



Effect of Spearmint Leaf Extract on Crown Rump Length of Pups of Swiss Albino Mice

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ABSTRACT

Introduction: Spearmint (*Mentha spicata* L), a member of Labiatae family, is used as herbal medicine for treatment of different gastrointestinal and urogenital diseases. **Aims & Objectives:** To evaluate the effect of spearmint leaf extract on crown rump length (CRL) of Swiss albino mice pups. **Place and duration of study:** This experimental study was conducted for 30 days in the Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore. **Material & Methods:** 21 female and 7 male mice were used for this study. Pregnant female mice were divided into three groups each having 7 animals. They were labelled as group A (control), group B (low dose experimental) and group C (high dose experimental). Group A received distilled water while group B and C received 3 & 6g/kg/day spearmint leaf extract respectively. On completion of gestation all the pups were delivered by hysterotomy. 28 pups from each group were selected randomly and labeled as A1 (control), B1 (low dose) and C1 (high dose experimental). Their CRL was measured and recorded. **Results:** CRL of pups in both experimental groups was decreased significantly with p-value <0.001. The difference among the experimental groups was also highly significant statistically with p-value <0.001. **Conclusion:** The present study reveals that spearmint leaf extract has a decremental dose dependent effect on CRL of Swiss albino mice pups.

Key words: Mentha Spicata Labiatae, herbal medicine, swiss albino mice, gestation, CRL.

INTRODUCTION

Spearmint (*Mentha Spicata* L) belongs to genus *Mentha*¹ and family Labiatae.^{2,3} Genus *Mentha* includes 25-30 species, some of them are spearmint, peppermint, curled mint, etc. Spearmint is the commonest mint having pungent taste.^{5,10} It is cultivated throughout the world⁵ but United States is its major producer.⁶ Small plant of spearmint has blue flowers that grow at the edges of the branches as narrow and long spikes.⁷ Common constituents of this plant are limonene, carvone, 1, 8-circole and volatile oils among which carvone is present in large amount.⁸ LD50 of spearmint extract is 13,606 mg/kg in adult Swiss albino mice.⁹

Different parts of spearmint plant are used as home remedy for certain diseases of gastrointestinal tract, starting from the oral cavity, it is used for treatment of dental carries, anorexia,¹⁰ nausea, vomiting,¹¹ hiccup,⁵ flatulence,⁵ bloody diarrhea¹² and hemorrhoids.¹³ It reduces the chemotherapy induced nausea and vomiting when used along with the other

antiemetic drugs.¹⁴ It is also helpful in treating some disorders of urogenital system for example in treatment of amenorrhea, dysmenorrhea¹⁰ and oliguria.¹³

Spearmint caused heartburn in humans (healthy volunteers) due to direct mucosal irritation without increasing the acid reflux or decreasing the pressure of lower esophageal sphincter¹⁵ and also produced dose dependent hepatic damage in male Wistar albino rats.⁸ In a human study spearmint had reduced the recurrence of nausea and vomiting induced by chemotherapy when given along with other antiemetic drugs.¹⁴

Histological sections of kidney tissues showed hydropic degeneration of epithelial cells, dilatation of tubules, enlargement of Bowman's capsule with focal infiltration of white blood cell in interstitial areas. Dose dependent atrophy of some tubules and glomeruli was also observed.¹⁶

As spearmint is used very commonly in nausea of pregnancy, the present study was designed to evaluate the effect of spearmint leaf extract on CRL of swiss albino mice pups.

MATERIAL AND METHODS

This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore.

Male and female mice were kept separately and acclimatized for 15 days. A twelve hours light and dark cycle was maintained at room temperature of 24 ± 2 °C.⁸ The food and water were provided to these mice ad libitum.

After acclimatization, one male and three female mice were kept together in a cage for a week for conception. Pregnancy was confirmed by presence of vaginal plug.

After conception female mice were randomly divided into three groups, A (control), B (experimental low dose) and C (experimental high dose), each having 7 female mice and were marked for identification with permanent markers of different colors.

Spearmint leaf extract was prepared from PCSIR laboratory, Lahore. Quantification of extract was done through Gas Chromatography-Mass Spectrometry (GC-MS) from Chemistry Department of Forman Christian College, Lahore.

Spearmint extract was given to the pregnant mice through gastric intubation.

GROUP A: Animals were given distilled water 15 ml/100 gm body weight per day for 15 days.

GROUP B: This was low dose experimental group, mice were given 20 g/L (3 g/kg body weight) of spearmint leaf extract for 15 days.

GROUP C: This was high dose experimental group, mice were given 40 g/L (6 g/kg body weight) of spearmint leaf extract. Duration of dosage was 15 days.

After euthanasia^{17,18} all fetuses were delivered through hysterotomy. 28 pups from each group were labeled accordingly as A1, B1 and C1. They were examined for congenital anomalies and their CRL i.e. length of the dorsal surface of the body of mice pups from external occipital crest to the center of anal opening¹⁹ was measured and recorded (Fig-1).

Statistical analysis:

The data was analyzed by using SPSS 20.0. Normality of data was tested by Shapiro Wilks Test. Mean, standard deviation, median and interquartile range were used as descriptive measures of crown-rump length of pups (a quantitative parameter). As assumption of homogeneity of variance wasn't met, Kruskal Wallis ANOVA was applied for comparison of variable in groups and Mann Whitney U test was applied for post hoc analysis. P-value ≤ 0.05 was considered as statistically significant.

