



## The effect of *Malva sylvestris* extract on blood protein and gamma interferon of *Candida albicans* infected mice

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### ABSTRACT

Various parts of *Malva sylvestris* have different medicinal properties such as antimicrobial activity. In this study the effects of *M.sylvestris* extract on protein electrophoresis pattern and gamma interferon of *C.albicans* infected mice was studied. Sixty female mice were divided randomly in six groups (three treatment groups, *Candida*, placebo, and control groups). Treatment groups received aquatic extract of *M.sylvestris* (50,100 and 200 mg/kg) for 20 days every other day via injection in peritoneum. *C.albicans* was injected once after sixth injection of extract. Results showed significant decrease in the amount of albumin in three treatment groups.  $\beta$ -globulin amount of 50 and 100 mg/kg groups and gamma interferon amount of all three treatment groups were increased significantly in proportion to control group. Clearing the body from pathogen organisms depends on cellular responses. It seems that *M.sylvestris* is capable of stimulating cellular immune response and can be used in studies about *C.albicans*.

**Keywords:** *Malva sylvestris*, *Candida albicans*, Mice

### INTRODUCTION

*Candida albicans* is from opportunistic pathogens which can sustain in people with weakened immune systems and cause diseases. This commensal yeast resides in the oral cavity, intestines and genitalia area and cause two types of infections depend on host: superficial infections such as oral or vaginal infections and cutaneous candidiasis that can be seen in healthy individuals, and deep infections including respiratory, gastrointestinal, urinary infections and Candidaemia in people with severe immune dysfunctions. *Candida albicans* is considered as the fourth most common cause of blood infection and the most common cause of death in America [1].

Limitations in the treatment of fungal diseases such few and expensive antifungal drugs, their side effects and drug resistance to these drugs has encouraged researchers to search for new antifungal drugs especially medicinal plants [2].

*Malva sylvestris* plant from Malvaceae family is a biennial plant with about 100 to 120 cm height. This plant is native to Europe, northern Africa, Asia and Mediterranean regions. All plant parts are used as Pharmaceuticals compounds but leaves and flowers are used more. Flowers of plant have 2 or 5 cm diameter without any special smell and taste like mucilage. This plant has been used throughout history as Food or Drug. *M.sylvestris* has many therapeutic uses including treatment of stomach ulcer and antioxidant, anti-cancer, skin repairing, anti-microbial and anti-inflammatory properties. Main active ingredient of *M.sylvestris* shoots are mucilage, flavonoids, tannins, phenolic compounds, ascorbic acid, carotenoids, tocopherol and anthocyanin. Plant in is used to treat inflammations, asthma, constipation, acne, hemorrhoids and disorders of the digestive and respiratory systems, and as analgesic [3, 4]. *M.sylvestris* has antifungal and antibacterial properties against a variety of important pathogens such as *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [5].

Phenolic compounds in *M.sylvestris* are able to reduce kidney damages caused by vanadium [6]. Today, herbal medicines are available in forms of Herbal tea, extract, powder, syrup, and ointment. The important point about herbal drugs is dosage [7].

This study was carried out to investigate the effects of *M.sylvestris* extract on plasma proteins and gamma interferon expression of *C.albicans* infected NMRI mice.

## EXPERIMENTAL SECTION

### Extraction

To prepare aquatic extract, 100 g of aerial parts powder was poured in a sterilized Erlenmeyer flask and ethanol 96% added to it to make a suspension. The mixture was shaken for 42 hours macerate. After filtering the Erlenmeyer flask contents using whatman paper and calculating the remaining amount of the extract in solution (2/118 gr), *M.sylvestris* concentration in the initial (mother) solution was determined. The obtained extract was diluted with sodium chloride 0.9; desired doses were prepared and were passed through 0.45 micron syringe filters.

### Laboratory animals

60 female mice from NMRI race weighing 25-30 grams were selected. Samples were kept for one month to adapt to environment. Mice had free access to standard food and water, natural light, temperature and humidity. The same situation continued during the injections.

