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Preliminary phytochemical and pharmacological (Antidiabetic) screening of *Cassia tora* Linn.

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Abstract

In the present study the methanol extracts and their ethyl acetate, n-butanol and dichloromethane fraction of seeds of *Cassia tora* Linn. were screened for phytochemical profiling and antidiabetic activity. After preliminary phytochemical investigations, methanol extract and its fractions were evaluated for activity employing single does and prolonged treatment in noramal and alloxan induced diabetic albino rats. All the extract were given orally of methanol extract at a dose 50, 100, and 200 mg/kg b. w. for fractions and 100 mg/kg b. w for their sub fraction of active extract. Glibenclamide was used as standard drug (mg/kg b. w. i.p.). The active n-butanol fraction of methanol extract was futher evaluated for antidiabetic activity and phytochemical characterization to isolate an active component. The active component was characterized using modern techniques like UV, IR, ^{1}H NMR and Mass etc. The present study shows that methanol extract at a doe's 200 mg/kg b.w. has significant antidiabetic activity in normal, acute as well as prolonged treatment compared to control. Among all fractions of methanol extract, the n-butanol fraction had more significantly reduced the blood glucose level after single does and prolonged treatment and nearly equal to standard Glibenclamide after prolonged treatment. The napthopyrone glycoside was isolated form the n-butanol extract of the seed of *cassia tora* as active constituent.

Key-Words: *Cassia tora*, Caesalpiniaceous, Alloxan, Antidiabetic activity, Napthopyrone glycoside.

Introduction

Diabetes mellitus is one of the common chronic diseases and contributor to the development of cardiovascular diseases. It is due to deficiency or a failure of normal action of insulin, which is responsible for the use of the sugar from the diet. The number of case of non-insulin dependent mellitus has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity, and again of population[1]. Insulin therapy is not enough cure such disorders. The present day insulin treatments when taken orally pose problems of certain side effects, broken up and digested by the gut enzymes & insulin resistance are still impervious to treatment. Compared with synthetic drugs, drugs derived from plants are more frequently considered to be less toxic with fewer side effects.

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There is an increasing demand by patients to use the natural products with antidiabetic activity[2]. Therefore, the search for more effective and safer natural antidiabetic agent devoid of adverse effect originating from plants, the seeds of *Cassia tora* (Linn) was chosen for more detailed investigation.

Cassia tora Linn. (Caesalpinaceae) is widely distributed in tropical and Asian countries. The seeds of *cassia tora* are reputed in Chinese medicine as vision-improving, antiasthenic, asperient and diuretic agents. *C. tora* have shown to possess various biological and pharmacological activities including antihepatotoxic, antiallergic, antimutagenic, antifugal, radical scavenging, and antimicrobial. Previous phytochemical investigation on the seeds of *C. tora* have resulted in isolation of several anthraquinone , naphthopyrone derivatives[3-6]. However, no pharmacological studies regarding antidiabetic activity have been carried out on the seeds of *C. tora* to date. In the present study, Naphthopyrone glucoside was isolated from the BuOH-soluble extract of the seeds of *C. tora.*

Material and Methods

General experimental procedure

All the solvents used for extraction, qualitative tests and TLC studies were distilled before use. Alloxan (Loba chemie, Mumbai, India) and Glibenclamide (Gift pack from Nicholas, Piramal, Mumbai) were used in this study. All other chemicals and reagents used were of analytical grade and obtained from Loba Chemie, Mumbai. Distill water was used throughout the experiment. Blood Glucose level of rats were determined using commercial glucose kit named Accu-Check Active purchased from Roche Diagnostics India Pvt. Ltd, Mumbai. Melting points were measured on an IA900 melting point apparatus. The TLC analysis was performed on glass plates (Silica get G, 0.25 mm layer thickness); compound was visualized UV visible light. The UV spectra were recorded on spectrometer by Shimadzu UV 1700 model in MeOH.

Plant Material

The seeds of *cassia tora* L. were collected from the different local areas of Gormi, Bhind (M.P.) during the month of Oct.-Nov. 2008. The seeds were identified and authenticated in the department of Botany, SMS Govt. Science College, Gwalior, (M.P.).

