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Original Research Article

In vivo antimicrobial efficiency of garlic extract against pulmonary infections; Histopathological and Biochemical study

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ABSTRACT

As microbes are massively risky and have gotten to be safe to about all advertised anti-microbials. Withinthe period of anti-microbial resistance, antimicrobial of natural origin give an successful and cheap alternative for combating resistant strains of Gram negative microorganisms which induce pneumonia e.g. (*P. aeruginosa*) *Pseudomonas aeruginosa* and (*K. pneumoniae*) *Klebsiella pneumoniae*. Cefotaxime is an antimicrobial used in control and treatment of anaerobic bacteria which may be administered with Gentamicin within the treatment of blended contaminations caused by anaerobic and oxygen consuming living beings. Combination treatment has corresponding components of activity. Gentamicin is bactericidal aminoglycoside antibiotic used mainly against Gram negative bacteria. Allium sativum in garlic extract has been known to have inhibitory action & and valuable as helpful specialist against numerous pathological diseases. Expanding Multidrug resistance of pathogens strengths to discover elective strategies for treatment of irresistible infections.

Methods: Ethical approval: Protocols were approved by the IRB of the Scientific Research Unit (SRU) at Inaya Medical College (IMC) RIYADH (KSA)

Results: *In-vitro* and *in-vivo* antibacterial presented effect for combination of antibiotics and FGE for fourteen days. Indicated a significant ($p < 0.05$) decrease of TNF-α and elevation in GPx of liver and kidney homogenate. Our data showed that FGH combined antibiotics could protect the liver and kidney against the histopathological and histochemical changes by blocking oxidative damages in addition to restorement of the antioxidant enzymatic profile.

Conclusion: FGE displayed the best effect on administration one hour before gentamicin and cefotaxime due to its anti-inflammatory and antioxidant effects. Moreover, the potential use of FGE as a prophylactic agent against multi drug resistant bacteria.

1. Introduction

As Pneumonia is the third deadly irresistible malady around the world, responsible for millions of passings per-year, it is subsequently fundamental to recognize the causative specialist to choose an compelling anti-microbial treatment. Irresistible infections caused by diverse sorts of pathogenic microorganisms have been controlled by utilize of accessible commercial antimicrobial drugs since final various a long time. Marvelous inappropriate utilize of anti-microbials has created (MDR) in a few pathogenic microbes. The multiple drug resistance is the most obstacles in viable treatment of irresistible maladies and to the controller of microbial pathogenicity.[1] (CAP) Community-acquired pneumonia is connected with tall dreariness and mortality universally.[2] Whereas various distinctive microbes can be dependable for CAP, Streptococcus pneumonia remains the common causative microorganism. A little sum of CAP is caused by Gram-negative microscopic organisms, particularly Pseudomonas

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aeruginosa and K.pneumoniae.[3,4] Aminoglycoside anti-microbial have long been used in antibacterial treatment. Gentamicin is an aminoglycoside anti-microbial. It is compelling against most of the life damage Gram negative bacterial pathogens.[5] Metronidazole is broadly utilized in human pharmaceutical for the treatment of anaerobic bacterial pathogens. In expansion to its anti-protozoa and bactericidal properties.[6] Cefotaxime could be a wide range β-lactam anti-microbial. It is considered to be comparable to ceftriaxone in terms of security and viability.[7] The chief issue relating to the treatment of Gram-negative microbial pathogens is their related with anti-microbial resistance, detailed as multidrug safe (MDR).[8] This makes the treatment of pneumonia caused by such pathogens a challenge. Pneumonia is common with long-term infection (>10 days).[9] Taking into contemplations the seriousness that will be related with CAP caused by Gram-negative microbes. Anti-microbials resistance issue, commonly since anti-microbial tend to lose their adequacy over time due to the engendering of resistance among bacterial microorganisms, mainly caused by the abuse and unseemly utilize of anti-microbial, as well as the extensive use of anti-microbial.[10] Within the period of anti-microbial resistance, Normal items are a primary source of modern normal treatment and seem substitute for anti microbials with moo inactive for resistance and might be utilized as an interchange pharmaceutical. Allium satvium in garlic FGH has been developed since old times and utilized as a flavor and flavoring, and due to its potential benefits in preventive and healing medication has been utilized in numerous societies. It has antimicrobial, hypolipidemic, antihypertensive, hypoglycemic, anticoagulant and hostile to- atherosclerotic nephroprotective and hepatoprotective impacts. Garlic is utilized as cure for overwhelming metal harming.[11] Garlic was put to consideration as a weapon against MDR microbes.[12] It prevents intracellular union of RNA and squares imperative proteins; inside microbes, organisms and infections.[13] This depicts the broad-spectrum action of garlic against distinctive bacterial and viral contaminations with moo potential for creating bacterial resistance since of the different destinations of activity.[14,15] The aim of the current study was directed to evaluate the antibacterial activity of new garlic extricate (FGE) against MDR bacterial species in-vitro and in-vivo models to survey its potential utilize besides examination of the conceivable ameliorative impact of garlic against histopathological and histochemical modifications actuated by combination of gentamicin and cefotaxime in rats.

