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Study of Fenugreek seed *(Trigonellafoenum-graecum L)* extract effect on cerebrum malformation in 18 and 20 days old fetus of diabetic rats

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Abstract
It has been proven that *Trigonellafoenum-graecum Lace* has hypoglycemic effects. This study investigated the effect of *Trigonellafoenum-graecum L* ethanolic extraction Cerebrum in 18 and 20days old fetus of diabetic mother.
In 2014, sixteen healthy female rats were divided into four groups (normal control, diabetic control, control Fenugreek seed and Fenugreek seed treatment). Diabetes was induced in four groups by Streptozotocin (50mg/kg/IP) and mating by male rat and vaginal plaque mentioned as a positive sign of pregnancy. After 18 and 20days rats were scarified and their fetuses were removed, their birth weight was recorded and Cerebrum simples were taken and fixed. Tissue sections were prepared by routine procedures and different histological parameters were examined.
The results show that in ratby18 days the number of cells in the white matter(1678/19 ± 182/2),the number of cells of gray matter(12014/45 ± 110/62) and the thickness of gray matter, white matter(2/14± / 27) and in ratby20 days the number of cells in the white matter(9871/71 ± 336/48),the number of cells of gray matter(22203/52 ± 728/41) and the thickness of gray matter, white matter(1.43 ± 0/03) in experimental group C has significant decrease, than experimental group A.
The present study demonstrates that alcoholic extract of Fenugreek seed possesses significant antihyperglycemic properties, thus suggesting its beneficial effect in the treatment of maternal diabetes.

Keywords: *Trigonellafoenum-graecum L* extract, maternal diabetes, foetuses, Cerebrum.

Introduction
Diabetes mellitus is one of the most common metabolic diseases of human beings. Mortality and morbidity increase in diabetics mainly because of the associated chronic complications such as nephropathy and atherosclerosis. It is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism [1].
Pancreas, by producing insulin allows the body to use glucose efficiently. However, with diabetes the pancreas insufficiently controls the insulin, causing blood sugar levels to rise [1]. Diabetic pregnancy is seen in over 8% of all pregnancies [2]. In diabetic mothers, during pregnancy placental transport of glucose and other nutrients intensifies due to an increased availability at the maternal site, resulting in their expansion in fetal and neonatal Macrosmia [3]. The elevated serum glucose concentration in mother, accompanied by hyperglycemia in the foetus, leads to degranulation of the fetal β-cells and results in fetal hypoinsulinemia. Indeed, in the majority of newborns of badly controlled diabetic mothers [blood glucose >16.7 mmol 1 44 −1] pancreatic β-cells show degranulation [4]. The risk for diabetes is significantly higher in the offspring of mothers who have non-insulin-dependent diabetes (Knowler *et al*. 1985). In addition, maternal diabetes increases the risk of hypoglycemia and other chemical imbalances such as low calcium and magnesium levels [1]. Data indicate that pre-gestational maternal diabetes is associated with strong teratogenic effects on the kidney, urinary tract, and heart and is also strongly associated with multiple congenital abnormalities [5]. The incidence of major malformations in women with type 1 diabetes is about 5%. Fetal anomalies account for almost half of prenatal deaths in diabetic pregnancies. Diabetes is not associated with increased risk for fetal chromosomal abnormalities [6]. One of the mammalian systems that are clearly impaired in diabetes is nervous system. Diabetes leads to numbness at the nerve endings [7]. Atherosclerosis in brain is another prominent change in diabetes. Studies have shown that obstruction of feeding vessels of nerves due to diabetes causes nerve bundles death and myelin destruction [8]. An increased number of malformations occur in infants born from mothers with maternal diabetes involving the central nervous system (CNS), the spinal column, the ribs, and

the urinary tract [9]. Specific types of anomalies in CNS which are linked to maternal diabetes are anencephaly, spina bifida, and hydrocephaly [6]. Maternal diabetes induced hyperglycemia and acute intracerebral hyperinsulinism reduces fetal brain neuropeptide Y concentrations [10]. Usually, foetuses with CNS anomalies or those exposed to adverse conditions which may affect CNS functioning take longer to habituate, or fail to habituate [11].

