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A STUDY OF PHARMACODYNAMIC DRUG INTERACTIONS BETWEEN *TRIGONELLA FOENUMGRAECUM* AND GLIBENCLAMIDE IN RATS

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ABSTRACT

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world. It is a deadly disease that affects an estimated 135 million people worldwide and numbers are increasing in rural and poor populations throughout the world. It is a condition in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. Present study aims to investigate the possible pharmacodynamic drug interactions between the routinely used natural condiment fenugreek and the commonly used antidiabetic drug glibenclamide in alloxan induced rats. For this study fenugreek (0.5 g/kg and 1 g/kg/p.o) and glibenclamide (0.45 mg/kg/p.o) were given to diabetic rats both alone and also in combination for 28 days. To assess the pharmacodynamic drug interactions, the parameters monitored were oral glucose tolerance test (OGTT), plasma glucose levels, aspartate aminotransferase (AST), alanine aminotransferase (ALT), liver glycogen and insulin levels. Administration of fenugreek and glibenclamide doesn't show any significant pharmacodynamic interactions. The results clearly indicate significant hypoglycaemic effect in oral glucose tolerance test, decrease in blood glucose, AST and ALT levels in diabetic rats when treated with fenugreek and glibenclamide both as alone and also in combination. Similarly, the effect of glibenclamide on liver glycogen and plasma insulin was also unaffected when given along with fenugreek. Moreover, the combination has shown good antioxidant property.

KEY WORDS

Pharmacodynamic drug interaction, alloxan, Trigonella foenumgraecum, fenugreek

INTRODUCTION:

Herbal medicine also called botanical medicine or phytomedicine refers to using plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio friendly plant-based products for the prevention and cure of different human diseases. Diabetes mellitus is considered as one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates ten years ago and this may double by the year 2030. Diabetes characterized by a relative or absolute insufficiency of insulin secretion, insulin dependent diabetes mellitus (IDDM) or concomitant resistance of the metabolic action of insulin on target tissues, non-insulin dependent diabetes mellitus (NIDDM)[1].

Insulin therapy affords glycemic control in IDDM, fatal hypoglycemia in the event of excess dosage, resistance due to prolonged administration,

limits its usage. Similarly, treatment of NIDDM patients with sulfonylureas and biguanides is always associated with side effects[2]. Hence, search for a drug with low cost, more potential, and without adverse side effects is being pursued in the several laboratories around the world. Many reports of herb drug interactions are sketchy and lack laboratory analysis of suspected preparations. Health-care practitioners should caution patients against mixing herbs and pharmaceutical drugs. Because physicians are likely to encounter patients who are using herbal remedies, they need to be aware of the purported effects of these products. They also need to be cognizant of the adverse effects of herbal remedies and the possibility of deleterious drug interactions[3]. Trigonella foenum-graecum commonly called as fenugreek is used to treat diabetes and glibenclamide is the most commonly used antidiabetic drug. Hence in the present study possible pharmacodynamic drug interactions between fenugreek and glibenclamide were assessed.

MATERIALS AND METHODS:

Animals

Wistar albino rats of 150-220g either sex were used for the study. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory condition (12 hr light and 12 hr dark cycle) and had free access to commercial pellet diet (Hindustan Lever Ltd., Bombay, India) with water ad libitum. The animal house temperature was maintained at 25 ± 2^{0}C with relative humidity at (50 ±15%). The study was approved by the Institutional Animal Ethical Committee of St.John College of Pharmacy (). Ethical norms were strictly followed during all experiments.

Collection and authentication of plant material:

The Trigonella foenum-graecum (fenugreek) seeds were collected from the local market of Warangal, Andhra pradesh and were authentified by Department of Botany, Kakatiya University, Warangal.

Preparation of aqueous extract

Fenugreek seeds were soaked overnight in water and were extracted with water in 1:3 ratio for 30 min by heating over a water bath. The extract was filtered, and wt/ml was randomly calculated and administered freshly to the experimental animals.

Chemicals:

Alloxan was procured from Hi Media Pvt Ltd., IND. Glibenclamide, was received as a gift drug from Aventis Pharma Ltd. All other chemicals used were of analytical grade obtained from Sd-Fine, India.

Instruments

UV-Visible spectrophotometer, electronic balance, homogenizer, centrifuge.

PHARMACOLOGICAL STUDIES

Oral glucose tolerance test

Wistar albino rats were fasted overnight and divided into four groups with 6 animals in each group. Group-I serves as normal. Group-II animals were treated with glibenclamide (0.45 mg/kg/p.o) to serve as standard. Group-III and group-IV animals were treated with glibenclamide and fenugreek (0.5 and 1 g/kg/p.o) respectively. The normal, standard and test groups were treated with drugs 30 minutes prior to the glucose load (3 g/kg/p.o). Blood samples were collected at 0, 15, 30, 60, 90, 120 and 180 min after glucose loading. Serum was separated, and glucose levels were measured immediately using standard procedures[4].

Anti-diabetic study-Interaction between glibenclamide and fenugreek

In the present study, diabetes was induced by subcutaneous injection of alloxan (100mg/kg)[5]. The alloxan was freshly prepared by dissolving 100 mg in 1ml of normal saline solution. The

animals were allowed to drink glucose solution overnight to overcome the alloxan induced hypoglycaemia. 48 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of >200 mg/dl were selected for the study. The rats were divided randomly into seven groups consisting of six rats each and the animals were treated for 28 days[6].

Group I: Served as control group received the 1% SCMC (2 mL/kg).

Group II: Served as disease control Alloxan (100mg/kg, s.c).

Group III: Received standard dug (Alloxan (100mg/kg, s.c) + Glibenclamide (0.45 mg/kg, p.o))

Group IV: Received low dose of fenugreek Alloxan (100mg/kg, s.c) + Fenugreek,(0.5 g/kg, p.o) to study the effect of low dose of fenugreek.

