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EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF FENUGREEK (TRIGONELLA FOENUM-GRAECUM) ON THE TESTICULAR TISSUE OF NEONATAL RATS OF DIABETIC MOTHERS

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ABSTRACT

Background and objectives: Incidence of abnormalities in various organs of fetus and infants of diabetic mothers are now well documented. Results of studies indicate anti-diabetic and anti-oxidant effects of fenugreek. The present study aimed to investigate the effects of the hydro-alcoholic extract of fenugreek on the testicular tissue of neonatal rats of diabetic mothers.
Materials and Methods: Sixty adult female Sprague Dawley rats were randomly divided into three healthy groups (including, control, Glibenclamide and fenugreek groups) and three diabetic groups (including, control, Glibenclamide and fenugreek groups) in this experimental study. Streptozotocin (STZ) was used to induce diabetes in the rats. Upon detection of pregnancy, 1000 mg/kg hydro-alcoholic extract of fenugreek was fed to healthy and diabetic fenugreek groups, 5 mg/kg Glibenclamide was fed to healthy and diabetic Glibenclamide groups, and healthy and diabetic control groups were given distilled water by gavage. After pregnancy and vaginal delivery, one month old neonatal rats of the six groups were selected and their testicles were removed after anesthetization. The serial sections of testicular tissue were studied in all the experimental groups. The prepared slides were then stained with Hematoxylin-Eosin (HE) and green Masson's trichrome (MT) staining methods. Blood glucose levels of the infants were also measured. Histomorphometric study was done using two micrometric standard methods (with slides and graded lenses) and also Olympus BX51 microscope and Olysia software.
Results: Administration of fenugreek extract to the diabetic pregnant rats (diabetic fenugreek group) significantly increased the mean number of spermatogonia cells and Leydig cells in one month old infants compared to the diabetic control group ($P<0.05$). However, the number of Sertoli cells and the number of seminiferous tubules of one month old newborns of diabetic rats in the diabetic fenugreek group did not show significant change compared to the diabetic control group. The mean diameter of seminiferous tubule and the thickness of testicular capsule of one month old infants of diabetic rats in the diabetic fenugreek group showed a significant increase compared to the diabetic control group ($P<0.05$).
Conclusion:
Administration of fenugreek extract as a rich source of antioxidant components to diabetic pregnant rats had a protective effect on the testicular tissue of neonatal rats born to diabetic mothers and improved qualitative and quantitative parameters of the testis.

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Introduction

Diabetes is one of the most important metabolic and endocrine diseases common in human communities that its prevalence is also increasing due to changes in lifestyle and dietary habits. The disease has two types including, insulin dependent diabetes mellitus (IDDM) or type 1diabetes and non-insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes. It

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causes many side effects, including: Nephropathy [1], Retinopathy [1], Neuropathy [2], and Cardiomyopathy [3]as well as damages to the reproductive system of adults, such as reduced pregnancy rate [4, 5], reduced diameter of the ovary [6], vaginitis, abnormal menstrual cycle, delayed oocyte maturation, decrease in the corpus luteum and increase in atretic follicle[7], ejaculation disorder, erectile dysfunction, decreased sperm motility, increased abnormally sperm, decreased number of Leydig cells and deformation of their core, and decreased number of Sertoli cells and their deformation [8, 9].

During embryonic development especially in the early stages, the fetus is influenced by hormonal and metabolic changes of mother's body. These changes can have significant effects on fetal growth and on the development of the different organs of the body. Excess amounts of blood glucose in pregnant women with diabetes are transferred to the fetus, resulting in stimulation of pancreatic β cells and increased insulin secretion by the pancreas of the fetus. This creates some complications that among them can mention to increased deposition of fat and abnormal fetal growth (fetal macrosomia) [9].

Uncontrolled diabetes, especially in the early stages of organ formation, in pregnant women causes birth defects. The prevalence of birth defects in infants of diabetic mothers is 2 to 4 times greater than that of the non-diabetic mothers. On the one hand, teratogenic effect of insulin-dependent diabetes can cause growth retardation, congenital malformations and fetal death [9]. Congenital malformations of infants of mothers with diabetes consist mainly of central nervous system disorders such as morphometric changes in the cerebellum [10], congenital abnormalities of the cardiovascular system, skeletal system and genitourinary system, and especially the lack of formation of the kidney [1, 2]. Also, calcium and magnesium balance between maternal and fetal blood is disrupted in pregnant women who develop gestational diabetes [11].This also causes congenital skeletal deformities in newborns [12].

