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Research Article

In Vivo Antimicrobial Activity of Ethanol Extract of Sumac (*Rhus coriaria*) on *Klebsiella pneumoniae*

Sarween Omar Taha
Department of Biology, Faculty of Science, University of Soran, Erbil, Iraq

Abstract: Eight isolates of *Klebsiella pneumonia* were obtained from different human specimens, identified through cultural, morphological and biochemical examination, in addition to API20E system. Susceptibility test to six antimicrobials were performed for all isolates. The isolates were grouped to four antibiogram, according to their resistance to tested antimicrobials; all isolates were resist to Ampicillin and Amikacin, or resist to at least one more antibiotics. The Minimum Inhibition Concentration (MIC) of ethanol extract for *Rhus coriaria* was determined (700 µg/mL). Sub-MIC was used as curing agents. Treating *Mus Musculus* mice (WBC count of 4.5×10^3) with Sub-MIC of *R. coriaria* extract, after nosily infected with more resistant isolate *K. pneumoniae* K4 and appearance of disease septum with 11.3×10^3 WBC count (after 5 days from infection). After treating the infected mice with plant extract and after 16 days from infection, the WBC count reduced to 5.1×10^3 µL which is in the normal range and the mice were healthy with good physiological behavior. While the infected mice and untreated with plant extract the WBC and other immunological parameters remain high even after 16 days from infection.

Keywords: Antimicrobial, ethanolic extract, *Klebsiella pneumoniae*, *Rhus coriaria*

INTRODUCTION

Plants are able to produce different compounds that used to protect themselves against different types of pathogens (Cowan, 1999). Interest in medicinal plants has revived as a consequence of current problems associated with the use of antibiotics (Emori and Gaynes, 1993).

There is a continuing search for new antimicrobials from other sources including plant extracts, these plants then emerged as compounds with potentially significant theatric application against human pathogen (Kathe *et al*., 2003). Most of the investigations show that medical plants used for treating many diseases that are caused by many pathogen, due to its chemical components (Hamilton-Miller, 1995; Toda *et al*., 1989).

R. coriaria (Anacardiaceae) commonly known as sumac is a wide bush that grow in all Mediterranean areas including Iraq, phytochemical in *R. coriaria* are being used as antibacterial, antidiarrheic, antidysentric, antihepatoxic, antiseptic, antispasmodic, antiviral, candidicide, anti-inflammatory, antioxidant (Duke *et al*., 2003).

This study was conducted in order to study the antimicrobial activity of ethanol extract of *R. coriaria* against *K. pneumoniae* in mice.

MATERIALS AND METHODS

Local bacterial isolates: Eight isolates of *Klebsiella pneumoniae* were obtained from different clinical specimens of patients were infected with pneumonia in Komary and Emergency hospitals in Erbil city, Iraq.

Antibiotics: Six antimicrobials: Ampicillin (Amp), Amikacin (Am), Chloramphenicol (Chl), Erythromycin (Ery), Lincomycin (Lin) and Kanamycim (Kan). These antimicrobials were used as disk-diffusion method by streaking the agar plate with bacterial inoculum, then the clear zone after incubation at 37°C for 24 h.

Identification of *Klebsiella pneumonia*: Cultural, morphological characteristics of isolates were studied, some biochemical tests were performed (Reynold, 2005), moreover API staph test were employed.

Extraction of *Rhus coriaria*: Alcohol extract were prepared according to Harborn *et al*. (1975), fifty gram of dried and pulverized powder using soxhelt extraction techniques, The hot extract wasconcentrated to dryness at 50°C in the vacuum oven. One gram portion of the sample was reconstituted in 100 mL of sterile distilled water and sterilized by membrain filtration.

Determination of MIC: The antimicrobial activity of ethanol extracts of *Rhus coriaria* was tested using serial dilution. *Klebsiella pneumoniae* S4 isolate inocula were standardized using standard curve prepared previously as recommended by Cruickshank *et al*. (1975). Bacterial suspensions were further diluted to obtain the 1×10^6 CUF inoculum. The Minimal Inhibitory Concentration (MIC) was defined as the lowest

concentration of antimicrobial extract that prevented visible growth. The confirmed spectrophoto metrically and by account of viable cells on nutrient agar (Atlas *et al*., 1995). The test was repeated three times.

Total WBC count: Blood samples were obtained from the heart of anesthetized mice and put in heparinized tubes, mixed well, them the WBC was counted using culture instrument and classical way (Thmel *et al*., 2004).

