The Bioscan 7(3) : 395-400, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INFLUENCE OF *TRIGONELLA FOENUM GRAECUM* (FENUGREEK) IN ALLOXAN INDUCED DIABETIC RATS

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KEY WORDS
Trigonella foenum graecum
Insulin
Glucose
Alloxan

Received on :
23.

Accepted on :
17.

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ABSTRACT
The present study was used to investigate the antidiabetic activity of *trigonella foenum graecum* in alloxan induced diabetic rats. The effect observed were compared with a known antidiabetic agent glibenclamide. In active pancreatic damage induced by alloxan (i.p.75mg/kg b.w), fenugreek seed aqueous extract (1300 mg/kg b.w) and ethanol extract (1g/kg b.w) adminstration significantly reduced the elevated level of blood glucose and increased the levels of serum insulin. It decreases the elevated levels of cholesterol, triglycerides, urea, uric acid, creatinine, glycosylated Hb, and increases the decreased levels of liver glycogen and protein. Also the histological examination of pancreas supported the antidiabetic effect of fenugreek.It is concluded that the aqueous and ethanol extract of fenugreek could act and possess good antidiabetic activity. Further *Trigonella foenum graecum* aqueous extract shows more significant results than *Trigonella foenum graecum* ethanol extract.

INTRODUCTION

Diabetes is a common endocrine disorder, affecting more than 100 million people world wide (6% of the population) (WHO/ Acadia, 1992) and the world health organization predicts this number will increase five fold in the near future (Grover *et al.,* 2002). Diabetes mellitus is primarily characterized by either lack of insulin or its action which causes derangement in the metabolism of carbohydrate, protein and lipid. This disease can be treated, but as yet it cannot be cured. People who are diabetic end up getting other medical problems as well with one thing in common - a problem with insulin (wild *et al.,* 2004). It is the leading cause of adult blindness and amputation, and a major cause of renal failure, heart attacks, and stroke. Diabetes is not one disease but rather is a heterogenous group of syndromes (Pamela, 1994).

Diabetes has been defined by the world health organization (WHO), on the basis of laboratory findings, as a fasting plasma venous glucose concentration greater than 7.8mmol/L (140mg/dl) or a concentration of 11.1 mmol/L (200mg/dl) or more two hours after a carbohydrate meal or two hours after oral ingestion of the equivalent of 75g of glucose, even if the fasting concentration is normal. Severe cases have persistent hyperglycaemia. (Zilva, 1988) Complications resulting from diabetes mellitus are the 3rd leading cause of death attributable to disease in the united states according to statistics complied by the national commission on diabetes.

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes mellitus. Plants are always an exemplary source of drugs, in fact many of the currently available drugs were derived either directly or indirectly from them. According to world ethnobotanical information reports, almost 800 plants may possess antidiabetic potential (Alarcon-Aguilara *et al.,* 1998).In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin and screening of more effective and safe hypoglycemic agents has continued to be an important area. In developing countries 80% of population are using traditional medicine in primary medical problems (Grover and Yadav, 2004). However, lots of herbs are now being used in the management of DM. Among herbs reported to possess anti-diabetic properties, *Trigonella foenum graecum* (fenugreek) is one of the best, in terms of efficacy and safety, history of traditional use, and results of research studies (kaczmar, 1998). *Trigonella foenum graecum* was commonly known as Fenugreek, Methi, Alhova, Bird's Foot, Greek Clover, Greek Hay. Fenugreek (*Trigonella Foenum-graecum*) is one of the oldest herbs known originating in the Mediterranean region and Asia (Azaizeh *et al.,* 2006).

MATERIALS AND METHODS

Plant material

The *Trigonella foenum graecum* is collected from Adhiparasakthi Agricultural college medicinal park, in kalavai-632506. Tamil Nadu-India. The *Trigonella foenum graecum* was dried and crushed in to fine powder formed.

Animals

Rats used in this experiment were male swiss Albino rats from our laboratory. The rats weighed between 150 to 200g were used. The rats were kept in animal house for ten days before starting the experiment. The animals were divided into seven groups of six rats in each group.

