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Original Article

Assessment of Antidiabetic Potential of Polyherbal Formulations

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ABSTRACT

Introduction: *Diabetes provides a major challenge to the present population globally. It is a major threat to global public health that is rapidly reaching epidemic scale. Plant based drugs are gaining importance to treat majority of human aliments including diabetes mellitus due to their less toxic effects.* ***Aims:*** *The present study was designed to assess the antidiabetic potential of polyherbal formulations in streptozotocin induced diabetic rats.* ***Methods:*** *Diabetes was induced by single intraperitoneal injection of streptozotocin (50 mg/kg) in male Wistar rats. Rats with fasting blood glucose levels ≥ 250 mg/dl after seven days of STZ administration were randomized into different groups and were treated with Formulation-1 (F1) and Formulation-2 (F2) in graded doses for 21 days. At the end of the study, blood glucose, lipid profiles were estimated. In addition, enzymatic and non-enzymatic liver antioxidant levels were also estimated. To elucidate the mode of action, we evaluated its effects on oral glucose tolerance test in normal rats and single-dose one day-study and multiple-dose twenty one day- study in diabetic rats.* ***Results:*** *The effect on the insulin level with the treatment by formulations suggests that the mode of action is a similar to that of Glibenclamide. Oral administration of F1 and F2 for 21 days significantly reduced blood glucose level in STZ induced diabetic rats. Both the formulations exhibited antihyperglycemic effect in glucose loaded rats and STZ induced rats. The blood glucose was significantly increased. Supplementation with F1 and F2 both with (250 and 500 mg/kg) showed reduction in the blood glucose levels and improved glucose tolerance, suggesting that there was an improvement in STZ-induced deleterious effects.* ***Conclusion****: This study reveals that F1 and F2 improved STZ-induced hyperglycemia, this effect may be mediated by interacting with multiple targets operating in diabetes mellitus.*

Key words: *Streptozotocin-diabetic rats, antihyperglycemic, formulation-1, formulation-2.*

INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute (or) relative deficiencies in insulin secretion and/or insulin action when fully express diabetes is characterized by fasting hyperglycemia. DM may be suspected (or) recognized clinically by the presence of characteristic symptoms such as excessive thirst, polyuria, pruritus, unexplained weight loss or one or more of the many complications associated with or attributable to the disease.[1]

India is the number one among the top 10 countries in the incidence of diabetes. In India, approximately 31.7 million people suffered from diabetes in 2000 and it is estimated that about 79.4 million people will be diabetic by 2030.[2]

India is the largest producer of medicinal herbs and is called 'Botanical Garden of the World'. In India, the use of different parts of herbs to cure ailments has been in vogue from ancient times. Medicinal herbs have been in use for thousands of years in one form or another like Ayurveda, Siddha, Unani etc.[3]

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Because of the side effects of the present anti-diabetic therapy, scope of alternative therapy in the form of herbal preparations is wide open.

We cannot get the desire result by selecting only one plant, therefore formulations from different plants are needed to get the desired response. This may be due to the adjuvant effect of other compound may help in enhancing the potency of the active compound resulting in additive or synergistic effect.[4]

In view of above information the present study has been undertaken to assess the antidiabetic of polyherbal formulation containing drugs from different herbs viz., Trigonellafoenum-graecum, Eugenia jambolana, Momordicacharantia, Sesame indicum, Allium sativum and Azadirachtaindica.

MATERIALS AND METHODS

Animals: Albino wistar rats weighing 150-250g were used for the present study. The animals were purchased from Mahaveer Enterprises, Hyderabad. They were maintained in the animal house of Luqman College of Pharmacy, Gulbarga for experimental purpose.

Preparation of Formulation:[5]

The ingredients were procured from commercial reputed Ayurvedic supplier and authenticated. All the ingredients were shade dried, powdered and mixed thoroughly in two different compositions as Formulation-1 (F1) and Formulation-2 (F2).

Table No 1: Composition of Formulation-1(F1) and Formulation 2 (F2).

Botanical name	Parts used	Quantity in Formulation 1	Quantity in Formulation 1
Trigonellafoenum-graecum.	Seeds	40 gms	20 gms
Eugenia jambolana.	Seeds	40 gms	40 gms
Momordica charantia.	Fruits	40 gms	40 gms
Sesamumindicum	Seeds	40 gms	20 gms
Allium sativum.	Bulbs	40 gms	20 gms
Azadirachta indica	Bark	40 gms	40 gms

The mixture was further boiled in distilled water at 1000 C for 60 minutes and filtered. The filtrate was evaporated to dryness, suspended in 0.5% Sodium Carboxymethyl cellulose (as vehicle) & used for subsequent experiments.