Insulin itself causes many abnormalities. With regard to past studies on the effects of medicinal herbs and thus their low risk compared to synthetic medicines, use of plants that are effective in reducing blood sugar seems to be the best way to control diabetes during pregnancy. Many types of research on anti-diabetic effects of medicinal plants have been carried out around the world that among them can mention to a comprehensive study performed by Indian scholars on more than 30 kinds of plant extracts [13].

Fenugreek (Trigonella foenum-graecum) is a plant of Fabaceae family. This plant is native to Iran. It grows in most parts of Iran, including Azerbaijan, Isfahan, Fars, Khorasan, and Semnan provinces and Damghan County. It is planted and consumed in Iran as an edible vegetable. Many studies have been conducted till now on anti-diabetic effects of fenugreek [14, 15 and 16]. The results of the current study also indicated anti-diabetic effects of this valuable plant. Fenugreek is able to reduce fasting blood sugar levels in animals [16]. The therapeutic effects of the plant are created with alkaloid content through the regulation of insulin secretion [16]. In addition, the antioxidant properties of fenugreek have also proven in the improvement of complications caused by diabetes [17]. These properties are due to the presence of compounds such as vitamins, flavonoids, terpenoids, carotenoids, coumarins, saponins, and sterols [18].

Given the anti-diabetic and antioxidant properties of fenugreek, the current research was performed to investigate the effects of the hydro-alcoholic extract of the plant on the testicular tissue of neonatal rats of diabetic mothers.

Methods

In this experimental study, 60 adult female Sprague Dawley rats weighing approximately 200-250 grams were prepared from Shiraz University of Medical Sciences. To adopt with environmental conditions (12 hours of light and 12 hours of darkness, at 23˚C), the rats were kept for a week in Animal House located in Department of Anatomy and Histology, School of Veterinary Medicine, University of Shiraz. Then the animals were divided randomly into six equal groups as follows:

The first (healthy control) group included infants of healthy mothers. The mothers did not receive the extract; the second (healthy fenugreek) group included infants of healthy mothers. The mothers were daily fed with fenugreek extract (1 g/kg) until delivery [19]; the third (health Glibenclamide) group included infants of healthy mothers. The mothers were daily fed with Glibenclamide (5 mg/kg) until delivery [20]; the forth (diabetic control) group included infants of diabetic mothers. The mothers did not receive the extract; the fifth (diabetic fenugreek) group included infants of diabetic mothers. The mothers were daily fed with fenugreek extract (1 g/kg) until delivery [19]; the sixth (diabetic Glibenclamide) group included infants of diabetic mothers. The mothers were daily fed with Glibenclamide (5 mg/kg) until delivery [20].

Streptozotocin (manufactured by Sigma Corporation, USA) was used for induction of diabetes. The medicine at a dose of 50 mg/kg was injected intraperitoneally. Blood sugar test was carried out before injection, 24 hours after injection (to confirm diabetes) and 10 days after injection (for stabilization of diabetes). Blood samples were obtained from the tail by making a small incision over the superficial veins of the tail end and glucose levels were measured using glucometer device. Blood sugar higher than 250 mg/dl was considered as the criterion of catching diabetes [21].

To prepare the extract, 1000 g of fenugreek plant (dried leaves and thin stems) was soaked in a mixture of 2 L of water and 96% ethanol for 48 hours. It was filtered through a filter paper and then the resultant was centrifuged at 3500 rounds per minute. To evaporate ethanol solution, the extract was then placed in an oven at a temperature of 45 ˚C for 2 days. Approximately20 g of the extracts were obtained at the end of the processes [22].

Each female rat of every six groups remained with the male for 24 hours for mating. The vaginal plug was examined next morning at 8 a.m. to 10 a.m. Observation of the plug was considered as day zero of gestation of the animal. After pregnancy and vaginal delivery, 5 male mice born in the first month was selected from each group. The testes were removed after

anesthetization and serial sections of testicular tissue were prepared in all the studied groups. The prepared slides were then stained with hematoxylin-eosin and green Masson's trichrome staining methods. Blood glucose levels of infants were also measured. Histomorphometric study was done using two micrometric standard methods (using slides and graded lenses) and also Olympus BX51 microscope and Olysia software.
The collected data were analyzed using SPSS software and one-way ANOVA and Duncan post hoc test.

