Proceedings S.Z.M.C. Vol: 37(1): pp. 36-40, 2023. PSZMC-874-37-1-2023

Embryo-hepatotoxic Potential of Spearmint Aqueous Extracts: A Histopathological Study

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ABSTRACT

Introduction: Spearmint, a member of lamiacae family, used in home remedies for the treatment of respiratory, GIT and other diseases.
Aims & Objectives: To observe the effects of spearmint aqueous extract on liver histology in Swiss albino mice pups.
Place and duration of study: The current experimental study was carried out in the Anatomy Department, Shaikh Zayed PGMI, Lahore over a period of 6 months.
Material and Methods: Adult mice (Swiss Albino mice) were used (7 male and 21 female). Female mice were divided and labeled into control group (A) and two experimental group i.e. low dose (B) and high dose (C) after conception; all these groups had 7 mice each. Distilled water was given to group A and leaves extract of spearmint was given to group B and C in doses of 3 & 6g/kg per day, respectively. After completion of pregnancy the pups were delivered by hysterotomy out of which 28 mice pups were randomly selected and marked as control/A1, low dose/B1 and high dose/C1 groups, respectively. The liver of all pups was dissected, slides made, and hepatocytes were examined for their shape, presence or absence of necrosis and vacuolization. Data was analyzed using SPSS software version 20.0,P-value<0.05 was taken as significant.
Results: Shape of hepatocytes was unchanged, but vacuoles and necrosis found in livers of freshly delivered pups with p-value <0.001whose mothers had received spearmint treatment during pregnancy. Difference among both spearmints treated groups was significant and p-value was 0.026.
Conclusion: Spearmint aqueous extracts administered to female mice during pregnancy in both low and high doses carry embryo hepatotoxic potential with in utero harmful effects on swiss albino mice pup offspring.

Keywords: Spearmint, labiatae, remedies, extract, shape, hepatocytes, vacuoles, necrosis.

INTRODUCTION

Members of Labiatae family include spearmint[1,2] commonly used for treatment urinary,[3] gastrointestinal[4] diseases, corn mint for headache, peppermint for respiratory diseases and other 25-30 species[5] which are also used as herbal treatment. Spearmint is mainly produced in United States[6]. The plant of Spearmint is small with pale blue flowers[7] and its constituents are carvone, limonene, volatile oil and 1, 8-cireole[8] etc. The main constituent of this plant is carvone[9].Spearmint is used as anti-cancer and antidiabetic herbal medicine[10]. LD 50 of carvone is 484.2 mg/kg in male swiss mice[11].Many parts of the plant especially the leaves are used for the treatment of diseases of respiratory system,[3] urogenital system like decreased urination[3] and obesity[4]. It is also used in treating gestational nausea and vomiting[12]. Flue and fever are treated by the home remedies of this plant[13]. Spearmint acts as an enzyme inducer i.e. it increases the activity of cytochrome P450 enzyme present in Liver[14]. It produced harmful effects on hepatocytes of male Albino rats[8]. These effects were the presence of necrosis and vacuolization in these hepatocytes along with congestion in veins and hepatic sinusoids. In human decreased the absorption of nonheme iron thus lead to anemia[15]. As spearmint showed harmful effects on adult liver, so this study was aimed to observe effects of spearmint's aqueous extract on liver histology of

swiss albino mice pups during intra embryonic development.

MATERIAL AND METHODS

Current study was performed in Anatomy Department, Shaikh Zayed PGMI, Lahore.

Initially both male and female animals were placed for acclimatization in separate cages for about fifteen days. 12 hours cycle (light/dark) with room temperature of about 24±2ºC was maintained. Animals were adequately provided with food/water.

Male and female mice were then placed together for approximately a week for conception. After confirmation of pregnancy these female mice were divided into A, B and C groups (control, low dose and high dose experimental groups respectively), each groups was having 7 female mice which were selected randomly.

Aqueous extract of spearmint was prepared in the PCSIR laboratory and its quantification was performed via GC-MS, at Chemistry Department, FC College in Lahore.

Aqueous extract and distilled water was given to these pregnant adult mice via gastric intubation from 6th day of pregnancy till term (because liver development starts on 8th day).

