

Decrease in Body Weight and Liver Weight of Pups in Swiss Albino Mice by Spearmint Leaves Extract

ASMA ZULFIQAR¹, SITWAT AMNA², ASMA SIDDIQUE³, SAQIB MANSOOR⁴, UZMA ALI⁵, KANWAL SHARIF⁶, MUHAMMAD SUHAIL⁷

¹Assistant Professor of Anatomy, Govt. Khawaja Muhammad Safdar Medical College, Sialkot

²Assistant Professor of Anatomy, Allama Iqbal Medical College, Lahore

³Associate Professor of Anatomy, Akhtar Saeed Medical & Dental College, Lahore

⁴Assistant Professor of Anatomy, Bakhtawar Amin Medical and Dental College, Multan

⁵Assistant Professor of Anatomy, Faisalabad Medical University, Faisalabad

⁶Assistant Professor of Anatomy, Continental Medical College, Lahore

⁷Professor and Head, Department of Anatomy, Amna Inayat Medical College, Lahore

Correspondence to: Asma Zulfiqar, Email: drasmazulfiqar@yahoo.com, Cell: 0321-2546772

ABSTRACT

Objective: To evaluate the effects of spearmint leaves extract on body weight, liver weight and RTWI of pups in Swiss albino mice.

Study Design: Experimental study

Place and Duration of Study: Anatomy Department, Shaikh Zayed PGMI, Lahore from 1st March 2014 to 30th June 2014.

Methodology: Seven male and 21 female mice were used. After conception female mice were divided into control (A), low dose (B) and high dose (C) experimental groups. There were 7 mice in each group. Group A was given distilled water where as group B was given 3g/kg/day and group C were given 6g/kg/day spearmint leaves extract. After 21 days (duration of pregnancy in mice) hysterotomy was done after euthanasia. Three groups of pups were made. These pups were selected randomly and marked as control, low dose and high dose experimental groups (A1, B1 & C1 respectively). Their body weights and liver weights were taken and recorded.

Results: Body weight, weight of liver and RTWI of pups in both the experimental groups were decreased significantly with $p < 0.001$ and difference between the experimental groups was also highly significant with $p < 0.001$.

Conclusion: The spearmint leaves extract decrease the body weight, weight of liver and RTWI of pups of Swiss Albino mice.

Keywords: Spearmint, Garden mint, Herbal tea, Swiss Albino mice, Conception, Body weight, Weight of liver, RTWI

INTRODUCTION

Garden mint or the spearmint is the commonest mint of Labiatae family.¹ It is frequently used in preparing medicine,² chutney and herbal tea.³ Genus *Mentha* is one of the largest genera of family labiatae.⁴ Some of its plants are used as medicine like spearmint, peppermint, tulsi etc.² This family is used for phytotherapy in Iran⁵ and European countries.⁴ Spearmint is used in candies, chewing gums⁶, mouthwashes⁷ and in home remedies for anorexia,⁸ hiccup,⁷ bloody diarrhea³ etc.

Spearmint acts by increasing the activity of liver enzymes like cytochrome P450 3A4.⁹ LD50 of its active ingredient carvone in mice is 923 mg/kg.¹⁰ There was reduced iron absorption in rats who received spearmint in different doses.¹¹ There was apoptosis in cervix and uterus of female rats when they were given spearmint tea.¹²

As it is proved by studies that spearmint is being used for nausea and vomiting during pregnancy³ in different forms, the current study was designed to evaluate the effects of spearmint leaves extract on body weight, liver weight and RTWI of pups in Swiss albino mice.

MATERIALS AND METHODS

This experimental study was conducted in Anatomy Department, Shaikh Zayed PGMI Lahore. Initially all animals (male and female) were kept in separate cages for acclimatization for almost fifteen days. 12 hours cycle (light/dark) with room temperature of around 24±2°C was maintained. Food and water was freely available for all mice. Both 21 female and 7 male mice were then kept together for conception. Vaginal plug was seen for confirmation of pregnancy, after seeing this plug the female mice were labelled and divided into control (A), low dose (B) and high dose (C) groups. All these groups had 7 female mice.

Spearmint leaves extract was prepared from the laboratory of PCSIR Lahore. Quantification of this extract was done via Gas Chromatography Mass Spectrometry or GC-MS from Chemistry Department, Forman Christian College Lahore.

Group A: received distilled water; dose was 15 ml/100g body weight/day.

Group B: was given 20 g/L i.e. 3 g/kg body weight of spearmint leaves extract.¹³

Group C: was given 40 g/L i.e. 6 g/kg body weight of spearmint leaves extract.¹³

The animals of all groups were euthanized on day 21 of gestation by giving sodium pentobarbital, an anesthetic agent and morphine, as an analgesic, intraperitoneally in standard doses for mice.^{14, 15}

After delivery through hysterotomy after euthanasia, 28 pups were randomly selected and labeled as A1, B1 and C1. Their weight was measured and recorded and then liver of all pups was dissected, measured and their weight was recorded.