Preparation of plant extract

Preparation of aqueous extract

10g of fenugreek Powders were soaked in 100g of boiled water for 6hrs then filtered through a seive and stored in dark bottles immediately. The aqueous extract was given at the dose of 1300mg of fenugreek/kg b.w.

Preparation of ethanol extract

100g of dry powdered of fenugreek was continuously extracted for 48h with 90% ethanol in a soxhlet apparatus. The collected extract was stored at 0-4ºC until used. The plant extract was pooled and evaporated to dry at 60ºC. The ethanol extract was given at the dose of 1g of fenugreek/kg b.w.

Experimental procedure

In this experiment total of 42 rats (24 diabetic surviving rats, 6 normal rats and 12 control rats) were used. They were divided into seven groups in each group six rats were selected. Group I was a normal untreated rats, group II was induced with alloxan (i.p. 75mg/kg b.w.). Aqueous extract of *Trigonella foenum graecum* was given at the dosage of 1300 mg/kg b.w./day for 21 days to normal rats of group III. Aqueous extract of *Trigonella foenum greacum* was given at the dosage of 1300 mg/kg b.w./ day for 21 days to diabetic induced rats of group IV. Ethanol extract of *Trigonella foenum graecum* was given at the dosage of 1g/kg b.w./day for 21 days to normal rats of group V. Ethanol extract of *Trigonella foenum graecum* was given at the dosage of 1g/kg b.w./day for 21 days to diabetic induced rats of group VI. Glibenclamide was given at the dosage of 600μg/kg b.w./ day for 21 days to diabetic induced rats of group VII.

Sample collection

At the end of treatment period the rats were allowed over night fasting, anaesthetized with ketamine 80 mg/kg b.w (i.p) and was killed by cervical decapitation.Blood was collected and used for the estimation of Glucose, Insulin and other biochemical parameters. Liver and Pancreas were dissected out. Washed in ice cold saline and used for further analysis. Pancreas was used for histopathological studies and liver was used for estimation of glycogen.

Histopathological studies

Histopathological evaluation was performed on pancreas tissue. Fresh pancreas tissue was excised and then fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Section were cut into 5μm thickness and stained with hematoxylin and eosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 10xs for histological changes. In histopathological study alloxan, induced animals (Group II) showed necrosis and reduction in the number of islets cells. The reduction and necrosis in pancreatic cell may be due to the decrease in the antioxidant defense in combating ROS mediated damage. The *Trigonella foenum greacum* aqueous and ethanolic extract treated animals showed regeneration of some the necrotic cells and decrease the cellular necrosis in the pancreas. It leads to increase in plasma insulin levels in treated groups.

Biochemical assay

Glucose and Insulin were estimated by the method of Sasaki and Matsui (1972) and Burgi *et al*. (1988). Urea and Uric acid were determined by the method of Natelson (1951) and Caraway (1965). Protein and Creatinine were studied by the method of Lowry (1951) and Win *et al*. (1977). Cholesterol and Triglycerides were estimated by the method of Parekh and Jung (1970) and Foster and Dunn (1973). Glycosylated Hb and Glycogen were assayed by the method of Sudhakar Nayak and Pattabiraman (1981) and Morales (1975).
Biochemical determination was carried out using shimadzu spectrophotometer.

Statistical analysis

ANOVA, statistical treatment applied under one way classification, changes were considered significant of the P values was <0.01, <0.05. The values expressed as mean $\pm$ SD.

RESULTS AND DISCUSSION

T. foenum-graecum seeds have been used as traditional medicines not only in diabetes but also in high cholesterol, inflammation and gastrointestinal ailments (Sharma *et al.,* 1990). The present study was focused in observing the hypoglycemic effect of *Trigonella foenum graecum* aqueous and ethanolic extract. The overall comparison of the antidiabetic effect of *Trigonella foenum graecum* aqueous extact with that of ethanolic extract was studied here.

The level of insulin in group II was decreased when compared to the normal group (Fig. 1). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed increased level of insulin when compared to the diabetic group.