Group A: was given only the distilled water.

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

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

After delivery through hysterotomy, 28 pups, randomly selected from each group and labeled as A1, B1 and C1. The livers of all pups were dissected immediately after birth, slides were made, and hepatocytes were examined for their shape, presence or absence of necrosis and vacuolization.

Statistical Analysis:

In current study Data was analyzed by SPSS statistical software version 20.0. Shape of hepatocytes, necrosis and vacuoles in hepatocytes were shown in percentages and frequencies. Comparison of the above variables among their groups was tested by Chi-Square statistical test.

RESULTS

Shape of Hepatocytes:

Shape of hepatocytes in control as well as the experimental groups was normal (Fig-1) so statistical analysis was insignificant for this parameter.

Vacuoles in Hepatocytes:

Histological slides of liver of control as well as spearmint treated mice pups were observed and presence or absence of vacuoles within the liver cells was examined which revealed 14 pups of group B1 (i.e. 50.0%) had vacuoles in hepatocytes whereas 22 pups (i.e. 78.6%) of group C1 had it. The B1 and C1 groups had higher number of mice pups with vacuoles when compared with group A (p-value was <0.001) as shown in Table-1, Fig-2 & Fig-3. Spearmint treated groups had significant difference as well between them (p-value was 0.026) shown in Table-2.

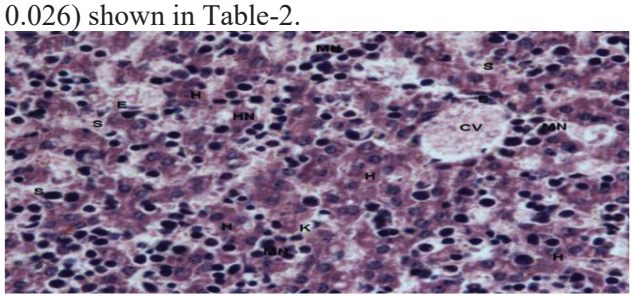

Fig-1: Photomicrograph of pup's liver of mice (group A1/control). showing:(H) Hepatocytes, (HN)Nucleus of hepatocytes, (K)Kupffer cells, (CV) Central vein, (MN)Mononuclear cells, (E)Endothelium of Sinusoids and central vein, (S)Sinusoids, (H & E, 40X)

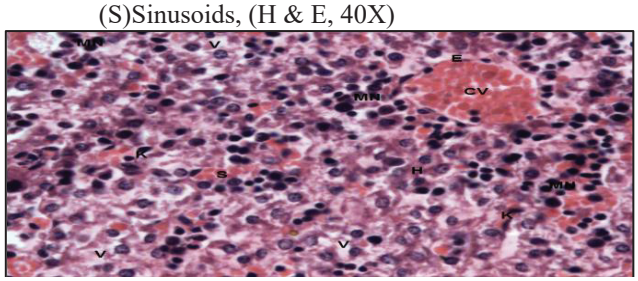

Fig-2: Photomicrograph of pup' liver of mice (group B1/low dose experimental) showing:(V) vacuolization of hepatocytes, (HN)Nucleus of hepatocytes, (H) Hepatocytes, (E) Endothelium of Sinusoids and central vein, (S) Sinusoids, (CV) Central vein, (K) Kupffer cells, (MN) Mononuclear cells, (H & E, 40X).

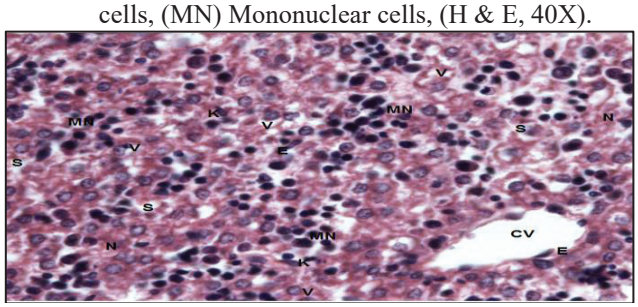

Fig-3: Photomicrograph of pup's liver of mice (group C1/high dose experimental) showing:(V) vacuolization of hepatocytes, (HN)Nucleus of hepatocytes, (H) Hepatocytes, (E) Endothelium of Sinusoids and central vein, (S) Sinusoids, (CV) Central vein, (K) Kupffer cells, (MN) Mononuclear cells, (H & E, 40X)

