

The Effects of Yarrow's (*Achillea millefolium*) Hydro Alcoholic Extract on Blood Proteins in Mice

¹Delaram Dadkhah and ²Mehrdad Modaresi
¹Isfahan Center, Payam e Noor University, Isfahan, Iran
²Department of Agriculture, Khorasgan Branch,
Islamic Azad University, Isfahan, Iran

Abstract: Yarrow (*Achillea millefolium*) means thousands leaves plant and belongs to Asteraceae family. Yarrow is a gramineous perennial plant which grows in mountainous regions of Europe, North Africa and East Asia (including North Iran and Alborz heights). This plant has many health benefits like treating problems of digestive system, respiratory system, cardiovascular system (stimulating blood circulation and lowering blood pressure) and genital-urinary system. In this study, the effect of Yarrow extract was evaluated on immunity parameters of blood serum in little female laboratory rats (Balb/C race). At first, samples were divided randomly in 5 groups: three treatment groups, one control group and one placebo group. Each group had eight members and all groups were kept in similar conditions. Hydroalcoholic extract was prepared in three doses including 50, 100 and 200 mg kg⁻¹ and was injected in peritoneum every 2 days for 20 days. Normal saline was injected to placebo group. All measurements were done using Protein Electrophoresis Method. Measured parameters were albumin amount, α -1 and α -2 globulin, β globulin, gamma globulin, albumin globulin ratio and total protein. Results showed that the amount of albumin, β globulin and total protein were increased in all three doses. A-2 were decreased in all treatments and α -1 were increased in all three doses but this increase was significant for doses 100 and 200 mg. Albumin globulin ratio was increased also in all treatments but it was significant only for 50 mg. Gamma globulin amount was increased in 50 and 100 mg but decreased a little in 200 mg. Results of this study showed that Yarrow extract caused significant changes in blood immune parameters and can affect immune system of body even in absence of antigenic factors.

Key words: Yarrow, immune factors, mice, electrophoresis parameters, globulin

INTRODUCTION

Yarrow (*Achillea millefolium*) is from Asteraceae family. Plants of this family are gramineous, perennial and have rhizomes with a 1 m straight stem. The origin of this plant is Northern hemisphere of world, especially mountainous regions of Europe and West lands of central and Southern Europe, East Asia and North Africa. The most distribution of this family is in temperate and cold regions of world. Yarrow grows in some Northern parts of Iran and Alborz heights, Urmia and Tabriz (Yassa *et al.*, 2007). Yarrow has a thin strait root with many thin branches. The upper part of root is the origin of many cylindrical stems. Stem is straight and simple and angular branches grow in different distances which have many beautiful leaves. Feathery small leaves of this plant cover the branch upward and leafy branches be derived in bottom. This plant has leaves without petiole, long and covered by downs which have many narrow cuts. These downy leaves are bayonet like with many pointy linear

pieces (Amirghofran and Karimi, 2001). Leaves have two side sacs filled by essence. There is many tiny combs like leaves which is the reason of thousands leaves name (Amirghofran and Karimi, 2001). White flowers of this plant are located at the end of stem as a capitulum. Numerous small capitulums have 4-8 mm length and 2-5 mm width and each of them has two types of flowers: tube flowers in the middle of capitulum and white tongue form type in margins. Tube flowers are more but smaller and in same color of the other type. In some forms of plant, these flowers (tongue form) are in purple or similar purple color, depending on living conditions. The numbers of tongue from flowers which are located as a hat on the top of flower are not >5 or 6 in each capitulum. All plant parts have a bitter penetrating smell. Yarrow species is smaller plant with white flowers but santolina have yellow flowers with penetrating fragrant smell. The fruit of Yarrow is achene, long and egg shaped. The weight of thousands grains is 0.15 g and grains are silver grey to yellow quadratic form 1.8-2 mm and with small wings.