Extraction of plant material[7]

The seed of *Cassia tora* was shade dried and powdered. The ground seeds of *Cassia tora* were shade dried and powdered. The ground seeds of *Cassia tora* (3 kg) were defatted with petroleum ether and then extracted with (5L.) of 95% methanol (40-60^0 C) in a soxhlet appratus for 17 – 18 hours. The combined solutions were then evaporated after the complete extraction to give a (258.25g) 11.65% yield with semisolid consistency and brownish black in colour. The methanol extract was suspended in H_2O (1L.) and successively extracted with ethy1 acetate, choloroform, n-butanol and dichloromethane to give 48.45 gm, 31.87 gm, and 20.40gm fraction respectively. Based on initial phytochemical and pharmacological testing the n-butanol fraction (35.5g) was chromatographed over silica gel (10x70 cm, 70-230mesh) as the stationary phase using Choloroform-ethyl acetate gradient (from 2.8:1.2 v/v) to yield 13 pooled fraction (Fraction 01-13). Fractions showing similar pattern of TLC were pooled together and finally 6 fractions were obtained. These fractions were further analyzed for their Phytochemical and pharmacological screening for antidiabetic activity.

Phytochemical investigations

The qualitative and TLC study were carried out for the all the extracts and fractions. The chemical tests and TLC study were performed with different reagents and a TLC study of extracts was carried out using silica gel G as stationary phase and chloroform : methanol ethyl acetate : phridine (8:2:3:1,v/v) and chloroform : Ethyl acetate (2:8:1:2, v/v) as mobile phase for methanol extract and n- butanol fraction respectively. Spots were observed under visible light and using vanillin H_2SO_4 as a detecting reagent.

Antidiabetic screening

Acute toxicity

The acute oral toxicity study was carried out as per guidelines set by Organization for Economic Co-operation and Development (OECD). The study protocol was approved by the animal Ethics Committee of the Institution

Preparation of dose

The methanol extract was formulated in suspension in distilled water using 2% PVP as suspending agent. 2% PVP has negligible effect on normal blood glucose level. The strength of the suspension was according to the does administered as 50, 100 and 200 mg/kg b.w. methanol and n- butanol fraction respectively and was expressed as weight of dried extract.

Preparation of standard drug

Gilibenclamide was used as reference drug for evaluating the antidiabetic activity. The drug (10 mg) was powdered and made into suspension in distilled water using 2% PVP as suspending agent. The strength of the suspension was prepared according to 10 mg/kg b. w.

Animals

Healthy wistar strain albino rats weighing about 100-200g were used for the study. The animal were kept in the experimental animal house of the department under standard environmental conditions and allowed free access to their respective normal laboratory chow diet and water ad libitum during entire period of experimentation. Alloxan monohydrate (120 mg/kg b.w.) freshly prepared in normal saline was injected intraperitonially to hyperglycemia.

Before starting the experiment, the animals were grouped according to their body weight. The diabetic animals were divided in to six groups in 6 rats each for preliminary investigation of antidibetic activity of methanol extract. The n- butanol fraction was divided in to the nine groups of six rats each. Group I and II served as normal and diabetic control and received normal saline while Group III was kept as standard drug treated Glibenclamide (10 mg/kg, orally) control. Three groups (Group IV, Group V and Group VI) were administered plant orally as methanol seeds extract of 50 mg/kg, 100 and 200 mg/kg repectively which were compared with standard glibenclamide group. The animal were kept fasting for 24 hr. with

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water ad libitum and injected intraperitoneally at does of 120 mg/kg. b. w. of alloxan monohydrate freshly prepared in normal saline solution. After 72 h of alloxan injection, the animals were tested for estimating the blood glucose level. The blood glucose level more than 200 mg/dl of blood was taken as consideration. The extract, standard drug Glibenclamide and vehicle were administered orally, every 24 hr for periods of 14 days. The blood samples were withdrawn from the tail vein puncturing with hypodermic needle before and 2, 4, 6 and 8 hrs. After administration of single dose (for acute study) and at the end of 14 day (prolonged treatment) and blood glucose levels were determined by using glucometer.[8]

Stastical analysis

All the data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test with control. The data are expressed as mean standard error mean as change in blood glucose level. The Dunnet's test was employed to analyze the results and p value less than 0.05 was considered statistical significant.

