

**HYPOGLYCAEMIC ACTIVITY OF *TRIGONELLA*
FOENUM GRAECUM SEED POWDER IN NORMAL AND
ALLOXAN INDUCED DIABETIC RATS**

T H E S I S

Submitted to the
**Maharashtra Animal and Fishery Sciences
University, Nagpur**

In partial fulfillment of the requirements for the Degree of

**MASTER OF VETERINARY SCIENCE
IN
VETERINARY PHARMACOLOGY AND TOXICOLOGY**

BY

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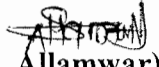
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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled “**HYPOGLYCAEMIC ACTIVITY OF *TRIGONELLA FOENUM GRAECUM* SEED POWDER IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS**” or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation been duly acknowledged.

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Mr. SANJAY SAYANNA ALLAMWAR has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled "HYPOGLYCAEMIC ACTIVITY OF *TRIGONELLA FOENUM GRAECUM* SEED POWDER IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS" submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination in the subject of **VETERINARY PHARMACOLOGY AND TOXICOLOGY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

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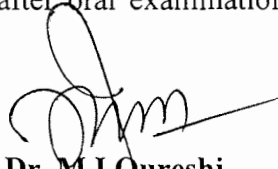
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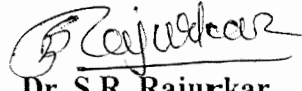
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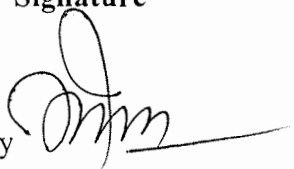


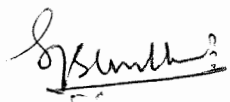
This is to certify that the thesis entitled “HYPOGLYCAEMIC ACTIVITY OF *TRIGONELLA FOENUM GRAECUM* SEED POWDER IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS” submitted by Mr. SANJAY SAYANNA ALLAMWAR to Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of MASTER OF VETERINARY SCIENCE in the subject of VETERINARY PHARMACOLOGY AND TOXICOLOGY has been approved by the Student’s Advisory Committee after oral examination in collaboration with the External Examiner.



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ACKNOWLEDGEMENT

I wish to extend my deepest appreciation and sincere gratitude to....

My honourable and enthusiastic Research Guide, Major Advisor and Chairman of Advisory Committee, **Dr. S.R.Rajurkar**, M.V.Sc., Ph.D., Associate Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, MAFSU, Parbhani for his never ending enthusiasm, rapidity, expert advice, valuable suggestions and constructive criticism. It has been privilege to work under his professional guidance.

I acknowledge with greatest regard that facilities provided for conducting the present research to **Dr. M.B. Gujar**, Associate Dean, College of Veterinary and Animal Sciences, MAFSU, Parbhani.

It gives me immense pleasure to express my deep sense of sincere gratitude towards my honourable teacher, **Dr. M.I.Qureshi**, M.V.Sc., Ph.D, Professor and for his charming personality, magnanimous nature, busy day to day routine and still ever fresh and smiling poise and for good guidance, constant supervision and excellent encouragement, placing the laboratory facilities during the whole research period.

I acknowledge deep sense of regards to the members of Advisory Committee, **Dr. P.R.More**, M.V.Sc., Ph.D., Associate Professor, Dept. of Vety. Pharmacology and Toxicology for their keen interest, necessary suggestions and invaluable help during research work. I feel extremely honoured for the opportunity bestowed upon me to work under his immortal guidance.

I acknowledge deep sense of regards to the member of Advisory Committee, **Dr. Abdul Aziz**, Professor and Head, Dept. of Vety. Microbiology, **Dr.G.B.Kulkarni**, Professor and Head, Dept. of Vety. Pathology for their keen interest necessary suggestions and invaluable help during research work.

I shall remain every grateful to most admirable persons is **Dr.C.S.Mamde**, Assistant Professor, Dept. of Anatomy, **Dr. G.R. Gangane**, Assistant Professor of Veterinary Pathology and **Dr. P.Yeotikar**, Assistant Professor, Dept. of Vety. Physiology for giving excellent suggestions and helping hand at every stage.

I would like to acknowledge my senior colleagues, Drs. D.P.Wale, R.M. Bankar and S.K.Suryawanshi for their keen interest and useful suggestions. I owe my special thanks to my favourite Department colleagues Drs. S.S.Gambhire and U.D.Bijegaonkar for their unfailing interest and co-operation during the course of this study. I feel immense pleasure in expressing my humble thankfulness to my junior friends Drs.P.V.Deshmukh, S.P.Surwase and Dipali Patil for bearing hardship for my work.

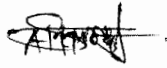
Special thank and acknowledge goes to my beloved and dear friends Drs. Rais Boss, Jinklod, Channa, Thakur, Vishal Abhijit, Chigure, Chilgunde, Kondre, Mahajan, Waghmare, Jinke, More, Shahed, Sawane, Salwe, Bhokare, Swapnil, Kailas Gite, Shankar, Karegaonkar, Kasralikar, Rohankar, Katkade, Suryawanshi, Jigalekar, Kalyankar, Wadje, Ambore, Totewad, Doifode, Kharmate, Randive, Balsaraf, Mundhe, Khesar, Sure, Balwantkar, Maroti (STD), Vijay Zambare, Shankar Udgire, Tekale, Pashamwar, Kawatwar, Suresh (Mamu), Kudmulwar, Mushir, Mustafa, Raju, Gangya, Hanmya, Venkya, Suresh, Santosh, Vijay, Prashant.

I am thankful to Shri Suryawanshi , Shri Pawar, Smt.Phulari and Smt.Rukhmini for their affectionately co-operation and assistance. My appreciation and thanks are to Mr. Shitalkumar Kadam for meticulous computer printing of this manuscript.

My beloved family members **Aai, Sow. Rukminibai, Baba, Sayana Tai, Sow. Shashikala, Bhauji, Shri Irranna, Bhau, Shankar, Vaini, Sow. Sandhya and cousin brother Hanmantrao, Vaini Sow. Shobha, Balaji (Bhau), Rukmini, Bablu, Sangita, Sneha** No praise is enough.

Place : Parbhani

Date : 27/10/05


(Allamwar S.S.)

LIST OF ABBREVIATIONS

%	Per cent
μ	Micron
ALT	Alanine Transaminase
@	At the rate of
BUN	Blood urea nitrogen
dl	Decilitre
GTT	Glucose tolerance test
g	Gram
IU	International unit
kg	Kilogram
l	Litre
mg	Milligram
min	Minute
S.E.	Standard error
w/v	Weight by volume

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Introduction

Chapter – 1

INTRODUCTION

Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion or insulin action or both resulting impairing metabolism of glucose and other energy yielding fuels such as lipids and protein. The first description of diabetes was given by an Egyptian physician Hesy Re about 3550 years ago.

Charak and Sushruta first brought to the notice that the urine of diabetic patient attracted ants due to its sweetness, hence named it as “Madhumeha”. It was Areticus a Roman physician who coined the word diabetes means “siphon” as diabetic patients show increased urination. Later the condition was named as diabetes mellitus. “Mellitus” is a latin word for sweetness. Traditional system of medicine contributed extensively for management and treatment of disease by using indigenous herbs.

The research in India since last four to five decades has thrown new lights on therapeutic potentialities of medicinal herbs for various ailments including diabetes. Sulfonylurea, biguanide and other synthetic derivatives are routinely used as oral substitutes for insulin. However, their long term use in diabetics is not free from side effects. Therefore, safe alternatives are needed

because of the inability of current available synthetic drugs or therapies to control all the related clinical and pathological aspects of diseases.

Rigveda, the oldest repositories of human knowledge written between 4500-1500 BC mentions the use of 67 plants for therapeutic use and Yajurveda enlists 81 plants whereas, Atharvaveda written during 1200 BC describes 290 plants of medicinal value. “Charak Samhita” describes 341 medicinal plants and “Sushrut samhita” (600 BC) mentions 395 medicinal plants (Handa, S.S. 1996).

Investigative studies on hypoglycaemic effects of medicinal herbs indicated usefulness of *Pterocarpus marsupium* (Sepaha and Bose, 1956 and Chakravarthy *et al.*, 1980), *Ficus bengalensis* and *ficus glomerata* (Gujral *et al.*, 1954; Shrotri and Aiman, 1960 a and Deshmukh *et al.*, 1960), *Gymnema sylvestre* (Mukerji, 1957; Gupta, 1961; Trivedi, 1963 and Gupta and Variyar, 1964). *Adhatoda vesica* (Modak and Rajarama Rao, 1966), *Tinospora cardifolia* (Gupta, 1964; Gupta, 1965 and Gupta *et al.*, 1967 a) and many others in diabetic patients.

Trigonella foenum graecum commonly known as “Methi”. The leaves of which is used as vegetable and the seeds have been used for numerous indications including labour induction, as digestive stimulant, general tonic to improve metabolism besides usingⁱⁿ diabetics (Kritikar and Basu, 1933; Sawant, 1974 and Basch *et al.*, 2003). The seeds of the plant have been investigated for hypoglycaemic, antioxidant, antilipidaemic and other properties in different

laboratory animals (Thakarns *et al.*, 2003; Hannan *et al.*, 2003 and Valette *et al.*, 1984).

Detailed study therefore was made to study the effect of seed powder in normal and diabetic alloxan induced rats. Attempts were also made to understand the possible mechanism of action for hypoglycaemic activity. In the view of the above study is planned with following objectives :

1. To study the effect of *Trigonella foenum graecum* seeds powder on normal wistar rats.
2. To know the hypoglycaemic effect of *Trigonella foenum graecum* seeds powder in alloxan diabetic rats.
3. To investigate the changes in biochemical parameters like serum glucose, serum cholesterol, triglyceride, blood urea nitrogen (BUN) and alanine transaminase (ALT).
4. To observe gross and histopathological changes in the organs.
5. To understand the possible mechanism of hypoglycaemic activity.



*Review of
Literature*

Chapter – 2

REVIEW OF LITERATURE

Major Indian population particularly those in rural areas largely depends upon traditional system of medicine. India is known for its richest source of medicinal herbs and 80 per cent people living in developing countries largely depends on traditional system of medicine for health care needs. Diabetes mellitus is a common, chronic metabolic disorder in man which is characterized by hyperglycaemia, disrupted metabolism of carbohydrates, protein and lipids. About 64 per cent of the total population of the world utilizes plants as drugs i.e. 3.2 billion people (Fransworth, 1990).

A large number of indigenous plants have been screened for their possible antidiabetic effect in animals. Some of them have been subjected to clinical trials in diabetic patients. The present chapter reviews the literature available on hypoglycaemic properties of medicinal plants including "*Trigonella foenum graecum*" selected for the present investigation.

2.1 Hypoglycaemic medicinal plants

A large number of plants have been successfully tested in laboratory which confirmed their blood sugar lowering activity besides other effects. In recent year W.H.O. had recommended 2500 herbs for testing their biopharmaceutic effect.

The earlier work on hypoglycaemic effect of medicinal plants was reviewed by Kirtikar and Basu (1933), Nadkarni (1954), Chopra *et al.* (1956), Sawant (1974), Handa (1996), Dahanukar (1995) and Rajurkar (2000).

The medicinal plants with encouraging hypoglycaemia in normal and diabetic animals are listed in Table 1.

Table 1 Indigenous plants producing hypoglycaemia in normal and diabetic animals

Plants	References
<i>Adhatoda vasica</i>	Modak and Rajarama Rao (1966)
<i>Allium cepa</i>	Arora and Stephan (1959); Brahmachari and Augusti (1961) and Mathew and Augusti (1975)
<i>Allium sativum</i>	Arora and Stephan (1959); Brahmachari and Augusti (1962b) and Kumar <i>et al.</i> (1999)
<i>Annona squamosa</i>	Shriwaikar <i>et al.</i> (2004) and Gupta <i>et al.</i> (2005)
<i>Azadirachta indica</i>	Pillai and Santhakumari (1981); Dixit <i>et al.</i> (1986); Chattopadhyay (1996); Bopanna <i>et al.</i> (1997); Bajaj and Srinivasan (1999)

Plants	References
<i>Bambusa dendrocalamus</i>	Bapat <i>et al.</i> (1969)
<i>Bougainvillea spectabilis</i>	Narayanan <i>et al.</i> (1984)
<i>Cassia auriculata</i>	Shrotri <i>et al.</i> (1963)
<i>Caserai esculenta</i>	Gupta <i>et al.</i> (1967a and b)
<i>Cannabis indica</i> (Bhang)	Soni and Gupta (1978)
<i>Coccinia indica</i>	De and mukerji (1953); Gupta (1962); Brahmachari and Augusti (1963); Trivedi (1963) and Vaishnav and Gupta (1995)
<i>Comiphora mukul</i>	Dahanukar and Harza (1995)
<i>Cryptostegia grandifolia</i>	Sharma <i>et al.</i> (1967)
<i>Cyamopsis tetragonoloba</i> Taub.	Jenkin <i>et al.</i> (1977); Pillai <i>et al.</i> (1980) and Bhandari and Sharma (1999)
<i>Dipteracanthus prostratus</i>	Dhawan <i>et al.</i> (1980)
<i>Dolichos lablab</i>	Srinivasan (1957)
<i>Ficus glomerata</i>	Gujral <i>et al.</i> (1954); Shrotri and Aiman (1960) and Gupta (1964)

Plants	References
<i>Ficus bengalensis</i>	Gujral <i>et al.</i> (1954); Deshmukh <i>et al.</i> (1960); Aiman (1961); Brahmachari and Augusti (1962a); Brahmachari and Augusti (1964) and Cherian and Augusti (1993)
<i>Gymnema sylvestre</i>	Guruswamy <i>et al.</i> (1959); Gupta <i>et al.</i> (1961); Trivedi (1963); Gupta and Variyar (1964) and Bopanna <i>et al.</i> (1997)
<i>Momordica charantia</i>	Kedar and Chakrabarti (1982); Qureshi (1988); Sarkar <i>et al.</i> (1996) and Viridi <i>et al.</i> (2003)
<i>Murraya koenigii</i>	Narayana and Sastry (1975); Khan <i>et al.</i> (1997); Yadav <i>et al.</i> (2002) and Tachibana <i>et al.</i> (2003)
<i>Pterocarpus marsupium</i>	Sepaha and Bose (1956); Harnath <i>et al.</i> (1958); Arora and stephan (1959); Gupta (1962); Trivedi (1963).
<i>Rivea cuneata</i>	Rajarama Rao and De (1952)
<i>Tinospora cardifolia</i>	Gupta (1964) and Gupta <i>et al.</i> (1967a)
<i>Trichosanthus dioca</i>	Dahanukar and Harza(1995) and Mukherjee <i>et al.</i> (2002)
<i>Vinca rosea</i>	Shrotri <i>et al.</i> (1963)

The active principles obtained from different herbs are regarded as blood sugar lowering compounds and are summarized in Table 2.