The scientist's have demonstrated evidences of insulinotropic and antidiabetic properties of 4 hydroxyisoleucine isolated from fenugreek seeds in glucose dependent manner. They suggested that antidiabetic effect of 4 hydroxyisoleucine was, atleast in part, from direct pancreatic beta cell stimulation. (Sauvaire *et al.,* 1991) (Broca *et al.,* 1999)

Fenugreek major free amino acid 4 hydroxyisoleucine stimulates insulin secretion from perfused pancreas invitro. (Al-Habbori and Raman, 1998)

The level of glucose in group II was increased when compared to the normal group (Fig. 2). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of glucose when compared to the diabetic group.

Fenugreek seed contains 45-60% carbohydrate, mainly mucilaginous fiber (galactomannans). Fenugreek seed contain galactomannan may be the cause of decrease in the blood sugar (Ali *et al.,* 1995).

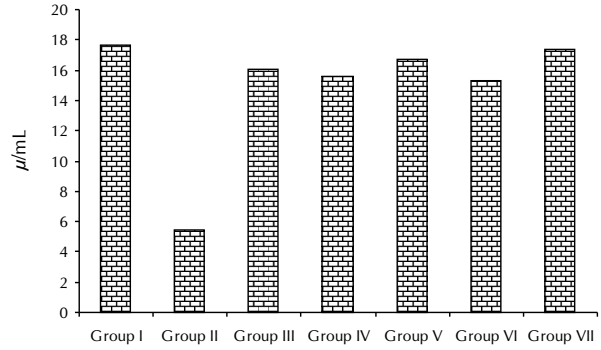

Figure 1: Levels of insulin in different groups of rats

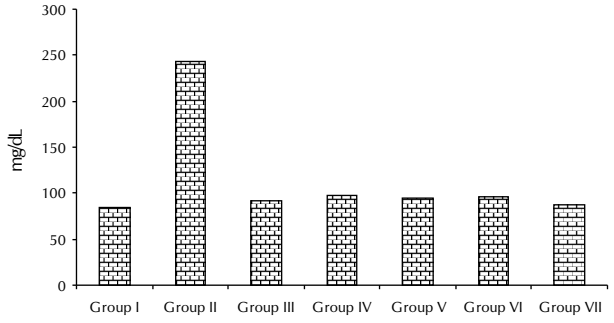

Figure 2: Levels of blood glucose in different groups of rats

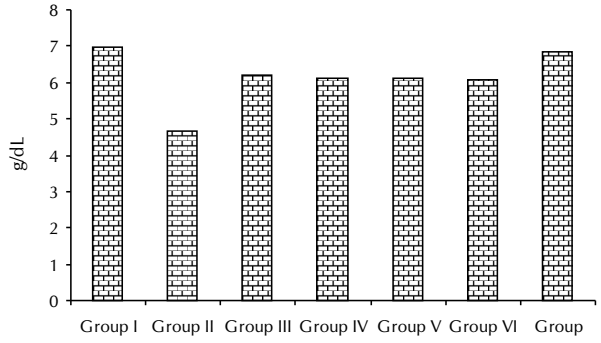

Figure 3: Levels of protein in different groups of rats

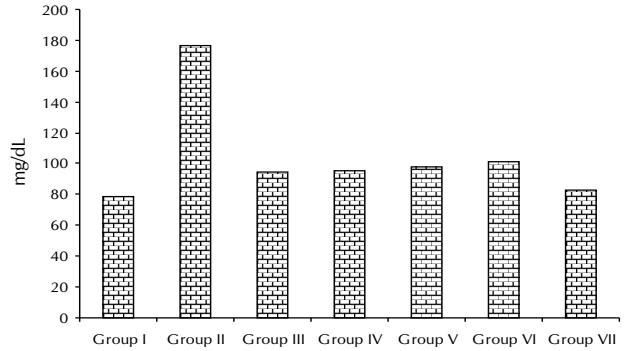

Figure 4: Levels of cholesterol in different groups of rats

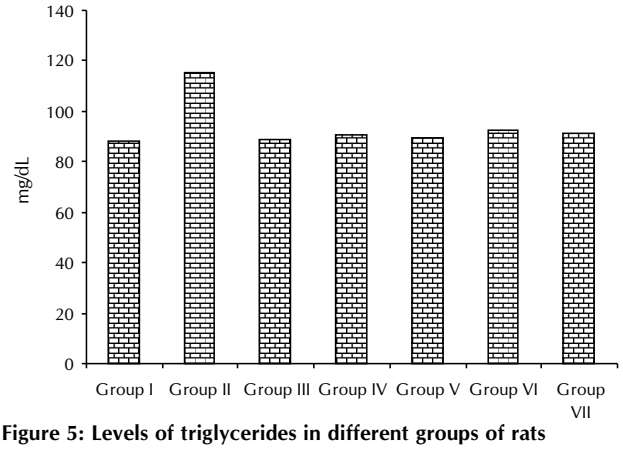

Figure 5: Levels of triglycerides in different groups of rats

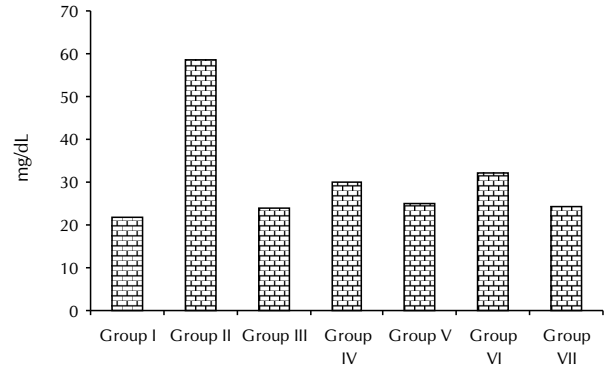

Figure 6: Levels of blood urea in different groups of rats

The seed fibers of fenugreek reduces the rate of glucose absorption and may also delay gastric emptying, there by preventing the rise in blood sugar levels following a meal (Gupta *et al.,* 2001).

Guar gum of fenugreek prevents the rapid uptake of glucose in the small intestine, aids in blood sugar retention in diabetic patients (Sharma *et al.,* 1996).

The level of protein in group II was decreased when compared to the normal group (Fig. 3). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed increased level of protein when compared to the diabetic group.

In diabetes the glucose was not utilized due to the insulin lack or insulin resistance so protein was utilized as energy source, so the level of protein decreased in group II diabetic rats.

The level of cholesterol in group II was increased when compared to the normal group (Fig. 4). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of cholesterol when compared to the diabetic group.

