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REGULAR ARTICLE

EFFECTS OF *TRIGONELLA FOENUM GRAECUM* ON THE LEUKOCYTES IN DIABETES MICE

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ABSTRACT

Immunological disorders is among the most frequent and serious complications of diabetes mellitus An inflammatory response is usually accompanied by increasing leukocyte activation. Numerous medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes. *Trigonella foenum-graecum* Linn. (*Leguminosae*) commonly known as fenugreek, is an annual herb, widely grown in India, Egypt, and Middle Eastern countries. It has been extensively used as a source of antidiabetic compound, from its seeds and leaves. The aim of this study was to investigate anti-inflammatory effects of fenugreek on streptozotocin - induced diabetic mice.

The animals were divided into four groups. Group I – control (C); Group II – diabetic untreated (DM); Group III –was administered purified from fenugreek *(T. foenum-graecum)* seeds (GII) in dose 100 $mg.kg^{-1}$ body weight/day for 15 days (GII), and Group IV – diabetic treated with fenungreek (D+GII). Thirty minutes after the last injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery.

Were measured in blood lymphocytes, monocytes, neutrophils and eosinophils in stained blood films.

The changes in analyzed parameters observed in the diabetes animals were also found to be significantly restored in fenugreek treated mice. The results demonstrated that extract of *Trigonella foenum-graecum* could be a potential herbal medicine in treating diabetes.

Keywords: immune system, diabetes mellitus, fenugreek (*Trigonella foenum-graecum*) seeds, leukocytes

INTRODUCTION

Chronic inflammatory illnesses such as diabetes, arthritis, and heart disease **(Xiong *et al.*, 2012).** Type 1 diabetes is an autoimmune disorder characterized by the specific destruction of the insulin secreting beta cells of the pancreatic islets by a certain population of autoreactive immune cells. The early stages of the disease process leading to the development of diabetes are characterized by insulitis, the infiltration of the pancreatic islets by mononuclear cells such as dendritic cells (DCs), macrophages and T cells. Since autoimmune response progresses many years before the onset of clinical diabetes, studies in T1D pathogenesis for the past several decades have been mainly focused on the role of adaptive immunity. T cells are believed to be the major effector cells responsible for beta cell destruction. CD8+ T cells have been found to be critical for disease pathogenesis in both T1D patients and NOD mice, a mouse model for spontaneous autoimmune diabetes. The slowly progressive course of autoimmune diabetes is almost certainly due to lobular nonsynchronous destruction of islet beta cells creating over time more and more pseudoatrophic islets (islets lacking all insulin producing beta cells but retaining islet cells producing glucagon, somatostatin, and pancreatic polypeptide) **(Eisenbarth, 2009).** Treatment of type 1 diabetes mellitus (DM1) has greatly improved but remains limited to combating the consequences of the disease. Immunosuppression can slow disease progression, but does not cure DM1. Immunotherapy attempts to protect remaining insulin-producing β cells and β cell function. Vaccination with β-cell specific antigens to stimulate tolerance and vaccination combined with immunotherapy (biologicals) are options for future therapy **(van der Torren and Roep, 2012)**.

Numerous medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes **(Khan *et al.*, 2012)**.

Preliminary trials have suggested possible hypoglycaemic, hypolipidaemic and immunomodulatory properties of the fenugreek plant. The seeds of fenugreek, *Trigonella foenum graecum*, commonly used as a spice in Middle Eastern countries and widely used in south Asia and Europe, are known to have anti-diabetic properties **(Basch *et al.*, 2003)**. Fenugreek lowers serum TG and total cholesterol, and hepatic lipid concentrations **(Annida *et al.*, 2004; Hannan *et al.*, 2003)**. The effect of fenugreek to lower hepatic lipids may be due to the potential of its seeds to modulate the activity of several enzymes, including those associated with glucose and lipid metabolism **(Raju *et al.*, 2001)**. Moreover, these effects of fenugreek may be due to the enhancement of biliary cholesterol excretion **(Stark and Madar, 1993)**. Fenugreek contains various alkaloids, flavonoids, and saponins, among which saponins are at the highest concentration in fenugreek. It has been demonstrated that some saponin components affect lipid metabolism, including liver and plasma TG and plasma cholesterol concentrations **(Li *et al.*, 2008).** However, the molecular mechanism underlying the effects of fenugreek and saponin on lipid metabolism is unclear. They contain an unusual amino acid (2S, 3R, 4S) 4-hydroxyisoleucine (4HO-Ile), so far found only in fenugreek, which has anti-diabetic properties of enhancing insulin secretion under hyperglycaemic conditions, and increasing insulin sensitivity **(Haeri *et al.*, 2012).** When fenugreek oil is given to alloxanized rats, notable reduction in renal toxicity besides improved hematological status and antidiabetic effect has also been accounted, which could be due to the immunomodulatory activity and insulin stimulation action of fenugreek **(Hamden *et al.*, 2010)**. The aim of this study was to investigate anti-inflammatory effects of fenugreek on streptozotocin - induced diabetic mice.

