Sys Rev Pharm 2020; 11(4): 243 – 246
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

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

Abbas M. Al-Sarraf[1], Yasmeen Ali Hussien[1*], Yahiya Ibrahim Yahiya[1], Hayder A. Al-Aubaidy[2]
[1]College of Pharmacy, University of Alkafeel, Al-Najaf, Iraq
[2]School 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 (peritoneal injection of Achillea fragrantissima oil extract at 10, 20 and 40 mg/kg of body-weight, (2/day for 10 days), respectively. Group IV has been injected with propylene glycol and used as an mg 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 Yahiyaa
College of Pharmacy, University of Alkafeel, Al – Najaf
Iraq
E-mail: Yasmeen.alamri@alkafeel.edu.iq

DOI: 10.31838/srp.2020.4.35

@Advanced Scientific Research. All rights reserved

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 flavones 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.

MATERIALS AND METHODS

Previous studies showed that the fragrantissema flowers contain tannin sglycosidessaponins-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% (LD50)

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]:

$$\text{Index} = \left[\frac{(\zeta_1 + \zeta_2 + ... + \text{m}\zeta_\text{n})\tau}{(\zeta_1 + 2\zeta_2 + ...\text{m}\zeta_\text{n})\rho}\right]$$

m : Dil - Exp

τ : Treated

ζ : Agglutint Scores

ρ : 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 4thgroup 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 at 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.

Acid	Percentage %
Caprylic	3.582
Capric	3.125
Lauric	5.261
Myristic	4.379
Palmitic	32.346
Stearic	5.231
Arachidic	4.231
Palmitoleic	2.135
Oleic	18.246
Linoleic	22.851
Arachidonic	0.882

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).

Groups	Doses	Hem agglutination index
Treated groups injected with extract	10 mg/kg	0.95 +_0.08
	20 mg/kg	0.42 _+ 0.07
	40 mg/kg	0.31 _+ 0.09
Control group	Propylene glycol	1.21 _+ 0.07

Also we conclude doses had apparent effects in reduction differences in swelling of the feet, see table (3).

Groups	Doses	Feet Swelling differences
Treated groups injected with extract	10 mg/kg	0.58 _+ 0.13
	20 mg/kg	0.45 _+ 0.18
	40 mg/kg	0.37 _+ 0.09
Control group	Propylene glycol	0.92 _+ 0.19

By the analysis and studying the effect of the extraction in the spleen-weight, it was noticed that the treated groups means were255.8/269.9/282.8mg/100g B/W, see table (4).

Groups	Doses	Spleen weight in mg/100 g body weight
Treated groups injected with extract	10 mg/kg	255.8_+ 2.72
	20 mg/kg	269.9_+ 2.23
	40 mg/kg	282.8_+ 3.13
Control group	Propylene glycol	220.7_+ 3.64

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 hem agglutination 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 AlKafeel to facilitate the progress of this project.

