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Original Research Paper

Effect of Pennyroyal (*Mentha pulegium*) Extract on Blood Parameters in Mice

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ABSTRACT

Fragrant pennyroyal (*Mentha pulegium*) from Lamiaceae family has many medicinal properties like haematopoietic enhancement. The effect of hydro alcoholic extract of pennyroyal on blood parameters of mice was studied on little laboratory mice from Balb/C race. Fifty mice were divided in five groups: control group, Placebo (0.6 cc of normal saline), and three extract treatment groups (50, 100, and 200 mg/kg/ 2days). Peritoneum injection was used for enforcing treatments. Blood samples were taken from mice hearts. Data were analysed using SPSS program at 5% probability level. Results showed that total number of white blood cells was increased significantly in third treatment group (200 mg/kg). Red blood cells also increased significantly in this group. Haemoglobin was increased in all treatment groups but haematocrit and blood indices (MCHC, MCH, MCV) did not show any significant effect. According to the results, pennyroyal extract can strengthen immune system in 200 mg/kg dose via increase in number of white blood cells and can affect haematopoietic via increase in number of red cells.

INTRODUCTION

Using pharmaceutical plants for medicinal purposes has a long history. Man has had to stick to plants for curing diseases and plants have been used as food or drug for treatment or prevention of diseases. Plants have been used mostly as fresh, brewed, or boiled long time ago but today, plus mentioned methods, they are used as basic matters of many chemical drugs (Zargari 1995). In most cases, treatment is related to specific matters of plants and definite amount of these matters are required to have the desired effect. It is important to know that amounts of mentioned matters are different in various plants following the climate which plant has been grown in. Pennyroyal (*Mentha pulegium*) is from Lamiaceae family which has a wide range of annual or perennial plants. Rectangle stem with simple, opposite or rarely amplexicaule leaves are from specifics of this family. Flowers are aggregated or located as packs along stem or in its end. This plant is herbaceous and perennial which lives widely in humid plains and borders of water streams. Leaves of this plant and flowering twigs have medicinal properties (Mir Haidar 2005). Various parts of pennyroyal have tannin, resin matters, pectin, sugar, and also essence which vary from 0.4% to 1% according to variety and growth environment (Flok 2000). This essence is soluble in most oil types and is from cyclohexanol or cyclohexanone groups (with different chemical structures). It has 75 to 90% of cetonic compounds especially pulegone ($C_{10}H_{16}O$), menton, isomenton, piperitenone, limonin, dipentene, menthol, hesperidin, diosmin and azolin (Ashtan 2003). Pennyroyal has been used in traditional medicine for treating inflammation, colic, and spasm. Extract, essence, and its oil have antimicrobial, antiviral, anti inflammation and anti scar effects and also is bile producer and anti spasm (Maliakal & Wanwimolruk 2001). Aquatic extract can destroy free radicals.

In this study, the effect of injecting hydro alcoholic extracts of pennyroyal (in 50, 100, 200 mg/kg doses) was studied on Syrian mice. By using injection method we were assured that animals received definite dose of extract.

MATERIALS AND METHODS

Fifty matured female mice from Balb/C race in weight range of 30±5 g were procured and kept under laboratory conditions (28-32°C and normal light period) for 50 days to adapt to the environment. After enforcing treatments, mice were kept in separate cages and had free access to food and water. The study was done according to international protocol of studies on laboratory animals.

Mice were divided in five groups randomly. The first group was control which was not injected. The second group was placebo group which received 10 injections in peritoneum of physiological serum (0.5 cc) for 20 days, every other day. Third to fifth groups were three treatment groups which received injections of extract (50, 10, 200 mg/kg) every other day in their peritoneum. Injections were given between 10 a.m. and 12 a.m. After 20 days, blood samples were taken and sent to the laboratory (Modaresi 2012). Data were analysed using SPSS program and one-way analysis of variance. Mean comparison was done using Duncan multiple ranges test.