Determination of acute Toxicity[6]

Acute oral toxicity studies for the formulations were carried out using OECD guideline 420 (modified, adopted 23rd march 2006

Antidiabetic Activity:

Effect of formulations in normoglycemic rats:

Acute study on normal rats:[7, 8]

The method of Bhopale et al., 2007 was followed. The animals were fasted for 18 hr. But allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 hr.), blood was withdrawn from the tip of the tail of each rat and the blood glucose was estimated with semi autoanalyzer. The normal rats were then divided into six groups of six animals each (n=6).

Group I- Vehicle 2% gum acacia (control) p.o.
Group II- Glibenclamide (2.5 mg/kg), p.o.
Group III- Aqueous extract of F1 (250 mg/kg), p.o.
Group IV- Aqueous extract of F1 (500 mg/kg), p.o.
Group V- Aqueous extract of F2 (250 mg/kg) p.o.
Group VI- Aqueous extract of F2 (500 mg/kg), p.o.

Blood glucose level was determined at 0 hour i.e. before drug administration, 0.5, 1, 2, 4, 8, 12, 16 and 24 hours after drug administration.

OGTT in normal rats:[9]

The method of Badole et al., 2006 was followed. Fasted rats were divided into seven groups of six rats each (n=6).
Group I - 2 % gum acacia (control), p.o.
Group II –2 gm/kg glucose, p.o.
Group III- Glibenclamide (2.5 mg/kg), p.o.
Group IV- Aqueous extract of F1 (250 mg/kg), p.o.
Group V- Aqueous extract of F1 (500 mg/kg), p.o.
Group VI- Aqueous extract of F2 (250 mg/kg), p.o.
Group VII- Aqueous extract of F2 (500 mg/kg), p.o.

After 30 min. of treatment, rats of all groups were loaded orally with glucose (2 gm/kg, p.o.). Blood samples were collected before and at 30, 90 and 150 min. after glucose administration.

Effect of formulations in STZ-induced diabetic rats:

Induction of diabetes[10]

STZ is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce DM in animals. Higher doses of STZ (60 mg/kg, i.p.) effectively induced diabetes in normal rats as reflected by severe hyperglycaemia, glycosuria (>2%), polyphagia, polydipsia and body weight loss when compared with normal rat.

Experimental design for Single-dose one-day study[11]

The diabetogenic rats, having blood glucose level more than 250 mg/dl were selected for the study. After an overnight fast, the diabetic treated animals were divided into six groups, each group contained six rats (N=6).

Blood samples were collected at 0, 0.5, 1, 2, 4, 8, 12, 16 and 24 hours after formulations/GLB administration. [single-dose one-day study] and blood sugar levels were measured by GOD-POD kit using semi autoanalyzer.

Group I-Diabetic control received 2% gum acacia, p.o.
Group II-Diabetic rats received Glibenclamide (2.5 mg/kg), p.o.
Group III- Diabetic rats received aqueous extract of F1 (250 mg/kg), p.o.
Group VI- Diabetic rats received aqueous extract of F1 (500 mg/kg), p.o.
Group V- Diabetic rats received aqueous extract of F2 (250 mg/kg), p.o.
Group VI-Diabetic rats received aqueous extract of F2 (500 mg/kg), p.o

Experimental design for Multiple-dose twenty one-day study[11]

The animals treated with respective doses of formulations and GLB were further treated for twenty one consecutive days [Multiple-dose twenty one-day study] in order to evaluate the chronic effect.

Group I – Normal control received 2 % gum acacia, p.o.
Group II—Diabetic control received 2% gum acacia, p.o.
Group III- Diabetic rats received Glibenclamide (2.5 mg/kg), p.o.
Group IV – Diabetic rats received aqueous extract of F1 (250 mg/kg), p.o.
Group V – Diabetic rats received aqueous extract of F1 (500 mg/kg), p.o.
Group-VI – Diabetic rats received aqueous extract of F2 (250 mg/kg), p.o.
Group-VII—Diabetic rats received aqueous extract of F2 (500 mg/kg), p.o

The results are depicted in table no.10 (Page no.69).

Estimation of biochemical parameters:[12]

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analyzed spectrophotometrically.

Statistical analysis:

The results are expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Dunnett's't' test using Graph Pad Prism version 4.0, USA.

RESULTS

Preparation of formulation:

Formuation-1 and Formulation-2 were prepared by simple percolation process. Distilled water was used for percolation process. The extractive value is mentioned in table No.2.

