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INVESTIGATING THE EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF (*PIMPINELLA ANISUM* L.) ON HAIR GROWTH

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ABSTRACT

In this study, the effects of above ground ethanol extracts (Pimpinella anisum L.) were assessed on hair growth. For this purpose, the prepared extract was treated as a positional solution on mice modified rear head area (dorsal) in 2%, 5%, 10% and 20% dosages. At the end of the 10th and 30th days, hair growth was assessed. The results showed significant differences between all the experimental groups and the control group ($p<0.001$). In 10% and 20% doses, there was Minoxidil effects. Based on these results, anise hydroalcohlic extract had a positive effect on the hair growth which is probably due to the effects of phytoestrogens in the plant.

Key word: Pimpinella anisum L., Hair growth, mice, Minoxidil.

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1. INTRODUCTION

Medicinal plants enjoy a high value and importance regarding the health and protection against disorders in the societies. This part of natural resources has a life duration equal to the human beings and was one of the most important sources of human food and medicine during their generations. From a historical viewpoint, plants have had a great importance in the development

of societies and extensive research has been done to find the products and natural ingredients of herbal medicine.

Hair is considered as a factor of beauty in human societies and hair loss on the head, eyebrows, and eyelashes is considered as an abnormality. Many people in every society suffer from hair loss. A large number of chemical and herbal drugs have been introduced so far. None of these drugs have produced efficient results. So, there is still a necessity for a strong drug for the treatment of reduced hair growth, hair loss and thinning hair. Male hormone is one of the reasons leading to hair loss so it cannot be seen in the females. Testosterone is the male sex hormone which causes hair loss. Anise plants contain phytoestrogens which may be a tunnel to solve the aforementioned problems.

2. MATERIALS AND METHODS

2.1. Animals

Mice weighing 30-25 grams were obtained from the Animal House School of Pharmacy in Zabol. Here, the animals are kept under 12 hours of darkness and 12 hours of light, a temperature range of 22-25C and enough humidity. Municipal tap water was provided for the animals. The mice were feed by a compressed food specialized for it.

The water was changed daily and they were given enough food. Helsinki Code of Ethics were applied to all animals to minimize harming animals. Each animal was used only once. Collection method Aeration part of the plant was collected on late October from Zabul medical research center. It was dried in the shadow and open air of the laboratory for a week and then was crushed. The plant was kept in a dry and cool environment with no light.

2.2. Plant material

In this study, for the preparation of the Hydroalcoholic extract, maceration with ethanol in 80 C was conducted. After extraction, the extracts were condensed at low temperature and pressure, and kept in a dark closed-door container until the test at a temperature of 7-4 degrees.

2.3. Dose determination

Due to lack of similar studies surveying the effects of hydro alcoholic anise plant on the hair growth, other studies conducted on the anise plant and the doses which make the plant to be positionally effective were consulted. Therefore, 2%, 5%, 10% and 20% dosages were selected.

2.4. Methods

A day before the start of the treatment, the hairs in the mice lumbar region (dorsal) was shaved by razor for 1.5 square centimeters in a way that no hair could be seen with the naked eye. The mice were divided into 6 groups of 5 animals and were treated with 70% alcohol, 2% extract, 5% extract, 10% extract, 20% extract and 5% minoxidil, respectively. Once a day, at 6 pm, 0.2 ml of the extract was dipped into the shaved area, and the area was massaged. It should be noted that the extract was shaken well before use. This was done for thirty days. In the tenth, twentieth and thirtieth days after the start of the experiment, the length of hair at the dorsal area was measured. In these days, the mice were first anesthetized by intraperitoneal injection of ketamine (75 mg per kg) and xylazine (8.6 mg kg). Then, 10 hairs were randomly separated from dorsal area with tweezers and the hairs' lengths were measured by caliper device. The effect of anesthetic substances on the hair growth is not established. So, from each mouse, 30 hairs were randomly isolated during the study from the roots by tweezers and then compared with the control and minoxidil groups.

2.5. Statistical Analysis

For comparison of results, Graphpad Prism 5.0 software was used. Groups of mice that should be compared were independent of each other and variable distribution in these groups followed normal distribution. Therefore, the appropriate statistical test to compare groups was ANOVA (one-way-ANOVA). After this test, post hoc tests (Newman-Keuls) were used to compare groups. $P<0.05$ was considered as the criterion for statistical significance.