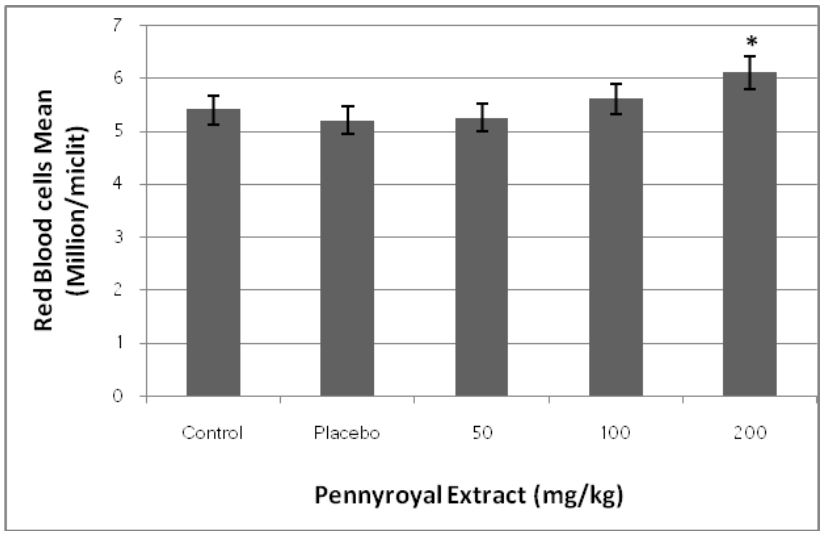

Fig. 1: Mean comparison of the number of red blood cells.

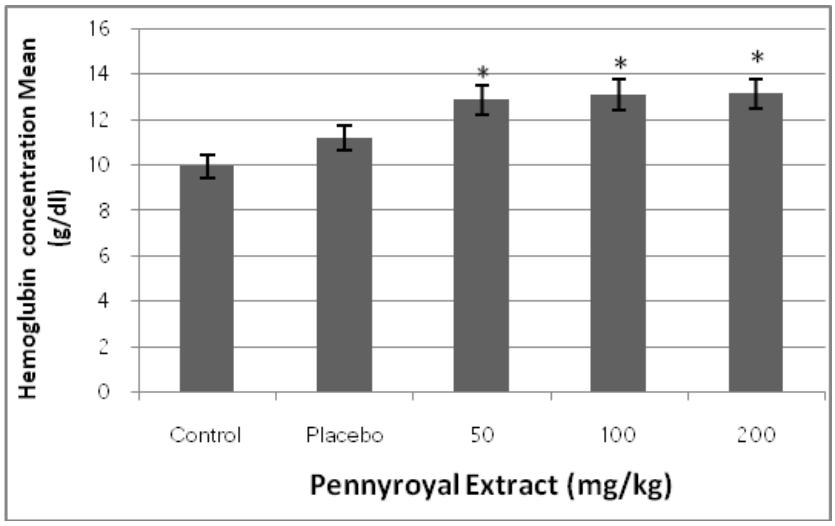

Fig. 2: Mean comparison of the hemoglobin amount of studied groups.

RESULTS

Mean comparison of groups showed significant increase in red blood cells of third experimental group (200 mg/kg) in comparison to control (Fig. 1).

The amount of haemoglobin was increased significantly in all the three treatments (50,100, 200 mg/kg) which are shown in Fig. 2.

Haematocrit amount was decreased but not significantly in all the studied groups. The amount of MCV was increased by first (50mg/kg) and second (100mg/kg) experimental groups, but not significantly. Also, third group (200 mg/kg) decreased the MCV not significantly (Fig. 3).

Mean comparison showed a little increase in MCH amount of first and second experimental groups (50 mg/kg, 100 mg/kg) and a little reduction in third group (200 mg/kg). The differences were not significant at 5 % probability level. MCHC amount of blood was increased in all the treatment groups (50, 100, 200 mg/kg) but not significantly. The amount of white blood cells was increased significantly in third group (200 mg/kg) (Fig. 4).

DISCUSSION

The number of red blood cells were increased in third group (200 mg/kg) significantly which is in agreement with results of O Neill et al. (2002). They reported in their study

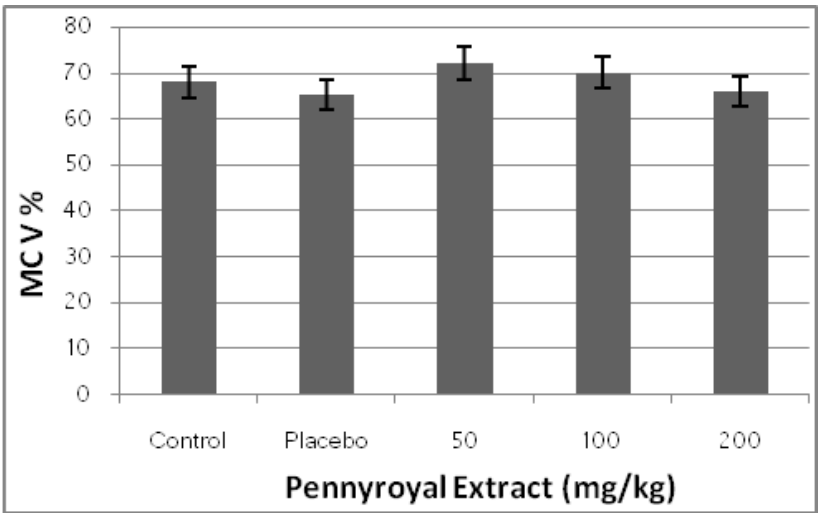

Fig. 3: Mean comparison of the MCV amount of studied groups.

