Comparison of the Effect of Aqueous and Alcoholic Extracts of *Rosmarins Officinalis* on the Macrophages of *Staphylococcus Aureus* (Invitro)

SabaaAbdAlSadaHadi[1], SihamJasim Mohsen[2]
[1, 2] College of Education for Girls - University of Kufa/Iraq

Abstract
The study was conducted for the purpose of evaluating the effect of rosemary plant (*Rosmarins officinalis*) on the efficiency and activity of polymorphnuclear cells (PMNs) with the phagocytosis process test. He tested the effect of the aqueous and alcoholic extract of the leaves of this plant. The results showed that when different concentrations of extracts are added, the percentage of the number of phagocytes increases by increasing the concentration compared to the control sample, as the concentrations of the rosemary alcoholic extract (150,100,50, 200) mg/ml increased the phagocytization coefficient in time (90,60) minutes, but it was an The concentrations of the rosemary aqueous extract (200,150,100,50) mg / ml led to an increase in the phagocytization coefficient, where the increase reached its maximum time (90,60) minutes, but it was an immean increase. The aqueous extract was the most efficient raw extract to increase the number of phagocytes.
Keywords: rosemary , phagocytosis , PMNs , Staphylococcusaureus

1. Introduction

Immunity has been a major health concern among consumers over the past decade and especially with the 2019 COVID-19 pandemic, increased interest in botanical foods, nutritional supplements and herbal extracts, among others that may confer significantly beneficial immunomodulatory effects, given that immunocompromised individuals are vulnerable. For the complications of disease infection, it has been recently concluded that these plant products and herbal extracts play important roles in enhancing immunity and helping to control infection (Arshadetal ., 2020), strengthening the defense systems in the body is one of the main factors that will protect and lead to recovery in case infection (Babichet al., 2020). In addition, the immune system is a highly complex biological network that has evolved to protect the host from various pathogens, such as bacteria, viruses, parasites, fungi, as well as cancer cells (Lange and Nakamura, 2020).

Among those medicinal plants, the rosemary plant, Rosmarinusofficinalis, of the Lamiaceae family, stands out. It is one of the medicinal plants that has been widely used since ancient times and has a lot of circulation due to the widening of its presence and its easy reproduction through roots or through cuttings, and it usually adapts to different environments in general (Al-Jubouri, 2014). It is grown in Central Asia, Southeast Africa, India, Australia, and southern Brazil (Yilan and Cristiane, 2010). It is also an important medicinal plant due to its biological efficacy, as it contains Rosmarinic acid and volatile oils by 1.5% (Amaniet al., 2017).

One of the most famous medicinal herbs and their essential oils that have immune properties is rosemary, Rosmarinusofficinalis, which is one of the richest herbs in biologically active compounds and health-promoting phytochemicals such as (carotenoids, flavonoids, stibenes, tannins, omega-3 fatty acids and other substances) (Guti´errezet al., 2019; Malaguranera , 2019; Gonzalez, 2010).

Aim of the study

This study aimed to demonstrate the importance of the rosemary plant in the immune system and its role in activating the immune-important cells that fight diseases, and therefore it can be prescribed as an immune-enhancing drug.

2. Materials and methods

Collecting plant leaves

The leaves of the rosemary *Rosmarinusofficinalis* were collected from one of the home gardens in the province of Najaf, and the plant was classified in the plant laboratory of the Department of Life Sciences in the College of Education for Girls / University of Kufa, after the leaves were cleaned well from the dust attached to them, they were washed with sterile water and then left to dry at room temperature. After drying the leaves, they were ground using an electric blender grinder to obtain a dry powder. The powder was placed in clean and dry plastic bags and kept in the refrigerator for later use.