Results

According to the results of this study, mean body weight of one month old neonatal rats of diabetic mothers in the diabetic fenugreek group showed a significant decrease compared to the diabetic control group ($P<0.05$). Administration of fenugreek extract in the diabetic fenugreek group significantly increased the mean number of Spermatogonia and Leydig cells of one month old infants compared to the diabetic control group ($P<0.05$). But the number of Sertoli cells and seminiferous tubules in the diabetic fenugreek group did not show significant change compared to the diabetic control group. The mean diameter of seminiferous tubule and thickness of testicular capsule in the diabetic fenugreek group showed significant increase compared to the diabetic control group ($P<0.05$).The mean volume and the weight of the testis in the diabetic fenugreek group did not show a significant change compared to the diabetic control group. However, the mean volume and the mean weight of the testis in the diabetic fenugreek group showed a significant decrease compared to the healthy control group ($P<0.05$). The mean blood glucose in the diabetic fenugreek group showed reduction compared to the diabetic control group, but the reduction was not significant.

	One month old infants of diabetic mothers			One month old infants of healthy mothers		
Groups / Parameters	Diabetic Fenugreek	Diabetic Glibenclamide	Diabetic Control	Healthy Fenugreek	Healthy Glibenclamide	Healthy Control
The number of spermtogonial cells (n/mm^2)	1607.86±118.32a	1673.19±104.22a	1345.01±89.17b	1807.04±111.39a	1758.21±98.16a	1799.43±85.74a
The number of Sertoli cells (n/mm^2)	429.86±24.71ab	441.16±30.57ab	394.81±22.37a	479.46±31.15b	493.28±38.27b	487.93±32.25b
The number of Leydig cells (n/mm^2)	385.43±26.41a	376.54±23.17a	316.25±19.95b	423.17±28.46a	417.39±22.18a	428.31±26.18a
The number of seminiferous tubule (n/mm^2)	17.01±4.12	16.93±3.24	17.89±2.43	15.86±3.75	16.32±4.01	15.37±3.19
Seminiferous tubule diameter (μ)	158.23±8.13a	156.91±9.06a	137.04±8.29b	172.35±12.49a	169.17±11.23a	165.47±10.39a
Testicular capsule thickness (μ)	25.08±2.39	24.29±3.09	23.86±2.73	27.45±2.83	26.94±3.11	27.86±2.43
Body weight (g)	74.16±5.13a	76.22±5.51a	88.66±6.72b	72.31±4.91a	66.11±6.72a	70.02±6.01a
Testicular weight (g)	0.24±0.02a	0.25±0.03a	0.23±0.04a	0.33±0.05b	0.35±0.04b	0.36±0.05b
Testicular volume (mm^3)	186.39±18.43a	190.29±26.32a	183.18±25.02a	324.18±31.44b	309.16±20.12b	332.14±30.15b
Blood sugar	115.27±6.43ab	106.32±7.75a	125.21±8.73b	91.37±6.91a	92.32±7.21a	94.62±8.21a

- According to Duncan test, the means available in each row, which at least have one letter in common, do not have significant difference with each other at %5 level.
- The means are presented as Mean±SD.
- $P<0.05$ is statistically considered significant.

Discussion

A significant decrease in the number of Leydig cells and Sertoli cells, reduction in the number of Spermatogonial cells, and a decrease in testicular volume and weight was observed in the one month old neonatal rats born to the mothers with diabetes compared to infants of healthy mothers. This is consistent with results of previous studies [2, 23 and 24]. The decrease in the number of Leydig cells and Sertoli cells and histological changes in seminiferous tubules of testicular tissue of neonatal rats born to diabetic mothers indicate that gestational diabetes can reduce spermatogenesis process in infants and can impair this phenomenon [2, 23 and 24]. By influence on the proliferation process of Sertoli cells in the seminiferous tubules during the

embryonic period, diabetes probably reduces spermatogenic activity in the tubules and finally reduces the height and the diameter of germinal epithelium, resulting in reduced weight and volume of the testis in infants [23, 25]. It is said that the efficiency of spermatogenesis in maturity period depends on the number of Sertoli cells in testicular tissue [26]. Since the proliferation of these cells merely occurs in pre-puberty periods [27], so diabetes can have significant effects on testicular tissue of infants.

