



Evaluation of Antibacterial and Cytotoxicity Activities of 5-Nitro Acetyl Salicylic Acid and 5-Bromo Acetyl Salicylic Acid Compounds

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Abstract

Introduction: Phenolic compounds have dramatic effects in treat different various diseases caused by pathogenic bacteria. From these compounds, substituted phenolic carboxylic acids carrying active chemical groups. Therefore the currently research was carried out to investigate and evaluate the antibacterial activity of 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid.

Material and Methods: Previously substituted carboxylic acids were biochemically tested by using the concentration of 0.1,1.0,10, and 100mg/ml of both 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid against two pathogenic bacteria which were represented by *Escherichia coli* and *Staphylococcus aureus*. Also cytotoxicity activities of the two phenolic carboxylic acids were estimated and evaluated by using hemolysis method of red blood cells.

Results: The concentration of 0.1,1.0,10,and 100mg/ml belonging to 5-nitro acetyl salicylic acid recorded inhibition zone diameters equal 8,23, 25 and 35mm against growth of *Escherichia coli* bacteria and *Staphylococcus aureus* respectively. Whereas the same concentration belonging to 5-bromo acetyl salicylic acid recorded 14,20,9 and 30mm and 19,22,15,and 20mm against growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. Concerning cytotoxicity activity of both 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid, there were no hemolytic results at concentration of 1:10,1:100,and 1:1000mg/ml.

Conclusion: The two phenolic acids represented by 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid showed excellent antibacterial activities against pathogenic bacteria. Also these acids had no toxic effect against red blood cells so these active phenolic acids can be used safety to treat various diseases caused by these micro-organisms.

Keywords: Antibacterial activity, Cytotoxicity, 5-nitro acetyl salicylic acid, 5-bromo acetyl ,salicylic acid, phenolic group.

Introduction

The medicinal importance of any drug comes from its biochemical activity to treat different diseases caused by infections by various

pathogenic micro-organisms such as bacteria, fungi and parasites ^{[1],[2]}. Several post and current studies were achieved to estimate the antibacterial potential of synthetic drugs and natural active

chemical materials isolated from medicinal plants. The medicinal actions of these drugs and metabolites chemicals belong to presence of various active and functional groups in their chemical structures such as carboxylic, phenolic, alkaloidic, hydroxylic, aldehydic and ketonic groups. Carboxylic acids are a class of organic compounds containing carboxyl as functional group^[3]. Also most of these acids contain phenolic groups in their chemical structures which are substituted by donor or acceptor groups. From these carboxylic acids are 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid where these acids contain substituted phenolic groups in the chemical structures belonging to these acids^[4]. Anti-microbial activity is the most accurate method for investigation the medicinal effect of chemical compounds on living cells of pathogenic micro-organisms then estimation of ability of these compounds to inhibit or end the biological role of these organisms leading to cure various diseases caused by these pathogenic micro-organism. Many studies were carried out to evaluate the antibacterial activity of synthetic and natural drugs which showed the great capability of these materials as therapies for various diseases^[5]. The current research was applied for evaluation of the medicinal effect of carboxylic acids represented by 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid on some pathogenic bacteria.

Materials and Methods

Preparation of Substituted Carboxylic Acid.

The carboxylic acids which are represented by 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid were prepared previously at ideal conditions^[6].

Pathenogenic Bacteria Preparation

Two strains pathogenic bacteria are *Escherichia coli* (negative towards Gram stain) and *Staphylococcus aurous* (positive towards Gram stain) were gotten by specified microbiologist at biology department in the microbiology laboratory

at the college of education for pure sciences at university of Basrah.

Culture Media Preparation

Mackoakey agar manitol sugar as a culture medium was prepared according to information determining by manufacturing company, and was supplied from India and the procedure of its preparation was achieved by a biologist at biology department, education college for pure sciences at university of Basrah.

Evaluation of Antibacterial Activity and Maximal Inhibitory Concentration of the Two Carboxylic Acids.

Several various concentrations of 5-nitro acetyl salicylic acid which were represented by (0.1 ,1.0 ,10 and 100 mg/ml) were carried out against growth of the pathogenic bacteria (*Escherichia coli* and *Staphylococcus aurous*) for estimation of inhibition zone diameters and maximal inhibitory concentration by using Mackoakey agar and maintol salt sugar as a culture medium according to diffusion method in poetry dishes. Then the concentrations treated with bacteria, where put in the incubator for 24 hours, after that the antibacterial activity and maximal inhibitory concentration were evaluated^{[7],[8]}. By the same method the antibacterial activity and maximal inhibitory concentration were carried out but by using 5-bromo acetyl salicylic acid compound.

Evaluation of cytotoxicity of carboxylic acids:

The cytotoxicity activity of the both carboxylic acids (5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid) was evaluated by using several concentrations of these acids, where 200 mg of each acid was dissolved in 10 ml of Ringer's solution then it was diluted to the ratios (1:1, 1:10, 1:100 and 1:1000 v/v). Negative control factor was carried out, contains Ringer's solution which was represented by normal saline whereas the positive control factor was used represented by tap water. The blood was centrifuged for 5min.with speed equal to 3000

rpm. Then 0.8 ml of each concentration of acids was put in sterilized test tube as Eppendropp tube contains an anti-clotting material, after that to each tube 0.2 ml of centrifugal blood was added and the total volume in each tube became 10 ml. The tubes were incubated in the incubator at 37°C for 30 min. and lately all tubes were tested to observe the hemolytic^[8].

