Effect of phenolic compounds of leaves extractes from Mentha spicata and Mentha longifolia on sex hormones level of female rats.

Khalid G. Al-Fartosi, Eman A. Al-Rekabi

Department of Biology, College of Science, University of Thi-Qar, Iraq.

Abstract:

The present work aimed to study the effect of phenolic compounds of Mentha spicata and Mentha longifolia on sex hormones level which including follicle-stimulating hormone (FSH), luteinizing hormone(LH), estrogene and progesterone in female rats.

Tow doses (200 and 400 mg/kg) from each plant were used and the animals were injected intraperitonially for 30 days as one dose/day. The results indicated that phenolic extractes of M. spicata and M. longifolia caused a significant increase ($P<0.05$) in plasma (FSH), (LH), estrogene and progesterone when compared with control group, which treated with normal saline.

Key Words: Mentha spicata, Mentha longifolia, phenolic compounds, FSH, LH, estrogene, progesterone.

Introduction:

Phenolic compounds are secondary metabolic compounds produced in plants, which possess in common an aromatic ring bearing one or more hydroxyl substituents C-OH, they are soluble in water , which sometimes combined with sugar molecule as glycosides and they are usually located in cell vacuoles (Harborne ,1984).

Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor and antimicrobial (Hertog, 1995; Rice-Evans et al., 1996; Middleton et al., 2000). Considering their bioactivity and presence in a wide range of vegetables, these substances are considered natural antioxidants and the vegetable source that it contains as functional food (McDonald et al., 2001).

Lamiaceae is one of the large plant families used as aframework to evaluate the occurrence of some typical

secondary metabolites (Wink, 2003). Most Lamiaceae accumulate terpenes and a range of other components, mainly in the epidermal glands of leaves, stems and reproductive structures. The genus Mentha includes (25–30) species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman et al., 2003). Mint has high levels of carvone, limonene and phenolics (Kumar & Chattopadhyay 2007).

The mint species have a great importance, both medicinal and commercial. Indeed, leaves, flowers and stems of Mentha spp. are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavour (Kothari and Singh, 1995; Moreno et al., 2002).

In addition, Mentha spp. has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its antinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities (Iscan et al., 2002; Moreno et al., 2002).

M. spicata (Spearmint) Labiatae is (30–100) cm long and has a strong odor. It has smooth or gray haired leaves. Its flowers are pale blue and collected at the edges of the branches as a long and narrow spike (Akdogan et al., 2004).

M. longifolia (horsemint) is a fast-growing, It can reach up to 1.5 m high. Strongly aromatic, The leaves (long and narrow with a sharp point) are usually coarsely hairy and the edges sparsely toothed, and they are formed in pairs opposite each other along the square-shaped stem. The small flowers of Mentha longifolia are crowded into spikes at the tip of the stems.Varying in colour from white to mauve(Codd, 1985).

The present study aimed to investigate the effect of phenolic compounds of Mentha spicata and Mentha longifolia on sex hormones level which including follicle-stimulating hormone (FSH), luteinizing hormone(LH), estrogene and progesterone in female rats.

Materials and Methods:

Plant material and powder preparation:

Fresh, spermint and horsmint were obtained from the local market in Thi-Qar province, Iraq. Each plant leaves were cleaned and dried under shade at room temperature (25°C), samples were ground to a powder form using electrical grinder, and the powder was kept in refrigerator in clean container until using.

Phenolic compounds reagents:

General phenols were detected according to (Jaffer et al.,1983).

Extraction of crude phenolic compounds:

Crude Phenolic compounds were extracted according to (Ribereau-Gayon, 1972) .

Experimental design:

The study was carried out on thirty mature female rats(Rattus norvegicus), age as 10-12 weeks and weighing between 180 - 200 gm were procured from Department of Biology, College of Science, Thi Qar University, Iraq. The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. The animals were divided into five equal groups, each group consist of (6) rats:

1- the first group(control group) was injected by (0.5ml/animal) from normal physiological saline (0.9% NaCl).

2- the second group was injected by (0.5ml/animal) of (200mg/kg) of M.spicata phenolic extract.

3- the third group was injected by (0.5ml/animal) of (400mg/kg) of M.spicata phenolic extract.

4- the fourth group was injected by (0.5ml/animal) of (200mg/kg) of M.longifolia phenolic extract.

5- the five group was injected by (0.5ml/animal) of (400mg/kg) of M.longifolia phenolic extract.

the animals were injected intraperitonially for 30 dayes as one dose daily.

Blood collection:

After thirty days of treatment, the animals were sacrificed. subsequently, the blood samples were collected by cardiac puncture, 5mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20ºC until the time of assay.

Hormone assay:

Serum samples were analyzed for FSH and LH concentrations, through solid phase ELISA based on the principle of competitive binding, using commercial kits from VEDALAB (France), while for measurement of estrogene and progesterone using kit from Bio Meriux(France).

Statistical analysis:

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ($P<0.05$) was considered to be statistically significant. and used to calculate least significant difference (LSD) values for the comparison of means following Steel et al., (1997).

Results and discussion:

The obtained results revealed a significant icreanse ($p<0.05$) in FSH and LH hormone level of the female rats treated with phenolic extractes of M. spicata at dose (200,400)mg/kg and M. longifolia at dose(200,400) mg/kg when compared with control group (table 1).

