ANTIMICROBIAL ACTIVITY OF ROYAL JELLY AGAINST E. COLI IN COMPARISON WITH THREE SELECTIVE ANTIBIOTICS

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ABSTRACT

Royal Jelly is very important for nutrition of larvae and the queen. It has many effect with the main property of royal jelly being active against different type of bacteria. In this study the suspected activity of royal jelly camper with activity of doxycycline on E. coli in vitro. The RJ showed the antimicrobial activity at a specific concentration reach 10 mg. by using SPSS version 24(T-test and ANOVA and Pearson correlation used to analyze results). If P-value ≤ 0.05 was considered significant. In this study, we wished to determine whether there are anti-microbial activities of royal jelly on E. coli activity, effect on newborn and to identify the possible implications of these factors to disease course. The gram negative bacteria E. coli was isolated and preserved as source from Al-Hussan Medical city and cultured on Eosin Methylene Blue agar then incubated at 37 o C for 18-24 hours. The sensitivity test was performed in in-vitro by making wells with diameter about (8 mm). The Minimum Inhibitory Concentration (MIC) was calculated by measuring the inhibition zone on nutrient agar. The royal jelly gave significant activity against E. coli. Doxycycline showed high significant activity. But the Gentamicin and doxycycline did not show any antibacterial activity. There is significant activity of Royal jelly against growth of E.coli

Keywords: Royal jelly, Doxycycline, E. coli

INTRODUCTION

Royal Jelly (RJ) consider as a food of nutritious value which can be produced by special glands called hypo-pharyngeal glands and used for feeding by larvae and queen bee. Larvae usually continuously fed with royal jelly buthe queen may be not [10]. RJ has special materials used for the development queen bee. These substance have a thick and milky white appearance, the PH is between3.6-4.2 and large amount of water” (about 60-70%), proteins (12-15%), carbohydrates (10-16%), lipids (3-6%), and traces of salts, vitamins, and free amino acids “[1-3]. RJ consider as the unsurpassed on eneutral-aceutical in both modern and also traditional medication because the high activity of antibacterial [4]

RJ consider as strong antimicrobial activity can effect on many pathogens. There are many studies founded certain material foundin RJ have ability to inhibit the activity and growth of microorganism like G+,G- bacteria and some viruses,fungi [5]. Aunique peptides of RJ give the antimicrobial activity lead to prevent pathogens from entrance to honeybees and protect it. Peptides and proteins in RJ composed simply structure and
not complicated imoieties modification or unregularly amino acids. Because its structure is special and simple make it very active antibiotic against huge number of pathogenic microorganisms \[6\]. It is hypothetically assumed, in nano-tubes and by using short artificial special peptides that give the ability to destroy the membranes of bacteria \[7\]. Most of study was done in vitro and that because after the ingestion of RJ many of its component may be destroyed especially the active components in digestive system of lab animals that may be as a results of changing of PH \[8\].

**MATERIALS AND METHODS**

**Identification of E. coli**

The gram negative bacteria E.coli was isolated and preserved as source from Al-Hussan Medical city and cultured on Eosin Methylene Blue agar then incubated at 37ºC for 18-24 hours(Figure1).

**Tests of Sensitivity**

After using of agar with different well diffusion as techniques for measuring antimicrobial activity of three antibiotics – Gentamicin, Doxycycline and Gentadoxycycline and RJ against *E. coli*.

**Royal Jelly Preparation**

Ten g of RJ were dissolved in 100ml of hot normal saline (w/v) as stock solutions.

**Preparation the antibiotics**

1. Gentamicin 80mg /2ml: taken the 0.8 ml of antibiotics and then completed to 10 ml distal water in order to getting (100 μg /ml) (stock solution),and from it premedicated the different concentration: 10, 20, 40, 60 and 80μg /ml.
2. Doxycycline 100 mg capsule: dissolving the capsule in 10 ml of distal water and then taken 0.1 ml of antibiotics and completed to 10 ml distal water in order to getting the (100μg / ml) and from it premedicated the different concentrations as follow : 10, 20, 40, 60 and 80μg /ml.
4. Gentadoxycycline: prepared in-vetro by combination of Gentamycin and Doxycycline as stock solution (100μg / ml) and from it premedicated the different concentrations as follow: 10, 20, 40, 60 and 80μg /ml

**Statistical Analysis**

SPSS statistical package version 24 used for all the statistical analysis. The independent sample –f-test and one- way ANOVA (analysis of variance) used to find values between groups and comparing as a result of less than or equal 0.05 and 0.01 to find P values.