2. Materials and Methods

The current study had been run in Inaya Medical Colleges in Research Unit and Microbiology Laboratory in the period between (Januarys –April 2019).

Ethical considerations; Research Protocols were approved by the IRB of the Scientific Research Unit (SRU) at Inaya Medical College (IMC) RIYADH (KSA).

Drugs, test strains, culture conditions; Gentamicin (80 mg / kg, I.M.)[16] and Cefotaxime (540 mg / kg, I.M).[17] Garlic (500 mg/kg, orally; local market.[18] *Pseudomonas aeruginosa (P. aeruginosa*), and *Klebsiella pneumoniae* (*K. pneumonia*) were the strains used. Test strains were isolated from induced pneumonic rats. Strains were identified using the VITEK 2® system and stored in tryptic — soy broth (Oxoid, England) with 20% glycerol at 80 °C.For propagation, bacteria werespread over sheep blood agar, incubated aerobically at 37 °C/24 h. Colonies were suspended in tryptic soy broth (TSB), incubated till the logarithmic growth phase, and then standardized according to EUCAST.[19]

3. Experimental animals and housing conditions

Animal care and all experimental procedures were carried out in accordance with international guidelines governing animal care and use for research. For this reason Fifty six Sprague Dawley rats weighing 200±10 gm. were used within the display study. Dawley rats were obtained from a nearby provider and the animals are gathered in isolated cages, in controlled temperature (23–25ºC), humidity (60%), light and dark cycles of 12 hours each. The animals were encouraged on standard pelleted eat less and water. Rats were kept for at slightest 3 days earlier to test methods and were ordinarily housed all through the observational period. The rats were particular pathogen free upon entry to office, and amid the observational period. Animal groups were caged in separate cages. Animals were divided into seven groups. (The First group) Worked as control, (The Second group): Expected FGH (500mg/kg.b.wt.), (The Third group) Received gentamicin (80 mg/ kg.b.wt.), (The Fourth group) Received cefotaxime (540 mg/kg.b.wt)., (The Fifth group) Received gentamicin and cefotaxime (The Sixthgroup) Received FGH one hour prior gentamicin (Seventh) Received FGH one hour before gentamicin and cefotaxime for 14 successive days. The agar well diffusion and broth micro dilution techniques were employed to test the antimicrobial activities of FGE and the tested two antibiotics against 2 Multi Drug Resistant's (MDR) strains; *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*). Animals were sacrificed; kidney and liver were removed for histopathological & histochemical parameters examination..

3.1. Animal grouping

After two weeks of accommodation, Fifty six Sprague Dawley rats were divided equally into seven groups (8 rats each).Group 1: Control, Group 2: 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 gentamicin and cefotaxime for 14 successive days, Group6: animals received FGH one hour prior gentamicin administration for 14 successive days and Group7: animals received FGH one hour before gentamicin and cefotaxime 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), Glutathione peroxidase and Tumor necrosis factor alpha (TNF-α).[20] Kidney and liver were inspected histopathologically and histochemically.[21]

3.2. Preparation of fresh garlic extract (FGE) and testing activity

Fresh garlic bulbs were obtained from a open food store, peeled, and homogenized utilizing sterile mortar and pestle. At that point sifted through a cheesecloth, centrifuged at 12,000 rpm for 10 min and sifted twice through a 0.22 lm channel (Millipore TM; MA, USA), to get crude garlic extricate. This spoken to the 100% concentration that was at that point weakened with sterile refined water to urge concentrations of 75%, 50%, and 25%, and after that put away within the fridge for ensuing employments.[22] FGE at diverse concentrations were tried for antibacterial action by the agar well dissemination test as depicted somewhere else.[23] Utilizing chloramphenicol (32 mg/mL) and sterile refined water as positive and negative controls, individually. Hindrance zones with distance across less than 12 mm were considered as negative for antibacterial action. (Figure 1)

3.3. Selection of garlic doses

The corresponding MIC values against *P. aeruginosa* and *K. Pneumonia* were prepared in human serum, then 20 ml were inoculated on a blank filter paper disk, placed on inoculated agar, incubated at 35–37 °C for 24 h and the diameter of the inhibition zone was determined. At the same time, rats were injected intraperitoneally with different doses (50, 100, 200 & 400 mg/kg) of FGE, 2-hours post-injection; and at 6-hours interval over 24 h; the antibacterial activity of serum garlic was estimated by the Kirby-Bauer method as previously described. The comparing MIC values against *P. aeruginosa* and *K. Pneumonia* were arranged in human serum, at that point 20 ml were inoculated on a clear channel paper disk, set on inoculated agar, incubated at 35–37 °C for 24 h and the breadth of the restraint zone was determined. At the same time, rats were infused internationally with distinctive measurements (50, 100, 200 & 400 mg/kg) of FGE, 2-hours post-injection; and at 6-hours interval over 24 h; the antibacterial action of serum garlic was evaluated by the Kirby-Bauer strategy as already depicted.[24]

