THE HYPOGLYCAEMIC EFFECT OF *Brassica juncea* (MUSTARD) AND *Trigonella foenum-graecum* (FENUGREEK) IN ALBINO RATS

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DECLARATION

I hereby declare that the thesis entitled **"THE HYPOGLYCAEMIC EFFECT OF *Brassica juncea* (MUSTARD) AND *Trigonella foenum-graecum* (FENUGREEK) IN ALBINO RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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PREETHY JOHN

CERTIFICATE

Certified that this thesis, entitled **"THE HYPOGLYCAEMIC EFFECT OF *Brassica juncea* (MUSTARD) AND *Trigonella foenum-graecum* (FENUGREEK) IN ALBINO RATS"** is a record of research work done independently by **Dr. Preethy John** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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We, the undersigned, members of the Advisory Committee of **Dr. Preethy John**, a candidate for the degree of **Master of Veterinary Science in Pharmacology and Toxicology,** agree that the thesis entitled **"THE HYPOGLYCAEMIC EFFECT OF *Brassica juncea* (MUSTARD) AND *Trigonella foenum-graecum* (FENUGREEK) IN ALBINO RATS"** may be submitted by **Dr. Preethy John** in partial fulfilment of the requirement for the degree.

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EXTERNAL EXAMINER

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1. INTRODUCTION

Diabetes mellitus is a major health problem and one of the leading causes of death in humans. It is projected that the incidence of diabetes is on rise. Present number of diabetics world wide is 150 million and this is likely to increase to 300 million or more by the year 2025. Major contribution will be from developing countries, particularly India. It is estimated that there are approximately 19.4 million diabetic individuals in our country and this is expected to touch 57.2 million in the year 2025 (King *et al.*, 1998). Reasons for this rise include modern life style, consumption of energy rich diet, obesity, higher life span etc. (Yajnik, 2001).

Diabetes mellitus has been recognized for centuries as a debilitating disease characterized by excretion of "sweet urine" (mellituria), polydypsia, wasting of tissue and development of ketoacidosis. It was known to ancient Indian physicians as 'Madumeha'. It is a disorder in which there is an imbalance between nutritional source and energy expenditure. It is caused either by an absence/deficiency of insulin produced by β cells of pancreatic islets or by insensitivity of target cells to insulin. The basic defect in diabetes mellitus is a deficiency of insulin leading to a decreased ability of the body to utilize glucose. This defect will produce the characteristic signs of diabetes (Adams, 2001).

Diabetes is clearly influenced by multiple and complex environmental and genetic factors and also their interaction. It may also be induced by a number of toxic substances and stress, which act either by interference with cellular utilization of glucose or by eliciting sympathetic discharges from the central nervous system (Ponnachan *et al.*, 1993). In short, this disease can be brought about by one or more predisposing factors viz, hereditary tendency, pancreatitis, neurogenic factors, stress, overfeeding and obesity, hyperfunction of the anterior pituitary gland and/or any factor causing degeneration of the islets of Langerhans.

In animals, diabetes mellitus occurs most frequently in dogs and cats, with an incidence of approximately 0.2-0.5 percent. Incidence rises with age and is most frequent in dogs of 8 years old and above. Some breeds have a genetic predisposition towards diabetes, like Miniature poodle, Scottish terriers, Rotweiler and Dachshund. Isolated cases have been reported in mules, ferrets, pigs, buffalos and monkeys. It is less frequently reported in ruminants and is often mild. Diabetes has also been reported in several breeds of birds (Stogdale, 1986). There are two major types of diabetes mellitus which are of interest in both human and veterinary medicine. They are type1 (Insulin Dependent Diabetes Mellitus/ IDDM) and type 2 diabetes (Non Insulin Dependent Diabetes Mellitus/ NIDDM). IDDM is an autoimmune disease of the pancreatic β-cell. This type is less common (only 5 percent of the total diabetes). NIDDM is a major health care problem. Abnormal insulin responses to glucose and insulin resistance of target peripheral tissues are the major features observed in type 2 diabetes (Adams, 2001).

Treatment of diabetes mellitus consists fundamentally of managing the diet and /or pharmacological therapies, which attempt to normalize metabolic activities, namely blood glucose levels. Pharmacological treatments are based on two types of drugs viz, insulin and oral hypoglycaemic agents such as biguanides and sulfonyl ureas (Trejo-Gonzalez *et al.*, 1996). Though biguanides and sulfonyl ureas are valuable in treatment of diabetes mellitus, their use is restricted due to their limited action, pharmacokinetic properties and accompanying side effects (Bailey *et al.*, 1989). More over these therapies only partially compensate for metabolic derangements seen in diabetes and do not necessarily correct the fundamental biochemical lesion (Taylor and Agius, 1988). These treatments do not effectively prevent the complications of diabetes like cardiopathy, nephropathy, neuropathy, cataract, hypertension etc. Insulin injection decreases glycaemia, but does not maintain physiologically normal blood glucose levels. Another drawback of insulin is that it cannot be used orally and continuous insulin injection have many side

effects and complications. So there is an increasing demand for natural products with antidiabetic property.

Now it has become necessary to look for an economical as well as therapeutically effective treatment, especially for usage in the developing and underdeveloped countries. Nature has been a source of medicine for thousands of years and plant-based system continue to play an essential role in the primary health care of 80 percent of the world's underdeveloped and developing countries (King *et al.*, 1998).

Research on medicinal plants is an important facet of biomedical research in India, because the country has an estimated number of 20,000 plant species of which 2,500 have medicinal value (Vohora, 1989). Plants have been the major source of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world. World ethnobotanic information about medicinal plants reports almost 800 plants used in the control of diabetes mellitus (Alarcon-Aguilara *et al*., 1998).

Compared to the synthetic drugs, herbal drugs are less expensive, easily available and free from side effects. In the case of synthetic drugs, their adverse side effects overshadow their potency and thus limit their usage. Hence it is appropriate at the present moment to turn the attention on remedies that may be found among indigenous herbs of this country. Even World Health Organization (WHO) suggested the need for investigating traditional methods of treatment of diabetes mellitus (WHO Expert Committee, 1980).

A large number of plant materials such as roots, barks, leaves, fruits and seeds of various plants are being used for years as indigenous remedy in diabetes mellitus in Ayurveda and folk lore medicine. Experimental studies as well as clinical trials have been conducted in few of the indigenous drugs. Some of them have been found to exert hypoglycaemic effect in normal as well as diabetes animals. Even though promising results have been obtained with some of these

indigenous drugs administered either alone or in combination, further experiments and trials are required to fully establish their utility.

Present study is directed towards investigation on the effectiveness of two medicinal plants namely *Trigonella foenum-graecum* (fenugreek) and *Brassica juncea* (mustard) commonly used in the Ayurvedic and folk lore medicine for the treatment of diabetes mellitus. In the present study, the effect of each of these drugs on blood sugar level of alloxan diabetic rats were investigated separately and compared with a standard oral hypoglycaemic drug, glibenclamide. The study is also aimed to compare the efficacy of these drugs at different doses.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ALLOXAN INDUCED DIABETES

Szkudelski (2001) reported that alloxan and its reduced form, dialuric acid established a redox cycle with the formation of superoxide radicals which dismutate to hydrogen peroxide and by Fenton reaction, hydroxyl radicals are formed. He also reported that the action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells.

Aleeva *et al.* (2002) estimated the count of pancreatic alpha and beta cells at various stages of alloxan–induced diabetic rats and found that alloxan decreased the count of insulin producing beta cells, but increased the number of glucagon secreting alpha cells in the pancreas by first week of diabetes.

Experiment conducted by Konrad *et al.* (2002) revealed that alloxan, a uracil analogue, almost completely blocked both glucosamine–induced and streptozotocin induced protein O-Glc N Acylation , suggesting that alloxan is an inhibitor of O-linked-N-acetyl glucosamine transferase (OGT).

Liu *et al.* (2002) suggested that administration of alloxan-streptozotocin combination reduced the dosage of each drug and decreased the toxic and side effects of each drug. This method has a high rate of success to induce type 1 diabetes.

The name 'alloxan' given by Wohler and Liebeg , is recorded as being derived from a combination of allantoin (a product of uric acid among others excreted by foetus into the allantois) and oxalsure (Oxaluric acid derived from oxalic acid and urea , found in urine).The remarkable discovery that a single

injection of alloxan can produce diabetes mellitus in laboratory animals was first reported by Dunn, J.S. and Mc Letchie, N. in 1942 (Mc Letchie, 2002).

Walde *et al.* (2002) stated that the glucose transporter-2 (GLUT-2) and glucokinase (GK) are target molecules for alloxan. The mRNA expression of beta-actin was also slightly affected with time after alloxan exposure.

2.2 INDIGENOUS DRUGS WITH HYPOGLYCAEMIC EFFECT

Ponnachan *et al.* (1992) reported that oral administration of fenugreek seed powder to alloxan injected rats maintained the glucose level similar to those treated with insulin. A significant decrease in liver glycogen was observed in diabetic rats which were reversed by fenugreek seed powder treatment. In the seed powder treated rats, the serum cholesterol levels were similar to those treated with insulin.

S-allyl cysteine sulphoxide (SACS), a sulphur containing amino acid of garlic which is the precursor of allicin and garlic oil was found to show significant antidiabetic effects in alloxan diabetic rats . Administration of SACS at a dose of 200mg/kg body weight significantly decreased the blood glucose and serum lipids (Sheela and Augusti, 1992).

Chattopadhyay (1993) observed that oral administration of alcoholic extract of *Ocimum sanctum* led to marked lowering of blood sugar level in normal, glucose fed hyperglycaemic and streptozotocin induced diabetic rats. The activity of extract was 91.55 percent and 70.43 percent of that of tolbutamide in normal and diabetic rats respectively.

Cherian and Augusti (1993) conducted a study on glycoside of leucopelargonidin isolated from the bark of Indian banyan tree (*Ficus bengalensis* Linn.) and demonstrated the hypoglycaemic and serum insulin raising effects in moderately diabetic rats, similar to the effects of a minimal dose of glibenclamide.