Guar gum of fenugreek was effective in the treatment of hypercholesterolemia (Sharma *et al.,* 1996) Fenugreek also

contains a biologically significant level of saponins. Saponins are known to have hypocholesterolemic effects. (Sharma, 1986; Sharma and Raghuram, 1990)

Generally plant protein appears to lower cholesterol level (James, 2004).

The level of triglycerides in group II was increased when compared to the normal group (Fig. 5). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of triglycerides when compared to the diabetic group.

The antihyperlipidemic properties of oral fenugreek seed powder has been suggested. (Basch, 2003) The scientist's showed the effect of fenugreek seeds and its extracts on plasma lipid profile on rabbits. (Al-Habori *et al.,* 1998) The plant protein in fenugreek is 26%, so it might exert a lipid lowering effect. (Sharma, 1986) The amino acid 4 hydroxyisoleucine present in fenugreek may also decrease the plasma triglyceride level.

The level of urea in group II was increased when compared to the normal group (Fig. 6). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of urea when compared to the diabetic group.

The urea level was increased in diabetes because the utilization of protein is increased leading to the formation of high level of urea.

The level of uric acid in group II was increased when compared to the normal group (Fig. 7). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of uric acid when compared to the diabetic group.

The uric acid in serum was increased in kidney diseases. The long term complications of diabetes was renal disease. So uric acid was increased in diabetes group. It will be returned to normal

The level of creatinine in group II was increased when compared to the normal group (Fig. 8). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of creatinine when compared to the diabetic group.

The creatinine in serum was increased in renal diseases. The long term complications of diabetes was renal disease. So creatinine was increased in diabetes group. It will be returned to normal value in fenugreek treated groups because fenugreek have protective effect on kidney during diabetes. (Singhal, 1982; Motawi, 1992)

The level of liver glycogen in group II was decreased when compared to the normal group (Fig. 9). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed increased level of liver glycogen when compared to the diabetic group.