3.4. Induction of pneumonic infections

Measuring weights for each group of rats employing a commercially accessible scale (AEadam, CT, USA) precise $\pm$ 0.1g, creatures in test and control groups were anesthetized with ethyl ether conveyed to animals by wetting of a 4x4 cloth and putting it interior a plastic holder with the animal. After sedation by visual perception, rats were mounted on a commercially accessible intubation stand (Kent Scientific^{TM}, CT, USA). Coordinate visualization of glottis and vocal cords was accomplished employing a commercially accessible otoscope. Roughly 1 mL of pre-prepared contamination strain of P. aeruginosa and K. Pneumonia was conveyed to trachea. After the strategy, animals were permitted to recover on a commercially accessible warmed mat (Kent Scientific^{TM}, CT, and USA) to anticipate hypothermia. After recovery from anesthesia, animals were put back in their particular cages. P. aeruginosa and K. Pneumonia were developed in TSB/1% glucose till the log stage. Bacterial concentration was decided by measuring the OD600 and comparing to the development curve developed by plot- ting OD600 of bacterial suspension and colony-forming units (CFU) plated on Mueller-Hinton agar. iverse bacterial dosages (106-1012 CFU) were infused into the animals. Over a 24 h period and at 6- hour's intervals, animal's body temperatures were measured and blood tests were collected from the tail and handled for add up to white blood cells (WBCs) check.

3.5. Histopathological and histochemical studies

Livers from the control group were fixed in 10% formalin buffered. Sections of paraffin were prepared and cut at 4μ thickness, stained with (H&E) Hematoxylin and Eosin.

3.6. Caspase 3 immunohistochemistry

Immunohistochemical staining performed on 4-μm, formalin-fixed, paraffin-embedded sections using caspase 3 antibodies at 1:50 dilution (DAKO, Carpentaria, CA). Antigen retrieval was performed in pH 8.0 for 30 minutes by steam heating the slides in a 1-mmol/L solution of EDTA. Staining was performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin detection system (DAKO). Positive and negative control sections had been used.

3.7. Evaluation of the stains

Caspase 3 expression in hepatocytes was evaluated according to H score[25,26] in fields distant from necrotic areas (40). Semi- quantitative analysis was performed as follows: (−): negative, (+): weak staining, (++): moderate staining, (+++): strong staining.[27]

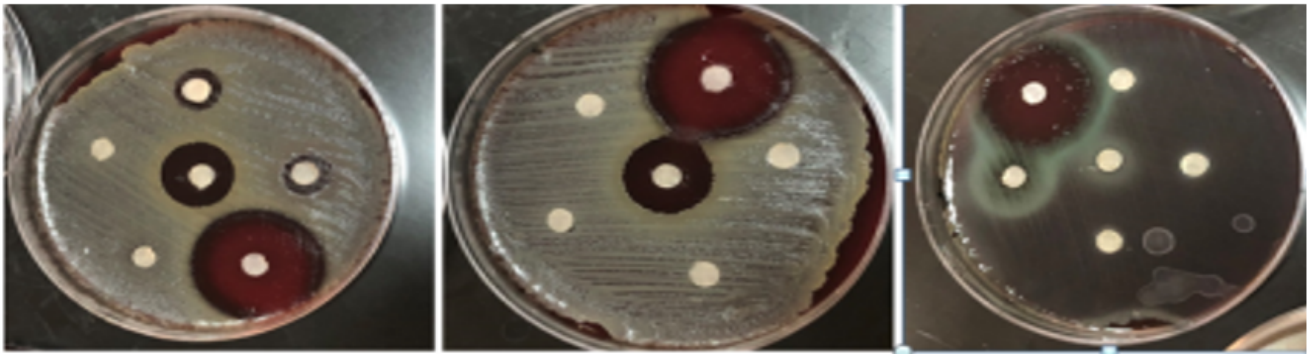

Fig. 1: Antibacterial activity of FGE at different concentrations on tested strains

3.8. Terminal harvest and processing of blood and tissues

In-vivo antimicrobial productivity of garlic treatment. One day after the conclusion of the test, rats were euthanized beneath aseptic conditions, blood tests and organs (liver and kidney) were collected from tainted groups. Blood and minced body organs (1gm each) were cultured for identifying the whole practical number by the spread plate procedure.[28]

3.9. Statistical analysis

Results were expressed as mean ± standard errors of the implies (S.E.M.). Comparison between more than two distinctive groups was carried out utilizing the one-way investigation of fluctuation (ANOVA) taken after by Turkey-Kramer's Different Comparison Test, where P<0.05 was considered significant.[29]

4. Results

4.1. Antibacterial activity of FGE

With the exception of P. aeruginosa and K. Pneumonia restraint zone estimation appeared a significant (P esteem <0.001) higher antibacterial movement with all test concentrations; compared to chloramphenicol; that was concentration and strain-dependent (Figure 2). The MIC values were found to be 32 mg/ml for P. aeruginosa while K. pneumoniae recorded 64 mg/ml.