Groups	Vacuoles in hepatocytes					
	Present		Absent		Total	
	%	N	%	N	%	N
A1	0	0	100	28	100	28
B1	50	14	50	14	100	28
C1	78.6	22	21.4	6	100	28

Table-1: Comparison for Status of Vacuoles in Pups' Hepatocytes in Control as well as Experimental Groups Exposed to Aqueous Extract of Spearmint

P-value < 0.001#

Chi-sq = 36.17

(I) Group	(J) Group	df	Chi-square	P-value
A1	B1	1	18.67	< 0.001#
	C1	1	36.24	< 0.001#
B1	C1	1	4.98	0.026*

Table-2: Group-wise Comparison for Status of Vacuoles in Pups' Hepatocytes in Control as well as Experimental Groups Exposed to Aqueous Extract of Spearmint.

Key:

A1	control group
B1	experimental, low dose group
C1	experimental, high dose group
df	degree of freedom
*	p-value <0.05 or significant difference
#	p-value <0.001or highly significant difference

Necrosis in Hepatocytes:

Mild necrosis of hepatocytes was present i.e. pyknosis, karyolysis in pups' hepatocytes of the two experimental groups. In group B1 14 pups (i.e.50.0%) where as in group C1 22 (I.e. 78.6%) pups had mild hepatocytes' necrosis. Both the experimental groups were found to have significantly higher number of pups with hepatocytes' necrosis when comparison was performed with control and p-value was <0.001 as shown in Table-3, Fig-4 & Fig-5. In spearmint treated groups the difference as shown in Table-4 had p- value of 0.026 which was statistically significant as well.

Groups	Necrosis in hepatocytes					
	Present		Absent		Total	
	%	N	%	N	%	N
A1	0	0	100	28	100	28
B1	50	14	50	14	100	28
C1	78.6	22	21.4	6	100	28

Table-3: Comparison for Status of Necrosis in Pups' Hepatocytes in Control as well as Experimental Groups Exposed to Aqueous Extract of Spearmint

P-value < 0.001#

Chi-sq = 36.17

(I) Group	(J) Group	Df	Chi-square	P-value
A1	B1	1	18.67	< 0.001#
	C1	1	36.24	< 0.001#
B1	C1	1	4.98	0.026*

Table-4: Group Wise Comparison for Status of Necrosis in Pups' Hepatocytes in Control as well as Experimental Groups Exposed to Aqueous Extract of Spearmint.

Key:

A1	control group
B1	experimental low dose group
C1	experimental high dose group
df	degree of freedom
*	p-value <0.05 or significant difference
#	p-value <0.001 or highly significant difference

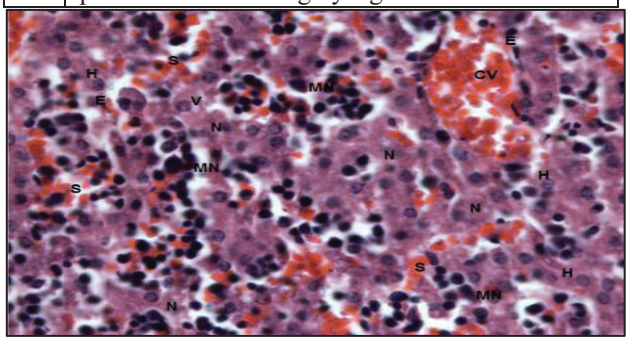

Fig-4: Photomicrograph of pup's liver of mice (group B1/low dose experimental) showing: (N) necrosis of hepatocytes, (HN)Nucleus of hepatocytes, (H) Hepatocytes, (E) Endothelium of Sinusoids and central vein, (S) Sinusoids, (CV) Central vein, (K) Kupffer cells, (MN) Mononuclear cells, (H & E, 40X).