The scientist's have reported that following exercise, the 4 hydroxyisoleucine, present in fenugreek seeds, increases the rate of glycogen synthesis in skeletal muscle. (Ruby *et al.,* 2005) Other studies have shown that fenugreek seeds and

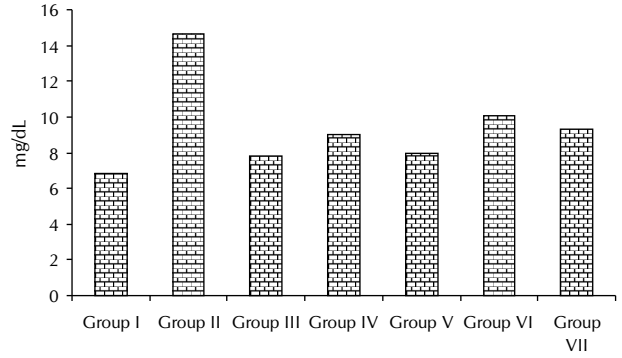

Figure 7: Graphical representation of levels of Uric Acid in different groups of rats

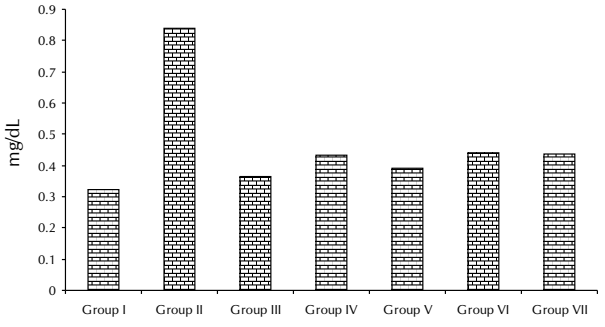

Figure 8: Graphical representation of levels of Creatinine in different groups of rats

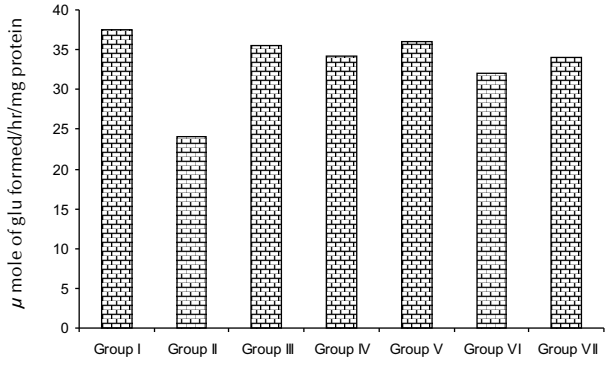

Figure 9: Graphical representation of levels of Glycogen in different groups of rats

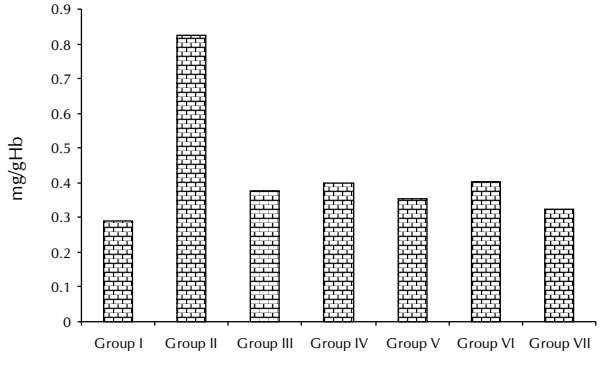

Figure 10: Graphical representation of levels of Glycosylated haemoglobin in different groups of rats