3. RESULTS

Findings Studing the effects of anise hydroalcohlic extract on hair growth at the end of the tenth day Administration of anise extract in different doses, 70% alcohol and 5% minoxidil, was done daily. In this test, at the end of the tenth day, 10 hairs were taken at random from each mouse.

Statistical analysis showed significant differences between the control group (70% alcohol) and all extract doses and minoxidil (Figure 1). Statistical analyzes show that there was a significant difference between the minoxidil and 2 and 5% extract, however, there was no significant difference between Minoxidil and 10 and 20% extract (Figure 1).

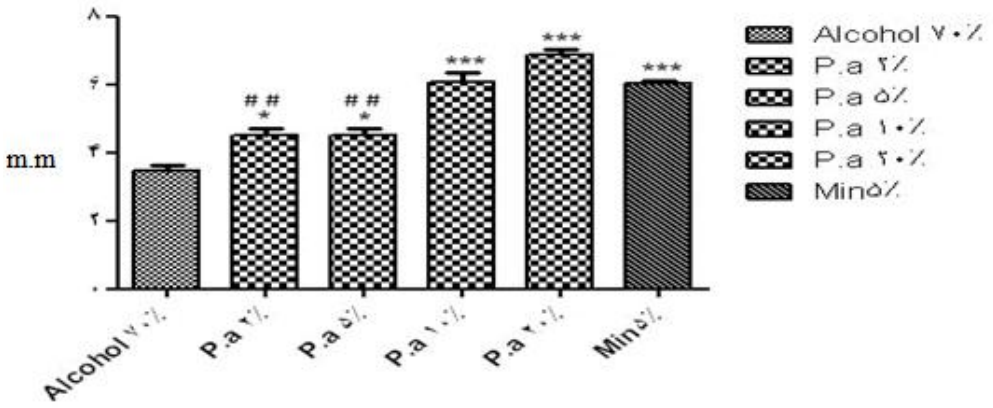


Fig.1. Examination of hydroalchoholic extracts effects of anise at the end of the 10th day

($p<0.05$) ($p<0.001$) Significant differences compared to the control groups (receiving alcohol 70 percent for 10 days). ($p<0.01$) Significant difference compared to minixidil.

Examining the effects of anise hydroalchoholic extract on the hair growth at the end of 20th day. Administration of anise extract in different doses, 70% alcohol and 5% minoxidil, was done once daily. In this test, at the end of the twentieth day, 10 hairs were cut at random from each mouse. Statistical analysis showed a significant difference between the control group and all doses of the plant and the minoxidil (Figure 2). Statistical analysis showed that the 2 and 5% extracts showed significant difference with the Minoxidil, but 10 and 20% groups showed no significant difference with minoxidil (Figure 2).

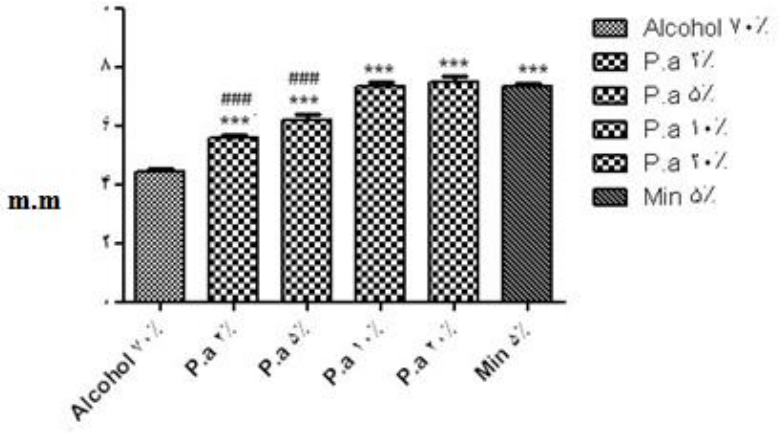


Fig.2. Examining the effects of anise hydroalchoholic extract at the end of 20th day

(P<0.001) Significant difference compared to the control group (receiving 70 percent alcohol for 20 days), (P<0.001) Significant difference with the minoxidil group

Examining the effects of anise hydroalchoholic extract on the hair growth at the end of 30th day.

Administration of anise extract in different doses, 70% alcohol and 5% minoxidil, was done daily. In this test, at the end of the thirtieth day, 10 hairs were cut at random from each mouse. Statistical analysis indicated a significant difference between the control group and all doses of the plant and minoxidil (Figure 3). Statistical analysis showed that 2 and 5% extracts had significant differences with Minoxidil but 10 and 20% groups had no significant difference with minoxidil (Figure 3).