Results and Conclusion

The results are expressed in the blood glucose level and their % reduction is presented in table 1 and 2. The Fig. 1 and 2 showed the effects of fraction in alloxan induced diabetic rates after a single does and Fig. 3 and 4 exhibited the effects of fraction after a prolonged treatment. The result obtain from the alloxan-induced diabetes indicated that does as 200 mg/kg b.w. of methanol extract showed more significant ($p<0.01$) antidiabetic activity in actuate as well as prolonged treatment compared to diabetic control. The result for were comparable with reference standard glibenclamide. On the basis of preliminary antidiabetic screenings of methanol extract, the fractions were further evaluated for their effect on blood glucose level. The order of sequence to produce antidiabetic activity in acute as well as prolonged treatment is Glibenclemide>n-butanol Fraction>Chlorofom fraction> Ethly acetate fraction>Dichloromethane fraction the research has been made to further evaluate the antidiabetic activity of n-butanol fraction of methanol extract of *Cassia tora* L.

Oral administration of friction F-f at100 mg/kg b.w. Resulted marked antidiabetic effect comparable to Glibenclamide (at 10 mg/kg b.w) acute as well as prolonged treatment. It has been observed that normal control and diabetic control has more similar blood glucose level throughout the experiment. The standard drug Glibenclamide showed a decrease level in 2, 4, 6 and 8th hr after a single dose treatment as 10mg/kg b.w. It showed the more significant effect on blood glucose level of alloxan induced diabetic rats in all 1 to 6 hr. after treatment as 226.83±2.786 at initial 0 hr. to 219.5±1.138, 213.83±0.792, 207.17 and 203.67± 0.7149 at 2nd, 4th 6th and 8th hr.and overall result showed 3.79% reduction to 14.59%.Fraction F-f has more significant effect (226.83±2.056 at initial to 216.50±0.619,210.67 and 208.33±0.61467 at 4th , 6th and 8th hr.) on blood glucose level of alloxan- induced diabetic rates as 12.85% at 8th hr. after a single dose. This value is nearly equal to that of Glibenclamide at 8th hr. 14.59%.

The blood glucose level of control groups did not show any significant change, whereas group III to IX, treated with Glibenclamide and six fraction of n-butanol (100mg/kg b.w.) respectively showed significant decrease when observed on 3, 7 and 14 day after treatment. On prolonged treatment, the effect of fraction F-f (221.67±0.667 at initial to 154.50±0.0670 at 14 day) was nearly equal to that of reference drug Glibenclamide (220.50±2.849 at initial to 138.67±0.558).

These finding clearly established that F-f fraction of n-butanol fraction exhibited more potent antidiabetic activity than all other fraction.

However, this claim demands further isolate antidiabetic principle component and characterization of constituent. The antraquinones, flavonoids, steroids, tanins, and tri-terpeniods were present in butanol fraction. The TLC profile showed the six different coloured spot with there R value using silica get G as a stationary phase and chloroform, Ethyl acetate in 2.8:1.2v/v ratio.

Phytochemical analysis of fraction F-f indicates the presence and its derivatives are present in the *Cassia tora* L. Identification of particular Flavonoids. Required to detailed spectroscopic studies.

The 14.75% isolate fraction F-f has yellowish orange in color with characteristic odour and bitter taste. This isolated compound was dissolves readily in chloroform, ether, and ethanol and is insoluble in water. The melting point has found to be 258-260ºC.

In the UV spectrum (menthol), the component showed the absorption maximum at 283 nm and an inflection in the 260-305-nm region corresponding to napthopyrone glycoside.

The mass spectrum of isolated fraction F-f gives the prominent parent peak at m/z 421.4 In the recent investigation, flavonol derivatives have been found to exhibit a significant in-vitro inhibitory activity against AGEs formation and blood glucose level.

On the basis of spectroscopic analysis it has been also confirmed that the napthopyrone glycoside are present in the seeds of *Cassia tora* L.

Therefore, this isolated component will be candidate for additional biological evaluation to further define their potential as therapeutic agents for diabetic complication being as a potent natural antidiabetic drug.