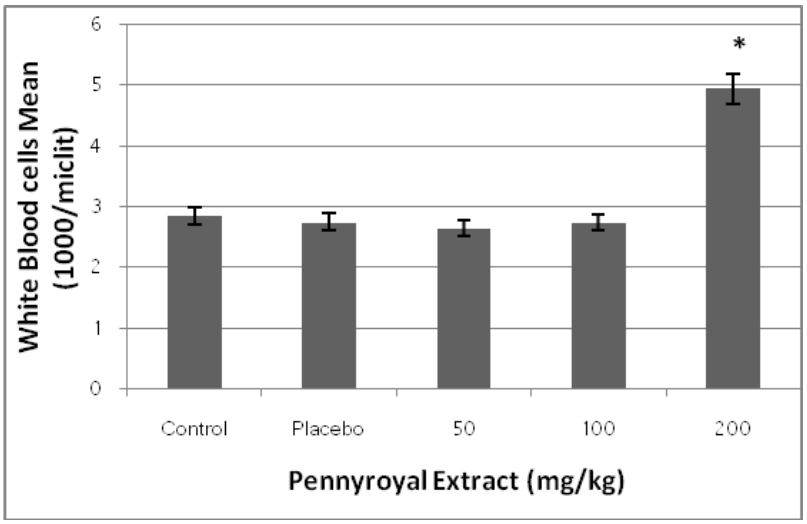

Fig. 4: Mean comparison of the number of white blood cells of studied groups.

that extracts of *Echinacea* plants and pennyroyal increased red blood cells of healthy horses. Haematopoietic is result of simultaneous and permanent reproduction and differentiation of cells from stem cells.

Considering the conducted researches and also mechanism of haematopoietic, increase in number of white blood cells by pennyroyal extract can probably be due to following reasons:

- With increase in mitosis division by affecting stem cells (pluripotent, myeloid multipotent and lymphoid).
- With increase in mitosis division of ancestral cells (Sarker & Nahar 2004).

Therefore, dose 200 mg/kg of pennyroyal extract has affected multifunctional haematopoietic stem cell and increased their multiplication. Division of these cells has led to production of colony forming unit, erythrocyte (CFU-E), which makes red blood cells.

Mean cell hemoglobin is a parameter which is affected by changes in amount of hemoglobin and red blood cells numbers. So, every factor which affects these items will change cell hemoglobin (Harrison 1994) but because of little changes in mentioned items red blood cell indices (MCV, MCH, MCHC) did not show any change.

Pennyroyal extract in 200 mg/kg dose increased white blood cell numbers and then neutrophils phagocytose and therefore stimulated and enhanced the immune system. In bone marrow, after divisions of myeloid multipotent cells,

colony forming unit myeloid (CFU-GEM) is made. These cells, which are from ancestral cells, are divided and make other ancestral cells including CFU-GEM, CFU-Baso, CFU-Eo. These cells are affected by growth stimulating factor of GM-CSF, IL-3, stem cell factor (CSF) and FLt-3L, and are stimulated and multiplicated. Considering mentioned subjects pennyroyal extract has affected probably chromosome 5 of T lymphocyte cells, endothelium and fibroblasts (producer cells of GM-CSF), and caused production of GM-CSF which this compound has affected CFU-GEM and stimulated its mitosis division that caused increase in white blood cells numbers (Sarker et al. 2004).

White blood cells defend the body against foreign agents and increase in their number will raise immune system activity (Harrison 1994). Pennyroyal is from plants which strengthen the immune system. Zi et al. (1997) showed in a study that polysaccharide component of some pharmaceutical plants has an important activity in immunity and strengthen phagocytosis in reticuloendothelial system and also stimulate interfrone production.

This study showed that peritoneum injection of pennyroyal hydroalcoholic extract was effective on blood parameters of Syrian mouse and confirmed its stimulating effect on haematopoietic system. Results also showed that the effect of pennyroyal extract was dose dependent.

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