The effect of diabetes on the brain suggests that it may lead to neurophysiological alterations, cognitive abnormalities, changes in both brain function and structure such as white matter hyperintensities, and the gray matter density changes in type I diabetes, which suggests that persistent hyperglycemia and acute severe hypoglycemia have an impact on brain structure [12]. In addition, white matter microstructure deficits were seen in type I diabetes [13]. Type 1 diabetes has had decreased gray matter and white matter in some parts of the cerebrum [14]. One study demonstrated that hyperglycemia prevents differentiation of cortical neurons and causes oxidative stress in diabetic pregnancies of rat [15]. Diabetes induces alteration in the dendritic morphology of cortical neurons [16], hippocampal neuronal apoptosis [17], and disturbs the proliferation and cell death of neural progenitors [18].

It is known that maternal diabetes can cause changes in the size and number of neurons in the spinal cord. This causes symptoms and irreversible damage on the offspring's nervous system [19]. Therefore, in pregnancies affected by diabetes there will be a higher risk of the development of type 2 diabetes so medical intervention is necessary [20].

Medicinal plants have been used in traditional medicine for many years and have a special role in the treatment of many diseases. So far, more than 1200 medicinal plants with effects in reducing blood sugar levels or reducing the complications of diabetes are known [21]. In this study Fenugreek seed extract was selected. Fenugreek seed (Trigonellafoenum-graecum) is an annual plant in the family Fabaceae. The hypoglycemic effect of Fenugreek seeds has been studied in many animal model systems as well as in humans, especially in IDDM and NIDDM patients. In one study it was shown that a broken fiber in fenugreek seed extract caused a significant effect on glucose homeostasis in animal models of type I diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin function [22]. Feeding fenugreek seed powder for 3 weeks has a protective role against histopathological abnormalities in tissues of rats that have been exposed to alloxan [23]. It has been proven that, compared with diabetic group, rats treated with Trigonellafoenum-graecum extract had an increase in body weight and a decrease in kidney /body weight ratio ($p<0.05$) and also, compared with diabetic group in a dose-dependent manner, rats treated with Fenugreek seed extract had lower blood glucose, glycated hemoglobin, triglycerides, total cholesterol and higher density lipoprotein-cholesterol ($p<0.05$) [24]. Currently, the main treatment of diabetes mellitus is using insulin and hypoglycemic drugs, but these compounds have numerous side effects. Identification of compounds with minimal side effects (especially in pregnancy) which reduce blood sugar and have no adverse effect on the foetus seems necessary.

The purpose of this investigation is to evaluate the possibility of Fenugreek seed extract treatment of congenital Cerebrum malformations in offspring of diabetic rats at day`s18 and 20compared to the offspring of mothers who did not receive the extract.

Materials and Methods

Ethanolic Extract of *Trigonellafoenum-graecum* (TFGSEE)

Fenugreek seeds were collected from a local market in Iran, May/2014 and botanically authenticated with voucher specimens that were deposited in the National Herbarium, Iran. Seeds were ground to fine powder by a cyclotec grinding machine. The powder (1000gr) was extracted with aqueous 96% ethanol, the extracted solution was evaporated, and a dark brown extract was obtained [1, 25, 26].

Animals

This is an experimental study, in which all ethics of working with laboratory animals was observed by ASPACA (American Society for the Prevention of Cruelty to Animals), in 2006.

16 Adult male Sprague-Dawley rats weighing 180-220 g, aged 3 to 4 months were used throughout this study. All institutional guidelines were adhered during the care and treatment of the animals used in the present study. Animals were maintained on a 12 h light/dark cycle at21 ± 2°C. A standard pellet diet and water were supplied ad libitum. The overall nutrient composition of the diet was36.2% carbohydrate, 20.9% protein, 4.4% fat and 38.5% fiber, with metabolisable energy content of 11.8 MJ/kg(2820 kcal/kg).

Induction of diabetes

Induction of type I diabetes was performed by a single intraperitoneal injection of overnight fasted rats (180-220g) with 50 mg/kg streptozotocin body weight freshly dissolved in 0.5 M-citrate buffer (pH 4.5) [7]. The blood glucose level was checked 7 days after streptozotocin administration. Animals having blood glucose levels 300-200mg/dl were considered as diabetic [25].