Results

Results of Antibacterial Activity of 5-nitro Acetyl Salicylic Acid:

The concentrations of (0.1, 1.0, 10 and 100 gm/ml) belonging to the compound 5-nitro acetyl salicylic acid have recorded inhibition zone diameters equal to (8, 25, 30 and 42 mm) against growth of *Escherichia coli* bacteria whereas the same concentrations have showed inhibition zone diameters equal to (8, 23, 25, 35 mm) towards growth of *Staphylococcus aureus* as in table (1).

Table (1) results of antibacterial activity of 5-nitro acetyl salicylic acid

Conc. (mg/ml)	Inhibition zone diameters (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
0.1	8	8
1.0	25	23
10	30	25
100	42	85

It was noticed that increase of concentration of 5-nitro acetyl salicylic acid has led to increase inhibition zone diameter then increasing of antibacterial activity leading to kill the most number of pathogenic bacteria for both strains belonging to microorganisms.

Results of Antibacterial Activity of 5-bromo Acetyl Salicylic Acid

The antibacterial activity recorded by using the compound of 5-bromo acetyl salicylic acid is illustrated in table (2). which show the medicinal action of this carboxylic acid on growth of both pathogenic bacteria which are represented by *Escherichia coli* and *Staphylococcus aureus*. The concentrations which were prepared belonging to

5-bromo acetyl salicylic acid gave inhibition zone diameters equal 30, 9, 20 and 14 mm against *E.coli* and 20,15,22 and 19 mm against *S. aureus* bacteria.

Table (2) results of antibacterial activity of 5-bromo acetyl salicylic acid

Conc. (mg/ml)	Inhibition zone diameters (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
0.1	14	19
1.0	20	22
10	9	15
100	30	20

Results of Cytotoxicity Activity of Phenolic Carboxylic acids:

The cytotoxicity activity of carboxylic acids represented by 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid, was estimated. In regard to 5-nitro acetyl salicylic acid, It was found that concentrations 1:10, 1:100 and 1:1000 mg/ml prepared from this acid had no toxic effect towards of hemolytic of red blood cells but only the concentration 1:1 mg/ml showed a positive toxicity against these cells as in table (3).Concerning the compound of 5-bromo acetyl salicylic acid also only the concentration represented by 1:1 mg/ml gave a positive effect towards hemolytic of red blood cells whereas the concentrations 1:10, 1:100 and 1:1000 didn't show any hemolysis effect of these cells as shown in table (4).

Table (3) Evaluation of cytotoxicity activity of 5-nitro acetyl salicylic acid

5-nitro acetyl salicylic acid conc. (mg/ml)	Hemolysis result
1:1	T+
1:10	NT-
1:100	NT-
1:1000	NT-
Control negative (blood + Ringer solution)	NT-
Control positive (tap water + blood)	T++++

T = Toxic, NT = Non-toxic

Table (4) Evaluation of cytotoxicity activity of 5-bromo acetyl salicylic acid

5-bromo acetyl salicylic acid conc. (mg/ml)	Hemolysis result
1:1	T+
1:10	NT-
1:100	NT-
1:1000	NT-
Control negative (blood + Ringer solution)	NT-
Control positive (tap water + blood)	T++++

T = Toxic, NT = Non-toxic

Discussion

Various researches ensured that the phenolic compounds and chemical substances carrying phenolic groups have high antibacterial activity because they contain hydroxyl groups (-OH) in their chemical structure which this active chemical group has ability for bonding with proteins hydrogen and this led to break of sulphuric and hydrogen bond existing in the tertiary structure of protein abundant in the bacterial cell ^{[11],[12]}. Also the phenolic groups are capable of destruction of cell wall then increase of its permeability for these compounds leading to denaturation of living cell proteins ^[13]. Different studies showed that the phenolic compounds have ability to bind with cell enzymes leading to inhibition of its biochemical potential. In addition to antibacterial activity, the chemical compounds containing phenolic groups have also anti-mutagenic and anti-carcinogenic activity ^{[14],[15]}. It is observed from table (2) that this carboxylic acid carrying phenol group has showed a very good antibacterial properties against both pathogenic bacteria. The most of phenolic compounds destruct the membrane and the wall of pathogenic bacteria cell, then leading to change the chemical composition of proteins, therefore chemical compounds which have phenolic groups are capable of having the antibacterial activity. Hydroxyl group which is abundant in the chemical structure of phenolic compounds has chemical ability to bind with hydrogen atoms of proteins leading to break the sulphur and hydrogen bonds existing in the tertiary structure of protein of the living cell of pathogenic bacteria ^{[16],[17]}.

Various researches ensured and proved the biochemical medicinal activity of phenolic compounds against growth of pathogenic micro-organisms including bacteria because these active chemical compounds inhibit the metabolism of nucleic acids which are represented by deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) by binding between phenolic hydroxylic groups with these acids and also between carboxyl group which existing in the chemical structure of carboxylic acid with nitrogenous bases represented by adenosine, thymine, guanine, cytosine and uracil which are present in nucleic acids ^{[18],[19],[20]}.

From tables (3,4), the both phenolic carboxylic acids can be carried out safely to treat the different diseases resulting from pathogenic bacteria specially *E-coli* and *S- aureus* ^{[21],[22]}. The biochemical necessity of investigation and evaluation of cytotoxicity action of chemical compound was determined by several different health reports ^{[23],[24]}.

Conclusion

The ted phenolic carboxylic acids represent 5-nitro acetyl salicylic acid and 5-bromo acetyl salicylic acid have been recorded an excellent activity to inhibit the growth of *E.coli* and *S.aureus* as pathogenic micro-organisms so these active carboxylic acid can be used as therapy for various diseases caused by these pathogenic bacteria.

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