Table 2 The hypoglycaemic principles isolated from the indigenous medicinal plants

Plants	Hypoglycaemic principle	References
<i>Allium cepa</i>	Allyl propyl disulphate	Mukerji (1957); Datta <i>et al.</i> (1942) and Augusti (1975)
<i>Adhatoda vasica</i>	Non-nitrogenous principle C ₂₄ H ₄₀ O ₄	Modak and Rajarama Rao (1966)
<i>Azadirachta indica</i>	Nimbidin (neutral substances)	Pillai and Santhakumari (1981)
<i>Coccinia indica</i>	Triterpenes	Dhanabal <i>et al.</i> (2004)
<i>Coccinia grandis</i> Linn.	Caffeic acid	Mukherjee <i>et al.</i> (2002)
<i>Cyamopsis tetragonoloba</i>	Gum-guar	Pillai <i>et al.</i> (1980)

Plants	Hypoglycaemic principle	References
<i>Ficus bengalensis</i>	Glycoside flavonoids A,B,C, B-leucoanthocyanin A,C-leucoanthocyanidin	Deshmukh <i>et al.</i> (1960) and Brahmachari and Augusti (1964)
<i>Ficus religiosa</i>	Phytosteroline (B-sistosteryl) (D-Glycoside)	Brahmachari and Augusti (1962 b) and Varshney <i>et al.</i> (1965)
<i>Gymnema sylvestre</i>	Gymnemic acid Gymnesians A-D (Triterpenoid saponins)	Mukerji (1957); Gupta (1962) and Trivedi (1963) Chattopadhyay (1998)
<i>Lianaria ramosissima</i>	Aminoglycoside	Deshmukh <i>et al.</i> (1961)
<i>Momordica charantia</i>	Charantin (Phytosterolin)	Lotlikar and Rajarama Rao (1966)

Plants	Hypoglycaemic principle	References
<i>Pterocarpus marsupium</i>	Pterostilbene 3, 4, dimethoxystilbene Flavonid fraction 1-epicatechin (flavonoid)	Harnath <i>et al.</i> (1958); Arora and Stephan (1959) Chakravarthy <i>et al.</i> (1980) Mukherji <i>et al.</i> (2002)
<i>Tinospora cardifolia</i>	Glycoside	Gupta <i>et al.</i> (1967a)
<i>Rivea cuneata</i>	Phytosterolins	Rajarama Rao <i>et al.</i> (1966)
<i>Swertia chirayita</i>	Swerchirin containing hexane fraction	Mukherjee <i>et al.</i> (2002)
<i>Syzygium cumini</i>	Peptidylglycan and oligosaccharide Glycoside and enzyme in seeds	Kelkar and Kaklij (1997) Mukherjee <i>et al.</i> (2002)

2.2 *Trigonella foenum graecum*

The plant "*Trigonella foenum graecum*" which is popular for its medicinal value and cultivated in many parts of India. The seeds are hot, with a

sharp bitter taste, tonic, antipyretic, anthelmintic, increase the appetite, cure leprosy, "vata" vomiting, bronchitis, piles, useful in heart disease (Ayurveda).

The plant seeds are suppurative, aperient, diuretic, emmenagogue, useful in chronic cough, dropsy (Yunani). The leaves are useful in external and internal swellings and burns, prevent the hair falling (Kirtikar and Basu, 1933; Chopra *et al.*, 1956; Nadkarni, 1954 and Sawant, 1974).

Swanston-flatt *et al.* (1989) studied the effect of twelve plants traditionally used in the treatment of diabetes mellitus in northern Europe. *Trigonella foenum graecum* (Fenugreek) was one of the twelve ingredients in the study. They observed nonsignificant effect of fenugreek on streptozotocin induced diabetic mice.

Bhardwaj *et al.* (1994) investigated control of hyperglycaemia and hyperlipidaemia by a plant product consisting of Guargum, Methi, Tundika and Meshasringi. They reported improvement in oral GTT in both control and diabetic groups on treatment with herbal powder. They found significant reduction in total cholesterol and LDL cholesterol in both groups.

The herbal powder was fed in 30 control (Healthy) and 30 non insulin dependent diabetes mellitus patients twice a day for a period of 4 weeks.

They also opined that the herbal powder can be effectively used to reduce post prandial blood glucose and LDL cholesterol in non insulin dependent diabetes mellitus patients as a long term measure.

Khosla *et al.* (1995a) investigated the effect of *Trigonella foenum graecum* (Fenugreek) on serum lipids in normal and diabetic rats. They administered unroasted and roasted fenugreek seed powder mixed with diet daily in a low dose of 2 gm/kg and high dose of 8 gm/kg body weight. They found significant fall in total serum cholesterol, triglyceride, LDL and VLDL cholesterol in normal rats fed with unroasted fenugreek seed powder. Further they also observed that the roasted form of fenugreek seed powder had pronounced hypolipidaemic action as compared to unroasted form. The diabetic rats fed with unroasted fenugreek seed powder at a higher dose of 8 gm/kg body weight showed significant fall in total serum cholesterol, triglyceride, LDL and VLDL cholesterol which was better as compared to the lower dose of 2 gm/kg body weight.

Khosla *et al.* (1995b) studied the effect of *Trigonella foenum graecum* (Fenugreek) in alloxan induced diabetic rats. The alloxan was administered in 5 per cent normal saline at the dose rate of 120 mg/kg body weight. The fenugreek seed powder was fed in two groups of rats at the dose rate of 2 g/kg and 8 g/kg body weight.

They observed 23.09% and 15.59% fall in blood glucose level of normal rats fed with fenugreek seed powder at 8 gm/kg body weight and 2 gm/kg body weight, respectively. However, the fall in the diabetic rats was observed to be 21.33% and 13.2% when the fenugreek seed powder fed for a period of 2 weeks. They opined that the roasted seeds of fenugreek had no effect on blood glucose levels in rats as compared to the unroasted form of fenugreek seeds.

Abdel-Barry *et al.* (1997) studied the effect of graded amounts (0.06, 0.2, 0.5 or 1 g/kg I.P. and 1, 2 or 8 g/kg orally) of the aqueous extract of *Trigonella foenum graecum* leaves given to both normal and alloxan diabetic rats. The treatment resulted in significant reduction of blood glucose concentration in both groups. The ethanolic extract produced no reduction in blood glucose concentration in normal rats, but intraperitoneal administration of 0.8 g/kg body weight of the ethanolic leaf extract to diabetic rats produced a significant reduction of blood glucose concentration at 2 and 24 h. only.

Bhandari *et al.* (1997) observed the effect of ethanolic extract of fenugreek (*Trigonella foenum graecum*) seeds on streptozotocin induced hyperglycaemic albino rats in comparison with those of glibenclamide used as a standard drug. The oral treatment of extract (200 mg/kg daily) for 20 days resulted in significant reduction in blood glucose and blood urea levels and an increase the serum protein and albumin levels.

Nada *et al.* (1997) evaluated the hypoglycaemic activity of traditional plant seeds preparation on male diabetic rats. They studied the plant seeds preparation (PSP) comprised of a mixture of equal portions of the powdered seeds of *Aloe vera* (*A. barbadensis*), *Comiphora myrrha* (*Commiphora molmol*), *Bryonia critica* (*B. cretica*), *Lupinus termis* (*L. albus*), *Trigonella foenum graecum* (*T. foenum graecum*) and Rawandi sini. The preparation was given at daily oral dose of a 10% decoction of PSP (100 mg in 1 ml per 100 g body weight) and glibenclamide (0.25 mg in 1 ml per 100 g body weight) in alloxanized male rats

for 8 weeks. They observed hypoglycaemic and hypocholesterolaemic activities of PSP in male alloxan diabetic rats, further the preparation reduced serum triglycerides, creatinine and AST level but had no effect on serum ALT level. They opined the fall in serum glucose level was sharper with glibenclamide than with PSP.

Alarcon-Aguilara *et al.* (1998) studied anti-hyperglycemic effect of plants traditionally used as antidiabetics. They investigated the antihyperglycaemic effects of 28 Mexican medicinal plants, each plant was processed in the traditional way and intragastrically administered to temporarily hyperglycaemic rabbits. They observed that out of 28 plants, only 8 plants significantly reduced the hyperglycaemic peak and the area under the glucose tolerance curve. They reported the 8 plants as *Guazuma ulmifolia*, *Tournefortia hirsutissima*, *Lepechinia caulescens*, *Rhizophora mangle*, *musa sapientum* (*M. paradisiaca*), *Trigonella foenum graecum*, *Turnera diffusa* and *Euphorbia prostrata*.

Al-Habori and Raman (1998) studied antidiabetic and hypocholesterolaemic effect of fenugreek. They concluded that the presence of saponin and high fibre content in fenugreek seeds is responsible for antihyperglycaemic effects readily from delayed gastric emptying caused by fibre content and components that inhibit carbohydrate digestive enzymes. Further they found reduction in the LDL and VLDL fractions of total cholesterol and

increase in HDL cholesterol in alloxan induced diabetic rats following treatment with fenugreek seed powder.

Gomez *et al.* (1998) studied the treatment of healthy adult alloxan pretreated individuals of *Anabas testudineus* with fenugreek seed extract by intramuscular injection. They observed significant reduction in serum glucose level (by 10.9%) and increased liver and muscle glycogen content (by 17.70 and 40.02% respectively) compared to controls, which were injected with saline solution (0.9% NaCl).

Vetrichelvan *et al.* (1998) studied the effect of *Trigonella foenum graecum* seed powder administered at 2 gm/kg alone or mixed with rifampicin (10 mg/kg) orally on the blood sugar concentration of alloxan induced diabetic rats. They observed hypoglycaemic effect of *Trigonella foenum graecum* seeds powder fed alone at the dose rate of 2 g/kg body weight in rats. However, they reported the loss of hypoglycaemic effect. When the rifampicin was fed following the administration of *Trigonella foenum graecum* seeds powder.

Nair *et al.* (2000) investigated the effect of feeding of fenugreek (*Trigonella foenum graecum*) at the dose rate of 20 g/day in non insulin dependent diabetes mellitus (NIDDM) male human beings. They observed the reduction in areas under plasma glucose response curves (AUC) in *Trigonella foenum graecum* treated individuals as against non treated group.

Anuradha and Ravikumar (2001) studied the influence of fenugreek seed powder on lipid peroxidation and antioxidant enzymes in normal and alloxan diabetic rats. They observed enhanced lipid peroxidation and increased susceptibility to oxidative stress with depletion of antioxidants in liver, kidney and pancreas of diabetic rats. However, the *Trigonella foenum graecum* seed powder fed at the dose rate of 2 g/kg body weight normalised the alterations.

The supplementation of *Trigonella foenum graecum* in normal rats increased the antioxidant status with reduction in peroxidation.

Puri *et al.* (2002) isolated an orally active principle called G_{II} from fenugreek seeds which elicited significant hypoglycaemic effect in alloxan diabetic albino rabbits. They further reported the administration of the G_{II} fraction to diabetic animals had significant influence on the serum insulin response to an oral glucose load, the response was greater in the treated than in untreated diabetic rabbits. They observed 74 per cent fall in the fasting blood glucose levels in severely diabetic rabbits after daily administration of G_{II} fraction for one month.

Vats *et al.* (2002) evaluated the hypoglycaemic effect of *Trigonella foenum graecum* Linn., *Ocimum sanctum* Linn. and *Pterocarpus marsupium* Linn. in normal and alloxanized diabetic rats. They observed that the alcoholic extract of *Trigonella foenum graecum* found to reduce the blood glucose level in normal rats (74.33 ± 4.77 to 60.56±1.9 mg/dl). However, the reduction in the alloxan induced diabetic rats was observed from 201.25±7.69 to 121.25±6.25 mg/dl.

Further they reported favourable effect of extract on glucose disposition in glucose fed hyperglycaemic rats.

Devi *et al.* (2003) studied the effect of fenugreek leaves on carbohydrate metabolism and liver enzymes. They observed that the supplementation of diet with fenugreek leaves had significant effect on hyperglycaemia, hypoinsulinaemia and glycosylated haemoglobin in streptozotocin induced diabetic rats. Further they reported the significant effect on carbohydrate metabolic enzyme, liver glycogen and an improvement in the body weight in diabetic rats.