The results showed a significant increase ($p<0.05$) in plasma estrogene hormone level of the female rats treated with phenolic extractes of M. spicata at dose(200,400)mg/kg and M. longifolia at dose 400 mg/kg, while the rats treated with phenolic extractes of M. longifolia at dose 200 mg/kg showed non significant increase in plasma estrogene hormone when compared with control group (table1).

Female rats treated with phenolic extractes of M. spicata at dose(200)mg/kg and M. longifolia at dose(200,400)mg/kg showed increase in plasma progestrone hormone, non significant when compared with control group, while the rats treated with phenolic extractes of M. spicata at dose(400)mg/kg showed a significant increase($p<0.05$) in plasma progestrone hormone when compared with control group (table 1).

Table 1 : Effect of phenolic compounds of Mentha spicata and Mentha longifolia on sex hormone levels of female rats.

Animal groups	FSH (mIU/ml) Mean ± S.E	LH (mIU/ml) Mean ± S.E	Estrogene (pg/mL) Mean ± S.E	Progestrone (mg/dL) Mean ± S.E
First group	3.50±0.1^{e}	3.10±0.03^{b}	17.50± 0.54^{b}	22.57±1.5^{b}
Second group	3.89±0.17^{d}	3.65±0.17^{a}	18.65±0.54^{b}	23.77±0.87^{ab}
Third group	4.47± 0.23^{a}	3.44±0.05^{a}	26.45± 1.74^{a}	23.46±0.71^{ab}
Fourth group	4.29± 0.08^{b}	3.62±0.06^{a}	28.49± 2.5^{a}	23.61±0.48^{ab}
Fifth group	4.03±0.30^{c}	3.46±0.07^{a}	24.60± 1.92^{a}	25.53±0.65^{a}
LSD	0.5	0.2	4.83	2.7

Values are means ± S.E.

Different letters refer to significant differences ($p<0.05$).

Same letters refer to No significant differences ($p<0.05$).

The results revealed increase in plasma follicle-stimulating hormone(FSH), luteinizing hormone(LH), estrogene and progesterone. From the results of this study, it seems that phenolic extractes of M. spicata & M. longifolia have a positive effect mainly on the gonads, stimulating the secretion of these gonadal hormones into circulation female rats. It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH and LH are regulated by the gonadotropic releasing hormone secreted by the hypothalamus.

This result agree with Ahmad et al. (2012) who observed that there was a significant increase of serum estradiol and progesterone in immature female rats treated with aqueous methanol extract of Flax seeds,and agree with Uboh et al. (2010) who observed that there was a significant increase of serum estradiol and progesterone in female rats, and testosterone in male rats treated with aqueous extract of P.Guajava leaves and ascorbic acid.

Sex hormones, particularly estradiol and progesterone in females, are produced primarily of the gonads under the influence of FSH and LH. The increases in the concentrations of sex hormones are known to exert positive feedback influence at the level of the pituitary gland, where they regulate the secretion of gonadotropins (Abraham et al., 1972; March et al.,1979).

Akdogan et al. (2007) reported that there was a significant increase in follicle-stimulating hormone, luteinizing hormone and estradiol and decrease in free testosterone levels in hirsute women treated with M. spicata extractes. In the menstrual cycle FSH, LH and estrogene levels increased progressively from menstrual phase to ovulation. In this study, the increase of these hormones levels after the post-treatment period could be due to physiological changes of the menstrual cycle.

In nonpregnant females with a normal menstrual cycle, the progesterone level remains relatively constant throughout the follicular phase of the menstrual cycle and the increases rapidly following the ovulation, while the estradiol secretion follows a cyclic biphasic pattern, with the highest concentration found immediately prior to ovulation (Baird, 1976; McNastty et al., 1976).

Imbalances or alterations in these hormones lead to irregularity in the ovarian functions and duration of estrous cycle (Shivalingappa et al., 2002). These hormonal imbalances may be caused by numerous chemical agents contained in plant extracts.

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تأثير المستخلصات الفينولية لنباتي النعناع Mentha spicata والبطنج Mentha longifolia في مستوى الهرمونات الجنسية لأناث الجرذان المختبرية

خالد كطع الفرطوسي إيمان علي الركابي

قسم علوم الحياة- كلية العلوم- جامعة ذي قار- العراق

الخلاصة:

تهدف الدراسة الحالية معرفة تأثير المستخلصات الفينولية لأوراق نباتي النعناع M. spicata والبطنج M. longifolia في مستوى الهرمونات الجنسية والتي تشمل الهرمون المحفز للجريبات FSH والهرمون اللوتيني LH والاستروجين والبروجسترون لأناث الجرذان المختبرية.

استعملت الجرعتان (200 و400) ملغم / كغم من المستخلصات الفينولية للنباتين وحقنت الحيوانات تحت البريتون لمدة 30 يوم بواقع جرعة واحدة يوميا. واظهرت النتائج ارتفاعا معنويا في مستوى الهرمون المحفز للجريبات والهرمون اللوتيني والاستروجين والبروجسترون مقارنة مع مجموعة السيطرة المعاملة بالمحلول الفسلجي.

الكلمات المفتاحية:

النعناع-البطنج-المستخلص الفينولي-الهرمون المحفز للجريبات-الهرمون اللوتيني-الاستروجين-البروجسترون.