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Research Article

Modulatory Effect of Fenugreek Loaded PLGA Nanoparticles on Lipofuscinogenesis in Pancreas of Alloxan Induced Diabetic Mice.

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ABSTRACT

Many traditional treatments have been recommended in the alterative system of medicine for the diabetes mellitus. Current research is now directed towards finding naturally occurring antidiabetic properties from plant origin. In Indian system of medicine *Trigonella foenum graecum* is an important medicinal plant and its leaves and seeds have been used in various ailments and as a health tonic. The purpose of this study was to examine the antioxidant activity of fenugreek seed extract (FSE) and its nanoparticles (FNPs) on body weight, pancreatic gland weight, lipid peroxidation and fluorescence product of the pancreas of alloxan induced diabetic mice. Adult albino male mice (*Mus musculus* L) were divided into four group's viz. i. Control Group: male mice were given subcutaneous injection of 0.15M acetate buffer pH 5.4 for 15 days. ii. Diabetic Group- mice were given single subcutaneous injection of alloxan 150 mg/kg body weight. iii. Diabetic → FSE: mice were given subcutaneous injection of FSE at a dose of 15mg/ kg body weight to diabetic mice for 15 days. iv. Diabetic → FNPs: mice were given subcutaneous injection of FNPs at a dose of 15mg/ kg body weight to diabetic mice for 15 days. Body weight and pancreatic gland weight was reduced in diabetic group but increased in fenugreek supplementary group. The end product of lipid peroxidation malondialdehyde (MDA) and fluorescence product were increased in diabetic group and after fenugreek administration the level of both the parameters reduced significantly. The results suggest that an anti-lipid peroxidation activity of fenugreek seeds and its nanoparticles, nanoparticles treated group showed better results than fenugreek seed extract treated groups indicating that the fenugreek nanoparticles are best antidiabetic and antioxidant as compared fenugreek seed extract.

Key words: Fenugreek seed extract, fenugreek nanoparticles, lipid peroxidation, fluorescence product, diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is now a worldwide disease and in particular, the number of young patients is increasing[1, 2]. The β cells in the pancreas are susceptible to ROS because they express very low levels of antioxidants[3]; therefore, it is considered that β cells are easily subjected to oxidative stress. It is well known that oxidative stress caused by ROS contributes to β cell death or dysfunction of the pancreas in type-1 diabetes[4].

Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin dependent diabetes mellitus (called alloxan Diabetes) in these animals, with characteristics similar to type- 1 diabetes in humans[5].

Management of DM may include lifestyle modifications, diet, exercise and long term use of hypoglycemic agents or insulin therapy[6]. It has been investigated that for a long time plants based herbal medicines or their extracts have been the major source of drugs for the treatment of DM in Indian medicine and other ancient systems, because plant products are frequently considered to be less toxic and more free from side effects than modern synthetic drugs[8]. Medicinal plants have become more important area of active research. Many herbs and plants have been described as possessing hypoglycemic activity when taken orally[7,9]. However, large floras are still waiting for investigation for their medicinal properties[10]. Medicinal plants possess antidiabetic potential or bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids, flavonoids and are confirmed to be effective in both preclinical and clinical studies[11, 12].

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family Leguminosae. The seeds of fenugreek are commonly used in India and in oriental countries as a spice in food preparations due to their strong flavor and aroma. The seeds are reported to have restorative and nutritive properties and to stimulate digestive processes[13]. Fenugreek seeds have been shown to have hypocholesterolemic effects in type 1 and 2 diabetes mellitus patients[14, 15] and alloxan induced diabetic animals [16, 17].

