ANTIDIABETIC ACTIVITY OF HF ON ALLOXAN INDUCED DIABETIC RATS

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Summary

The antidiabetic activity of the HF consisting of three well-known herbs for diabetes namely, *Piper betel, Butea monosperma* and *Trigonella foenum graecum* was investigated in normal and alloxan induced diabetic rats. Effect of herbal formulation (HF) (100 mg/kg and 200 mg/kg, p.o) for 12 days on the level of serum glucose, total cholesterol, triglycerides, serum SGPT/SGOT levels and body weight in diabetic rats were evaluated. The short term study including Glucose tolerance test, determination of blood glucose level of normal rats and diabetic rats were also evaluated. Treatment for 12 days with formulation alleviated body weight loss in diabetic rats. Administration of the formulation for 12 days significantly (p<0.01and p<0.05) decreased serum glucose, total cholesterol, triglycerides and SGPT/SGOT levels. In short term study, both normal and diabetic rats significantly (p<0.01) lowered blood glucose level at both doses in dose dependant manner. In glucose tolerance test, both the doses significantly (p<0.01) reduced the external glucose load. A comparison was also made between the action of HF and glibenclamide (STD) (4 mg/kg, p.o), the standard antidiabetic drug. The antidiabetic activity of the formulation was similar to that observed for STD, confirming its antidibetic potential.

Keywords: Herbal formulation; Antidiabetic activity; *Piper betel; Trigonella foenum; Butea monosperma*

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Introduction

Diabetes is a serious illness with multiple complications and premature mortality, accounting for at least 10 % of total healthcare expenditure in many countries. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025[1]. Many oral hypoglycaemic agents such as biguinides and sulfonylurea's are available along with insulin for the treatment of diabetes mellitus, but these synthetic agents can produce serious side effects, and in addition, they are not suitable for use during pregnancy [2, 3, 4, 5]. Therefore, search for safe and more effective agents has continued to be an important area of active research. Drugs with multiple mechanisms of protective actions, including antidiabetic properties, may be one way forward in minimising the complications of the disease in human being.

The antidiabetic properties of *Piper betle*, *Trigonella foenum graecum*, and *Butea monosperma* were earlier investigated and were found to possess antidiabetic property [6, 7,8]. Some of the ingredients were also found to produce significant reduction in blood glucose levels and lipid profiles. The present study was aimed to investigate the effect of HF on blood glucose level, serum lipid profiles, serum SGOT /SGPT levels and body weight on normal as well as alloxan diabetic rats.

Materials and Methods

Plant material

The alcoholic extracts of seeds of *Trigonella foenum*, leafs of *Piper betle* and flowers of *Butea monosperma* were gifted by Greenchem herbals, Bangalore (India)

Experimental Animals

Healthy adult Wistar rats aged 2 to 3 months, weighing about 180-250 gm were obtained from Agharkar research institute, Pune. Animals were acclimatized for 8 days after their arrival, under standard laboratory conditions of light and temperature and fed on pellets and water *ad libitum*.The Institutional animal Ethics Committee constituted according to the provisions of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India approved the protocol of this study.

Chemicals

The Alloxan monohydrate was gifted by Explicit chemicals, Pune (India).
The standard glibenclamide was procured from local supplier. The diagnostic kits for estimation of serum glucose, cholesterol, triglyceride, SGPT/SGOT levels were procured from Accurex Diagnostic Pvt Ltd, Mumbai (India), Vital Diagnostics Pvt Ltd, Thane (India) and Span diagnostic Ltd, Surat (India).

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, fasted overnight were divided into four groups (n=5) and were orally fed with the alcoholic extracts in increasing dose levels of 100, 500, 1000 and 3000 mg/kg [9]. The rats were observed continuously for 2 h for behavioural, neurological and autonomic profiles and after 24 and 72 for any lethality [10].

Induction of diabetes

Diabetes was induced by intraperitoneal injection of alloxan at a dose of 150 mg/kg body weight. The alloxan solution was freshly prepared; kept on ice and injected immediately. Non-fasting blood samples were collected via retro-orbital sinus by using sterilized glass capillary and used for measuring blood glucose levels by Glucose-oxidase peroxidase (GOD/POD) method. Only those rats, which showed blood glucose levels above 200 mg/dl, were considered diabetic and selected for the study.