Group V: Received high dose of fenugreek Alloxan (100mg/kg, s.c) + Fenugreek,(1 g/kg, p.o) to study the effect of high dose of fenugreek

Group VI: Received Alloxan (100mg/kg, s.c) + Glibenclamide(0.45 mg/kg, p.o) + Fenugreek(0.5 g/kg, p.o) to study the effect of fenugreek (LD) when given with glibenclamide.

Group VII: Received Alloxan (100mg/kg, s.c) + Glibenclamide(0.45 mg/kg, p.o) + Fenugreek (1g/kg, p.o) to study the effect of fenugreek (HD) when given with glibenclamide.

Collection of blood samples

The blood samples were withdrawn on 7th, 14th, 21st and 28 th day from the retro orbital plexus of rats under anesthesia using a glass capillary tube after a fast of 6 hrs and the blood was centrifuged (2,500 rpm for 10 min) to get serum. The serum was used for biochemical estimation of blood glucose, aspartate aminotrasferase (AST), alanine aminotransferase (ALT), liver glycogen levels and insulin levels.

Estimation of blood glucose

Blood glucose was estimated by using glucose kit obtained from Span Diagnostics.

***Method:** GOD-POD method[7]*

***Principle:** Glucose oxidase (GOD) oxidizes glucose to gluconic acid and H_2O_2. In presence of enzyme peroxidase, released H_2O_2 is coupled with phenol and 4-aminoantipyrine (4-AAP) to form coloured quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.*

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucokinase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + 4-\text{AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye}$$

Aspartate amino transferase (AST):

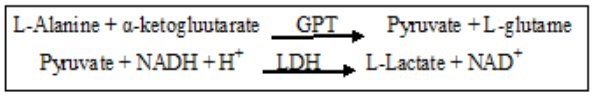
AST levels in serum were estimated using GOT/AST test kit using IFCC method without pyridoxal phosphate. Glutamate oxaloacetate transaminase (GOT) also known as Aspartate aminotransferase (AST) is a transaminase, GOT catalyses the transfer of the amino group of L-aspartate to α-ketoglutarate to give L-glutamate. GOT is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles and kidneys. Damages to cells of these tissues induce GOT increase in serum. The levels were measured at 340 nm[8, 9,10].

L-Aspartate + α-ketogluutarate $\xrightarrow{\text{GOT}}$ Oxaloacetate + L-Glutamate
Oxaloacetate + NAD + H^+ $\xrightarrow{\text{MDH}}$ L-Malate + NAD^+

Alanine amino transferase (ALT):

Alanine amino transferase (ALT) levels in serum were estimated using ALT test kit using IFCC method. Glutamate pyruvate transaminase (GPT) also known as alanine amino transferase (ALT) is a transaminase. GPT catalyses the transfer of the amino group of L-alanine to α-ketoglutarate to give L-glutamate. The highest levels are found in the liver and the kidneys, and

in smaller amounts in heart and skeletal muscle. GPT concentration is increased when hepatic cells are damaged (liver cells necroses or injury of any cause). The levels were measured at 340 nm[10, 11, 12].



ESTIMATION OF LIVER GLYCOGEN

Animals were decapitated and cut open to excise the liver. The liver was stored in normal saline. A 200mg sample of liver was weighed on a torsion balancing finely ground with 20% TCA in a homogenizer[13]. The precipitate of proteins was filtered off and the clear filtrate was submitted for the following analysis.

Procedure

In a colorimeter tube 2 ml of liver extract was added to 3 ml of iodine reagent. After mixing the optical density was read in a photometer at 650 nm against a blank, obtained by adding 2 ml of 5% TCA to 3 ml of reagent in the same way. The amount of glycogen was read from a calibration curve.

ESTIMATION OF INSULIN LEVELS

Blood sample was withdrawn in a heparinized capillary tube from the retro-orbital venous plexus under ether anaesthesia. Plasma insulin content was measured by radioimmunoassay technique using insulin RIA kit.

ESTIMATION OF ANTIOXIDANT PARAMETERS

Preparation of homogenate

The animals were sacrificed, and pancreas was isolated and weighed, the homogenate is prepared as follows:

Procedure

Excised pancreas was cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in ice cold 10 mM tris HCL buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and non-vascular matter in a sediment able form. It was then centrifuged in cooling centrifuge at 5000 rpm at 20ºC temperature and clear supernatant was separated and used to estimate superoxide dismutase (SOD), catalase, reduced glutathione(GSH) and lipid peroxidation (LPO).

In vivo antioxidant parameters

a) Superoxide dismutase (SOD)[14]

Principle

Rate of auto oxidation of epinephrine and the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8 – 10.2, O_2 generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2 introduced. The auto oxidation of epinephrine proceeds by at least two distinct pathways only one of which is free radical chain reaction involving O_2 and hence inhabitable by SOD.

Procedure

0.5 ml of sample was diluted with 0.5 ml of distilled water, to this 0.25 ml ethanol, 0.5 ml of chloroform (all reagents chilled) was added the mixture was shaken for one minute and centrifuged at 2000 rpm for 20 minutes. The enzymatic activity in supernatant was determined. To 0.05 ml of carbonate buffer (0.05 M, pH 10.2) and 0.5 ml of EDTA (0.49 M) was added. The reaction was initiated by the addition of 0.4 ml of epinephrine and the change in optical density/min was measured at 480 nm. SOD activity was expressed as units / mg protein change in optical density/min. 50% inhibition of epinephrine to adrenochrome transition by enzyme is taken the enzyme unit. Calibration

curve was prepared by using 10 – 125 units of SOD.

b) Catalase[15]

Principle

In UV range H_2O_2 can be followed directly by the decrease in absorbance (O.D 240) per unit time is a measure of catalase activity.