The antidiabetic and secretagogue effects of a leucocyanide derivative , namely dimethyl ether of leucocyanidin -3-O-β-O-galactosyl cellobioside isolated from the bark of Banyan tree (*Ficus bengalensis* Linn.) were reported by Kumar and Augusti (1993). This flavonoid compound demonstrated 76 percent activity of tolbutamide at a dosage of 250 mg/kg body weight, in alloxan induced moderately diabetic rats.

Cakici *et al.* (1994) examined the hypoglycaemic effect of orally administered extracts of *Momordica charantia* fruits in normoglycaemic or cyproheptidine–induced hyperglycaemic mice. They reported that the aqueous extract reduced the fasting glucose levels of both hyperglycaemic or normoglycaemic mice.

Kato and Miura (1994) were of the opinion that the methanolic extract of rhizomes of *Polygonatum officinale* reduced the blood glucose of normal mice and streptozoticin induced diabetic mice in 4 hours after intraperitoneal administration.

Khan *et al.*(1995) studied the effect of *Murraya koenigii* and *Brassica juncea* on carbohydrate metabolism using rats and found that both showed significant hypoglycaemic action. They also found that both the plants exerted their hypoglycaemic effect by enhanced glycolysis, glycogenesis and decreased glycogenolysis.

Medina *et al.* (1994) opined that the administration of the decoction of Juniper berries (120 mg total berries/kg) to streptozotocin induced diabetic rats for 24 days resulted in a significant reduction both in blood glucose levels and in the mortality index, as well as the prevention of loss in body weight

Kumari and Augusti (1995) reported that S-methyl cysteine sulphoxide (SMCS) isolated from onion (*Allium cepa*) had a stimulatory effect on the β cells of the pancreas in moderately diabetic rats. They also found that the antioxidant

properties of sulphoxide group and a possible lipotropic effect of a liable methyl group present in SMCS having some role to enhance the antidiabetic property.

Treatment of normoglycaemic rats with the aqueous extract of *Orthosiphon staminus* at a dose of one g/kg showed a significant decrease in blood glucose concentration. The effect of the extract in streptozotocin induced diabetic rats was comparable to that of glibenclamide (Mariam *et al.*, 1996).

Experiments conducted by Sharma *et al.* (1996) revealed that oral administration of 50 percent ethanolic extract of *Cinnamomum tamala* leaves significantly lowered the plasma glucose levels in normoglycaemic and streptozotocin hyperglycaemic rats. The extract also exhibited antihypercholesterolemic and antihypertriglyceridemic effects in streptozotocin-hyperglycaemic rats.

The hypoglycaemic activity of a purified extract from prickly pear cactus (*Opuntia fuliginosa* Griffiths) was evaluated on streptozotocin-induced diabetic rats by Trejo- Gonzalez *et al.*(1996). They concluded that the control of diabetes by this plant can be achieved with oral daily doses in the range of 1mg/kg body weight.

Abdel-Barry *et al.* (1997) tested the hypoglycaemic activity of the aqueous and alcoholic extracts of fenugreek leaf on normal and alloxan diabetic rats. Graded amounts (0.06, 0.2, 0.5, 1g/kg i/p and 1, 2, 8 g/kg p.o) of the aqueous extract produced a significant reduction of blood glucose concentration. But the ethanolic extract produced no reduction in blood glucose level in normal rats. Intraperitoneal administration of 0.8g/kg of ethanolic extract to diabetic rats produced a significant reduction of blood glucose concentration.

Administration of Neem seed kernel powder (NP) alone (500mg/kg) as well as the combination of NP (250mg/kg) with glibenclamide (0.25mg/kg) ~~significantly~~

significantly decreased the concentration of blood glucose in alloxan diabetic rabbits (Bopanna *et al.*, 1997)

Farias *et al.* (1997) observed that trans- Dehydrocortinin (t-DCTN) isolated from the bark of *Croton cajacara* had a significant hypoglycaemic activity in alloxan-induced diabetic rats but not in normal rats.

Hsu *et al.* (1997) isolated Paeoniflorin, 8-debenzoyl paeoniflorin from the dried roots of *Paeonia lactiflora* which produced a significant blood sugar lowering effect in streptozotocin treated rats.

Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of the aqueous and alcoholic extracts of *Caesalpinia bonducella* seed in normal and diabetic rats were studied by Sharma *et al.* (1997). They found that both the extracts exhibited hypoglycaemic activity at a lower dose of 100mg/kg in normal rats and also produced an antihyperglycaemic effect in diabetic rats. The hypoglycaemia produced by the aqueous extract was of prolonged duration as compared to the ethanolic extract.

Teotia and Singh (1997) concluded that administration of 2.5g almond (*Prunus amygdalus*) seed and its proportionate fractions, namely 1.22g defatted seed and 1.28g oil to 3 groups of albino rats, have a definite hypoglycaemic action during a 2 month period. The active factor seemed to be a non oil fraction, which is partly soluble in ethyl ether only.

Single doses of unroasted seeds (60 percent and 80 percent) of *Cajanus cajan* on administration to normal as well as alloxanised mice showed significant reduction in serum glucose levels. In case of roasted seed, there was a significant increase in serum glucose levels. Amalraj and Ignacimuthu (1998) stated that roasting of seeds at high temperature for half an hour resulted in total loss of hypoglycaemic principle present in the seeds.

Noor and Ashcroft (1998) suggested that *Tinospora crispa* was able to cause a reduction in blood glucose level in moderately diabetic rats and the hypoglycaemic effect was probable due to its insulinotropic activity.

Prince and Menon (1998) observed that oral administration of 2.5g and 5.0g/kg body weight of the aqueous extract of the seeds of *Syzigium cumini* (Jamun) for six weeks resulted in a significant reduction in blood glucose in alloxan diabetic rats.It also prevented decrease in body weight. They found that Jamun seed extract was more effective than glibenclamide.

Ahmad *et al.* (2000) reported that oral administration of the flavonoid content of *Cuminum nigrum* seeds caused a hypoglycaemic effect at a dose range of 0.5-1.5g/kg in both normal and alloxan diabetic rats.

Leaf extract (LE) and seed oil (SO) of *Azadirachta indica* at a dose rate of 500mg/kg and 5ml/kg respectively produced significant hypoglycaemic effect in normal as well as diabetic rats (Khosla *et al.*, 2000).

Experiments conducted by Raphael *et al.* (2000) on antidiabetic avtivity of *Phyllanthus niruri* revealed that alcoholic extract of the plant significantly reduced the blood sugar in normal rats and in alloxan diabetic rats.

Srinivas *et al.* (2000) reported that an aqueous extract of the leaves of *Raphanus sativus* (Radish) have shown marked/significant antidiabetic effect on streptozotocin induced models of both insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM).

According to Dash *et al.* (2001) aqueous extract of the leaves of *Lantana camara* produced significant reduction of blood glucose concentration between two to four hours of administration in alloxan induced hyperglycaemic rats and normoglycaemic rats.

Administration of aqueous extract of *Catharanthus roseus* (*Vinca rosea*) flower and leaf was found to regulate the blood sugar level in alloxan diabetic rats. Its hypoglycaemic activity was through β- cell rejuvenation, regeneration and stimulation (Ghosh, 2001).

Kelkar *et al.* (2001) tested the seedling parts and callus cultures of onion for their antidiabetic activity by feeding tissue extracts to diabetic rats. The results indicated much higher antidiabetic activity in callus cultures when compared to natural bulbs of onion.

Aloe vera leaf pulp extracts showed hypoglycaemic activity on IDDM and NIDDM rats whereas *Aloe vera* leaf gel extract showed hyperglycaemic activity on NIDDM rats. So Okyar *et al.* (2001) suggested that pulps of *Aloe vera* leaves devoid of the gel are useful in the treatment of non-insulin dependent diabetes mellitus.

Rao and Rao (2001) were of opinion that the aqueous extract of *Syzigium alternifolium* seeds have maximum blood glucose lowering effect in both normal and alloxan diabetic rats. The hypoglycaemic effect of both ethanol and hexane fractions were significantly less than that of aqueous extract of the seed.

Investigations were carried out by Sachdewa *et al.* (2001) to evaluate the effect of aqueous extract of *Hibiscus rosa sinensis* leaves on blood glucose level and glucose tolerance. Repeated administration of the extract at an oral dose of 250mg/kg significantly improved glucose tolerance using rats. The peak glucose level was obtained at 30 minutes of glucose load, thereafter a decreasing trend noted upto 120 minutes. The hypoglycaemic activity of the leaf extract was comparable to tolbutamide.

In alloxan diabetic rabbits, *Alpinia galanga* did not produce significant reduction in blood glucose, but produced fall in blood glucose level in normal rabbits (Akthar *et al.*, 2002).

Andallu and Varadacharyulu (2002) studied the antihyperglycaemic and antioxidant role of mulberry leaves in streptozotocin induced diabetic rats and found that mulberry leaves possessed antihyperglycaemic effect and that was comparable with glibenclamide.

Arun and Nalini (2002) reported that administration of turmeric or curcumin to diabetic rats reduced the blood sugar level significantly.

The study conducted by Babu *et al.* (2002) revealed that the alcohol extract of *Cassia kleinii* leaf exhibited concentration dependent antihyperglycaemic effect in both glucose fed hyperglycaemic and alloxan induced diabetic rats.

Bilbis *et al.* (2002) opined that the aqueous extract of *Arachis hypogea* caused a significant decrease in fasting blood glucose in normal and alloxan induced diabetic rats. It also caused a reduction in serum triglyceride, total cholesterol, High Density Lipoprotein(HDL) and Low Density Lipoprotein(LDL).

Grover *et al.* (2002) reported that *Brassica juncea* in diet at a strength of 10 and 15 percent showed significant antihyperglycaemic effect in alloxan rats, but not in streptozotocin rats.

Korkmaz and Gurdal (2002) investigated hypoglycaemic effects of panicles of *Artemisia santonium* in normal and alloxan–induced diabetic rabbits and found that 2ml/kg aqueous extract significantly reduced blood glucose level.

Pandey and Khan (2002) reported that feeding of alloxan diabetic rats with diets containing 15 percent powdered unextracted seeds containing water soluble

gummy fibre, 15 percent powdered defatted seeds from which lipid and saponins were removed and 6 percent water soluble gummy fibre isolated from *Syzigium cumini* seeds significantly lowered blood glucose levels and improved oral glucose tolerance.