Determination of blood glucose level

Blood samples were collected from retro-orbital sinus [11]. The samples were analysed for blood glucose content by using glucose-oxidase method [12].

Composition

Each 100 mg of HF contains:

Herb used	Parts used	Type of extract	Qty (mg)
Piper betle	Leafs	Alcoholic	30.77 mg
Butea monosperma	Seeds	Alcoholic	30.77 mg
Trigonella foenum	Flowers	Alcoholic	38.46 mg

Effect of HF on normal fasted rats

Effect on blood glucose level

Overnight fasted normal rats were randomly divided into four groups (n=5) were orally administered Group I, 1 ml of D.W., Group II, HF 100mg/kg, Group III, HF 200 mg/kg and Group IV, 4 mg/kg of STD respectively. Blood samples were collected from the retro-orbital sinus prior and 1, 2, and 3h after treatment. Blood glucose level was determined by the glucose oxidase method.

Glucose tolerance test in Normal rats

Oral glucose tolerance test is a standard procedure, involving monitoring blood glucose levels over a period of time following a glucose load. It is used in the diagnosis of diabetes and in experiments where a test drug is assessed for hypoglycemic activity. Overnight fasted normal wistar rats were randomly divided into four groups (n=5). These rats were orally administered as Group I, 1 ml of D.W., Group II, 100 mg/kg of HF, Group III, 200 mg/kg HF and Group IV, 4 mg/kg of STD. The rats of all the groups were loaded with glucose solution (1g/kg, p.o.) 30 min after the administration of the HF or glibenclamide. Blood samples were collected from retro-orbital sinus under anaesthetic ether inhalation (To minimize distress) at 0.5, 1 and 2h after glucose loading.

Effect of HF on alloxan induced diabetic rats

Single-Dose Short Term Study

Overnight fasted alloxan induced diabetic rats were randomly divided into five groups (n=5) on 3rd day after alloxanization, were orally administered Group I,1 ml of D.W (Served as control), Group II, 1 ml of D.W. (Served as diabetic control), Group III ,HF (100mg/kg), Group IV, HF (200 mg/kg) and Group V, STD (4 mg/kg). Fasting blood glucose level was estimated prior and 1, 2 and 3h after treatment.

Multi-Dose Long Term (12 Days) Study

The diabetic animals, divided into four groups (*n*=5) were orally administered vehicle, HF (100 mg/kg and 200 mg/kg) and STD (4 mg/kg), respectively for 12 days. The fasting blood glucose levels were estimated on days 0, 5 and 12. The effects of administration of formulation on diabetic rats were estimated on the 12th day. Serum SGOT/SGPT levels, serum lipid profiles, and change in body weight were assessed in the diabetic rats treated with formulation and compared with diabetic control and normal animals.

Statistical Analysis

Numerical results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test $P < 0.01$ and $P<0.05$ being the criterion for statistical significance.

Results

Acute Toxicity Studies

In the acute toxicity study, the administration of the HF at various doses did not elicit any mortality up to 3000 mg/kg body weight in rat. Even at this high dose there were no gross behavioural changes or any clinical symptoms observed.

Effect of HF on normal fasted rats

Effect on blood glucose level

In order to study the optimum effective dose of HF on the blood glucose level, different doses (100 mg/kg and 200 mg/kg) were administered orally to overnight-fasted healthy rats. The onset of hypoglycemic activity of the formulation at 100 mg/kg and 200 mg/kg was evident within 1h as shown in Fig.1. There was significant ($p<0.01$) reduction in blood glucose level at both the selected doses at 2 and 3 h. maximum reduction in blood glucose level was found to be at 100mg/kg at 3 h. The hypoglycemic effect of the HF at 100 mg/kg and 200 mg/kg was found to be comparable to that of STD (4 mg/kg).

