

Study of Embryotoxicity of *Mentha piperita* L. During Organogenesis in Balb/c Mice

Estudio de la Embriotoxicidad de *Mentha piperita* L. Durante la Organogénesis en Ratones Balb/c

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SUMMARY: *Mentha piperita* (Labiatae), commonly known as peppermint is a native Iranian herb which is used in folk medicine for various purposes. This study was carried out to reveal the teratogenic effect of *Mentha piperita* on mice fetuses. In this experimental study, pregnant Balb/c mice divided to four groups. Case group received 600 (treatment I) and 1200 (treatment II) mg/kg/day the hydroalcoholic extract of *Mentha piperita* during 6-15 of gestational days and one control group received normal saline during GD6-GD15 by gavages and other control group did not receive any matter during 6-15 of gestational days. Mice sacrificed at GD18 and embryos were collected. Macroscopic observation was done by stereomicroscope. 20 fetuses of each group were stained by Alizarin red-S and Alcian blue staining method. The Mean weight of fetuses decreased in treatment groups rather than control ($P < 0.05$) but CRL there was no significant difference between treatments and controls groups. In the treatment I (600 mg/kg/day) and treatment II (1200 mg/kg/day), normal saline and control group, no gross congenital malformations were observed in fetuses. Treated fetuses also had no delayed bone ossification as determined by Alizarin red-S and Alcian blue staining method. This study showed that the hydroalcoholic extract of *Mentha piperita* (600 and 1200 mg/kg/day) has no teratogenic effect in mice fetuses if used continuously during embryonic period.

KEY WORDS: *Mentha piperita*; Teratogen; Bone ossification; Developmental toxicity; Mice.

INTRODUCTION

More than 80% of the people in the world population currently rely on traditional medicines and most of these therapies involve the use of plant extracts (Zhang, 2002). Recent reports indicate a wide use of medicinal herbs by pregnant women (Hepner *et al.*, 2002).

Mentha piperita is a herb, which is commonly, used in folk medicine, in Iran, turkey, India, the Middle East, Europe and Canada for flatulent colic, appetite, to relieve abdominal pain, fever, nausea and vomiting and digestion (Zargari, 1996; Westfall, 2004; Starbuck, 2001).

Also *M. piperita* is used for preventing vomiting and morning sickness in pregnant women (Westfall). *M. piperita* contains volatile oil, menthol, menthon, methofman, and limonene (Adkogan *et al.*, 2004a; Baser, 1993).

The chemical components of leave extract and oil of *M. piperita* vary with plant maturity, geographical region

and processing conditions (Ruiz Del Castillo *et al.*, 2003; Xu *et al.*, 2003).

Previous studies have shown antiviral, antibacterial and anti fungal effects of *M. piperita* (Minami *et al.*, 2003; Schuhmacher *et al.*, 2003; Choi *et al.*, 2003; Azuma *et al.*, 2003; Duarte *et al.*, 2005; Tampieri *et al.*, 2005).

Also, Inoue *et al.* (2002) and Satsu *et al.* (2004) reported anti allergic effects of this herb. Furthermore, previous studies have been shown anti-inflammatory, analgesic and antispasmodic effects of *M. piperita* (Atta & Alkofahi, 1998; Göbel *et al.*, 1995, 1996; Juergens *et al.*, 1998; Asao *et al.*, 2003; Hiki *et al.*, 2003; Micklefield *et al.*, 2003). Also Tate *et al.* (1997) reported anti nausea effect of this herb.

Some studies reported that the extract of *M. piperita* reduce symptoms in dyspepsia (Madisch *et al.*, 2001; Rösch *et al.*, 2002) and IBS patients (Kline *et al.*, 2001).

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Furthermore, a study reported the histopathological effect of *Mentha piperita* on white matter of cerebellum and proximal convoluted tubules in animal model (Thorup *et al.*, 1983; Spindler & Madsen, 1992). It was reported that the oil of *Mentha piperita* has a genotoxic effect on human lymphocytes (Lazutka *et al.*, 2001).

Recent researches have shown that spearmint tea has antiandrogenic properties in both animals and females (Adkogan *et al.*, 2007; Güney *et al.*, 2006) and anti spermatogenic activity in rodents (Adkogan *et al.*, 2004; Sampaio, 2004).

On the other hand some researches showed the chemoprotective, antimutagenic and anti carcinogenic effects of *M. piperita* (Samarth *et al.*, 2006; Samarth & Kumar, 2003; Samman *et al.*, 1998). In regard to the use of *Mentha piperita* for preventing vomiting and morning sickness in pregnant women there is a lack of studies about teratogenic effects of *Mentha piperita*. This investigation was carried out to reveal the teratogenic effect of *M. piperita* on Balb/c mice fetuses.

MATERIAL AND METHOD

Time and setting. The study was performed in 2008 at the Faculty of Medicine, Gorgan University of Medical Sciences. Approval for this study was gained from the Animal Care and Ethics Committee of the Gorgan University of Medical Sciences.