In vivo effect of *Rhus coriaria*:

Animals used in the experiment: Adult male laboratory-mice *Mus musculus* (White albino) weighing 30-35 g, 10-12 week in age bred in our animal house in the ambient environmental condition, they were kept singly in metabolic cages under room temperature 22-24°C.

In vivo antimicrobial activity: Nosely Infected mice with *Klebsiella pneumonia* S4 isolate of suspention containing 5×10^6 cells mL (Song *et al*., 1997). The micewere randomly divided into 3 groups, each containing 5 animals. When the symptoms of disease appeared, animals were administered the sub-MIC 0f plant extract twice dailyorally for 6 consecutive days:The first group (disease control) received tap water only, the second group was infected with bacterial suspension and the third group infected with bacteria which received the sub-MIC of plant extract. Enumeration of S4 isolate cells in the blood was performed according to Goden *et al*. (2005) and Kuo *et al*. (2005) during 0, 4, 8 and 12 days from infection. The frequencies of WBC and deferential WBC were evaluated (Thmel *et al*., 2004) for 4 consececuative days and the animals were observed for 12 days from the day on which the disease was induced and the death rate was recorded.

RESULTS AND DISCUSSION

Eight isolates of *Klebsiella pneumoniae* were obtained from different clinical specimens of patients were infected with pneumonia in Komary and Emergency hospitals in Erbil city, Iraq. These isolates were identified culturally, morphologically and biochemically. The API 20E system was performed to support the identification process.

According to the type and number of antimicrobial resistance *K. pneumoniae* isolates were grouped to 4 antibiogram (Table 1), the range of resistance was either all resist 100% to Amp and Am, or resist to at least one antibiotic among six antimicrobials used. All isolates are multiresistant (resist to more than two antibiotics).

The MIC of alcoholic *Rhus coriaria* extract 700 µg/mL for K4 isolate (Table 2), cleared that alcoholic extract of *R. coriaria* has in Vitro antimicrobial activity.

To evaluate the effect of *Rhus coriaria* extract on *K. pneumonia*e in Vivo, laboratory mice were nosily infected with *K. pneumuniae,* after (5) days the symptoms of disease appeared (Animals behavior, mortality, colored hair from white to yellow) and the mice become weak. The total WBC count was 9.7×10^3 cell/mL and 11.3×10^3 for infected only and infected and treated with plant extract respectively, comparing with 4.6×10^3/mL for the control treatment. Abnormal enlargement of respiratory tract was observed, comparing with uninfected mice. After 8 days from infection the total WBC raised to 14.4 for infected mice and the value of WBC decreased from 11.3 to 9.5 for infected and treated with plant extract, while for the control remain 4.6×10^3.

After 12 days from infection the value of total WBC for infected untreated raised to 16.3×10^3, while for infected and treated mice decreased to 7.3, then to 5.1 after 16 days from infection andthe mice become healthy. Nutrophil raised after infection the mice from 2.79 and 3.05 for infected untreated and treated to 7.9 and 10.05 for untreated and treated mice respectively, after 8 days from infection the amount of nutrophyl decreased to 7.9, 6.3 then 3.58 when infected mice treated with extract of R. co, on the other hand for infected mice the nutrophyle raised up to 11.85 in day 16 from infection. Other immunologicalparameters affected when the mice infected with *K. pneumoniae.*

These findings have cleared demonstrated that the clearance of *K. pneumonia* from the blood of infection mice by sub-MIC of alcoholic extract was zero, as compared with the infected untreated mice even after 16 days from infection the number was 500 cell mL. Furthermore it was more effective than other treatments.

DISCUSSION

In order to examine the effect of alcoholic extracts of *R. coriariain vivo* on reduction of antibiotic resistant action, laboratory mice *Mus musculus* were used and infected nosily with 5×10^6 CFU/mL suspension of *K. pneumoniae* S4 isolate. After five days of infection, the symptom of disease appeared on infected mice, such as swallowing, lung raised to out the body and they become weak and the total WBCs count raised from 4.5×10^3 cells µL to 11.3×10^3 cells µL as shown in (Table 3) the type of leukocytes also elevated, neutrophil raised from 3.054×10^3 cells µL to 10.05×10^3 cells µL l. The WBCs used as an immunological parameters to determine the case of infection (Provan *et al*., 2004), while normal range of total WBCs were 4.2×10^3 cells µL (Hoffman *et al*., 2000, Provan *et al*., 2004), because the main type of phagocytic cells which is required to participate in the phagocytosis in the ingestion of foreign bodies (like bacterial cells) are neutrophil and macrophage (Kern, 2002; Henderson