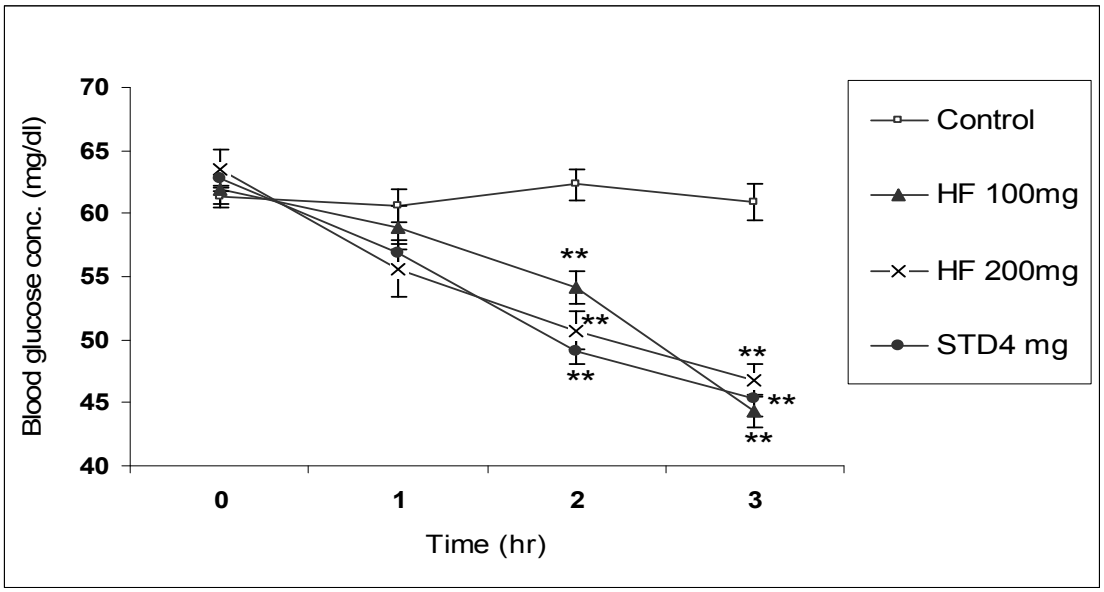

Fig. 1: Effect of HF on blood glucose level in normal fasted rats n=5, ** (p<0.01)

Glucose tolerance in normal rats

The blood glucose levels of the normal rats reached a peak at 30 min after the oral administration of glucose (1 g/kg, p.o) and gradually decreased to the pre-glucose load level (Fig.2). Both the doses selected significantly (p<0.01) improved the GTT up to 2h. Both the doses and STD prevented the rise in blood glucose level significantly (p<0.01) at ½ h, after glucose administration.

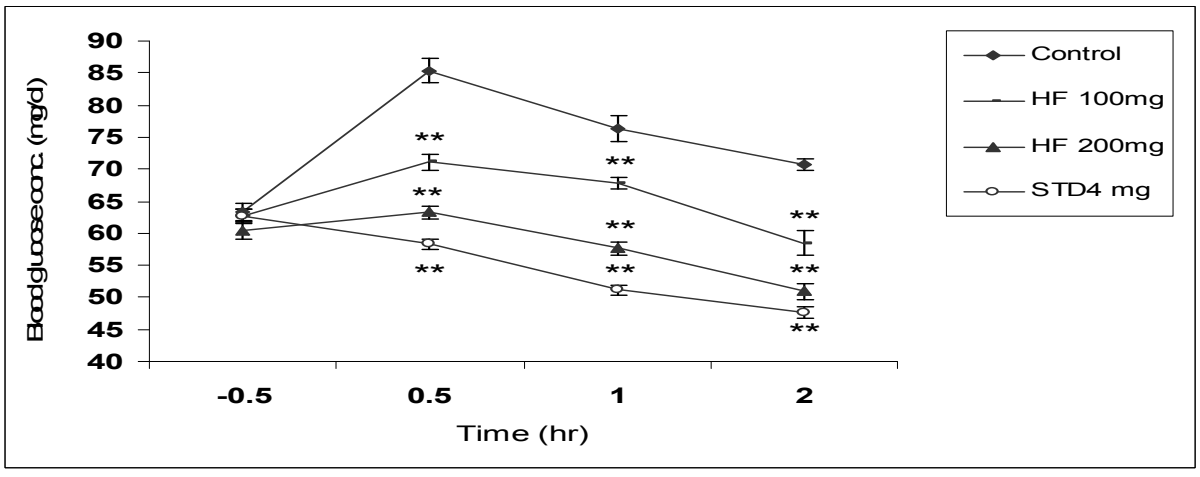

Fig. 2: Effect of HF on Glucose tolerance in normal rats n=5, ** (p<0.01)