MATERIAL AND METHODS

The experiments were carried out on male mice with average body weight 25-26g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water. Animals were divided into four groups of ten mice each. Group I – control (C), Group II – diabetic untreated (DM), Group III –was administered purified from fenugreek (*T. foenum-graecum*) seeds (GII) in dose 100 $mg.kg^{-1}$ body weight/day for 15 days (GII), and

Group IV – diabetic treated with fenungreek (D+GII). Fenugreek seeds were administered orally.

Diabetes mellitus (DM) was induced in groups II, and IV and received a single i.p. injection of streptozotocin - STZ, 60 mgKg-1 body weight, freshly dissolved in 0.1 M citrate buffer (pH 4.5). DM was verified by measuring blood glucose in tail nick blood samples. Mice with non fasting blood glucose levels of ≥20 mmol/L after 48 h of STZ injection or greater and symptoms of polyuria, polyphagia, and polydipsia were considered diabetic.

Thirty minutes after the last injection animals all experimental groups were anaesthetized and decapitated. The blood samples were collected from the carotid artery. Were measured in blood leukocytes, lymphocytes, monocytes, neutrophils and eosinophils in stained blood films.

The results were expressed as means ± standard deviation. The statistical analysis of the results was carried out with Statistica program version 9. The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analysed using one-way ANOVA followed by post hoc analysis with Tukey test. Statistical significance was defined at $p<0.01$.

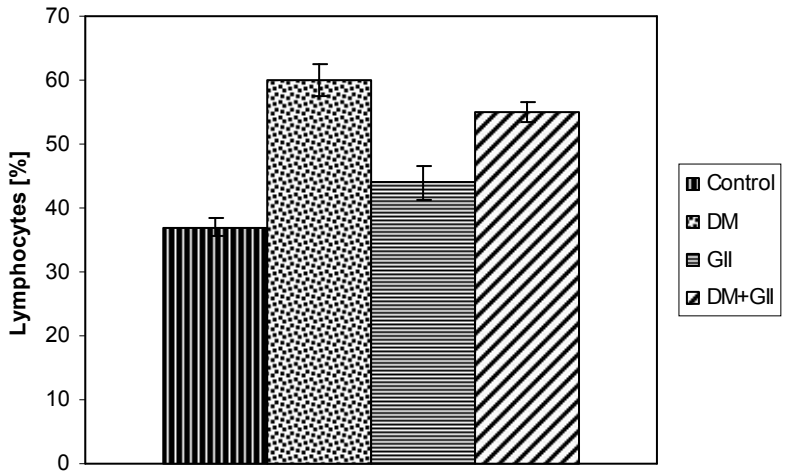
RESULTS AND DISCUSSION

Diabetes mellitus is a complicated metabolic disorder that has gravely troubled the human health and quality of life. Conventional agents are being used to control diabetes along with lifestyle management. However, they are not entirely effective and no one has ever been reported to have fully recovered from diabetes. Type 1 diabetes (T1D) is an autoimmune disorder characterized by the specific destruction of the insulin secreting beta cells of the pancreatic islets by a certain population of autoreactive immune cells. The early stages of the disease process leading to the development of diabetes are characterized by insulitis, the infiltration of the pancreatic islets by mononuclear cells such as dendritic cells (DCs), macrophages and T cells.