Preparation of hot aqueous extract of rosemary

A weight of (20) gm of the dry powder of the leaves was taken and mixed with (400) ml of hot sterilized distilled water and placed in a water bath at a temperature of (45) C and (100) rpm and left for a period of (5) hours, then Get out of the water bath and put it in room temperature for (24) hours, after that the mixture was filtered using several layers of sterile medical gauze to get rid of plant residues, then centrifuged at a speed of

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(3000) cycle / minute for (10) minutes, then the extract was dried using the electric oven with heat (40) C and kept in the refrigerator at a temperature of (4) C until use (Adzuet al., 2001)

Prepare the alcoholic extract of rosemary

Harborne, (1984) adopted the method of preparing the alcoholic extract of rosemary with making some modifications to suit the research requirements, as (10) grams of the powder were placed in the thimble of the extraction thimble in the Apparatus Soxhlet apparatus, then (100) ml of ethanol with a concentration of (70%) was added to it was extracted for a period of (24) hours at a temperature of (40-45) C, then the sample was dried in the oven at a temperature of (40-45) C, and from it the storage solution was prepared.

The qualitative lists used to investigate the active substances present in the hot aqueous extract and the alcoholic extract of the rosemary plant

1-Detection of glycosides

This test was conducted by adding (1) ml of the crude extract to (2) ml of Benedict's reagent, and then placed in the water bath for several minutes until the appearance of a blue color that indicated the presence of glycosides (Mahmoud and Khalil, 2010).

2: Detection of tannins

(1) ml of the plant extract was added to the potassium hydroxide detector (1% KOH), as the appearance of a white color is evidence of the presence of tannins (Treas and Evans, 1989).

3-: Detection of Flavonids

A volume of (4) ml of ethyl alcohol with a concentration of (95%) was added to (1) ml of plant extract in a glass test tube, and the test tube was placed in a boiling water bath for (25) minutes, and after it was removed from the water bath, drops of hydroxide solution were added Potassium (0.5) M, and the appearance of a dark color indicates a positive test (the presence of flavonoids) (Aisha *etal.*, 2015).

4- Detection of phenols

(3) ml of the plant extract was added to (2) ml of iron chloride (1%), and a bluish-yellow color appeared indicating the presence of phenols (Atlas et al., 1995).

5- Detection ofTerpenoids

A volume of (5) ml of the plant extract was taken and added to a mixture of (2) ml of chloroform and (3) ml of concentrated sulfuric acid, and the appearance of a reddish-brown middle layer indicated the positiveness of the test (presence of terpenes)(Eluyode and allabi, 2017).

Roswell Park Institute–1640 Tissue Culture Media (PRMI-1640):

Prepare flow according to manufacturer's instructions. Lab (England), then (10%) genetic calf serum was added to it, which was inhibited by heat (56) C for (30) minutes, and (10) ml of antibiotics solution (penicillin and streptomycin) was added at a concentration of (10) mg / ml, adjusting the pH of the medium to (7.2) using drops of sodium bicarbonate, sterilized through fine filters (0.45) micrometers, then distributed in sterile tightly closed tubes, and kept at a temperature of (20) C until use in immunological experiments.

Preparation of AB blood group serum

The serum was prepared according to the method of Hudson and Hay, (1980) when blood was drawn from the vein of a healthy person with blood type AB and placed in a dark tube that did not contain an anticoagulant, the tube was left at room temperature for (30) minutes for the purpose of coagulation, the thrombus was lifted and the tube was quickly discarded (2000) cycle / minute for (15) minutes, the upper layer (serum) was taken and kept at a temperature of (-20) C. It was used in the examination of phagocytosis as palatable substances**-**.Preparation of Staphylococcus aureus suspension:

It was prepared according to what was mentioned in Cech and Lehrer, (1984) when it was planted on Manitol Salt agar medium for the purpose of ascertaining its purity, it was incubated for (24) hours at a temperature of (37) C, and (2-3) colonies were transferred by means of a sterile carrier to a beaker containing (50) ml From the sterile liquid Brain-Heart Infusion medium, then incubated for a period of (24) hours at a temperature of (37) C. After the end of the incubation, the medium was centrifuged at a speed of (3000) rpm for a period of (15) minutes, then the precipitate was washed with Hanks Balanced Saline Solution (HBSS) and then suspended in the same solution. Then the cells were counted for the purpose of calculating the total number using (Haemocytometer), adjusting the number to (106 x 8) cells/ml, the cells were killed by boiling the suspension for an hour in a water bath, and it was used in the phagocytosis test.

Isolation of polymorphnuclear cells (PMNs) from peripheral blood

Blood was obtained from healthy donors throughout the period of immunological experiments.