Table No 2: The percentage yield of formulations

Sl. No.	Formulations	Percentage yield
1.	Formulation-1	16.50 %
2.	Formulation-2	15.50 %

Pharmacological Evaluation:

Acute oral toxicity studies: Animals showed good tolerance to single doses of formulation-1 and formulation-2 in doses as high as 2000 mg/kg b.w. and were non-lethal. We selected 250 and 500 mg/kg b.w. of formulation-1and formulation-2 to test the anti-diabetic and antioxidant effect. Further, both the doses of formulation-1and formulation-2 did not produce any noticeable signs of toxicity (behavioural changes) and mortality after once daily administration for fourteen-days.

Effect of formulations in normoglycemic rats:

Acute study on normal rats:

The hypoglycemic effect of F1 and F2 were investigated in normal rats and the results were expressed in Table 3 and Fig 1.

The extract of F1 and F2 at the dose of 250 mg/kg and 500 mg/kg exhibited significant reduction (P<0.01 and P<0.001 respectively) in SG level over the period of 12 hr. compared to normal control group.

Table No: 3: Effect of F1 and F2 on blood glucose level in normal rats

Groups	Percentage decrease in blood glucose level mg/Dl								
	0 hr	0.5 hr	1 hr	2 hr	4 hr	8 hr	12 hr	16 hr	24 hr
Control	83.9 ± 2.01	6.70 ± 0.67	10.2 ± 1.90	13.4 ± 2.11	13.9 ± 1.58	14.0 ± 1.7	15.4 ± 1.78	13.4 ± 0.60	15.2 ± 2.25
Standard Glibenclamide 2.5mg/kg	83.3 ± 2.55	13.9 ± 2.11**	24.2 ±1.93***	34.3 ±2.04***	42 ±2.41***	46.6 ± 1.74***	51 ±1.84***	24.5 ± 2.50**	16.4 ± 3.16
F1 250 mg/kg	74.5± 1.87	3.31 ± 1.45	6.93 ± 0.78	18.2 ± 1.14	27.3 ± 1.73**	29.4 ± 1.70**	35 ± 1.44**	17.69 ± 2.41	10 ± 0.90
F1 500mg/kg	84.7 ± 3.29	5.72 ± 0.73	23.7 ± 1.03***	27.6 ±1.74***	33.7 ±1.61***	40.2 ± 1.78***	45.9 ±1.84***	21.52 ± 2.51**	16.4 ± 3.16
F2 250 mg/kg	91.11 ±1.89	3.86 ±0.07	5.62 ±0.10	11.48 ±0.10	15.66 ±0.11***	23.44 ±0.11***	32.40 ±0.01**	16.54 ±0.15***	7.35± 0.10
F2 500 mg/kg	78.4 ± 1.68	3.55 ± 1.32	14.7 ± 0.59**	23.9 ±1.16***	31.2 ±1.36***	36 ± 1.25***	41.2 ±1.64***	20.82 ± 2.49	9.99 ± 1.44

Values are mean SEM; n=6
**P<0.01, ***P<0.001 compared with normal control

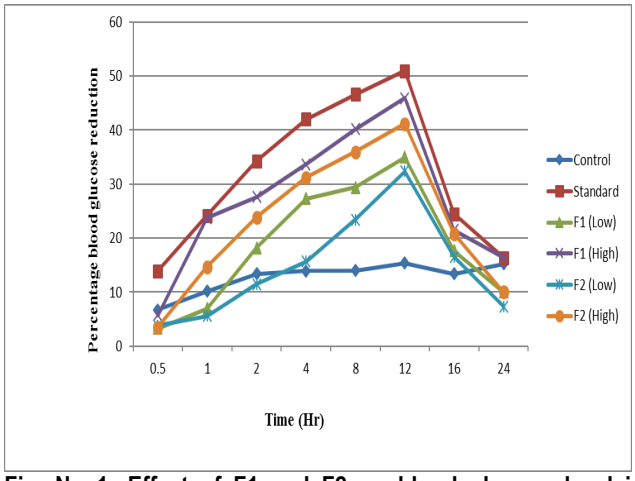

Fig. No 1: Effect of F1 and F2 on blood glucose level in normal rats

500 mg/kg of F1and F2 showed maximum blood glucose level reduction at 12th hour (45.9±1.84 % and 41.2±1.64 respectively P<.001) compared to the normal control. The reduction in blood glucose levels at 1st, 2nd, 4th, 8th and 16th hrs. Was also significant, as compared to the normal control.