The methanol extract of stem bark of *Ficus racemosa* at doses of 200 and 400mg/kg p.o exhibited significant hypoglycaemia in normal and alloxan–induced diabetic rats. The activity was comparable to that of glibenclamide (Rao *et al.*, 2002).

The results of the experiment conducted by Revilla *et al.* (2002) demonstrated that the water extract of the ariel parts of *Equisettum myriochaetum* showed a hypoglycaemic effect in type 2 diabetic patients, 90 minutes after its administration.

Sabu and Subburaju (2002) reported that an aqueous leaf extract of *Cassia auriculata* was found to lower the serum sugar level in normal rats. The extract was also found to inhibit the body weight reduction induced by alloxan administration.

Vetrichelvan *et al.* (2002) suggested that alcohol extract of *Celosia argentea* seeds reduced the increase of blood glucose in alloxan induced diabetic rats and also the extract prevented a decrease in body weight in alloxan induced diabetic rats.

Sharma *et al.* (2003) found that ethanolic extract of *Eugenia jumbolana* seeds when given orally to sub diabetic for one day, mild diabetic for seven days and severe diabetic for 15 days significantly lowered the fall in fasting blood glucose and also produced fall in peak blood glucose.

2.3 *Brassica juncea* (Mustard) - Other effects

Topical administration of mustard oil increased the temperature of the paw skin by 2-3°C and developed a slight oedema. The findings of Lippe *et al.* (1993) indicated that endothelium derived nitric oxide (NO) plays a mediator role in the vasodilator component of mustard oil induced neurogenic inflammation on the rat paw skin where an increase in vascular permeability does not seem to involve NO directly.

The influence of *Murraya koenigii* (Curry leaf) leaf and *Brassica juncea* (Mustard) seeds on the levels of lipids, faecal bile acids and natural sterols in rats administered 1,2-dimethyl hydrazine (1,2-DMH) were studied by Khan *et al.* (1996). The levels of cholesterol and phospholipids decreased. Bile acids and neutral sterols showed a sharp increase in liver and faeces. Morphological and histological studies revealed that incidence of neoplasms in the colon and intestine were significantly low in spices fed groups.

Tripathi *et al.* (2001) concluded that feeding mustard meal as protein supplement reduced growth rate and induced iodine deficiency. Carcasses of lambs fed with mustard meal have more fat and less protein.

Fung *et al.* (2002) found that both *Brassica juncea* chitinase with two chitin binding domains (BjCHI1) and deletion derivative of BJCHI 1 lacking one chitin binding domain (BjCHI 2) showed invitro antifungal activity towards *Trichoderma viridae*, causing reductions in hyphal diameter, hyphal branching and conidia size.

Mandal *et al.* (2002) reported a novel trypsin inhibitor from Indian mustard *Brassica juncea* (BjTI) that is unique in being the precursor of a 2S seed storage protein.

The combined effects of acetic acid and mustard flour were investigated by Rhee *et al.* (2002) to ascertain their impact on *Eschericia coli* O157:H_7 (*E. coli* O157:H_7) stored at 5 and 22°C. They found that the numbers of *E. coli* O157:H_7 were reduced much more rapidly at 22°C. Significant reduction in population of *E.coli* O157:H_7 was enhanced with the addition of one percent acetic acid.

According to Yokozawa *et al.* (2002), oral administration of isorhamnetin diglucoside, a major flavonoid compound from mustard leaf (10 or 20mg/kg body weight/day for 10 days) reduced serum levels of 5-(hydroxyl methyl) furfural (5-HMF) which is an indicator of oxidative stress in diabetic rats. In addition, after oral administration the thiobarbituric acid reactive substance levels of serum liver and kidney mitochondria declined significantly.

The ability of sinapic acid isolated from *Brassica juncea* to scavenge peroxynitrite (ONOO (-)) which is a potent cytotoxic species was studied by Zou *et al.* (2002). Spectrophotometric analysis revealed that sinapic acid suppressed the formation of ONOO (-) mediated tyrosine nitration through an electron donation mechanism.

2.4 *Trigonella foenum-graecum* (FENUGREEK) – OTHER EFFECTS

Petit *et al.* (1993) reported that chronic administration of fenugreek seed extract enhanced food consumption and motivation to eat in rats and also induced hyperinsulinaemia and hypocholesterolaemia.

Stark and Madar (1993) suggested that the ethanol extract derived from fenugreek had the ability to inhibit taurocholate and deoxy cholate absorption in a dose dependent manner in rats. Reduction in plasma cholesterol levels ranged from 18-26 percent and tendency for lower concentration in liver was also noticed.

According to Panda *et al.* (1999), the administration of fenugreek seed extract (0.11g/kg body weight for 15 days) to both mice and rats significantly decreased serum triiodothyronine (T_3) concentration and T_3/T_4 ratio, but increased T_4 levels and body weight. A significant reduction in superoxide dismutase (SOD) activity was also observed.

Khosla *et al.* (1995) are of the opinion that when fenugreek was administered in the form of unroasted and roasted seeds in low (2g/kg) and high (8g/kg) to normal and alloxan-diabetic rats, there was a significant fall in various serum lipids like total cholesterol, triglycerides, LDL and Very Low Density Lipoprteins (VLDL) cholesterol in normal rats and decreased their raised levels and increased HDL cholesterol in diabetic rats.

Platel and Srinivasan (2000) noticed that fenugreek enhanced pancreatic lipase activity and stimulated chymotrypsin in albino rats.

Anuradha and Ravikumar (2001) studied the influence of fenugreek seed powder supplementation in the diet on lipid peroxidation and antioxidant status in normal and alloxan diabetic rats. It was found that the seed powder normalised the alterations exhibited by diabetic rats like the enhanced lipid peroxidation and increased susceptibility to oxidative stress associated with the depletion of antioxidants in liver, kidney and pancreas. In normal rats, supplementation resulted in increased antioxidant status with reduction in peroxidation. They suggested that the soluble portion of the seeds could be responsible for the antioxidant property.

Studies conducted by Sur *et al.* (2001) revealed that the intraperitoneal administration of the alcoholic extract of the fenugreek seed both before and after the inoculation of Ehrlich ascites carcinoma (EAC) cell in mice produced more than 70 percent inhibition of tumor cell growth. Treatment with the extract was

found to enhance both the peritoneal exudate cells and macrophage cell counts. The extract also produced a significant anti-inflammatory effect.

Zia *et al.* (2001) opined that the aqueous, methanol and chloroform extracts of *Trigonella foenum-graecum* caused significant mortality of *Meloidogyne javanica* larvae. The methanol soluble fraction eluted from pure distilled water showed the highest (>92 percent) nematicidal activity compared with the fractions eluted from pure methanol and different ratios of chloroform.

A survey on head lice, pediculosis and treatment of lice with natural plant extract was conducted by El-Basheir and Fouad (2002). One hundred infested patients (90 females and 10 males) with different age and hair length were treated with mixed cream from four plants-*Lawsonia alba* L., *Trigonella foenum-graecum*, *Hibiscus cannabis* and *Artemisia cina*. The head lice completely disappeared with in a week among these patients.

Devasena and Menon (2002) investigated the modulatory effect of fenugreek seeds on circulatory lipid peroxidation (LPO) and antioxidant status during Dimethyl hydrazine(DMH) induced colon carcinogenesis in male Wistar rats. They reported that fenugreek exerts chemopreventive effects by decreasing circulatory LPO and enhancing antioxidant levels.

The effect of fenugreek seeds compared to Omeprazole was studied by Pandian *et al.* (2002) on ethanol-induced gastric ulcer in rats. The aqueous extract and gel fraction isolated from the seeds showed significant ulcer protective effects. The cytoprotective effect of the seeds seemed to be due to the antisecretory action and the effects on mucosal glycoprotein. The fenugreek seeds also prevented the rise in lipid peroxidation induced by ethanol by enhancing antioxidant potential of gastric mucosa thereby lowering mucosal injury. Histological studies revealed that

the soluble gel fraction derived form the seeds was more effective than Omeprazole in preventing lesion formation.

Bin-Hafeez *et al.* (2003) evaluated the immunomodulatory activity of aqueous extract of *Trigonella foenum-graecum* in mice. At doses of 50 and 100mg/kg, a significant increase in organ weight of thymus was observed. They recorded significant increase in lymphoid organ cellularity. The extract elicited a significant increase in delayed type of hypersensitivity. Humoral immunity as measured by plaque forming cell showed an elevated response at a dose of 100mg/kg. In the hemagglutination titre test, plant extract showed modulatory effect on all doses. It elicited a significant increase in phagocytic index and phagocytic capacity of macrophages.

Hibasami *et al.* (2003) reported that protodioscin (PD) isolated from fenugreek displayed strong inhibitory effect on human leukaemia (HL-60) cells, but weak inhibitory effect on human stomach cancer (KATO III) cells.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The experiment was conducted in fifty six male albino rats weighing 150-200g. The rats were procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. They were maintained on identical feeding and managemental practice in the laboratory for one week before the commencement of study. The experiment was carried out during a period of 45 days.

3.1.1 Experimental Group Design

Fifty six rats were randomly divided into seven groups, each group comprising of eight animals.

Group I (T_0)	Normal control, no treatment was given.
Group II (T_1)	Alloxan (Diabetic) control, no treatment was given.
Group III (T_2)	Diabetic, feed incorporated with *Brassica juncea* seed powder at a dose of 2g/kg body weight from day 16 to day 45 (30 days).
Group IV (T_3)	Diabetic, feed incorporated with *Brassica juncea* seed powder at a dose of 8g/kg body weight from day 16 to day 45 (30 days).
Group V (T_4)	Diabetic, feed incorporated with *Trigonella foenum-graecum* seed powder at a dose of 2g/kg body weight from day 16 to day 45 (30 days).
Group VI (T_5)	Diabetic, feed incorporated with *Trigonella foenum-graecum* seed powder at a dose of 8g/kg body weight from day 16 to day 45 (30 days).
Group VII (T_6)	Diabetic, feed with 0.5 mg glibenclamide / day/ animal from day 16 to day 45 (30 days).

