Some Immunological Evaluations of Propolis in Albino Male Mice

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Summary:
Propolis is a complex resinous bee product that has a wide range of biological activities. In the present investigation, two oral doses (10 and 20 mg/kg/day) of propolis (ethanol extract) were evaluated immunologically in albino male mice (80 animals) through three types of experiments. In the first, the propolis was tested alone, while in the second and third experiments, propolis was given before and after the immune suppressive drug MMC (pre- and post treatments, respectively). The three experiments were paralleled with three negative controls, in which the propolis was replaced with distilled water. In the first experiment, the dose 10 mg/kg of propolis enhanced the parameters investigated, and a significant increase was observed in the total count of leucocytes (10.7 vs. 7.8 x 10^3 cells/cu.mm.blood), lymphocytes (7.0 vs. 5.3 x 10^3 cells/cu.mm.blood), neutrophils (2.9 vs. 2.1 x 10^3 cells/cu.mm.blood), monocytes (0.5 vs. 0.3 x 10^3 cells/cu.mm.blood) and eosinophils (0.3 vs. 0.1 x 10^3 cells/cu.mm.blood), PI (15.2 vs. 10.8%), PFC (72 vs. 38%), AR (0.84 vs. 0.57 mm) and DTH (0.68 vs. 0.40) as compared to negative controls. Much more enhancements were observed in the dose 20 mg/kg. In the second and third experiments, a similar picture was drawn in the interaction of propolis (pre- and post-treatments) with MMC, in which the propolis extract was able to modulate the immune suppressive effect of MMC, and this was dependent on the type of treatment and dose, and again, the dose 20 mg/kg was more effective in this respect.

Key words and Abbreviations: Arthus reaction (AR), Delayed type hypersensitivity reaction (DTH), Mitomycin C (MMC), Phagocytic index (PI), Plaque forming cells (PFC), Propolis and Sheep red blood cells (SRBC).

Introduction:
Propolis, a traditional remedy since ancient times in many countries, is a complex resinous bee product with a physical appearance that varies widely. The colour may be creamy, yellow, green, light or dark brown. Some samples have a friable hard texture, while others may be elastic and gummy (1, 2). Chemically, more than 300 constituents have been identified in different propolis samples (3), and such wide variation is directly related to bud exudates collected by bees from various flowers (4, 5, 6, 7). Moreover, the constituents can be varied due to climate, season, location and year, and therefore its chemical formula is not stable (4, 8, 9). The most important pharmacologically active constituents in propolis are flavonoids (flavones, flavonols, and flavonones), phenolics and aromatics, but flavonoids are thought to account for much of the biological activity in propolis. Investigating the active components revealed that propolis is effective against some viruses, bacteria and fungi, moreover, other beneficial biological activities (i.e. anti-oxidants) have also been suggested (10, 11, 12, 13, 14, 15). Therefore, propolis has attracted the attention of many scientists since the late 1960's, and during the last four decades, investigations have been active in revealing the chemical composition, biological activity, pharmacology and therapeutical uses of propolis. However, its activity on immune response is not well-documented, and accordingly, the present study was designed with the aim to evaluate some immunological effects of a sample of Iraqi propolis in albino male mice.

Materials and methods
1. Extraction of Propolis
Propolis samples were collected from an Apiary located in Al-Tarmiya (a region 60 km north-east Baghdad) in different seasons during the period 2003-2004, and stored at 4°C. For the purpose of extraction, one gram of propolis was cut into small pieces, and extracted at room temperature with 50 ml of 70% ethanol using ultrasonic bath (Decon FS 300, England) for 90 minutes. Then, the ethanol extract was evaporated at 50°C until dryness, and the resulted deposit was dissolved in distilled water to prepare the doses 10 and 20 mg/kg (16).

2. Laboratory Animals
Albino male mice (Mus musculus) were the tested animals, which were 9-10 week old at the beginning of experiments, and their weight was 21 ± 3 gram. They had free excess to water and food (ad libitum) during experiments.
total number of animals was 80 mice.

