Journal of Traditional and Complementary Medicine 8 (2018) 506–514

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Preliminary clinical assessment and non- toxicity evaluation of an ayurvedic formulation BGR-34 in NIDDM

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ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form
29 November 2017
Accepted 29 November 2017
Available online 3 April 2018

Keywords:
BGR-34
Berberis aristata
NIDDM
Indian system medicine
Type 2 diabetes

ABSTRACT

In view of the overall health impact of NIDDM, inventers understand the necessity of improving glycemic control in adults with type 2 diabetes. BGR-34 provides an effective treatment option for adults with type 2 diabetes who have been inadequately controlled on lifestyle with or without other oral hypoglycemic agents (OHGAs) such as metformin, sulfonylurea, or a glitazones. BGR-34 is an appropriate option to consider for addition to a managed care drug formulary. Treatment with BGR-34 produced clinically relevant and statistically significant reductions in all three key measures of glucose control studied —FPG, PPBG and HbA1c— when compared with placebo. BGR-34, showed the promising result with respect to glycemic parameters in NIDDM patient with a significant reduction in fasting blood sugar by 34.3%, postprandial blood sugar 35.5% & glycosylated haemoglobin by 20.31% as compared to placebo group showing a reduction by 13.2%, 10.9% & 10.87% respectively. The trial has also been registered to CTRI, India. This study has been registered in the clinical trial registry-India.

1. Introduction

Diabetes is nowadays considered as a global epidemic with 415 million patients recorded in 2015, and around 642 million patient expected by 2040.[1,2] Diabetes is categorized into two main types insulin-dependent (IDDM) and non-insulin dependent (NIDDM). The most widely drug used for treatment of NIDDM, are metformin, glimepiride, repaglinide, pioglitazone, sitagliptin, and acarbose.[3] The use of these oral hypoglycaemic drugs may be effective in controlling blood glucose level, but may not prevent all the complications of diabetes.[4] Keeping in view the possibility of above side effect, World Health Organization Expert Committee on diabetes has encouraged that traditional medicinal herbs should be investigated on large scale for discovering safe and effective oral anti-diabetic agents. Therefore, there is a clear need for exploring alternate source of anti-diabetic drugs. The present study was directed to polyherbal anti-diabetic drug BGR-34, developed by Council of Scientific & Industrial Research, India (CSIR). It is the combination of *Berberis aristata*, *Tinospora cordifolia*, *Pterocarpus marsupium*, *Gymnema sylvestre*, *Rubia cordifoila* & *Trigonella foe-numgraecum* already known for controlling diabetes mellitus.[5–10] Aimil Pharmaceuticals (India) Ltd is licensee for manufacturing and marketing of BGR-34 worldwide. The Pre-clinical studies of BGR-34, produced promising outcomes on diabetes induced experimental rats without producing adverse effect on liver, heart and kidney (unpublished data CSIR).

The aim of the present study is, to evaluate the safety & clinical efficacy of BGR-34 with following objectives:

i. To setup the safe limits of BGR-34 dosage through acute and sub-acute toxicity in rats.

Abbreviations: NIDDM, noninsulin-dependent diabetes mellitus; CSIR, council of scientific & industrial research, india; CTRI, clinical trial registry-India; OHGAs, other oral hypoglycemic agents; FPG, fasting plasma glucose; PPBG, post-prandial blood glucose; HbA1c, glycosylated haemoglobin; OECD, organization for economic co-operation and development; CPCSEA, committee for the purpose of conduct and supervisions of experiments on rats; TLC, TOTAL leukocyte count; DLC, differential leukocyte count; Hb, haemoglobin; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxaloacetate transaminase; ALP, alkaline phosphatase; BBN, total bilirubin; HDL, high-density lipoproteins; OPD, out Patient Department.

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

https://doi.org/10.1016/j.jtcme.2017.11.004

ii. To estimate the anti-diabetic efficacy of BGR-34 in Indian NIDDM patients on blood glucose regulation.

2. Materials and methods

2.1. Plant material collection

The medicinal plants (Table 1) were procured, from the local herbal market and authenticated in-house by Dr. H.B Singh, former chief scientist, Raw Materials Herbarium and Museum, NISCAIR, New Delhi. Authenticated voucher samples of raw material were preserved in research and development section of Aimil Pharmaceuticals (I) Ltd.