4.2. In-vivo induction of infections and antimicrobial activity of FGE

Doses of 1.0 $\times 10^6$ and 1.0 $\times$ 10^8 CFU were adequate enough to induce infections by *P. aeruginosa* and *K. pneumonia*, respectively confirmed by leukocytosis after 12. Furthermore Comparing the inhibition zones achieved by the MIC and test doses, doses of 100 and 200 mg/kg produced comparable results sustained for 24 h. Systemic infections with *P. aeruginosa* and *K. pneumonia* were induced in rats via intraperitoneal injection. The artificial infection induced by the pathological microorganisms indicated that, groups treated with FGE recorded survival rate of 90%. For groups treated with gentamycin and Cefotaxime recorded 100% mortality by the 4th day. No growth was detected for the group treated by FGE in both infections. However, for groups treated by FGE with Gentamycin and or FGE with Gentamycin and cefotaxime, low growth levels were detected. As showed in Figure 3 in concentrations of 100 and 200 mg/KG Normal histological structure of the respiratory duct, alveoli and arteries in lung performed, normal kidney showing renal corpuscles, proximal and distal tubules and normal liver showing hepatocytes.

4.3. I-Effect of fresh garlic extract on MDA, GPx and TNF-α of kidney and liver in rats treated with gentamicin, cefotaxime.

(1) and (2) illustrated that, Gentamicin or cefotaxime groups induced a significant decrease in GPx and increase in MDA and TNF-α versus control group, whereas oral fresh garlic extract administration prior gentamicin or cefotaxime or gentamicin with cefotaxime groups induced a significant increase in GPx and decrease in MDA and TNF-α as compared to gentamicin, cefotaxime, gentamicin and cefotaxime groups respectively.

Effect of fresh garlic extract on histopathological picture of kidney in rats treated with gentamicin and or cefotaxime

The results in Figure 4 showed that, normal kidney section histological structure of normal control and garlic groups, gentamicin group showed severe inflammatory cells infiltration with dilatation of blood vessels and perivascular edema as well as severe tubular degeneration with eosinophilic casts formation in renal tubules, cefotaxime group showed severe congestion in glomerular tuft at the cortex and severe hemorrhage in between the degenerated tubules at the corticomedullary portion, gentamicin and cefotaxime group, showed Focal inflammatory cells infiltration was detected in between the degenerated and

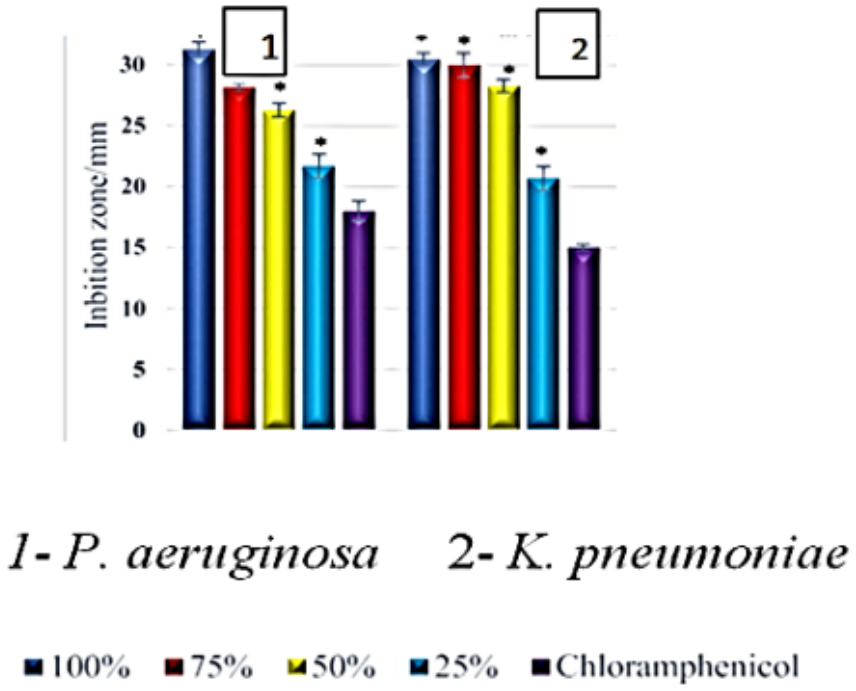

Fig. 2: Antibacterial activityof FGE

Table 1: Effect of fresh garlic extract on MDA and GPx of kidney and liver in rats treated with gentamicin and cefotaxime (Mean ± 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±0.477e	36.96±1.944a	22.24±0.425d	55.94±2.438a
(2) FGE	30.43±0.413	31.61±2.430ab	22.23±0.542d	34.04±8.424b
(3) Gentamicin(Gen.)	86.80±4.371a	4.610±0.407c	78.68±5.612a	7.360±0.785c
(4) Cefotaxime (Cef.)	37.36±1.205d	24.32±2.809ab	31.16±0.344cP	36.47±2.430ab
(5) Gen. + Cef.	74.49±0.595	21.40±1.946b	66.08±1.566b	31.61±2.430b
(6) Gar.+ Gen.	48.81±1.548c	26.75±4.656ab	37.212±2.007c	36.47±2.430ab
(7)Gar + Gen +Cef	36.65±1.004de	35.01±2.381a	28.48±2.224d	53.50±2.809a