Data analysis was done by using SPSS 20. Normality of this data was tested by using Shapiro Wilks Test. Kruskal Wallis ANOVA test was applied for the comparison of these groups and the Mann Whitney U test was applied for the post hoc analysis. $P \leq 0.05$ was considered significant statistically.

RESULTS

The average body weight of pups for control group (A1) was recorded as 1.72±0.07gm, for low dose experimental group (B1), 1.42±0.07gm and for high dose experimental group (C1) it was 1.32±0.11gm (Table 1). The mean body weight of pups was highest for group A1 and lowest for group C1, the difference was highly significant with p -value < 0.001 (Table 2, Fig. 1). When group wise comparison was made, the difference between control group (A1) and both experimental groups (B1 & C1) was highly significant with $p < 0.001$. There was highly significant difference between low dose experimental group (B1) and high dose experimental group (C1) with $p < 0.001$ (Table 2). This showed that the difference among the groups was dose dependent.

Weight of liver in pups of control group was recorded as 0.056±0.005 g, in low dose experimental group 0.049±0.004 g and in high dose experimental group 0.039±0.004 g. The liver weight also had highest mean and median for group A1 and lowest for group C1 (Table 3, Fig. 2). Pair wise comparison showed that difference of group A1 from groups B1 and C1 was highly significant with $p < 0.001$. Similarly difference between B1 and C1 groups was also highly significant with $p < 0.001$ (Table 4). This result also proved that the difference in weight of liver among the groups was dose

The RTWI value for pups of group A1 was calculated as 3.24 ± 0.16 , for B1 (low dose experimental) group it was 3.39 ± 0.14 whereas for C1 (high dose experimental) group it was 2.92 ± 0.14 . The difference among three groups was significant with $p < 0.001$ as well as the pair wise comparison was that difference of group A1 from groups B1 and C1 was highly significant with p-value of < 0.001 . Similarly difference between B1 and C1 groups was also highly significant with p-value of < 0.001 (Table 5, Fig. 3).

Table 1: Body weights of pups in control and the experimental groups exposed to spearmint leaves extract, at the time of dissection

Group	Body weight of pups (gm)			
	Mean \pm SD	Median	Q1	Q3
A1	1.72 \pm 0.07	1.77	1.64	1.78
B1	1.42 \pm 0.07	1.43	1.41	1.47
C1	1.32 \pm 0.11	1.35	1.31	1.38

Table 2: Pair-wise comparison of body weights of pups in control and experimental groups exposed to spearmint leaves extract at birth (by using Tukey's Test)

(I) Group	(J) Group	Mean Difference (I-J)	Standard Error	P-value
Group A1	Group B1	0.291	0.023	<0.001**
	Group C1	0.398	0.023	<0.001**
Group B1	Group C1	0.106	0.023	<0.001**

**Highly significant difference ($p < 0.001$)

Table 3: Weight of Liver of pups in control and the experimental groups exposed to spearmint leaves extract, at the time of dissection

Group	Weight of liver (gm)			
	Mean \pm SD	Median	Q1	Q3
A1	0.056 \pm 0.005	0.060	0.050	0.060
B1	0.049 \pm 0.004	0.050	0.050	0.050
C1	0.039 \pm 0.004	0.040	0.040	0.040

Table 4: Pair-wise comparison of liver weights of pups in control and the experimental groups exposed to spearmint leaves extract (by using Mann-Whitney U test)

(I) Group	(J) Group	Mann Whitney U	Z-Approx	P value
Group A1	Group B1	144.0	-4.82	<0.001**
	Group C1	0.0	-6.82	<0.001**
Group B1	Group C1	48.0	-6.32	<0.001**

Table 5: Pair Wise Comparison of Relative Tissue Weight Index for Liver of Pups in Control and Experimental Groups Exposed to Spearmint Leaves Extract (by Using Mann Whitney U Test)

(I) Group	(J) Group	Mann Whitney U	Z-Approx	P value
Group A1	Group B1	153.5	-3.93	<0.001**
	Group C1	45.5	-5.69	<0.001**
Group B1	Group C1	7.5	-6.32	<0.001**

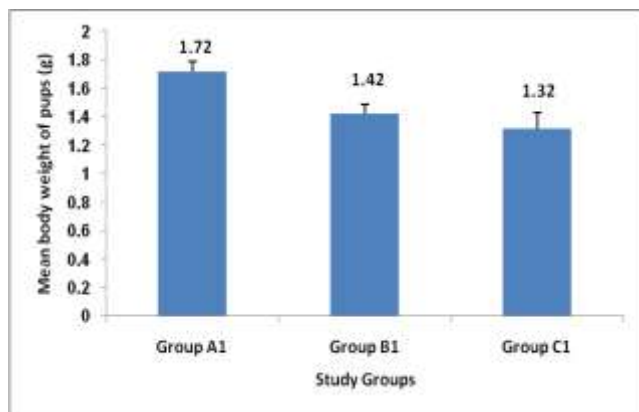


Fig. 1: Comparison of mean body weight (g) of pups of control group and the experimental groups exposed to spearmint leaves extract