3. Laboratory Methods
Total and differential counts of leucocytes, phagocytic index (PI), plaque forming cells (PFC), Arthus reaction (AR) and delayed type hypersensitivity reaction (DTH) were the parameters of immunological evaluations. The PI was assessed using peritoneal phagocytes, and the target of phagocytosis was heat-killed yeast (*Saccharomyces cervisiae*). To carry out the PFC, AR and DTH, the animals were immunized intraperitoneally with 0.1 ml of 5% sheep red blood cells (SRBC), and further two booster doses were given in days 5 and 9. In day 12, the left foot pad was injected with 0.1 ml of 5% SRBC. At the same time the right foot pad was injected with phosphate buffer saline in a similar manner. Four hours later, the thickness of both pads was measured using a vernier, and the difference represented AR index. Twenty-hours later the measurement was repeated to assess the DTH index. After that, the animal was dissected and the spleen cells were obtained, and incubated with SRBC in agarose gel in vitro at 37°C. An hour later, a diluted (1:10) guinea pig serum was added, and a further incubation for 30 minutes was carried out to visualize the spleen cells that have produced anti-SRBC antibody. These cells (PFCs) were recognized by a zone of lysis, and their percentage represented the PFC index. Detailed of these methods are presented by Hudson and Hay (17).

4. Experimental Design
Two oral doses of propolis extract were investigated (10 and 20 mg/kg/day). These doses were evaluated to test their effect in the animals for the investigated parameters through three types of experiments. In the first, the animals were given a daily single dose of propolis for seven days, and in day 8, they were dissected to assess total and differential counts of leucocytes and PI. For this experiment, negative (dosed with distilled water) and positive (dosed with the immune suppressive drug mitomycin C; MMC, 5rng/kg) controls were included. In the second, propolis was given for six days (single dose/day), followed by a single dose of MMC in day 7 (pre-treatment). In the third, a single dose of MMC was given in day 1, while a single dose/day of propolis was given in the next six days (post-treatment). In day 8, the animals were immunized with SRBC to assess AR, DTH and PFC. It is worth to mention that day 8 represents day 1 in the immunization protocol described in the forthcoming section of laboratory methods. The latter two experiments were paralleled with two control groups, in which the propolis was replaced with distilled water for each experiment.

5. Statistical Analysis
The data were presented in terms of means ± standard errors (S.E.), and significant differences between means were assessed by the least significant difference (LSD) using the computer programme SPSS.

Results:
The 10 mg/kg dose of propolis elevated significantly the count of leucocytes (10.7 vs. 7.8 x 103 cells/cu.mm. blood), lymphocytes (7.0 vs. 5.3 x 103 cells/cu.mm. blood), neutrophils (2.9 vs. 2.1 x 103 cells/cu.mm. blood), monocytes (0.5 vs. 0.3 x 103 cells/cu.mm. blood) and eosinophils (0.3 vs. 0.1 x 103 cells/cu.mm. blood) in the treated animals compared to negative controls. The 20 mg/kg dose of propolis behaved in a similar manner, but the enhancement of these counts was much better (Table 1).

The negative controls showed a PI of 10.8%, which was significantly higher than the corresponding value in the positive controls (6.3%), however, a much more significant increase was observed in the 10 and 20 mg/kg doses of propolis (15.2 and 18.4%, respectively) (Table 1).

Both types of hypersensitivity reactions (AR and DTH) scored a significant increased response in the doses 10 (0.84 and 0.68 mm, respectively) and 20 (0.90 and 0.68 mm, respectively) mg/kg of propolis, as compared to the negative (0.57 and 0.40 mm, respectively) and positive (0.42 and 0.32 mm, respectively) controls (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative Controls</th>
<th>Propolis (10mg/kg)</th>
<th>Propolis (20mg/kg)</th>
<th>Positive Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean + Standard Error</td>
<td></td>
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</table>
Giving propolis before MMC (pre-treatment) modulated the immune suppressive effect of MMC, especially the dose 20 mg/kg, which enhanced the investigated parameters (Table 2).

### Table 2: The effect of propolis-MMC interaction (pre-treatment) on some immunological parameters in albino male mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Leucocyte Count x 10^6 (cell/cu.mm.)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10.5 ± 0.28 a</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>6.8 ± 0.15 a</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.1 ± 0.08 a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.4 ± 0.10 a</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.2 ± 0.03 a</td>
</tr>
<tr>
<td>Phagocytic (%)</td>
<td>14.00 ± 0.8 a</td>
</tr>
<tr>
<td>Plaque Forming Cell (%)</td>
<td>69.00 ± 3.3 a</td>
</tr>
<tr>
<td>Arthus Reaction (mm)</td>
<td>0.84 ± 0.04 a</td>
</tr>
<tr>
<td>Delayed Type Hypersensitivity (mm)</td>
<td>0.58 ± 0.04 a</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>8</td>
</tr>
</tbody>
</table>

Different letters in the same raw: Significant difference (P < 0.05)

A similar augmentation was drawn when the propolis extract was given after MMC (post-treatment), and again the dose 20 mg/kg was the most effective in this regard (Table 3).

