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Research article

Angelica archangelica extract ameliorates the oxidative stress induced by *Pseudomonas aeruginosa* in rats

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Keywords: *Angelica archangelica*, *Pseudomonas aeruginosa*, Antibacterial, Antioxidant.

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Abstract

This study investigated the influence of *Angelica archangelica* roots aqueous extract on *Pseudomonas aeruginosa* bacteria. The antimicrobial activity of different concentrations of *Angelica* was assessed in vitro on two strains of yeast and two strains of fungi. *P. aeruginosa* antibiotics resistance was confirmed against three antibiotics. Results demonstrated that *Angelica* had very high activity on the growth of *P. aeruginosa.* To confirm this activity in vivo, four groups of male wistar albino rats were used. Control group received water only, infection group subjected to bacterial infection for two weeks, treatment group subjected to bacterial infection for two weeks followed by oral administration of *Angelica archangelica* roots extract for two other weeks and protection group subjected to bacterial infection with oral administration of *Angelica archangelica* extract for two weeks. At the end of experiment, histopathological examination of kidney and liver specimens was carried out; also blood samples were collected to measure biochemical and oxidative stress parameters. Amelioration of the histological images of both the liver and kidney were observed in both group 3 &4. Biochemical results showed significant enhancement in all parameters measured in group 3 & 4 as compared to group 2. Results were more abundant in group 4 than group 3. In conclusion, *Angelica archangelica* roots aqueous extract has the potential to remove the oxidative stress caused by *P. aeruginosa* which resulted in enhancement of histopathological and biochemical results.

Introduction

Pseudomonas aeruginosa; gram negative bacteria attributed to the genus pseudomonas is a widespread microorganism. *P. aeruginosa* has toxic and pathogenic effects and is responsible for several nosocomial and community acquired infectious diseases [1]. This is related to the high mortality rate in infected patients [2]. Pseudomonas species has the ability to transmit form one organ to another. If it persists in the intestine, it can move to the liver, spleen and mesenteric lymph nodes in mice treated with streptomycin antibiotic [3].

The increase in usage of multi antibiotics in fighting the activities of many microorganisms has led to the emergence of multi drug resistant microorganisms [4]. This antibiotics resistance leads to toxicity and consequently decline in the use of these drugs. *P. aeruginosa* has the ability to live in different environments and is resistant to most antibiotics due to its capability to form biofilms [5]. Although vaccination with microbial antigens could be an effective method for protective host responses in most infectious diseases, in this organism vaccination is unsuccessful due to the immune deficiency [6].

In traditional medicine, there was a trend to use plants in the treatment of burns and infectious diseases. This field of medicinal plants treatment returned again to kill bacteria which are resistant to most antibiotics [7].

Phototherapy research; a new field that attracted investigators interest in recent years to use medicinal plants extracts to fight these multi resistant microorganisms [8]. That is considered as a promising source of drugs and a new approach of treatment [9]. Hence, a number of natural products have been chosen and approved as antimicrobial drugs. As more pathogens have resistance against most antibiotics, more research is needed to find highly effective natural products to kill these pathogens [10].

Angelica is widespread and well known plants that were used in medical treatment for centuries. It is a nonchemical antioxidant. *Angelica archangelica* [Family: Umbelliferae (Apiaceae)] is an exceedingly planted Angelica grass that has been applied in traditional medicine as a medication for assorted disorders like: headaches, backaches, asthma, skin diseases and digestive troubles. Archangelica comes from the Greek word 'arkhangelos' (=arch-angel), due to the myth that it was the angel Gabriel who told of its use as a medicine [11]. Due to its chemical constituents including essential oils, organic acids, steroids, coumarines and flavonoids, diverse pharmacological actions of the plant root partition have been studied. Angelica archangelica root was reported to have analgesic, antiseptic, anti-inflammatory, antifungal, antispasmodic, and anti-cancer cell proliferative properties as well as calcium channel blocking and calcium binding activity. Angelica archangelica root contains several essential oils such as; pynene, limonene and coumarin that some scientists proved that the previous component may accelerate antioxidant effects. [12].

Catechins cause damage of the cell membrane of the bacteria, inhibition of the synthesis of fatty acids and inhibition of enzyme activity. Also, they have anti-inflammatory effect (specially the inflammation caused by oxidative stress) [13]. Bacterial cell membrane damage inhibits the ability of the bacteria to bind to host cells [14] and restrains the bacterial capability to bind to each other to biofilms formations which are strong effect in pathogenesis. Also, bacterial membrane injury results in the bacteria inability to secrete toxins [15].

The goal of this research was to examine the effect of *Angelica archangelica* roots water extract on the oxidative stress caused by oral bacterial infection of rats (protective and treatment effects). The bacteria used in this study, *P. aeruginosa*, were obtained from the microbiology lab, National Research Center, Dokki, Egypt.

Material and methods

Plant extraction preparation

The roots of *Angelica archangelica* (whole root) were provided by Mr. Haraz (Famous oldest Egyptian herbs and folk medicine manufactures and supplies, Cairo, Egypt). Air dried *Angelica archangelica* roots were crushed. Boiled distilled water was added to roots. After cooling, juice was filtered three times. The obtained solution was used in a base of body weight as 5 ml for each kg body [16]. In this study water extract was used because it is easy to prepare and contains all components required in the roots of *Angelica archangelica*.

