TOXICITY OF TRIGONELLA FOENUM GRAECUM (FENUGREEK) IN BONE MARROW CELL PROLIFERATION IN RAT

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ABSTRACT

Fenugreek has a wide range of medical applications and its medicinal use has been clear in several studies, however, few studies are available on effects on haematopoietic stem cell of bone marrow. The goal of the present study was to investigate the effect of Fenugreek on fetal macroscopic diameters and microscopic bone marrow cell histological changes in its teratogenic dosages. Fenugreek decoction was dissolved in 1.5 milliliter distilled water and injected intraperitoneumly in three dosages of 0.8 g/kg, 1.6 g/kg, and 3.2 g/kg for three groups of Wistar female rats mated by Wistar male. For another group (as control group) only 1.5 milliliter distilled water was injected. Bone marrow tissue was prepared from rat fetus and was cut using a microtome and stained with hematoxylin and eosin. Sections were evaluated for changes using light microscope. LD_{50} for the measurement of teratogenic dosage of fenugreek was 4.1 and 3.5 g/kg in female and male rat, respectively. There was a positive relation between the injected drug dosage and fetal mortality rate. Among all fetal diameters, ear to ear diameter was decreased in groups received Fenugreek decoction. The severity of stem cell histological changes caused by 3.2 g/kg drug injection was lower than distilled water injection and in evaluation of other cells, differences in the severity of histological changes across three groups with different drug dosages and control group was detected. Fenugreek in teratogenic dosages can decrease the severity of bone marrow cell proliferation and increase fetal mortality rate.

Keywords: Fenugreek, bone marrow, fetal mortality, herbal medicine.

INTRODUCTION

Fenugreek *(Trigonella foenum graecum)* is a plant from the family Leguminosae cultivated in some countries such as India, Africa, Egypt, Morocco, and occasionally in England. Fenugreek is an old medicinal plant and has been commonly used as a traditional food and medicine. The seeds are reported to have restorative and nutritive properties (Khosla *et al.,* 1995) and its nutrient composition is moisture, protein, fat, saponins, and dietary fibers (Ikeuchi *et al.* 2006). In addition, wide range of its medicinal applications were identified and its medical use for the treatment of inflammation, tumors, cardiovascular diseases, renal insufficiency, infections, and metabolic disorders has been clear in several studies (Muralidhara *et al.,* 1999; Petit *et al*., 1995; Sharma *et al*., 1990; Puri *et al*., 2002; Pandian *et al.* 2002; Tahiliani and Kar, 2003; Sur *et al.,* 2001; Ahmadiani *et al.,* 2001). It has been also shown that at the stated dose, it increases the bone marrow cell counts indicating its stimulatory effect on blood cells especially macrophages (Bin-Hafeez *et al.,* 2003). Furthermore, one of the nutritional profiles of Fenugreek seed is iron and may influence the iron absorption (Fenugreek Trigonella Foenum-Graecum, 2008).

However, a few studies were available about the effects of Fenugreek and its toxicity on immunomodulatory effects and haematopoietic stem cells of bone marrow. Also the mechanisms of these effects have not been clear. The goal of the present study was to investigate the effect of *Fenugreek* on fetal macroscopic diameters and microscopic bone marrow cell histological changes in its teratogenic dosages.

MATERIALS AND METHODS

Decoction of Fenugreek plant

Fenugreek plant used in this study was supplied by Tehran central market and its decoction was prepared from dry leaves.

Animal selection

Four groups of Wistar female rats weighing 200-250g (Razi serum production institute, Iran) and each group included three rats were maintained in new cages with the temperature of 22°C to 26°C; humidity of 45-55 percent one week before pairing and were given food and water. For mating, in each cage, one Wistar male rat was placed beside female rats in the evening and then rats were separated in the morning of next day. Smear test was used to prove the mating.

Drug administration

Fenugreek decoction was dissolved in 1.5 milliliter distilled water and used in three dosages of 0.8 g/kg, 1.6

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g/kg, and 3.2 g/kg for three groups, respectively in one time on day 10 of mating. For another group (as control group) only 1.5 milliliter distilled water was injected. Injection was done intraperitoneally (Sur *et al*., 2001) for all groups. One day before the delivery, pregnancy ended (Pregnancy ended at 20 days).