Satyanarayana *et al.* (2003) evaluated the hypoglycaemic activity of herbal preparations in normal and diabetic rabbits. Herbal preparations containing leaf juice of *Catharanthus roseus* and the seed powder of fenugreek treated with *C. roseus* (0.5, 0.75 and 1.0 ml/kg) and fenugreek (50, 100 and 150 mg/kg) at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h after being fasted for 18 h. They observed the percentage blood glucose reduction produced by the combination of *C. roseus* (0.5 ml/kg) and fenugreek (50 mg/kg) was more than the sum of their individual per cent blood glucose reduction in both normal and diabetic rabbits.

Venkatesan *et al.* (2003) studied the effect of oral administration of fibernat (Cocktail of fenugreek seed powder, guar gum and wheat bran) on lipid metabolism and cholesterol homeostasis in rats. They found that fibernat intake significantly increased apo B, E. receptor expression in rat liver as reflected by an

increase in the maximum binding capacity of the apo B, E receptor to LDL and VLDL. Further they observed 1.5 fold increase ⁱⁿ the activity of hepatic triglyceride lipase in fibernat fed rats as compared to those fed with atherogenic diet alone.

Annida and Stanely Mainzen Prince (2004) reported lowered lipid profile in streptozotocin induced diabetic rats (—) in comparison with rats treated with 0.5 g/kg and 1 g/kg dose level of fenugreek leaves.

Broca *et al.* (2004) extract insulinotropic agent ID-1101 (4-hydroxy isoleucine) from fenugreek seeds. The ID-1101 an amino acid was observed to contain glucose dependent – insulin stimulating activity in rats. They also observed that the ID-1101 ^{could} improve insulin resistance through an increase in peripheral glucose utilization rate in sucrose, lipid fed rats. Further they found chronic ID-1101 treatment significantly reduced the insulinemia in type 2 diabetic rats and reduced the progression of hyperinsulinemia in insulin resistant obese zucker fa/fa rats.

Mondal *et al.* (2004) observed the hypoglycaemic effect of *Trigonella foenum graecum* seeds in streptozotocin induced diabetic rats. They reported the fall in fasting blood glucose level (FBG) in *Trigonella foenum graecum* treated diabetic rats. However, the FBG level was observed to be 24.33 m mol/lit in diabetic untreated rats as against 9.89 m mol/lit in *Trigonella foenum graecum* treated rats and 5.21 m mol/lit in normal healthy rats.

Yadav *et al.* (2004) observed ^{that} the *Trigonella foenum graecum* seed powder to be effective in preventing the hyperglycaemia in alloxan induced diabetes in rats. They observed that the *Trigonella foenum graecum* seeds powder (TSP) alongwith the sodium-orthovanadate (SOV) ^{exerted} positive effect on altered lipid metabolism and reported the TSP and SOV to be effective in reducing the triglycerides and total cholesterol level in blood serum of alloxan induced diabetic rats, the combination was fed for a period 21 days. They also observed an increase in total lipids by 48% and 55% in liver and kidneys of diabetic rats in comparison to nearly normal level in SOV and TSP fed rats.

Talpur *et al.* (2005) investigated the ability of various combinations of essential oils such as fenugreek, cinnamon, cumin, oregano etc. They examined the effects of these natural products on Zucker fatty rats (ZFRs) and spontaneously hypertensive rats (SHRs). They observed combined essential oils lowered the circulating glucose levels and systolic blood pressure in both ZFRs and SHRs, suggesting that these natural products are enhancing insulin sensitivity.



*Material &
Methods*

Chapter – 3

MATERIAL AND METHODS

The study was carried out to evaluate, the hypoglycaemic response of an indigenous medicinal plants. *Trigonella foenum graecum* (Fig.1) in normal and alloxan induced diabetic rats. The seeds were locally obtained and made into powder by grinder and was used for experimental study. The details of methods used are described in this chapter.

3.1 *Trigonella foenum graecum*

Trigonella foenum graecum a member of family “Leguminosae” is most widely cultivated throughout the country. The plant is known by different vernacular names in India which are as follows :

Language	Vernacular name
Bengali	Haenugraeb, methi, methica
English	Fenugreek, Greek Hayes
Gujrati	Bhaji, methi, methini
Hindi	Methi, muthi
Marathi	Methi
Sanskrit	Methi, Bahuparni, Dipani, Jyoti, Chandrika, Kunchika
Tamil	Vendayam, ventayam
Telagu	Mentikura, mentulu
Urdu	Methi



Fig.1 Specimen of plant – *Trigonella foenum graecum*

3.1.1 Botanical description

Family	Leguminosae
Plants and stem	Nearly smooth, erect, annual. Stipules not toothed
Leaves	2-2.5 cm long, oblanceolate-oblong, toothed; used internally and externally for their cooling properties
Flowers	1-2 number of flowers, axillary, sessile, calyx- teeth linear, corolla much exerted
Seeds	Small, oval to square in shape, yellow coloured, sharp bitter in taste; carmin, tonic, aphrodisiac; seeds are hot and dry.

3.2 Processing of plant material

The seeds of the plant, *Trigonella foenum graecum* were procured from the local market and identified by taking help of botanist. The seeds were cleaned and made into fine powder (Fig.2 and 3) by grinding using domestic mixer and was used for the experiment. The seeds powder was fed @ 2 g/kg body weight in experimental animals.

3.3 Experimental animals

Fifty weanling wistar rats of both the sex were procured from Raj Biotech, (India) Ltd., Talekar Wasti, Wing, Taluka; Khandala, Dist. Satara. All

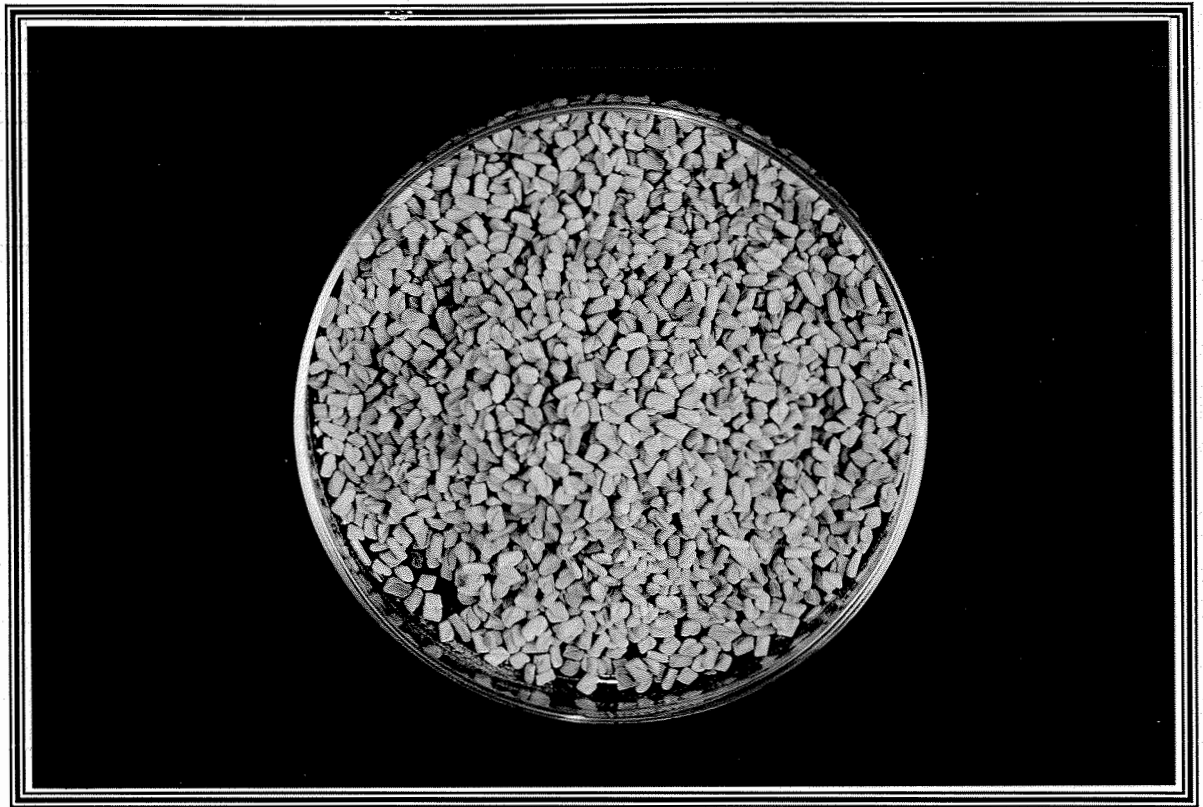


Fig. 2 Seeds of plant – *Trigonella foenum graecum*

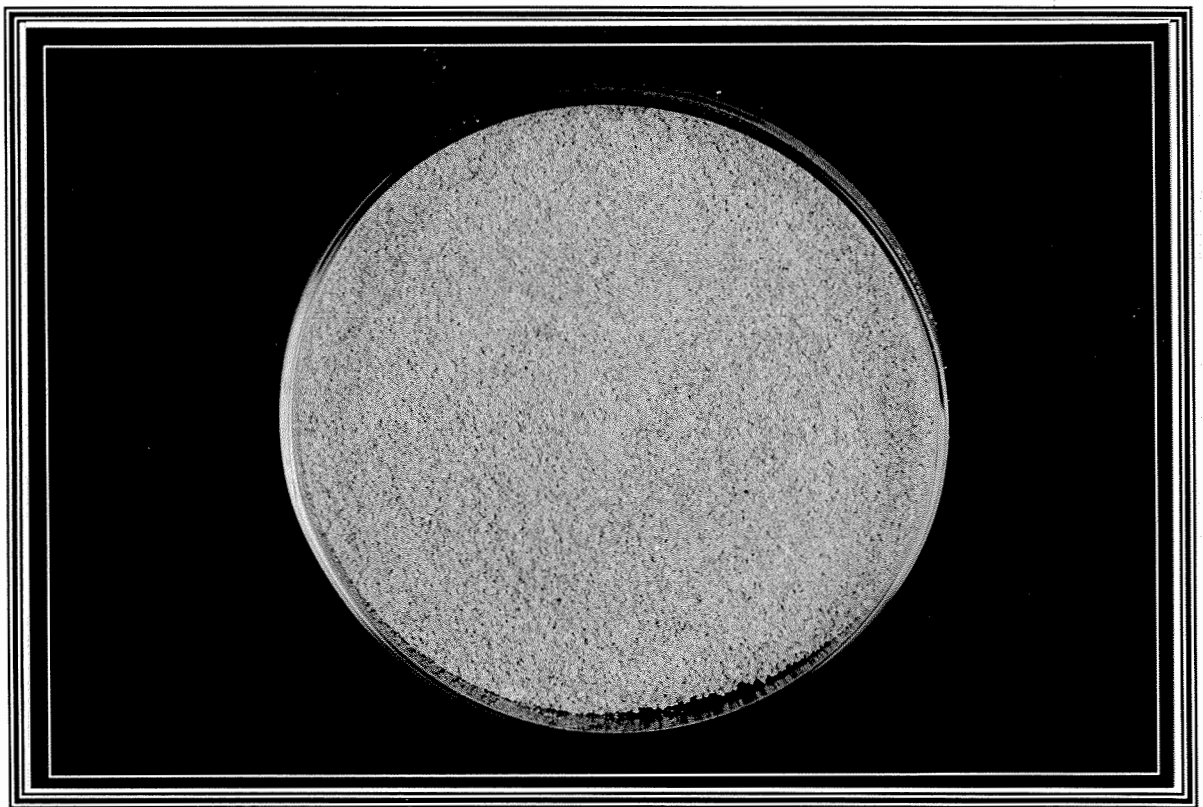


Fig. 3 Powdered seeds of plant - *Trigonella foenum graecum*

the rats were acclimatized to the experimental condition for period under investigation.

3.4 Induction of diabetic condition

All the rats after a period of acclimatization were used for the present investigation. Diabetes was induced in thirty rats using intra peritoneal injection of alloxan monohydrate (SD-Fine Chem Ltd., Mumbai) @ 120 mg/kg body weight by dissolving in 5 per cent physiological saline. These were divided into 5 groups summarized in Table3.

Table 3 Groupwise treatment of normal and diabetic rats

Group No.	No.of rats	Treatments
I	10	Normal (control)
II	10	Normal rats fed with fenugreek seed powder @ 2g/kg body weight
III	10	Diabetic control rats (Alloxan induced)
IV	10	Diabetic rats fed with fenugreek powder @ 2g/kg body weight
V	10	Diabetic rats treated with glibenclamide @ 600 µg/kg body weight

3.5 Animal feed

All the rats used in present experiment were maintained in standard laboratory conditions and were fed with rat feed procured from Pranav Agro

Industries Ltd., Pune. Fresh and pure water was supplied adlibidum to all the experimental animals.

3.6 Feeding schedule

All the animals were fed in a fixed schedule as below :

3.6.1 Control Group

All the ten (10) rats in group were fed with normal rat feed.

3.6.2 Non-diabetic treatment group

All the ten (10) rats in this group served as non-diabetic treatment group and fed with normal diet containing *Trigonella foenum graecum* seed powder @ 2 g/kg body weight.

3.6.3 Diabetic, untreated group

The ten (10) diabetic animals in this group were fed with rat feed only and no treatment was given to these animals.

3.6.4 Diabetic, plant treated group

The ten (10) diabetic wistar rats in this group served as treatment group and fed with normal diet containing *Trigonella foenum graecum* seed powder @ 2 g/kg body weight.

3.6.5 Diabetic, drug treated group

The ten (10) diabetic Wistar rats in this group were fed with normal rat feed and standard hypoglycaemic drug glibenclamide (Daonil, Aventis) @ 600 µg/kg body weight by oral route.

3.7 Glucose tolerance test

Oral glucose tolerance test was performed in six rats in each group before the commencement of treatment. The rats were fasted over night and glucose was orally administered at the rate of 2 g/kg body weight. The blood samples were collected from orbital plexus at 0, 30, 60, 90, 120, 150 and 180 minutes and glucose levels were measured immediately on 61st day again oral glucose tolerance test was repeated after over night fasting.