Every plant can produce about 3000-4000 grains and they can germinate in maximum 3 cm depth. The most important components of Yarrow are essential oils (Chamazulene, Sabinen and Beta caryophyllene, proazolens and other sesquiterpene lactones, Caffeic acid, Salicylic acid and flavonoids (Rotin, Apigenin, Luteolin, and acacetin) (Saeidnia *et al.*, 2005)). Yarrow is one of the most important pharmaceutical plants which has been used frequently in ancient medicine for diseases generally and for curing scars and burning specifically (Saeidnia *et al.*, 2009). One of the most important therapeutic properties of Yarrow extract is its antibacterial effect on wide range of pathogens of human and animals. It has anti hemorrhoid and anti spasm effects and its herbal infusion is useful for indigestion plus stomach gas and increase in stomach acid. It has many effects on nervous system, heart and their problems because of having tannin and bitter, fragrant matters (Sharififar *et al.*, 2009). It is useful for curing simple diarrheas, hemorrhoids with bleeding and for stopping bleeding and is also effective in repulsing kidney stones because of having diuretic properties. This plant has anti fever effect and its brewed in water or alcohol can heal skin scars and breast problems. Essence of Yarrow is useful for curing neuro pains and rheumatic diseases. Tozyo *et al.* (1994) proved antibacterial, cytotoxic, anti cancer and anti inflammatory effects of this plant in their study and ascribed its anticancer effect to sesquiterpenes. Studies of Alcaraz and Ferrandiz (1987) showed anti oxidant and anti inflammatory effects of aerial parts of Yarrow. Anti inflammatory effect of extant flavonoids is because of its role in arachidonic acid metabolism. In this study, by emphasizing traditional and herbal medicine, effect of Yarrow extract on immune system of rats as an appropriate pattern was studied to introduce the best effective one.

MATERIALS AND METHODS

Experimental animals: Mices (Balb/C race) were used in this study which were in 30 ± 5 g weight range. Forty mice were kept in various cages for 40 days to reach desired weight and maturity. Among this period and also during injection, animals received similar food, water, temperature ($28-32^{\circ}\text{C}$) and natural light period. About 10 days before injection, samples were divided to 5 groups randomly and were kept in separate cages. Eight mice were kept from Balb/C race in each cage so that the mean weight was 30 ± 5 g.

Experimental groups

Control groups: To have base amount of blood protein parameters, this group was kept in similar condition of treatments but without injection.

Placebo group: To assure of lack of injections effect in results, 0.5 cc of normal saline was injected to members of this group.

Treatment groups (1-3): The 0.5 cc of hydroalcoholic extract of Yarrow was injected every other day in doses of 50, 100 and 200 mg/kg/2 days for 20 days.

Statistical analysis: Mean comparison of data was done using one way variance analysis and Duncan test ($p < 0.05$) and using SPSS program.

RESULTS AND DISCUSSION

Mean comparison of α -1 globulin at 5% probability level showed increase in concentration of this protein for second (100 mg kg^{-1}) and third (200 mg kg^{-1}) groups in proportion to control. Figure 1 shows mean comparison results. Mean comparison of α -2 globulin at 5% probability level showed decrease in its concentration among all experimental groups. Results are shown in Fig. 1. Mean comparison of α -globulin of various experimental groups showed significant ($p < 0.05$) increase in protein of treatments in proportion to control group. Figure 1 shows these results.

There was not any significant difference in concentration of gamma globulin among various experimental groups at 5% probability level but first and second group had an increase whereas third group showed a little decrease. The ratio of albumin to globulin was increased in all three treatments ($50, 100$ and 200 mg kg^{-1}) but this increase was only significant for first group ($p < 0.05$). Mean comparison of total protein at 5% probability level, showed significant increase in all treatment groups (Fig. 2).