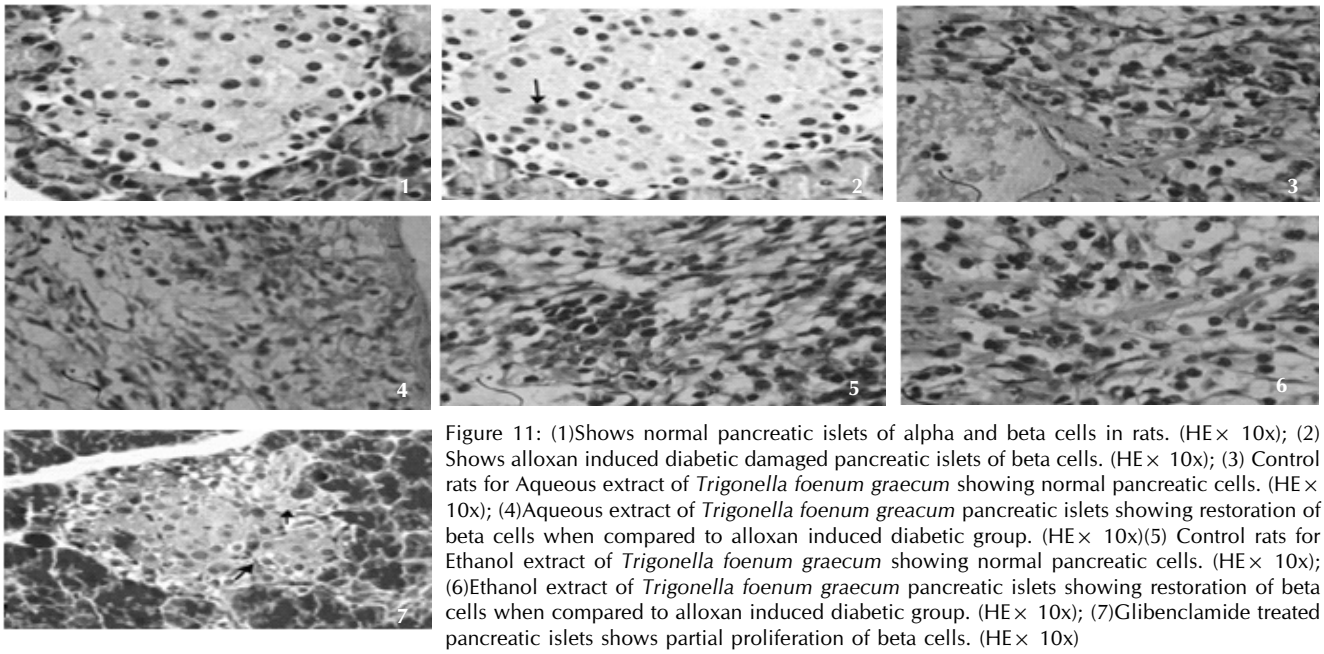

Figure 11: (1)Shows normal pancreatic islets of alpha and beta cells in rats. (HE× 10x); (2) Shows alloxan induced diabetic damaged pancreatic islets of beta cells. (HE× 10x); (3) Control rats for Aqueous extract of *Trigonella foenum graecum* showing normal pancreatic cells. (HE× 10x); (4)Aqueous extract of *Trigonella foenum greacum* pancreatic islets showing restoration of beta cells when compared to alloxan induced diabetic group. (HE× 10x)(5) Control rats for Ethanol extract of *Trigonella foenum graecum* showing normal pancreatic cells. (HE× 10x); (6)Ethanol extract of *Trigonella foenum graecum* pancreatic islets showing restoration of beta cells when compared to alloxan induced diabetic group. (HE× 10x); (7)Glibenclamide treated pancreatic islets shows partial proliferation of beta cells. (HE× 10x)

leaves prevent liver glycogen depletion in STZ induced diabetic rats. (Vats *et al.,* 2003; Devi *et al.,* 2003)

The level of glycosylated hemoglobin in group II was increased when compared to the normal group (Fig. 10). The group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract showed decreased level of glycosylated hemoglobin when compared to the diabetic group.

Group III & V show nearer values of normal group. It shows the non toxic effect of aqueous and ethanolic extract of fenugreek respectively. Group VII was treated with glibenclamide standard diabetic drug. It shows the nearer values of normal group.

The effect of fenugreek extract in diabetic induced rats were compared with standard glibenclamide diabetic drug. From the results we concluded that the aqueous and ethanolic extract of fenugreek have antidiabetic activity. But aqueous extract have more significant effect than ethanolic extract.

ACKNOWLEDGEMENTS

The authors wish to express sincere thanks to the Management of Adhiparasakthi College of Arts & Science, G.B. Nagar, Kalavai- 632506, Vellore, Tamil Nadu, India.

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