$H_2O_2 \rightarrow H_2 + O_2$

$RDOH \rightarrow H_2O + ROH + A$

Decomposition of H_2O_2 = Decrease in absorbance at 240 nm.

Dilute homogenate 20 times with Phosphate buffer pH 7.0

c) Reduced Glutathione (GSH)[16]

Procedure

To 1ml of sample 1ml of 10% TCA was added. The precipitated fraction was centrifuged and to 0.5 ml supernatant, 2 ml DTNB was added. The final volume was made up to 3 ml with phosphate buffer. The colour developed was read at 412 nm. The amount of glutathione was expressed as µg of GSH/mg protein reduced glutathione was used as standard (100 µg/ml).

d) Lipid peroxidation (Malondialdehyde formation)[17]

Procedure

2 ml of sample was mixed with 2 ml of 20% trichloroacetic acid and kept in ice for 15 min. The precipitate was separated by centrifugation and 2 ml of samples of clear supernatant solution were mixed with 2 ml of aqueous 0.67% thiobarbituric acid. This mixture was heated on a boiling water bath for 10 minutes. It was cooled in ice for 5 min and absorbance was read at 535 nm. The values were expressed as nm of MDA formed/mg of protein. Values are normalized to protein content of tissues.

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Tukey test using computer based fitting program (Prism, Graph pad 5.0). Statistical significance was set accordingly.

RESULTS:

Effect on glucose tolerance

The blood glucose levels in the control group (G-I) increased upto 60 min after glucose load. Group II animals treated with glibenclamide (0.45 mg/kg, p.o) didn't show significant increase in the blood glucose levels upon glucose load. Group III and IV treated with combination of glibenclamide and fenugreek (0.5 g/kg and 1 g/kg, p.o) also prevented raise of blood glucose levels upon glucose load, when compared with control animals (G-I). This suggests that hypoglycemic effect of glibenclamide is unaffected when given in combination with fenugreek. Moreover, the combination was found to show additive effect (Table 1).

Table No 1: Effect of Trigonella foenum graceum and glibenclamide on oral glucose tolerance test

GROUP	Blood Glucose levels(mg/dl)					
	O minutes	15 min	30 min	60 min	120 min	180 min
Control	90.8 ± 1.485	192 ±2.576	229.4 ± 3.938	252.9 ± 2.041	205 ± 1.589	175 ± 2.038
Glibenclamide (0.45 mg/kg, po)	94.12 ± 2.350	149.4 ±1.787^{a***}	132.2 ± 1.035^{a***}	143.5 ±1.948^{a***}	130.2 ± 2.380^{a***}	110 ± 0.933^{a***}
Glibenclamide (0.45 mg/kg, po) + fenugreek (ld) (0.5 g/kg, po)	102.5 ± 1.212	129.4 ± 1.076^{b**}	135.9 ± 1.976 ^{b***}	149.8 ± 1.618 ^{b***}	119.8± 1.631^{b**}	93.85± 0.253^{b***}
Glibenclamide + fenugreek (hd) (1 gm/kg, po)	97.32 ± 1.153	91.03 ± 1.390^{b***}	99.12 ± 0.928^{b**}	121.81 ±0.66^{b***}	104.3 ± 1.142^{b***}	91.00± 0.580^{b***}

Values are expressed as Mean ± SEM of 6 animals.

*Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, . **p<0.01, ***p<0.001, ns – Non-significant.*

Effect on blood glucose levels

Animals treated with alloxan (100 mg/kg, s.c) (G-II) alone showed a significant increase in blood glucose levels on 0, 7th and 14th day when compared to normal animals (G-I) (p<0.001). Treatment with glibenclamide (0.45 mg/kg, p.o) (G-III) caused a significant decrease in blood glucose levels on 7th and 14th day when compared to the disease control group (G-II) (p<0.001). Administration of fenugreek at two different doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) showed a significant dose dependent decrease in blood glucose levels on day 7 and 14 when compared to the disease control group (G-II) respectively (p<0.001). Animals when treated with glibenclamide (0.45 mg/kg, p.o) and fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-VI and VII) also showed significant decrease in blood glucose levels when compared to control group (G-II) on day 7 and 14 respectively (p<0.001). The decrease in blood glucose levels were within the normal range, which indicates that the combination of glibenclamide and fenugreek neither produce neither severe hypoglycaemia nor effects antidiabetic activity of glibenclamide (Table 2).

Table No 2: Effect of Trigonella foenum graceum and glibenclamide on serum glucose levels

GROUP	TREATMENT	Blood Glucose Levels(mg/dl)		
		0^{th} day	7^{th} day	14^{th} day
I	Normal	78 ± 2.121	80 ± 1.826	74 ± 1.826
II	Alloxan (100 mg/kg,s.c)	310 ± 6.377^{a}	280 ± 7.292^{a***}	297 ± 2.944^{a***}
III	Alloxan(100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po)	302 ± 1.581	87.4 ± 3.555^{b***}	83 ± 0.816^{b***}
IV	Alloxan (100 mg/kg,s.c) +Fenugreek (0.5 g/kg, po)	299.5 ± 1.041	199 ± 2.483^{b***}	110 ± 3.853^{b**}
V	Alloxan (100 mg/kg,s.c) +Fenugreek (1g/kg, po)	297 ± 2.287	127 ± 2.345^{b**}	98.5 ± 3.116^{b***}
VI	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po) + Fenugreek (0.5 g/kg, po)	296.5 ± 2.872	85 ± 1.909^{b***}	84.5 ± 1.531^{b***}
VII	Alloxan (100 mg/kg,s.c) +Glibenclamide + Fenugreek (1g/kg, po)	296.8 ± 1.181	84.5 ± 1.771^{b***}	82.2 ± 2.698^{b***}

Values are expressed as Mean ± SEM of 6 animals.

Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test".

*Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII. **p<0.01, ***p<0.001, ns – Non-significant*

Table No 3: Effect of Trigonella foenum graceum and glibenclamide on serum alanine aminotransferanse (ALT) levels

GROUP	TREATMENT	Serum ALT Levels		
		0^{th} day	7^{th} day	14^{th} day
I	Normal	14.8 ± 1.225	15.3 ± 1.052	16.1 ± 1.102
II	Alloxan (100 mg/kg,s.c)	16.5 ± 0.213^{a}	58.5 ± 1.277^{a***}	59.2 ± 1.466^{a**}
III	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po)	16.32 ± 0.124	35.2 ± 1.184^{b***}	38.9 ± 0.816^{b***}
IV	Alloxan (100 mg/kg,s.c) +Fenugreek (0.5 g/kg, po)	15.9 ± 0.373	15.2 ± 1.277^{b***}	14.8 ± 1.251^{b***}
V	Alloxan (100 mg/kg,s.c) +Fenugreek (1 g/kg, po)	16.1 ± 0.271	15.3 ± 1.541^{b**}	13.2 ± 1.290^{b***}
VI	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po) + Fenugreek (0.5 g/kg, po)	15.3 ± 1.331	15.8 ± 1.791^{b**}	15.2 ± 0.918^{b***}
VII	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po) + Fenugreek (1 g/kg, po)	15.4 ± 1.639	15.9 ± 1.012^{b***}	16.3 ± 1.320^{b***}

Values are expressed as Mean ± SEM of 6 animals.

*Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII. **p<0.01, ***p<0.001, ns – Non-significant*

Effect on serum ALT levels

In the group treated with alloxan (100 mg/kg, s.c) (G-II) a significant increase in ALT levels were observed when compared to the normal group (G-I) ($p<0.001$). The group III rats treated with standard drug glibenclamide (0.45 mg/kg, p.o) showed a significant decrease in ALT levels on 7^{th} and 14^{th} day, when compared to the disease control group (G-II) ($p<0.001$). Treatment with fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) showed a significant decrease in ALT levels on 7^{th} and 14^{th} day when compared to the disease control group (G-II) ($p<0.001$). The groups VI and VII treated with both glibenclamide (0.45 mg/kg, p.o) and fenugreek (0.5 g/kg and 1 g/kg, p.o) also showed a significant decrease in ALT levels on 7^{th} and 14^{th} day when compared to the disease control group (G-II) ($p<0.001$). This suggests that combination of fenugreek and glibenclamide favours decrease in ALT levels back to normal, than glibenclamide alone (Table-3).

Effect on serum AST levels

Rats treated with alloxan (100 mg/kg, s.c) (G-II) showed a significant increase in the AST levels when compared to the normal group (G-I) ($p<0.001$). Glibenclamide (0.45 mg/kg, p.o) treated group (G-III) showed a significant decrease in AST levels on 7^{th} and 14^{th} day, when compared to the disease control group (G-II) ($p<0.001$). The groups IV and V receiving fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) showed a significant decrease in the AST levels on 7^{th} and 14^{th} day when compared to the disease control group (G-II) ($p<0.001$). When fenugreek (0.5 g/kg and 1 g/kg, p.o) in two doses was administered along with glibenclamide (0.45 mg/kg, p.o) (G-VI and VII) plasma AST levels were significantly reduced on 7^{th} and 14^{th} day when compared to the disease control group (G-II) ($p<0.001$). This indicates that combination of fenugreek and glibenclamide has additive effect in decreasing the raised AST levels.

Table No 4: Effect of Trigonella foenum graceum and glibenclamide on serum aspartate aminotransferase (AST) levels

GROUP	TREATMENT	Serum AST Levels		
		0^{th} day	7^{th} day	14^{th} day
I	Normal	50.5 ± 1.181	55.3 ± 1.835	59.8 ± 1.047
II	Alloxan (100 mg/kg,s.c)	62.3 ± 0.834^{a}	94.5 ± 1.538^{a***}	98.3 ± 1.734^{a***}
III	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po)	61.7 ± 0.659	63.4 ± 1.404^{b***}	63.7 ± 0.922^{b***}
IV	Alloxan (100 mg/kg,s.c) +Fenugreek (0.5 g/kg, po)	61.2 ± 0.947	44.5 ± 1.480^{b**}	48.9 ± 1.264^{b***}
V	Alloxan (100 mg/kg,s.c) +Fenugreek (1 g/kg, po)	60.15 ± 0.483	43.9 ± 1.418^{b***}	48.9 ± 2.056^{b**}
VI	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po) + Fenugreek (0.5 g/kg, po)	59.3 ± 0.479	59.2 ± 1.736^{b**}	51.7 ± 1.047^{b***}
VII	Alloxan (100 mg/kg,s.c) +Glibenclamide (0.45 mg/kg, po) + Fenugreek (1 g/kg, po)	58.8 ± 0.495	59.3 ± 1.446^{b***}	52.3 ± 1.667^{b***}