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Table 1: Effect of n- butanol fractions on blood glucose levels in Alloxan induced diabetic rats after single dose

***P*< 0.05- Significant, ** *P*< 0.01- More significant Vs. Diabetic control
SEM: Standard Error Mean, n= Number of animals in each group (6)

Groups		Blood Glucose Level mg/dl (Mean ± SEM)				
		Initial	Time (h) after treatment			
			2 hrs.	4 hrs.	6 hrs.	8 hrs.
I	Control	98.83 ± 0.3073	99.17 ± 0.3074	99.33 ± 0.333	100.17 ± 0.1409	101.50 ± 0.3418
II	Diabetic Control	225.50 ± 2.473	228.17 ± 0.9098	231.67 ± 0.7601	236.83 ± 1.353	238.67 ± 0.7149
III	Diabetic+ Glibenclamide 10 mg/kg	226.83 ± 2.786	219.5 ± 1.138** (3.79%)	213.83 ± 0.792** (7.76%)	207.17 ± 0.7373** (12.52%)	203.67 ± 0.7149** (14.59%)
IV	Diabetic+ Fraction F-a (100 mg/kg)	228.83 ± 2.906	225.34 ± 2.372 (1.24%)	224.33 ± 1.687 (3.16%)	222.50 ± 0.8931* (6.05%)	220.83 ± 0.4773* (7.47%)
V	Diabetic+ Fraction F-b (100 mg/kg)	227.17 ± 1.641	225.92 ± 1.565 (0.98%)	225.62 ± 1.302* (2.61%)	223.33 ± 0.6424* (5.70%)	221.83 ± 0.9098* (7.05%)
VI	Diabetic+ Fraction F-c (100 mg/kg)	229.17 ± 2.512	226.17 ± 1.887 (0.87%)	225.89 ± 1.669 (2.49%)	225.25 ± 0.9940* (4.88%)	224.33 ± 1.430* (6.05%)
VII	Diabetic+ Fraction F-d (100 mg/kg)	228.17 ± 2.023	226.23 ± 0.7923 (0.84%)	225.97 ± 0.6708 (2.46%)	225.67 ± 1.039 (4.71%)	224.83 ± 1.195* (5.79%)
VIII	Diabetic+ Fraction F-e (100 mg/kg)	228.67 ± 2.140	226.65 ± 1.682 (0.67%)	226.45 ± 1.400* (2.25%)	226.13 ± 1.056* (4.52%)	225.86 ± 1.078* (5.36%)
IX	Diabetic+ Fraction F-f (100 mg/kg)	226.83 ± 2.056	221.45 ± 0.918* (2.95%)	216.50 ± 0.619** (6.54%)	210.67 ± 0.4179** (11.04%)	208.33 ± 0.6146** (12.85%)

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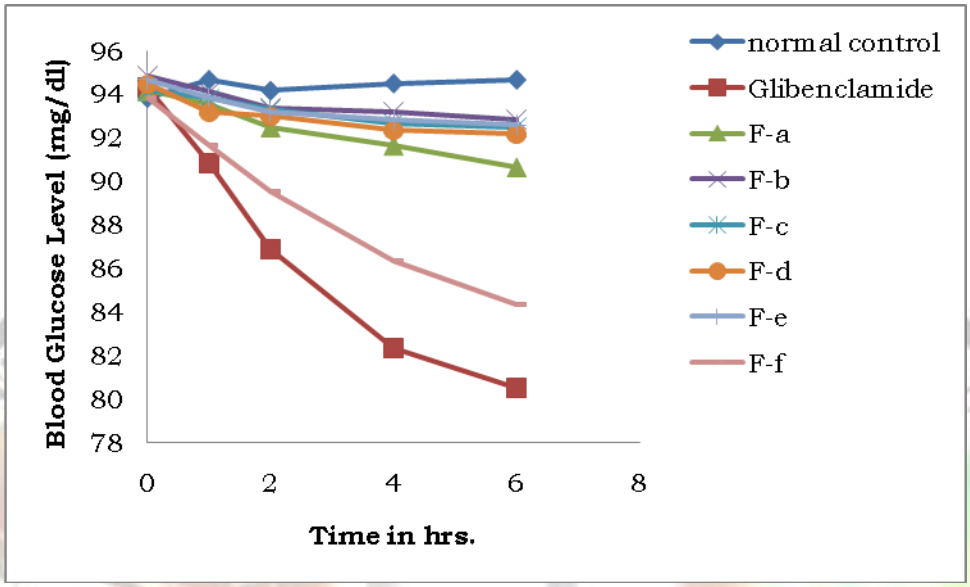