In vitro antimicrobial activities

Four kinds of pure strains of bacteria, *Bacillus subtilis*-NRRL B-941(G+ve), *Staphylococcus aureus* NRRL B-767 (G+ve), *Pseudomonas aeruginosa* NRRL B-23 (G-ve), *Escherichia coli*-NRR-B 210(G-ve), two strains of yeast *Saccharomyces cerevisiae* Y-2034 and *Candida albicans* NRRL Y-477, and two strains of fungi *Aspergillus niger* NRRL -3 and *Fusarium oxysporum* NRRL 26406 were plated out in nutrient agar medium for bacteria and yeast, malt extract agar medium for fungi and were employed as described by Harrigan [17].

The antimicrobial activity was determined according to Barry [18]. For bacteria and yeast, the liquid media were used for 18 to 24 h (Harvested at the logarithmic phase of growth, at absorbance 600 nm). For fungi, the liquid media were used for approximately 72 h and the spore density of each fungus was adjusted at 595nm.

The inhibition zone method was prepared and adapted using filter paper discs of 0.5 cm diameter. The solid media were sterilized and divided at 50ºC into 30 ml portion in sterile petri-dishes of 15cm diameter. For the inoculums of the tested organism, 1.0 ml of cells ($2x10^8$ CFU/ml) for bacteria and yeast, and for fungi 10^5 spores/cells were added to the surface of the medium. Aliquots of different concentrations of *Angelica* extract (20, 40, 60, 80, 100 and 120 g/l) were placed on filter paper disks. Disks were placed carefully on the surface of the inoculated solid medium. The petri-dishes were placed in the refrigerator for 2 h to permit good diffusion and then transferred to an incubator at 37ºC for 24 h for the bacteria and at 28ºC for 72 h for fungi and yeast growth. The plates were then examined and the inhibition zones produced by different concentration of *Angelica* were determined. Rimactane as antibiotic (200 µg/disc) and Flucoral as antifungal (200 µg/disc) were used as antibiotic reference.

Pseudomonas aeruginosa Antibiotic resistance assessment

According to Barry [18] as mentioned above, different antibiotics such as Rimactane**,** gentamicin, piperacillin and ZOSYN (piperacillin and tazobactam) (200 µg/disc) were used for the detection of *Pseudomonas aeruginosa* resistance.

The pathogenesis of pseudomonal infections is multifactorial and complex. *Pseudomonas* species are both invasive and toxigenic. The 3 stages are (1) bacterial attachment and colonization, (2) local infection, and (3)

bloodstream dissemination and systemic disease. The importance of colonization and adherence is most evident when studied in the context of respiratory tract infection in patients with cystic fibrosis and in those that complicate mechanical ventilation. Common symptoms of the infections include: skin: Rashes, which may consist of pus-filled pimples, eyes: Pain and redness, Soft tissue: Discharge of green pus and a sweet, fruity smell and other possible symptoms: diarrhea, or urinary tract infection.

In-vivo assay

Forty eight male Albino rats (10-12 weeks old) weighing about 120 to 150 gm were obtained from animal house, National Research Centre, Giza, Egypt). They were kept under standard laboratory conditions with *ad-libitum* access to food and water in well-ventilated cages made of galvanized zinc plates and ate bread and drunk water. Before experiment, the rats divided into four cages under stable temperature, suitable light and good feeding for seven days before experiment, after that body weight determined and observe the health status and psychological behavior of the rats to begin the experiment. Experimental animal design was previously approved by the National Research Center ethical committee.

(Male rats tolerate the infection pain in the stomach than females because male rats aren't as hormonal as females—because isn't that what they always say—and are therefore more reliable in terms of getting data and they don't have estrous cycles that can complicate pharmacology; and in many fields, there is a significantly larger body of literature and data sets on).

The animals were divided into four groups of twelve each as follows;

Group 1 (G1): Control group; with daily water administration only.

Group 2 (G2): Infection group; subjected to bacterial infection alone for two weeks in a dose of 1.3X107 CFU/ml/animal (One ml of bacterial suspension/day).

Group 3 (G3): Treatment group; subjected to bacterial infection for two weeks as above followed by oral administration of *Angelica archangelica* roots water extract (One ml of plant water extract/day) for two weeks.

Group 4 (G4): Protection group; subjected to bacterial infection with concurrent oral administration of *Angelica archangelica* roots water extract for two weeks.

At the end of experiment, blood samples were collected from the retro-orbital venous plexus of all animals into heparinized tubes. The plasma obtained after centrifugation (3000 rpm for 10 min at 4°C) was used for malondialdehyde (MDA) and catalase determination. Erythrocytes were washed three times in phosphate buffered saline (PBS) solution. Lysed erythrocytes were prepared by addition of four volumes of ice-cold distilled water. Cell membranes were removed by centrifugation at 8500 rpm for 20 min, and the supernatant was used for the assay of GSH concentration and antioxidant enzymes activities. According to the antioxidant assays, appropriate phosphate buffers of pH 7 for glutathione peroxidase and pH 8.5 for SOD were added to the hemolysate samples; therefore, the antioxidant enzymes do not lose their activities.

Histopathological examination of kidney and liver tissues

Kidney and liver specimens of all animals were dissected immediately after death and fixed in 10% neutral-buffered formal saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol (70% - 80% - 90% and finally absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55°C and embedded in hard paraffin. Serial sections of 6 µm thick were cut and stained with Hematoxylin and eosin [19] for histopathological investigation. Ten micrometer sections cut from paraffin tissues stained with hematoxylin-eosin (H&E) and the investigation of the tissues was done by light microscope. Images were captured and processed using Adobe Photoshop version 8.0.

Biochemical assays

For biochemical analysis specially manufactured kits were used.