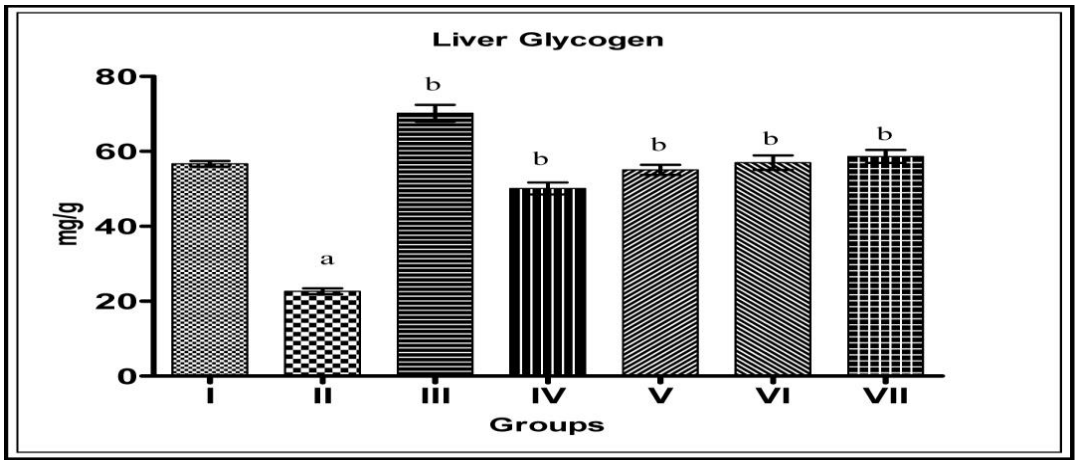
Values are expressed as Mean ± SEM of 6 animals.

Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII. **p<0.01, ***p<0.001, ns – Non-significant

Effect on liver glycogen

At the end of the study in the group treated with alloxan (100 mg/kg, s.c) (G-II) a significant depletion in liver glycogen levels were observed when compared to the normal group (G-I) ($p<0.001$). Treatment with standard drug glibenclamide (0.45 mg/kg, p.o) (G-III) significantly increased liver glycogen levels on 14th day when compared to the disease control group (G-II) ($p<0.001$). Administration of fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) for 14 days also showed a significant increase in liver glycogen levels when compared to the disease control group (G-II) ($p<0.001$). When the diabetic rats were co-administered with fenugreek (0.5 g/kg and 1 g/kg, p.o) and glibenclamide for 14 days (0.45 mg/kg, p.o) the liver glycogen levels were significantly increased when compared to the disease control group (G-II) ($p<0.001$). The results suggest that combination of glibenclamide and fenugreek doesn't effect the benefit of glibenclamide on liver glycogen (Figure 1).

Fig.1. Effect of Trigonella foenum graceum and glibenclamide on Liver glycogen levels

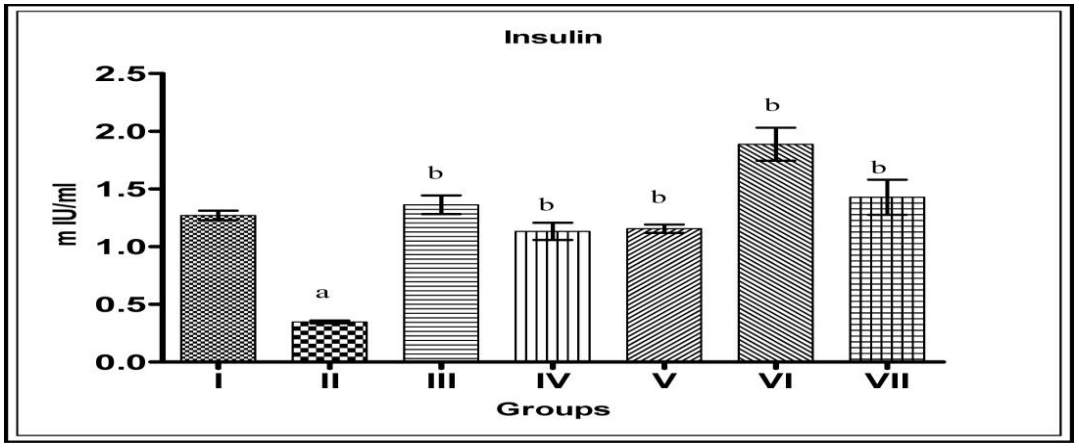


All values shown are mean ± SEM and n = 6.

Values are expressed as Mean ± SEM of 6 animals.

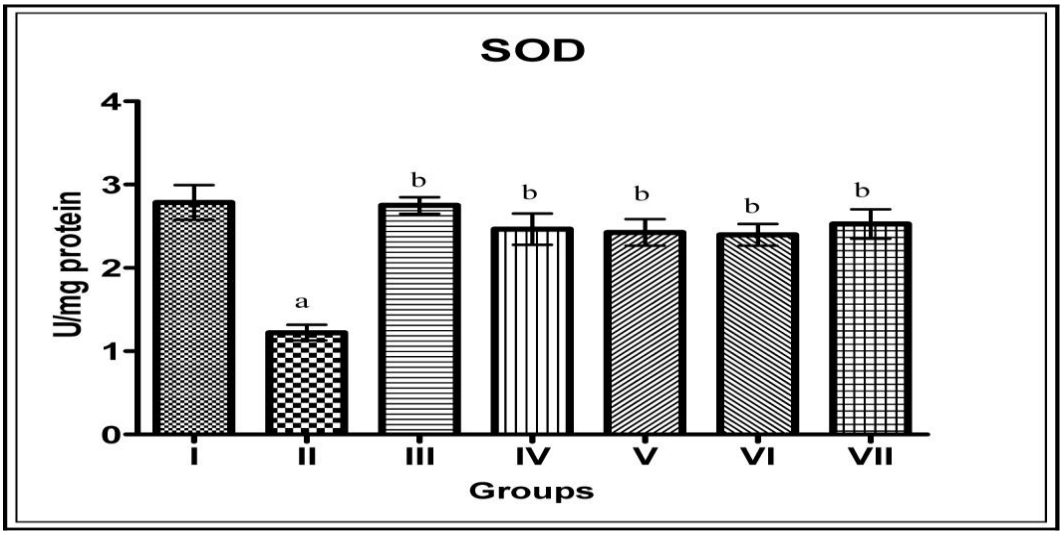
Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Fig.2. Effect of Trigonella foenum graceum and glibenclamide on serum insulin levels