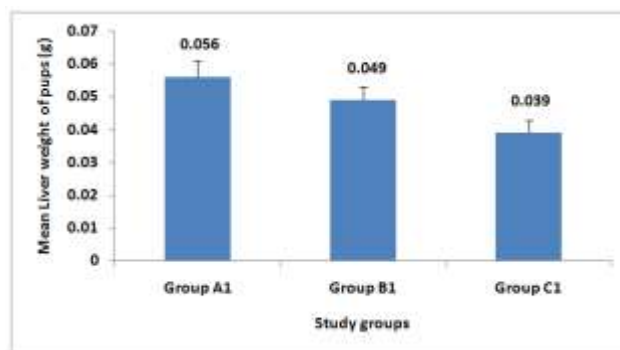


Fig. 2: Graphic comparison of liver weight (gm) of pups of group control group and the experimental groups exposed to spearmint leaves extract

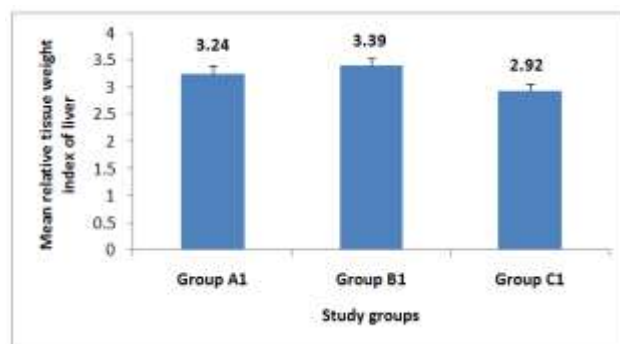


Fig. 3: Comparison of RTWI of pups of control group and the experimental groups exposed to spearmint leaves extract

DISCUSSION

Spearmint, or podina, is commonly used in diets in East Asia and India.¹⁴ Its use in toothpaste, candies, gums, etc. It is also very common. By increasing the activity of cytochrome enzyme 9, it decreases the concentration of many hormones in the blood.¹⁴

In present study the mean body weight of pups of control group was measured as 1.72 ± 0.07 g, in low dose experimental group 1.42 ± 0.07 g and for high dose experimental group it was 1.32 ± 0.11 g (Table 1). The comparison among the control and experimental showed that there was marked decrease in body weights of experimental groups. The difference was highly significant statistically with $p < 0.001$ (Table 2). Similar reduction in body weight of pups was seen in a study conducted on dill (*Anethum graveolens* L.) which was given to female rats in low and high doses. Decreased body weight was seen in these pups because of the harmful effects of this herb on embryo at cellular as well as molecular levels. It also contains phytoestrogens which interfere with synthesis and degradation of Estrogen.¹⁵

In another study¹⁶ dill/soya seeds extract decreased the body weight by increasing serotonin levels and suppressing the intake of food when given orally to adult obese rats for 5 weeks. Serotonin increased levels in blood would cause stimulation of satiety centers in brain, which lead to weight reduction. In another study also performed on adult male rats upon giving essential oils containing carvone, they showed decrease in the body weight significantly with $p < 0.05$.¹⁷ Different studies have proved that spearmint increases lipid peroxidation,¹³ which itself is a cause of decreased fetal weight.¹⁸

On the contrary, there was no significant change in the mean body weight of adult female rats, when 20 g/L spearmint tea was given to these rats for 30 days.¹² Mugisha¹⁹ and Nozhat et al²⁰ also reported that there was no change in body weight of male rats treated with spearmint extract in low doses.

In the present study, the liver weight was also decreased in experimental groups. The weight of liver of group A1 (control) had the highest mean and median, where as group C1 (high dose

experimental) had the lowest mean and median values (Table 3). There was highly significant difference between the weight of liver of control and both experimental groups were significant with $p < 0.001$ (Table 4, Fig. 2).

In a study conducted by Lasrado et al.²¹ in 2014, reported an increase in weight of liver, heart, pituitary, thyroid and salivary glands in adult rats by use of spearmint extract for 90 days. Weight of liver and some other organs was increased in adult diabetic rats when they received water and ethanolic extract of dill for 15 days. The mechanism of such changes was because of inflammation of the organ.²² In another study, Liver weight gain was also seen in male rats who received carvone containing essential oils. This increase in liver weight was due to increased levels of serum cholesterol and triglycerides, which may lead to fatty liver.¹⁷

RTWI was also calculated in the present study and it showed that the group B1 (low dose experimental) had highest mean and median values than group A1 (control) and C1 (High dose experimental). The difference among three groups was highly significant with $p < 0.001$ (Table 5 Fig. 3). Similar result was also observed in a study on adult rats treated with high dose of spearmint extract that showed increase in RTWI.²¹ Another study showed that RTWI for liver was also increased in adult rats where limonene was administered in various doses, this increase in RTWI might be due to enzyme induction by limonene.²²

CONCLUSION

The leaves extract of spearmint decreases body weight, weight of liver and RTWI of pups of Swiss Albino mice. Therefore, use of spearmint in all forms must be reduced during pregnancy so that there will be minimal chance of such complications

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