Glibenclamide showed its significant effect from 1st hour after the treatment. It showed maximum reduction in blood glucose level at 12th hour (51±1.84, P<0.001). The reduction in blood glucose level at 1st, 2nd, 4th, 8th and

16th hour was also significant (P<0.001) as compared to the normal control group.

Testing of formulations in STZ-induced diabetic rats:

Single-dose one-day study:

A single dose of F1 (250 and 500 mg/kg) and F2 (250 and 500mg/kg) treatment exhibited reduction in SG levels at different time intervals compared to basal levels (0hr). However, 500 mg/kg of F1 and F2 treated animals showed significant percentage reduction (P<0.001)in SG levels (50.52±0.13 and 49.60±0.11 respectively) over 12 hr. post treatment compared to their basal levels. Also it reduced blood glucose level significantly at 1st, 2nd, 4th, 8th& 16th hr. as compared to the diabetic control group (P<0.001). The onset of action started from the 1st hour.

Administration of GLB showed significant reduction (P<0.001) in SG levels with maximum reduction (57.52±0.15) at 12 hr. compared to their basal levels. The reduced blood glucose level was significant at 1st, 2nd, 4th, 8th& 16th hr. as compared to the diabetic control group (P<0.001). The onset of action of Glibenclamide started from the 1st hour.

Furthermore, single dose treatment of F1 and F2 (250 mg/kg) showed significant reduction (P<0.001) in SG levels at different time intervals as compared to diabetic control group. These data suggested that, F1 and F2 exhibited significant hypoglycemic activity in STZ-induced diabetic rats.

The results are depicted in Table 4 and Fig 2

Multiple-dose twenty one-day study:

Repeated administration of F1 and F2 (250 mg/kg) for 21 days showed significant reduction in SG levels (130.79±5.10 and 147.43±5.32 respectively, P<0.001) compared to basal values (0 day). However, it was more marked in animals treated with a dose of 500 mg/kg of F1 and F2 (125.79±8.44 and 139.19±4.15 respectively, P<0.001). On 21st day, higher doses of F1 and F2 showed greater percentage reduction in glycemia compared to diabetic control and their potency is comparable to GLB treated diabetic rats (105.79±5.9, P<0.001).

Multiple-dose twenty one-day study suggested that both the tested doses of F1 and F2 showed better anti-diabetic activity. The results are depicted in Table no.5 and Fig 3

DISCUSSION

In vivo anti-diabetic study:

Diabetes mellitus (DM), is a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main causes of morbidity and mortality within the next 25 years. Interest in the evaluation of plant products to treat DM is growing because plants contain many bioactive substances with therapeutic potential. In recent years, several authors have evaluated the efficacy of different medicinal plants in the DM. To our knowledge, this is the first detailed study in which a follow up was carried out to investigate the potency of aqueous extracts of Formulation-1 (F1) and Formulation-2 (F2) with different compositions containing Trigonella foenum-graecum, Eugenia jambolana, Momordicacharantia, Seasumindicum, Allium sativum and Azadirachta indica on the glucose levels, lipid profile, non-enzymatic and enzymatic antioxidants in STZ-induced diabetic rats.

Table No. 4: Effect of F1 and F2 on blood glucose level in STZ induced diabetic rats (single dose one day study)

G	Percentage blood glucose reduction mg/dl								
	0 hr.	0.5 hr.	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	16 hr.	24 hr.
Control	250.6±0.33	0.51±0.04	0.97±0.02	1.14±0.04	1.48±0.16	2.66±0.13	2.44±0.14	1.67±0.11	0.81±0.60
Standard Glibenclamide 2.5 mg/kg	263.1±0.26	21.27±0.01	32.96±0.12**	37.56±0.12**	47.67±0.12**	51.56±0.10**	57.52±0.15**	34.38±0.11	7.50±0.13
F1 250 mg/kg	261.2±0.43	8.55±0.07	18.06±0.01*	28.62±0.07*	31.50±0.01*	37.53±0.17*	47.75±0.15	29.52±0.12	4.63±0.13
F1 500 mg/kg	265.1±0.37	13.35±0.05	23.73±0.02**	34.61±0.13**	41.50±0.12*	43.59±0.20**	50.52±0.13**	30.45±0.13	8.51±0.16
F2 250 mg/kg	252.4±0.66	2.76±0.07	11.31±0.12*	25.82±0.10*	26.39±0.13*	37.70±0.14**	45.61±0.15**	25.59±0.13	6.29±0.86
F2 500 mg/kg	257.5±0.90	9.34±0.09	17.75±0.06**	30.38±1.01**	33.48±0.14*	39.51±4.90**	49.60±0.11**	26.53±0.15	5.36±0.12

values are mean SEM; n=6 **P<0. 01, ***P<0.001 compared with the diabetic control.