2.2. Preparation of BGR-34

BGR-34 was prepared at Aimil Pharmaceuticals (I) Ltd as per methodology mentioned in know how document received from CSIR- NBRI-CIMAP, India.

2.3. Toxicity studies

An acute and sub-acute oral toxicity studies were conducted in accordance with the Organization for Economic Co-operation and Development[11–13] Guideline 425 and 407 respectively. The experimental protocol has been approved by the Institutional Animal Ethics Committee of Shree Dhanvantry Pharmaceutical Analysis and Research Centre Pvt. Ltd. with the Experimental Protocol Approval Number SDPARC/IAEC/2015/046 and SDPARC/IAEC/2015/051 respectively prior to the initiation of the study. Experiments were performed as per the instructions prescribed by the Committee for the Purpose of Conduct and Supervisions of Experiments on Rats (CPCSEA), Ministry of Environment and Forest, Government of India.

2.4. Experimental rats

Female albino Wistar (Mahaveer Enterprises, Hyderabad) weighing 180–200 g ± 20 were maintained under standard laboratory conditions of temperature (22 ± 3 °C) and humidity 30–70% with 12 h day: 12 h night cycle. Rats had free access to water and rodent pellet diet (Hindustan Lever Ltd, Bangalore, India).

2.5. Acute oral toxicity study

Acute oral toxicity study of BGR-34 was carried out in 15 adult female Wistar as per the[11,12] test guidelines 425. All rats were dosed orally once in a stepwise manner i.e next higher dose level was administered to next animal after observation of the previous animal for any mortality for 48 h. Dose levels were progressed in geometric progression with the factor of 2. Dosing was started by oral administration of 250 mg/kg bw of BGR-34 to Ist test animal. As no mortality was observed in Ist animal when observed for 48 h, next animal was treated with 500 mg/kg bw dose and observed in a similar manner and so on up to 2000 mg/kg bw. A total of 5 rats were tested, at test dose 2000 mg/kg bw and observed for any clinical sign of toxicity for a total of 14 days. Dosage progression has been depicted in Table 2.

Table 1
Composition: each tablet contains following ingredients.

Drug name	Botanical name	Part used
Daruharidar	*Berberis aristata*	Stem
Vijaysar	*Pterocarpus marsupium*	Heart wood
Gudmar	*Gymnema sylvestre*	Leaf
Manjeeth	*Rubia cordifolia*	Root
Methika	*Trigonella foenum graceum*	Seed
Giloy	*Tinospora* cordifolia	Stem

2.6. Sub-acute oral toxicity

For sub-acute toxicity study, a total of 50 Wistar (25 males and 25 females) were used. They were subdivided into 5 groups of 10 rats each (5 males and 5 females), according to[13] guideline 407. The first group (G1), was the control group which received distilled water while the next three test groups (G2, G3, G4), were served BGR-34 orally at the doses of 1500; 750 and 375 mg/kg bw/day respectively the equivalent high, normal and low dosage for. The duration of treatment and observation for these first four groups was 28 days. The last group, called satellite group/reversible group (G5) was treated in similar manner with high dose level (1500 mg/kg bw/day) for 28 days and further observed without medicine for next 14 days post-treatment for the reversibility, persistence, or delayed occurrence of toxic effects of BGR-34 and were sacrificed on 43 day. During this period, all the rats were observed daily for signs of toxicity and mortality. The changes in body weight, food and water intake and clinical signs were also observed and recorded.

2.7. Blood analysis

On the end of dosing and observation period, blood was collected from retro orbital sinus from all rats for hematological study viz haematocrit (%), haemoglobin (gm %), Total leukocyte count (TLC) and Differential leukocyte count (DLC) were estimated by following method of Docie.[14]

2.8. Clinical biochemistry

To investigate major toxic effects in tissues and, specifically, effects on kidney and liver, blood samples obtained from all rats just prior to killing the rats for biochemical examinations were performed at the end of the test period: the analysis of blood glucose,[15] serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT)[16,17]; alkaline phosphatase (ALP), total bilirubin (BBN),[18] Urea[19]; total protein; albumin[20] and creatinine[21]; Cholesterol, HDL Cholesterol[22] and Triglyceride[23] were estimated in serum.