Numerous medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes. Plants are of enormous medicinal importance, they are being extensively explored for their use against diabetes. Herbal drugs can be quite acceptable as these drugs are known to cause less adverse

effects **(Modak *et al.*, 2007)**. They are quite popular in developing countries **(Ali *et al.*, 2006).** The increased admiration of herbal medicines for diabetes may be due to the side-effects associated with the conventional antidiabetic drugs. Preliminary trials have suggested possible hypoglycaemic, hypolipidaemic and immunomodulatory properties of the fenugreek plant **(Ramadan *et al.*, 2011).** *Trigonella foenum-graecum* (fenugreek) is native to areas from the Eastern Mediterranean region to Central Asia and Ethiopia **(Morton, 1990)**. It has long been used in Ayurvedic and Chinese medicine **(Bash *et al.*, 2003)**. Fenugreek lowers serum TG and total cholesterol, and hepatic lipid concentrations **Annida *et al.*, 2004)**. The effect of fenugreek to lower hepatic lipids may be due to the potential of its seeds to modulate the activity of several enzymes, including those associated with glucose and lipid metabolism **(Raju *et al.*, 2001)**. Moreover, these effects of fenugreek may be due to the enhancement of biliary cholesterol excretion **(Trinh *et al.*, 2001)**. Fenugreek contains various alkaloids, flavonoids, and saponins, among which saponins are at the highest concentration in fenugreek. It has been demonstrated that some saponin components affect lipid metabolism, including liver and plasma TG and plasma cholesterol concentrations **(Li *et al.*, 2008)**.

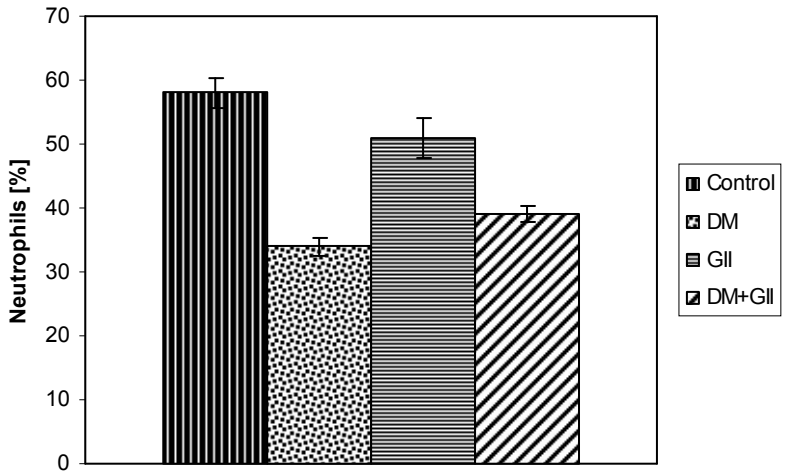
Some studies show an increase in the concentration of lymphocytes following exercise due to the recruitment of lymphocytes NK, cells T and B from the periphery of the body **(Bruunsgaard and Pedersen, 2000).** The administration of purified from fenugreek (*Trigonella foenum-graecum*) seeds (GII) in dose 100 mg.kg-1 body weight/day for 15 days to STZ-induced diabetic mice led to significant decreases in the count of lymphocytes versus DM group. The changes were statistically significant ($p \leq 0.001$). The untreated diabetic animals showed significantly raised lymphocytes activity as compared to the control group. The administration of GII however, significantly restored lymphocytes activity, and levels in the diabetic mice to near control ($p \leq 0.01$). The results are shown in Fig. 1



*p≤0.001; **p≤0.01

Figure 1 Effect of fenugreek *(Trigonella foenum-graecum)* seeds (GII) in dose 100 mg.kg^{-1} body weight/day for 15 days on lymphocytes in STZ-induced diabetic mice(DM). Values are given as means ± SD of at least ten values