Fig. 1: Effect of n-butanol fractions on blood glucose levels in normal rats after single dose

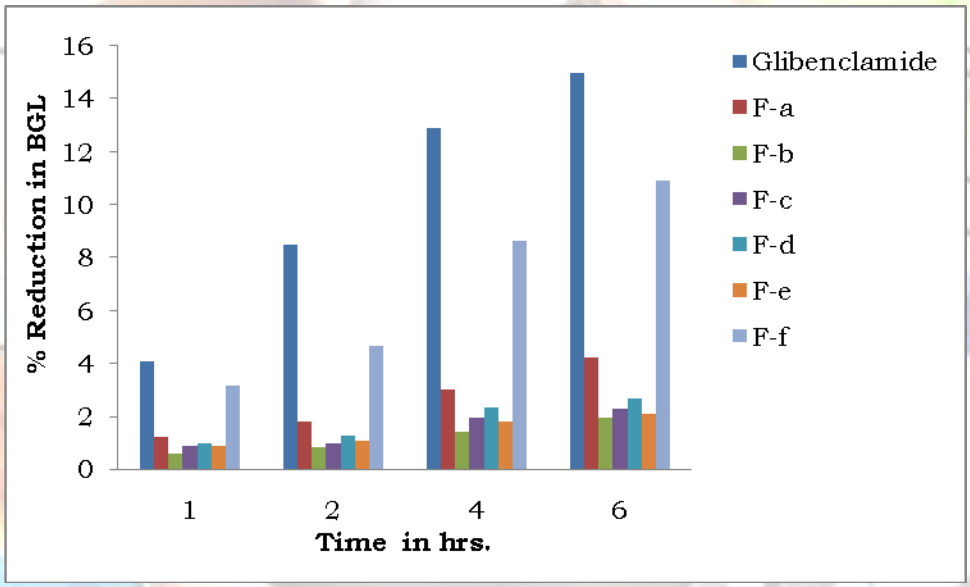

Fig. 2: % reduction of blood glucose levels in normal rats

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Table 2: Effect of fractions on blood glucose levels in Alloxan-induced diabetic rats after prolonged treatment

******P*< 0.05- Significant, ****** *P*< 0.01- More significant Vs. Diabetic control,
SEM: Standard Error Mean, n= Number of animals in each group (6)

Groups		Blood Glucose Level mg/dl (Mean ± SEM)				
		Initial	Days after treatment			
			1	3	7	14
I	Control	98.67 ± 0.333	99.17 ± 0.4014	100.50 ± 0.8851	101.33± 0.8028	99.84 ± 1.195
II	Diabetic Control	219.17 ± 1.851	232.33 ± 1.282	239.17 ± 0.4014	241.33± 0.7149	245.17 ± 1.046
III	Diabetic+ Glibenclamide 10 mg/kg	220.50 ± 2.849	198.83 ± 0.703** (14.41%)	176.33 ± 0.988** (26.27%)	154.50 ±1.057** (35.97%)	138.67 ± 0.558** (43.43%)
IV	Diabetic+ Fraction F-a (100 mg/kg)	221.33 ± 1.054	215.67 ± 0.7601* (7.17%)	207.83 ± 10515* (13.43%)	201.17± 1.851* (16.64%)	193.83 ± 0.946** (20.94%)
V	Diabetic+ Fraction F-b (100 mg/kg)	222.83 ± 1.108	220.83 ± 0.793 (4.94%)	218.17 ± 2.056* (8.78%)	215.33± 1.874* (10.77%)	211.17 ± 3.468** (13.86%)
VI	Diabetic+ Fraction F-c (100 mg/kg)	221.83 ± 0.4773	218.50 ± 0.846* (5.95%)	213.33 ± 1.520* (10.80%)	207.50 ± 0.7188 (14.01%)	201.83 ± 1.973** (17.67%)
VII	Diabetic+ Fraction F-d (100 mg/kg)	222.17 ± 0.7941	219.50 ± 1.057* (5.52%)	215.33 ± 1.406* (9.96%)	210.67 ± 0.615* (12.70%)	204.83 ± 2.212** (16.45%)
VIII	Diabetic+ Fraction F-e (100 mg/kg)	222.30 ± 1.893	220.33 ± 1.994* (5.16%)	217.33 ± 1.706* (9.13%)	214.67± 1.333* (11.04%)	209.50 ± 3.575** (14.54%)
IX	Diabetic+ Fraction F-f (100 mg/kg)	221.67 ± 0.667	206.50 ± 1.784** (11.12%)	199.50 ±1.384** (20.34%)	175.0 ±1.335** (27.48%)	154.50 ± 0.670** (36.98%)