PMNs were isolated according to the method of Cech and Lehrer, (1984) and the following agencies: Blood was drawn from healthy donors and placed in a sterile plastic tube containing heparin (500 international units / ml) as an anticoagulant, and a dextran solution (6%) to precipitate red blood cells at a rate of (3 ml per 10 ml of blood), then the contents of the tube were gently mixed. And placed in the incubator at a temperature of (37) C for a period of (45) minutes.

It was observed that the lower two layers contain red blood cells (neglected) and the upper layer contains plasma rich in white blood cells. It was transferred to another sterile tube and the cells were washed with Hanks balanced salt solution (HBSS) twice at a capacity of (1500) revolutions / min for (10) minutes. Cells were suspended in PRMI-1640 culture medium. The cells were counted according to the method

(Hudson and Hay 1980), by using trypan blue dye (0.2%) by means of a Haemocytometer slide under a light microscope, as the stained cells were considered dead, but the cells that did not take the dye are live cells.

Effect of an aqueous and alcoholic rosemary extract on the phagocytosis of *Staphylococcusaureus* (Invitro)

The method of Cech and Lehrer, (1984) was adopted by my agencies:-

The suspension of polymorphonuclear cells (PMNs) was prepared at a rate of (106 x 2) cells/ml , and the suspension of killed bacteria was prepared according to paragraph (3-9) at a rate of (106 x 8) cells/ml.

An aqueous and alcoholic extract of rosemary was prepared at two concentrations (50 and 100) mg/ml, each separately in sterile plastic tubes according to the required concentration (taking into account the final volume of each tube). Two control tubes were prepared free of the extract.

Added to each tube (0.25) ml of cell suspension (PMNs) and (0.25) ml of bacterial suspension at a ratio of (4:1), respectively, and (0.25) ml of AB blood group serum.

The tubes were incubated at a temperature of (37) C in a water bath rocking at a speed of (10) cycles/min for periods of time (30, 60, 90 and 120) minutes.

At the end of each time period, swabs from each concentration were prepared on clean glass slides with (3) repetitions for one concentration, left to dry and fixed with methyl alcohol for (5-10) minutes and stained with Kamsa dye (20) minutes, examined with an oil lens and counted (200) cells To calculate the Phagocytic Index (PI) phagocytosis coefficient:

$$\text{phagocytosis coefficient PI} = \frac{\text{The number of phagocytic cells}}{\text{Total cell count (phages + non-phages)}} * 100$$

3. Results and Discussion

Table (1) shows the rates of phagocytic index (PI) for PMNs cells treated with concentrations (50, 100, 150, 200) mg/ml for each of the alcoholic and aqueous extracts of gel corona for different periods of time compared to the control, as it is noted from the table that the coefficient of The phagocytosis increases gradually with time, forming a significant level until it reached its maximum at (60 and 90) minutes, then it decreased again within 120 minutes and for all concentrations used. From that, it is noted that there is a close relationship between the time factor and the phagocytosis coefficient, and that the efficiency of the cells for phagocytosis increases over time, while it begins after the minute. 90 by losing their ability to devour, and Loaketal (1990) justified this by increasing the concentration of lysosomal enzymes and toxic substances that are produced by the cells themselves as a result of this process or by cell death as a result of exposure to continuous work pressure, and through the results of statistical analysis it was found that there is a significant increase in the level of (P≥0.05) with increasing the concentration and for all the times used in the experiment, as a significant increase was noted by ((P≥0.05) for all concentrations of the water and alcohol extract compared with the control at time (60 and 90) minutes.

Picture (1) shows the process of devouring Staphylococcus aureus bacteria. The concentration (200) mg/ml for both the aqueous and alcoholic extracts continued to have the same effect, as it was observed that there were no significant differences between the aqueous and alcoholic extracts for a time of (60 and 90) minutes.

Polymorphonuclear white blood cells (PMNs) and mononuclear cells in the blood, which become macrophage cells after reaching the tissues, are among the first defense means when germs enter or inflammation occurs, and the effectiveness and activity of these cells can be measured through the process of phagocytosis and their ability to get rid of germs And foreign bodies entering the body is known as the process of phagocytosis (Hyde, 1996).