The selected rats were randomly allocated into 4 groups (A, B, C and D).

Group A: (normal control) (NC): Healthy mothers were given normal saline.

Group B: (control Fenugreek seed extract) (NTFGSEE): Healthy mothers were given Fenugreek seed extract at a dose of 1,000 mg / kg daily.

Group C: (diabetic control) (DC): Diabetic mothers were given normal saline.

Group D: (Fenugreek seed extract treatment) (DTFGSEE): Diabetic mothers were given Fenugreek seed extract at a dose of 1,000 mg/kg daily.

Female animals of four groups in oestrus stage were caged with male rat for mating. Mating was confirmed by vaginal plug observation [27]. Each group included 4 rats and animals were given the extract orally by an intragastric tube once daily for 21 days. The stock solution was prepared for multiple groups, such that 1 mL of extraction was administered per day for each animal.

At day`s18 and 20of pregnancy, two rats of each groups were killed. After getting their foetuses, the rats were placed in appropriate fixative (bufferd formalin 5% for light microscope and glutaraldehyde 2% for electron microscope). Then the cerebrum was collected from foetus of all rats and the weight of neonates was measured.

Histomorphometric study

All tissue samples were fixed in 5% buffered formalin fixative for histopathological investigations and subsequently embedded in paraffin. Sections (5 microns thickness) were stained with Hematoxylin & Eosin staining and Green Masson's trichrome techniques. Sections were observed with Olympus BX51 microscope for evaluation of histomorphometric parameters such as:

1) Thickness of gray matter (μm). 2) Thickness of white matter (μm). 3) The number of cells in the Gray matter per unit (mm^2). 4) The number of cells in white matter per unit (mm^2). 5) The ratio of gray matter to white matter.

Thicknesses of gray matter and white matter were measured by ocular micrometer and Olympus BX51 light microscope using Olysia software. The number of cells per unit (mm^2) in both white and gray matters, and the ratio of gray matter to white matter were counted by ocular graticule and Olympus BX51 light microscope using Olysia software.

Statistical analysis

All values were expressed as mean ± standard deviation (SD).Significant differences among the groups were determined by one way analysis of variance (ANOVA) followed by Duncan's test to analyze the difference. The Statistical Package for Social Sciences (SPSS) 13.0 software was also used for data analysis. Values of $P \leq 0.05$ were taken as statistically.

Results

The result showed that the STZ (50 mg/kg) induced diabetic mellitus in rats and blood glucose reached to 468 ± 19.322mg/dl. Administration of Fenugreek seed extract (1000mg/kg) reduced the blood glucose significantly so that it reached 123.5 ± 4.342 mg/dl and 121.2 ± 4.258 mg/dl at the end of 18 and 20 days ($p<0.05$). Mean bodyweight of offspring in group C were increased 5/27±0/45gram and group D were significantly decreased 4/30±0/54gram and 4/34±0/53gram at 18 and 20 days. (Table 1, 2)

Discussion

Type II diabetes is one of the most common metabolic diseases in the world and its rate is increasing, the most important characteristic of this disease is chronic hyperglycemia [28]. In recent years, various plant extract have been claimed to be useful for the cure of diabetes mellitus, but few of them tested for their effects on body tissue of diabetic patient. In present study, we investigated the antidiabetic effect of *Trigonellafoenum-graecum* L ethanolic extract on embryo of STZ- induced female diabetic rats. STZ is a compound commonly used for the induction of type I diabetes in experimental rats. STZ caused diabetes by rapid depletion of βcell in pancreas Langerhans Island, which leads to a reduction of insulin release [29].

In diabetic pregnancy, maternal glucose transport to fetal blood via the Placenta [1] and increase in fetal blood glucose may result in diabetic neuropathy in fetus, as diabetes leads to neuropathy in adult [30]. Hyperglycemia effectively makes more substrate available for aerobic glycolysis in the brain, leading to acidosis [31] and enhanced oxygen free radical formation by reduction in levels of protective endogenous antioxidants [32].