Reduced Glutathione (GSH) concentration

GSH concentration was determined spectrophoto metrically according to the method of Beutler [20]. The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Plasma Malondialdehyde (MDA) concentration

MDA concentration was determined spectrophoto metrically by the method of Satoh [21]. Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resulting pink product can be measured at 534 nm.

Superoxide dismutase (SOD) activity in cell lysate

SOD activity in cell lysate was determined spectrophotometrically by the method of Nishikimi *et al.* [22]. The principle of this assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro-blue tetrazolium dye. The percent inhibition directly proportional to SOD activity was calculated.

Erythrocyte glutathione reductase (GSH-R) activity

GSH-R activity was determined by the method of Goldberg and Spooner [23].

Catalase determination

Catalase enzyme reacts with a known quantity of H_2O_2 [24]. The reaction is stopped after exactly one minute with catalase inhibitor.

$$2H_2O_2 + \text{Catalase} \longrightarrow 2H_2O + O_2.$$

Glutathione Peroxidase determination

In the presence of peroxidase (HPR), any remaining H_2O_2 reacts with 3,5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample [25].

$$2H_2O_2 + \text{DHBS} + \text{AAP} \xrightarrow{\text{HPR}} \text{Quinoneimine Dye} + 4H_2O.$$

GST Activity

GST activity was determined spectrophotometrically by method of Habig et al. [26]. The biodiagnostic GST assay kit measures total GST activity (Cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinirobenzene (CDNB) with reduced glutathione (GSH). The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.

Liver Enzymes

Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined by the method of Reitman and Frankel [27].

Creatinine

Creatinine in alkaline solution reacts with picric acid to form a colored complex [28]. The amount of the complex formed is directly proportional to the creatinine measured at 492 nm.

Statistical Analysis

Data were presented as the mean + Standard error (SE) values. One way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Post Hoc and the least significant difference (LSD) tests using a statistical package for social science (SPSS version 20). $P < 0.001$ was considered as statistically significant.

Result

Antimicrobial activities

The antimicrobial activities are shown in Tables (1 & 2). The results demonstrate that *Angelica* possessed antimicrobial activities against the tested microorganisms except *A. Niger and F. oxysporum. Angelica* had very high activity on the growth of *P. aeruginosa, E. coli, B. subtilis, Staph. aureus, S. cerevisiae* respectively. Similarly, the effect of commonly used Rimactane as antibiotic and Flucoral as antifungal was tested against these microorganisms for comparison with *Angelica*. The highest antimicrobial activity 11.7 mm was observed in *P. aeruginosa* and with increasing the concentration of *Angelica* the zone of inhibition increased till reached 24.8 mm.

Therefore, the activities were depending on *Angelica* concentrations. Also *P. aeruginosa* was highly affected by *Angelica* as an antimicrobial agent when compared with the antibiotic Rimactane.

Antibiotic resistance

Pseudomonas aeruginosa is a notoriously difficult organism to control with antibiotics as seen in Table 3. The zone of inhibition varied among them. However, they all showed weak inhibition when compared with *Angelica* (24.8 mm).

Table 1. Inhibition zones (mm) of microbial growth (bacterial species) by different concentrations of Angelica.

Concentrations of Angelica (g/l)	Inhibition zone (mm)			
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***
20	0.6±0.22	2.8±0.23	11.7±0.44	2.3±0.66
40	1.2±0.44	4.4±0.15	15.5±0.31	3.6±0.45
60	1.4±0.25	5.5±0.37	18.6±0.44	6.2±0.23
80	1.8±0.43	7.7±0.66	20.3±0.72	6.4±0.86
100	2.3±0.27	9.8±0.34	24.8±0.35	7.2±0.33
120	2.1±0.43	9.8±0.03	24.8±0.33	7.4±0.64
Rimactane (200 µg/disk)	16.3±0.54	18.3±0.33	2.6±0.21	23.6±0.11

Table 2. Inhibition zones (mm) of microbial growth (yeast and fungal species) by different concentrations of *Angelica*.

Concentra-tions of Angelica (g/l)	Inhibition zone (mm)			
	Saccharomyces cerevisiae	*Candida albicans*	*Aspergillus niger*	*Fusarium oxysporum*
20	1.2±0.46	1.2±0.43	0	0
40	1.5±0.83	3.3±0.13	0	0
60	2.6±0.47	6.5±0.22	0	0
80	3.9±0.13	7.6±0.22	0	0
100	6.2±0.66	9.8±0.14	0	0
120	6.5±0.73	9.8±0.03	0	0
Flucoral (200 µg/disk)	25.2±0.33	27.2±0.52	24.6±0.11	21.6±0.41

Table 3. Antibiotic resistance.

Antibiotic (200 µg/disk)	Inhibition zone(mm)
Rimactane	2.6±0.21
Gentamicin	6.5±0.83
Piperacillin	7.2±0.46
ZOSYN (piperacillin and tazobactam)	8.6±0.47

In vivo results

During the experiment, several external signs were observed which include animal appetite, weight, morbidity and mortality and other features.

The animals in all groups showed no decline in their rate of feeding i.e, there was not any significant change in their appetite. Moreover, no diarrhea was observed in the different groups. On the other hand, variation in the daily weight of different groups as compared with the control group ($P < 0.05$) was observed. In group 2 (G2), infected rats with *P. aeruginosa*, there was significant loss in their weight during the period of oral administration of bacteria ($P < 0.01$) compared with healthy control group (G1), but in protected group (G3) and treated group (G4), there were only slight difference in their weight compared to healthy control group (G1).