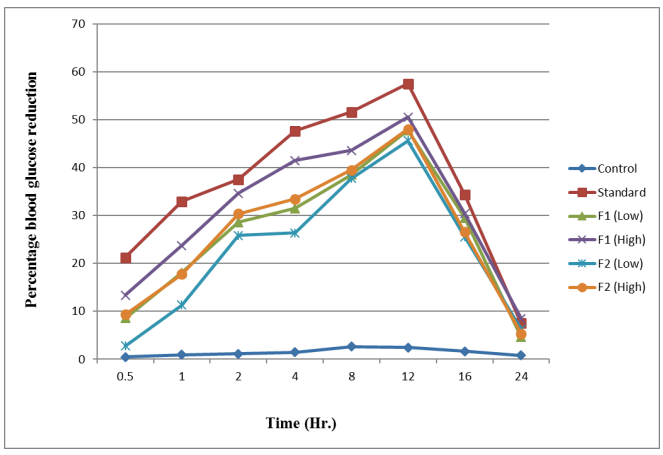

Fig. No. 2 Effect of F1 and F2 on blood glucose level in STZ induced diabetic rats (single dose one-day study)

Table. 5: Effect of F1 and F2 on blood glucose level in STZ induced diabetic rats (multiple-dose twenty one-day study)

Groups	Blood glucose level mg/dl			
	0 day	7th day	14th day	21st day
Control –ve	80.43± 1.676	91.61±2. 58	86.44±1.88	80.44±1.77
Control +ve	240.20 ±5.167	273.018± 6.197	286.9±6.72	262.29±6.4 6
Standard Glibenclamide 2.5 mg/kg	258.00 ±4.496	195.80±8 .610***	128.87±9.8 3***	105.79±5.9 ***
F1 250 mg/kg	259.18 6±4.99 7	225.80±1 3.51	167.9±12.1 ***	130.79±5.1 0***
F1 500 mg/kg	268.11 6±5.54 0	214.3±9. 76***	148.97±4.1 6***	125.79±8.4 4***
F2 250 mg/kg	258.09 7±4.46 3	233.92±5 .45	163.05±5.7 7***	147.43±5.3 2***
F2 500 mg/kg	263.08 1±6.67 9	213.21±4 .25***	151.97±4.1 5***	139.19±4.1 5***

Values are mean ± SEM; n=6
***P<0.001 compared with the diabetic control.

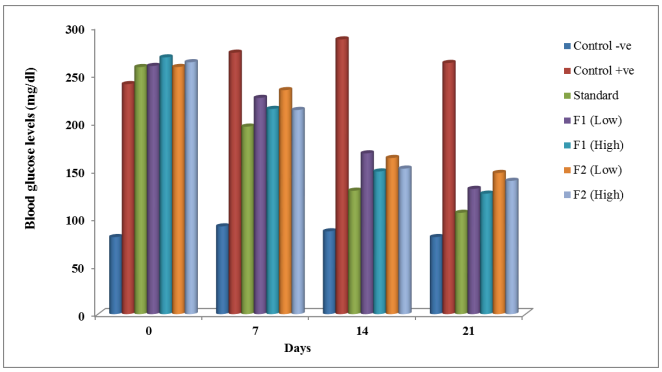

Fig 3: Effect of F1 and F2 on blood glucose level in STZ induced diabetic rats (multiple-dose twenty one-day study)

The present data indicates that F1 and F2 significantly reduced hyperglycemia in both single-dose one-day and multiple-dose twenty one-day diabetic studies and were comparable to standard Glibenclamide. This could be mediated by improving the glycemic control mechanisms (extra-pancreatic) and increasing insulin secretion from remnant pancreatic cells in diabetes rats.

CONCLUSION

These results demonstrate the strong hypoglycemic activity of formulation-1 and formulation-2 (250 mg/kg and 500 mg/kg) with different composition in STZ induced diabetic model.

So it may be concluded from the results of our present study that a combinations of several herbal plants exert the significant antidiabetic effects. These could be due to different types of active principles, each with single or diverse range of biological activities, which shows synergistic action as antidiabetic drug. This study emphasized the need to carry out in-depth pharmacological evaluations of herbal formulations and ascertain their claims in the light of modern scientific understanding such that their potentials may be tapped for better use as alternate and safe herbal drugs.

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