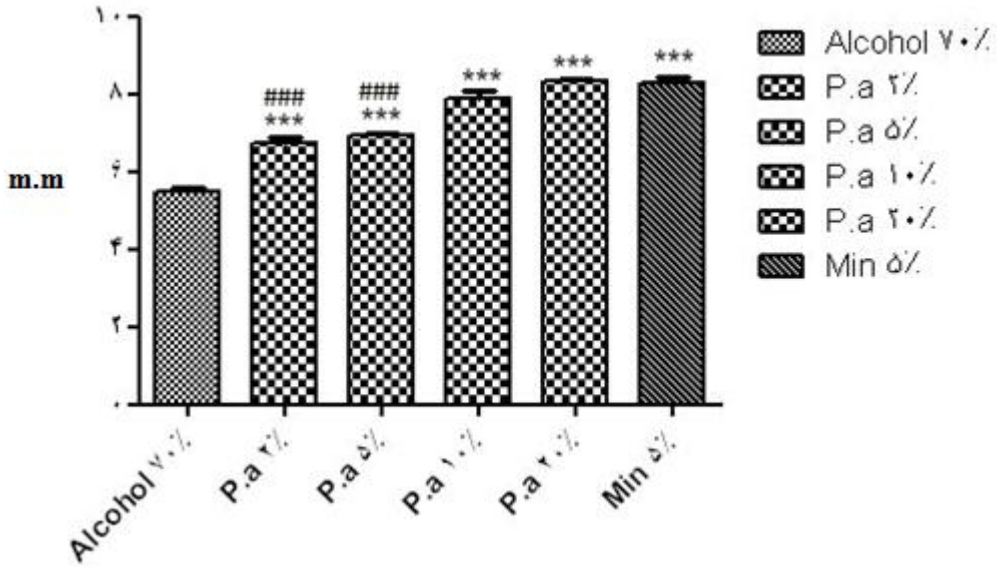


Fig.3. Examining the effects of anise hydroalchoholic extract at the end of 30th day

(P<0.001) Significant difference compared to the control group (receiving 70 percent alcohol for 30 days), (P<0.001) Significant difference with the minoxidil group

Hair growth rate compared at different doses of the hydroalchoholic anise extract on the tenth, twentieth and thirtieth days.

The increase in hair growth was measured in ten-day intervals. It was shown that during the first 10 days of the experiment the highest growth rate happened. This is probably due to better drug delivery as a result of little hair in the lumbar area (Figure 4).

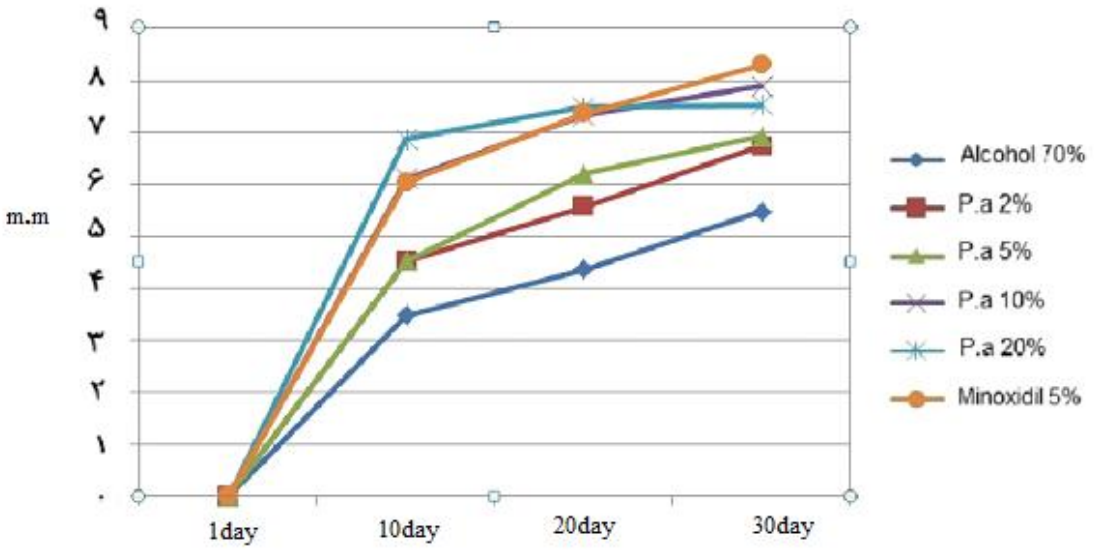


Fig.4. Compares the amount of hair growth in the control group, different doses of the extract and 5% minoxidil on the tenth, twentieth and thirtieth days