In this study, the effect of *P. aeruginosa* gastrointestinal infection as well as the protective and therapeutic influences of *Angelica archangelica* were assessed using histopathological and biochemical investigations.

Histopathological alterations in different groups

Kidney and liver tissues from all groups were histopathologically examined to show the different changes in tissues among infected, protected and treated groups as compared with normal tissues.

Examination of kidney tissues revealed normal structure in healthy control group (G1) as shown in Figure 1. Whereas interstitial hemorrhage and vascular degeneration of epithelial lining cells of many tubules were observed in infected group (G2) Figure 2. In the treated group (G3) Figure 3, the kidney tissues showed the decrease in interstitial hemorrhage and slight vascular degeneration. The interstitial hemorrhage was still observed in the protection group to a greater degree than in the treated group (G4) Figure 4.

Liver tissues of healthy control group (Figure 5) appeared normal and there was no distorted tissue observed, but the liver tissue of infected group (Figure 6) showed massive dilation of blood vessels with fibrosis, cellular infiltration and the normal architecture of the tissue was markedly distorted. The liver tissues of treated group (Figure 7) with *Angelica archangelica* extract (daily dose for 14 days) showed normalized tissue structure except slight dilation of blood sinusoid. The protection group liver (Fig 8) showed mild cellular infiltration and very slight fibrous tissue.

Biochemical investigations

Effect of *P. aeruginosa* oral administration on oxidative stress markers, liver enzymes and kidney functions in experimental rats was investigated (Table 4). The protective and therapeutic influences of *Angelica archangelica* on the same parameters were evaluated.

The results describe the effect of ingested *P.aeurginosa* on the biochemical parameters and shows, in infected groups, increase in MDA and creatinine levels in addition to increase in glutathione peroxidase, ALT and AST activities. On the other hand, there is a decrease in reduced glutathione and glutathione reductase, glutathione S-transferase and catalase activities with highly significant values compared with healthy control groups.

In treated and protected groups, there were significant enhancement in the results ($P<0.001$) compared to infected group but the removal of oxidative stress caused by *P. aeruginosa* in protected group is greater than that in the treated group.

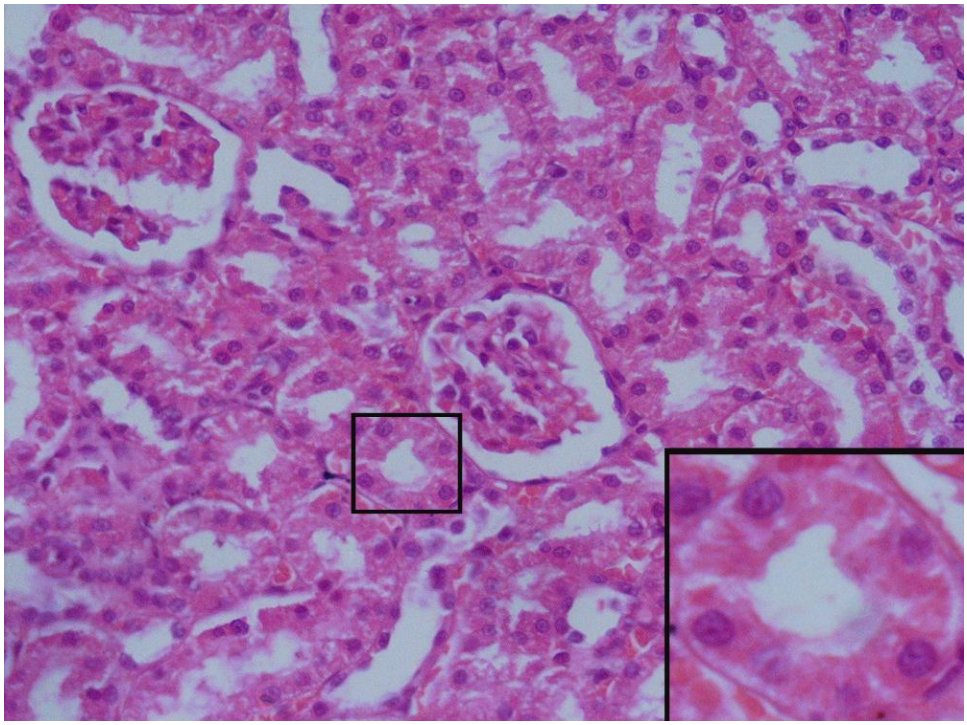


Figure 1. A photomicrograph of a section of normal kidney tissue shows the normal structure of this tissue being formed of glomeruli embedded in between tubules. The right lower corner shows - at a higher magnification - the epithelial cells lining the tubules (Hx & E X 200, 500).