3.7.1 Hypoglycaemic activity

The mean per cent fall in serum glucose was calculated from the formula ;

$$\frac{\text{IBS} - \text{FBS}}{\text{IBS}} \times 100$$

Where;

IBS = Mean initial fasting serum glucose level

FBS = The mean of the post treatment readings

The peak fall in serum glucose was calculated from the same formula but with FBS as one of the post treatment readings where fall was maximum.

3.8 Observations

All the animals in control and treatment groups were observed for general behaviour, blood biochemical alterations, gross and histopathological changes.

3.8.1 General behaviour

All the animals were observed for general behaviour, activity and alertness prior and the at the end of experimental period.

3.8.2 Bio-chemical estimation

Two ml of blood was collected from each animal from orbital plexes by using ether anaesthesia. Taking all the aseptic precautions on day zero (0 day) i.e. prior to experiment but after confirmation of induction of diabetes in respective treatment groups. Day thirtyth (30th day) and on day sixtyth (60th day) i.e. on completion of experiment. The serum was collected and assessed for the blood biochemical parameters, viz.

1.	Glucose	GOD-POD	End point	Span Diagnostics
2.	Triglyceride	Enzymatic	End point	Span Diagnostics
3.	Cholesterol	Enzymatic	End point	Span Diagnostics
4.	ALT	Modified -IFCC recommended UV kinetic	Kinetic	Span Diagnostics
5.	BUN (^{Blood} urea nitrogen)	Mod-Berthlot method	End point	Crest Bio systems

The biochemical estimations were carried out by Auto Span reagent kits on auto analyser slim (SEAC) .

3.8.3 Gross pathological investigation

All the experimental animals were sacrificed at the termination of experiment and the vital organs were observed for gross pathological alterations, if any.

3.8.4 Histopathological observation

The tissues of target organs viz., liver, kidney and pancreas were collected. The tissue samples thus collected were preserved separately for histopathological examinations in 10 per cent formalin solution (Neutral buffer formal saline) for liver and kidney and Bouin's solution for pancreas.

The fixed tissues were cut into pieces of required thickness and processed by routine paraffin sections were cut at 3-5 μ thickness and stained by conventional procedures using haematoxyline and eosin (Singh and Sulochana, 1997) and Gomoris method (Singh and Sulochana, 1997) for pancreas.

3.9 Statistical analysis

All the data for blood biochemical parameter ^{were} analysed by Factorial Randomised Block Design (FRBD) as suggested by (Panse and Sukhatme, 1967).



*Results &
Discussion*

Chapter – 4

RESULTS AND DISCUSSION

The study was carried out to assess the efficacy of *Trigonella foenum graecum* seed powder on biochemical parameters like serum blood glucose, triglyceride, cholesterol, alanine transaminase and serum urea nitrogen levels of normal and alloxan induced diabetes in wistar rats.

4.1 Induction of diabetes

4.1.1 Glucose tolerance test (GTT)

Induction of diabetes was confirmed by performing the glucose tolerance test in Groups II to V rats on day 'zero' i.e. prior to treatment and the glucose tolerance status was performed in Groups I to V rats at the termination of the experiment on day sixtyth.

Table 4-5 and Fig.4 summarise the mean blood glucose level in Group II animals on day 0 and 60th of experiment at different time intervals.

The blood glucose levels (mg/dl) were observed at 0 h (92.42 ± 2.47), 30 Min. (160.28 ± 3.54), 60 min (142.31 ± 2.88), 90 min (128.17 ± 1.15), 120 min (120.22 ± 1.07), 150 min (110.69 ± 1.77) and 180 min (99.15 ± 3.76) on day 0 of the experiment, respectively.

Table 4 : Effect of *Trigonella foenum graecum* seed powder on oral glucose tolerance test in normal fasting rats*

Sr. No.	Treatment	Blood glucose level in mg/dl (Mean \pm S.E.)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
1.	Normal rats (0 day) Glucose @ 2 g/kg body weight	92.42 \pm 2.47	160.28 \pm 3.54	142.31 \pm 2.88	128.17 \pm 1.15	120.22 \pm 1.07	110.69 \pm 1.77	99.15 \pm 3.76
2.	Normal treated with seed powder (60 day) **Glucose @ 2 g/kg body weight	72.95 \pm 1.99	138.00 \pm 2.55	129.00 \pm 1.99	121.46 \pm 1.00	101.85 \pm 1.95	92.60 \pm 2.37	79.80 \pm 1.26

* Only six rats from the Group II were subjected to GTT

** Glucose was given 30 min after administration of seed powder (2g/kg body weight orally)

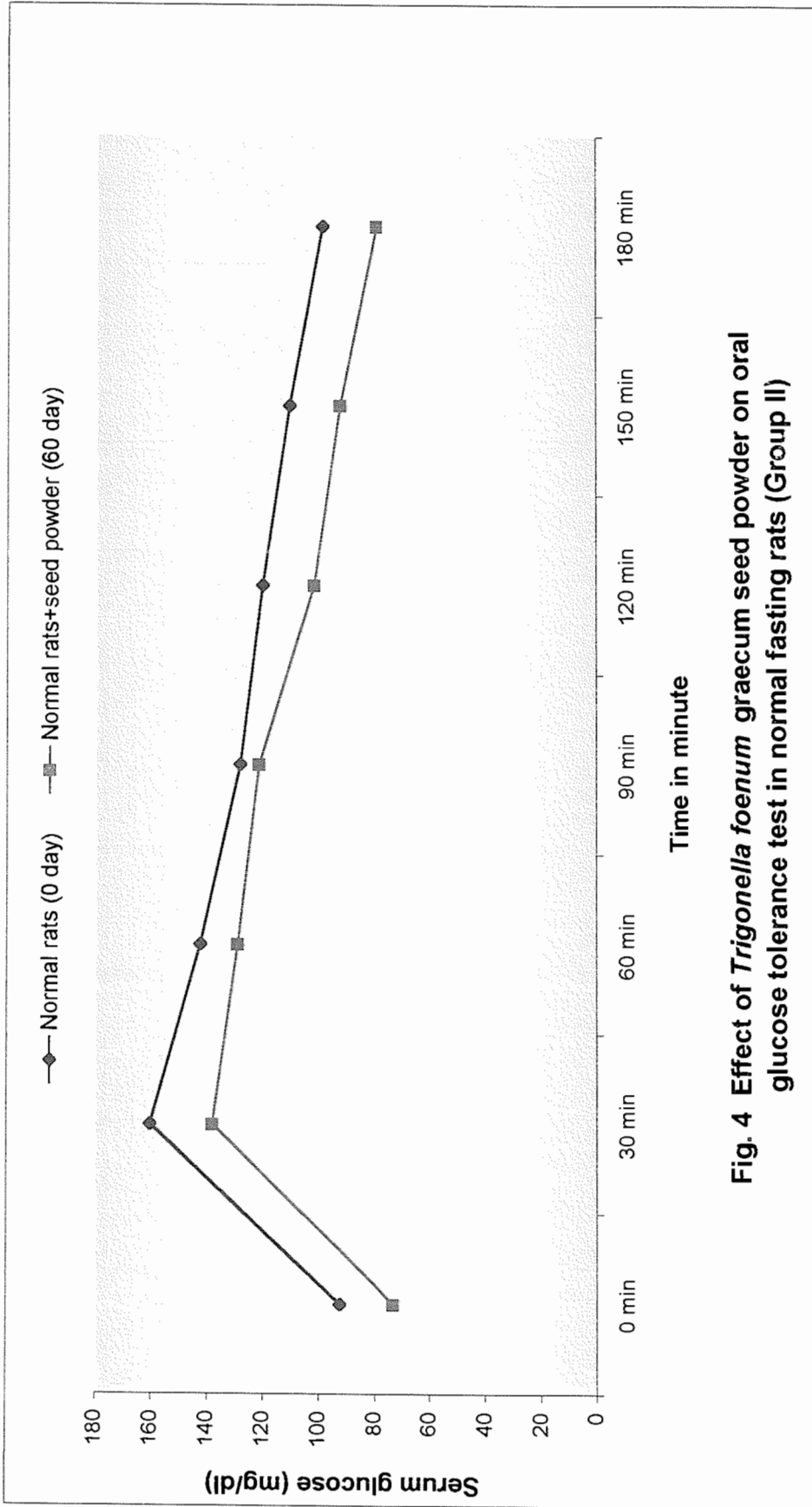


Fig. 4 Effect of *Trigonella foenum graecum* seed powder on oral glucose tolerance test in normal fasting rats (Group II)

Table 5 : Effect of *Trigonella foenum graecum* seed powder on per cent changes in blood glucose on glucose tolerance in normal fasting rats

Sr. No.	Treatment	Post treatment per cent rise in blood glucose level in mg/dl					
		30 min	60 min	90 min	120 min	150 min	180 min
1.	Normal rats (0 day)	67.86	49.89	35.75	27.8	18.27	6.73
2.	Normal treated with seed powder (60 day)	65.05	56.05	48.51	28.9	19.65	6.85

The readings on day 60th were 72.95±1.99, 138.00±2.55, 129.00±1.99, 121.46±1.00, 101.85±1.95, 92.60±2.37 and 79.80±1.26 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

The per cent change in this group animals on day 0 ranged between 67.86 to 6.73 however, it was observed to be 65.05 to 6.85 on day 60th of the experiment.

Table 6-7 and Fig.5 summarise in blood glucose levels during GTT in Group III animal. The glucose level on day 0 were 232.40±2.29, 313.69±8.72, 335.59±4.20, 320.76±2.34, 287.45±4.22, 282.05±3.46 and 275.46±4.28 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

However, glucose levels on day 60th was observed to be 253.72±2.72, 277.39±2.46, 367.01±1.91, 352.63±1.57, 328.01±2.70, 319.13±2.22 and 309.76±2.42 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

The per cent change in these group rats ranged between 103.19 to 43.06 per cent on day 0 which was observed to be 113.29 to 56.04 on day 60th.

Table 8-9 and Fig. 6 summarise in blood glucose levels during GTT in Group IV animal. The glucose levels on day 0 were 220.99±3.18, 290.18±7.90, 315.04±5.38, 300.90±4.79, 291.48±5.98, 285.54±5.16 and 236.01±8.38 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

Table 6 : Oral glucose tolerance test in alloxan diabetic fasting rats (Group III)*

Sr. No.	Treatment	Blood glucose level in mg/dl (Mean \pm S.E.)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
1.	Diabetic control (0 day) Glucose @ 2 g/kg body weight	232.40 \pm 2.29	313.69 \pm 8.72	335.59 \pm 4.20	320.76 \pm 2.34	287.45 \pm 4.22	282.05 \pm 5.46	275.46 \pm 4.28
2.	Diabetic control (60 day) Glucose @ 2 g/kg body weight	253.72 \pm 2.72	277.39 \pm 2.46	367.01 \pm 1.91	352.63 \pm 1.57	328.01 \pm 2.70	319.13 \pm 2.22	309.76 \pm 2.42

* Only six rats from the Group III were subjected to GTT

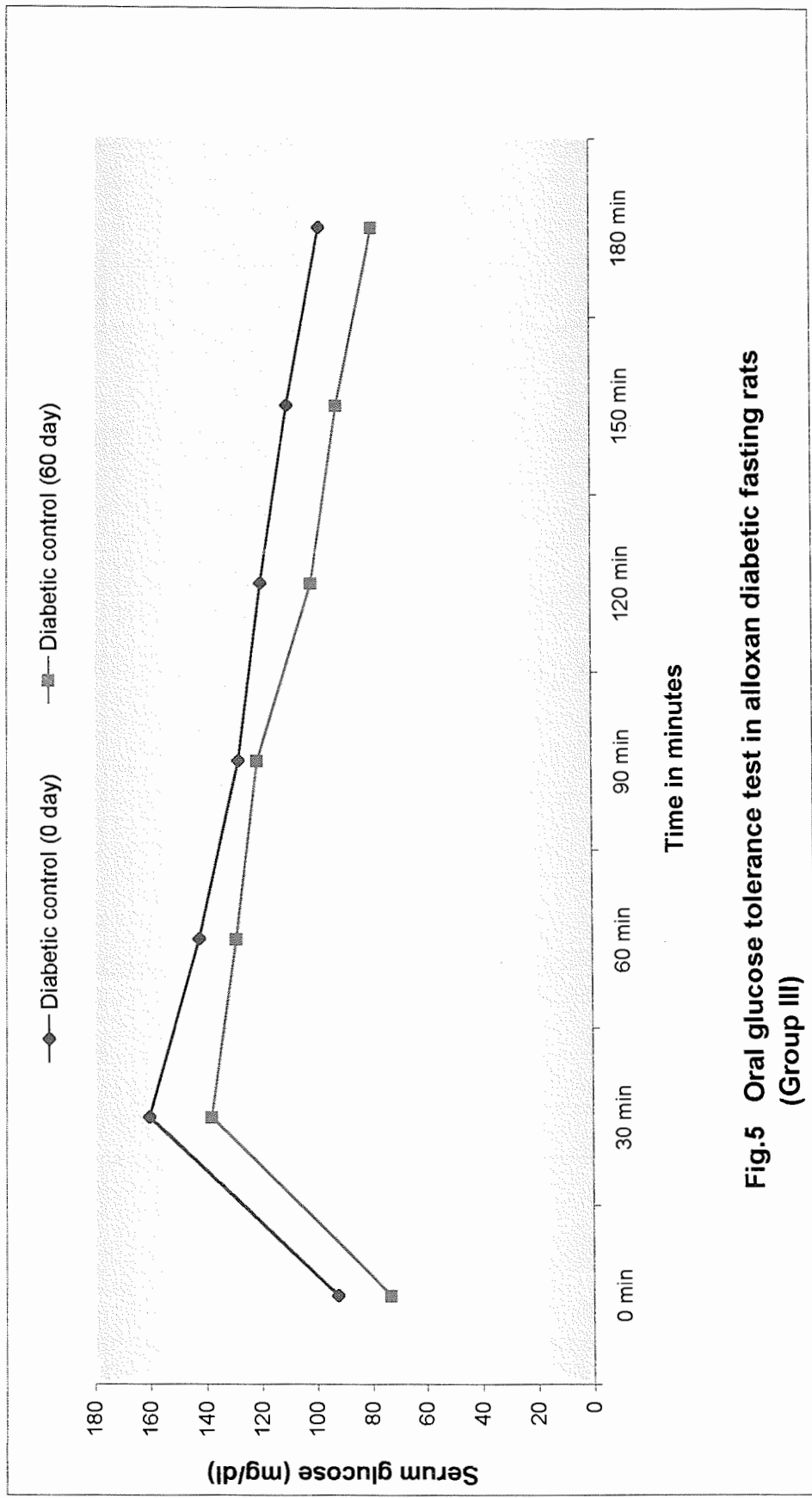


Fig.5 Oral glucose tolerance test in alloxan diabetic fasting rats (Group III)