Materials. Plant material. *Mentha piperita* leaves were collected from cultivated plant, from suburb of Gorgan, Northern Iran.

Methods. Preparation of plant extract. The aerial parts of *Mentha piperita* were reduced to small pieces, dried in a circulating air stove and powdered in a grinder. The powdered material was then macerated using a hydroalcoholic (60°) solvent for 48 hours. The ethanol was removed by vacuum distillation and the resulting residue was filtered and concentrated at 40C° to make a jelly-like material. In addition to thin layer chromatography and purity tests (foreign matter, total ash, acid insoluble ash and water insoluble ash) for qualification analysis, monosaccharide-linked another reagent assay (spectrophotometry) have been carried out to determine the concentration of polysaccharides in *Mentha piperita* leaves for standardization of the extract.

At the time of administration, the prepared powder of the extract was solved by the saline and the mice were treated with the solution.

The animals used in this study were experimental, 28-30 gram weight, and 7-8 week old virgin female and mature male Balb/c mice. The males were part of the animal house breeding stock with confirmed mating experience. Dry food pellets and water were provided *ad libitum* with animal house conditions maintained at 20-22°C, 65-68% relative humidity, and a 12 h: 12 h photoperiod (lights on 0700-1900h). Two females were caged with a male of the same strain overnight. The presence of vaginal plug the next (following) morning confirmed that mating had taken place and was designated as day zero of pregnancy (Gestation Day 0: GD 0). Females that did not mate within 2 estrus cycles were excluded from the study.

Pregnant mice were randomly divided into the two experimental groups (600 mg/ kg/day and 1200 mg/ kg/day of *Mentha piperita* extract) and the two control groups. 12 mice in the two experimental groups received 600 mg/ kg/day and 1200 mg/ kg/day orally of *Mentha piperita* extract respectively. One control group received normal saline orally, from GD 6 to GD 15, by oral intubation. The other control group did not receive normal saline. On GD 18 the pregnant mice were sacrificed under chloroform anesthesia and uterus was opened and umbilical cord cut close to the fetus; each fetus and placenta were then weighed. Each fetus was assigned a number according to its position in the uterine horn, starting with number one at the ovarian end of the left uterine horn. Fetuses were assessed as either alive or dead and any resorption was noted. All live fetuses were measured crown-rump length (CRL), Bi-parietal diameter (BPD) and were examined externally for 'Qlformations or deviations from normal growth as described. Also, each of the fetuses was weighed by sensitive electronic measurement serrations PT 210 German and observed for gross malformations by stereo research microscope, Blue Light US. Fetuses were eviscerated and the skin removed to facilitate stain penetration. Skeletal staining of fetuses was performed by the Alcian Blue- Alizarin Red S method. Differences in body weight, bi-parietal diameter (BPD) and crown rump length (CRL) between controls and treatment groups were analyzed using a one-way ANOVA. A value of $P < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

During the whole experiment, no maternal deaths and behavioral changes were recorded in any group. The treated females consumed as much food and water as the controls and gained comparable weight.

Table I. Number of live, absorbed fetuses, BPD, CRL, weight of fetuses in controls and treatments. *p<0.05 compared to controls.

| Groups | Live Fetuses (n) | Fetuses (n) | | BPD (mm) | CRL (mm) | Weight (gr) |
|----------------------------------|------------------|-------------|--|-----------|------------|-------------|
| | | Absorbed | | | | |
| Control | 54 | 2 | | 7.25±0.02 | 22.96±0.38 | 1.43±0.61 |
| Control (Normal Saline) | 38 | 3 | | 7.20±0.04 | 22.85±0.22 | 1.41±0.02 |
| Treatment I (600 mg/kg Mentha) | 45 | 6 | | 7.15±0.12 | 22.38±0.22 | 1.29±0.02* |
| Treatment II (1200 mg/kg Mentha) | 52 | 1 | | 7.05±0.05 | 22.03±0.16 | 1.37±0.02* |

There were no signs of maternal toxicity due to *Mentha piperita* treatment. No signs of toxicity were noted in any of the animals. All pregnant animals appeared healthy at sacrifice. The weight of fetuses in treatment I (1.29 ± 0.02) and treatment II (1.37 ± 0.02) were lower than controls ($p < 0.05$) (Table 1). Also BPD in the treatment I group (7.15 ± 0.12) and treatment II group (7.05 ± 0.05) was not significantly lower than the control (7.25 ± 0.02) groups (Table 1). Crown rump length in treatment and controls was similar.

Implantation and number of fetuses in the left horn (55%) of uterine of the treatment group was higher than the right horn (45%). While in the control group this percent was similar, 48% in the right and 52% in the left uterine horn. Major congenital malformations in fetuses were not found in treatment and control groups. Alizarin red S and Alcian blue staining did not show skeletal malformations and delayed bone ossification in the treated groups as comparing with control groups.

