Effect of Hydro Alcoholic Leaf Extract of *Myrtus communis* on Pituitary- Gonad Axis in Adult Male Rat

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Abstract

Myrtus communis has been identified as a holy plant for ages and a great verity of medicinal properties have been described for it in literature. Today, there is a number of Myrtus drugs in Iran market including: Myrtoplex cream, Myrtex Solution, Aftoplex, Dineh Inhaler Powder, Rectol cream and so on. These in complete sentence properties may be due to the presence of different chemical compounds such as Tanin, Flavinoid, Saponin, Ascorbic acid, 1,8-Cineole, Myricetin and Delta-cadinene.Since *Myrtus communis* is used to treat sexual impotent in some areas, the present study was performed to evaluate the effect of hydro alcoholic *Myrtus communis* leaf extract on pituitary – gonad axis in adult male rat. Forty adult male rats wistar strain were selected and randomly divided into five groups; control(n=8) which received no treatment, sham which received distilled water as a solvent(n=8), and three experimental groups (1,2and3)(n=8 for each of group) which received 0.75, 1.5and 3 mg/kg *Myrtus communis* leaf extract respectively. The extracts were delivered through meal for 21 days. After the last prescription of extract, the animal were unconscious and a blood was taken blood from their heart. Then considered the concentration of FSH, LH and testosterone by radio immunoassay (RIA) method. The obtained results were analyzed based on spss, Excel, one –way variance and post-hoc statistical programs , and significant at the level ($p< 0.05$) take in consideration. The results of hormonal examination indicated the addition of 1.5 and 3 mg/kg body weights of *myrtus communis* leaf extract showed a significant increase in the level of testosterone ($p< 0.05$) but concentration of LH, FSH hormones showed no significant difference. The *Myrtus communis* leaf extract causes an increase in the testosterone hormone ,that probably is related the compounds as Flavinoid, Ascorbic acid and Myricetin (by inhibition of aromatase activity) , Linoleic acid, Oleic acid and Palmitic acid (by inhibition 5alpha reductase activity) and 1,8-Cineole, Delta-cadinene (the cytochrome-P450 Inducer).

Keywords: *Myrtus communis, Sex hormones, Rat*

Introduction

Myrtus.communis belongs to the family of myrtaceae, order myrtales, and subclass Rosidae.Myrtus (ancient greek name) is a dominant genus of family myrtacea. This genus, which consists of evergreen shrubs or small trees, has been further divided into 100 species, widely distributed in the warmer parts of the world (Fahim *et al*.,2009). The plant *M.communis* linn. Known as true myrtle, is an evergreen shrub or small tree with dense foliage, which may grow more than 5 meter (about 16.5 feet) high. It is native to

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Mediterranean area. (Elfellah *et al*.,1984). The leaves are in cross rows, thick, aromatic due to oil- bearing glands, which are strongly scented when crushed, balsamic homeostatic and tonic (Chiej,1984) myrtul, a volatite oil found in most parts of the plant, was formerly used as an antiseptic and tonic (Genders,1994). The flowers are white and solitary, with a length of about 1.8 cm, born on short stalks (Davis ,1990). The fruit is a purplish black berry with many seeds. It can be eaten fresh when ripe or can be dried as it was once used as an aromatic food flavoring, especially in the Middle East and can also be made into an acid drink (Bown ,1995). *Myrtus communis* has different chemical compounds including: Tanin, Saponin, flavinoid, ascorbic acid. In addition to mentioned compounds, *Myrtus communis* has Terpinolene percent of all extract compounds include its main part, also placed Cineole (Paoletta *et al*.,2008) percent and the terpineol (Campbell and Kurzer,1995) percent and linalyl acetate percent (Chevallier,1996). The other main compounds are limonene, toluene, Delta- cadinene, and 1,8- Cineole and Gamma terpinene, myrcene, myrtenil acetate, camphene, Linoleic acid, Oleic acid, palmitic acid. (James and Duke,2005)