Table 7 : Per cent changes in blood glucose on glucose tolerance in diabetic control rats (Group III)

Sr. No.	Treatment	Post treatment per cent rise in blood glucose level in mg/dl					
		30 min	60 min	90 min	120 min	150 min	180 min
1.	Diabetic control (0 day)	81.29	103.19	88.19	55.05	49.65	43.06
2.	Diabetic control (60 day)	23.67	113.29	98.91	74.29	65.41	56.04

Table 8 : Effect of *Trigonella foenum graecum* seed powder on oral glucose tolerance test in fasting diabetic rats fed with seed powder (Group IV)*

Sr. No.	Treatment	Blood glucose level in mg/dl (Mean \pm S.E.)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
1.	Diabetic rats (0 day) Glucose @ 2 g/kg body weight	220.99 \pm 3.18	290.18 \pm 7.90	315.04 \pm 5.38	300.90 \pm 4.79	291.48 \pm 5.98	285.54 \pm 5.16	236.01 \pm 8.38
2.	Diabetic rats \pm seed powder (60 day) **Glucose @ 2 g/kg body weight	117.82 \pm 1.90	181.17 \pm 7.95	246.82 \pm 11.52	226.04 \pm 5.64	185.63 \pm 2.54	154.71 \pm 3.74	120.03 \pm 2.80

* Only six rats from the Group IV were subjected to GTT

** Glucose was given 30 min after administration of seed powder (2 g/kg body weight orally)

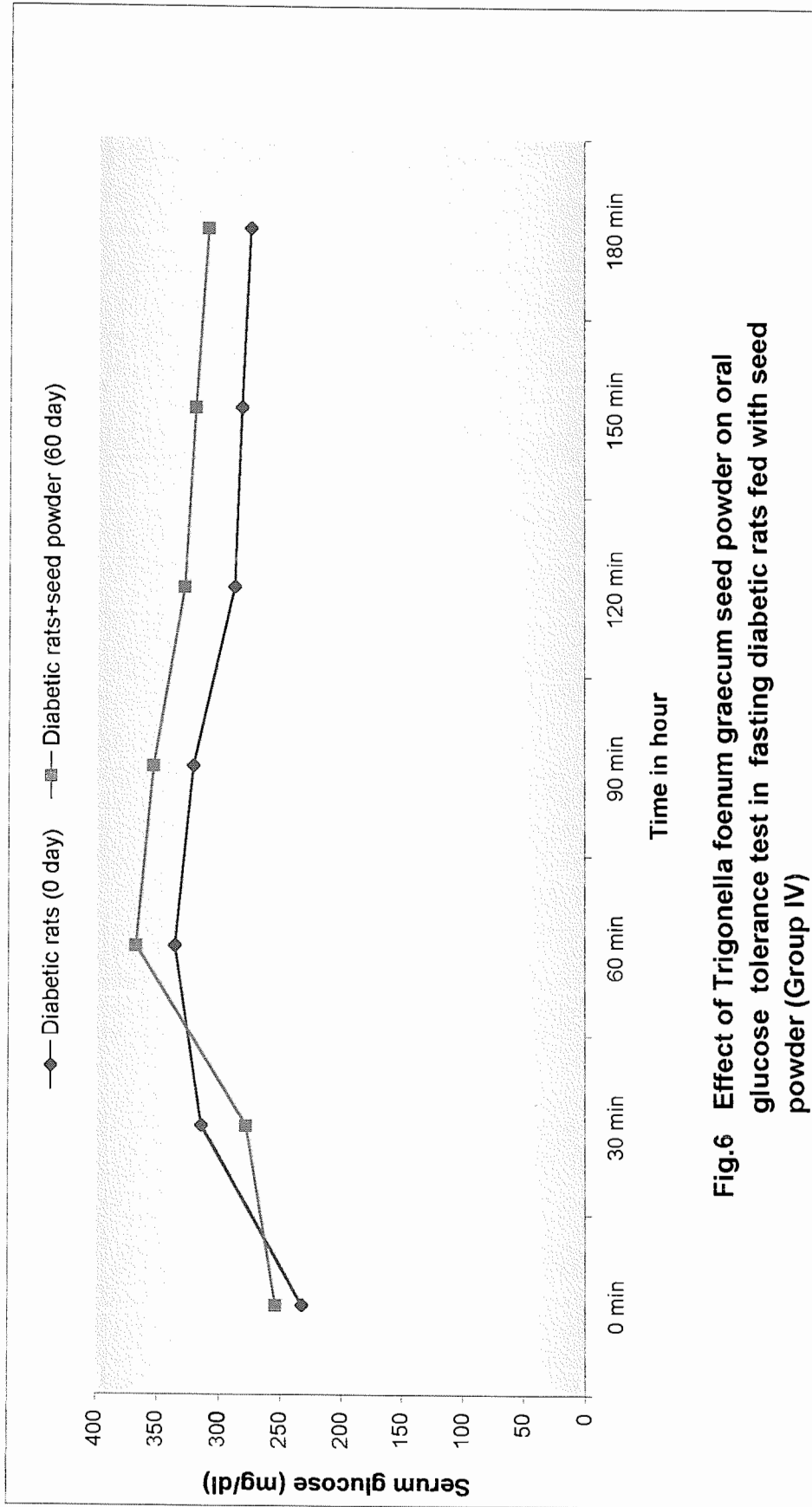


Fig.6 Effect of Trigonella foenum graecum seed powder on oral glucose tolerance test in fasting diabetic rats fed with seed powder (Group IV)

Table 9 : Effect of *Trigonella foenum graecum* seed powder on per cent changes in blood glucose on glucose tolerance in diabetic rats fed with seed powder (Group IV)

Sr. No.	Treatment	Post treatment per cent rise in blood glucose level in mg/dl					
		30 min	60 min	90 min	120 min	150 min	180 min
1.	Diabetic rats (0 day)	69.19	94.05	79.91	70.49	64.55	15.02
2.	Diabetic rats ± seed powder (60 day)	63.35	129.0	108.22	67.81	36.89	2.21

However, glucose levels on day 60th were 117.82±1.90, 181.17±7.95, 246.82±11.52, 226.04±5.64, 185.63±2.54, 154.71±3.74 and 120.03±2.80 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

The per cent change in these group rats ranged between 94.05 to 15.02 per cent on day 0 which was observed to be 129.0 to 2.21 on day 60th.

Table 10-11 and Fig.7 summarise in blood glucose levels during GTT in Group V animal.

The glucose level on day 0 were 217.85±3.13, 278.97±3.68, 311.96±4.99, 299.88±6.81, 272.14±2.20, 259.31±3.23 and 247.77±2.68 mg/dl at 0, 30, 60, 90, 120, 150 and 180 min, respectively.

However, the blood glucose levels (mg/dl) was observed at 0 h (120.10±2.55), 30 min (147.34 ±2.62), 60 min (179.45±3.44), 90 min (160.21±1.08), 120 min (121.76±2.63), 150 min (109.67±3.97) and 180 min (91.18±3.90) on the day 60th of the experiment, respectively.

The per cent change in this group animals on day 0 ranged between 94.11 to 29.92. However, it was observed to be 59.35 to 10.43 on day 60th of the experiment.

From the present results it is evident that there is no alterations in the tolerance of glucose in Group II rats (healthy, treated with *Trigonella foenum graecum*). However, improvement in the glucose tolerance was observed in

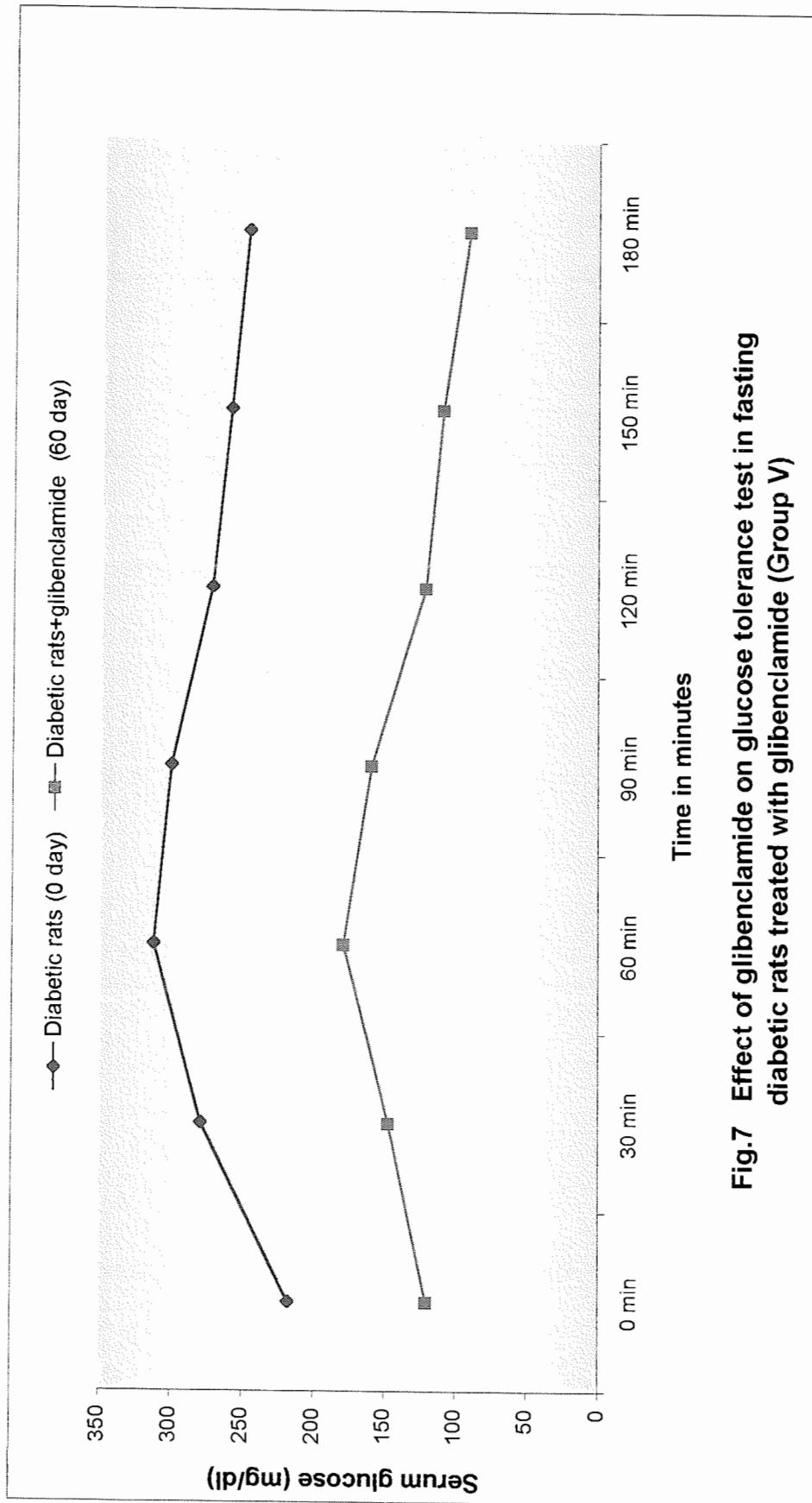


Fig.7 Effect of glibenclamide on glucose tolerance test in fasting diabetic rats treated with glibenclamide (Group V)

Table 11 : Effect of glibenclamide on per cent changes in blood glucose on glucose tolerance in diabetic rats treated with glibenclamide (Group V)

Sr. No.	Treatment	Post treatment per cent rise in blood glucose level in mg/dl					
		30 min	60 min	90 min	120 min	150 min	180 min
1.	Diabetic rats (0 day)	61.12	94.11	82.03	54.29	41.46	29.92
2.	Diabetic rats \pm glibenclamide (60 day)	27.24	59.35	40.11	1.66	10.43 \downarrow	28.92 \downarrow

Group IV rats (alloxan diabetic, treated with *Trigonella foenum graecum* @ 2 g/kg body weight) and in Group V rats (alloxan diabetic, treated with glibenclamide @ 600 µg/kg body weight).

The improvement in the treatment group is because of treatment with *Trigonella foenum graecum* in Group IV and with glibenclamide in Group V.

4.2 Observations

All the animals were observed for general behaviour, blood biochemical alterations, gross and histopathological changes.

4.2.1 General behaviour

All the animals used in present experiment were observed to be fresh and active prior to the alloxan injection.

Animals in all the groups except Group III were observed to be active throughout the experimental period. However, rats in Group III (diabetic untreated) were observed to be less active and exhausted and with deteriorating physique.