Effect Single-Dose Short Term Study of HF on alloxan induced diabetic rats (Effect on blood glucose level)

A single-dose administration of the HF (100 mg/kg and 200 mg/kg, p.o.) on 3rd day after alloxanization, showed significant ($p<0.01$) reduction in blood glucose level and also the onset of activity after 1 h interval as shown in Fig.3. The reduction in the blood glucose level was found to be dose dependant. Maximum reduction in blood glucose level was seen at dose of 200 mg/kg after 2 h of the dose administration. STD (4 mg/kg) produced a significant reduction ($p<0.01$) in blood glucose level compared to diabetic control at 1, 2 and 3h.

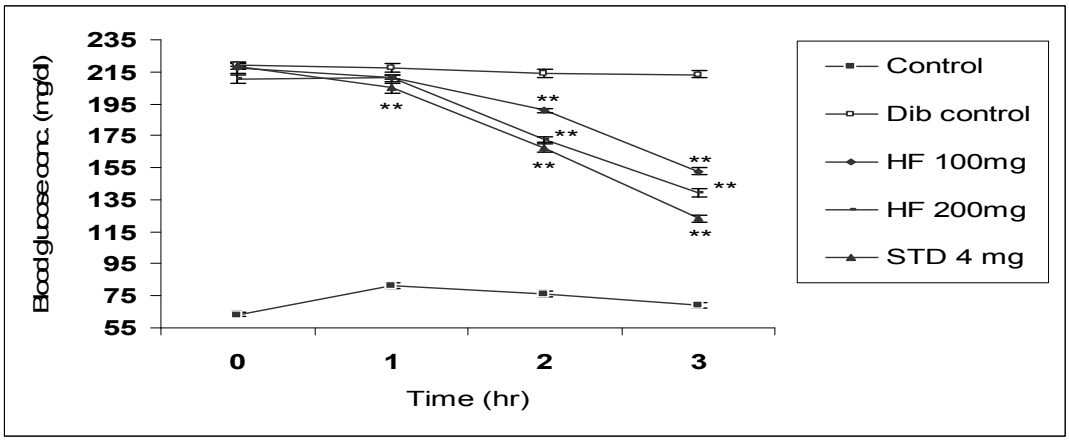

Fig.3: Effect of single-dose short term treatment of HF on alloxan induced diabetic rats n=5, ** ($p<0.01$)

Multi-dose long term study of HF on alloxan induced diabetic rats

1. Effect on blood glucose level

In the diabetic rats treated with the HF at 100 mg/kg and 200 mg/kg, the glucose level decreased steadily to 185.60 mg/dl and 171.04 mg/dl on the 12 day of treatment respectively as shown in Fig.4. STD (4 mg/kg) lowered the basal glucose level steadily and was 161.30 mg/dl on the 12 day of treatment. The antidiabetic effect of the HF at 200 mg/kg was similar to that of STD (4 mg/kg) while that of 100 mg/kg was slightly lower.