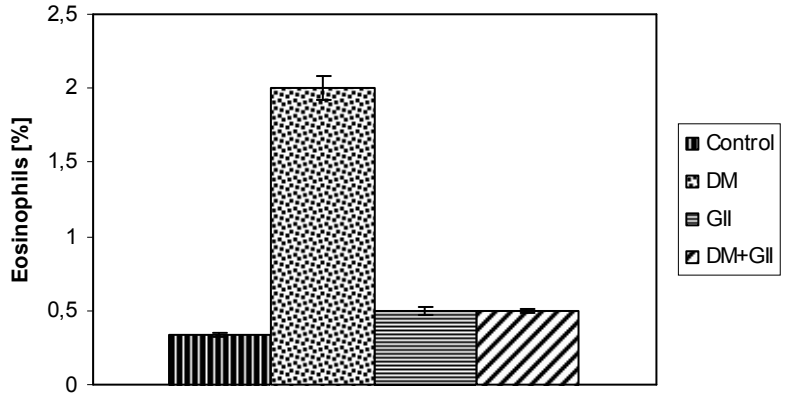
In our study, diabetes decreased neutrophils count. Neutrophils of diabetic patients do not increase their bactericidal activity in response to the same intensity of infection as compared to nondiabetic patients **(Moss *et al.*, 2000).** Diabetes decreases the affinity of the neutrophils with the endothelial cells. This effect, on the other hand, protects the lungs from neutrophils migration, thus decreasing its oxidative production. The increase in blood neutrophils after fenugreek administration may be a result of minor adherence of cell in target tissues (Fig. 2)



*$p \leq 0.001$; **$p \leq 0.01$

Figuer 2 Effect of fenugreek *(Trigonella foenum-graecum)* seeds (GII) in dose 100 mg.kg^{-1} body weight/day for 15 days on neutrophils in STZ-induced diabetic mice(DM). Values are given as means ± SD of at least ten values

Simultaneously the contents of eosinophils was significantly reduced ($p \leq 0.001$) after administration of once daily for 15 days GII compared to the diabetic group. Eosinophils are also altered in stress conditions. Was observed a huge increase in the number of eosinophils during the course diabetes (Fig.3).



*$p \leq 0.001$

Figure 3 Effect of fenugreek *(Trigonella foenum-graecum)* seeds (GII) in dose 100 mg.kg^{-1} body weight/day for 15 days on eosinophils in STZ-induced diabetic mice(DM). Values are given as means ± SD of at least ten values

Elevation of inflammation markers is frequently reported in diabetic organisms with increase in subpopulations of monocytes, which may contribute to the development of atherosclerosis **(Patiño *et al.*, 2000)** On the other hand, the exercise usually produced a decrease in some subpopulations of monocytes count. Nevertheless, in our study diabetes didn't caused alteration in the monocytes percentage (Fig.4).

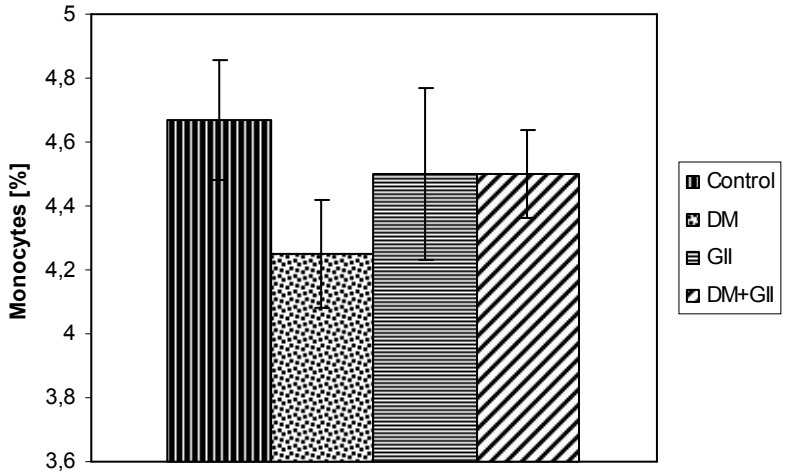


Figure 4 Effect of fenugreek *(Trigonella foenum-graecum)* seeds (GII) in dose 100 mg.kg^{-1} body weight/day for 15 days on monocytes in STZ-induced diabetic mice(DM). Values are given as means ± SD of at least ten values

In a study carried out by Hamden et al., 2010 it was shown that fenugreek oil in the amelioration of diabetes, hematological status, and renal toxicity which may be attributed to its immunomodulatory activity and insulin stimulation action along with its antioxidant potential **(Hamden *et al.*, 2010).** Our study showed that experimental diabetes induces metabolic damages and alters, at least in part, the differential leucocytes count.

CONCLUSION

In summary, administration of fenugreek *(Trigonella foenum-graecum)* seeds (GII) was able to improve metabolic and immunological aspects in the experimental diabetic mice.

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