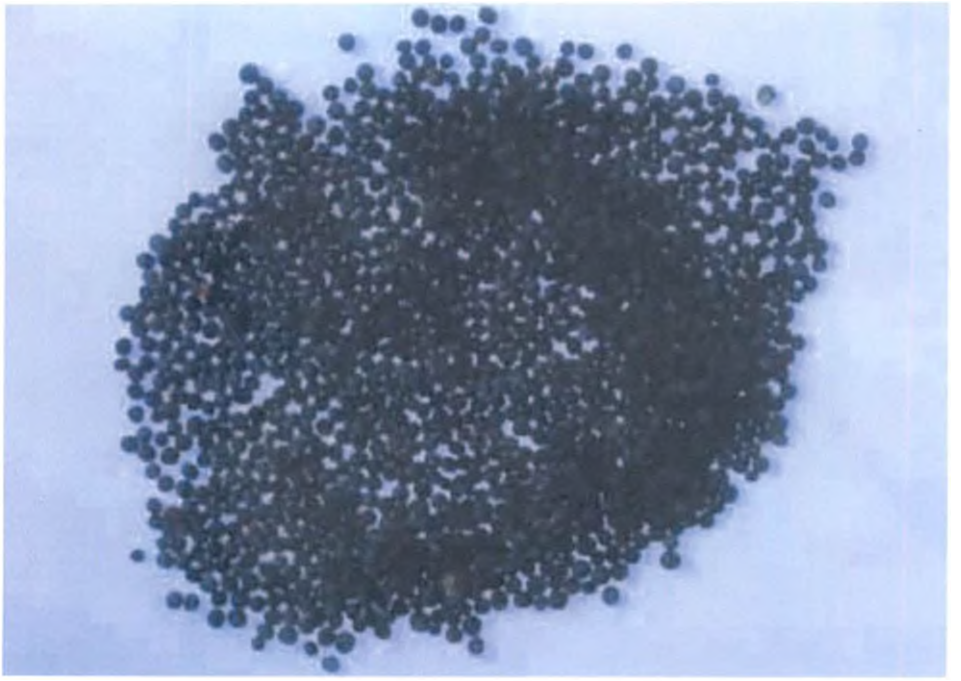

Fig 1. ***Brassica juncea*** **(Mustard) Seed**

Fig 2. ***Trigonella foenum-graecum*** **(Fenugreek) seed**

3.2 PROCEDURE FOR INDUCTION OF DIABETES

All the animals were fasted overnight and their body weights and blood glucose were estimated on the next day (zero day) morning. Rats were given alloxan* at 100mg, 120mg, 150mg, 180mg/kg body weight subcutaneously so as to fix up a convenient dose for the experiments. At 100mg dose hyperglycaemia was not produced. At 120mg /kg dose there was remarkable increase in blood glucose without much toxic reactions. Doses at 150mg and above were found to be toxic and some of the animals died within 2-4 days after the injection. The toxic symptoms noticed were off feed, diarrhoea, convulsions, paralysis and abdominal distension in addition to normal symptoms of diabetes.

After fixing up the effective dose at 120mg/kg body weight, alloxan was accurately weighed and 10% w/v solution in distilled water was prepared (so that 1ml of solution contained 100mg of alloxan). All the treatment groups except the normal control (T_0) were made diabetic by subcutaneous injection of alloxan monohydrate at a rate of 120 mg/kg body weight. After 16 days, blood glucose was estimated using O-toluidine method. Only treatment rats showing moderate hyperglycemia (200-250 mg/100ml) were selected for specific drug treatment.

3.3 PREPARATION AND ADMINISTRATION OF DRUGS

3.3.1 *Brassica juncea* (Mustard)

The dried seeds (Fig.1) were taken and powdered well in a blender. T_2 and T_3 were administered the fine seed powder with feed daily at a dose of 2g/kg and 8g/kg body weights respectively for 30 days.

3.3.2 *Trigonella foenum –graecum* (Fenugreek)

Well dried seeds (Fig.2) were pulverized in a blender to get fine powder. Treatment groups T_4 and T_5 were administered fenugreek seed powder well mixed with the diet daily at a dose of 2g/kg and 8g/kg body weights respectively for 30 days.

* sd fine –CHEM Ltd. Boisar.

3.3.2 Glibenclamide

Tab Daonil* (5mg) was powdered and administered with feed to treatment group T_6 at a rate of 0.5 mg /animal /day for 30 days.

3.4 COLLECTION OF BIOLOGICAL SAMPLES

3.4.2 Blood

Blood was collected retro orbitally from the inner canthus of the eye under light ether anaesthesia using sodium heparinised capillary tubes (Micro Hematocrit Capillaries). Blood was collected in fresh vials containing sodium fluoride (10 mg/ml blood) and disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA, 1mg/ml) as anticoagulant/antiglycolytic agents (Glucose disappears rapidly from the whole blood due to its enzymatic break down to lactic acid. This can be prevented by addition of sodium fluoride).

3.4.3 Serum

Blood was collected in fresh vials without any anticoagulant and kept at room temperature for one hour. After that, it was centrifuged at 2000 rpm for 20 minutes and serum was separated and was used for triglyceride and cholesterol estimations.

3.4.4 Liver

Liver samples were collected after sacrificing the animals on the 46th day of the experiment and liver glycogen was estimated by Anthrone's method.

3.5. ESTIMATION OF BIOCHEMICAL PARAMETERS

3.5.1 Estimation of blood sugar

The blood sugar was estimated by O-toluidine method as cited by Hyvarinen and Nikila (1962).

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* Hoechst Marion Roussel

3.5.1.1 *Preparation of protein free blood*

The presence of protein interferes with many chemical determinations, primarily the analyses for compounds containing amino/amido nitrogen and those involving reduction or oxidation of a metal ion. Proteins should be removed before such analysis can be made.

The proteins of whole blood, plasma or serum are precipitated by many metal acids. Tungstic acid is the most common deproteinising agent in use.

Preparation of reagents

Tungstic acid reagent

Dissolved 1g poly vinyl alcohol in 100ml distilled water with gentle warming. Cooled and transferred to a one litre volumetric flask containing 11.1g sodium tungstate, previously dissolved in 100ml distilled water and mixed by swirling. In separate vessels, added 2.1 ml concentrated sulphuric acid to 300ml distilled water. After mixing thoroughly, it was added to the tungstate solution in the volumetric flask. Mixed and diluted to one litre with distilled water.

Procedure

Pipetted out 1.8ml of tungstic acid in a test tube and added 0.2ml of blood to the same and mixed well. Centrifuged at 3000rpm for 10 minutes and the supernatant was collected for further estimation.

3.5.1.2 *Determination of Glucose*

Principle of O-toluidine method

Glucose react with O-toluidine in glacial acetic acid in the presence of heat to yield a blue green N-glycosylamine, the absorbance of which was measured at 625nm.

Preparation of reagents

O-toluidine reagent

O-toluidine was distilled to get a colourless solution. To five grams of thiourea, added 90ml distilled O-toluidine and diluted to one litre with glacial acetic acid and stored in an amber coloured bottle.

Glucose standard (100 mg/ml)

Dissolved one gram pure anhydrous glucose in one litre water containing 1.5g benzoic acid.

Procedure

Pipetted out 2.5ml of O-toluidine reagent into a test tube and transferred 0.5ml of the deproteinised blood to the same. To set blank, 0.5ml of distilled water was added instead of the deproteinised blood to another test tube containing O-toluidine reagent. The standard was set by adding 0.05ml glucose standard and 0.45 ml distilled water. Mixed thoroughly and heated for 10 minutes in a boiling water bath. Then cooled by placing them in a cold water bath. The optical densities (OD) were read at 625nm in a spectrophotometer.

Calculation

$$\text{Glucose(mg/100ml of blood)} = \frac{\text{OD of sample}}{\text{OD of standard}} \times 100$$

3.5.2 Estimation of cholesterol

Cholesterol level in serum was estimated by enzymatic CHOD-PAP method (Allain *et al.*, 1973) using kit from Agappe Diagnostics.

Principle

Two thirds of cholesterol present in the blood is esterified. The esterified cholesterol is oxidized by cholesterol esterase (CHE) to form cholesterol and fatty acid. Cholesterol is again oxidized in the presence of cholesterol oxidase (CHO) to

cholesterol-3-one and hydrogen peroxide. Phenol and 4-amino antipyrene then combines with hydrogen peroxide by oxidative condensation in the presence of peroxidase to produce red coloured quinone. The intensity thus produced is directly proportional to cholesterol concentration.

Procedure

Blank, standard and sample were prepared as follows

	Blank	Standard (200mg/ml)	Sample
Working standard	1ml	1ml	1ml
Standard	10µl		
Serum			10µl

Mixed and read the optical density in spectrophotometer at a wave length of 505nm.

Calculation

$$\text{Cholesterol concentration (mg \%)} = \frac{\text{O.D of sample}}{\text{O.D of standard}} \times 200$$

3.5.3 Estimation of Triglyceride

Triglyceride level in serum was estimated by GPO-PAP method (Nussel and Arav, 1975) using kit from Agappe diagnostics.

Principle

Triglycerides are hydrolysed by lipase and liberated glycerol is phosphorylated with the help of glycerol kinase in presence of ATP to glycerol 3-phosphate. Glycerol 3-phosphate is then oxidized in presence of glycerol phosphate oxidase (GPO) to dihydroxy acetone phosphate and hydrogen peroxide. 4-chlorophenol and 4-amino

antipyrene then combines with hydrogen peroxide by oxidative condensation in presence of peroxidase to produce red coloured quineneimine. The intensity of colour thus produced is directly proportional to triglyceride concentration.

Procedure

Blank, standard and sample were prepared as follows

	Blank	Standard (200mg/ml)	Sample
Reagent	1ml	1ml	1ml
Standard		10µl	
Sample			10µl

Mixed well and read the optical density at 500nm after five minutes incubation at 37°C.

Calculation

$$\text{Triglycerides (mg\%)} = \frac{\text{O.D of sample}}{\text{O.D of standard}} \times 200$$

3.5.4 Estimation of Liver glycogen by Anthrone's method (Narasimhan, 1971)

Principle

The liver tissue is digested with potassium hydroxide (KOH) and the digesta is treated with anthrone reagent*. The sulphuric acid medium of the anthrone reagent causes dehydration of the sugar to a furfural derivative which condenses with anthrone to form a blue coloured compound. The colour produced is compared with a standard in a spectrophotometer at 620 nm.