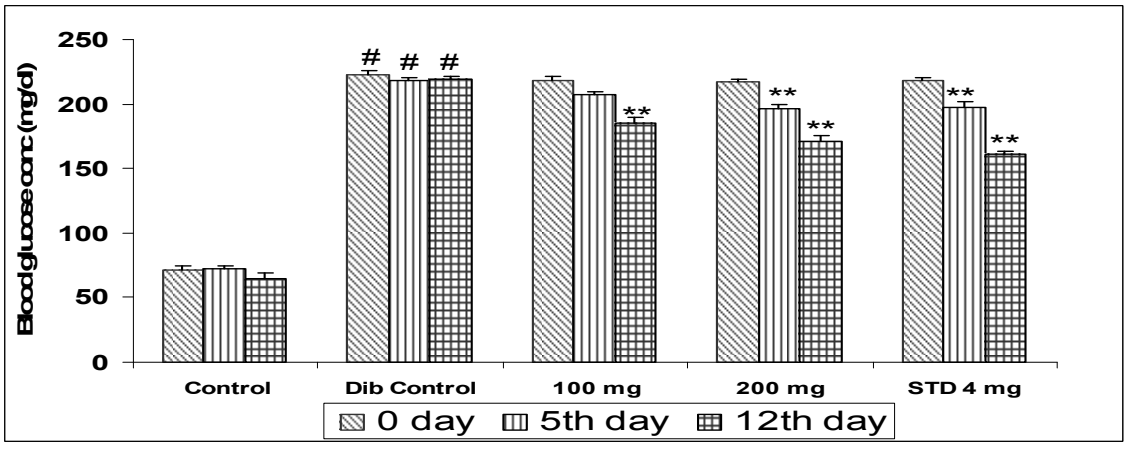

Fig. 4 Effect of multi-dose long term treatment of HF on blood glucose level of alloxan induced diabetic rats n=5, ** ($p<0.01$), and # ($p<0.05$)

2. Effect on serum lipid profiles

There is significant (p<0.01) increase in serum lipids such as Cholesterol and Triglycerides in alloxan diabetic rats. Administration of HF at dose 100 mg/kg and 200 mg/kg significantly (p<0.01 and p<0.05) improved these parameters (Fig 5). The dose 100 mg/kg was highly significant (p<0.01) as compared to 200 mg/kg (p<0.05) in reducing triglyceride level.

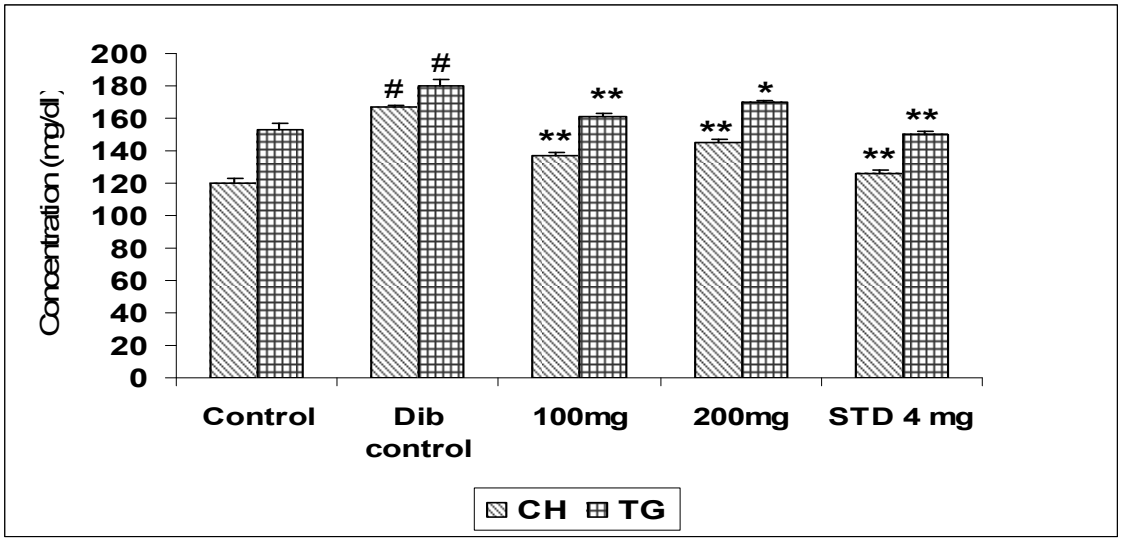

Fig. 5: Effect of multi-dose long term treatment of HF on serum lipid profiles of alloxan induced diabetic rats n=5, ** (p<0.01), and # (p<0.05)

3. Effect on serum SGPT and SGOT levels

Serum SGOT and SGPT levels were elevated significantly (p<0.01) in alloxan induced diabetic rats as compared to normal rats (Fig.6). Alloxan diabetic rats when treated with the HF (100 mg /kg and 200 mg/kg) and STD(4 mg/kg) there was a significant reduction (p<0.01 and p<0.05) in the elevated levels of SGOT and SGPT. Maximum reduction was observed at the dose of 200 mg/kg.