Ballester et al. (2005) reported that diabetes can lead to impairment in spermatogenesis process by an FSH-dependent mechanism and can reduce sperm count [25]. Furthermore, it was demonstrated that an increase in glucose levels shows its effect on the Leydig cells and Sertoli cells of diabetic rats directly by damaging mitochondria and smooth endoplasmic reticulum [28].

The results of this study also showed significant weight gain in one month old infants of diabetic mothers compared to the infants of healthy ones. The weight gain of these infants is due to the increased transportation of glucose and other nutrients from mother to fetus through the placenta [29]. In this case, a large amount of excess fat is stored in the shoulders and the trunk of the infants [30]. Treatment with the hydro-alcoholic extract of fenugreek is able to modify weight gain in infants of diabetic mothers.

Enhancement of spermatogonial cell counts in infants of diabetic mothers treated with fenugreek extract and Glibenclamide was statistically significant compared to the diabetic control group. Atrophy of the seminiferous tubules and decrease of the spermatogenic cells are the signs of morphological abnormalities in spermatogenesis. Thickening of seminiferous tubule basement membrane plays an important role in the reduction of spermatogenesis [31]. Basement membrane thickness is increased by diabetes and sperm production decreases by the disease. Reduced number of Sertoli cells also leads to decreased production of spermatogonial cells [24]. So fenugreek extract could prevent the reducing process of the number of spermatogonia and Sertoli cells in neonatal rats of diabetic mothers.

Studies have shown that oxygen free radicals play an active role in damages of sex cells. Hyperglycemia, on the one hand, increases production of oxygen free radicals and on the other hand, reduces the production of antioxidants in cells. By weakening the protective mechanisms related to cells, hyperglycemia increases those damages caused by oxidative stress [32]. It has been also stated that antioxidant concentration in hyperglycemia conditions decreases significantly in fetal cells [33]. The results of the current research showed that the administration of the hydro-alcoholic extract of fenugreek as an herbal antioxidant can somewhat reduce the effects of hyperglycemia in infants of diabetic rats. However, it cannot completely neutralize the effects. Fenugreek antioxidant effects have proved in the study of Jannat et al. (2002). Fenugreek has also recommended by this study as an important alternative for treatment of complications of antioxidant system damages caused by diabetes (by Streptozotocin injection) [17].

Conclusion

According to the findings obtained in this study, it can be concluded that administration of the hydro-alcoholic extract of fenugreek to pregnant rats with diabetes has a protective effect on the testicular tissue of neonatal rats born to mothers with diabetes due to the presence of compounds with antioxidant properties that can reduce free radicals. The extract can also improve qualitative and quantitative parameters of the testis.

References

1. Mills J., et al. Malformation in infants of diabetic mothers occur before the seventh gestational week: implications for treatment. Diabetes. 1979; 28: 292-293
2. Philipps AF., et al. Effects of chronic fetal hyperglycemia up on oxygen consumption in the ovine uterus and conceptus. J clin invest. 1984;74(1): 279-286.
3. Rajasekaran S., et al. Antioxidant effect of Aloe Vera gel extract in Streptozotocin – induced diabetes in rats. Pharmacol. Reports. 2005;57: 90-96.
4. Pedersen., et al. Congenital malformation in newborn infants of diabetic women. Lancet. 1964; 1124-1126.
5. Savion S., et al. Diabetes teratogenicity is accompanied by alteration in macrophages and T cell subpopulation in the uterus and lymphoid organs. IntImmunopharmacol. 2004; 4(10-11): 319-27.
6. Khaksar Z., et al. Ovarian histomorphometric changes in adult offspring of diabetic rat mothers. Comp.Clin.Pathol. 2013;22(2): 239–244
7. Dianmond MP., et al. Effects of streptozotocin- and alloxan- induced diabetes mellitus on mouse follicular and early embryo development. J. Reprod. Fertil. 1989; 86(1): 1-10.
8. Khaksar Z., et al. Alteration in gonadal and testicular cells of male fetuses and neonates of diabetic rats. Comp.Clin.Pathol. 2013; 22(6): 1111–1115
9. Vignon F., et al. Comparative study of semen in diabetic and healthy men. Diabetes Metab. 1991; 17: 350-4.
10. Khaksar Z., et al. Effect of Maternal Diabetes on Cerebellum Histomorphometry in Neonatal Rats. JSSU. 2010; 18 (1):56-63**.**
11. Hematian H., et al. Evaluation of maternal diabetes effects on Lumbosacral portion of spinal cord in neonate rats by morphometry. Journal of Shahrekord University of Medical Sciences 2010; 12(1):53-59.