2.9. Histopathological studies

After collecting a blood sample, the vital organs (brain, heart, lung, kidney, and liver) were excised and their wet weight was taken as soon as possible after dissection to avoid drying. These

Table 2
Dosage progression for LD $_{50}$ determination of BGR-34 in single dose oral toxicity study.

Day	Animal no.	Dose (mg/kg bw)	Outcome
1	1	250	No death
3	2	500	No death
5	3	1000	No death
7	4	2000[a]	No death
9	5	2000[a]	No death
11	6	2000[a]	No death
13	7	2000[a]	No death
15	8	2000[a]	No death

[a] 2000 mg/kg bw is the limit test dose.

organs were then preserved in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The stained sections were examined under a bright field microscope for any cellular damage or change in morphology.[24]

2.10. Clinical study

A clinical trial of the anti-diabetic potential of BGR-34 was conducted in accordance as per the Indian Council of Medical Research guidance document, 2006[25] on conducting trials of ayurvedic substances. The experimental protocol has been approved by the ethical committee on human safety trial and the study was conducted at Aggarwal hospital, New Delhi, India, between 6/10/2014 to 6/4/2015 on OPD basis. This study has been registered in the clinical trial registry-India (CTRI Registration number: CTRI/2016/11/007476).

2.11. Study design

Sixty four NIDDM outpatients attending the Aggarwal hospital, New Delhi, India were selected based on inclusion and exclusion criteria. They were served with placebo one month duration (the one month run period with dietary and life style schedule to be followed). The patient were randomised divided into BGR 34 as test groups and Triphala as placebo groups. Patients underwent clinical examination and biochemical investigation on day 1 and at monthly intervals. Adverse drug reaction (eg. headache, dizziness, nausea, vomiting)/effects if any were also recorded during study period. The study protocol was approved by the hospital's institute's Ethics Committee with the Protocol Approval Number AH/IEC/NBRMAP-DB01/6.4.2014. Informed written consent was obtained from all study participants. If they so desired, patients were free to withdraw from the study.

2.12. Inclusion criteria

The diabetes patients between age 25–60 years, either male or female diagnosed with type 2 Diabetes mellitus, and characterized by fasting plasma glucose (FPG) > 126 mg/dL or 2 h post-prandial blood glucose (PPBG) > 200 mg, selected for present study.

2.13. Exclusion criteria

Patients on Insulin, and with acute infections or chronic debilitating diseases, tuberculosis, malignancy, HIV infection etc. were excluded from the clinical study. Pregnant and lactating women, patients with concomitant severe illness requiring other medication, patients with severe hypertension and the patients having the history of severe unstable angina, myocardial infarction, renal failures were excluded from the study.

2.14. Follow-up and assessment

All subjects underwent clinical examination and evaluation of blood sugar levels on entry and at monthly intervals for the 4 month study period as per assessment method illustrated in Fig. 1. At each monthly visit, subject evaluations were based on symptoms, fasting plasma glucose (FPG) and post-prandial blood glucose (PPBG). The glycosylated haemoglobin (HbA1c), was done at the day 1 and day 120.

2.15. Primary and secondary outcome measure

The primary end-point was symptomatic relief, reduction and control of NIDDM symptoms, polyuria, nocturia, polyphagia, polydipsia, pain calf muscle, burning sensations of soles and palm, general fatigue, loss of weight, decreased libido, itching on genital, blurred vision delayed healing of wound and quality of life at: 0, 30, 60, 90, 120 days. The assessment was done on the basis of specially prepared Performa for assessing.

2.16. Statistical analysis

Data was arranged in MS Excel. Student-t test was used to compare the difference in mean values between the two groups. Paired *t*-test has been used for within group analysis. For every outcome variable, results are presented as mean ± SD (standard deviation), p-value <0.05 was considered statistically significant. STATA 12.0 (STATA Corp, Houston, TX, USA) statistical software has been used for data analysis.

3. Results

3.1. Acute oral toxicity study

In Acute oral toxicity studies, test drug administered rats were safe at the limit test dose of 2000 mg/kg body weight with no signs and symptoms of toxicity and mortality.

3.2. Sub-acute oral toxicity

3.2.1. Changes in body weight, water, and food intake

After administration of test drug (2000 mg/kg), significant changes occurred in body weight, water, and food intake in female and male rats. Both the control and treated rats appeared healthy throughout the study period (Figs. 2 and 3).