Yarrow has anti inflammatory and liver protection properties which are because of its flavonoid compounds. Yaeesh announced that this plant had liver saving efficiency which was probably because of prohibiting calcium channels. In that study, protection effect of Yarrow was studied against d-galactosamine and lipopolysaccharide in rats. Results showed that Yarrow extract decreased their mortality from 100-40%. Also, these rats showed modified structure, lack of parenchyma shrinkage and reduced cell swelling of liver. Albumin is a transition protein which can join with organic and mineral ligands like bilirubin, thyroxin, penicillin, cortisol, estrogen, calcium and magnesium. Then, reduction in its concentration can be followed with important results from pharmacokinetic (drug transportation) and because Yarrow increases blood albumin, it can have positive effects on pharmacokinetic. Albumin increasing is

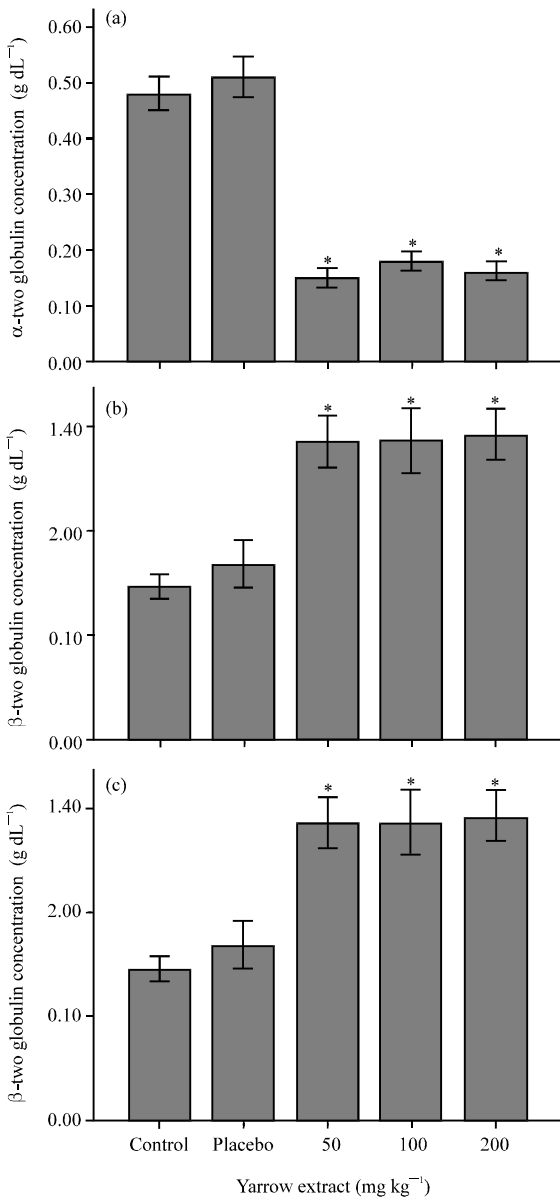


Fig. 1a-c: Mean comparison of α -1, α -2 and β -globulin percentage

important clinically too because it has the most important role in producing plasma's colloid osmotic pressure then decrease in its amount which can be due to production problems or losing it cause serious problems in vessels osmotic pressure and this process is shown as environmental edema. Albumin was increased in all experimental groups of this study and considering the increasing role of Yarrow on albumin researchers can say that Yarrow probably prohibits environmental edema.

The main part of α -1 globulin is α -1 anti trypsin. This protein controls a wide range of serine proteases and

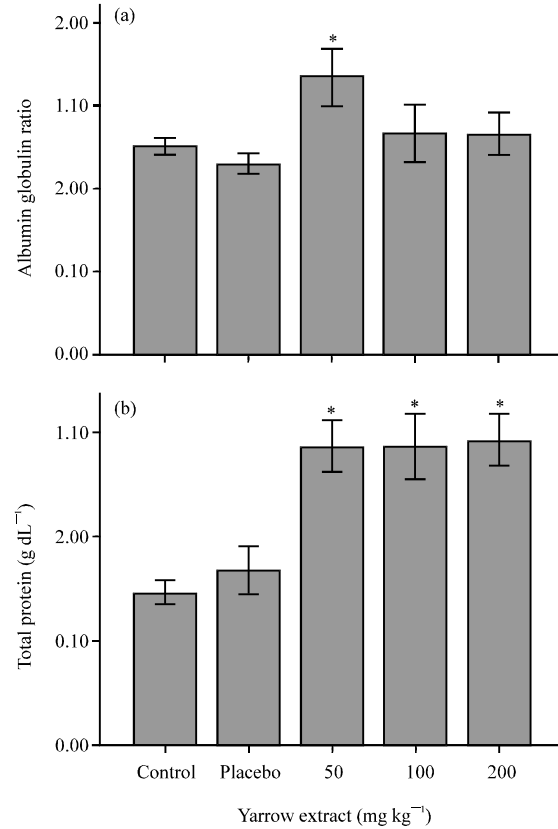


Fig. 2a, b: Mean comparison of albumin globulin ratio and total protein's concentration