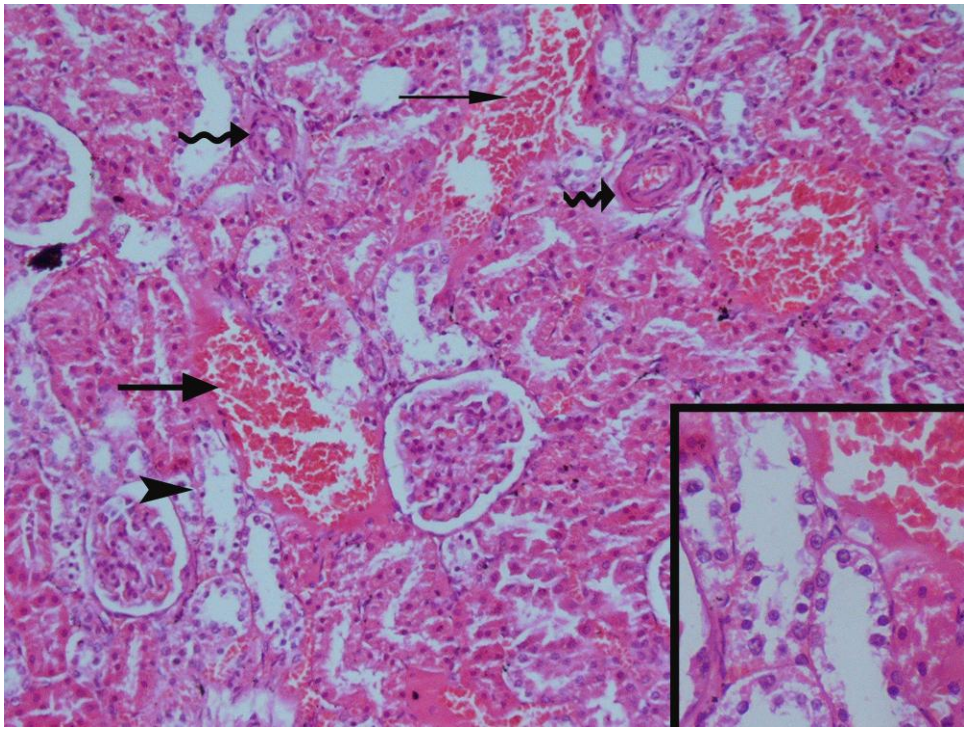


Figure 2. A photomicrograph of a section of infected kidney tissue shows marked interstitial hemorrhage (arrows), thickening of the blood vessels' walls (wavy arrow) and vacuolar degeneration of epithelial lining cells of many tubules. The right lower corner of the figure shows – at a higher magnification – vacuolar degeneration in tubular cells (Hx & E X 100, 200).

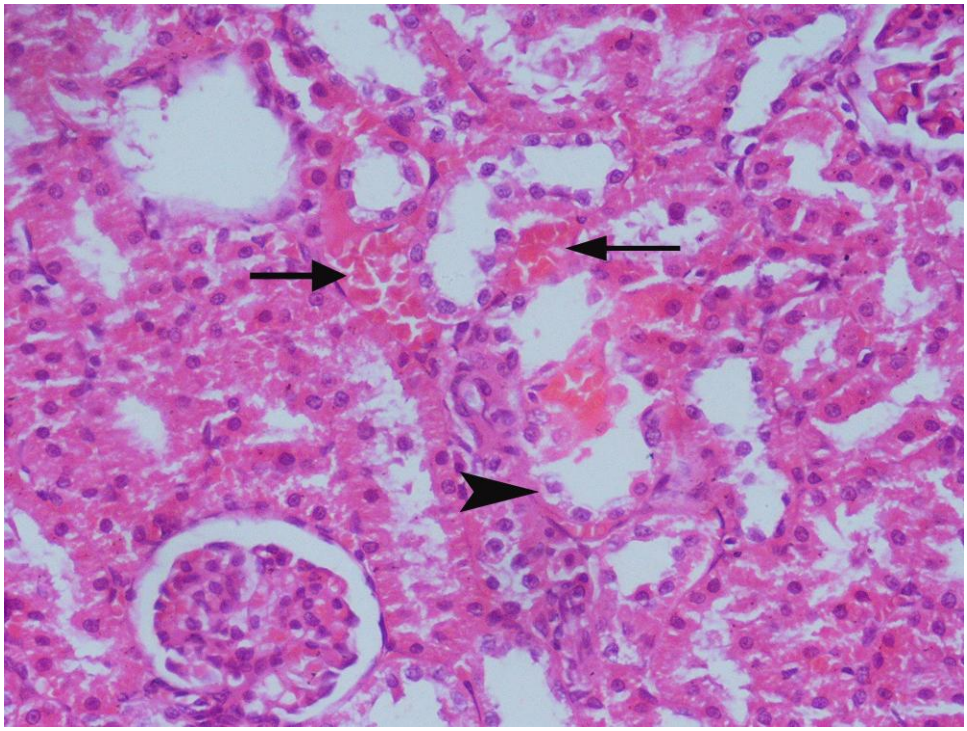


Figure 3. A photomicrograph of a section of treated rat's kidney tissue shows marked decrease in interstitial hemorrhage (arrow). Most of the tubular lining epithelial cells appear normal except for a few cells that still show vacuolar degeneration (arrowhead) (Hx & E X 200).