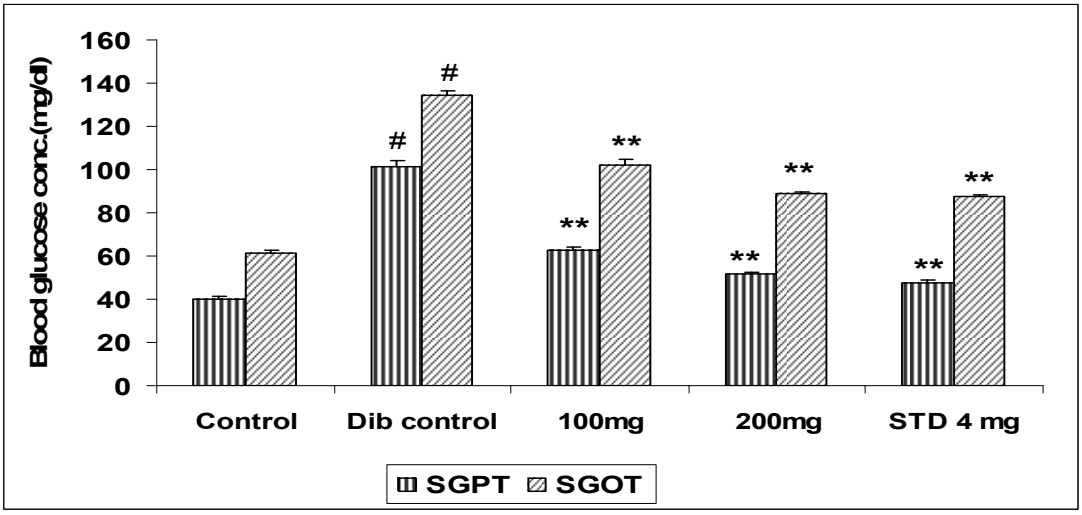

Fig. 6: Effect of multi-dose long term treatment of HF on serum SGPT and SGOT levels of alloxan induced diabetic rats n=5, ** (p<0.01), and # (p<0.05)

4. Effect on body weight

Treatment with the HF and STD reversed the decrease in body weight of diabetic rats in comparison to the untreated control rats (Fig.7). On 5th day of treatment HF at dose of 100 mg/kg showed significant (p<0.01) improvement in body weight, while 200 mg/kg did not show any significance level. On 12th day of treatment the HF at a dose of 100 mg/kg was highly significant (p<0.01) than that of 200 mg/kg and similar to that of glibenclamide. The effect of 200 mg/kg was found to be less significant (p<0.05).

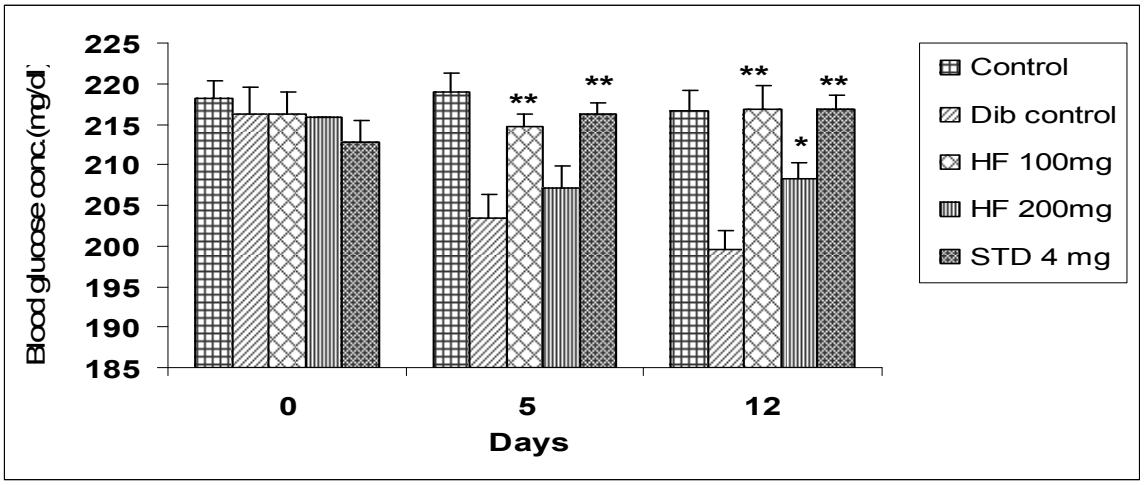

Fig.7 Effect of multi-dose long term treatment of HF on body weight alloxan induced diabetic rats n=5, ** (p<0.01), and # (p<0.05)

Discussion

Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide, they reduce blood glucose in normoglycaemic animals [13,14]. Some other plants act like biguanides such as metformin which is an antihyperglycaemic compound; they do not affect blood glucose in normal state [15, 16, 17, 18].