*Central Drug house (P) Ltd, Bombay, New Delhi.

Preparation of reagents

1. 30%KOH solution: Dissolved 300g of reagent grade potassium hydroxide pellets in distilled water in a beaker. Cooled and transferred to one litre volumetric flask and diluted to the mark
2. 95% sulphuric acid: Mixed one litre of concentrated sulphuric acid with 50ml distilled water and cooled.
3. 0.2% Anthrone reagent: The reagent was prepared by dissolving 0.2 g anthrone in 100ml, 95% sulphuric acid. The reagent was prepared fresh whenever required.
4. Standard glucose solution (20µg/ml): The stock standard was prepared by dissolving one gram highest purity anhydrous glucose in saturated benzoic acid solution and diluting to 100ml with the same. The working standard was prepared by diluting one ml of the stock standard to 500ml with distilled water.

Procedure

Approximately 0.5g of liver was taken in a test tube containing 3ml of 30% KOH solution. The tissue was digested by heating the tube for 20 minutes in boiling water bath. The sample was then cooled and quantitatively transferred to a 50ml volumetric flask and diluted to the mark with distilled water. After thorough mixing, 5ml of the solution was transferred into a second 50ml volumetric flask and diluted to the mark.

Unknown: 5ml of digesta prepared at the end of final dilution of 50 ml.

Standard: 5ml of glucose working standard.

Blank: 5ml of distilled water.

The unknown, standard and blank (5ml each in labeled test tubes) were kept in a cold water bath and added 10 ml of anthrone reagent to each of the three test tubes from a fast flowing burette. Mixed the reagents by swirling the test tubes. After cooling, covered the mouth of test tubes with glass stoppers and heated for 10 minutes

in boiling water bath. Then immediately cooled by placing them in cold water bath. The readings were taken against the blank at 620nm in a spectrophotometer.

Calculation

$$\text{Liver glycogen (g\%)} = \frac{Ax}{As} \times \frac{100}{1.11} \times \frac{500}{5} \times \frac{100}{\text{Wt. of tissue in g}} \times \frac{1}{10^6}$$

Ax : Reading of unknown

As: Reading of standard

Concentration of standard in μg =100.

Correction factor for conversion of glucose to glycogen= 1/1.11

Dilution factor =500/5= 100.

Factor for expressing the value in %= $100/10^6$

3.5 STATISTICAL ANALYSIS OF DATA

The data obtained were statistically analysed by using one way Analysis of Variance for comparison between groups and student *t* test for within groups as described by Snedecor and Cochran (1985). The results are expressed as mean ± standard deviation

Results

4. RESULTS

The results obtained for various parameters viz, blood glucose, serum cholesterol, serum triglyceride and liver glycogen of rats in various groups during the course of experiment are presented in Tables 1 to 9 and are graphically represented in Fig. 3 to 8.

4.1 BLOOD GLUCOSE LEVEL

Blood glucose level was estimated before alloxan administration (zero day), after 16 days of alloxan administration and last day of the experiment (45^{th} day). The individual and mean values are presented in Tables 1 to 5. The values are graphically represented in Fig.3, 4 and 5. Table 6 shows percentage difference in blood sugar level after various treatments.

The levels of blood glucose of the rats in all groups prior to alloxan administration were not significantly different. The values obtained were 93.98±15.04, 83.61±9.49, 86.59±16.82, 75.88±11.00, 77.43±9.25, 81.92±13.77, 83.04±11.23 mg/100ml blood. Diabetic control rats (group II) remained persistently diabetic throughout the course of the study (Table 2). Normal controls (group I) did not show significant variation in the blood glucose through-out the experimental periods whose values are presented in Table1. Group VII (Glibenclamide at the rate of 0.5mg/animal) and group I did not differ significantly and the blood glucose levels obtained were 106.70±15.55 and 94.18±14.49 mg/100ml respectively after 30 days of treatment. Groups IV, V, VI showed significant reduction ($P<0.05$) in blood glucose level and had values of 195.57±20.33, 177.46±12.16, 125.30±14.44 mg/100ml respectively after 30 days of treatment. The blood sugar values obtained for animals fed with mustard at the rate of 2g/kg (group III) after 30 days was 226.21± 18.29 mg/100ml which did not significantly differ from that of group II. At the end of 30 days, blood glucose levels were decreased by 3.3, 3.12, 12.7, 21.18, 39.14, and 46.38 percent respectively for groups II to VII.

Table 1. Blood glucose level of normal control,mg/100ml

Animal No	Blood Glucose Level (mg/100ml)		
	Zero day	16th day	45th day
1	75.52	75	77.04
2	87.04	86.9	87.44
3	88.89	89.06	88.94
4	95.19	94.79	94.97
5	85.19	85.42	85.93
6	98.52	97.92	97.49
7	94.81	95.31	95.48
8	126.67	125.52	126.13
Mean ± SD	93.98± 15.04^{A}	93.74± 14.74^{B}	94.18± 14.49^{E}

Table 2. Blood glucose level of diabetic control, mg/100ml

Animal No.	Blood Glucose Level (mg/100ml)		
	Zero day	16th day	45th day
1	89.45	235.57	226.46
2	73.37	228.87	220.63
3	81.41	214.95	204.76
4	96.48	242.78	232.28
5	92.46	245.88	244.97
6	79.90	238.14	229.63
7	68.84	210.31	201.06
8	86.93	227.32	216.40
Mean ± SD	83.61± 9.49^{A}	230.48±12.73 ^{A}	222.02±14.54^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 3. Effect of Mustard seed powder administered at two different doses on blood glucose level, mg/100ml

Animal No.	Mustard (2g/kg body weight) (Group III)			Mustard (8g/kg body weight) (Group IV)		
	Zero day	16th day	45th day	Zero day	16th day	45th day
1	68.23	217.87	210.20	94.97	248.95	219.90
2	97.40	244.68	237.76	76.38	232.11	198.96
3	69.79	231.91	219.90	87.44	241.05	209.38
4	103.65	238.72	230.10	65.33	204.21	170.83
5	98.44	245.96	235.71	66.83	201.05	166.15
6	67.19	201.70	190.82	78.39	245.79	213.02
7	81.25	243.83	239.80	63.82	214.74	180.73
8	106.77	249.79	245.41	73.87	235.79	205.73
Mean ± SD	86.59 ± 16.82^{A}	234.31 ± 16.62^{A}	226.21 ± 18.29^{A}	75.879 ± 11.00^{A}	227.961 ± 18.72^{A}	195.574 ± 20.33^{B}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 4. Effect of Fenugreek seed powder administered at two different doses on blood glucose level, mg/100ml

Animal No.	Fenugreek (2g/kg body weight) (Group V)			Fenugreek (8g/kg body weight) (Group VI)		
	Zero day	16th day	45th day	Zero day	16th day	45th day
1	64.80	212.23	163.83	62.20	201.55	101.90
2	71.43	246.81	192.55	79.30	231.44	136.19
3	83.16	232.98	178.72	89.84	222.16	130.00
4	90.82	248.40	189.89	73.98	207.22	110.95
5	76.02	228.19	173.40	66.67	214.43	116.67
6	87.76	232.98	179.79	101.22	245.88	144.29
7	77.04	219.15	157.45	86.99	225.77	125.71
8	68.37	242.02	184.04	95.12	237.63	136.67
Mean ± SD	$77.43 \pm 9.25^{\overline{A}}$	$232.85 \pm 12.85^{\overline{A}}$	$177.46 \pm 12.16^{\overline{C}}$	$81.92 \pm 13.77^{\overline{A}}$	$223.26 \pm 15.11^{\overline{A}}$	$125.30 \pm 14.44^{\overline{D}}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 5. Effect of glibenclamide (0.5mg/animal) on blood glucose level, mg/100ml

Animal No.	Blood Glucose Level (mg/100ml)		
	Zero day	16^{th} day	45^{th} day
1	76.19	229.95	108.76
2	79.04	242.17	118.04
3	97.64	220.81	120.62
4	91.90	209.12	89.18
5	61.90	201.02	80.93
6	87.14	235.03	125.26
7	90.50	224.87	110.31
8	80.00	215.23	100.52
Mean $\pm$ SD	83.04 ± 11.23^{A}	222.28 ± 13.63^{A}	106.70 ± 15.55^{E}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 6. Percentage difference in blood glucose level

Normal Control	Diabetic control	Mustard		Fenugreek		Glibenclamide
		2g/kg	8g/kg	2g/kg	8g/kg	0.5mg/animal
0.47%	3.30%	3.12%	12.7%	21.18%	39.14%	46.38%