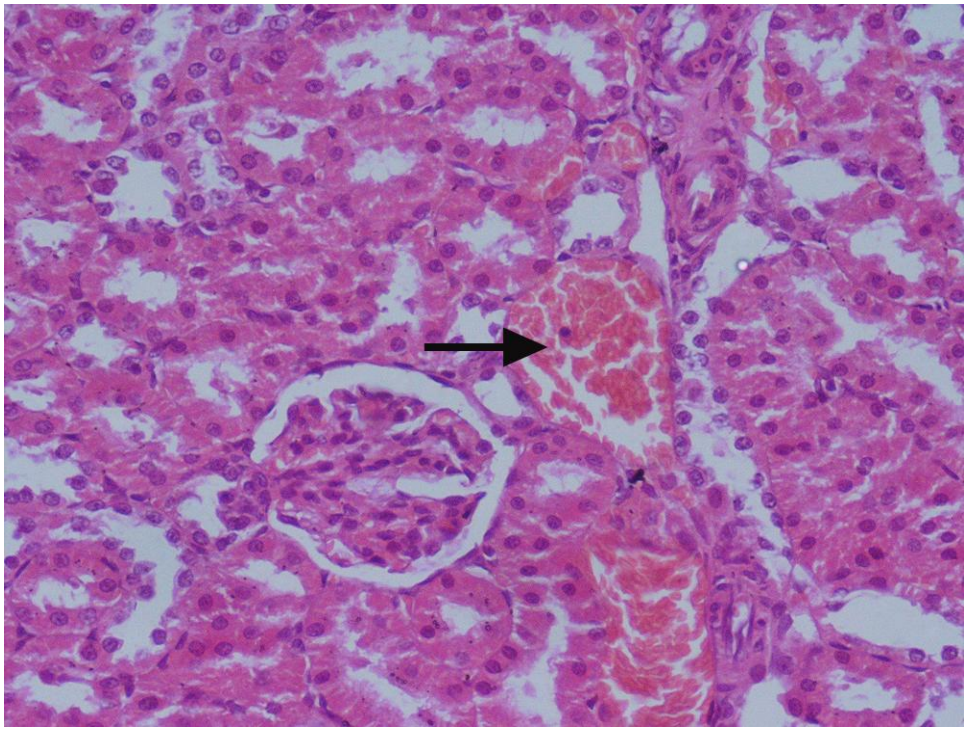


Figure 4. A photomicrograph of a section of protected kidney tissue shows normalization of tubular lining cells, while the interstitial hemorrhage is still observed in a greater degree than in the treated group (arrow) (Hx & E X 200).

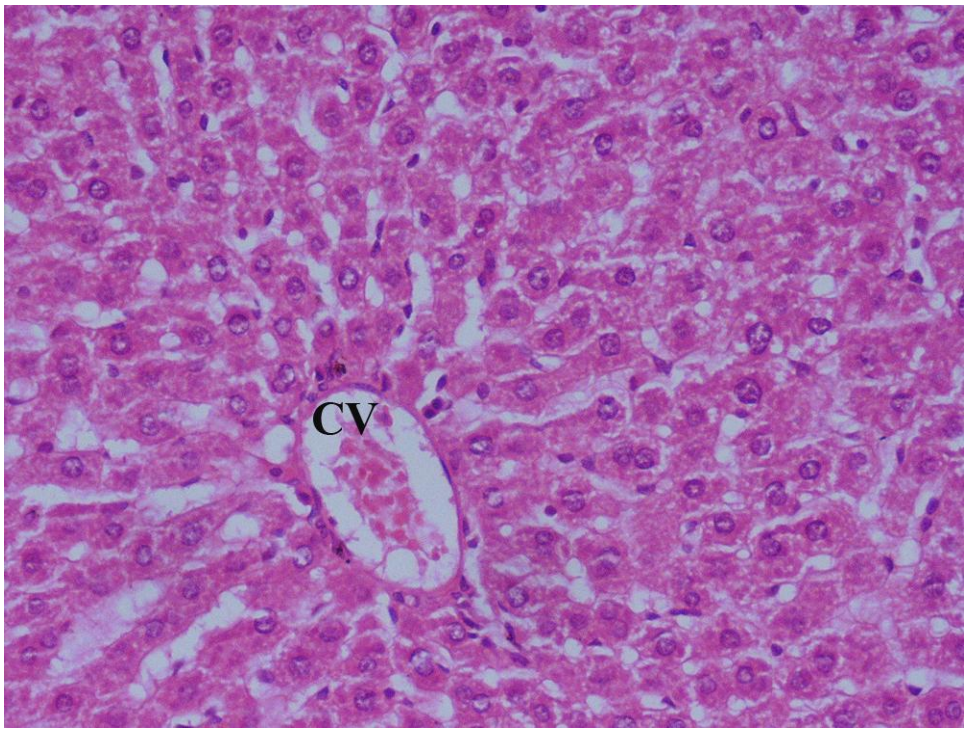


Figure 5. A photomicrograph of a section of normal liver tissue shows the central vein (CV) and the hepatocytes arranged in cords radiating from the central vein (Hx & E X 200).