### Treatment groups

- Control group: this group didn't receive any injection and was used as a criterion for comparing CBC amounts of treatment groups.
- Placebo group: members of this group received sodium chloride 0.9 injections to study the effects of injection shock.
- Candida group: members of this group received  $1 \times 10^6$  cfu/ml of *Candida albicans* ATCC 1677, (0.5 McFarland) once to induce treatment infection.
- Treatment groups: received 10 injections of *M.sylvestris* hydroalcoholic extract in 50, 100, 200 mg/kg doses for 20 days. Suspension of *Candida albicans* ( $1 \times 10^6$  cfu/ml) was injected between fifth and sixth injections of the extract.

All injections were done in peritoneum. After the injections, blood samples were taken and collected in tubes with EDTA and were centrifuged for 5 minutes at  $2500 \times g$ .

### Measuring protein of plasma

Protein of plasma was measured using plasma protein electrophoresis kit (Cellogel, Malta chemetro, Italy). After spotting samples on gel and band formation, coloring was done and each band was determined by densitometry.

### Measuring cytokine gamma interferon

Gamma interferon was evaluated using Elisa test and kit (murine INF- $\gamma$  Elisa kit) made by Boster biological Technology, CA, USA by sandwich enzyme- Linked Immunosorbent Assay.

### Statistical analysis

Obtained data were analyzed using one way ANOVA at 5% probability level, and using SPSS<sub>13</sub> program.

## RESULTS AND DISCUSSION

Mean comparison results of plasma protein at 5% probability level showed that has  $\alpha$ -1 globulin and  $\alpha$ -2 globulin not significant change in all groups.

Albumin concentration was decreased in candida and treatment groups significantly ( $p < 0.05$ ) in proportion to control group (Fig. 1).

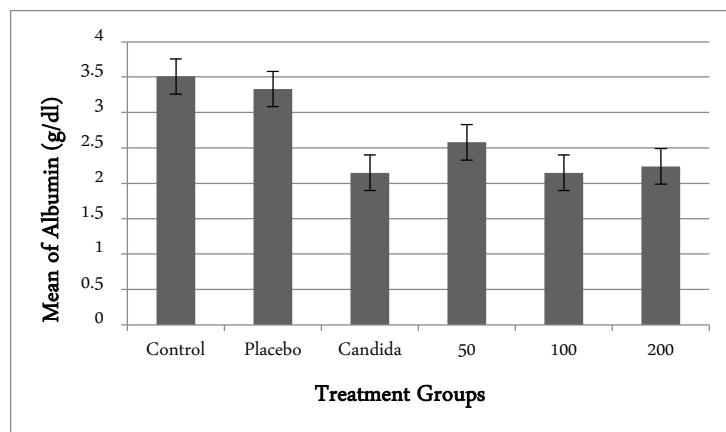


Figure 1: Effect of *Malva sylvestris* extract on mean comparison of Albumin in all groups

Beta globulin amount of candida group and 50 and 100 mg/kg groups were increased significantly ( $p < 0.05$ ) in proportion to control group (Fig. 2).

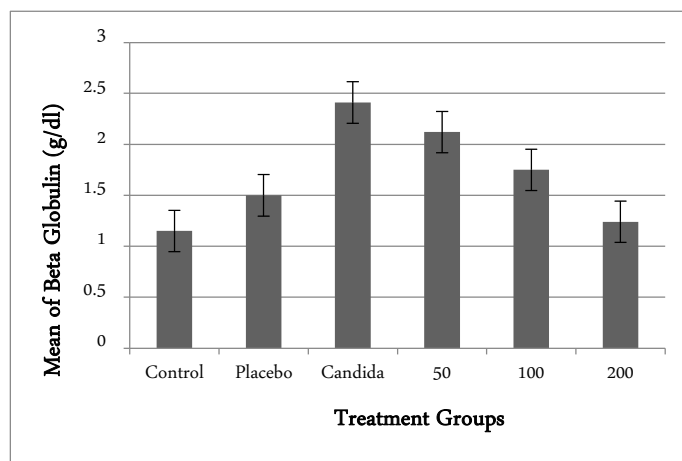


Figure 2: Effect of *Malva sylvestris* extract on mean comparison of  $\beta$ -globulin in all groups

The amount of gamma globulin was increased significantly ( $p < 0.05$ ) in candida group (Fig.3).