M.communis linn has been traditionally used as antibiotic, antiseptic, astringent, antidiabetic, homeostatic and tonic. The fruit is carminative, used for treating bronchitis, diarrhea, dysentery, hemorrhage and internal ulceration (Alem *et al*.,2008)). The plant is also taken internally for the treatment of urinary infection, digestive problems, vaginal discharge, bronchial congestion, sinusitis and dry coughs(Bialymstoku *et al*.,2004). A decoction is employed as a mouthwash in cases of aphthae (George *et al*.,1972). The leaves are effective ointment, both fresh and dried, in the treatment of acne, eczema, wounds, ulcers, gum infections and hemorrhoids, while the myrtle oil is used with advantage, in place of dried leaves, as a flavoring for culinary purposes. The leaves are also a rich source of flavonoids, and especially of myricetin glycosides. (Hinou *et al*.1988).

Several investigations have revealed the strong antibacterial activity of the leaves extract (Feisst *et al*.,2005). The oligomeric acylphloroglucinol, myrtucommulone-A, showed strong antibacterial and anti-inflammatory properties, while a powerful antioxidant activity was observed for its lower homologue semimyrtucommulone (Rosa *et al*.,2003). Today, there is a number of Myrtus drugs in iran market including: Myrtoplex cream, Myrtex Solution, Aftoplex, Dineh Inhaler Power, Rectol cream and so on. The present study was performed to evaluate the effect of hydro alcoholic *Myrtus communis* leaf extract on pituitary – gonad axis in adult rat.

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Materials and Methods

The extract was provided through percolation method. The solvent was made up of 50% of water and 50% of ninety-six percent ethanol. After grinding the leaves, the power was mixed with the solvents for 72 hours. Then, the filtered complex was centrifuged with 4500 RPM. The outcome of the process was then condensed and desiccated into a dried brown substance.

The subjects were 40 adult male wistar rats weighing 200-220 gram and 2.5-3 month old, provided by the animal breeding center of Islamic Azad University of Kazerun and kept there too. The temperature was 26±2 centigrade, and the light period was 12 hours light and 12 hours darkness. The research started at late may to the end of September 2009. There was no control over the subject water or food. The rats were kept in poly carbonate cages (15 x 25 x 40 steel roof).

There were 8 rats in each cage. The floor was covered with sawdust and was cleaned every three days. Animals were divided into five groups including eight rats weighing an average of 200 gram. Control group: that only received water and food, 8 rats. Sham group: which received 0.2 ml distilled water(as a solvent) daily, and experimental groups, one, two and three which received 0.75, 1.5 and 3mg/kg myrtus extract every day respectively. The extract was delivered to subjects trough their meal. The study lasted for 21 days. Animals were anaesthetized by inhaling method and helping the Ether 24 hours after the latest prescription of extract. The animal thorax cleaved and 5 ml blood from the left ventricle was obtained the obtained blood sample was centrifuged in any case for 15 minutes and with speed of 3000 RPM. Concentration of FSH, LH and testosterone were considered and determined from radioimmunoassay (RIA) using the kentron mechanism. The used kit was prepared for the testosterone hormone from Germany IBL company and for LH and FSH hormones from America Monobind Company. The obtained results were analyzed based on spss, Excel, analysis on- way variance and posthoc statistical programs, and the level of significance was ($p<0.05$) take in consideration.

Results

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Considering the hormone level a significant increase was evident in the amount of testosterone hormone of experimental groups (2,3). However no significant difference was observed in the LH and FSH hormone level of experimental groups (table 1).

Table 1. comparison of FSH, LH, Testosterone hormones plasma concentration mean after the prescription of *myrtus communis* leaf extract in adult male rats.