RESULTS

The normality of CRL of pups of all groups revealed skewed distribution when tested by Shapiro Wilks test with p-value <0.001 (highly significant, Table-1) and homogeneity of variance was heterogeneous with p-value 0.016 (Table-2). The mean CRL of group A1 was 2.84 ± 0.14 cm, where as in groups B1 and C1 it was 2.58 ± 0.10 cm and 2.38 ± 0.12 cm respectively (Table-3).

The mean and median of CRL were highest for group A1 and lowest for C1. The difference was found highly significant among three groups with p-value <0.001 (Table-4, Fig-2), when pair wise comparison was made, the difference between control group and both experimental groups was highly significant with p-value <0.001 . There was highly significant difference between B1 and C1 with p-value <0.001 (Table-5).



Fig-1: Photograph of pup of Swiss albino mice, showing its CRL measurement.

CRL of pups (cm)	Group	Shapiro-Wilks		
		Statistic	Df	Sig.
	Group A1	.815	28	<.001**
	Group B1	.850	28	<.001**
	Group C1	.851	28	<.001**

Table-1: Test of Normality for CRL of Pups in Control and Experimental Groups

	Levene Statistic	df1	df2	Sig.
CRL of pups (cm)	4.380	2	81	.016*

Table-2: Test of Homogeneity of Variance for CRL of Pups in Control and Experimental Groups

Groups	Crown-rump length of pups (cm)				
	Mean	SD	Median	Q1	Q3
Group A1	2.84	±.14	2.80	2.70	3.00
Group B1	2.58	±.10	2.60	2.50	2.70
Group C1	2.38	±.12	2.40	2.30	2.50

Table-3: CRL of Pups in Control and Experimental Groups, at the Time of Dissection

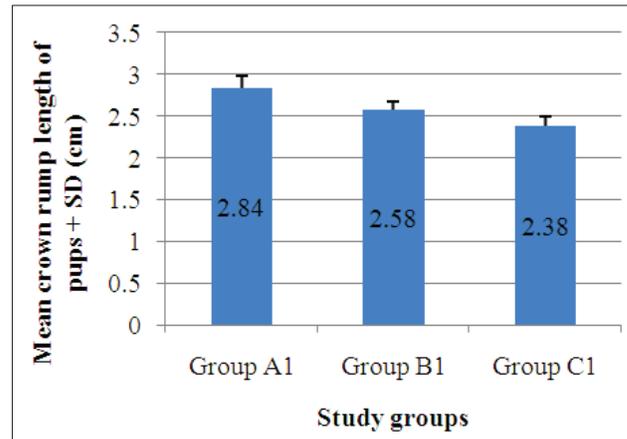


Fig-2: Graphic comparison of CRL (cm) of pups of control group and the experimental groups, exposed to spearmint leaf extract.

Group	N	Mean Rank	Kruskal Wallis ANOVA		
			Chi-square	df	P-value
Group A1	28	68.32	63.9	2	< 0.001**
Group B1	28	42.29			
Group C1	28	16.89			
Total	84				

Table-4: Comparison of CRL of Pups in Control and Experimental Groups (by using Kruskal Wallis ANOVA)

(I) Group	(J) Group	Mann Whitney U	Z-Approx	P-value
Group A1	Group B1	61.0	-5.56	< 0.001**
	Group C1	0.0	-6.50	< 0.001**
Group B1	Group C1	67.0	-5.47	< 0.001**

Table-5: Pair Wise Comparison of CRL of Pups in Control and Experimental Groups (By Using Mann Whitney U Test)

Key

- df degree of freedom
- F f-test (Ratio of variances)
- Sig. significance (P-value)
- ** highly significant difference (p-value < 0.001)
- * significant difference (p-value < 0.05)
- SD standard deviation
- Q1 middle of first half of data
- Q2 median
- Q3 middle of second half of data

DISCUSSION

Spearmint is a common constituent of East Asian and Indian diet.²⁰ It is used in medicines, candies and chewing gums etc.^{20,26} It is used as herbal medicine for certain diseases of GIT²⁵ and urogenital system.^{11,13} Spearmint increases the activity of cytochrome P450²¹ and decreases the blood concentration of some hormones.^{20,22}

In the current study CRL of pup was recorded and found decreased in both experimental groups. The mean CRL was lowest for group C1 and low in group B1 when compared with group A1. This difference was highly significant statistically with p-value < 0.001 (table-4), the difference between B1 and C1 was also highly significant with p-value < 0.001 (table 5). This showed that the result was dose dependent.

Similar findings were observed by Malinezaman et al in 2014 that showed the CRL of all pups of rats was significantly decreased in both low dose and high dose experimental groups, where adult pregnant rats received dill/soya seeds extract for 10 days i.e. for two consecutive estrous cycles, whereas untreated male rats were used for mating in this study (the active ingredient of soya is also carvone).²³ The effect of soya on growth of fetus may be attributed to its effects on molecular and cellular levels.²⁴

CRL of pups was unchanged when measured in pups of spearmint treated male rats where spearmint was given in doses of 10, 20 & 40 mg/kg per day for 45 days and after administration of last dose these rats were cohabitated with female rats (untreated) for mating.^{25,26}

CONCLUSION

The results of present study showed that spearmint leaf extract caused reduction in CRL of pups of Swiss albino mice when given in low and high doses. Hence it is recommended that the use of spearmint leaf and its products should be minimized during pregnancy in humans as well to avoid such complications.

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