3.2.2. Clinical signs of toxicity

Rats were observed at 24 h interval up to 28 day for any clinical signs of toxicity. Three male and 2 female rats of group G2 and G5 showed piloerection at 21 day. The reversal in piloerection was observed in all male and female rats of group at G5 during the observation period of additional 14 days post treatment. G1, G3 and G4 did not show any clinical sign of toxicity entire observation period.

3.2.3. Hematological analysis

The effect of test drug on hematological parameters are given in Tables 3 and 4. There was no significant ($P < 0.05$) difference in TLC, Hb, neutrophil, lymphocyte, monocyte and eosinophil between test drug group and control group.

3.2.4. Biochemical analysis

The effect of test drug on kidney function and liver function parameters are given in Tables 5 and 6. Groups G2 and G5 showed significantly decrease in SGPT level as compared to normal control group in both male and female rats. However test groups and G1 had non-significant differences in SGOT levels. Alkaline phosphatase level in the test groups and control group did not show any significant difference. Male rats of G2 (high dose group) showed significantly low bilirubin compared to control group however, no such changes was observed in G2 (high dose group) female rats. Triglyceride level was found significantly decreased for males in G2 (high dose group) but non-significant decrease for female rats. There was no significant difference between test drug group and their respective control group for serum urea, serum creatinine, serum albumin, total serum protein, serum cholesterol, serum glucose and serum basophil.