REFERENCES

1. Bach, J.F., Immunosuppressive therapy of autoimmune disease, Trendes pharmacol, Sci., 1993; 14: 213-216.
2. Barry, J.M., Immunosuppressive drugs in renal transplantation, A review of regimens, Drugs, 1992; 44: 554-566.
3. Goodman, L.S. and Gilman, A., The pharmacological Basis of Therapeutics, 13th ed., Mc Graw –Hill, New York, 2017.
4. Winkeistein, A. Principles of immunosuppressive therapy, Bull-Rheum, Dis, 1971; 21:627.
5. Jabs DA, Rosenbaum JT, Foster S., Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel, Am J Ophthalmol.2000; 130: 492-513.
6. Rakhmankulov, U. Sh.; Kasymov, Z.; Sidyakin, G.P., The presence of gamma lactones in species of family Asteraceae of flora of Soviet central Asia RASTIT RESUR. 1979; 15(1): 79-89.
7. Nemeth, E., Achillea species used medicinally in Hungary, Israel Journal of Plant Science, 2010, 58, pp.279-289.
8. Soltan, M.M., Zaki, A.K., Antiviral screening of forty-two Egyptian medicinal plants, Journal of Ethno pharmacology, 2009;126, 102–107.
9. Elmann, A., Mordechay, S., Erlank, H., Telerman, A., Rindner, M., Ofir, R. Antineuroinflammatory effects of the extract of Achillea fragrantissima, BMC Complementary and Alternative Medicine, 2001;11, 98.
10. Choucry, M.A. Chemical composition and anticancer activity of Achillea fragrantissima (Forssk.) Sch. Bip, (Asteraceae) essential oil from Egypt, Journal of Pharmacognosy and Phototherapy, 2017;9(1), pp.1-5.
11. Hifnawy MS, Rashwan OA, Rabeh MA., Comparative chemical and biological investigations of certain essential oils belonging to families Asteraceae, Lamiaceae and Graminae, Bull Fac Pharm Cairo Univ, 2001;39: 35-53.
12. Eissa TF, González-Burgos E, Carretero ME, Gómez-Serranillos MP, Compositional analysis and in vitro protective activity against oxidative stress of essential oils from Egyptian plants used in traditional medicine, Nat Prod Commun. 2014;9(9):1377-82.
13. Hammad, H.M., Albu, C., Matar, S.A., Litescu, S.C., Al Jaber, H.I., Abualraghib, A.S. and Afifi, F.U., Biological activities of the hydro-alcoholic and aqueous extracts of Achillea biebersteinii Afan, (Asteraceae) grown in Jordan, African Journal of Pharmacy and Pharmacology, 2013;7,1686-1694
14. El-Ashmawy, I. M. Anti-Inflammatory and cyclooxygenases inhibitory effects of Asteraceae, rich in flavonoids and tannins, Ejpmr, 2017;4(12), 96-102.
15. Hasona AN, Qumani MA, Alghassab TA, Alghassab MA and Alghabban AA., Ameliorative properties of Iranian Trigonella foenum-graecum L, seeds and

Punica granatum L, peel extracts in streptozotocin-induced experimental diabetic guinea pigs., Asian Pac J Trop Biomed.2017;2; 7(3): 234–239.

16. Ageel, A.M., Mossa, J.S., Al-Yahya, M.A., Al-Said, M.S. and Tariq, M., Experimental studies antirheumatic crude drugs used in Saudi traditional medicine, Drug-Exp-Clin-Res, 1989; 15(8): 369-372.
17. Kota, S., Kota, S., Meher, L., Tripathy, P., Sruti, J., Modi, K. Pheochromocytoma with renal artery stenosis: A case-based review of literature (2012) Journal of Cardiovascular Disease Research, 3 (1), pp. 36-39. DOI: 10.4103/0975-3583.91601
18. Barel, S.; Segal, R. and Yashphe, J., The antimicrobial activity of the essential oil from Achillea fragrantissima, J Ethnopharmacol, 1991; 33(1-2):187-191.
19. Harborne, J.B., Harborne JB. Photochemical Methods, a Guide to Modern Technique of Plant Analysis. London: Chapman and Hall. 1998.
20. Jaffer, H.J., Jawad, A.M., Naji, A and Al-Naib, A. Photochemical and biological screening of some Iraqi plants, Fitoterapia Lix 229. 1983.
21. Jiang Chang; Yanqing Xia; Suping Ma; Xuan Fang; Minzhuo Sun. Improvement of saponification extraction method for fatty acids separation from geological samples, Acta Geochim, 2016; 35(2):148–155.
22. Lorke, D., A new approach to tropical acute toxicity testing, Arch. Toxicol,1983;53: 275-287.
23. Turner, R.A. and Hebborn, P., Screening methods in pharmacology, Vol. II, Academic press, New York., 1971.
24. Liew, F., Regulation of delayed type hypersensitivity to pathogens and alloantigen. Immunology Today. 1982; 31, 18-23.
25. Semkova, I.; Gencheva, G.; Mirchera, J. and Nikolova, M. Kototifen, effect on immune system, Medico-Biologic Information, 1989;3: 23-26.
26. Trevor, E. Davis. Glucocorticoids suppress T cell function by up regulating microRNA 98, Arthritis Rheum, 2013 Jul; 65(7): 1882–1890.
27. Evan, W.C., Pharmacognosy .14th ed. Pp 250-251 WB Saunders company Limited , London.1999