Sample preparation and histopathological analysis

In the first stage, bone marrow tissue was prepared from rat fetus as different sections (with 3 cm diameter) and immediately fixed in 10% formal saline solution for 24h. Then, these sections were directly dehydrated in a graded series of ethanol concentrations (50, 70, 80, and 96%) and then embedded in paraffin (Abdel-Barry *et al.* 2000). Thin sections (4-5 µm thick) were cut using a microtome and stained with hematoxylin and eosin. Sections were evaluated for changes using light microscope.

Determination of LD_{50} and fetal mortality

For calculation of fetal mortality after embedding, the number of reabsorbed alive fetus was recorded and fetal mortality rate was calculated. For determination of teratogenic dosage of injected drug, the LD_{50} calculated by probit analysis (Finney, 1971).

Macroscopic evaluation

We evaluated and compared some fetal macroscopic indices such as body weight, crown-rump length, bi-parietal diameters included ear to ear and nasal to occipital diameters.

Microscopic evaluation

In this stage, histological changes related to cell proliferation were evaluated and graded as none (0), mild (+1), moderate (+2), and severe (+3). Bone marrow cell evaluation included stem cells, haematopoitic cord, proerytroblasts, miloblasts, lymphoblasts, mega-caryocytes, reticulocytes, colony formation units, and sinusoids.

STATISTICAL ANALYSIS

Results were reported as the mean ± standard deviation (SD). Differences in mean scores were tested by one-way ANOVA test or Kruskal-Wallis test. P values of 0.05 or less were considered statistically significant. All statistical analyses were performed by using SPSS version 13 (SPSS Inc., Chicago, IL, USA) for windows.

RESULTS

For the measurement of teratogenic dosage of drug, LD_{50} was calculated and it was 4.1 g/kg in female rat and 3.5 g/kg in male rat.

Fetal mortality rate was increased in injected drug dose (fig. 1). The results of the measurement of fetal diameters were summarized in table 1. There was a positive relation between the increase of drug doses and decrease in the fetal ear to ear diameter; however, other fetal diameters were not dependant to the drug dosage increasing.

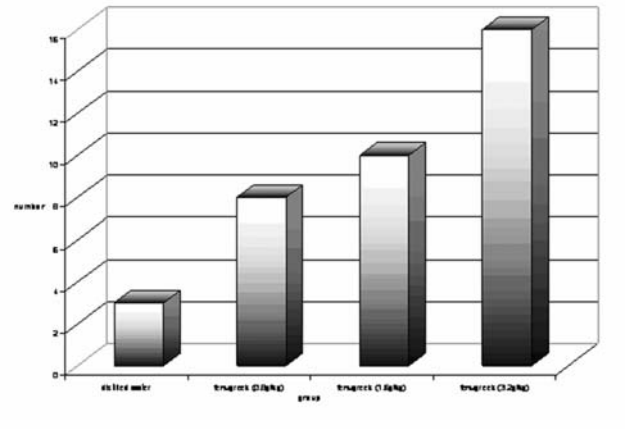

Fig. 1. Fetal mortality in the groups injected with different dosages of fenugreek and control group.

In histological evaluation of stem cells, no difference between the effects of distilled water and drug injection with the dosage of 0.8 g/kg (P=0.820) and 1.6 g/kg (P=0.180) was found, but the severity of stem cells histological changes caused by 3.2 g/kg drug injection was lower than distilled water injection (P<0.001). In evaluation of other cells of bone marrow (figs. 2-5), significant differences in the severity of histological changes between three groups with different drug injection and control group was detected (table 2). Except for the changes in haematopoitic cord, in other types of bone marrow cell, significant decrease in the severity of histological changes with the increase of drug dosages was found (table 2).

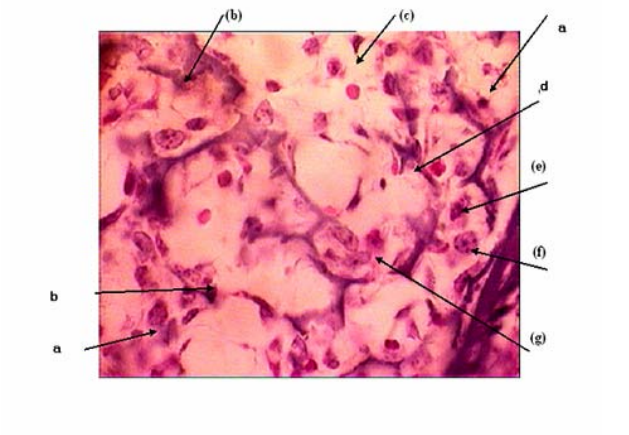

Fig. 2: Bone marrow cell proliferation in distilled water group (a. Miloblast, b. Colony formation unit, c. Reticulocytes, d. Proerytroblasts, e. Lymphoblasts, f. Megacaryocytes, g. Haematopoitic cord)

