STUDYING OF ANTIBACTERIAL EFFECT FOR WATERY AND
ALCOHOLIC EXTRACTS OF PIPER NIGRUM L FOR SOME
PATHOGENS IN VITRO AND IN VIVO

Afrah J. Abd1, *Noor S. Aboud2, Haifaa J. Hassan3
1Al-Muthana University, Faculty of Veterinary Medicine
2Al-Muthana university, Faculty of Science
3Al-Qadisiya University, Faculty of Veterinary medicine.

ABSTRACT
This study was designed to evaluate the effects of the watery and alcoholic extracts from leaves of pepper nigrum L in vitro on ten pathogenic factors in addition to studying the antibacterial effect of watery leaves extract toward proteus vulgaris in vivo. Results showed that the watery and alcoholic extracts from leaves pepper nigrum L have antibacterial activity against both Gram positive (staphylococcus aureous, streptococcus pneumonia) and Gram negative bacteria (klebsilla pneumonia, proteus vulgaris, Escherichia coli, vibrio cholera and Salmonella paratyphi) in addition to candidia albicanes. However watery extract of pepper nigrum was more potent than alcoholic extract against pathogenic factor. According to results proteus vulgaris was the more sensitive pathogenic factor toward pepper nigrum L, thus watery extract was chosen to injected intraperitonialy in the laboratory rabbits, which cause morphological and pathological degenerative lesion for hepatic, renal tissues in addition to changes in hepatic function indicators, cardiac and renal profile tests which include total serum bilirubin, aspartame aminotransferase, alanine aminotransferase, alkaline phosphatase, gama-glutamle transaminase for hepatic indicator, while urea, creatinine, uric acid, potassium and creatinine kinase for renal tests while cardiac tissue and indicators that includes trponine, myoglobin and CK –MP, were did not affected any mater. Watery extract of pepper nigrum L cause significant improvement (p≤ 0.01) in the value both renal and hepatic tests for injected rabbits. histopathological studies confirm these results which include regeneration of degenerative lesion portal area and moderate congestion renal medulla and cortex tissue.
Key words: pepper nigrum L, antibacterial, in vitro, in vivo, Proteus vulgaris.

INTRODUCTION
Since the introduction of antibiotics, there has been tremendous increase in the resistance of diverse bacterial pathogens\(^{(1,2)}\). This shift in susceptibility greatly affects our ability to successfully treat patients empirically, plant derived products have been used for medicinal purposes for centuries, at present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments\(^{(3)}\). They are also extensively used, particularly, in many Asian, African and other countries. Green pepper (piper nigrum L) is an important spices of commerce valued for it's aroma and pungency, green pepper has two major phenolic compounds which are oxidized to black pigment during the drying by pepper polyphenole oxidase, the occurrence of these compounds in green berries being anew natural product not reported earlier\(^{(4)}\).

Green pepper is regarded as powerful antioxidants due to the presence of vitamins A, B6, B9, and C, as they help in neutralizing free radicals in the body that are likely to cause serious damage to the cells during their movements, also it help in providing relief from pain and inflammation, including patients who suffering from rheumatoid arthritis or osteoarthritis, green peppers are a boon for asthma patients since the phytonutrients present help in relaxing the airways and reducing wheezing, also it contain twice the amount of vitamin C than oranges, that make it highly useful for reducing the formation of blood clots, which in turn lower the risks of stroke and heart attack; the amount of homocysteine, a toxic by-product of biochemical processes in the body, can be decreased by consuming vitamins B6 and B9. As such, green pepper is a rich source of these essential nutrients\(^{(5)}\).

Green peppers are loaded with fiber that reduces the degree of exposure of colon cells to bacteria and toxins, also they are a good source of vitamin B6 that assists in several bodily functions, right from metabolism to the healthy functioning of the immune and nervous system, green peppers aid in strengthening teeth and bones, carrying oxygen throughout the body and proper nerve functioning. Apart from these, green peppers also help in maintaining the pH level in the body\(^{(6)}\).