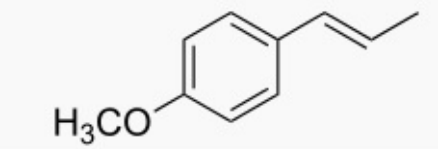
4. DISCUSSION

In this study, the effect of anise plant extract on hair growth in mice was evaluated. The results indicated that doses of 10% and 20% were significantly ($p < 0.001$) more effective compared to the control group (70% alcohol) and no significant difference was observed regarding the control group (Minoxidil 5%).

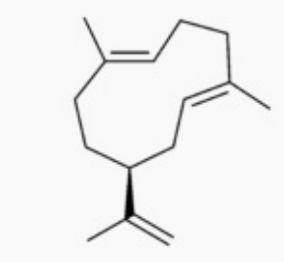
The major components of the plant are volatile compounds such as anethol, eugenol, Anisalids and non-volatile compounds such as Astragl, stigmasterol and more. Anatole is one of the volatile materials. Kang et al showed that Anatole has anti-inflammatory properties (1). The research on areata hair loss indicated that anti-inflammatory compounds improve the disease conditions (2,3). Tabanka et al in a paper also showed that this compound has similar phytoestrogens effects. Estrogen plays an unknown role in the hair growth. Hair follicles have both A and β estrogen receptors. Estrogen can stimulate hair growth in the men, while in women it inhibits the growth of hair length.

Precursor of androgen can be converted to estrogen in the hair follicle. Estrogen influences on the 5-alpha-reductase enzyme and the dihydroxy level of testosterone changes. This increases hair

growth in men. As all the male mice which were used were male, this mechanism can be used for hair growth in the men which has an almost similar effect to that of finasteride (4).



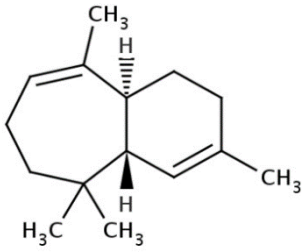
The other solution which can affect hair growth is germacrene.



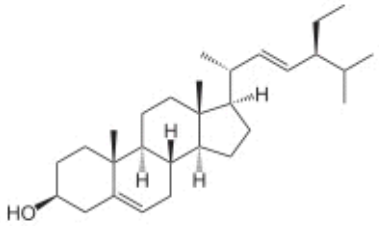
It is a sesquiterpene which is a type of terpene with three isoprene units (C5H8) and its general formula is $C_{15}H_{28}$.Terpenes are organic material which are abundant in the nature and are the main essence in most of the plants (5).

Positive effects of sesquiterpene on hair growth found in valerian (Nardostachys jatamansi DC) have been proven (6). Germacrene anti-androgenic effects in the Curcuma aeruginosa Roxb is proven and six other sesquiterpenes are available in this plant. If the extract is kept at high temperatures, its anti-androgenic effects reduce greatly (7). Antiandrogen can increase hair growth (8).

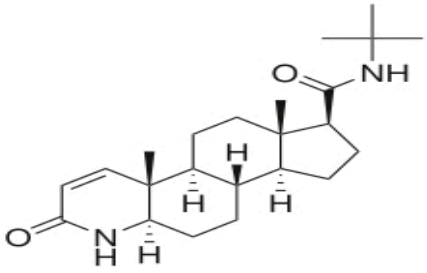
The other solution in P.anisum is gama-himachalen. The composition of the plant is also available in Cedrus atlantica. In our current study, the results showed that the plant had a good effect on the hair growth. So, more studies are needed to investigate the effects of gama-himachalen on hair growth.



Another combination which may have a favorable effect on the hair growth is a phyto sterols called stigmasterol. Stigmasterol has estrogenic properties (9).



Since this hormone is similar to estrogen, it is likely that this material can have a good effect on the hair growth. In a study, Bloom and his colleagues compared minoxidil and alphatradiyol (that is structurally similar to estrogen) and found that alphatradiyol can slow hair loss (10).



Other combinations of anise plant were studied in different papers and no relationship was seen with hair growth.

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