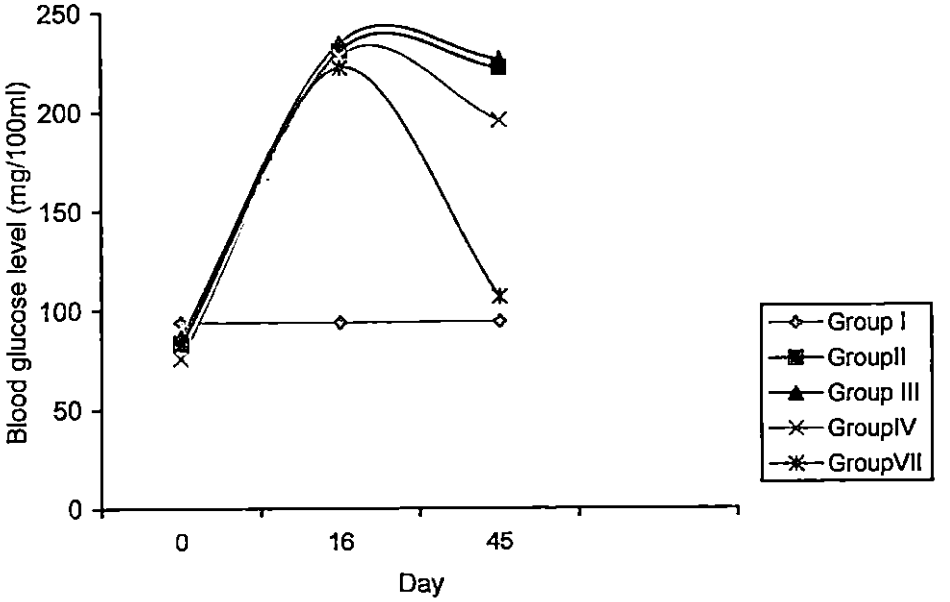

Fig.3. Effect of Mustard seed powder on blood glucose level

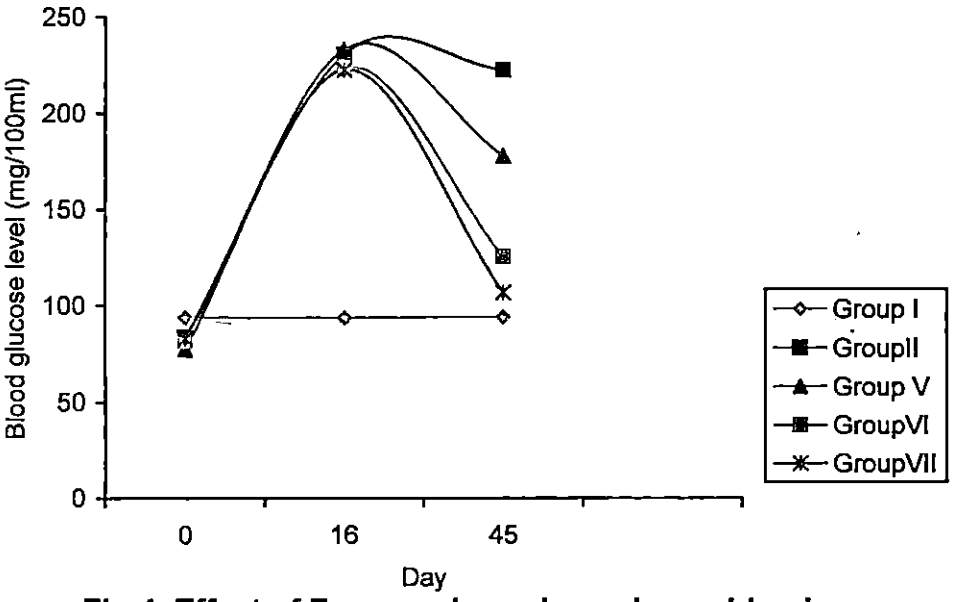

Fig.4. Effect of Fenugreek seed powder on blood glucose level

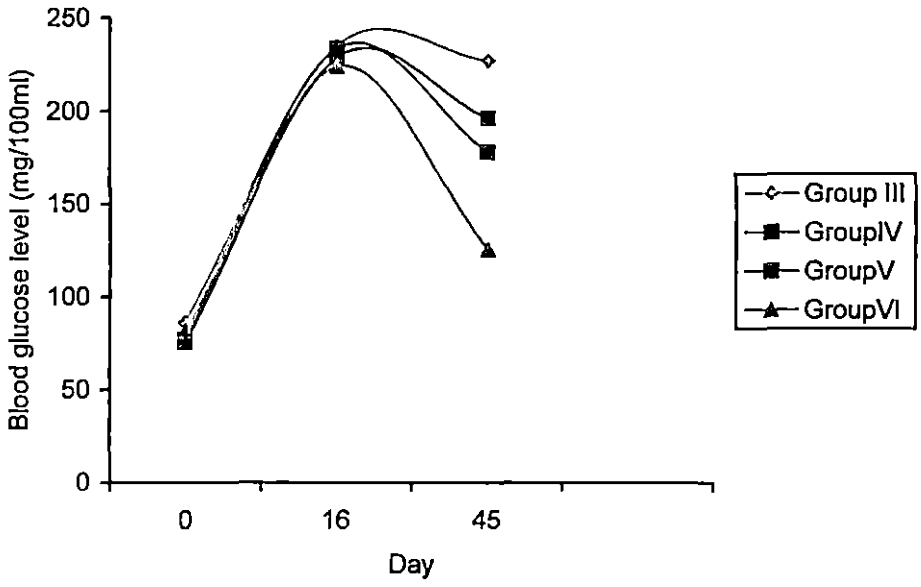

Fig.5. Comparison of the hypoglycaemic effect of Mustard and Fenugreek

4.2 SERUM CHOLESTEROL LEVEL

Serum cholesterol (mg%) estimated on 45th day of the experiment is presented in Table 7. The results are graphically represented in Fig. 6. Animals of group II remained hypercholesterolaemic (166.46±15.72 mg%) through-out the period of study. There was significant ($P<0.05$) decrease in the cholesterol levels of groups IV, V, VI compared to group II. The cholesterol level of group V was intermediate to group IV and VI with values reading 96.53±15.41, 118.01±15.52 and 85.19±14.31 mg% respectively. Group III had the value 130.49±15.33 mg% which was not significantly different from group II. Minimum serum cholesterol level was shown by group I and group VII and the values were 76.64±18.38 and 74.72±19.77mg% respectively.

4.3 SERUM TRIGLYCERIDE LEVEL

The effect of treatments on serum triglyceride estimated on 45th day of experiment is shown in Table 8 and is graphically represented in Fig. 7. All the treatment groups except group III showed significant ($P<0.05$) reduction in serum triglyceride level when compared to diabetic control (group II). Group II and group III exhibited a maximum serum triglyceride level which read as 162.08±25.04 mg% and 133.14±25.65 mg% respectively. There was no significant difference between group IV and group V which had the values 121.14±24.22 and 100.83±24.35 mg% respectively. The serum triglyceride level was lowest in group I and VII ie, 74.68±17.68 mg% and 74.06±17.36 mg% respectively.

4.4. LIVER GLYCOGEN LEVEL

Liver glycogen values obtained after 30 days of experiment (45th day) are presented in Table 9 and Fig. 8. The minimum liver glycogen level found was 1.87± 0.11g/100 g% in group II kept as diabetic control. Normal control (group I) rats differed significantly ($P< 0.05$) from group II and they showed maximum level of liver glycogen which was 2.51±0.13 g%. The other groups (groups III to VII)

Table 7. Effect of treatments on serum cholesterol, mg%

Animal No	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
1	49.23	165.43	118.10	150.39	85.39	70.00	65.15
2	72.31	162.14	134.48	113.39	128.09	90.77	78.79
3	73.85	152.26	121.55	120.47	93.63	83.08	101.52
4	83.89	173.66	126.72	104.45	104.12	70.77	59.09
5	53.08	199.18	130.17	110.79	89.14	78.46	52.27
6	90.77	171.19	111.21	125.98	95.13	114.62	106.82
7	87.69	148.97	141.38	109.45	76.40	82.31	69.70
8	102.31	158.85	160.34	118.90	100.37	91.54	64.39
Mean ± SD	$76.64 \pm 18.38^{\overline{E}}$	$166.46 \pm 15.70^{\overline{A}}$	$130.49 \pm 15.33^{\overline{A}}$	$118.01 \pm 15.52^{\overline{B}}$	$96.53 \pm 15.41^{\overline{C}}$	$85.19 \pm 14.31^{\overline{D}}$	$74.72 \pm 19.77^{\overline{E}}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 8. Effect of treatments on serum triglyceride, mg%

Animal No	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
1	48.73	173.47	106.77	152.12	79.12	49.80	75.96
2	61.39	162.59	146.62	125.10	136.26	102.77	85.02
3	77.22	135.37	121.53	132.82	101.10	10.40	89.90
4	81.65	182.99	130.83	95.75	120.88	57.71	53.66
5	54.43	193.20	145.11	82.63	84.98	75.89	47.39
6	90.51	179.59	90.98	142.86	109.90	117.00	96.29
7	87.34	121.77	157.14	105.02	61.54	90.12	79.44
8	96.20	147.62	166.17	132.82	112.82	104.35	64.81
Mean ± SD	$74.68 \pm 17.68^{\overline{D}}$	$162.08 \pm 25.04^{\overline{A}}$	$133.14 \pm 25.65^{\overline{A}}$	$121.14 \pm 24.22^{\overline{B}}$	$100.83 \pm 24.35^{\overline{B}}$	$87.26 \pm 23.92^{\overline{C}}$	$74.06 \pm 17.36^{\overline{D}}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

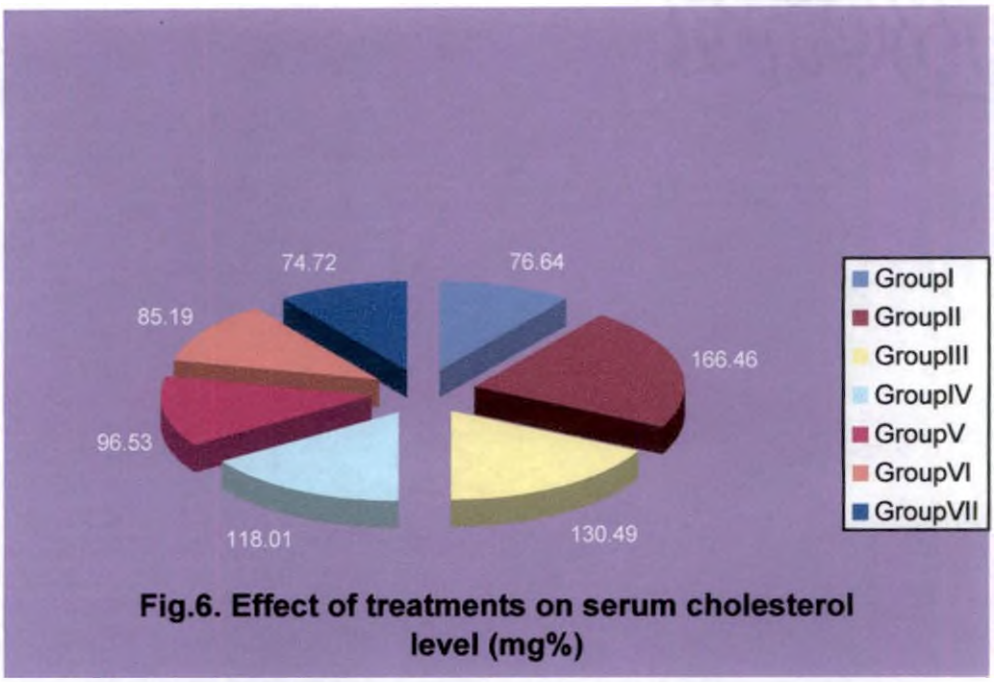

Fig.6. Effect of treatments on serum cholesterol level (mg%)