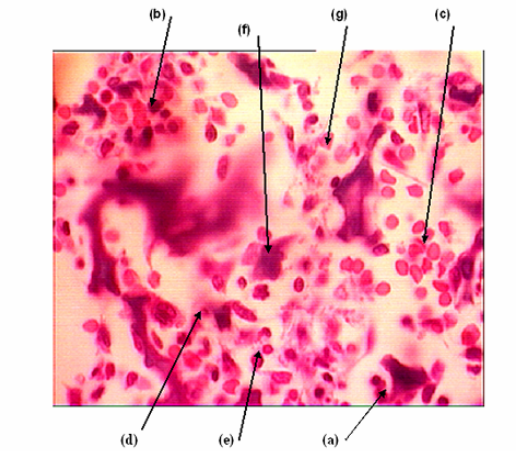

Fig. 3: Bone marrow cell proliferation in 0.8 g/kg fenugreek injection group (a. Miloblast, b. Colony formation unit, c. Reticulocytes, d. Proerytroblasts, e. Lymphoblasts, f. Megacaryocytes, g. Haematopoitic cord).

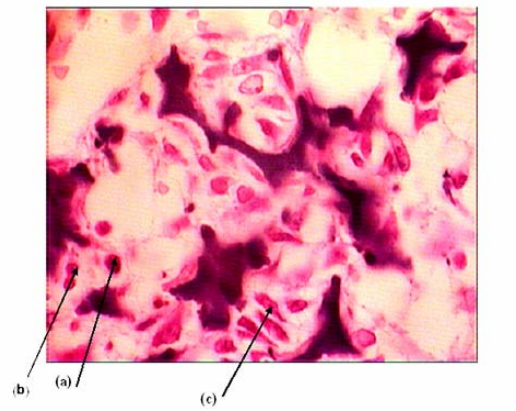

Fig. 4: Bone marrow cell proliferation in 1.6 g/kg fenugreek injection group (a. Miloblast, b. Lymphoblasts, c. Megacaryocytes).

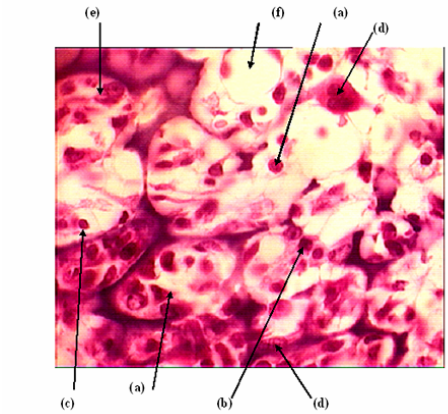

Fig. 5: Bone marrow cell proliferation in 3.2 g/kg fenugreek injection group (a. Miloblast, b. Colony formation unit, c. Lymphoblasts, d. Megacaryocytes, e. Haematopoitic cord, f. sinosoid)

DISCUSSION

The toxic effects of Fenugreek on male and female reproductive systems and also its adverse effects on developing fetus have been shown in previous studies. In a study by Kassem *et al*. (2006) a significant reduction in fetuses developing due to the reductions of both fetal and placental weights at 20 days of gestation and litter size was observed. This result was proven histopathologically by the observation of proliferative changes of the endometrial glands (Kassem *et al.* 2006). Similarly, in the present study, we also showed the side effects of this drug on the decrease of the fetal ear to ear diameter and increase of fetal mortality rate. However, the effects of Fenugreek on bone marrow proliferation and its side effects on the normal histological pattern of bone marrow cell have not been clearly demonstrated. In the present study, we found the side effects of high dose Fenugreek especially 3.2 g/kg dosage on the majority types of bone marrow cell. These side effects can be occurred following to the different etiologies. *In vivo* investigations on animal models showed the impairment of peripheral conversion of thyroid hormones by the use Fenugreek seed extract so that administration of Fenugreek (0.11 g/kg daily for 15 days) to male rats could lead to the changes in the level of thyroid hormones (Kelly 2000). Furthermore, a stimulatory effect on bone marrow cellularity was observed in normal rats continuously infused with thyroid hormones. Results of studies on bone marrow were expressed in absolute numbers of total nucleated erythroid cells per milligram of femoral marrow at the beginning and after 8 hours of continuous infusions (Malgor *et al.* 1975). Therefore, differences in histological changes in bone marrow cell proliferation may be caused by the effects of Fenugreek on thyroid hormones secretion.