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

Table 2: Effect of fresh garlic extract on TNF-α of kidney and liver in rats treated with gentamicin and cefotaxime (Mean ± SE) (n = 8)

Groups	Kidney TNF-α(Pg/g.t.)	Liver TNF-α (Pg/g.t.)
(1) Control(Cont.)	46.6±2.02d	46.6±2.02d
(2) FGE	52±5.77d	52±5.77d
(3) Gentamicin(Gen.)	228.7±10.04a	228.7±10.04a
(4) Cefotaxime (Cef.)	94±3.05c	94±3.05c
(5) Gen. + Cef.	156±5.13b	156±5.13b
(6) Gar.+ Gen.	172±3.05b	172±3.05b
(7)Gar + Gen +Cef	71.33±10.84cd	71.33±10.84cd

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

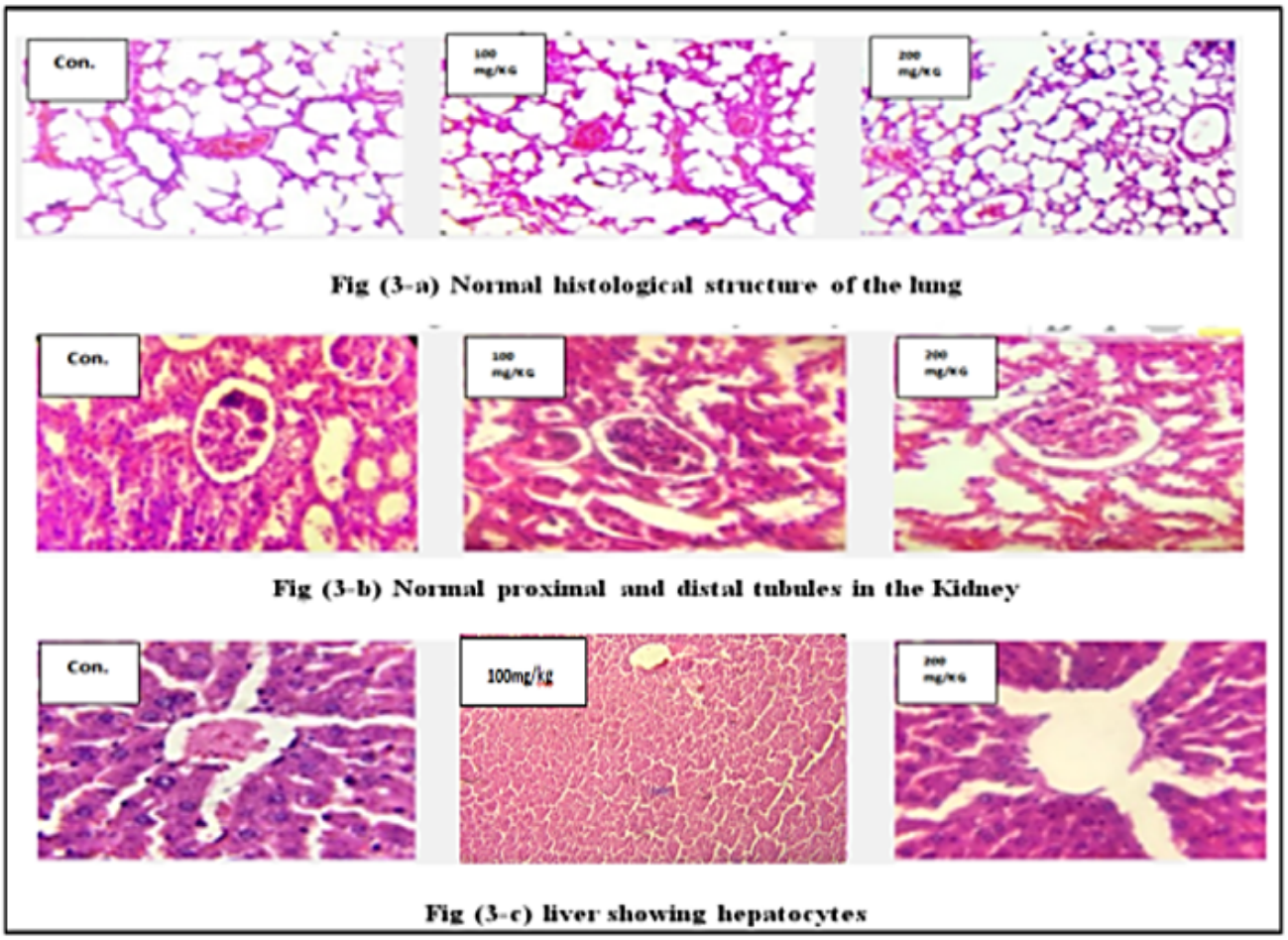

Fig. 3: a): Normal histological structure of the lung; **b):** Normal proximal and distal tubules in the Kidney; **c):** Liver showing hepatocytes

cystically dilated ducts at the corticomedullary portion, Kidney section of rat treated with garlic, gentamicin and cefotaxime group, showed perivascular few inflammatory cells infiltration with normal glomeruli renal tubules.