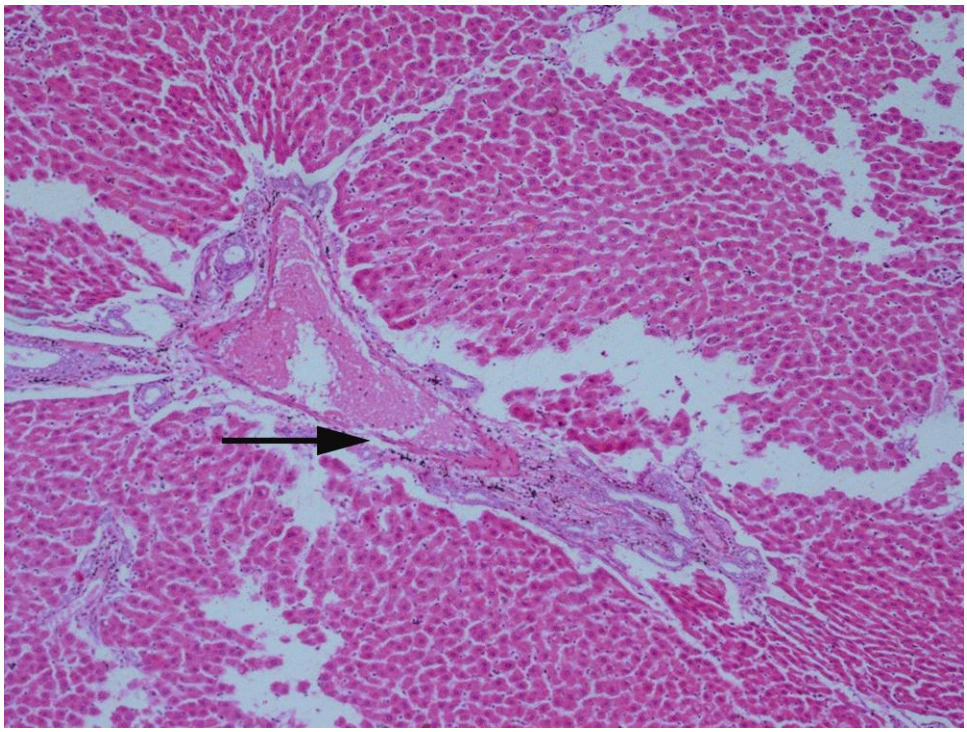


Figure 6. A photomicrograph of a section of infected liver tissue shows massive dilatation of blood vessels with fibrosis and cellular infiltration around (arrow). The normal architecture of the tissue is markedly distorted (Hx & E X 100).

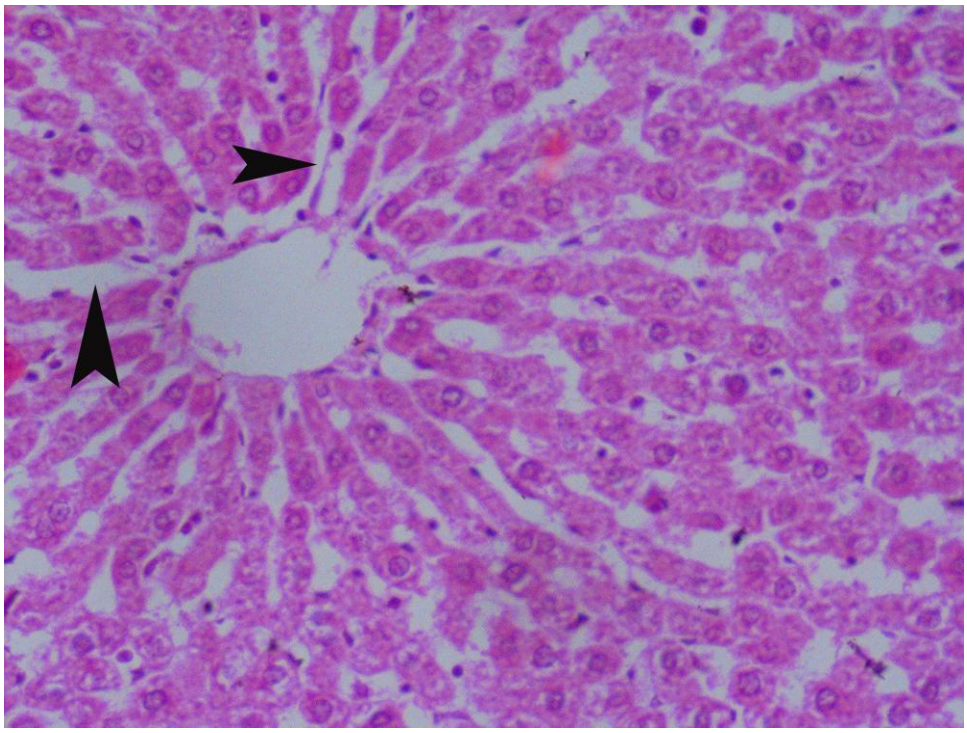


Figure 7. A photomicrograph of a section of treated rat's liver tissue shows normalization of the tissue structure except for slight dilatation of blood sinusoids (arrowhead) (Hx & E X 400).

Table 4. Shows the oxidative stress markers, liver enzymes and kidney function in different groups (G1, G2, G3 and G4).

Parameter	Control Group 1 (n=12)	Bacterial infection Group 2 (n=12)	Treated group Group 3 (n=12)	Protective group Group 4 (n=12)
MDA (nmol/ml)	3.2±0.391	10.2±0.695 P < 0.0001	3.82±0.603 P < 0.0001	4.594±0.449 P < 0.0001
SOD (U/gHb)	1440.45±76.119	6176.627±357.039 P < 0.0001	1962.633±301.837 P < 0.0001	2291.406±233.254 P < 0.0001
GSH (mg/dl)	4.652±0.496	1.349±0.252 P < 0.0001	3.788±0.299 P < 0.0001	3.236±0.264 P < 0.0001
GPx (mU/ml)	77.358±5.239	153.382±7.757 P < 0.0001	86.524±5.427 P < 0.0001	95.5±8.532 P < 0.0001
GR (U/L)	12.675±0.652	4.91±0.566 P < 0.0001	11.245±0.459 P < 0.0001	10.225±0.313 P < 0.0001
GST (U/L)	23.35±1.211	7.845±0.783 P < 0.0001	21.083±0.715 P < 0.0001	19.803±0.755 P < 0.0001
Catalase (U/L)	66.434±2.34	22.679±1.694 P < 0.0001	60.994±2.388 P < 0.0001	56.315±2.292 P < 0.0001
ALT (U/ml)	26.07±2.344	47.31±2.397 P < 0.0001	29.558±2.291 P < 0.0001	33.341±2.393 P < 0.0001
AST (U/ml)	34.716±2.51	66.4±2.358 P < 0.0001	37.6±1.645 P < 0.0001	42.241±1.538 P < 0.0001
Creatinine (mg/dl)	0.845±0.064	2.261±0.239 P < 0.0001	1.055±0.063 P < 0.0001	1.33±0.102 P < 0.0001

Discussion

P. aeruginosa naturally lives in the environment, so it is associated with human infection [29, 30]. *P. aeruginosa* is gram-negative bacteremia and is considered as the fourth cause of primary hospital acquired infections. *P. aeruginosa* produces a number of toxic substances that have been implicated in its pathogenicity [31]. The bacterium is a pragmatic pathogenic organism, primarily causing nosocomial infections in immunocompromised patients [32, 33]. It has the ability to give rise to a wide-spectrum of infections as disruption of normal

physiological function occurs including deteriorated epithelial barriers [34], depleted neutrophil production [35], altered mucociliary clearance [36] *P. aeruginosa* is scarcely correlated with chronic infections in formerly healthy patients [37, 38]. It is highly antibiotic resistant and can cause high frequencies of mortality [39].