Many of peppers' phytochemicals have antioxidant abilities. This means they can help neutralize free radicals in the body, which damage cells. So they may help prevent or reduce
symptoms of certain diseases; similar to hormones, some phytochemicals also act as messengers in the body, pepper also contain Lutein and zeaxanthin, which may slow the development of eye diseases, such as cataracts or macular degeneration. In addition to Beta-carotene, which may help protect against certain types of cancer, such as breast cancer in women before menopause, Lycopene, which may decrease the risk for ovarian cancer (7).

AIM OF STUDY
This study was used to investigate the effect of green pepper on G+ve and G-ve bacteria with candidia albicanes in addition to investigate therapeutic effect of this plant on hepatic, urinary and cardiac systems integrity of laboratory rabbits.

MATERIALS AND METHOD
3-1-Collection of peppernigrum plant
Plant material leaves of peppernigrum was obtained from the agricultural collage garden from the period 15 December to 15 April after cleaning the leaves from the dust; they were put in oven to dry then crushed to produce powdered material.

3-2-Preparation of the plant extracts
3-2-1-The aqueous extracts
fifty gm from the dry powder of the medicinal plants were put in conical flask size 1000ml, 450ml of distilled water were add, left on hot plate with magnetic stirrer at 25C˚ for two days. Suspension was filtrated by filter paper 0.45mm diameter type Whattman No.2. Extract was dried in oven at 45C˚, then weighted by using sensitive balance, kept in the refrigerator at 4C˚ for usage in another time (8).

3-2-2-The alcoholic extracts
Fifteen gm powder of medicinal plant was dissolved in 200ml methanol 80%, put in thumble in the soxhelt apparatus and left seven hour at 60C˚ the mixture filtrate by Whatman No.1 filter paper, then evaporate using the rotary vacuum evaporator to fluid, dried at oven 45C˚ (9).

3-2-3-Preparation Of Bacterial Suspension
Bacterial suspension obtained from postgraduates diagnosed isolated( E coli, staphylococcus aureus , pseudomonasaeroginasa ,proteusvulgaris ,klebsiella pneumonia, streptococcus pneumonia,vibrio cholera ,candidia albicanas , salmonella typhium ) were
prepared on Muller Hinton Broth and incubated at 37c for 24 hours, then 1micron was taken from each bacterial suspension and diffused on Muller Hinton agar By using L-shape spreader, then 3 equal distant wholes were mode inside the plates for putting different plant extract concentration (50,75, 100)% plate were incubated at 37c for 24 hours; the effect of plant extract on bacteria was calculated by Minimum Bactericidal concentration (MBC)\(^{10} \). 

3-3- Animals
Clinically healthy six month old about1.5 kilofemale white newzealand rabbitswere used in the experiment (rabbits are divided to tow groups control group administrated food and tap water and injected with \(10^3\) proteus vulgaris suspension (as this bacteria consider the more susceptible to this plant extract ) intraperitonially and treatment group T2 administrated food and tap water and injected with \(10^3\) proteus vulgaris suspension intraperitonially to induceurinary tract infection then injected with watery extract ofpiper nigrum Lafter 36 hrs of signspresentation, plant extract was administrated intraperitonially in the form of two dose 500 mg daily for three weeks.

3-4- Sample collection
At 10 weeks whole blood was collected via cardiac puncture from anasthesized (ketamine 50mg/kg-xylazine 10mg/kg) in EDTA tubes.Rabbits, then euthanized with a single cardiac injection fatal plus (concentrated pentobarbital, 360 mg/kg), blood was collected in EDTA tube, liver, cardiac and kidney tissues were collected for histological studies.

3-5- Blood chemistries
Blood urea nitrogen, creatinine, creatinine kinase, uric acid, calcium, Serm bilirubin, Aspartame aminotransferase, Alanine aminotransferase, Alkaline phaspatase, Gama-glutamle transaminase, Trponine, Myoglobin Ck-Mpconcentration in plasma were determined using commercially available kit (sigma)

3-6-Statistical analysis
Mean±SE was used to describe variables. All data are analyzed using ANOVA table and Duncan's multiple range test was used to determine if the means were significantly (P\(\leq\)0.01) different or not\(^{(11)} \).
RESULTS