The hydrophobic character of fenugreek results in pharmacokinetic restrictions such as low absorption and bioavailability by oral route, extensive metabolism and rapid elimination[18]. Biodegradable polymeric nanoparticles are extensively used to improve the therapeutic properties of various drugs and bioactive compounds. The reasons for the widespread use of PLGA are its biodegradability, biocompatibility and the fact that

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Table 1: Effect of fenugreek nanoparticles on body weight (gm) and weight of pancreas (mg) of alloxan induced diabetic mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr. No.	Treatment (n=5)	Weight of animal (gm)	Statistical Significance	Weight of pancreas (mg)	Statistical Significance
1	Control	28.5296 ± 2.1605		193.875 ± 15.597	
2	Diabetic	22.2893 ± 2.5344	1:2, P<0.01	123.875 ± 12.3917	1:2, P< 0.01
3	Diabetic → FSE	26.4085 ± 1.6625	2:3, P< 0.01 2:4, P<0.01	158.375 ± 19.9423	2:3, P< 0.01 2:4, P<0.01
4	Diabetic → FNPs	27.2975 ± 0.9374	3:4, P-non-significant	185.75 ± 9.8089	3:4, P< 0.01

Table 2: Effect of fenugreek nanoparticles on the level of total lipid peroxidation (n mol MDA / mg wet tissue) and fluorescence product (µg/ mg wet tissue) of alloxan induced diabetic mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr. No	Treatment (n=5)	Total lipid peroxidation	Statistical Significance	Fluorescence product	Statistical Significance
1	Control	23.8163 ± 4.2049		0.004133±0.000945	
2	Diabetic	43.096 ± 2.9609	1:2, P<0.01	0.00917± 0.000859	1:2, P< 0.01
3	Diabetic → FSE	28.7426 ± 4.7865	2:3, P<0.01	0.003921±0.000993	2:3, P< 0.01
4	Diabetic → FNPs	23.3104 ± 3.9876	2:4, P<0.01 3:4, P-non-significant	0.003529±0.000856	2:4, P<0.01 3:4, P< 0.01

drug products containing PLGA have been approved for parenteral use by regulatory authorities around the world[19]. So to find out whether fenugreek nanoparticles will be more useful or FSE in diabetes we have synthesized PLGA nanoparticles. We have synthesized the fenugreek loaded PLGA nanoparticles, previously nobody has synthesized and we have reported 1st time these fenugreek nanoparticles.

MATERIAL AND METHODS

Preparation of fenugreek seed extract

Fenugreek seeds were collected from the Mahatma Phule Krushi Vidyapeeth, Rahuri. They were (10g) were cleaned and ground into a fine powder using a grinding machine. Ethanol was used for extraction by soxhelt extraction method[20]. The extract was evaporated to dryness under reduced pressure at 60^0 C by rotary evaporator. Extract was placed in dark bottle and stored at -8^0C.

Synthesis of fenugreek loaded PLGA nanoparticles (FNP's)

Fenugreek loaded PLGA based nanoparticles was prepared using oil in water single emulsion solvent evaporation process[21].

Animals

Male albino mice (*Mus Musculus* L.) were used for present study. They were bred and reared in departmental animal house (1825/PO/EReBi/S/15/CPCSEA) in separate cages under proper conditions of light, temperature and humidity. They were supplied with Amrut mice feed (Pranav Agro industries) and water *ad libitum.*

Experimental design: Mice were divided in to four groups:

1) Control Group: Three months male mice were given subcutaneous injection of 0.15m acetate buffer pH 5.4 for 15 days.

2) Diabetic Group: Three months male mice were given subcutaneous injection of alloxan 150 mg /kg body weight for 15 days [22].

3) Diabetic → FSE: Three months male mice were given subcutaneous injection of fenugreek seed extract at a dose of 15 mg /kg body weight to diabetic mice for 15 days [23].

4) Diabetic → FNPs: Three months male mice were given subcutaneous injection of fenugreek nanoparticles at a dose of 15mg/ kg body weight to diabetic mice daily for 15 days.

After completion of the dose, the mice were killed by cervical dislocation; pancreas were dissected out, blotted and weighed. The pancreas tissue was homogenized by using mixture containing 75 mM phosphate buffer (pH7.04), 1mM ascorbic acid, 1mM ferric chloride and 0.001 ml chlortetracycline (10ppm).

Measurement of body weight of mice

Mice were weighed before starting experiment, during respective treatment and also after completion of each treatment. The record of these observations was maintained.

