefotaxime group, showed focal necrosis (n) in the hepatic parenchyma, dilatation and congestion in the central vein (cv) and dilatation and congestion in portal vein (pv) and dilatation in bile duct (bd) with inflammatory cells infiltration in portal area, metronidazole group, showed dilatation and congestion were detected in central vein (cv), dilatation and congestion were detected in portal vein (pv) with

multiple number of newly formed bile ducts in portal area (bd), gentamicin and cefotaxime group, The portal area showed severe dilatation and congestion in the portal vein as well as oedema with few inflammatory cells infiltration surrounding the dilated bile ducts, , showed sever dilatation in the central vein (cv) by haemolysed blood with granular degeneration (d) in the hepatocytes gentamicin, cefotaxime and metronidazole group. Garlic and gentamicin group, showed dilatation and congestion in central vein (cv) and mild degeneration (d) in the hepatocytes and congestion in portal vein (pv) with inflammatory cells infiltration (m) in the portal area, dilatation of the central vein (cv) with normal hepatocytes garlic and cefotaxime group. Garlic, gentamicin and cefotaxime group, showed oedema(o) with few inflammatory cells infiltration (m) in the portal area, garlic, gentamicin, cefotaxime and metronidazole group, showed few inflammatory cells infiltration (m) in the portal area with normal hepatocytes.

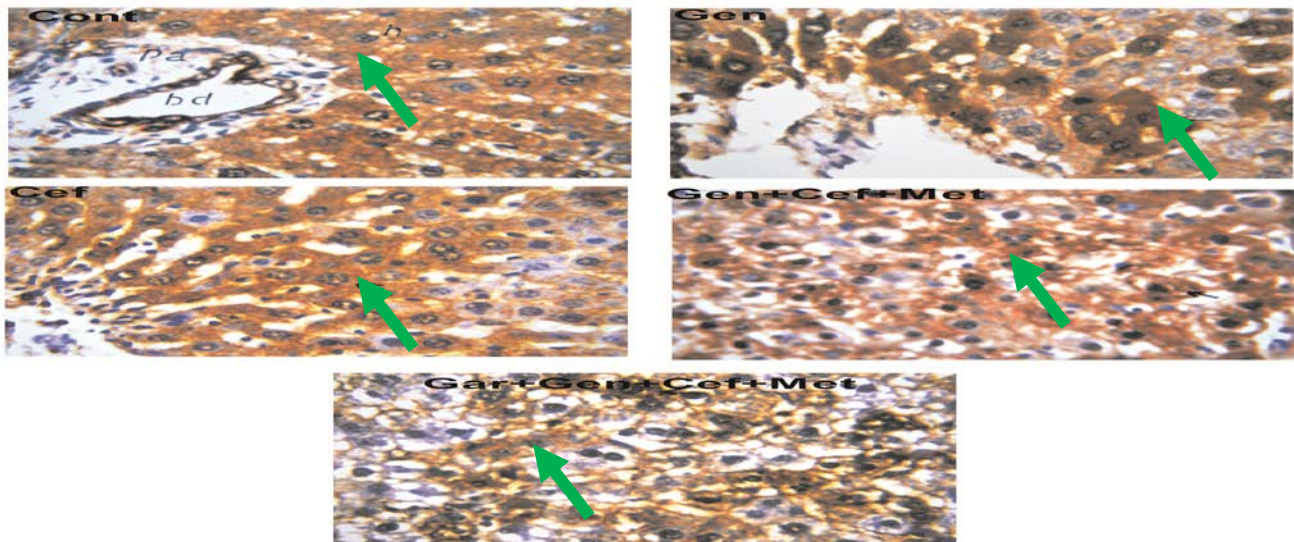
IV- Effect of fresh garlic on the severity of Immunohistopathological reaction using caspase-3 in kidney of different experimental groups.

The immunohistopathological Figures displayed that, kidney of rats intramuscular administrated gentamicin or cefotaxime or gentamicin, cefotaxime and metronidazole groups showed severely positive immunoreaction using caspase-3 antibody, whereas garlic administration attenuates these changes.



V- Effect of fresh garlic homogenate on the severity of histochemical reaction using caspase-3 in liver of different experimental groups.

The histochemical Figures displayed that, kidney of rats intramuscular administrated gentamicin or cefotaxime or gentamicin, cefotaxime and metronidazole groups showed severely positive immunoreaction using caspase-3 antibody, whereas garlic administration attenuates these changes.