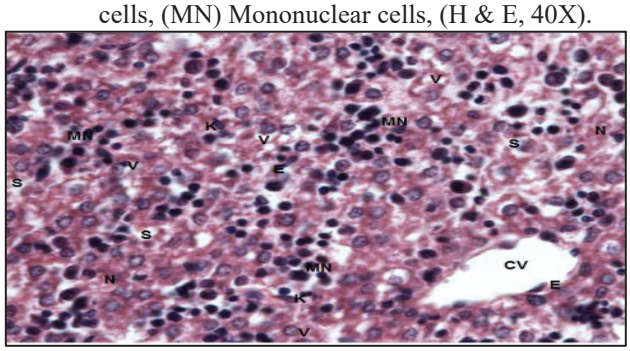

Fig-5: Photomicrograph of pup's liver of mice (group C1/high dose experimental) showing:(N) necrosis of hepatocytes, (HN)Nucleus of hepatocytes, (H) Hepatocytes, (E) Endothelium of Sinusoids and central vein, (S) Sinusoids, (CV) Central vein, (K) Kupffer cells, (MN) Mononuclear cells, (H & E, 40X).

DISCUSSION

M. spicata labiatae or spearmint is the 3rd commonest flavoring agent used in the world[16]. Its excessive use is harmful because it produces free radicals when metabolized, leading to oxidative

stress[8]. It produced damage in uterus[7] and kidneys[9] when given in experimental doses. In adult rats there was necrosis of hepatocytes and tissue degeneration when spearmint was given in the form of herbal tea[8].

The current study revealed embryo-hepatotoxic potential of aqueous spearmint extracts in Swiss albino mice pups exposed to maternal ingestion in low and high doses. Hepatocytes of each group revealed normal shape with vacuolar presence in hepatocytes of both spearmint treated groups. C1 group was found to have higher number of pups with vacuoles in hepatocytes (p-value 0.026, Table-1). The difference in all groups was found to be statistically significant and p-value was <0.001 as shown in Table-2.

The findings of research by Ramosa et al on limonene induced liver injury in adult female rats were similar to the results obtained in the present study on embryos he found changes like: hydropic degeneration of hepatocytes, may be due to the intracellular edema or the formation of microvacuoles in these cells, the clear spaces in cytoplasm also called microvacuoles, were formed because of the actions of the toxic agents, that lead to intracellular retention of sodium and accumulation of water.[17]The above vacuolization may developed due to excessive glycogen accumulation in hepatocytes and it may appeared as an adaptive response of the hepatocyte to resist against consequent damage produced by these toxic agents[18].

In present study foci of necrosis were observed in liver of pups of the two experimental groups, these results were highly significant, p-value <0.001, (Table-4). The similar findings were seen by Akdogan, in that research there was degeneration along with necrosis in adult rat liver who received given spearmint in the form of tea for 30 days.[8]Necrosis of liver tissue was also seen in adult rats who were treated with limonene for 45 days and doses were 25mg & 75mg/kg per day[17]. Necrosis of hepatocytes in these animals occurred because there was impairment in homeostasis of cell, that lead to water influx and transportation of extracellular ions within the cells. All this occurred because there was increased ROS and cytoplasmic calcium ion, along with depletion of ATP and Na-K-ATP as pump damage. Activation of few protease enzymes like calapin, cathepsin etc was also involved in this process of cellular degeneration[8]. The cellular necrosis is characterized by swelling of organelles, rupture of cell membrane with spillage of cell contents into surrounding[19]. The initial necrotic changes, which were reversible, like cytoplasmic swelling with dilatation of intracellular organelles along with blebbing of cell membrane due to cytoskeleton damage[20]. The late necrotic changes like, mitochondrial depolarization, lysosomal breakdown, increase in the bleb formation in cell membrane followed by rupture of these blebs and in the end, there is opening of different ion channels that lead to death in these cells[21].

CONCLUSION

The results of current study revealed embryo-hepatoxic potential of aqueous extracts of spearmint caused no change in shape of hepatocytes but produced necrosis and vacuolization of hepatocytes, when its low and high doses were administered to gestating female mice. Therefore, its use may be minimized in pregnancy in human as well.

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