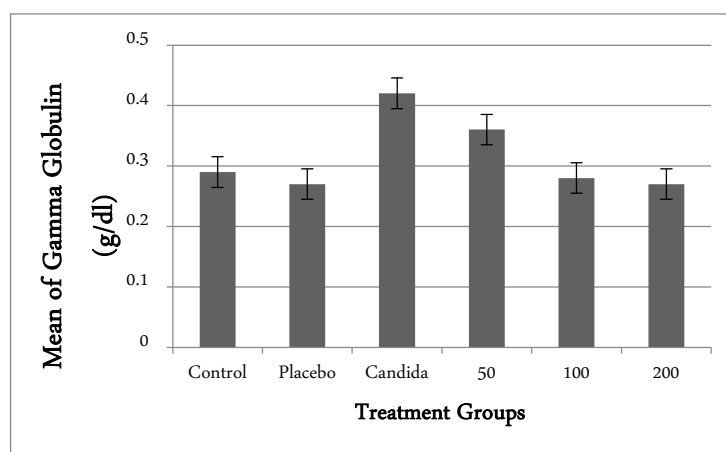


Figure 3: Effect of *Malva sylvestris* extract on mean comparison of Gamma globulin in all groups

Albumin/globulin ratio was decreased in candida and treatment groups significantly ( $p < 0.05$ ) in proportion to control group (Fig. 4).

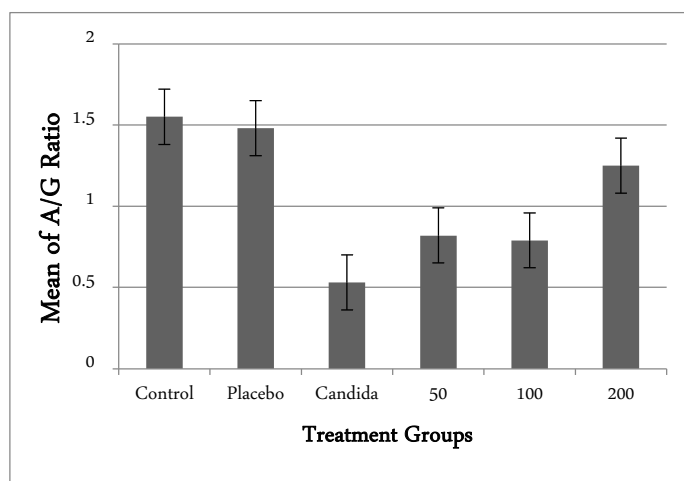


Figure 4: Effect of *Malva sylvestris* extract on mean comparison of A/G in all groups

Gamma interferon expression of candida group was decreased significantly ( $p < 0.05$ ) whereas it was increased in 50, 100 and 200 mg/kg groups significantly ( $p < 0.05$ ) in proportion to control group (Fig. 5).

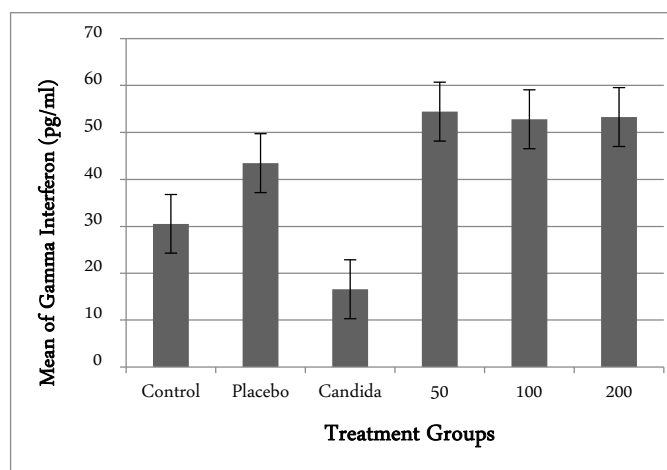


Figure 5: Effect of *Malva sylvestris* extract on mean comparison of Gamma interferon in all groups