Result illustrated here indicate that both of crude extracts from the *paper nigrum* L leaves showed anti-bacterial activity against either G+ve bacteria (*staphylococcus aureous*, *streptococcus pneumonia* or G-ve bacteria (*vibrio cholera*, *klebsilla pneumonia*, *proteus vulgaris*, *Escherichia coli* and *salmonella paratyphi*), however the crude extract of *Paper nigrum* L. were more potent antibacterial agent against G+ve bacteria than *candidia* and G-ve bacteria. The finally results related with *proteus vulgaris* appear high sensibly for 50,75,100% concentration off watery extracts and MBC reach about 20,25 and 27ml respectively, while MBC for alcoholic extract present more resistance for *proteus vulgaris* bacteria. Result also illustrate that *psendomonas aeroginosa* appear more sensitive toward both extract for *Paper nigrum* L. and MBC for 50,75,100% concentration reach about 24,25, and 20 respectively while MBC for alcoholic extract reach about 20,22 and 23ml for 50,75, and 100 concentration respectively. Other pathogenic G–ve bacteria like *vibrio* appear sensitive for watery extract of *paper nigrum* L leaves extract and MBC for 50,75,100% concentration reach about 10,15,16ml concentration 17,19,20 ml for 50,75 and 100% concentration respectively. *Candida* sensitivity was weak toward watery extract of *paper nigrum* L and MBC reach about 10,10,18 ml for 50,75 and 100% concentration respectively, while MBC for alcoholic concentration were 13,15,21 ml for 50,75 and 100% concentration respectively. *Salmonella* resistant was intermediate toward watery leave extracts for *paper nigrum* L and MBC for 50,75 and 100% concentration of watery leave extract reach 10,16 and 18 ml respectively, while for alcoholic extract MBC about 12,15 and 19 ml for 50,75 and 100% respectively. *Staphylococcus aureus* sensitivity results were more potent than *vibrio cholera* and *salmonella typhimurim*, *vibiro cholera* and *condidia*, and MBC reach about 20,25 and 10 ml for 50,75 and 100% concentration respectively, while there was highly resistant toward all alcoholic leave extract concentration. *E coli* resistant sensitively was intermediate toward watery leaves extracts of *paper nigrum* L and MBC reach about 15,14,10 ml for 50,75 and 100% concentration while there was clear resistance toward all concentration of *paper nigrum* L leaves alcoholic extracts. *klepsella pneumonia* resistance were high toward watery leaves extracts of *paper nigrum* L and MBC reach about 10,10,18 ml for 50,75 and 100% concentration also clear resistance present toward all alcoholic leaves extract for *paper nigrum* L. *Streptococcus pneumonia* appear intermediate resistance toward watery extract for *paper nigrum* L leaves and MBC was about 16,17 and 19 ml for 50,75 and 100% concentration while this bacteria was resistant to all alcoholic leave extract concentration as explained in figure bellow:
According to results the larger MBC was for *proteus vulgaris* toward watery extract of *paper nigrum* L. and this bacteria was selected to inject intraperitonially then treated with watery leaves extract for paper nigrum L, after 48 hrs of urinary signs presentation.

Results invivo indicate presence of significant variation (p≤0.01) in the level of total serum bilirurin GPT,GOT, Alkaline phosphatase and GGT of hepatic profile indicators for G2 animal group than G1 animal group while results correlated with renal profile tests illustrates presence of significant variation (p≤ 0.01) in the level of creatinin, creatinine kinase and potassium while blood urea nitrogen and uric acid have some improvement but don’t reach significant variation (p≥ 0.01). cardiac profile tests represent no significant variation between both groups as explained bellow in table 1:

**Table (1) represent the effect of paper nigrum L. watery extract on**

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>G1 animal group</th>
<th>G2 animal group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver function tests</strong></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>bilirurbin</td>
<td>0.339±0.016 a</td>
<td>0.756 ±0.01 b</td>
</tr>
<tr>
<td>GGGOT</td>
<td>1.428±22.72 a</td>
<td>1.125±39.07 b</td>
</tr>
<tr>
<td>SGPT</td>
<td>0.552±32.03 a</td>
<td>1.025±64.62 b</td>
</tr>
<tr>
<td>Alkaline phosphatan</td>
<td>0.755±37.08 a</td>
<td>1.184±69.55 b</td>
</tr>
<tr>
<td>GGT</td>
<td>0.468±7.359 a</td>
<td>0.3097±13.64 b</td>
</tr>
<tr>
<td><strong>Renal profile test</strong></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>B.U.N</td>
<td>42.1.5528 a</td>
<td>44.64.6.993 a</td>
</tr>
<tr>
<td>creatinin</td>
<td>0.7235 ± 0.028 a</td>
<td>1.196 ± 0.036 b</td>
</tr>
<tr>
<td></td>
<td>Creatinine kinase</td>
<td>Uric acid</td>
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<td>-------------------------</td>
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</tr>
<tr>
<td></td>
<td>131.8 ± 1.685 a</td>
<td>2.29 ± 0.092 a</td>
</tr>
<tr>
<td></td>
<td>409.6 ± 2.3 b</td>
<td>2.28 ± 0.115 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cardiac profile test</th>
<th>percentage</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin I</td>
<td>0% positive</td>
<td>0% positive</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>0% positive</td>
<td>0% positive</td>
</tr>
<tr>
<td>Tropomycin</td>
<td>0% positive</td>
<td>0% positive</td>
</tr>
<tr>
<td>CK-MP</td>
<td>0% positive</td>
<td>0% positive</td>
</tr>
</tbody>
</table>

Result are expressed as mean ± standard error.

* a : represent no significant variation

Different letters between groups refer to significant variation under propability (p ≤ 0.01)

Degree of freedom 1.9.

These results are also supported by histopathological examination. Histopathological results related with G1 animal group reveal hypertrophy and increase the thickness of proximal and distal convoluted tubules with blood accumulation in their lumen. Presence of cystic dilatation in the cortex with inflammatory cells, decrease the lumen of proximal, distal convoluted tubules and henli loop, ascending and descending branches also are the same. Renal corpuscle increase more than normal size, some of them are distracted. Disappearance of limited line between proximal and distal convoluted tubules also present. Medullary region characterized by presence of hemorrhage between the collecting ducts and presence of RBC in their lumen as explained in figures bellow

![Fig1:A](image_url)
Photomicrographs of haematoxylin and eosin stained sections of rabbit kidney; (A, B, C and D) A: hypertrophy and increase the thickness of proximal and distal convoluted tubules with blood accumulation in their lumen. B, C: Renal corpuscle increase more than normal size, Disappearance of limited line between cells of renal tubules. D: Medullary region include presence of hemorrhage between the collecting ducts with RBC in their lumen. (A, B, C, D: H&E, 100×).
Results related with G2 animal group illustrate that moderate regeneration for cells of collecting ducts, proximal and distal convoluted tubules, disappearance of congestion in the interstitial spaces for cortex of kidney as explained in figures below.

Photomicrographs of haematoxylin and eosin stained sections of rabbit kidney; (A&B) presence of reveal moderate regeneration for cells of collecting ducts, proximal and distal convoluted tubules, disappearance of congestion in the interstitial spaces for cortex of kidney. (A, B: H&E, 100×).

Histopathological alteration for G2 animal group hepatic tissues referred to presence of hemorrhage in liver parenchyma with presence of cystic dilatation, accumulation of
inflammatory cells around the portal area, congestion of blood vessels as explained in figures bellow:

**Fig3: A**

**Fig3: B**

**Fig3: C**

Photomicrographs of haematoxylin and eosin stained sections of rabbit liver; (A, B and C) A, B: presence of hemorrhage in liver paranchyma with presence of cystic dilatation, C: accumulation of inflammatory cells around the portal area (A, B: H&E, 10×).
Histopathological section of G2 animal group reveal nearly presence of normal hepatocyte with moderate congestion, neither red blood cells nor cystic dilation are present as explained bellow:

![Histopathological section of G2 animal group](image)

**Fig4:** (A) Photomicrographs of haematoxylin and eosin stained sections of rabbit liver; presence reveal moderate regeneration with moderate congestion and dilation of central vein less degenerative signs in hepatocytes also present (H&E:40×).