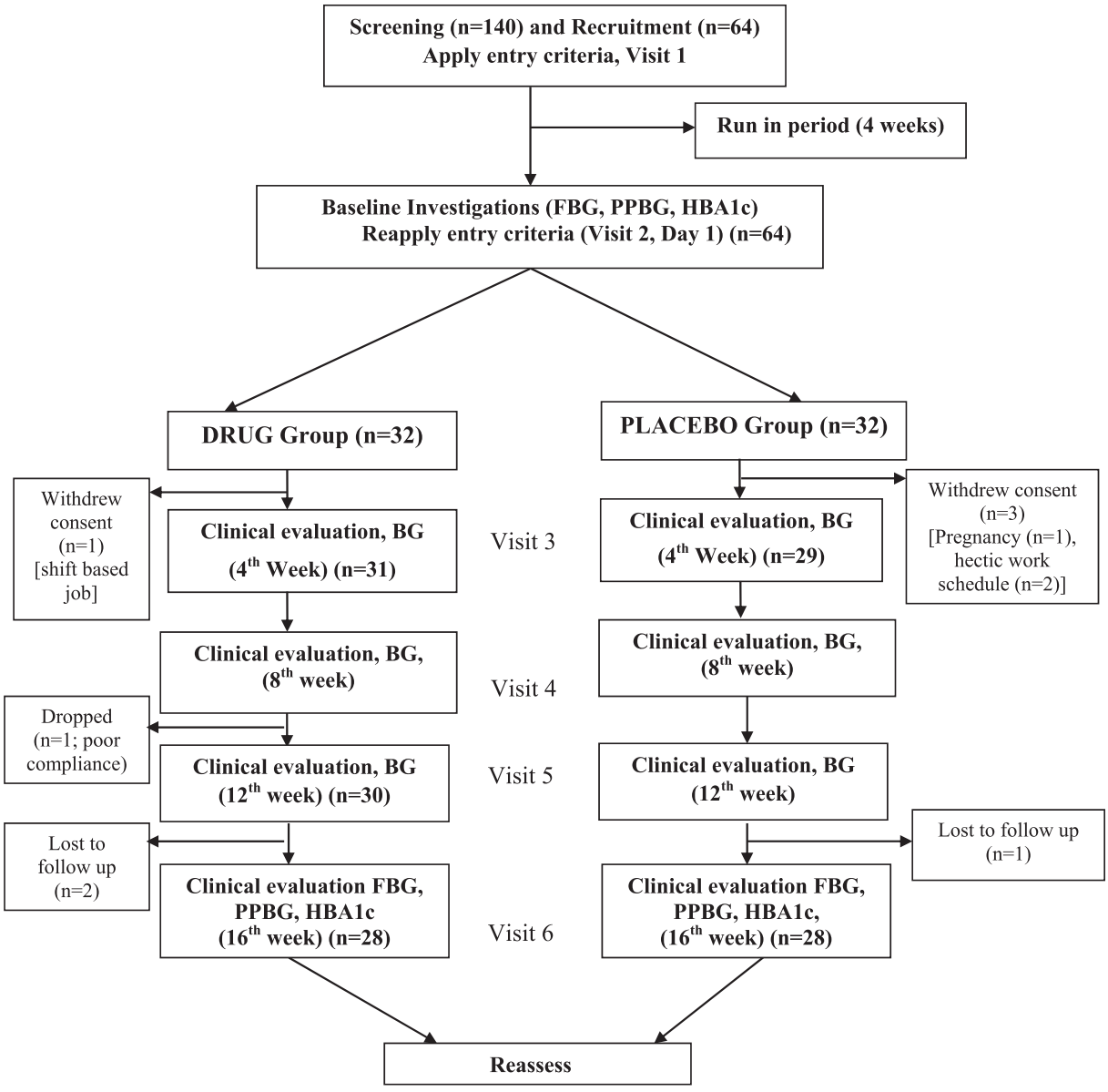

Fig. 1. Assessment Method: Study design and patient recruitment process. BG; Blood glucose, FBG; Fasting blood glucose, PPBG; Post prandial blood glucose, HBA1c; Glycosylated haemoglobin.

3.2.5. Relative organs weight

There was no significant difference ($P < 0.05$) for organs like kidney, liver, spleen, testes, ovaries, heart, lungs and brain was observed between treated male and female groups compared to G1 (control group).

3.2.6. Gross necropsy

Mild pin point haemorrhage in lungs and pneumonitis was observed in 1 female rat of G2 (high dose treated group). No other macroscopically abnormality was found in observed organs of treated groups.

3.2.7. Histopathology

Mild alveolar histiocytosis was observed (Fig. 4 G) in 1 female rat of G 2 and pneumonitis was observed (Fig. 4 B) in 1 male animal of G 2, which may be attributed to repeated dosage administration. No abnormality was seen in any of the treated groups of high dose treated group as compared to background vehicle control (Fig. 4 P).

3.3. Clinical study

Fifty six patients (30 male and 26 females) with type 2 diabetes mellitus participated in the study, out of 64 total enrolled. There were 28 patients in the test drug group and 28 patients in the placebo group. The mean age of patients for drug and placebo group was 47.9 ± 6.7 years and 49.7 ± 5.9 years respectively. Average body weight in the drug group and placebo group was 67.04 ± 8.6 kg and 70.1 ± 6.9 kg respectively. This clinical trial was registered to CTRI, India with registration no CTRI/2016/11/007476.

3.4. Fasting blood glucose (FBG)

Biochemical results of all patients were analyzed before and after completion of the study. Blood sugar fasting showed significant reduction ($p = 0.001$) from 196.0 ± 32.7 mg/dL to 129.3 ± 33.3 mg/dL in test drug treated group as compared to placebo group where fasting blood sugar reduced from

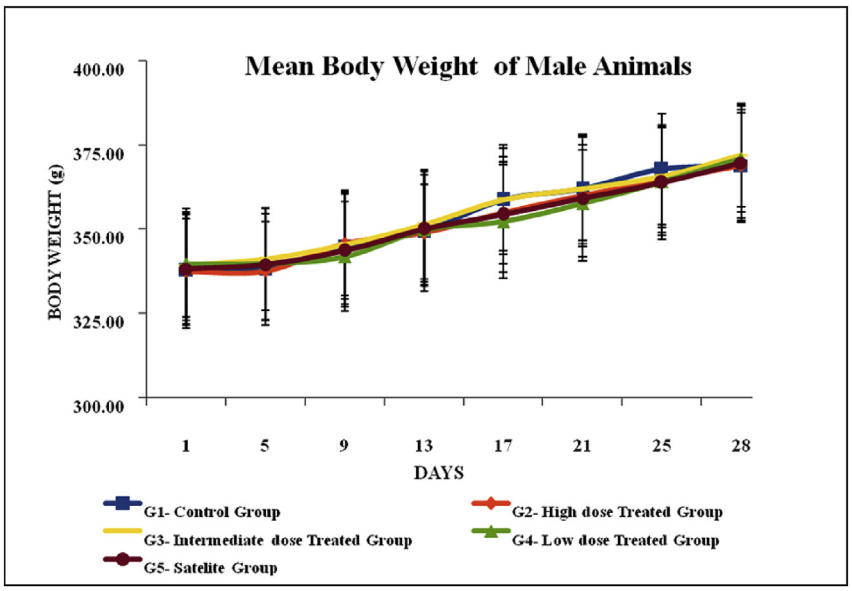

Fig. 2. Mean body weight of male rats during the treatment period.