4.4. Effect of fresh garlic extract on histopathological picture of liver in rats treated with gentamicin and or cefotaxime

The results in Figure 5 showed that, 1-is the Liver section of normal control, 2- garlic groups, there were no histopathological alteration and showed normal histological structure of the central vein and surrounding hepatocytes, 3-gentamicin group, showed severe dilatation in central vein with degeneration in the hepatocytes and severe dilatation and congestion in portal vein with inflammatory cells infiltration as well as dilatation in the bile ducts, severe congestion in portal vein as well as severe inflammatory cells infiltration in portal area and degeneration in the hepatocytes. 4- cefotaxime group, showed focal necrosis in the hepatic parenchyma, dilatation and congestion in the central vein and dilatation and congestion in portal vein and dilatation in bile duct with inflammatory cells infiltration in portal area, metronidazole group, showed dilatation and congestion were detected in central vein, dilatation and congestion were detected in portal vein with multiple number of newly formed bile ducts in portal area, gentamicin and cefotaxime group, The portal area showed severe dilatation and congestion in the portal vein as well as edema with few inflammatory cells infiltration surrounding the dilated bile ducts, Garlic and gentamicin group, showed dilatation and congestion in central vein and mild degeneration in the hepatocytes and congestion in portal vein with inflammatory cells infiltration in the portal area, gentamicin and cefotaxime group, showed edema with few inflammatory cells infiltration in the portal area.

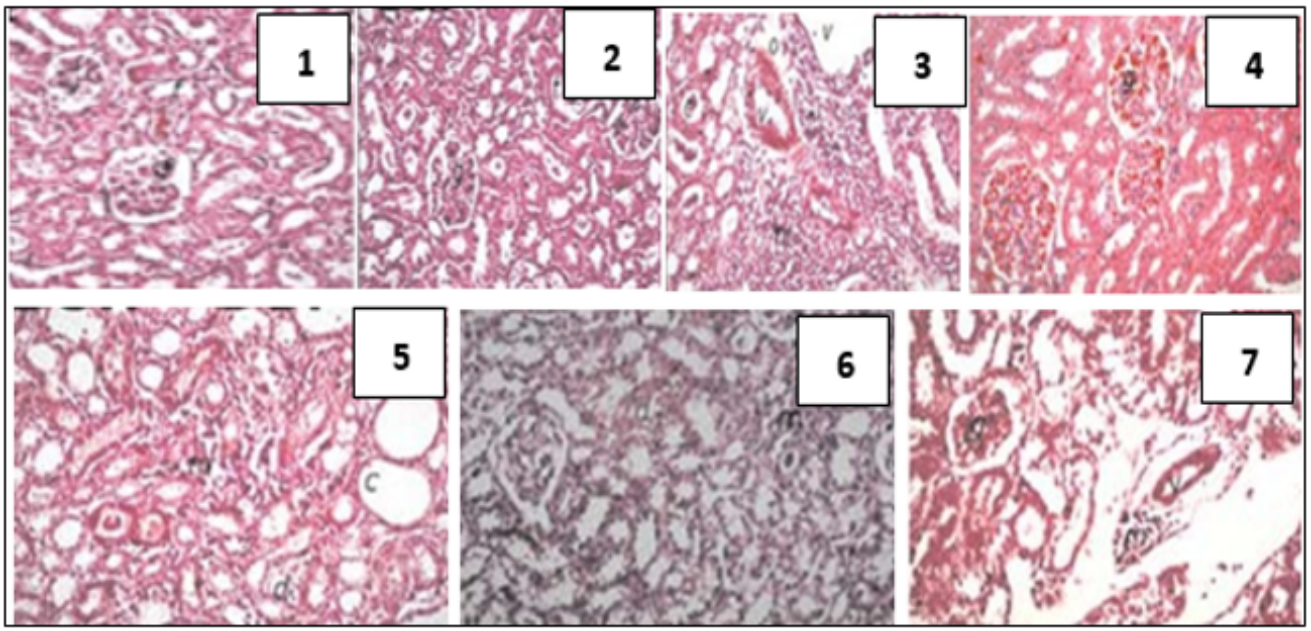

Fig. 4: 1: Control, 2: animals received FGH 3: animals received gentamicin, 4: animals received cefotaxime, 5: animals received gentamicin and cefotaxime, 6: animals received FGH one-hour prior gentamicin and 7: animals received FGH one hour before gentamicin and cefotaxime. Effect of fresh garlic extract on histopathological picture of kidney in rats treated with gentamicin and or cefotaxime