DISCUSSION

The findings of this developmental toxicity study showed that the *Mentha piperita* extract did not cause any major birth defects and delayed bone ossification in fetuses if used continuously during the embryonic period. There are very rare studies on the effect of *Mentha piperita*. Inoue study in Turkey showed that the *M. piperita* has a cytotoxic effect on rats (Inoue *et al.*, 2001). Romero-Jiménez & Campos-Sánchez (2005) reported that *M. piperita* oil has a cytotoxic effect and it induced the changes in chromatids chromosomes.

Furthermore, Lazutka *et al.* reported that *M. piperita* oil according to Smart method has a cytotoxic effect.

Gordon *et al.* (1987) reported that cytotoxic effect of *Mentha piperita* can be related to high level of pulegone which is a toxic substance or menthol.

Furthermore in a study, Akdogan *et al.* (2004b) showed that *M. piperita* decreased testosterone level and increased LH, FSH concentration in male rats. Also, he reported that this herb has effect on hypophyseal-testis hormonal axis causing alterations in germinal layer of seminiferous tubules in adult male rats thus Akdogan *et al.* concluded that *M. piperita* has antispermatogenic effect in rats.

In the literature we did not find any article about teratogenic effect of this plant. In our study we did not observe any birth defect or bony deletion in fetuses treated with *Mentha piperita* extract. Morphologic cytotoxic and hormonal alterations of this herb may be due in intracellular or DAN of cells. Also the different effects of *M. piperita* in different studies can be due to various chemical components of leave or oil extract of *M. piperita* which depend on plant maturity, species, geographical region and processing conditions (Ruiz del Castillo *et al.*; Xu *et al.*)

In this study, the fetuses of the treatment group had decreasing weight.

The decreasing weight of fetus in treated experimental groups may be due to the blocking of cell growth which may be due to genotoxic effect of this herb. Also the decreasing weight of fetuses in treated experimental groups can be due to reabsorbing of extra cellular liquid in the fetus. because extra cellular liquid in the fetus makes up the main part of weight and volume of fetuses. This mechanism was explained in previous studies which reported that saffron or crocin with reabsorbing of extra cellular liquid in the fetus causes decreasing weight in fetuses (Golalipour *et al.*, 2008; Garcia-Olmo *et al.*, 1999).

The growth of the fetus during intrauterine life is reflected in the weight at birth. Fetal growth is largely determined by the availability of nutrients from the mother, as well as placental capacity to supply these nutrients in sufficient quantities to the fetus. Of course there is evidence that both placental volume and the rate of placental growth may influence fetal size. Also the decreasing of weight of fetuses can be due to blocker effect on cell growth.

This study showed that *Mentha piperita* extract has no teratogenic effect in mice fetus if it is used continuously during embryonic period, although this herb caused decreasing weight of fetuses. Therefore, regarding our results we suggest that pregnant women avoid high consumption of *M. piperita* during the organogenesis period.

Further study is needed to determine the exact mechanisms of decreasing fetus weight due to *Mentha*

piperita consumption in organogenesis period.

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RESUMEN: *Mentha piperita* (Labiatae), comúnmente conocida como menta, es una hierba nativa de Irán, que se utiliza en la medicina tradicional para diversos fines. Este estudio fue realizado para descubrir el efecto teratogénico de la *Mentha piperita* en fetos de ratones. Los ratones Balb/c preñadas fueron divididas en cuatro grupos. Los grupos recibieron 600 (tratamiento I) y 1200 (tratamiento II) mg/kg/día del extracto hidroalcohólico de *Mentha piperita* durante los días 6-15 de gestación (DG), mientras que un grupo control recibió solución salina normal durante los DG 6-15 vía oral y otro grupo control sano no recibió sustancia durante los DG 6-15. Los ratones fueron sacrificados el DG 18, recolectando los fetos. Se realizó la observación macroscópica mediante un estereomicroscopio. 20 fetos de cada grupo se tiñeron por el método de rojo de alizarina-S y azul de Alcían. La media de peso de los fetos disminuyó más en los grupos de tratamientos que los controles ($p < 0.05$), pero CRL no presentó diferencias significativas entre los tratamientos y los grupos control. En los fetos del grupos tratamiento I (600 mg/kg/día), tratamiento II (1200 mg/kg/día), solución salina normal y control no se observó ninguna malformación congénita grave. Los fetos tratados tampoco tuvieron osificación ósea retrasada según lo determinado por el método de rojo de alizarina-S y azul de Alcían. Este estudio mostró que el extracto hidroalcohólico de *Mentha piperita* (600 y 1200 mg/kg/día) no tiene efectos teratogénicos en fetos de ratones al ser utilizado continuamente durante el período embrionario.

PALABRAS CLAVE: *Mentha piperita*; Teratógenos; Osificación ósea; Toxicidad para el desarrollo; Ratones.

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