Therefore, maternal diabetes results in malformation of this region. Neuropathy of numerous nerves like sciatic nerve has been reported in diabetic rat's foetus [30, 33]. Malformations in this region of brain may occur due to neuropathy [4, 34]. Diabetes mellitus is associated with moderate cognitive deficits and neurophysiological and structural changes in the brain, a condition that may be referred to as diabetic encephalopathy. Maternal diabetes leads to white matter hyperintensities and gray matter density changes in foetus [12]. Maternal diabetes leads to fetal hyperbilirubinemia [6], which could result an encephalopathy which named Kernicterus [35]. Some studies have shown that flavonoids are able to decrease plasma glucose level [36]. Quercetin inhibits glucose transporter (GLUT2), so diminishes glucose intestinal absorption [37]. Caffeoylquinic acid (chlorogenic acid) is a specific inhibitor of glucose-6-phosphate translocase and reduces hepatic glucose production [38], thus decrease blood glucose level and HbA1C [39]. Plant antioxidants are able to restore and regenerate pancreatic β cells.

Conclusion

According to biochemical and histological findings of previous studies and the results of this study it is concluded that one of the mechanisms of hypoglycemia Fenugreek seed extract is reconstruction and repair of pancreatic islets and subsequent increase in insulin levels. The mean number of cells in gray matter and white matter increased in diabetic rat's foetus that fed Fenugreek seed extract compared to diabetic rat's foetus group Indicating extract beneficial effect in the treatment of maternal diabetes. It has been proven that administration of Soluble dietary fiber fraction to normal, type 1 or type 2 diabetic rats significantly improved oral glucose tolerance, and also, total remaining unabsorbed sucrose in the gastrointestinal tract of non-diabetic and type 2 diabetic rats following oral sucrose loading was significantly increased by Fenugreek seed extract.

Thus, in this study the therapeutic effect of Fenugreek seed extract was demonstrated, and due to the limited side effects it could be a possible new therapeutic treatment in type-1 diabetes.

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Table1: The mean parameters (± S.E) of spinal cord in four testing groups at age 18 days old

Weight(gr) Blood glucosetransverse	Transverse diameter	vertical diameter (central canal)	Vertical diameter	number of cells diameter (central canal)	number of cells in white matter	volume ratio of gray in gray matter	matter to white matter
Group A	3/12±/11	71.3 ± 4.258	1377/11±141/44	38/21±2/11	932/42±85/1972/68±5/231728/57±184/59	12473/92±142/75	2/34±/49
Group B	3/23±/21	69.2 ± 3.136	1321/32±162/21	37/25±1/97	937/12±79/67 76/97±6/66	1718/12±182/37	12479/82±135/23 2/38±/50
Group C	4/07±/43*	475 ± 19.689*	1312/56±123/57*	31/75±1/39*	923/19±80/56* 85/86±4/32*	1678/19±182/25*	12014/45±110/62* 2/14±/27*
Group D	3/27±/20	112.7 ± 4.679	1351/76±147/2335/42±2/12	962/13±89/09	78/31±5/731715/15±171/68	12304/25±124/82	2/31±/28

Values are demonstrated with mean± SD. significant difference between DC and other groups demonstrated with*sign (P<0.05).

Table2: The mean parameters (± S.E) of spinal cord in four testing groups at age 20 days old

Weight(gr)Blood glucosetransverse	Transverse diameter	Vertical diameter (central canal)	Vertical diameter	number of cells diameter (central canal)	number of cells in white matter	volume ratio of gray in gray matter	matter to white matter		
Group A	4/02±/59	70.4 ± 4.661	1369/14±139/56	37/32±2/17	940/24±84/17	74/75±5/65	10871.88±376.16	25734.62±840.58	1.76±0.09
Group B	4/03±/42	65.6 ± 3.853	1358/57±151/33	38/23±1/54	948/19±94/59	74/94±7/66	10845.14±373.48	25717.86±664.34	1.75±0.11
Group C	5/27±/45*	468 ± 19.322*	1303/43±134/43*	32/86±1/35*	918/16±84/24*	87/93±4/24*	9871.71±336.48*	22203.52±728.41*	1.43±0.03*
Group D	4/30±/54	123.5 ± 4.342	1349/52±133/28	36/58±2/33	951/11±90/03	77/42±5/86	10598.72±355.9924516	32±625.751	71±0.08

Values are demonstrated with mean± SD. significant difference between DC and other groups demonstrated with*sign (P<0.05)