The different herbs of the present HF have been used in the treatment of diabetes mellitus without any discernable toxic effects. However, when used in combination of these herbs for any investigation, it is necessary to evaluate its potential toxic effects. Hence various doses of the HF were administered orally to normal healthy rats following which different clinical signs and symptoms including mortality were evaluated. Our findings indicate the HF was found to be devoid of any toxic symptoms and did not induce mortality. Hence our acute toxicity results clearly suggest HF is safe for investigation.

In preliminary screening of hypoglycemic activity, the HF (100 mg/kg and 200 mg/kg) and STD (4 mg/kg) has elicited hypoglycemic activity in normal rats. Further, the HF and STD pre-treatment altered the pattern of glucose tolerance curves in normal rats. Therefore hypoglycemic effect of STD has been attributed to an enhanced activity of β-cells of the islets of Langerhans, thereby enhancing the secretion of insulin [19]. Hence, it may be presumed that the HF might exert its effect on glucose metabolism through similar mechanism(s) as that of glibenclamide.

Alloxan, a β-cytotoxin, induces "chemical diabetes" in a wide variety of animal species through damage of insulin secreting cells [20]. Alloxan has been observed to cause a massive reduction in the number of the islets of Langerhans and induce hyperglycemia [21]. Intraperitoneal administration of alloxan (150 mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and loss of body weight when compared with untreated normal rats. Single-dose (short term) study with the HF (100 mg/kg and 200 mg/kg) and STD (4 mg/kg) showed a significant decrease in blood glucose level at 2-3h after treatment.

Biochemical parameters were evaluated following multi-dose long term treatment (12 days) with the HF. The 12 days treatment of alloxan diabetic rats with the HF (100 mg/kg and 200 mg/kg) and STD (4 mg/kg) induced a significant reduction in blood glucose, Serum lipid profiles such as triglyceride & cholesterol levels and serum SGPT & SGOT levels. The reduction produced in the above mentioned bio-chemical parameters by HF was almost similar to that of the STD especially at the dose of 200 mg/kg.

We have noticed elevated serum lipids in alloxan diabetic rats. Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease [22]. The marked increase in serum triglycerides and cholesterol observed in diabetic rats is in agreement with the findings of [23]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [24,25]. Hypertriglyceridemia is also associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and insulin intolerance[26]. In our study, administration of the extract to the alloxan induced diabetic rats significantly ($p < 0.05$) improved these parameters. The observed hypolipidaemic effect may be because of decreased cholesterogenesis and fatty acid synthesis.

Many researchers have reported increased transaminases (SGOT and SGPT) activities in the serum of diabetic animals. The increased levels of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes and may also be responsible for the increased gluconeogenesis and ketogenesis observed in diabetes [27,28]. The results showed that serum SGPT & SGOT levels increased in diabetic control rats when compared with non diabetic control rats, the administration of the HF (100 mg/kg and 200 mg/kg) and STD(4 mg/kg) similarly decreased SGPT and SGOT levels when compared with diabetic rats. This might suggest the protective action of the formulation and STD in reversing any organ damage due to induction of experimental diabetes that is manifested by elevation in the levels of SGOT and SGPT.

We have registered decrease in body weight in alloxan diabetic rats. When HF (100mg/kg and 200mg/kg) was administered to animals given alloxan, the weight loss was reversed. The ability of HF to protect body weight loss seems to be a result of its ability to reduce hyperglycaemia. It is possible that the formulation might act through both, pancreatic and extra-pancreatic mechanism(s), i.e. stimulating the insulin secretion from β-cells of islets of Langerhans, accelerating glycogen synthesis in liver, glucose uptake and peripheral glucose utilizing process.

Conclusion

The study concludes that the HF elicits hypoglycaemic/antidiabetic effects in both normal and experimentally induced hyperglycaemic alloxan rats. Further, HF treatment can significantly alter the pattern of glucose tolerance in normal rats. Hence, the study demonstrate that herbal product exhibits promising anti diabetic activity and helps to

maintain in good glycemic and metabolic control and may have the ability to prevent long-term complications largely through its protection via anti-lipidemic activity.

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