4.2.2 Blood biochemical observations

Two ml of blood was collected from orbital plexus of each animal and the serum was processed for blood biochemical alterations on days 0, i.e. prior to

the treatment and on days 30th and 60th of post treatment period. The observations are interpreted as under.

4.2.2.1 Serum glucose level

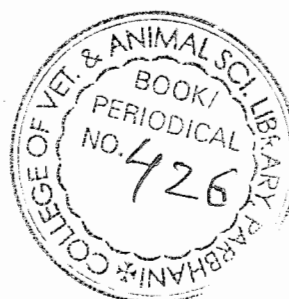
Table 12 and Fig.8 summarised the mean serum glucose levels in all the five group rats on days 0, 30th and 60th of post treatment period.

Serum glucose level in all the five groups of rats on day 0 i.e. prior to the treatment raised between 98.00 ± 2.22 to 266.45 ± 10.35 mg/dl.

The serum glucose level in control group rat was observed to be with in normal physiological limit throughout the experiment and readings were 98.00 ± 2.22 , 99.66 ± 1.36 and 104.25 ± 1.80 mg/dl on day 0, 30th and 60th respectively. No statistically significant alteration were observed.

The serum glucose level in Group II animals i.e. normal healthy rats treated with *Trigonella foenum graecum* @ 2 g/kg body weight was observed to be 101.97 ± 1.56 , 83.26 ± 2.24 and 72.61 ± 2.05 mg/dl on days 0, 30th and 60th respectively.

Statistically significant reduction ($P < 0.01$) in the serum glucose level was observed on day 30th and 60th of post treatment period when compared to that of day 0 readings. However, statistically nonsignificant reduction was observed on day 60th when compared to be 30th day readings.



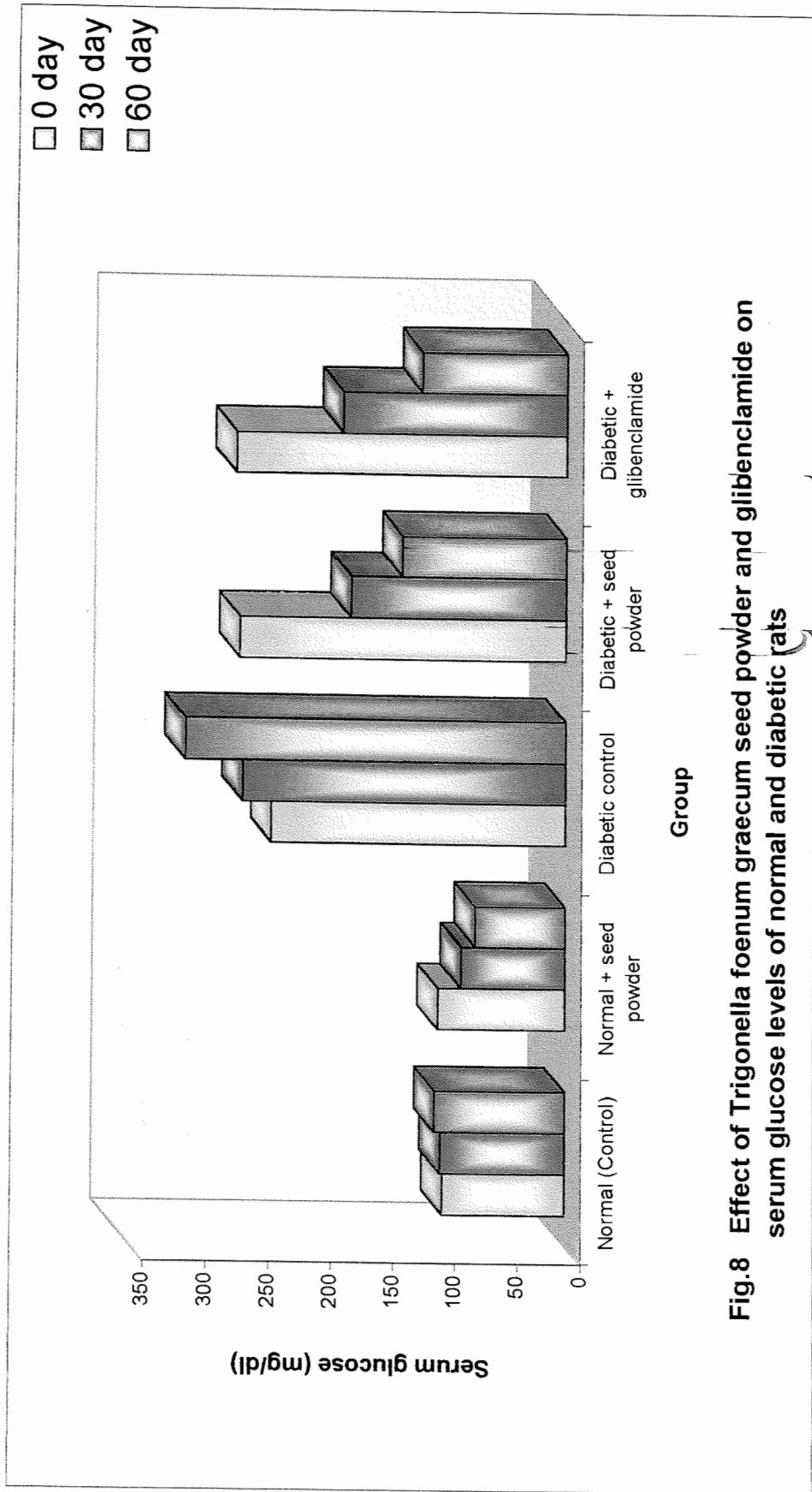


Fig.8 Effect of Trigonella foenum graecum seed powder and glibenclamide on serum glucose levels of normal and diabetic rats

The serum glucose levels in Group III animals (diabetic untreated) were observed to be 237.04 ± 5.43 , 259.96 ± 7.07 and 305.57 ± 5.82 mg/dl on day 0, 30th and 60th of post treatment period respectively.

Statistically significant increase ($P < 0.01$) in the serum glucose level was observed on day 30th and 60th when compared to that of day 0 readings. The statistically significant ($P < 0.01$) elevation was also observed in day 60th reading when compared to that of day 30th reading. The gradual increase in the serum glucose level is indicative of uncontrolled diabetic status.

Group IV rats were treated with *Trigonella foenum graecum* seed powder @ 2 g/kg body weight. The serum glucose levels in these group rat was observed to be in statistically significant ($P < 0.01$) reduction and was observed 262.83 ± 6.23 , 174.05 ± 4.51 and 133.07 ± 3.95 mg/dl on day 0, 30th and 60th respectively.

Statistically significant ($P < 0.01$) reduction was observed in the serum glucose level of day 30th and 60th when compared to that of pretreatment level, however, significant reduction was also observed on day 60th when compared to that on day 30th.

Group V rats which received the standard drug glibenclamide @ 600 μ g/kg body weight and served as standard drug treatment group.

Statistically significant reduction ($P < 0.01$) was observed in the serum glucose level in this group animals on day 30th and 60th when compared with that

pre treatment level. Statistically significant reduction ($P < 0.01$) was also observed in the serum glucose level of this group rat on day 60th when compared with that on day 30th and the levels were 266.45 ± 10.35 , 181.46 ± 4.74 and 117.18 ± 2.46 mg/dl on days 0, 30th and 60th respectively.

4.2.2.2 Glucose : mean and peak per cent changes

The mean and peak glucose per cent changes are summarised in Table 13 and Fig.9.

The mean per cent change in the serum glucose level of Group I (control group) animals was observed to be elevated by 4.03 per cent which indicated rise of 6.3 per cent in peak per cent.

The mean per cent change in serum glucose of Group II (normal healthy rats fed with *Trigonella foenum graecum* seed powder) animals was observed to be reduced by 23.57 per cent which corresponded to 28.79 per cent fall in the peak per cent alterations.

However, an increase in the glucose mean per cent (19.28 per cent) was observed corresponding to 28.09 per cent elevation in peak per cent alterations in Group III animals which served as diabetic untreated group.

Group IV rats (diabetic and treated with *Trigonella foenum graecum* seed powder @ 2 g/kg body weight) showed 41.59% reduction in the mean per cent glucose levels which corresponded to 49.35 per cent reduction in the peak per cent of serum glucose.

Table 13 : Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum glucose levels showing mean and peak per cent changes in normal and diabetic rats

Group No.*	Treatment	Mean per cent changes	Peak per cent changes
I	Normal (Control)	4.03↑	6.3↑
II	Normal ± <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	23.57 ↓	28.79 ↓
III	Diabetic control (Alloxan induced)	19.28 ↑	28.09 ↑
IV	Alloxan diabetic ± <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	41.59↓	49.35↓
V	Alloxan diabetic ± glibenclamide (@ 600µg/kg body weight)	43.95↓	56.02↓

* Each group contained ten rats

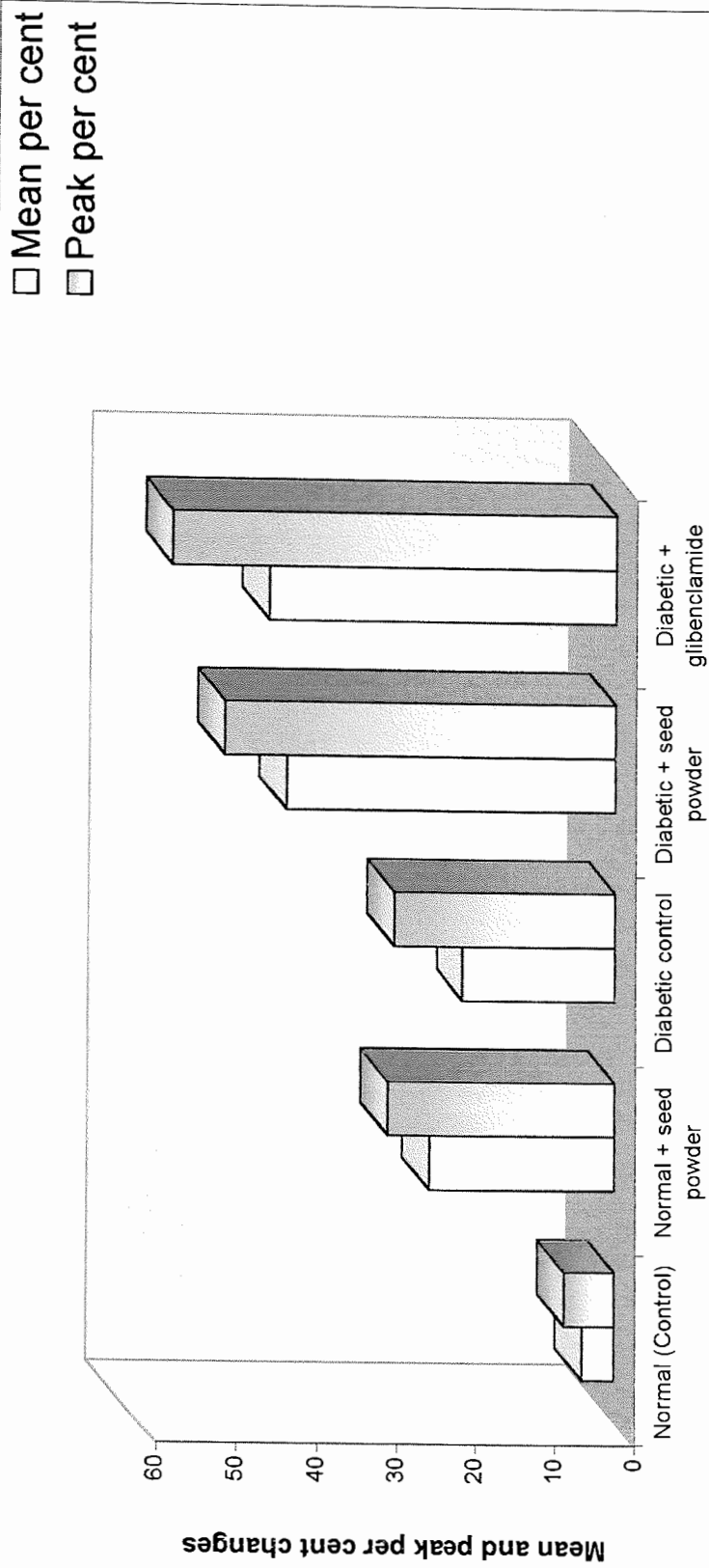


Fig.9 Effect of Trigonella foenum graecum seed powder and glibenclamide on serum glucose levels showing mean and peak per cent changes in normal and diabetic rats

The Group V rats (diabetic treated with glibenclamide @ 600 µg/kg body weight) showed reduction of 43.95 per cent in the mean serum glucose per cent change which was corresponding to 56.02 per cent fall in peak per cent change.

Khosla *et al.* (1995) reported the same results while studying the efficacy of *Trigonella foenum graecum* in rats when fed for period of 2 weeks. They also have reported 15.59 per cent fall in normal and *Trigonella foenum graecum* fed rats. However, 13.2 per cent fall was observed in the blood glucose level when the diabetic rats fed with 2 g/kg dose level of *Trigonella foenum graecum*.

Abdel-Barry *et al.* (1997), Al-Habori and Raman (1998) and Broca *et al.* (2004) reported the hypoglycaemic activity of *Trigonella foenum graecum* in rats. Broca *et al.* (2004) opined that the hypoglycaemic activity in *Trigonella foenum graecum* may be because of an increase in peripheral glucose utilization and / or by decreasing the hepatic glucose production.

4.2.2.3 Serum triglyceride level

The mean serum triglyceride levels in the control and all the four treatment groups rat are summarised in Table 14 and Fig.10.

The mean serum triglyceride levels in control group rats were 79.57±1.69 and 80.14±1.99 on day 0 i.e. prior to the treatment and on day 60th of post treatment period respectively.