It has been clearly shown that the intestinal disaccharidase activity and glucose absorption can be decreased and gastrointestinal motility increased by the administration of a soluble dietary fiber fraction of Fenugreek and thus it can decrease serum glucose, increased liver glycogen content and enhanced total antioxidant status (Hannan *et al.* 2007). Also, Fenugreek administered at 2 and 8 g/kg dose orally significantly reduced the blood sugar both in normal and diabetic rats (Khosla *et al.* 1995). In addition, the metabolism of bone marrow is directly dependant to glucose (Vaccari *et al.* 1958). Therefore, decrease in glucose absorption in bone marrow may lead to metabolic disturbances of bone marrow cell and enzymatic dysfunction in bone marrow cell proliferation.

It was also suggested that estrogen can regulate B lymphocyte development in mouse bone marrow and its deficiency causes a marked increase in bone marrow cell (Masuzawa *et al.* 1994). The p45 NF-E2 turn-on 3b-HSD gene and encodes an enzyme for regulation of all steroid hormone biosynthesis. 3b-HSD induces the estrogen

Table 1: Fetal diameters in Fenugreek and distilled water groups

Characteristics	Control group	Fenugreek group		
		0.8 g/kg	1.6 g/kg	3.2 g/kg
Fetal weight	6.50±0.18	6.50±0.17	6.45±0.19	6.42±0.21
Crown-rump length	39.38±0.39	39.40±0.39	39.41±0.19	39.40±0.40
Ear to ear diameter	8.02±0.18	8.01±0.16	7.97±0.14	7.94±0.13 *
Nasal to occipital diameter	14.93±0.33	14.93±0.32	14.93±0.39	14.83±0.28

Data are indicated as mean±SD, * P value<0.05

Table 2: The severity of histological bone marrow cells proliferation in Fenugreek and distilled water groups*

Characteristics	Control group	Fenugreek group		
		0.8 g/kg	1.6 g/kg	3.2 g/kg
Stem cells	2.75±0.45	2.67±0.51	2.33±0.51	1.42±0.90 **
Haematopoitic cord	2.67±0.49	1.83±0.40 **	1.50±0.54 **	1.08±1.16 **
Proerytroblasts	2.67±0.49	1.17±0.40 **	0.67±0.51**	0.17±0.38 **
Miloblasts	2.75±0.45	1.67±0.51**	1.33±0.51**	0.33±0.49 **
Lymphoblasts	2.58±0.51	1.67±0.51**	1.33±0.51**	0.33±0.49 **
Megacaryocytes	2.75±0.45	1.50±0.54 **	0.67±0.51**	0.08±0.28 **
Reticulocytes	2.67±0.49	1.33±0.51**	1.83±0.40	1.25±0.45 **
Colony formation units	2.75±0.45	1.83±0.40 **	1.33±0.51**	0.00±0.00 **
Sinusoids	0.00±0.00	0.67±0.51**	1.67±0.51**	1.42±0.51**

Data are indicated as mean±SD
*Histological changes related to cell prolifration were graded as none (0), mild (+1), moderate (+2), and severe (+3).

producing, in the form of estradiol, in megakaryocyte cells. It has been shown that the addition of exogenous estradiol can increase pro-platelet, while the inhibition of estradiol receptors blocked pro-platelet in live mice (Nagata *et al.* 2003). Furthermore, Fenugreek seed is a source of the steroidal saponin diosgenin. It has been demonstrated that both progesterone and estrogen (estradiol [1,3,5 (10)-estratrien-3, 17 β-diol]) levels were lower in the Fenugreek-fed females as compared with those in the control animals (Kassem *et al.* 2006). In view of the role of estradiol on bone marrow formation and also the presence of steroidal saponin diosgenin in Fenugreek seed, it seems that the administration of Fenugreek in high dosages may adversely influence the bone marrow cell proliferation.

In spite of the fact that above probable mechanisms and relationships may explain the bone marrow changes after high dose administration of Fenugreek, more studies for demonstration of these mechanisms are needed.

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