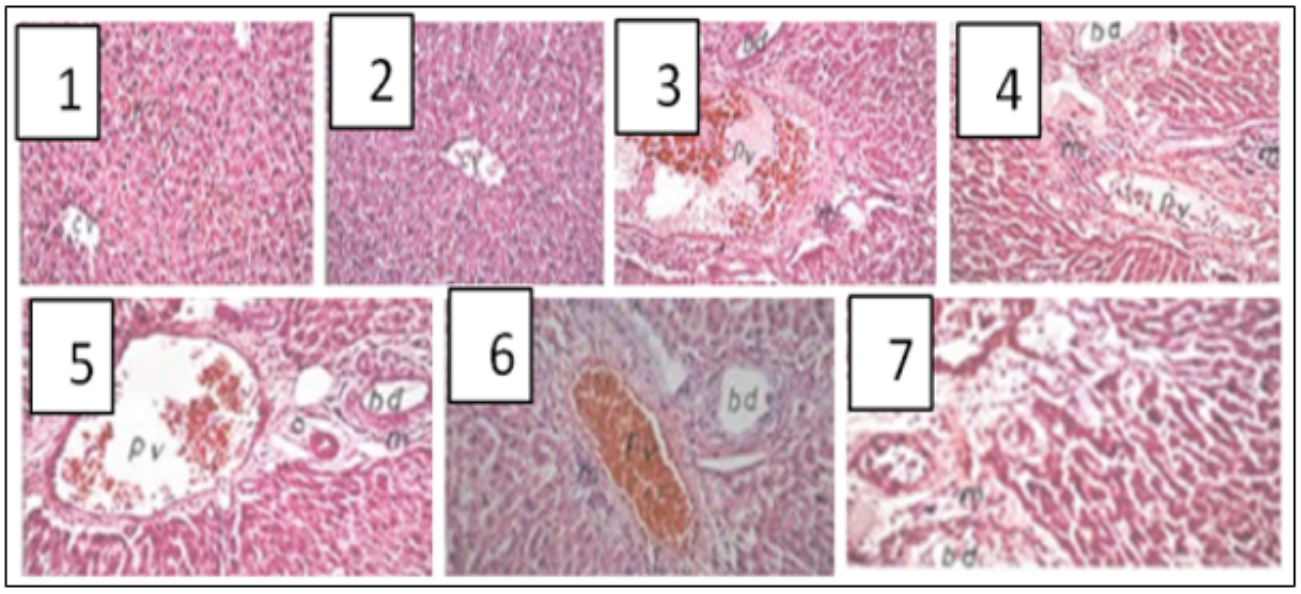

Fig. 5: 1: Control**,** 2: animals received FGH 3: animals received gentamicin, 4: animals received cefotaxime, 5: animals received gentamicin and cefotaxime, 6: animals received FGH one-hour prior gentamicin and 7: animals received FGH one hour before gentamicin and cefotaxime. Effect of fresh garlic extract on histopathological picture of liver

4.5. Effect of FGE on the severity of Immunohistopathological reaction using caspase-3 in Liver and kidney of different experimental groups.

Caspase 3 plays a pivotal role in liver damage etiology and administration of gentamicin or cefotaxime for rats exhibited positive immunoreaction using caspase-3 antibody, whereas garlic administration weakens these changes.

5. Discussion

The current study is coordinated to consider the potential utilize of garlic within the treatment of pneumonia. New Garlic is broadly utilized characteristic supplements to make strides and keep up health of people.[30] FGE is dynamic against diverse organisms that are safe to antimicrobial activity and the amalgamation of FGE with antimicrobials leads to fractional and add up to synergism.[31] Garlic is utilized as antimicrobial, against- atherosclerotic, cure for overwhelming metal harming and anticoagulant, antihypertensive, hepatoprotective, hypolipidemic and Nephroprotective.[6] Organosulfur compounds normally display in garlic give the premise for creative sources of novel anti-microbials.[32] Abd El-Aziz and Kandeel[33] and Ramakrishnan et al.[34] expressed that, Poisonous quality of (gentamicin) aminoglycosides was related to tissue harm and advancement of receptive oxygen species. Moreover, organisms encompasses a part of antioxidative defense instruments for controlling receptive oxygen species anticipating cellular harm, counting lessening glutathione (non- enzymatic) and superoxide dismutase, glutathione reductase, glutathione S-transferase, catalase and glutathione peroxidase (enzymatic guards). Bansal et al.[35] detailed that, GPx ubiquitously exists both in cytosol and mitochondria of the hepatocytes. Saydam et al.[36] said that peroxidase in Glutathione can viably look free radicals and other oxygen species through nonenzymatic and enzymatic conjugation with diminished glutathione. Priuska and Schacht[37] expressed that, controls of oxidative push is controlled by the cellular (Turf, GPx) chemicals and nonenzymatic glutathione calculate. These results confirmed by Nasr and Saleh[38] 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 current study has clearly demonstrated the capability of gentamicin (80 mg / kg /day) to induce stress in rat liver and Kidney, as clearly confirmed by the significant elevation in TNF-α and a significant decline of GPx. These results agreed by Hosni et al.[39] who concluded that gentamicin induced a significant increase in AST, ALT, ALP, creatinine and urea and significant decrease in GSH and CAT of Liver and Kidney homogenates. Administration of Gentamicin to rats has been originated to enhance the production of H2O2 in mitochondria, as a result of the increase in the production of superoxide anions.[40] Both (H2O2 and Superoxide anion) may interact to form unstable reactive radical, i.e. hydroxyl radical. The collection of H2O2 and hydroxyl radicals lead to gentamicin nephrotoxicity by actuating mesangial cells compression, altering the filtration surface zone, these alterations leading to the decrease of the glomerular filtration rate. Like so, gentamicin treatment has initiated a solid amassing of oxidants (MDA and NO) within the kidney whereas Grass exercises and GSH substance were essentially diminished.[41]