Table 14 : Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum triglyceride levels of normal and diabetic rats

Group No.*	Treatment	Serum triglyceride level in mg/dl (Mean \pm S.E.)	
		Pre-treatment (0 day)	Post-treatment (60 day)
I	Normal (Control)	79.57 \pm 1.69	80.14 \pm 1.99
II	Normal \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	84.97 \pm 1.89 ^a	69.65 \pm 2.62 ^b
III	Diabetic control (Alloxan induced)	81.95 \pm 1.75 ^a	142.85 \pm 3.29 ^b
IV	Alloxan diabetic \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	80.23 \pm 0.65 ^a	110.67 \pm 4.26 ^b
V	Alloxan diabetic \pm glibenclamide (@ 600 μ g/kg body weight)	82.32 \pm 1.28 ^a	99.06 \pm 2.50 ^b

* Each group contained ten rats

a, b superscripts shows significant difference in row (P<0.01)

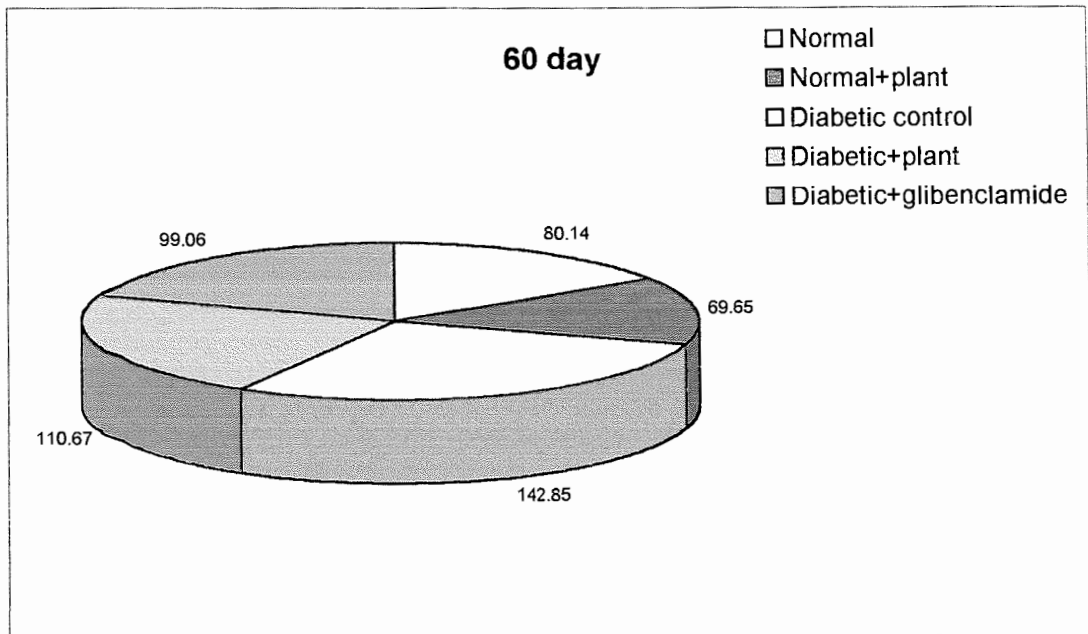
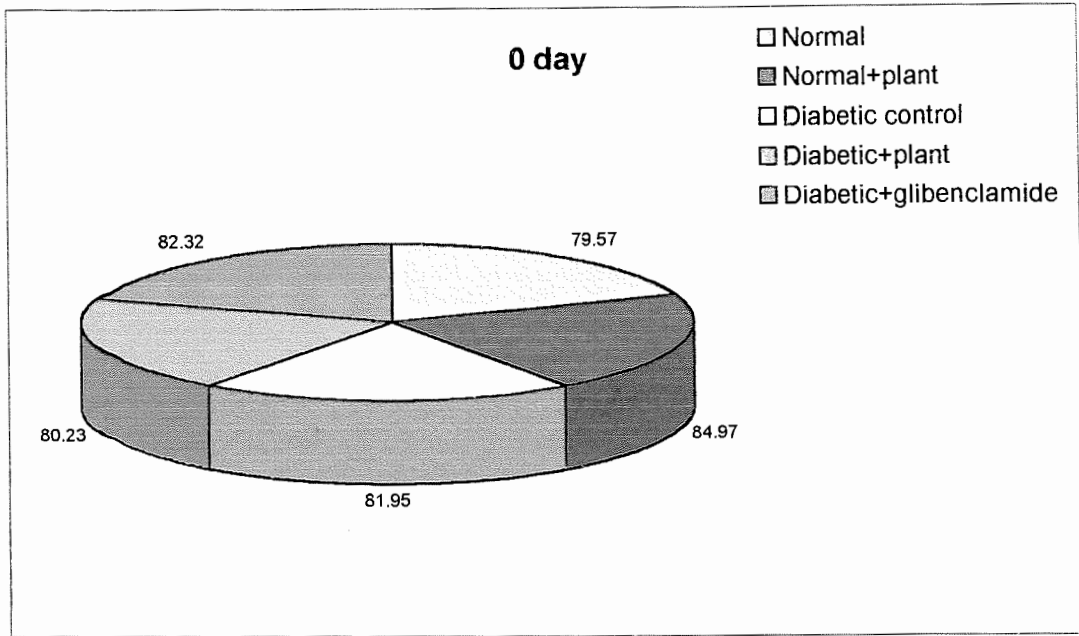


Fig.10 Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum triglyceride levels of normal and diabetic rats.

No statistically significant alteration was observed in the serum triglyceride level of control group animals.

Group II animals showed statistically significant reduction ($P < 0.01$) in the mean triglyceride level which was observed to be 84.97 ± 1.89 mg/dl on day 0 and 69.6 ± 2.62 mg/dl on day 60th of the post treatment period.

Statistically significant elevation ($P < 0.01$) in the mean triglyceride level (142.85 ± 3.29 mg/dl) was observed on day 60th when compared to its 0 day reading (81.95 ± 1.75 mg/dl) in diabetic and untreated (Group III) rats.

Statistically significant increase ($P < 0.01$) in the mean serum triglyceride level was also observed in Group IV and Group V animals.

An increase of mean serum triglyceride level from 80.23 ± 0.65 to 110.67 ± 4.26 mg/dl was observed in Group IV animals. However, an elevation from 82.32 ± 1.28 to 99.06 ± 2.50 mg/dl was noticed in Group V animals treated with standard drug glibenclamide on day 0 and 60th of post treatment period.

Khosla *et al.* (1995a) reported the hypolipidaemic effect of *Trigonella foenum graecum* when fed with @ 2 g/kg body weight in normal rats. However, they also observed an increase in the triglyceride levels in diabetic and treated with *Trigonella foenum graecum* rats. However, an increase in the triglyceride level was observed to be significantly lowered than that observed in diabetic control rats. However, the triglyceride level was observed to be 100.6 ± 3.79 mg/dl in diabetic treated rats as against 144.0 ± 6.17 mg/dl in diabetic control rats after

experimental period of 2 weeks. Venkatesan *et al.* (2003), Annida *et al.* (2004) and Yadav *et al.* (2004) reported the similar results when the experiment were carried out in rat.

4.2.2.4 Serum cholesterol level

The mean serum cholesterol levels in all five groups animals used in the present investigation are summarised in Table 15 and Fig.11.

No statistically significant alteration was observed in control (Group I) animals where the serum cholesterol level was observed to be 70.38 ± 1.29 and 71.23 ± 1.27 mg/dl on days 0 and 60th respectively.

Statistically significant reduction ($P < 0.01$) from 70.55 ± 2.43 to 57.15 ± 2.03 mg/dl in the mean serum cholesterol level was observed in Group II animals on day 0 and 60th of post treatment period.

Statistically significant elevation ($P < 0.01$) in mean serum cholesterol level was observed in all the III, IV and V group animals.

The diabetic untreated animals from Group III show^{ed} statistically significant increase ($P < 0.01$) in serum cholesterol level which was observed to be 76.78 ± 1.58 , on day 0 and 146.67 ± 2.65 mg/dl on day 60th i.e. at the termination of the experiment.

Table 15 : Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum cholesterol levels of normal and diabetic rats

Group No.*	Treatment	Serum cholesterol level in mg/dl (Mean \pm S.E.)	
		Pre-treatment (0 day)	Post-treatment (60 day)
I	Normal (Control)	70.38 \pm 1.29	71.23 \pm 1.27
II	Normal \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	70.55 \pm 2.43 ^a	57.15 \pm 2.03 ^b
III	Diabetic control (Alloxan induced)	76.78 \pm 1.58 ^a	146.67 \pm 2.65 ^b
IV	Alloxan diabetic \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	74.21 \pm 1.92 ^a	88.73 \pm 2.14 ^b
V	Alloxan diabetic \pm glibenclamide (@ 600 μ g/kg body weight)	79.48 \pm 1.59 ^a	97.87 \pm 2.18 ^b

* Each group contained ten rats

a, b Superscripts shows significant difference in row (P<0.01)

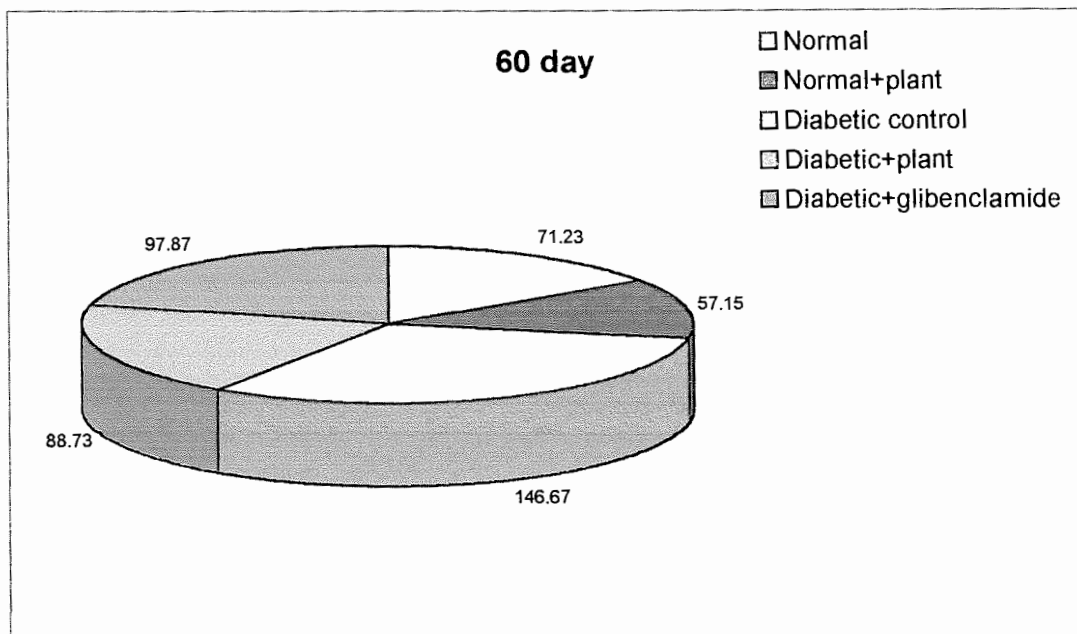
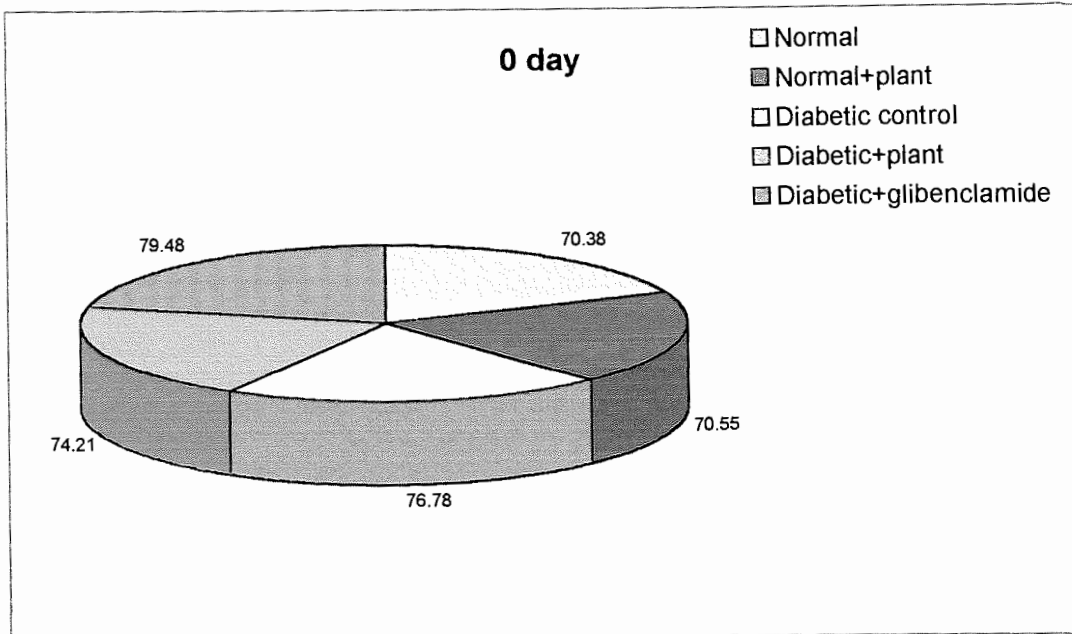


Fig.11 Effect of *Trigonella foenum* graecum seed powder and glibenclamide on serum cholesterol levels of normal and diabetic rats.

Group IV rat (diabetic fed with *Trigonella foenum graecum* @ 2 g/kg body weight) showed an increased cholesterol level (88.73 ± 2.14) mg/dl on day 60th as compared to 74.21 ± 1.92 mg/dl on day 0 of the experiment.

