Comparative Activity of *Peganum harmala* Seeds Extract and Rifampicin Against *Brucella abortus* Experimentally Infection in Mice

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**ABSTRACT**

Forty two mice were divided into six groups which were treated with Rifampicin for successive three days and water extract of *Peganum harmala* for six days, then inoculated with $5 \times 10^5$ CFU/ML of *B. abortus* all animals sacrificed after 21 days and concern on total white blood cells count, spleen and liver indices, delayed type hypersensitivity reaction and *Brucella* enumeration in spleen.  

The result showed a significant increasing of white blood cells, delayed type hypersensitivity and prophylactic index in comparison with infected alone.

**INTRODUCTION**

*Brucella abortus* is a facultative intracellular bacterium that infects humans and domestic animals. *Brucella* replicates in host mononuclear phagocytes, and survival in phagocytic cells allows the bacterium to escape the extracellular mechanism of host response such as complement and antibodies (1). *Brucella* are frequently able to survive and multiply in these cells because they inhibit the bactericidal myeloperoxidase-peroxide-halide system by releasing 5′-guanosine and adenine (2).

*Brucella* are transport into the lymphatic system and may replicate there locally they, also may replicate in the kidney, liver, spleen, breast tissue or joints, causing both localized and systemic infection granuloma may accompany extracellular replication of the bacteria especially in the liver and spleen (3). CD$^+$ T-cells play an important role in the protection against *Brucella* infection, either by activating CD$^+$ T-cells or secreting cytokines that mediate macrophages activation among these was interferon-γ (4). Immunopotentiation of the body immune system is obtained from mice treated with interferon pass a good immunological effect (5). Some studies demonstrated the ability...
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of *Peganum harmala* seed extract to induce immunostimulation against bacteria, fungi and parasites (6) in this study *P. harmala* water extract was used versus the treatment with Rifampicin the drug were assessed for their ability to eradicate bacteria from the spleen a target organ in *Brucella* infection.

**MATERIALS AND METHODS**

**Animals:**
Males of BALB/c mice, 6-8 Weeks of ages were obtained from institute for Embryos and Infertility Research, Al-Nahrain University.

**Drugs:**
Rifampicin (Misson viva care limited) was dissolved in sterilized water and orally administrated by gavage in a dose of (4.2 mg/kg) for three successive day's.

**Bacteria:**
*Brucella abortus* Biotype-1 was used in infection experiment.

*P. harmala* extract.
Water extract: of harmala seed prepared as described by Adaay et al., 1989 (7) and given in a dose of 500 µg/20 gm for six successive day's according to (8).

**Experimental Protocol:**
The animals were divided into six groups with seven animals in each group as in the following:

1- Group of animals treated with Rifampicin.
2- Group of animals treated with Rifampicin and infected with *Brucella*.
3- Group of animals treated with *P. harmala*.
4- Group of animals treated with *P. harmala* and infected with *Brucella*.
5- Group of animals treated with 1 ml of sterilized PBS and served as a Positive control.
6- Group of animals treated with 1 ml of sterilized PBS and served as a negative control.

All groups, except treated alone and negative control were inoculated intraperitoneally with 5x10^5 CFU/ml of *Brucella* according to method of (4).

All groups of animals were sacrificed after 21 days post infection and concern on the following parameters according to Oliveira *et al* (9)

1- Total count of white blood cells
2- Spleen and liver indices
3- Delayed type hypersensitivity.
4- *Brucella* enumeration in spleen.

**Total and differential count of white blood cells:**

Peritoneal fluid cellular influx, after treatment with *P. harmala* and infection with *Br. abortus* was differentially enumerate. thick smear were stained with Giesmsa, than lymphocyte, neutrophil, monocytes, and Eosinophils were differentially counted according to Gravey *et al* (10).