The diminish of GPx action in kidney as appeared in our result can be related with the extreme tubular degeneration, the essential location of medicate aggregation, since this chemical is synthesized nearly exclusively in tubular cells.[42] FGE deliberates a defensive impact to cells against oxidative harm by expanding the levels of catalase and superoxide dismutase antioxidant proteins.[43] The current study reported that the antioxidant impacts of FGE because it is related with its capacity to expel responsive oxygen species, to upgrade endogenous anti-oxidation frameworks, and to hinder the arrangement of lipid peroxides and the oxidation of moo thickness lipoproteins (LDLs).[44] Caspase 3 is pivotal in liver damage etiology, apoptosis induction and processing[45] the study is directed to record that in caspase-3- stained tissues; the strongest apoptotic expression was in the gentamicin rat group. Masjedi et al.[46] stated that, Garlic suppress caspase-3 protein synthesis with stimulation of reduced glutathione and improves histopathological picture of pancreas of diabetic patient. FGE allicin induced cell death in human hepatoma cells through either autophagy or apoptosis and might be a potential novel complementary gene therapeutic agent for the treatment of apoptosis-resistant cancer cells.[45] The histopathological and immunohistochemical pictures in our study displayed the best effect of garlic administration one hour before gentamicin and cefotaxime due to its antioxidant, anti-inflammatory and anti-apoptotic effects. In-vitro evolution of the FGE revealed its antibacterial activity; against P.aurginosa and k. pneumonia clinical bacterial strains. Furthermore, in-vivo antibacterial activity was evaluated against P. aeruginosa and K. pneumonia. FGE showed potent antibacterial activity against tested strains that was concentration and strain-dependent. FGE allicin interferes with crucial bacterial enzymes through the inactivation of thiol group.[47,48] Other investigators[49,50] didn't record prominent antibacterial activity for either the aqueous or alcoholic extract of FGE by the disk diffusion methods. The antibacterial activity of fresh garlic was found to be superior to garlic oil and powder and butylated hydroxyanisole.[51]

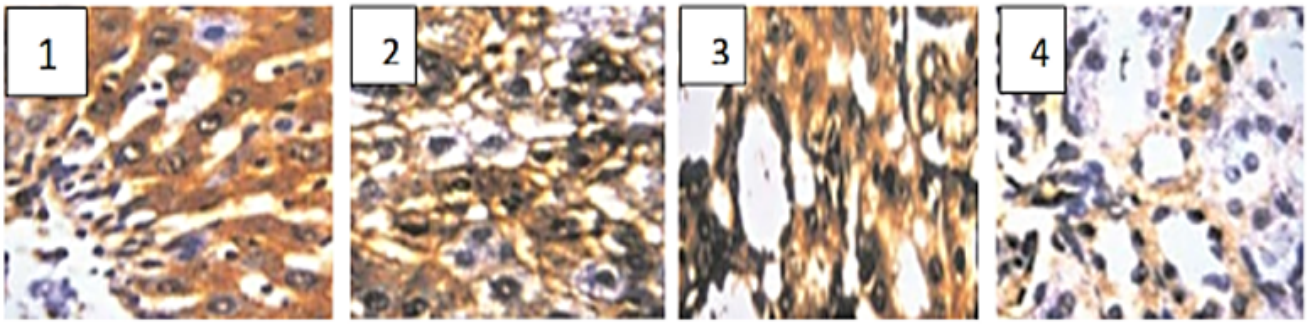

Fig. 6: Histochemical figures for liver after administration ofgentamicin or cefotaxime showed positive immunoreaction using caspase-3 antibody, whereas in 2-garlic administration weakness the Histochemical change, 3-The immunohistopathological figures for when administrate garlic it weaknessthe immunohistopathological changes; Effect of FGE on the severity of Immunohistopathological reaction using caspase-3 in Liver and kidney

6. Conclusions

FGE revealed a unique antibacterial activity against clinical microorganisms (P. aurginosa and k. pneumonia); in the two models in-vitro and in-vivo without detected toxic effects. Moreover FGH could protect the liver and kidney against the histopathological and histochemical alterations by blocking oxidative damages.

7. Acknowledgement

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