Statistically significant elevation ($P < 0.01$) was also observed in Group V animals (diabetic treated with glibenclamide @ 600 μ g/kg body weight) and the levels were observed to be 79.48 ± 1.59 and 97.87 ± 2.18 mg/dl on day 0 and 60th respectively.

Khosla *et al.* (1995a) reported the reduction in total cholesterol level in normal rats fed with 2 g/kg dose level of *Trigonella foenum graecum* for a period of 2 weeks. However, they also reported an increase in the total cholesterol level in diabetic and treated with *Trigonella foenum graecum* rats. The increase was observed to be significantly less in treated group as against diabetic non treated group. However, the results in the present experiments are in compared with results of Venkatesan *et al.* (2003), Annida *et al.* (2004), Yadav *et al.* (2004) and Al-Habori and Raman (1998).

An increase in lipid profile (triglyceride and total cholesterol levels) in diabetic uncontrolled group may be because of excessive mobilization of fatty acids from adipose tissue because of availability of insufficient level of insulin. However, reduction in the triglyceride and cholesterol levels in *Trigonella foenum graecum* and glibenclamide treated animals may correlate with the improved insulin status due to its hypoglycaemic action.

4.2.2.5 Serum alanine transaminase (ALT)

The serum alanine transaminase level in control and all the four treatment animals are summarised in Table 16.

The control group and animals in Group II i.e. healthy animals fed with *Trigonella foenum graecum* @ 2g/kg body weight and in Group IV, diabetic fed *Trigonella foenum graecum* @ 2 g/kg body weight showed statistically nonsignificant alterations in the mean serum ALT levels where the levels were observed to be 50.90±2.87, 53.83±3.26, 61.69±2.54 mg/dl on day 0 and 54.01±2.95, 54.96±4.24 and 64.62±2.06 mg/dl on day 60th in Group I, II and IV respectively.

Statistically significant increase ($P<0.01$) in serum ALT level was observed in diabetic untreated animals from Group III where the elevation was observed to be 91.75±3.62 mg/dl on day 60th from 65.71±3.73 mg/dl on day 0 of the experiment.

Statistically significant elevation ($P<0.01$) was also observed in diabetic and glibenclamide treated animals on day 60th which was observed to be 78.28±3.50 mg/dl when compared to that on day 0 (62.79±2.36 mg/dl).

An increase in the alanine transaminase (ALT) level in diabetic untreated group may be because of affection in liver metabolism. However, the increase ALT level in Group V animals i.e. diabetic and treated with standard drug glibenclamide may be because of the effect of glibenclamide on liver or use of drug alloxan for the induction of diabetes. However, a detail study is required.

Table 16 : Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum ALT levels of normal and diabetic rats

Group No.*	Treatment	Serum ALT level in $\mu\text{g/dl}$ (Mean \pm S.E.)	
		Pre-treatment (0 day)	Post-treatment (60 day)
I	Normal (Control)	50.90 \pm 2.87	54.01 \pm 2.95
II	Normal \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	53.83 \pm 3.26	54.96 \pm 4.24
III	Diabetic control (Alloxan induced)	65.71 \pm 3.73 ^a	91.75 \pm 3.62 ^b
IV	Alloxan diabetic \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	61.69 \pm 2.54	64.62 \pm 2.06
V	Alloxan diabetic \pm glibenclamide (@ 600 μg /kg body weight)	62.79 \pm 2.36 ^a	78.28 \pm 3.50 ^b

* Each group contained ten rats

a, b superscripts shows significant difference in row (P<0.01)

4.2.2.6 Serum urea nitrogen

The serum urea nitrogen levels (mg/dl) in all the five group rat summarised in Table 17.

The Group I (control) animals showed statistically non-significant change in the serum urea nitrogen on day 60th of post treatment period when compared to that on day 0. The values were observed to be 20.57 ± 0.63 and 21.67 ± 0.62 mg/dl on day 0 and 60th respectively.

Statistically significant reduction ($P < 0.05$) level was observed in group II rats where, the values were reduced to 19.79 ± 0.58 mg/dl on day 60th as compared to 22.92 ± 1.0 mg/dl on day 0 of the experiment.

Statistically significant increase was observed in Groups III, IV and V animals where, the serum urea nitrogen levels on day 60th were 38.09 ± 1.78 , 26.27 ± 1.52 and 31.75 ± 0.87 mg/dl when compared to 0 day readings as 21.85 ± 0.83 , 20.82 ± 0.53 and 22.45 ± 0.97 mg/dl in Group III, IV and V respectively.

The alterations in serum urea nitrogen level was observed to be statistically significant ($P < 0.05$).

An increase in the serum urea nitrogen level was observed to be reduced in normal rats fed with *trigonella foenum graecum*. An elevation in serum urea nitrogen level was observed in group III, IV and V rats. However, the

Table 17 : Effect of *Trigonella foenum graecum* seed powder and glibenclamide on serum urea nitrogen levels of normal and diabetic rats

Group No.*	Treatment	Serum urea nitrogen level in mg/dl (Mean \pm S.E.)	
		Pre-treatment (0 day)	Post-treatment (60 day)
I	Normal (Control)	20.57 \pm 0.63	21.67 \pm 0.62
II	Normal \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	22.92 \pm 1.0 ^a	19.79 \pm 0.58 ^b
III	Diabetic control (Alloxan induced)	21.85 \pm 0.83 ^a	38.09 \pm 1.78 ^b
IV	Alloxan diabetic \pm <i>Trigonella foenum graecum</i> (@ 2 g/kg body weight)	20.82 \pm 0.53 ^a	26.27 \pm 1.52 ^b
V	Alloxan diabetic \pm glibenclamide (@ 600 μ g/kg body weight)	22.45 \pm 0.97 ^a	31.75 \pm 0.87 ^b

* Each group contained ten rats

a, b superscripts shows significant difference in row (P<0.05)

serum urea nitrogen level in diabetic and *trigonella foenum graecum* treated rats (Group IV) was observed to be within physiological limits.

An increase in the serum urea nitrogen level may be because of the drug alloxan used for induction of diabetes.

4.2.3 Pathological changes

All the animals from all the five groups were sacrificed at the termination of the experiment and were subjected to the gross and microscopic examination.

4.2.3.1 Gross pathological changes

There were no gross pathological changes to naked eyes in visceral organs of the experimental groups of rats.

4.2.3.2 Histopathological changes

Histoarchitectural studies on liver, kidney and pancreas were conducted in almost all experimental rats.

Liver: In most diabetic control rats hepatocytes showed vacuolization and occasional pyknosis of nuclei. Occasional vacuolization was observed in liver of few rats belonging to Group IV. There was no appreciable histopathological change in liver of rats of Group V. The liver of Group I and II rats were histologically normal.

Kidney : The kidneys of almost^{all} rats did not showed any observable histopathological changes.

Pancreas :The pancreas of Group III rats histopathologically revealed minimum to mild degenerative changes in islets of Langerhans characterized by pale cytoplasmic beta cells. Minimum vacuolization was also observed. Three rats showed occasional necrobiotic to necrotic changes in beta cells of pancreas.

The pancreas of Group IV, V rats on subjection to histopathology revealed focal vacuolization and minimum degenerative changes. Exceptⁱⁿ Group I and Group II the pancreas of other group rats showed histopathological changes. However, in Group IV and Group V the intensity and involvement of organs in respect to histopathological changes were less as compared to Group III. Rats of Group I and Group III showed normal histoarchitecture of liver, kidney and pancreas.



Conclusions

Chapter – 5

CONCLUSIONS

Trigonella foenum graecum seed powder was assessed for its hypoglycaemic activity in normal and diabetic **Wistar** rats. From the results of the present experiment it can be concluded that :

1. The *Trigonella foenum* graecum seed powder @ 2 g/kg body weight when fed for a period of 60 days improves glucose tolerance in alloxan induced diabetic rats.
2. The *Trigonella foenum* graecum seed powder lowers the blood glucose level in normal, healthy **Wistar** rats.
3. The *Trigonella foenum* graecum seed powder contains hypoglycaemic activity which can lower blood glucose level in diabetic rats
4. The *Trigonella foenum* graecum seed powder @ 2 g/kg body weight reduces the triglyceride level in normal healthy rats.
5. The *Trigonella foenum* graecum seed powder @ 2 g/kg can also reduce the triglyceride level in alloxan induced diabetic rats.

6. The effect of *Trigonella foenum graecum* seed powder @ 2 g/kg body weight on blood glucose level is comparable to that of glibenclamide @ 600 µg/kg body weight.
7. The *Trigonella foenum graecum* seed powder can reduce the total cholesterol level in normal healthy Wistar rats.
8. The *Trigonella foenum graecum* seed powder treatment can reduce the total cholesterol level in diabetic Wistar rats when compared to that of diabetic untreated rats.
9. The effect of *Trigonella foenum graecum* seed powder @ 2 g/kg body weight on total cholesterol level is comparable to the effect of glibenclamide @ 600 µg/kg body weight.
10. Glibenclamide @ 600 µg/kg body weight can increase the serum ALT level in alloxan induced diabetic rats.
11. The treatment of *Trigonella foenum graecum* seed powder and glibenclamide may lead to an elevation in the serum urea nitrogen when used for 60 consecutive days in Wistar rats.
12. The treatment of *Trigonella foenum graecum* seed powder @ 2 g/kg body weight may increase the serum urea nitrogen level in normal healthy Wistar rats when fed for 60 days.



Summary

Chapter – 6

SUMMARY

An experiment was carried out to assess the hypoglycaemic activity of *Trigonella foenum graecum* seed powder when fed @ 2 g/kg body weight for 60 consecutive days in normal and alloxan induced diabetic Wistar rats. The results were also compared against the standard allopathic ^{antidiabetic} compound glibenclamide when fed @ 600 µg/kg body weight.

Fifty Wistar rats were randomly divided into five groups, each group containing ten rats and served as experimental animals. Diabetes was induced using alloxan monohydrate injected intraperitoneally @ 120 mg/kg in Group III to V animals.

Diabetes was confirmed by conducting glucose tolerance test on day 0 of the experiment. Group II and Group IV animals were treated with the *Trigonella foenum graecum* seed powder @ 2 g/kg body weight. Where, Group II animals served as normal, treated group and Group IV animals were diabetic and treated animals.

The Group V animals received standard allopathic hypoglycaemic drug glibenclamide @ 600 µg/kg body weight. The experiment ^{was} carried out for a period of 60 days. The observations viz. glucose tolerance test, serum glucose level, triglycerides, cholesterol, alanine transaminase, serum urea nitrogen, gross

and histopathological changes were studied during the experimental period of 60 days.

Rats in control as well as four treatment groups did not show any abnormal and treatment related behavioural changes throughout the period of experiment.

No alterations was observed in the glucose tolerance of Group II animal on day 60th when compared to that on day 0. The glucose tolerance was observed to be reduced at 60th, 90th, 120th, 150th and 180th min of glucose tolerance test in Group III animals. However, glucose tolerance was observed to be improved at 120th, 150th and 180th min in *Trigonella foenum graecum* seed powder treated group (Group IV) and at 30th, 60th, 90th, 120th, 150th and 180th min of glucose tolerance test in Group V animals treated with glibenclamide.

NO significant change was observed in serum glucose level of control group animals. However, the serum glucose level in Group II, IV and V animals was observed to be significantly lowered ($P < 0.01$) on day 30th and 60th of post treatment.

The serum glucose level in Group III animals i.e. diabetic and untreated group was observed to be significantly higher ($P < 0.01$) on day 30th and 60th when compared to that on day 0.

The mean per cent fall in the serum glucose level was observed to be comparable in Group IV animals i.e. treated with *Trigonella foenum graecum*

seed powder @ 2 g/kg body weight against Group V animals treated with glibenclamide @ 600 µg/kg body weight.

No significant alterations were observed in serum triglyceride level of rats from control group. Statistically significant reduction in triglyceride level was observed in Group II animals i.e. normal and treated with *Trigonella foenum graecum* seed powder @ 2 g/kg body weight. Statistically significant elevation ($P < 0.01$) was observed in triglyceride level of Group III, Group IV and Group V animals. However, the elevation in glibenclamide treated animals were observed to be less when compared to that of Group IV animals.

No significant alterations were observed in total cholesterol level of Group I animals. Group II animals showed statistically significant reduction ($P < 0.01$) in total cholesterol level on day 60th when compared that on day 0.

Statistically significant elevation ($P < 0.01$) in total cholesterol level was observed in Group III, Group IV and Group V animals. However, the elevation was observed to be less in Group IV animals treated with *Trigonella foenum graecum* seed powder @ 2 g/kg body weight as against Group V treated with glibenclamide @ 600 µg/kg body weight.

No statistically significant alterations in serum ALT level was observed in Group I, Group II and Group IV animals. However, significant elevation ($P < 0.01$) in the serum ALT level was observed in diabetic untreated animals from Group III and diabetic, glibenclamide treated animals from Group V.

No statistically significant alterations in serum urea nitrogen was observed in Group I animals. However, statistically significant elevation (P<0.05) in the serum urea nitrogen was observed in Group III, IV and V animals.

Statistically significant reduction (P<0.05) in the serum urea nitrogen was observed in Group II animals.

No visible gross pathological changes were observed in the visceral organs. The microscopic changes revealed pyknosis and vacuolization in liver and pancreas. The kidneys did not showed any observable histopathological changes in the treatment group rats.



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Vitae

VITAE

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He participated in National Service Scheme (N.S.S.) unit of Parbhani Veterinary College and enrolled as a member of N.S.S. special campaign programmes. He is well conversant with computer application and passed MS-CIT certificate on computer concepts with B grade. He participated actively in sports and cultural programmes during undergraduate programme.