Table 1: Antibiogram groups and number of *K. pneumoniae* isolate

Antibiogram	Amp	Am	Chl	Ery	Lin	Kan	Isolate no	No. of antibiotic resistance
A1	+	+	-	-	-	-	1, 3, 4	2
A2	+	+	-	-	+	-	5, 2	3
A3	+	+	+	-	-	-	7, 8	3
A4	+	+	+	+	+	+	6	6

Table 2: The MIC of alcoholic extract of *R. coriaria* on *K. pneumoniae*

	Concentration of *R. coriaria* µg/mL									
Plant extract	100	200	300	400	500	600	700	800	900	1000
Alcoholic extract	1.32	1.28	1.19	1.09	1.08	0.96	0.52	0.21	0.21	0.07

Table 3: Some immunological characteristics of mice infected with *K. pneumoniae*

Treatments	T. WBCs×10^3	NE×10^3	Ly×10^3	Mo×10^3	Eo×10^3
At zero time from infection					
A	4.60	3.05	1.17	0.37	0.00
B	4.30	2.79	1.07	0.34	0.00
AB	4.50	3.05	1.17	0.37	0.00
After 5 days from infection					
A	4.60	3.05	1.17	0.37	0.00
B	9.70	7.96	1.02	0.72	0.00
AB	11.30	10.05	1.18	0.10	0.00
After 8 days from infection					
A	4.60	3.05	1.17	0.37	0.00
B	14.40	7.13	0.61	0.69	0.00
AB	9.50	7.96	1.02	0.72	0.00
After 12 days from infection					
A	4.60	3.05	1.17	0.37	0.00
B	16.30	13.84	1.32	0.94	0.00
AB	7.30	6.31	0.09	0.58	0.00
After 16 days from infection					
A	4.60	3.05	1.17	0.37	0.00
B	13.60	11.85	1.63	0.21	0.00
AB	5.10	5.58	0.98	0.51	0.00

A: Control; B: Infected mice with *K. pneumonia*; AB: Infected mice with *K. pneumonia* and treated with plant extract; TWBCs: Total white blood cells; Ne: Netrophyl; Ly: Lymphosite; Mo: Monosite; Eo: Eosinophil

and Oyston, 200; Ernst and Stendahl, 2006), so during infection with bacteria, the range of neutrophils increased comparing with the control. While basophile and eosinophil are role inimmunity, eosinophil increasing in cancer and parasitic infections (Bain and Flower, 1996). High level of basophils generally corresponds to an active allergic response (Wikipedia the Free Encyclopedia, 2010).

After three days of administration, all of the infected mice were examined to number of total WBCs and differential leukocyte count to check the effects of alcoholic extracts at 700 µg mL, the total WBCs of infected mice were treated with SMIC only was 5.1×10^3 cells µL and differential leukocyte count decreased when compared with control group.

At the end of the experiment when the animals were examined for TWBCs and other immunological parameters, the results indicated that for infected mice the total WBC raised to 16.3×10^3, while for infected and treated with plant extract was 5.1×10^3 due to the effect of *R. coriaria* extract.

During infection all of the infected mice and the control examined to total bacteria in the blood (Table 3) represent count of viable bacteria presented in theblood. After 5 days from infection viable bacteria increased to 166 and 333 for infected with bacteria only and infected mice treated with plant extract respectively. After 8 days from infection in the extract treated mice, bacterial number decreased considerably to 166, then to zero up to the end of experiment, while in the infected untreated mice the number was 5×10^2 and then increased to 1×10^3 cells mL after 12 days, then decreased to 5×10^2 at the end of the experiment.

These findings have cleared demonstrated that the clearance of *K. pneumoniae* from the blood of infection mice by alcoholic extract was zero, as compared with the infected untreated mice even after 16 days from infection the number was 5×10^2 cell mL. Furthermore it was more effective than other treatments.

The effect of *R. coriaria* extract maybe due to that sumac is rich in tannin and other components and the antimicrobial activity of tannin is well documented (Chung *et al*., 1998; Abu-shanab *et al*., 2005; Gulmez, *et al*., 2006). The ethanolic extract displayed broad spectrum of activity, science G^+ and G^- bacteria were inhibited with *R. coriaria* extracts (Abu-Shanab *et al*., 2005).

This study demonstrates that extract of sumac may be a potential source of natural, safe and cheep alternative to synthetic and chemical antimicrobials.

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