because of its high control power and simple penetration to tissues plays an important role in tissues protection. The most important role of it is protecting lungs from tissue destruction due to proteases especially elastase neutrophils. Deficit of α -1 anti trypsin is related to amphisem and kind of liver disease. α -1 anti trypsin is one of the serum glycoproteins which is increasing in response to acute inflammation but this increase has low clinical importance and is not specific to a particular disease. In a research on cinnamon, α -1 globulin was increased significantly in 200 mg kg⁻¹ which is in agreement with the results (Modaresi, 2011). In another study, significant increase of it was obtained by blackseed extract (Modaresi, 2011). All of these studies were conducted on little laboratory rats. In this study also, the amount of α -1 globulin was increased in 100 and 200 mg experimental groups. Permeability of glomerular capillaries in nephritic syndrome by losing the other small proteins causes increase in amount of α -2 macro globulin ten times or more. In this disease, proteins with low molecule weight (especially albumin) are being filtrated and excreted through the urine and reduction in albumin

and α -1 globulin is visible in electrophoresis pattern but the amount of proteins with high molecular weight like α -1 macro globulin is being increased ten times or more.

CONCLUSION

Considering the result, researchers can conclude from increase in α -1 globulin and albumin and also decrease in α -2 globulin that probably increasing amounts of Yarrow have controlled α -2 globulin synthesis without causing change in glomerular capillaries permeability (Mansoureh *et al.*, 2003). Transferrin is the largest part of β -globulin and plays main role in iron metabolism in body. This glycoprotein has high tendency to connect to iron and transfers it to tissues. Even though the amount of this iron is <1% of body's total iron, this is the most important iron reservoir in body. Connecting transferrin to iron makes an environment with low levels of free iron and this prohibits bacteria growth. In a study on saffron and cinnamon, β -globulin showed significant increase. Considering the results and the other studies, researchers can say that probably increasing amounts of Yarrow (concentrations >50 mg kg⁻¹) can have a positive dose dependent role in iron metabolism of Syrian rat (Lopes *et al.*, 2005). No significant changes in immunoglobulin amount of studied groups show that immune system has not been stimulated in any of groups and Yarrow injection has not received any response from receiver groups. All drugs should be evaluated in terms of their effects on the immune system. Reduction in globulin level of serum can be an indicative of reduction in immunoglobulin production (Modaresi, 2012). Even though reduction in serum level of anti bodies is a relatively insensitive index, antibody response for controlling the immune system must be evaluated but it doesn't mean that Yarrow is not effective on immune system of rats suffering from some diseases. Pand and Foller showed in their studies that immune responses of body was increased by probiotic containing regimes which this is in agreement with studies of Yakhkeshi *et al.* (2012). They showed in their study that Yarrow (3% of diet) could strengthen immune system of birds. Stimulating immune system by probiotics leads to increase in T cells, phagocytic cells and level of serum proteins. Also, using vegetative drugs can stimulate immune system via increase in activity of vitamin C. In a study on ginger, there was not seen any significant change in amount of immunoglobulin of studied groups. Sasaki *et al.* (1989) showed in their study that glycosylated derivative of caffeic acid was responsible of various biological activities such as suppression of cellular and humoral immunity. Yarrow extract has much of

this matter and then it can be preventive of immune system. The albumin globulin ratio was increased a little in 2 and 3 groups, probably because of high increase in α -1 globulin and β -globulin amount. The amount of albumin and globulins and also the ratio of these proteins show us the liver function. Significant increase in albumin amount in all three experimental groups and increase in albumin globulin ratio (significant for first group and non significant for second and third groups) show that Yarrow injection increases liver activity. Dugenci *et al.* (2003) conducted a study on rain bow trout with some vegetative drugs with 0.1 and 1% for 2% of body weight for 3 weeks. In that study, the lowest plasma protein was obtained in group with 0.1% extract and 1% group had the highest protein. These results confirm that extant flavonoids of this plant have the ability of immune system induction. Probably, flavonoids can strengthen the immune system because of their antioxidant quality and gathering free radicals.