**RESULTS**

The activity of anti-bacterial which showed in a table (1) give highly significant differences (P≤0.05) of RJ at concentration 10mg/ml (1.12± 0.2)(Figure2 and 3). The antibiotic (Doxycycline) consider as superior of other antibiotics with MIC at 10 μg/ml that give highly significant differences (p≤0.05) (2.10±0.07) againstG-ve bacteria (*E. coli*) that found as sensitive to these bacteria, but the both antibiotics (Gentamicin and Gentadoxycycline) it doesn’t clear to show any significant differences. The *E.coli* give resistance at sensitivity tests of antibacterial activity of antibiotic (Gentamicin), begin from low concentration 10 μg/ml (1.62±0.10) until high concentration100μg/ml (2.35±0.03) with significant difference (p≤0.05). the same results appear for antibiotic (Gentadoxycycline) that prepare from combination of Gentamicin and Doxycycline, when not give any
significant differences (p≤0.05) after using the sensitive tests of the antibiotic at concentration 10 μg/ml (1.85±0.10) and 100 μg/ml (2.65±0.05) against *E. coli*.

Table 1: Inhibition zone diameter (by mm.) for different concentration of Royal Jelly and three antibiotics against *E. coli*

<table>
<thead>
<tr>
<th>Concentration of Royal Jelly (mg/ml)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
<td>I</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of Antibiotics (μg/ml)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>Aa</td>
<td>Aa</td>
<td>Ca</td>
<td>Da</td>
<td>Ea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Ac</td>
<td>Bc</td>
<td>Cc</td>
<td>Da</td>
<td>Ec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentadoxycycline</td>
<td>Ab</td>
<td>Ab</td>
<td>Bb</td>
<td>Bb</td>
<td>Db</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Any different in capital refer to significant differences between groups horizontally (P≤0.05), these values represented by Mean ± SE.
DISCUSSION

The antibacterial activity of RJ against *E.coli* show statistically significant difference and that due to presence of several substance in RJ that inhabit E.coli like Trans-10-hydroxy-2-decenoic Acid (10-HAD) and that agreed with many study which found the inhibition zone and activity of RJ against microorganism of 10-
HAD which found the activity of acid to inhibit the growth of G+ve bacteria (Micrococcus pyogenes) and G-ve bacteria (*E. coli*)[9]. Other investigation that founded the activity of RJ to microorganism and compare the antimicrobial potential that found the soluble fatty acids, these compounds contain high quantity of 10-HDA, and ether-insoluble fractions which included Royalisin. And when examined the effect of these fraction against different types of bacteria especially *E.coli*[10]. In this study we found the *E. coli* give a resistance to Gentamicin that may be due to several pathways of resistance which is can be caused by plasmid-associated synthesis of enzymes (for example, acetyl transferases, nucleotidylyltransferases, and phosphotransferases) that modify and inactivate aminoglycoside antibiotics. Each of these enzymes has its own aminoglycoside specificity and decreased uptake of drug when the oxygen-dependent transport system for aminoglycosides or porin channels are absent, and therefore, cross-resistance is not an invariable rule[11]. Antibiotic (Doxycycline) has high antibacterial activity against *E. coli* by numbering of inhibition zone among them (Gentamicin, Gentadoxycycline and RJ), due to this drug binds reversibly to the 30S subunit of the bacterial ribosome, thereby blocking access of the amino acyl-tRNA to the mRNA-ribosome complex at the acceptor site, and as broad-spectrum, bacteriostatic antibiotics, the tetracyclines are effective against gram-positive and gram-negative bacteria as well as against organisms other than bacteria[11,12]. Gentadoxycycline gives low activity against *E. coli*, where it is less than doxycycline and more than Gentamicin by the measurement of inhibition zone through agar well diffusion technique, because of Gentamicin is bacteriocidal effect of antibacterial while Doxycycline is bacteriostatic effect, both of them protein synthesis inhibitors and bind to the 30S subunit of the bacterial ribosome[12].

**CONCLUSION**

There is significantly activity of Royal jelly against growth of *E.coli*.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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