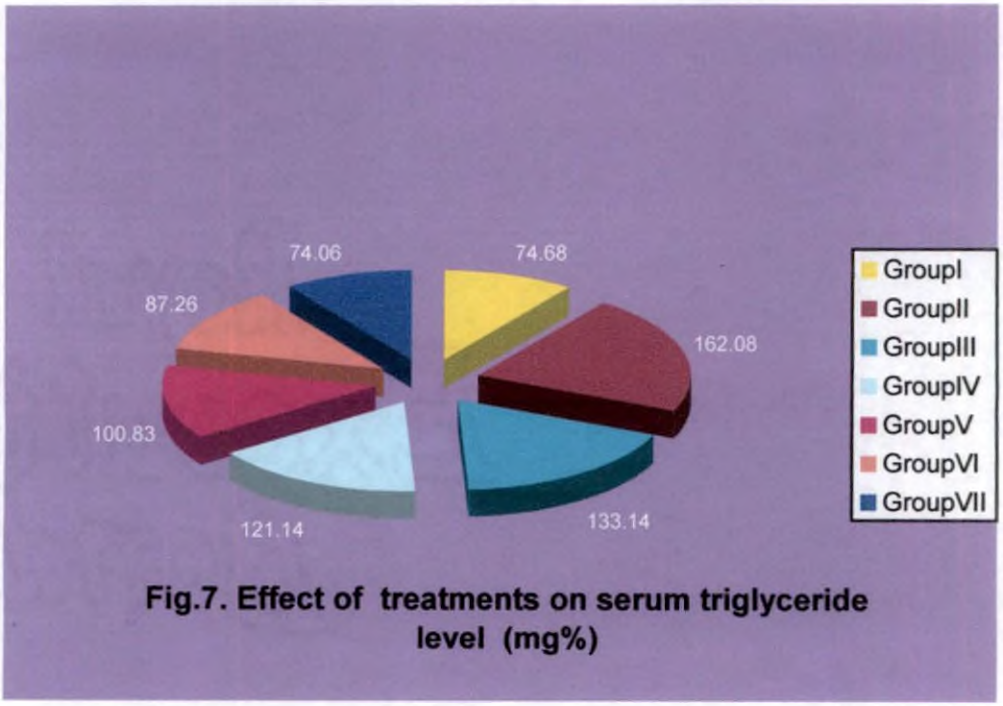

Fig.7. Effect of treatments on serum triglyceride level (mg%)

($P<0.05$) showed significant increase in liver glycogen compared to group II. The liver glycogen levels of group VI and group VII had no significant difference (2.163 ± 0.05 and 2.176 ± 0.05 g% respectively). Animals of group III had the value 1.92 ± 0.06 g%. In group IV and group V, the liver glycogen values were 1.99 ± 0.07 and 2.06 ± 0.04 g% respectively.

Table 9. Effect of treatments on liver glycogen, g%

Animal No	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
1	2.24	1.89	1.96	1.91	2.1	2.19	2.23
2	2.49	1.89	1.87	1.98	2.01	2.16	2.15
3	2.49	1.97	1.95	1.95	2.05	2.23	2.16
4	2.55	1.71	1.94	2.08	2.02	2.12	2.21
5	2.46	1.71	1.92	2.09	2.07	2.23	2.19
6	2.58	1.88	2.01	1.94	2.05	2.09	2.09
7	2.67	2.01	1.85	2.04	2.12	2.18	2.17
8	2.56	1.93	1.84	1.96	2.03	2.21	2.1
Mean ± SD	$2.51 \pm 0.13^{\overline{F}}$	$1.87 \pm 0.11^{\overline{A}}$	$1.92 \pm 0.06^{\overline{B}}$	$1.99 \pm 0.07^{\overline{C}}$	$2.06 \pm 0.04^{\overline{D}}$	$2.16 \pm 0.05^{\overline{E}}$	$2.18 \pm 0.05^{\overline{E}}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

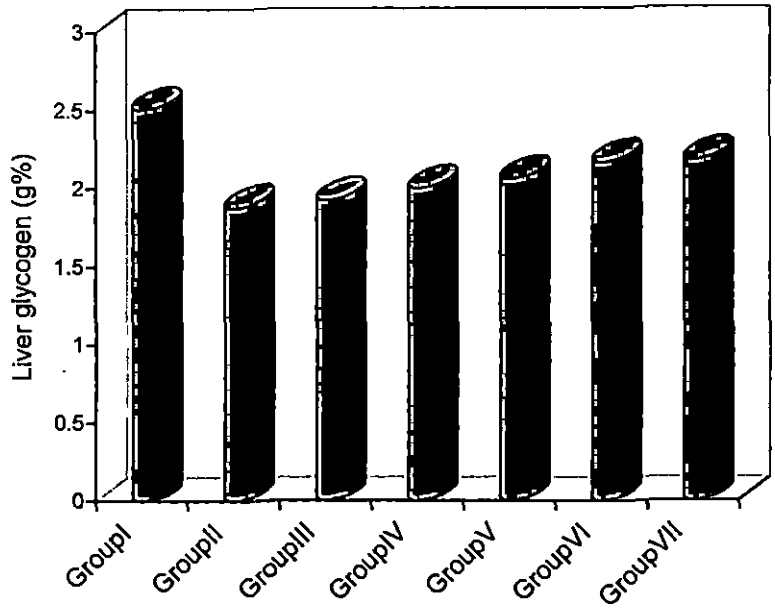

Fig.8. Effect of treatments on liver glycogen

Discussion

5. DISCUSSION

The present study was carried out to assess the hypoglycaemic activity of seed powders of *Brassica juncea* (mustard) and *Trigonella foenum-graecum* (fenugreek) at two different doses in alloxan induced diabetic rats and to compare their efficacy with the standard oral hypoglycaemic agent, glibenclamide. The public health impact of the increase in diabetes is potentially enormous because of the high incidence of complication of diabetes, which include retinopathy, nephropathy, neuropathy and macrovascular disease. These problems are responsible for the erosion of quality of life and productivity as well as significant mortality (Miles, 2003).

In the present study, group I served as normal control and group II as diabetic control. Group III and IV were fed with diets mixed with mustard seed powder at doses 2g/kg and 8g/kg body weight respectively for 30 days. Similarly fenugreek seed powder at doses 2g/kg and 8g/kg body weight respectively was given to groups V and VI. Group VII was treated with glibenclamide at 0.5mg/animal/day for 30 days. During the course of the experiment, changes in various parameters viz, blood glucose, serum triglyceride and cholesterol and liver glycogen were studied.

Results obtained from the study indicated that the agents under study viz, fenugreek seed powder at both doses and mustard seed powder at 8g/kg reduced blood glucose, serum cholesterol, serum triglyceride and increased liver glycogen levels. But the level of these parameters in animals treated with mustard at 2g/kg body weight remained similar to that obtained for diabetic control.

5.1 BLOOD GLUCOSE LEVEL

Initial average blood glucose levels prior to alloxan administration were similar among the seven groups. After 16 days of alloxan administration, the blood glucose levels of the rats selected for study were significantly higher ie, administration of alloxan led to about three fold elevation of blood glucose level. The glucose levels of diabetic rats were maintained at a higher level through-out the

study. A similar effect was observed when the mustard seed powder was administered at a dose of 2g/kg body weight. Fenugreek seed powder at doses 2g/kg and 8g/kg bodyweight were found to exert highly significant reduction in blood glucose. Hypoglycaemic effect was more pronounced with higher dose. The mustard seed powder at 8g/kg body weight also manifested hypoglycaemic property which was however less when compared to treatment with fenugreek seed powder. It caused a 12.7 percent reduction in blood glucose level. Results obtained are presented in Tables 1 to 6 and Fig. 3 to 5.

Diabetes inducing activity of alloxan has been previously reported by many workers (Raphael *et al.*, 2000; Babu *et al.*, 2002; Sharma *et al.*, 2003). Szkudelski (2001) attributed the mechanism of alloxan action to the formation of highly reactive hydroxyl radicals which causes rapid destruction of insulin secreting β cells of islets of Langerhans.

In the present study, glibenclamide caused 46.38 percent reduction in blood glucose level in diabetic rats. In a study conducted by Sheela and Augusti (1992) it was noted that feeding of glibenclamide caused nearly 50 percent fall in blood glucose in alloxan diabetic rats.

Khosla *et al.* (1995) reported that hypoglycaemic effect of fenugreek seed powder was dose related. He noticed a fall of 21.33 percent with high dose (8g/kg) and 13.2 percent with low dose (2g/kg) after two weeks of treatment. He suggested that hypoglycaemic effect of fenugreek is partly because of its high dietary fibre content and high viscosity. In the present study, blood glucose level was decreased by 39.14 percent and 21.18 percent with high and low doses respectively after 30 days of fenugreek administration.

Ponnachan *et al.* (1992) observed that oral administration of aqueous suspension of fenugreek seed powder to alloxan injected rats maintained the glucose level near to control rats in 30 days. The study indicated that the seed powder is having an action similar to insulin which normalises the altered metabolic pathways.

Leaves of fenugreek also have been shown to have antihyperglycaemic effect. Abdel-Barry *et al.* (1997) suggested that oral and intraperitoneal administration of aqueous extract of fenugreek leaves produced a significant reduction of blood glucose concentration.

Several probable mechanisms have been suggested to explain the mechanism of action of fenugreek.

Gupta *et al.* (1999) observed that the activities of glucose-6-phosphatase and fructose 1,6-biphosphatase were lowered by fenugreek seed powder.

Puri *et al.* (2002) were of opinion that the hypoglycaemic effect of fenugreek seeds may be mediated through stimulating insulin synthesis and /or secretion from the β–pancreatic cells of Langerhans. The effect may also be by increasing sensitivity of tissues to available insulin.