### Table 3: The effect of propolis-MMC interaction (post-treatment) on some immunological parameters in albino male mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Leucocyte Count x 10^6 (cell/cu.mm.)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.7 ± 0.34 a</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>5.5 ± 0.22 a</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.0 ± 0.2 6 a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.1 ± 0.01 a</td>
</tr>
</tbody>
</table>
Some Immunological Evaluations of Propolis in Albino Male Mice
Ali Gassan Majed

Chinese origins, in which, it has been suggested confirmed using propolis samples of Brazilian and due to its anti-radical activity. This view has been anti-oxidants, and its anti-oxidants properties are aromatic products are anti-oxidants (22, 23). For this reason, they are flavonoids, phenolics and pharmacologically active constituents of propolis terms of chemical constituents. The most important intensive research, and the effects are explained in biology of plant natural products and their chemistry of plant natural products and their cure different ailments (21). Therefore, the food and shelter but also served the humanity to health care of its people. They not only provided about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people. They not only provided food and shelter but also served the humanity to cure different ailments (21). Therefore, the chemistry of plant natural products and their biological effects have been the potential of intensive research, and the effects are explained in terms of chemical constituents. The most important pharmacologically active constituents of propolis are flavonoids, phenolics and aromatics, and it is well known fact that these products are anti-oxidants (22, 23). For this reason, propolis is considered as being a natural source of anti-oxidants, and its anti-oxidants properties are due to its anti-radical activity. This view has been confirmed using propolis samples of Brazilian and Chinese origins, in which, it has been suggested that some propolis compounds are absorbed in the intestine and enter the blood circulation, where they act as hydrophilic anti-oxidants and increase tissue concentration of vitamin C (24, 25). Both actions have the potential to enhance the immune functions. These effects have been further questioned, but in terms of cytokines, which are the main directors of immunological programmes, and can potentate the immune response positively and/or negatively. However, this subject is better understood if considers the cells that produce cytokines. It has been demonstrated that propolis increases the ratio of CD4+/CD8+ cells, which are the main producer of these cytokines, and an increase in their ratio is in favour of immune enhancement (19, 26, 27).

In agreement with the forthcoming findings, the leucocyte count was significantly increased in all groups of propolis-treated mice, an observation which suggests that propolis stimulate tissue generation in mammals (28, 29). Such suggestion has been confirmed in vitro in cultured cells by other group of investigators. The total count of leucocytes gives an overall picture of the immune system function, but the differential count may specify some functions. The neutrophils and monocytes were significantly increased. These two types of cells are involved in an important innate immune function that is phagocytosis. The PI was significantly increased; therefore propolis affected these cells numerically and functionally. These results have been encouraged to explain the anti-tumour effect of propolis by modulating the production of some complement components and cell-surface markers, and the effect has been ascribed to the production of two cytokines (IL-1 and 11-2) (19, 30).

To investigate the humoral and cellular immune responses of the acquired immune system in mice-treated with propolis, AR and DTH were respectively determined. Both assays were in favour of an immune stimulation. Such findings have been confirmed in chickens infected with viruses, and treated with some bee products. The AR is an antibody-mediated phenomenon, and the antibody production is carried out by activated B-lymphocytes (31, 32). While, DTH is mediated mainly by T-lymphocytes. The lymphocyte count in the present study was significantly increased, but unfortunately, it was

| Eosinophils | 0.1 ± 0.01 a | 0.3 ± 0.10 b | 0.3 ± 0.02 b |
| Phagocytosis (%) | 10.00 ± 0.7 a | 14.00 ± 0.8 b | 15.40 ± 0.5 b |
| Plaque Forming Cell (%) | 42.81 ± 3.2 a | 61.60 ± 7.6 b | 61.20 ± 4.7 b |
| Arthus Reaction (mm) | 0.48 ± 0.02 | 0.68 ± 0.04 b | 0.72 ± 0.04 a |
| Delayed Type Hypersensitivity (mm) | 0.50 ± 0.02 a | 0.57 ± 0.05 a | 0.62 ± 0.02 a |

Number of Animals

8 8 8

Different letters in the same raw: Significant difference (P < 0.05)
not possible to characterize these cells in terms of their types. In the present study, it was found that PFCs were significantly increased in mice treated with propolis extract, and such increase was evident in the three investigated groups. This result is in agreement with Scheller et al. investigation (33). However, the PFC assay strongly supports the findings of AR.

In conclusion, propolis may be considered as an immune stimulating agent, as it enhanced the function of mouse immune system, and it was also able to modulate the immune suppressor effect of MMC. However, it is too early to generalize such findings, and further investigations are required to understand the immunological effects of propolis. These investigations must concentrate to understand the profiles of cytokines, and to define the lymphocytes in terms of their CD markers.

References:


