The Protective Effects of Achillea fragrantissima on Immune Response in Mice Model: A Pilot Study

Abbas M. Al-Sarra1, Yasmine A. Hussien1, Yahiya Ibrahim, Yasmeen Y. Ibrahim, Hayder A. Al-Aubaidy2
1College of Pharmacy, University of Alkafeel, Al-Najaf, Iraq
2School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Bundoora, VIC, Australia 3086

Correspondence Author E-mail: yasmeen.alamri@alkafeel.edu.iq

Article History: Submitted: 26.01.2020 Revised: 25.03.2020 Accepted: 11.04.2020

ABSTRACT
The main objective of the present research is to assess the effects of Achillea fragrantissima on humoral & cellular immunity in the rat model. Achillea fragrantissima flowers are subject to much biochemical analysis with the aim of extracting fatty acids, whether saturated or unsaturated. The lethal dose 50 of the oil extract was 192.5 mg/kg under the surface of the skin, and 687.8 mg/kg oral and a total of 120 mice were included in this research as this group was divided into three study groups (i) intraperitoneal injection of Achillea fragrantissima oil extract at 10, 20 and 40 mg/kg of body-weight, (ii) day for 10 days, respectively. Group IV has been injected with propylene glycol and used as an mgr Ah control. There was significant improvement in the hem agglutination index in the 3 studied groups when comparing to the control-group (P<0.05). This was associated with a reduction in the feet swelling difference (P<0.01), and a significant increase in the spleen weight (P<0.01), in the 3 studies-groups compared with controlling-group. These effects appeared to be dose dependent. Achillea fragrantissima oil extract appears to possess immunoprotected effects (both humoral and cellular immunity) in mice model.

Keywords: Achillea fragrantissima; oil extract; Hem agglutination index; Feet swelling difference; Spleen weight.

Correspondence: Yasser Ibrahim, Yahiya College of Pharmacy, University of Alkafeel, Al – Najaf Iraq
E-mail: yasmeen.alamri@alkafeel.edu.iq
DOI: 10.31838/srp.2020.4.35

INTRODUCTION
In this research, clinical immunosuppressant have been used extensively to treat many immune disorders in order to inhibit the immune response [1], and this type of inhibitor is considered the most widely used, especially in circumstances of organ-transplantation, and the prevention of degenerative illness in children and in the manipulation of auto-immune disorders [2]. The group of drugs has been divided into four main types, in terms of the nature and mechanism of their movements [3]: the adrenal cortex, stimulants, antibody reagents, add chemical medications to this, as it is workable for these medications to forestall isolating cells, and lessen the absolute number of little lymphocytes Antibodies [4] are hindered, consequently deferred extreme touchiness and the amalgamation of immunoglobulin Gs (IgGs). Additionally we might want to call attention to that there is a connection between the viability of the calming medication and its immunosuppressive impact, for instance in corticosteroids, it has the two impacts [5]. In the existing research, Achillea fragrantissima oil extract has been used as an immunosuppressant drug. It contains 83% volatile oil, from four to five % tannins and an amount of flavonoids. Four types of flavonies have been separated from extracts of metabolic plants, and lactones have also been isolated in the genus of this plant [6]. The desert is growing and has been used as a medicinal plant for many years. Achillea contains around 130-140 species of perennial plants worldwide [7]. In addition, volatile oil can be used as an effective broad-spectrum antimicrobial [7-8] and plant extracts to reduce fever, headache and weakness [8] and has been extracted from a number of previous studies, this plant can develop neurodegenerative diseases [9] and have anti-cancer activities [10]. In addition, it was reported that the gaseous forms of A. fragrantissima have insecticidal and rodenticidal activities but when inhaled can have a soothing effect to treat bronchitis and spasms [11], Furthermore, the anti-inflammatory, antioxidant and anti-proliferative effects of A. fragrantissima were confirmed by different pharmacological studies [12-13]. Moreover, anti-protozoal activities were demonstrated towards Trypanosome evansi [14]. It was additionally demonstrated to be powerful in diminishing blood glucose levels in diabetes [15] and treating intense irritation, ailment and joint pain [16]. The significant oil removed is viable against and microscopic organisms and regarded growths [17]. The present paper aims to evaluate the immune protective effects (both humoral and cellular immunity) of Achillea fragrantissima mice model.

MATERIAL AND METHODS
Previous studies showed that the fragrantissima flowers contain tannin sglycosidesaponins-resins-alkaloids and coumarins in different concentrations [18-19].

Compliance with Ethical Standards
In the current study, animals were kept in the animal home, at the University of Kafeel, at a controlled temperature (25 ± 20 °C) and humidity (60-65%), light 12H alternately: 12 dark sessions and mice were fed the standard Chao diet with water and were approved On study by the Institutional Animal Care and Use Committee (IACUC - 2018/11) at Al-Kafeel University, Najaf, Ashrafieh, Iraq.

Preparing fatty extraction and determining its acids
Oil extraction to determine the composition of the saturated ad unsaturated fatty acids were performed after drying and extracting the A. fragrantissima flower using Ether and Soxhlet extractor at 40°C, using rotating-evaporator and dissolving the extraction in propylene-glycol.
Measurement of lethal dose 50% (LD 50)

Oral admission & sub-cutaneous intromission method were used to determine LD50 (21). The animals which died were computed in each group within a complete day hours.

Study Protocol

120 mice were divided into three study groups and one control group (30 mice in each group). Study groups (1, 2, and 3) were injected with A. fragrantissima oil extract at (10, 20 & 40 mg/kg body-weight, respectively), two per day for 10 days.

The extract and its effect on humoral-immunity

Red blood cells were extracted for sheep and used as an antigen after washing three times, adding ten cells to the brine and injecting them into all groups 3 days after extraction injection and after 10 days, then collecting samples and isolating sera furthermore, presented to a temperature of 56 °C for 30 minutes. Then weaken the serum shortcoming with 0.5 ml of saline arrangement and set in the northern vessels and 0.5 ml of red platelets washed at 5% per opening and blended and brood for 3 hours at 37 °C

Hem-agglutination was computed via measurement of its score from [22]:

\[
\text{Index} = \frac{\left(\zeta_1 + \zeta_2 + \ldots + m \zeta_n \right) r}{\left(\zeta_1 + 2\zeta_2 + \ldots + m \zeta_n \right) \rho}
\]

\(m\) : Dil - Exp

\(\tau\) : Treated

\(\zeta\) : Agglutin Scores

\(\rho\) : Controls

Extraction and its effect on cellular-immunity

Treatments of the delayed-hyper-sensitivity, forty rats were classified to 4-groups. The three first-groups were administered with ten-twenty-forty mg/kg in the pertoneum for 10 days (2/day), the 4th group was added with propylene glycol as a control-group, and then sheep-red-blood cells were administered with a dose of ten by eight in half ml of Sal-Sol on the R-foot of the R-sub-cutaneous of the posterior leg, on the final-day of the extraction administration, while the L-foot was administered with half ml of Sal-Sol, after complete day hours, the reaction was computed by Vernier and the variance among the swelling in the right-foot and the left-foot was computed [23].

Extraction and its effect on spleen-weight

Four groups of mice were separated into 4-groups, as the first-three groups received by administration doses of extracting ten-twenty-forty mg/kg of body-weight, for 10-days, while the control-group received the propylene glycol injection after that it received All groups were Red-Blood-Cells at a dosage 10x9 (2/day) from the 4th day of the extraction the injection until the 10th day after that, the rats were killed, their spleen weighed, and the spleen weight was calculated in milligrams/100 grams [24].

Statistical analysis

Data analysis has been carried out by using SPSS program. All values were expressed as mean ± SEM. A linear-model using 2 ways ANOVA followed by potshot analysis was carried out to search significant-effects of intervention on mean-changes in the variables between the 3 studied groups, results will be considered significant if P equal or < than 5%.

RESULTS

Through experimentation and analysis, it turns out that the extraction was acidic at a rate of 5.3. The proportion of the extraction about 4.8% of dry-weight, 100-fracture was 65%, and the results showed that the proportion of un-infected were 35%, saturated-fatty-acids 54.13%, un-saturated-fatty-acids 45.87%. The following table (1) shows the percentage of individual saturated and Tran’s fatty acids table.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic</td>
<td>3.582</td>
</tr>
<tr>
<td>Capric</td>
<td>3.125</td>
</tr>
<tr>
<td>Lauric</td>
<td>5.261</td>
</tr>
<tr>
<td>Myristic</td>
<td>4.379</td>
</tr>
<tr>
<td>Palmitic</td>
<td>32.346</td>
</tr>
<tr>
<td>Stearic</td>
<td>5.231</td>
</tr>
<tr>
<td>Arachidic</td>
<td>4.231</td>
</tr>
<tr>
<td>Palmitolec</td>
<td>2.135</td>
</tr>
<tr>
<td>Oleic</td>
<td>18.246</td>
</tr>
<tr>
<td>Linoleic</td>
<td>22.851</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>0.882</td>
</tr>
</tbody>
</table>

Through the toxicological study, it was found that the toxic LD50 study by mouth was 687.8 mg/kg B/W and sub-cutaneous injection was 192.5mg/kg B/W. The results also showed the effect of the extraction on humoral-immunity had clear-effects in reduction edge-agglutination index by an average of 0.31/0.42/0.95 for each of the treated-groups at a rate of 10,20,40 mg/kg B/W , table (2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Hemagglutination index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated groups injected</td>
<td>10 mg/kg</td>
<td>0.95 _+ _0.08</td>
</tr>
<tr>
<td>with extract</td>
<td>20 mg/kg</td>
<td>0.42 _+ _0.07</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>0.31 _+ _0.09</td>
</tr>
<tr>
<td>Control group</td>
<td>Propylene glycol</td>
<td>1.21 _+ _0.07</td>
</tr>
</tbody>
</table>

Also we conclude doses had apparent effects in reduction differences in swelling of the feet, see table (3).
By the analysis and studying the effect of the extract in the spleen-weight, it was noticed that the treated groups means were 255.8/269.9/282.8 mg/100g B/W, see table (4).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Spleenweight inmg/100g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated groups injected with extract</td>
<td>10 mg/kg</td>
<td>265.8, 2.73</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>265.9, 2.33</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>282.8, 3.13</td>
</tr>
<tr>
<td>Control group</td>
<td>Propylene glycol</td>
<td>220.7, 3.64</td>
</tr>
</tbody>
</table>

**DISCUSSION AND CONCLUSION**

A summary of the research paper and the current study are only an experimental study in the animal model to assess the effect of the A. Fragrantissima plant on measuring and evaluating the extent of the immune response.

1. The results showed, through analyzing these results, that the extraction had apparent effects.
2. The results and analysis showed that the extract showed the effect of decreasing in the hemagglutination index.

**The reasons behind the conclusions drawn**
- Due to the existence of stimulants, as these have the ability to cause quick and severe decreasing lymphocytes.
- Existence of some flavonoids in this plant.

**ACKNOWLEDGEMENTS**

Authors would like to acknowledge the technical support received from the staff at the La Trobe University, Bundoora, VIC, Australia (Dina A. Jamil) and College of Pharmacy, University of AIKafeel to facilitate the progress of this project.

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