Seeds of *Brassica juncea* have been shown to exert hypoglycaemic effect in rats (Khan *et al.*, 1995). They found that mustard seed powder exerted its hypoglycaemic activity by enhancing glycolysis, glycogenesis and decreasing glycogenolysis.

Grover *et al.* (2002) reported that *Brassica juncea* in diet at a strength of 10 percent and 15 percent showed significant antihyperglycaemic effect in alloxan rats, but not in streptozotocin–diabetic rats.

5.2 SERUM CHOLESTEROL

A decrease in the serum cholesterol was observed in all treatment groups except those treated with mustard seed powder at a dose of 2g/kg body weight (Table 7 and Fig 6). Fenugreek seeds showed a dose dependent decrease in serum cholesterol level ie, 42.01 percent fall with low dose and 48.82 percent fall with high dose. However effect of fenugreek seed powder is not as effective as with glibenclamide which showed 55.12 percent reduction in cholesterol level.

Sheela and Augusti (1992) reported an increase in serum lipids in alloxan-induced diabetes. According to Prince and Menon (1999), the marked

hyperlipidaemia that characterized the diabetic stage is a consequence of the uninhibited action of lipolytic enzymes on the fat depots.

Ponnachan *et al.* (1992) were of opinion that in fenugreek seed powder treated rats, serum cholesterol levels were reversed to that of control rats. Khosla *et al.* (1995) suggested that a marked fall was observed in total serum cholesterol in normal rats treated with unroasted form of fenugreek seed.

Adams (2001) suggested that insulin has an antilipolytic activity which may result in hypocholesterolaemia. Puri *et al.* (2002) suggested that fenugreek seeds act through stimulating synthesis/ secretion of insulin from pancreatic β-cells. Hypocholesterolaemic activity of fenugreek seeds may also be due to its insulinomimetic activity.

5.3 SERUM TRIGLYCERIDE

All the treatment groups except those treated with mustard seed powder at 2g/kg body weight showed a decrease in serum triglyceride (Table 8 and Fig. 7). The effect of mustard seed powder at 8g/kg body weight was comparable to that produced by fenugreek seed powder at 2g/kg. Hypolipidaemic effect of fenugreek seed powder was dose dependent, the decrease being more with a high dose (46.16 percent) as compared to low dose (37.79 percent).

In a study conducted by Khosla *et al.* (1995) similar results were obtained with fenugreek seed powder. They attributed the hypolipidaemic effect of fenugreek to the rich fibre content.

Kumar and Menon (1992) suggested that level of lipids is usually raised in diabetes and such elevation represents a risk factor for coronary heart disease.

Prasanna (2000) observed that serum triglyceride and cholesterol were significantly decreased in hyperlipidaemic patients when treated with fenugreek seed powder.

5.4 LIVER GLYCOGEN

In the present study, glycogen content of liver was decreased markedly in diabetic animals. In all the treatment groups, liver glycogen was found to be increased (Table 9 and Fig 8). Treatment with fenugreek seed powder at 2g/kg and 8g/kg body weight significantly increased hepatic glycogen content, but lesser than normal controls indicating that the defective glycogen storage was not completely corrected by the herb. This is in agreement with earlier findings made by Vats *et al.* (2003). He found that hepatic glycogen content decreased by 75 percent in streptozotocin induced diabetic rats and the alteration was partly prevented by fenugreek seeds.

Ponnachan *et al.* (1992) reported that a significant decrease in liver glycogen was observed in alloxan diabetic rats which was reversed by fenugreek seed powder.

Hardman and Limbird (2001) opined that glibenclamide stimulate insulin release from pancreatic β-cells. Insulin inturn stimulates the storage of glucose in liver as glycogen and also inhibits the breakdown of glycogen.

Khan *et al.* (1995) noticed that administration of 10 percent mustard seed enhanced the rate of glycogenesis in alloxan treated rats which is responsible for the increase in the concentration of hepatic glycogen.

Diabetes mellitus is characterised by partial or total deficiency of insulin resulting in derangement of carbohydrate metabolism and a decrease in activity of glycolytic enzymes resulting in depletion of liver glycogen (Murphy and Anderson, 1974). It also impair the capacity of the liver to synthesise glycogen. It is well known that hyperlipidaemia has an association with atherosclerosis which is vastly increased in diabetes.

Results of the present study confirms the glucose lowering and hypolipidaemic effects of fenugreek seed powder at both low and high doses. In the case of mustard seed powder, hypolipidaemic and hypoglycaemic effect were only detectable in animals administered 8g/kg body weight. So fenugreek seed powder

was found to be a better hypoglycaemic agent than mustard seed powder as observed in the present investigation. The potential therapeutic effects of fenugreek and mustard seeds can be utilized for providing dietary management of diseases like diabetes mellitus, coronary heart disease etc.

Summary

6. SUMMARY

The present study was conducted to assess the hypoglycaemic effect of seed powders of *Trigonella foenum-graecum* (fenugreek) and *Brassica juncea* (mustard) at two different doses on alloxan induced diabetic rats and to compare their efficacy with the established oral antidiabetic drug, glibenclamide.

The experiment was conducted in fifty six male albino rats weighing 150-200g. They were randomly divided into seven groups, each group comprising of eight animals. Group I served as normal control. All the groups except normal control were made diabetic by subcutaneous injection of Alloxan monohydrate at a rate of 120mg/kg body weight. Only those rats showing moderate hyperglycaemia (200-250mg/100ml) were selected for further study. Group II was kept as diabetic control. Rats of group III and IV were fed with diets mixed with mustard seed powder at dose rates of 2g/kg and 8g/kg body weight respectively for 30 days. Feed mixed with fenugreek seed powder at doses of 2g/kg and 8g/kg body weights were given to group V and VI respectively for 30 days. Group VII was given the standard drug, glibenclamide at the rate 0.5mg/ animal/ day for 30 days.

Parameters like blood glucose, serum cholesterol, serum triglyceride and liver glycogen were studied. Blood glucose was estimated on zero day, 16^{th} day and 45^{th} day. The other three parameters were estimated after 30 days of treatment.

The maximum decrease in blood glucose level was observed in rats treated with fenugreek at the rate of 8g/kg body weight but not so effective as glibenclamide. Fenugreek seed powder showed a dose dependent reduction in blood glucose level. Mustard seed powder at the rate of 8g/kg body weight also possessed hypoglycaemic effect but less when compared with fenugreek seeds. There was no significant reduction in blood glucose level of rats treated with mustard seed at the rate of 2g/kg body weight.

The peak serum cholesterol level was found in group II and group III where as group I and group VII had the minimum level. Mustard seed powder at the rate of 8g/kg body weight and fenugreek seed powder at both high and low doses showed significant hypocholesterolaemic effect. Among them, mustard seed powder at the rate of 8g/kg body weight possessed least effect while fenugreek seed powder at the rate of 8g/kg body weight possessed the maximum.

Serum triglyceride levels were highest in group II and group III. Minimum levels were shown by group I and group VII. A maximum decrease in serum triglyceride was noticed in group VI. Fenugreek seed powder at the rate of 2g/kg body weight and mustard seed powder at the rate of 8g/kg body weight was found to have same hypolipidaemic effect.

Liver glycogen levels were lowest in group II. Fenugreek seed powder at the rate of 8g/kg body weight could produce an increase in liver glycogen which was comparable to glibenclamide. A significant increase in liver glycogen was found in groups III, IV and V.

In the present study, mustard showed only a mild hypoglycaemic activity when compared to fenugreek seed powder. So it can be best utilized only in the case of mild diabetic patients controlled on exercise and diet. As they have been consumed safely for over centuries by people, safety and efficacy are assured.

The results of the present study clearly demonstrated that fenugreek seed powder has a good hypoglycaemic effect. These findings suggest that the fenugreek seeds should be promoted as a dietary supplement for diabetes as well as those prone to getting diabetes.

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THE HYPOGLYCAEMIC EFFECT OF *Brassica juncea* (MUSTARD) AND *Trigonella foenum-graecum* (FENUGREEK) IN ALBINO RATS

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was undertaken to assess the hypoglycaemic activity of seed powders of *Brassica juncea* (mustard) and *Trigonella foenum-graecum* (fenugreek) at two different doses (2g/kg and 8g/kg body weight) in alloxan diabetic rats and to compare their efficacy with standard oral hypoglycaemic drug, glibenclamide.

The experiment was conducted in fifty six male albino rats for a period of 45 days. Rats were divided into seven groups of eight each. Group I served as normal control and group II as diabetic control. Seed powders of mustard and fenugreek at low and high doses were given to group III to group VI respectively for 30 days from 16^{th} day onwards. Glibenclamide at a rate of 0.5mg/ animal /day was fed to group VII. Blood glucose level was estimated at zero day, 16^{th} day and 45^{th} day of the experiment. Serum cholesterol, serum triglyceride and liver glycogen were estimated at 45^{th} day of the experiment.

Fenugreek seed powder caused a maximum decrease (39.14 percent) in blood glucose level at 8g/kg body weight. But it is not effective as glibenclamide which caused a 46.38 percent reduction. At 2g/kg body weight, fenugreek caused 21.18 percent reduction. Mustard at 8g/kg can also act as a hypoglycaemic agent, but less effective when compared to fenugreek seed powder. No significant reduction was noticed with the low dose of mustard. This showed that fenugreek seed powder at both doses and mustard at high dose are having potent hypoglycaemic effect.

Higher level of serum cholesterol and triglyceride were noticed in group II ie, 166.96$\pm$15.70 mg% and 162.08$\pm$ 25.04 mg% respectively. The levels of group III animals were comparable to group II. All the other treatment groups showed a significant reduction in serum cholesterol and triglyceride levels. But the reduction is not as effective as that caused by glibenclamide. Fenugreek seed powder showed a better hypolipidaemic effect than mustard seed powder.

Low level of liver glycogen was observed in group II. Among the treatment groups, group VII showed a peak level of liver glycogen. The liver glycogen level of

group VI was comparable to that of group VII ie, 2.16± 0.05 g% and 2.17± 0.05 g%. respectively. Other treatment groups also showed an increase in liver glycogen.

From the study, it can be concluded that fenugreek seed powder at both doses and mustard seed powder at high dose are potent hypoglycaemic agents, but their effect is less when compared to glibenclamide.