Pseudomonas aeruginosa is responsible for 10–15 % of the nosocomial infections worldwide [40]. Often these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. P. aeruginosa represents a phenomenon of antibiotic resistance, and demonstrates practically all known enzymic and mutational mechanisms of bacterial resistance [41].

According to screening studies of antimicrobial activity of plant extracts and natural products, higher plants act as essential and important source of new antimicrobial natural components [42]. As antibacterial medications are going through a crisis due to the quickly accelerating antibiotic resistance, new antimicrobial agents are urgently needed against resistant strains from the pool of bacteria which continuously undergo genetic changes. In addition to possible antimicrobial properties against human pathogens, it is also of interest to determine whether these extracts or compounds possess activity against plant pathogens [43].

P. aeruginosa has permanently been deemed to be a hard goal for antimicrobial chemotherapy. However, the entire sequencing of a *P. aeruginosa* wild-type strain has extended a big deal of beneficial information, regarding not only its pathogenicity, but also its resistance power [44]. Several possible mechanisms stand behind *P. aeruginosa* resistance to many types of antibiotics including its outer-membrane low permeability, the constitutive expression of various efflux pumps, and the production of antibiotic-inactivating enzymes (e.g., cephalosporinases) [45]. Moreover, *P. aeruginosa* also has a noteworthy capability to gain new antibiotics resistance mechanisms. This may due to the big size and the inconstancy of its genome, and to its distribution in aqueous media, which act as a reservoir for bacteria with other resistance bacterial genes [46].

In this study, the first step carried out was the *in vitro* antibacterial activity assay [47]. As observed, *Angelica* roots had strong antimicrobial activity against different bacteria and yeasts as mentioned above. Although *P. aeruginosa* was resistant to different antibiotics like Rimactane, gentamicin, piperacillin and ZOSYN, it was greatly affected by *Angelica*. Many reports focused on the antibiotic sensitivity patterns of *P. aeruginosa* and highlighted the problem of antibiotic resistance [45, 48]. The high antimicrobial activity of *Angelica* may be attributed to the main constituents of the essential oils of *Angelica* which possess antimicrobial effect against different microorganisms [49]. Its potent antibacterial activities are mainly attributed to its coumarin content [43]. Coumarins produced by the plant are considered as a defense mechanism against microrganisms [50, 51].

Coumarin compounds could be beneficial to the plants themselves as natural anti-pathogenic compounds, and for human as dietary supplements depending on their mild antimicrobial and anti-inflammatory effects, and as reference compounds in various bioactivity tests [52].

Also this study, histopathological investigation of the kidney and liver tissues in infected group with *P. aeruginosa* showed marked damage caused by bacteria. On the other hand, kidney and liver tissues in rats treated with *Angelica* extract showed recovery of these tissues and protective groups showed normal tissues appearance as compared with normal group. These results proved the effect of *Angelica* extract as antibacterial herb where it inhibited the pathogenic action of *P. aeruginosa*.

Yeh et al [53] found that *angelica archangelica* inhibited the formation of malondialdehyde in homogenates of mouse liver both in vitro and in vivo. *Angelica archangelica* had cytoprotective effect against chronic ethanol-induced hepatotoxicity, possibly through the inhibition of the production of oxygen free radicals that cause lipid peroxidation, and hence indirectly protects the liver from oxidative stress. In treatment and protection groups, the concentration of malondialdhyde was decreased. These results illustrate the antibacterial action of Angelica extraction. In this study, malondialdhyde concentration increased in the infected rats group compared with normal healthy group showing the pathogenic effect of *p. aeruginosa* which led to lipid peroxidation as a result of oxidative stress which appears due to bacterial infection.

One of the most notable observations in the present study was the antioxidant effect of *Angelica Archangelica* where in both treated and protected groups, there were significant enhancement in the results compared to infected group but the removal of oxidative stress caused by *P. aeruginosa* in protected group was greater than that in the treated group. The levels of antioxidants in protective group appeared higher than that of treated group.

Clinically, *P. aeruginosa* bacteremia looks like other forms of gram negative sepsis; with hypotension, fever, refractory shock, adult respiratory syndrome, and renal failure as common symptoms [39]. In our study, the levels of liver enzymes and kidney function tests in the infected group were significantly higher as compared with the normal group. These results prove the hazardous effect of *P. aeruginosa* on hepatic and renal functions. In the treatment and protection groups, these functions were improved as compared with infected group.

Conclusion

In conclusion, this study showed the benefit of *Angelica Archangelica* extract as antibacterial against

Pseudomonas aeruginosa bacteria which is a strong antibiotic resistant bacterium. Also, *Angelica Archangelica* extract protect and treat the damage in infected liver and kidney tissues and this extract effective to protect and treat the oxidative stress caused by *Pseudomonas aeruginosa* bacterial infection.

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