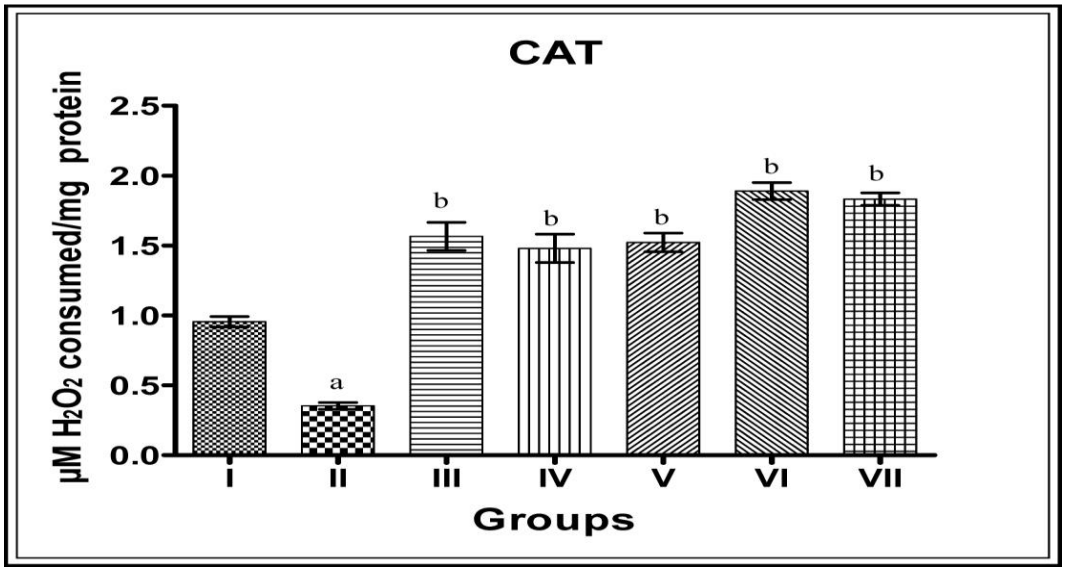
Values are expressed as Mean ± SEM of 6 animals. Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Fig.3. Effect of Trigonella foenum graceum and glibenclamide on Superoxide Dismutase (SOD) levels in Pancreas



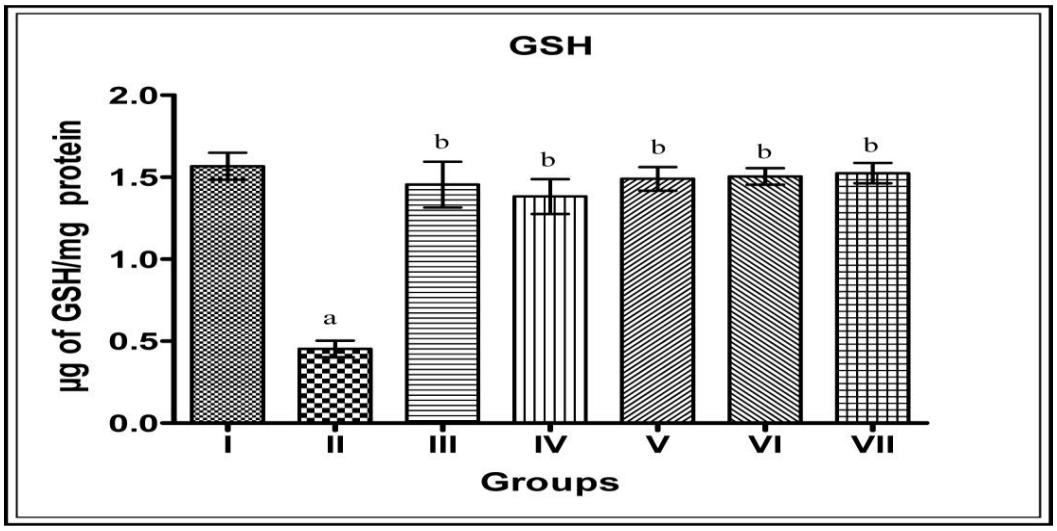
Values are expressed as Mean ± SEM of 6 animals. Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Fig.4. Effect of Trigonella foenum graceum and glibenclamide on Catalase (CAT) levels in Pancreas



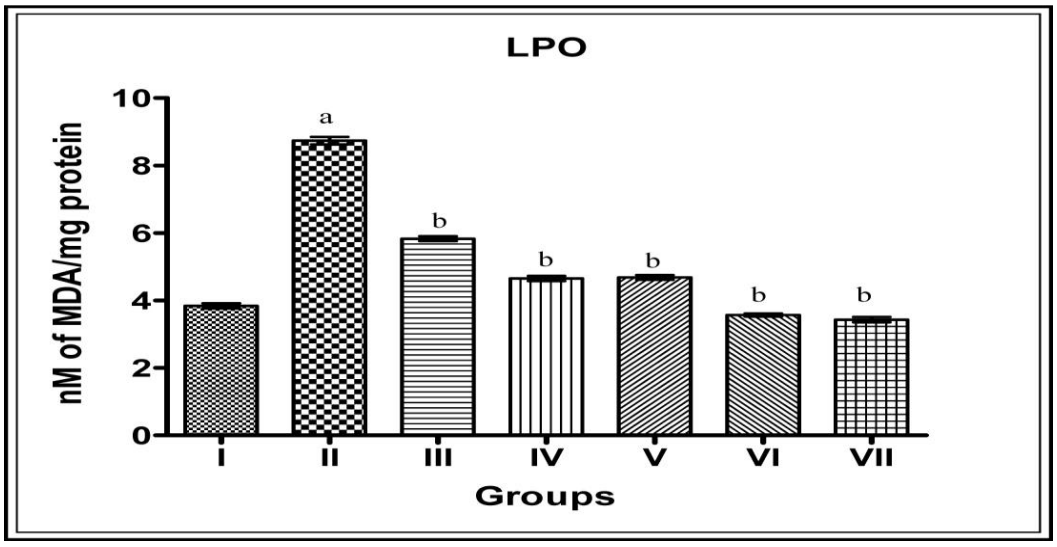
Values are expressed as Mean ± SEM of 6 animals. Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Fig.5. Effect of Trigonella foenum graceum and glibenclamide on Reduced glutathione (GSH) levels in Pancreas