**Spleen and Liver indices:**

All animals were weighed at the end of experiment time their spleen and liver were aseptically removed and weighed. The Organ Index was calculated according to the following equation (10).

\[
\text{Organ Index} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 1000
\]

**Delayed type hypersensitivity reaction:**

*Brucella* allergens were prepared according to (9) using Rough *Br. abortus*. Then, the concentration were 60 µg/ml, then mixed with equal volume of complete Freunds adjuvant, mice were injected with 0.2 ml of *Brucella* allergen in the left leg. The right leg was injected with 0.2ml PBS as a control, the foot pad thickness was measured with a Vernier Caliper after 24 hr, the difference in the thickness between the *Brucella* allergens injected foot and control as delayed type hypersensitivity.

**Brucella enumeration in the Spleen and Prophylactic Index:**

One mg of the spleen was individually homogenized in the sterilize PBS and each prepared volume was seeded on Trypticase Soy agar (Difco) plate *Brucella* were enumerated after six days at 37°C in 5% CO₂ according to (11). The prophylactic index was calculated according to this formula:

\[
\text{Prophylactic Index} = \frac{\text{No. of colony in treated and infected group}}{\text{No. of colony in untreated infected group}} \times 100
\]

**Statistical analysis**

ANOVA test was used to compare the results.
RESULTS AND DISCUSSION

I: Total White Blood Cell and Differential Count:
Table (1) shows the changes in white blood cells count in animals treated with Rifampicin, *P. harmala* and infected with *Brucella*. The numbers of all treated groups were significantly increased (p<0.05) it reached to 8280, 9500 cells /cumm in groups treated with Rifampicin and *P. harmala* seeds extract respectively. Also the number of white blood cells was increased in groups treated with Rifampicin, *P. harmala* seeds extracts and infected with *Brucella* which reached to 8100 , 8890 cells /cu mm respectively.

II: Weight of spleen and liver and their Indices.
Table (2) shows the changes in weight and indices for spleen and liver in animals post infection. The liver Index was 6.32 , 7.65 in groups treated with Rifampicin, *P. harmala* and infected, 5.37, 7.38 in groups treated with Rifampicin and *P. harmala* uninfected then untreated control (7.80) and negative control (5.42).
The splenic Index was 2.62 and 1.90 in groups treated with Rifampicin and *P. harmala* and infected, 1.05 and 1.60 in groups treated with Rifampicin and *P. harmala* uninfected with *Brucella* respectively, in infected untreated control (1.20) and negative control (1.0).

III: Estimation of delayed type hypersensitivity reaction.
Table (3) shows the changes of delayed type hypersensitivity in groups treated with Rifampicin, *P. harmala* extract and infected with *Brucella*. It which reach 0.41 mm in comparison with treated uninfected group 0.32 and 0.32 mm respectively while the positive and negative control reached 0.26 and 0.25 mm respectively.