12. Williams and wilkins. The pregnant, diabetic and her newborn.Problems and Management. 1977; Baltimore MD, USA Pedersen, J. 2nded.
13. Kar A. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetes rats: Journal of Ethnopharmacology. 2003; 84(1): 105-108.
14. Jamal A., et al. Hypoglycaemic and antihyperglycaemic effects of Trigonellafoenum-graecum leaf in normal and alloxan induced diabetic rats. Journal of Ethnopharmacology.1997; 58 (3): 149-155.
15. Khosla P., et al. Effect of Trigonellafoenumgraecum (Fenugreek) on blood glucose in normal and diabetic rats.Indian J PhysioPharmacol. 1995; 39(2): 173-4.
16. Xue Wan-Li., et al. Effect of Trigonellafoenum-graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr. 2007; 16: 422-426.
17. Kaviarasan S., et al. In vitro studies on antiradical and antioxidant activities of fenugreek seeds.food chemistry. 2007; 103(1):31-37.
18. Genet S., et al. Alteration in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues:effect of vanadate and fenugreek. Molecular and celluar biochemistry. 2002; 236(1-2):7-12.
19. Zia T., et al. Evaluation of the oral hypoglycemic effect of Trigonellafoenumgraecum L. (methi) in normal mice. Journal of Ethnopharmacology. 2001; 75(2-3): 191-5.
20. Hinata S., et al. Regulation of glucokinase gene expression in cultured rat islet cells: the inhibitory effects of T3 and glucagons and the stimulatory effect of glibenclamide. Diabets Research. 1993; 26(1): 13- 23.
21. Sancheti S., et al. Antihyperglycemic, antiyperlipidemic, and antioxidant effects of Chaenomelessinensis fruit extract in streptozotocin induced diabetic rats. Eur Food Res Technol 2010; 231:415-421.
22. Erdemoglu N., et al. Anti-inflammatory and antinociceptive assessment of plants used as remedy in Turkish folk medicine. Journal of Ethnopharmacology 2003; 89(1): 123-129.
23. Khaki A., et al. Protective effect of quercetin on spermatogenesis in streptozotocin- induced diabetic rat. J Med Plan 2009; 8:57-64.
24. Rossi GI, Aeschlimann M. Morphometric studies of pituitary gland and testes in rats with streptozotocin-induced diabetes. Andrologia 1982; 14: 532-42.
25. Ballester J., et al. Insulin- dependent diabetes affects testicular functions by FSH and LH- linked mechanisms. J Androl. 2005; 25:706-719.
26. Guyton, Arthur C.; Hall, John E. Textbook of Medical Physiology. 11th ed. Philadelphia: Elsevier Saunders; 2006; 972- 976.
27. Griswold MD. The central role of sertoli cells in spermatogenesis. Semin Cell Dev Biol 1998; 9(4): 411-6.
28. Borland K. Mita M. The actions of insulin- like growth factor I and II on cultured sertoli cells. Endocrinology 1984; 114: 240-6.
29. Jones CW. Gestational diabetes and its impact on the neonate. Neonatal Network 2001; 20(6):17-23.
30. Cuningham FG, Lolo KG, Blome AL, Hat JC. William's obstetrics. 22th ed. McGraw-Hill: New York; 2005; 1170-1187.
31. Cameron DF., et al. Morphological alteration in the testes from diabetic man and rat. Diabetes 1982; 31:11A.
32. Moudi B., et al. Biochemical and Histological Study of Protective Effect of Sodium Tungstate on Oxidative Stress Induced by Streptozotocin in Pancreas of Diabetic Rats. J Iran Anat. Sci. 2008; 5(21-22):279-292.
33. Ornoy A., et al. Role of ROS in the diabetes induced anomalies in rat embryos in vitro. Teratology. 1999; 60(6):376-386.