Results related with cardiac tissue for both animal groups indicate presence of normal appearance of cardiac muscle tissue, clear pathological lesions did not be recorded as explained in figures bellow:

![Histopathological section of cardiac tissue](image)
DISCUSSION

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (12). Antimicrobial activity of herbs has been known and described for several centuries (13). Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi (14,15,16). Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (17). *Capsicum annuum* L. belonging to family, Solanaceae, is commonly consumed all over the world as spices it has been known to possess medicinal properties like anti-inflammatory, analgesic, stomachic. The plant is native to America and cultivated in different parts of tropical and subtropical parts of world. *C. annuum* L. owes its medicinal properties to capsaicinoids primarily capsaicin present in highest proportion in placental tissue connecting seeds to fruits, in the fruit itself but not avoidable in seeds (18).
Our results refer to presence of broad spectrum antimicrobial effect of watery and alcoholic green pepper extracts, Bioautographic tests demonstrated that capsaicin was the main antimicrobial component. At least two other non-polar components of green pepper extract also contributed in the antimicrobial activity also pepper is known to be antimicrobial agent due to presence of phenolic compound against antimicrobial pathogen the compound 3,4-dihydroxyphenyl ethanol glucoside (A), and 3,4-dihydroxy-6-(N-ethylamino) benzamide (B). The compound A is more effective than compound B, and this may be due to unstable nature for compound B. (19)

Our results also refer to significant variation in the level of liver function tests for G2 animals group than G1 animals as present in the level of ALT and AST for G1 group as compared with G2 groups, these enzymes which involved in intermediary metabolism and stored in hepatocytes are released when hepatocytes are actually damaged, increased in serum concentration of these enzymes provide important clues to involvement of hepatocytes, ALT and AST are sensitive test of hepatocytes injury although often referred to as liver function test they don't measure hepatocytes but instead hepatocytes damage decrease values of these enzymes for G2 may be due to antioxidant activity for green pepper antioxidant in reduction of oxidative damage due to Capsanthin which suppressed hydroperoxide formation as well as beta carotene, lutein, and zeaxanthin, Intereststringly, capsanthin decomposed more slowly than the other carotenoids and the radical scavenging effect of capsanthin was found to last longer. Also, the capsanthin esterified partially and/or totally with fatty acids (mono-and/or diesterified capsanthin), isolated from paprika color, suppressed oxidation of methyl linoleate in a similar manner as non esterified capsanthin. This finding suggests that the radical scavenging ability of capsanthin was not influenced by esterification, that is, the ability would contribute to the polyene chain, especially conjugated keto group. It was first found that esterified (monoenesterified and diesterified) capsanthins also were good radical scavengers. (21). Results also refer to significant increment in alkaline phosphatase for G1 group as compared with antioxidant groups G2, alkaline phosphatase is related with liver dysfunction this may be due oxidative damage, antioxidant activity for green pepper play important role in improvement of liver integrity (22).

Our results also refer to significant variation in the level of renal profile tests for G1 animals group than G2 animals as present in the levels of urea, creatinine, uric acid, creatinine kinase and potassium. *Proteus volguris* responsible for Urease production which leads to
precipitation of organic and inorganic compounds, which leads to struvite stone formation. Struvite stones are composed of a combination of magnesium ammonium phosphate (struvite) and calcium carbonate-apatite. Struvite stone formation can be sustained only when ammonia production is increased and the urine pH is elevated to decrease the solubility of phosphate. Both of these requirements can occur only when urine is infected with a urease-producing organism such as Proteus. Urease metabolizes urea into ammonia and carbon dioxide: Urea $\rightarrow$ $2 \text{NH}_3 + \text{CO}_2$. The ammonia/ammonium buffer pair has a pK of 9.0, resulting in the combination of highly alkaline urine rich in ammonia$^{(23)}$. Green pepper have antimicrobial phenolic compound that responsible for significant improvement for G2 animal group.

CONCLUSION
Our results indicate that watery and alcoholic extract of pepper nigrum L have antibacterial activity against pathogenic factors, the watery extract of pepper nigrum L was more affective. Proteus vulgaris cause morphological and histopathological degenerative lesion for hepatic, renal but no cardiac tissues. watery extract of papper nigrum L. papper nigrum L have protective effect against Proteus vulgaris pathogen.

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