The role of the plant in stimulating the immune system lies in its activation of phagocytic cells, including granular leucoytes, which are the most abundant white blood cells and the first line of defense for the body, including neutrophils, as a rapid response is generated by these cells when bacteria invade it, where neutrophils surround the bacteria and digest them. Internal endocytosis by lysosomal enzymes present in the cytoplasmic granules and this process is called phagocytosis (Power, 1989).

The role of cells in phagocytosis and killing them outside the body may be attributed to the effective substance santonin contained in these extracts or to the glycosides, phenols and tannins present in the extracts, which may act as factors that attract cells towards bacteria and then adhere to them. Also, the essential oils contained in the rosemary plant have an important role in affecting Receptors on the surfaces of phagocytic cells act as palatable opsinazaion substances to accept bacterial cells present in the culture medium, and the role of the active compounds can be counted in giving energy to the cells to help them devour the bacteria and secrete lysosomes on them (Twaij and AL-Badr, 1988).

It should be noted that there is a discrepancy between the effect of the crude extracts, where it is clear that the aqueous extract was more efficient than the alcoholic extract in affecting the efficiency of cells, and the reason for this is due to the nature of the compounds present in each crude extract and their ability to stimulate phagocytic cells to perform their function (Shoiebet al., 2003).

Table (1): Effect of aqueous and alcoholic rosemary extract on the bolus of *Staphylococcus aureus*						
Extract	transaction aggregates	Phagocytosis factor (%) mean±SD				
		Time of exposure				Multivariate p-value
		30 min	60 min	90 min	120 min	
Aqueousrosemary	the control	51.31±0.02 e	56.14±0.12 d	61.63±0.07 e	47.3±0.03 e	LSD=0.175 0.0001*
	50 (mg/mL)	54.16±0.15 d	73.5±0.21c	70.56±0.1 d	67.11±0.09 d	
	100 (mg/mL)	56.1±0.10 c	74.62±0.1b	72.57±0.09 c	66.66±0.13 c	
	150 (mg/mL)	56.29±0.04 b	77.3±0.11 a	74.65±0.06 b	68.1±0.10 b	
	200 (mg/mL)	60.05±0.09 a	77.12±0.13 a	78.18±0.16 a	69.31±0.02 a	
LSD		0.165	0.250	0.185	0.156	
p-value		0.0001	0.0001	0.0001	0.0001	
Alcoholic rosemary	the control	51.31±0.02 d	56.04±0.07 e	61.65±0.05 e	47.21±0.11e	LSD=2.224 0.0001*
	50 (mg/mL)	55.22±0.19 c	65.61±0.14 d	66.24±0.09 d	63.56±0.11 d	
	100 (mg/mL)	56.08±0.07 a	67.08±0.08 c	69.36±0.04 c	64.04±0.07 c	
	150 (mg/mL)	55.62±0.11 b	66.58±0.11 b	70.47±0.41 b	65.56±0.19 b	
	200 (mg/mL)	55.11±0.1 c	71±0.01 a	73.22±0.1 a	64.59±0.08 a	
LSD		0.207	0.167	0.356	0.219	
p-value		0.0001	0.0001	0.0001	0.0001	

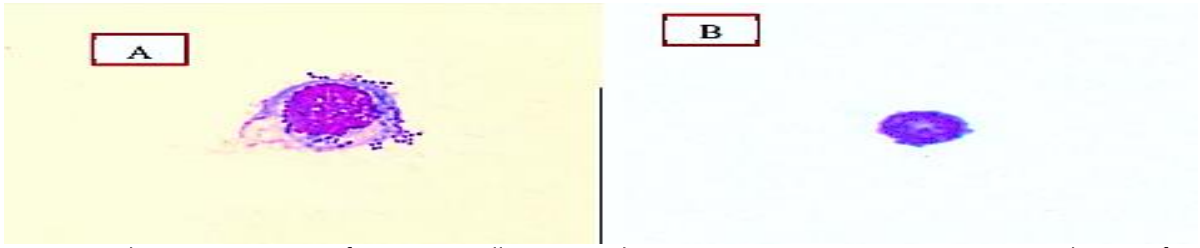

Image (1): A: phagocytic (PMNs) of S. aureus cells. B: non-phagocytic (PMNs) using Giemsa stain with magnification of X1000

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