According to results albumin concentration and albumin to globulin ratio were decreased in candida and treatment groups significantly ( $p < 0.05$ ). Reduction in albumin/globulin ratio shows reduction in albumin amount or increase in globulin amount. As it was explained, albumin synthesis is decreased in liver diseases and plasma of patients with liver diseases shows often lower albumin/globulin ratio. Increased liver enzymes ALP, AST and ALT in serum indicate liver damages. Liver disease and systemic infections caused by *Candida albicans* increase ALP level and decrease the amount of albumin [8]. Aquatic extract of *M.sylvestris* in 400 and 800 mg/kg doses didn't affect the amount of liver enzymes ALP, AST and ALT [9].

Also, non-polysaccharide compounds of plant cell walls such as oxalic acid have the ability to bind with certain metal ions and therefore make them unavailable biologically. Furthermore, these compounds will probably change the amount and the ratio of albumin and can affect serum protein electrophoretic map because of liver damages and by affecting protein transport systems. *M.sylvestris* has this compound [10].

Histological studies and measurement of liver enzymes showed that hydroalcoholic extract of *M.sylvestris* in 300 and 600 mg/kg doses protected liver against liver paracetamol disease [7].

It is believed that *M.sylvestris* is useful for nourishing and cleansing the liver [10]. The amount of gamma globulin was increased significantly ( $p < 0.05$ ) in candida group in proportion to control group and treatment groups. Globulins of gamma region which include various anti bodies of humoral immune system are increased in many infections and immunological diseases [9]. Immunoglobulins are from humoral specific immunity. Beta globulin

amount of candida group and 50 and 100 mg/kg groups were increased significantly ( $p < 0.05$ ) in proportion to control group. Considering that transferrin and hemopexin are from blood cells of beta area and since transferrin is the largest component of beta globulin, this protein transfers ferric ions from iron stores or mucosal iron to bone marrow. Regulation of mRNA translation for transferrin is carried out by blood iron and hepatocytes iron in liver. Total protein is the ratio of all extant proteins in serum and this ratio is changed based on the changes of plasma proteins [9]. Gamma interferon expression of candida group was decreased significantly ( $p < 0.05$ ) whereas it was increased in all treatment groups significantly ( $p < 0.05$ ) in proportion to control group. Th<sub>1</sub> Cells (CD<sup>4+</sup>) play important role in regulating the immune system to respond to fungal infections and activating cytokines and anti-fungal agents. Protection against *Candida albicans* depends on IL<sub>2</sub> the production, gamma interferon and requires the coordination of several cytokines such as IL<sub>12</sub>. Possibility of infection is related to Th<sub>2</sub>, (CD<sup>4+</sup>) cells which cause production of IL<sub>4</sub> and IL<sub>10</sub> and interfere in performance of Th1 cells and gamma interferon. Gamma interferon plays the most important role against fungal infections which its existence is necessary for IL<sub>12</sub> response against candida infections [6]. In a research on *M.sylvestris*, the plant increases the IL<sub>12</sub> gene transcription and gamma interferon, also activates macrophages and Th<sub>1</sub> and disabled IL<sub>4</sub> transcription [10]. Gamma interferon inhibits the growth of *Candida albicans* by production of nitric oxide (NO). This cytokine removes *Candida albicans* by activating necessary macrophages of nitrogen reactions [8].

### CONCLUSION

High amount of gamma interferon in treatment groups and showed that *M.sylvestris* plant is important to induce and enhance cellular immune responses. Clearing pathogenic organisms from the body depends on cellular responses. It seems that *M.sylvestris* which is capable of stimulating cellular immune responses can be used in studies about *Candida albicans*. It is suggested to study a wider range of *M.sylvestris* extract doses on *Candida albicans* infection and the immunity of mice. Also, since *M.sylvestris* is known in traditional medicine as a liver tonic, changes in liver enzymes of *Candida albicans* infected mice can be studied.

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