IV: Total Colony count and Prophylactic Index.
Table (4) shows the changes in number of colony count. The number was significantly decreased (P<0.05) in both groups treated with Rifampicin and *P. harmala* until they reached to 58.70 and 89.4 x 10⁴ CFU/ml in comparison with the a positive control 290 x 10⁴ CFU/ml.
Table -1: Total and differential counts of white blood cells in mice treated with Rifampicin and *P. harmala* seeds water extract and infected with *B. abortus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Count ± SD</th>
<th>Differential count ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutrophils</td>
</tr>
<tr>
<td><strong>Rifampicin treatment</strong></td>
<td>8280 ± 300</td>
<td>65 ± 0.7</td>
</tr>
<tr>
<td><strong>Rifampicin treated and infected with Brucella</strong></td>
<td>8100 ± 40</td>
<td>61 ± 2.4</td>
</tr>
<tr>
<td><strong>P. harmala treatment</strong></td>
<td>9.500 ± 218</td>
<td>89 ± 1.4</td>
</tr>
<tr>
<td><strong>P. harmala treated and infected with Brucella</strong></td>
<td>8890 ± 301</td>
<td>75 ± 2.0</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>8720 ± 104</td>
<td>63 ± 0.9</td>
</tr>
<tr>
<td><strong>Negative control</strong></td>
<td>7940 ± 212</td>
<td>54.4 ± 1.6</td>
</tr>
<tr>
<td><strong>LSD</strong></td>
<td>810</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Table -2: Spleen and liver indices of mice treated with *P. harmala* seeds water extract and Rifampicin then infected with *B. abortus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spleen Index</th>
<th>Liver Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rifampicin treatment</strong></td>
<td>1.005</td>
<td>5.73</td>
</tr>
<tr>
<td><strong>Rifampicin treated and infected with Brucella abortus</strong></td>
<td>2.62</td>
<td>6.32</td>
</tr>
<tr>
<td><strong>P. harmala treatment</strong></td>
<td>1.60</td>
<td>7.38</td>
</tr>
<tr>
<td><strong>P. harmala treated and infected with Brucella abortus</strong></td>
<td>1.90</td>
<td>7.65</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>1.20</td>
<td>7.80</td>
</tr>
<tr>
<td><strong>Negative control</strong></td>
<td>1.00</td>
<td>5.42</td>
</tr>
<tr>
<td><strong>LSD</strong></td>
<td>0.45</td>
<td>0.79</td>
</tr>
</tbody>
</table>
Table -3: Effect of *P. harmala* seeds water extract and Rifampicin on delayed type hypersensitivity elicited in the foot pad swelling of mice treated and infected with *B. abortus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DTH Mean ± SD</th>
<th>DTH Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin treatment</td>
<td>0.32 ± 0.10</td>
<td>48</td>
</tr>
<tr>
<td>Rifampicin treated and infected with <em>Brucella abortus</em></td>
<td>0.36 ± 0.07</td>
<td>53</td>
</tr>
<tr>
<td><em>P. harmala</em> treatment</td>
<td>0.31 ± 0.03</td>
<td>38</td>
</tr>
<tr>
<td><em>P. harmala</em> treated and infected with <em>Brucella abortus</em></td>
<td>0.41 ± 0.19</td>
<td>82</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.26 ± 0.15</td>
<td>4.2</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.25 ± 0.04</td>
<td>0</td>
</tr>
<tr>
<td>LSD</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

Table -4: Total Colony Count (x10^4) CFU/ml and prophylactic index in mice treated with *P. harmala* seeds water extract and Rifampicin then infected with *B. abortus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD CFU/ml</th>
<th>Prophylactic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin treatment</td>
<td>58.70 ± 7.9</td>
<td>79.75</td>
</tr>
<tr>
<td><em>P. harmala</em> treatment</td>
<td>89.4 ± 7.5</td>
<td>69.17</td>
</tr>
<tr>
<td>Positive control</td>
<td>290 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

The results of the experimental study with *P. harmala* showed immune enhancing activities for murine immune system which included multiple pathways. Among these was its ability to stimulate lymphocyte and other subset of white blood cells (Table 1). The activity of stimulation occurs by stimulating macrophages to secrete amonokines (8). In this study the results demonstrated that the spleen and liver weight were reduced to nearly normal weight when the infected animal treated with Rifampicin and *P. harmala* seed water extract. These results are in agreement with Oliveria (9) who treated mice with ribosomal vaccine.

Also the results are consistent with Mohammed (12) who treated mice infected with *Br. abortus* using Immunoferon daily, for six days.

*Brucella* colony enumeration in spleen (Table 4) indicated an increase in the prophylactic index in groups treated with Rifampicin and *P. harmala* water extract due to inhibitory effect of Rifampicin to DNA dependent RNA synthesis as it has bacteriocidal effect to brucellosis (13).

The increase in spleen and liver weight or size in untreated animals is correlated with proliferation of *Brucella* in the reticulo-endothelial system. Similar results were reported by Vitas *et al* (14) when treated *Brucella* infection mice with outer membrane protein in mice.
The low level of bacterial specific delayed type hypersensivity reaction is correlated with disease progression in mice. This may be due to its ability to suppress the cellular coordination (2). While groups treated with Rifampicin and \textit{P. harmala} expressed delayed type hypersensivity.

This indicate that \textit{P. harmala} extract has the ability to activate the macrophages and increase proliferation of the lymphocyte and induce a secretion of lymphokines (8).

REFERENCES