Values are expressed as Mean ± SEM of 6 animals. Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Fig.6. Effect of Trigonella foenum graceum and glibenclamide on Lipid peroxidation (LPO) levels in Pancreas



Values are expressed as Mean ± SEM of 6 animals. Statistical significance test for comparisons were done by one-way ANOVA, followed by "Dunnet's Multiple Comparison Test". Comparisons were done between a) Goup I vs Group II and b) Group II vs Group III, IV, V, VI, VII.

Effect on insulin levels

Alloxan (100 mg/kg, s.c) induced diabetic rats (G-II) showed a significant decrease in plasma insulin levels when compared to the normal group indicating destruction of pancreatic beta cells (G-I) (p<0.001). Administration of glibenclamide (0.45 mg/kg, p.o) (G-III) showed a significant increase in plasma insulin levels when compared to the disease control group (G-II) (p<0.001). The groups (G-IV and V) receiving fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) showed a significant increase in plasma

insulin levels when compared to the disease control group (G-II) (p<0.001). The groups VI and VII rats treated with both fenugreek (0.5 g/kg and 1 g/kg, p.o) and glibenclamide (0.45 mg/kg, p.o) also showed a significant increase in plasma insulin levels when compared to the disease control group (G-II) (p<0.001) (Figure 2).

Effect on in-vivo antioxidant parameters

Superoxide dismutase (SOD)

The rats treated with alloxan (100 mg/kg, s.c) (G-II) showed a significant decrease in SOD levels when compared to the normal group (G-I) (p<0.001). The groups receiving glibenclamide (0.45 mg/kg, p.o) (G-III), fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) and combination of glibenclamide and fenugreek (G-VI and VII) showed a significant increase in SOD levels when compared to the disease control group (G-II) (p<0.001) (Figure 3).

Catalase

Diabetic rats treated with alloxan (100 mg/kg, s.c) (G-II) showed a significant decrease in catalase levels when compared to the normal group (G-I) (p<0.001). The groups treated with glibenclamide (0.45 mg/kg, p.o) (G-III), fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) and combination of glibenclamide and fenugreek (G-VI and VII) showed a significant increase in catalase levels when compared to the disease control group (G-II) (p<0.001) (Figure 4).

Reduced glutathione (GSH)

In the group treated with alloxan (100 mg/kg, s.c) (G-II) a significant decrease in GSH levels were observed when compared to the normal group (G-I) (p<0.001). The groups receiving glibenclamide (0.45 mg/kg, p.o) (G-III), fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) and combination of glibenclamide and fenugreek (G-VI and VII) showed a significant increase in GSH levels when compared to the disease control group (G-II) (p<0.001) (Figure 5).

Lipid peroxidation (LPO)

In the animals treated with alloxan (100 mg/kg, s.c) (G-II) a significant increase in LPO levels were observed when compared to the normal group (G-I) (p<0.001). The groups treated with glibenclamide (0.45 mg/kg, p.o) (G-III), fenugreek at both doses (0.5 g/kg and 1 g/kg, p.o) (G-IV and V) and combination of glibenclamide and fenugreek (G-VI and VII) showed a significant decrease in LPO levels when compared to the disease control group (G-II) (p<0.001) (Figure 6).

DISCUSSION

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycaemia. It is a global health problem and considered as a major risk factor of the immature morbidity and mortality worldwide. The significant symptoms are polyuria, polyphagia, polydypsia[18]. The drugs used for treating this disease are either too expensive or have undesirable side effects. Treatment with sulphonylureas and other drugs are also associated with side effects. However, for a number of reasons complementary medicine has grown in popularity in recent years. Often herbs may be used together because the combination is more effective and may have fewer side effects. The popularity of herbal medicinal products makes it important to understand potential interactions between herbs and prescribed drugs. Millions of people today use herbal therapies along with prescription and non-prescription medications. Although considered natural, many of these herbal therapies can interact with other medications, causing either potentially dangerous side effects and/or reduced benefits from the medication. Currently, there is very little information published on herb-drug interactions while the use of herbs is progressively growing across the world. As there is large belief that herbal medicines are safe to use, it needs to be

understood that depending on the amount and potency of the pharmacologic principles contained in the herbal preparation, potential exists for herb-drug interaction to occur when the herbal product is consumed with the modern day medicine[19].

Hence our study aims to investigate the possible drug interactions between the routinely used natural condiment Trigonella foenum graceum (fenugreek) and the commonly used oral antidiabetic drug glibenclamide in alloxan induced diabetic rats. In the present study alloxan is used to induce diabetes which is considered as a suitable experimental model for type-2 diabetes mellitus. It is a cyclic urea compound which induces diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells and causes cell death[20]. Glibenclamide is the most widely used antidiabetic drug around the world which belongs to the second-generation sulphonyl ureas. It causes inhibition of ATP-dependent potassium channel in the pancreatic β-cells which alters the resting potential of the cell, leading to calcium influx and stimulation of insulin secretion[21]. The net effect is increased responsiveness of β-cells to glucose, resulting in more insulin release.

In oral glucose tolerance test, there is decrease in blood glucose levels in treated rats when compared with control group. Administration of both fenugreek along with glibenclamide prevented raise in blood glucose levels suggesting that the hypoglycaemic effect of glibenclamide is unaffected when given in combination. In antidiabetic study, administration of fenugreek decreased the blood glucose levels in diabetic rats. Treatment with glibenclamide along with fenugreek also decreased the blood glucose levels but does not produce severe hypoglycaemia in the combination. The hypoglycemic effects of fenugreek have been attributed to several mechanisms. An in-vitro study demonstrated that the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered[22]. In human studies, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors. Fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism[23,24]. The increase in the activities of plasma ALT and AST indicate that alloxan induced diabetes might cause hepatic dysfunction. Treatment with glibenclamide decreases the plasma AST and ALT levels when compared to the control group but increased levels of AST and ALT when compared to the normal group. The combination of glibenclamide and fenugreek favoured the decrease of AST and ALT to the normal levels. Hence, this combination may be useful to overcome the idiosyncratic hepatotoxicity of glibenclamide[25].

Glycogen synthesis in rat liver and skeletal muscle is impaired in diabetes[26]. Diabetes induces an increase in synthase catalytic efficacy. The specific activity of phosphorylase is decreased in diabetic rats. In our study also liver glycogen level was found to be low in alloxan treated rats similar to the earlier reports[26]. Treatment with glibenclamide enhanced the liver glycogen content significantly when compared with diabetic control group; this is due to the ability of glibenclamide causing reactivation of the glycogen synthase system. Administration of glibenclamide along with fenugreek increased the liver glycogen content suggesting that the

combination doesn't affect the benefit of glibenclamide on liver glycogen stores i.e the combination might not interfere with reactivation of glycogen synthase system.

Insulin levels decreases in alloxan treated rats. Sulphonylurea derivatives stimulate the ß-cell, not only during hyperglycemia but also in normo- and hypoglycaemic conditions, thus increasing the intraislet insulin levels[27]. Treatment with glibenclamide and fenugreek alone increased the plasma insulin levels when compared to the control group. Treatment with fenugreek and glibenclamide in combination also increased the plasma insulin levels significantly. Several studies reported that the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells[22]. So, in our study increase in plasma insulin levels in groups treated individually and in combination might be due to the presence of 4-hydroxyisoleucine in fenugreek and inhibition of ATP-dependent potassium channel in the pancreatic β-cells of glibenclamide. The results suggest that combination of fenugreek with glibenclamide doesn't interfere with insulin releasing effect of glibenclamide.

In recent years, much attention has been focussed on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications[28]. Diabetes increases the production of tissue damaging by glucose autooxidation and /or nonenzymatic protein glycosylation. Reactive oxygen species can be eliminated by a number of enzymatic and non-enzymatic antioxidants, thus protecting tissue / organ damage from oxidative stress. In the present study, antioxidant parameters were estimated in homogenates of pancreas in all groups. Superoxide dismutase is considered as a primary enzyme since it is involved in the direct elimination of reactive oxygen species[30]. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals[31]. The levels of SOD were reduced in alloxan treated rats and treatment with fenugreek and glibenclamide both alone and also in combination increased SOD levels indicating that they improve the antioxidant status. Catalase is an enzymatic antioxidant, a heamoprotein which catalyses the reduction of hydrogen peroxide to water and oxygen and protects the tissue from highly reactive hydroxyl free radicals[32]. The reduced activity of catalase in pancreas was observed during diabetes which may result in deleterious effects due to accumulation of superoxide anion radicals and hydrogen peroxide. The activity of catalase was increased in alloxan induced diabetic rats treated with glibenclamide and fenugreek both alone and in combination indicating antioxidant property of fenugreek and glibenclamide. Reduced glutathione is a potent free radical scavenger GSH within the islet of β-cell and is an important factor against the progressive destruction of the β-cell following partial pancreatectomy. Depletion of GSH in diabetes results in enhanced lipid peroxidation. Treatment with fenugreek and glibenclamide both alone and in combination resulted in the elevation of the GSH levels, which might have protected the cell membrane against oxidative damage. Malondialdehyde (MDA) is an end product of lipid peroxidation, a non-enzymatic antioxidant, present in less concentration scavenging hydroxyl free radicals[33]. In our study, increase in MDA was observed in diabetic rats and when treated with glibenclamide and fenugreek LPO levels were reduced. This decrease in LPO levels can be contributed to the increase in SOD, Catalase and GSH levels. The combination of glibenclamide and fenugreek shows additive antioxidant property. The soluble part of the seed might be

responsible for its antioxidant property in fenugreek as per the earlier reports. Glibenclamide prevents the progression of reactive oxygen species (ROS) mediated damage. Oral hypoglycaemic agents may be effective for glycemic control but they do not appear to be effective in entirely preventing the progression of ROS mediated organ damage[34]. As combination of fenugreek and glibenclamide has shown additive antioxidant property, this combination may be beneficial in preventing progressive diabetic complications.

From the above observations it can be concluded that combination causes hypoglycaemia in oral glucose tolerance test and decreased blood glucose levels in diabetes. But the decrease in blood glucose levels was never below the normal range indicating safety of the combination and also lack of any pharmacodynamic drug interaction between fenugreek and glibenclamide. Similarly, no interaction was observed either on insulin or hepatic glycogen levels. Moreover, the combination has shown significant antioxidant property, which might be helpful to prevent end organ damage. Hence, fenugreek can be consumed by diabetic patients on glibenclamide therapy without any necessity of dose adjustment along with effective control on blood glucose levels. Further studies are obviously required to rule out any pharmacokinetic interactions between glibenclamide and fenugreek.

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