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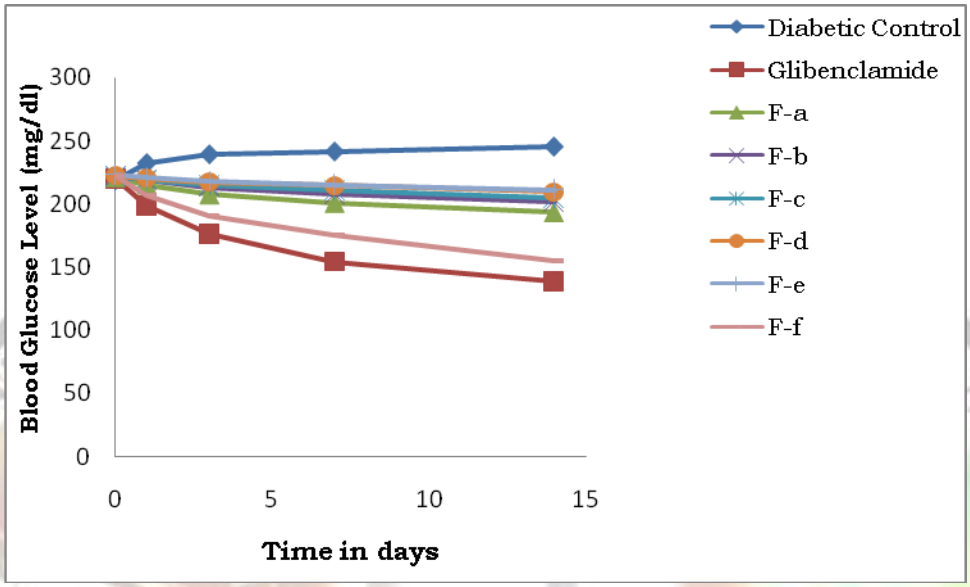

Fig.3: Effect of fractions on blood glucose levels in Alloxan induced diabetic rats after prolonged treatment

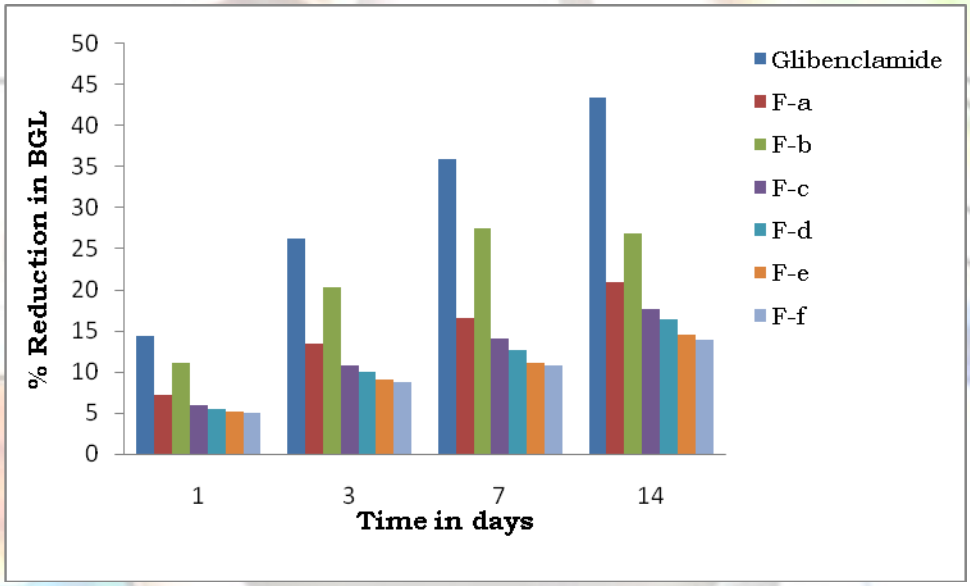

Fig. 4: % Reduction of blood glucose level in Alloxan induced diabetic rats