III. DISCUSSION

Garlic is one of the widely used natural supplements to maintain and improve health of humans (14). It is active against microorganisms that are resistant to antibiotics and the combination of garlic extracts with antibiotics leads to partial and total synergism (15). Garlic is used as a spice antimicrobial, hypolipidemic, antihypertensive, hypoglycemic, anti-atherosclerotic, hepatoprotective, Nephroprotective and antidote for heavy metal poisoning and anticoagulant (4).

Abd El-Aziz and Kandeel (16) reported that, Toxicity of aminoglycosides (gentamicin) was associated with tissue damage and generation of reactive oxygen species.

Ramakrishnan et al.(17) reported that, Organism has a lot of antioxidative defense mechanisms for controlling reactive oxygen species preventing cellular damage, including the non-enzymatic (mainly reduced glutathione) and enzymatic defenses (including superoxide dismutase, glutathione reductase, glutathione S-transferase, catalase and glutathione peroxidase). GPx ubiquitously exists both in cytosol and mitochondria of the hepatocytes *Bansal et al. (18)*. Glutathione peroxidase can effectively scavenge free radicals and other oxygen species through nonenzymatic and enzymatic process by conjugation with reduced glutathione *Saydam et al.(19)*.

Priuska and Schacht (20) stated that, oxidative stress is mainly regulated by the cellular enzymatic (Cat., SOD, GPx) and nonenzymatic glutathione factor.

These results confirmed by *Nasr and Saleh (21)* who found that, garlic has an ameliorative effect against cisplatin-induced oxidative stress and renal damage through its antioxidant, anti-inflammatory and antiapoptotic properties.

The present study has clearly demonstrated the ability of gentamicin (80 mg / kg /day i.m for 14 days) to induce stress in rat kidney and liver, as evidenced by the significant elevation in TNF- α and a significant decline of GPx. These results supported by our previous study *Hosny et al. (22)* who reported that gentamicin induced a significant increase in AST, ALT, ALP, urea and creatinine and significant decrease in GSH, CAT and SOD of kidney and liver homogenates

Gentamicin administration to rats has been found to enhance the production of H₂O₂ in mitochondria, as a result of the increase in the production of superoxide anions (23). Superoxide anion and H₂O₂ may interact to form a reactive and unstable radical, namely a hydroxyl radical. This radical is formed by the reaction between H₂O₂ and Fe²⁺ (24). The accumulation of H₂O₂ and hydroxyl radicals lead to gentamicin nephrotoxicity by inducing mesangial cells contraction, altering the filtration surface area, these modifications leading to the decrease of the glomerular filtration rate. In this way, gentamicin treatment has induced a strong accumulation of oxidants (MDA and NO) in the kidney while SOD activities and GSH contents were significantly decreased (25).

The decrease of GPx activity in kidney as shown in our result could be associated with the severe tubular degeneration, the primary site of drug accumulation, because this enzyme is synthesized almost exclusively in tubular cells (26). Garlic confers a protective effect to kidney cells against oxidative damage by increasing the levels of endogenous antioxidant enzymes such as superoxide dismutase and catalase (27).

It has been reported that the antioxidant effects of garlic are associated with its ability to remove reactive oxygen species, to enhance endogenous anti-oxidation systems, and to inhibit the formation of lipid peroxides and the oxidation of low density lipoproteins (LDLs) (28).

Our results agreed with (29) who recorded that in caspase-3-stained tissues, the strongest apoptotic expression was in the gentamicin group compared with N-acetylcysteine - receiving group.

Masjedi et al.(30) stated that, Garlic suppress caspase-3 protein synthesis with stimulation of reduced glutathione and improves histopathological picture of pancreas of diabetic patient.

Gentamicin increased caspase-3, but metformin inhibited the expression of caspase-3 (38). A significant increase in caspase-3 activity was observed in cells treated with gentamicin (31).

Regarding to our results of histopathological and immunohistochemical pictures. These results displayed the best effect of garlic administration one hour before gentamicin, cefotaxime and / or metronidazole due to its antioxidant, anti-inflammatory and anti-apoptotic effects.

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AUTHORS

First Author – Hosny Abd El Fadil, Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Second Author – Abdel Alim F. A, Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Third Author – Raslan, Y. A, Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Fourth Author – Amany M. El-Garhy, Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Fifth Author – Ahmady Y. Kamare, Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Corresponding Author: E-mail: kamare_ahmady@yahoo.com. , National Organization for Drug Control and Research, NODCAR, Egypt; 29, Cairo, Egypt; e-mail: amanygd@yahoo.com; Tel.: (00202) -33933804 - 35851278; Fax: (00202)- 35855582