Data are presented mean ± SEM (X±SEM)

Groups	*Myrtus communis* leaf extract (mg/kg)	Testosterone (ng/ml)	LH (mIu/ ml)	FSH (mIu/ml)
Control	-	2.76 ± 0.31	0.76 ± 0.24	23.50 ± 2.04
Sham	Solvent	3.03 ± 0.30	0.50 ± 0.10	25.95 ± 2.87
Experimental group 1	0.75	3.06 ± 0.25	0.72 ± 0.21	26.15 ± 3.19
Experimental group 2	1.5	4.22 ± 0.30*	0.37 ± 0.06	25.22 ± 2.69
Experimental group 3	3	4.63 ± 0.38*	0.52 ± 0.10	26.37 ± 1.91

* significantly different at p<0.5 level, (compared with the control group).

Discussion

As table 1 reveals, in experimental groups (1 and 3) a significant increase in the level of testosterone was observed hormone. Which may have a different reason. Flavinoid is one of the present compound in the *myrtus communis* leaf extract (Amensour *et al*.,2009). Flavinoid causes an increase in the testosterone hormone serum level to inhibit the activity of enzymes that participate in the testosterone metabolism including: Aromatase and 5 alpha reductase (Appendino *et al*.,2002). The research showed that flavinoid inhibit the performance of 5 alpha reductase. Thus, they inhibit to convert testosterone to dihydro testosterone and as a result, leads to an increase rate of testosterone hormone (Guyton, 2010).

Ascorbic acid (vitamin c) is another compound of *myrtus communis* (amount 82 mg per 100gr desiccated leaves of plant) (Yoshimura *et al*.,2008). Research showed that ascorbic acid can cause the control of aromatase enzyme activity, which seems to be increase by pH. The proper pH is 7.5 for aromatase activity which seems to be very sensitive to pH changes. Since aromatase enzyme cause androgen to convert to estrogen its restriction can cause an increase in the androgen ie, testosterone serum level. In general ascorbic acid has anti-infertility property .On the other hand, compounds such as linoleic acid, oleic and palmitic acid in *myrtus communis*, the inhibition of 5 alpha reductase. Control of this enzyme decrease the conversion of testosterone to dihydro testosterone (active from of the hormone in the tissue). Which ultimately causes as increase in testosterone level (Praveen and vayail, 2003).

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Myricetin is another compound of *myrtus communis*, which inhibit the activity and controls the aromatase enzyme and finally, causes, an increase in testosterone hormone serum level. (Ipo and Andrzej, 2002)

1,8 cineol another monoterpen, is a main compound of *myrtus communis*. Cineol causes the induction of Cytochrome p450. On the other hand Cytochrome p450 play a basic role in the laydige cells at testosterone biosynthesis (cholesterol convert to pergenanolon) direction, and family its induction cause, increase in the testosterone biosynthesis. Delta Cadinene is another *myrtus communis* compound, able to induce testosterone. It cause an increase in the testosterone serum level. Also Delta cadinene can induce cytochrome p450, and ultimately cause in the testosterone hormone serum level. (Hayder *et al.*,2008).

Mytrus communis has numerous chemical compounds including: myrtocummulone, semimyrtucommulone, tannin, terpinolen, linalil acetate, gama terpinen, myrsen, myricitrin, delta cadinene, camphon with have strong anti-oxidant properties. Since anti-oxidant properties control super oxid and hydroxyl radical; it causes the spermatogenesis natural trend to continue. Moreover, antioxidant compounds have the vitaminoid effects which increase Testosterone level and decreases estrogen level. (Feisst *et al*.,2005).

Considering the hormone situation of research samples, the amount of LH and FSH serum revealed no significant change in the amount of concentration of LH, FSH hormones in the experimental group (table 1) It can be concluded that since feedback mechanism of FSH is not the only factor cause the action testicle steroids inhibin, Activin and follistatin influence GnRH in the FSH concentration regulation it may cause the modification of the FSH concentration
(James and Duke, 2005).

Conclusion

Myrtus communis leaf extract causes an increase in testosterone hormone, which is probably related to compounds including flavonoid (inhibition 5 Alpha Reductase and Aromatase), ascorbic acid, myricetin (inhibit aromatase) and linoleic, oleic acid and palmitic acid as the compounds of controller 5 Alpha reductase, 1,8 cineol and delta cadinene (Cytochrome p450).

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