Measurement of pancreatic gland weight of mice

The mice from respective groups were killed by cervical dislocation after completion of treatment. Pancreas dissected out, dried with the help of blotting paper and wet weight of gland was measured using digital scale balance. The record of these observations was maintained.

Determination of total lipid peroxidation [24]

Tissue homogenate was prepared in chilled mortar using 75mM potassium phosphate buffer pH 7.04 containing 1mM ascorbic acid and 1mM ferric chloride and the total lipid peroxidation was estimated.

Measurement of fluorescence product [25]

The lipofuscin granules from pancreas were extracted using chloroform: Methanol mixture (2:1 v/v). The fluorescence was measured by using quinine sulphate as a standard by using photofluorometer.

All values were expressed as mean ± S.D. statistical analysis was carried out by one way ANOVA, Tukey's HSD test.

RESULTS

The average body weight and gland weight was 28 gm and 193 mg respectively at the beginning of the treatment. Diabetic mice showed a progressive reduction in body weight and gland weight as compared to control group. The body weight and pancreatic gland weight in FSE treated diabetic mice were significantly increased as compared to diabetic mice. (Table no. 1). The total lipid peroxidation and fluorescence product in pancreas was increased in diabetic group as compared to control and increase was significance (1:2, $P < 0.01$), while it was decreased significantly in Diabetic → FSE and Diabetic → FNPs (2:3, $P < 0.01$; 2:4, $P < 0.01$) as compared to diabetic group. The MDA and fluorescence product was decreased in Diabetic → FNPs as compared to Diabetic → FSE (Table 2).

DISCUSSION

In our study, we observed decrease in body weight, pancreas weight, while significant increase in fluorescence product and concentration of MDA, a secondary product of LPO, in pancreas of alloxan treated diabetic mice. Alloxan is a beta cytotoxin induces diabetes in a wide variety of animal species by damaging pancreatic B cells resulting in decrease in endogenous insulin release which lead to decrease glucose utilization by the tissues and a resultant diabetic (Hyperglycemia) condition.

Strain (1991) has observed disturbance of antioxidant defence system in DM i.e. oxidative stress play an important role in pathogenesis of diabetes[26]. Oxidative stress is the result of excessive free radical production. These free radicals are scavenged by various antioxidants enzymes[27-29]. Unscavenged free radicals exert peroxidative effect on polyunsaturated fatty acids of the membrane of cells as well as cell organelles[30]. Malondialdehyde is formed in extensive membrane lipid peroxidation. These peroxidized membranes are digested by lysosomes. The free radicals also bring about damage to lysosomes and lysosomal enzymes, making them inefficient which turn into residual bodies[31-33]. These are lipofuscin granules. As lipofuscin granules are auto fluorescent they lead to increase in fluorescence product also[34]. Lipofuscin is often called age pigment and considered a hallmark of aging. This is not only because the amount of lipofuscin increases with age, showing an almost linear dependence[35], but also and more importantly because the rate of lipofuscin accumulation correlates negatively with longevity[36-39].

In the present study the level of lipid peroxidation and fluorescence product was decreased after treatment of fenugreek seed extract and fenugreek nanoparticles. These results suggest that FSE and FNPs administration in diabetic mice reduce LPO and MDA product possibly by decreasing free radical formation and increasing antioxidant. The administration of an antioxidant such as fenugreek seed extract may ameliorate tissue dysfunction since antioxidants are known to improve tissue integrity[7, 40, 41].

The reduction in the lipid peroxidation and fluorescence level in pancreas of mice after receiving fenugreek extract and its nanoparticles indicated that it is having free radical scavenging capacity which helps to prevent cellular damage and thereby reduce lipid peroxidation. It concludes that, fenugreek seeds are having antioxidant and anti peroxidative properties. The better results seen in fenugreek nanoparticles treated group than fenugreek seed extract treated groups, it clearly indicates that the fenugreek nanoparticles is best antidiabetic and antioxidant than fenugreek seed extract.

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