REFERENCES

- Alcaraz, M.J. and M.L. Ferrandiz, 1987. Modification of arachidonic metabolism by flavonoids. *J. Ethnopharmacol.*, 21: 209-229.
- Amirghofran, Z. and M.H. Karimi, 2001. Cytotoxic activity of thymus vulgaris *Achillea millefolium* and *Thuja orientalis* on different growing cell line. *Med. J. Islamic Republic Iran*, 15: 149-154.
- Dugenci, S.K., N. Arda and A. Candan, 2003. Some medicinal plants as immunostimulant for fish. *J. Ethnopharmacol.*, 88: 99-106.
- Lopes, F.C.M., F.P. Benzatti, C.M.J. Junior, R.R.D. Moreira and I.Z. Carlos, 2005. Effect of the essential oil of *Achillea millefolium* L. in the production of hydrogen peroxide and tumor necrosis factor- α in murine macrophages. *Braz. J. Pharm. Sci.*, 41: 401-405.
- Mansoureh, S., T. Asemaneh and M.H.S. Mojtaba, 2003. Effect of dormancy breaking treatments on *Achillea millefolium*. *Pajouhesh-Va-Sazandegi*, 15: 56-57.
- Modaresi, M., 2011. The effect of Cinnamon extract on serum proteins levels of male Balb/c mice. *JANUARY*, 16: 444-452.
- Modaresi, M., 2012. A comparative analysis of the effects of Garlic, Elderberry and Black Seed extract on the immune system in mice. *J. Ani. Vet. Adv.*, 11: 458-461.
- Saeidnia, S., A.R. Gohari, F. Kiuchi and G. Honda, 2005. *In vitro* Anti-epimastigote activity of some Iranian medicinal plants. *Iran. J. Pharm. Res.*, 2: 101-103.
- Saeidnia, S., N. Yassa, R. Rezaeiipoor, A. Shafiee, A.R. Gohari, M. Kamalinejad and S. Goodarzy, 2009. Immunosuppressive principles from *Achillea talagonica*, an endemic species of Iran. *DARU*, 17: 37-41.

- Sasaki, H., H. Nishimura, T. Morota, M. Chin and H. Mitsuhashi *et al.*, 1989. Immunosuppressive principle of *Rehmannia glutinosa* var. *hueichingensis*. *Planta Med.*, 55: 458-462.
- Sharififar, F., S. Pournourmohammadi and M. Arabnejad, 2009. Immunomodulatory activity of aqueous extract of *Achillea wilhelmsii* C. Koch in mice. *Indian J. Exp. Biol.*, 47: 668-671.
- Tozyo, T., Y. Yoshimura, K. Sakurai, N. Uchida, Y. Takeda, H. Nakai and H. Ishii, 1994. Novel antitumor sesquiterpenoids in *Achillea millefolium*. *Chem. Pharm. Bull. (Tokyo)*, 42: 1096-1100.
- Yakhkeshi, S., S. Rahimi and H.R. Hemati Matin, 2012. Effects of yarrow (*Achillea millefolium* L.), antibiotic and probiotic on performance, immune response, serum lipids and microbial population of broilers. *J. Agric. Sci. Tech.*, 14: 799-810.
- Yassa, N., S. Saeidnia, R. Pirouzi, M. Akbaripour and A. Shafiee, 2007. Three phenolic glycosides and immunological properties of *Achillea millefolium* from Iran, population of Golestan. *DARU, J. Pharm. Sci.*, 15: 49-52.