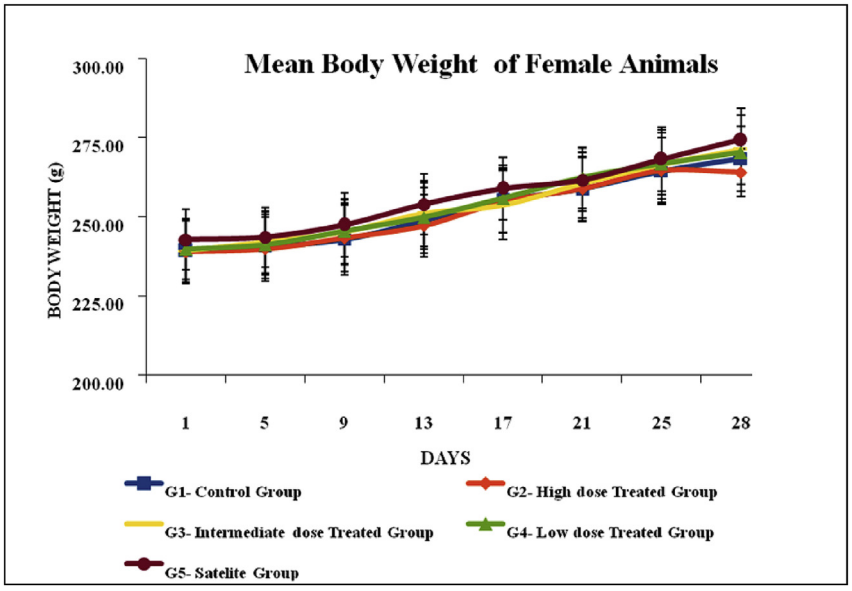

Fig. 3. Mean body weight of female rats during the treatment period.

Table 3
Hematological parameters in female rats.

Group	Days				
	G1- Control	G2- High dose	G3- Intermediate dose	G4- Low dose	G5- Satellite
WBC	6240 ± 588.72	6220 ± 572.19	6820 ± 376.03	6740 ± 1585.43	7820 ± 1098.81
HGB	13.78 ± 0.63	13.50 ± 0.39	13.80 ± 0.70	12.12 ± 1.22	13.04 ± 0.64
NEUT	7.00 ± 0.45	6.20 ± 1.02	8.60 ± 1.57	8.90 ± 1.63	8.20 ± 0.86
LYMPH	81.40 ± 0.86	82.80 ± 1.24	79.20 ± 3.31	82 ± 2.51	78.6 ± 3.61
MONOCYTES	11.00 ± 1.10	11.60 ± 1.1	12.40 ± 1.44	11.2 ± 1.44	12 ± 0.71
EOSINOPHIL	1.40 ± 0.25	1.60 ± 0.25	1.20 ± 0.20	1 ± 0.32	1.6 ± 0.25
BASOPHIL	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Data has been expressed as Mean ± SEM.

187.2 ± 43.3 mg/dL to 162.9 ± 41.6 mg/dL. The percent reduction in the test drug treated group was highly significant ($p < 0.001$) as compared to the placebo group (Table 7).

Table 4
Hematological parameters in male rats.

Group	Days				
	G1- Control	G2- High dose	G3- Intermediate dose	G4- Low dose	G5- Satellite
WBC	8440 ± 559.10	7920 ± 609.43	9080 ± 868.60	8160 ± 1523.67	9460 ± 1523.67
HGB	14.62 ± 0.42	12.44 ± 0.98	12.16 ± 0.38	13.94 ± 0.39	14.08 ± 0.36
NEUT	7.60 ± 1.12	7.00 ± 0.32	6.20 ± 0.37	7.40 ± 1.69	7.20 ± 1.77
LYMPH	80.40 ± 1.99	80.40 ± 0.68	82.20 ± 1.02	78.60 ± 2.38	76.40 ± 2.38
MONOCYTES	10.00 ± 2.38	10.60 ± 0.75	10.40 ± 0.51	12.00 ± 0.51	12.00 ± 1.14
EOSINOPHIL	2.00 ± 0.63	2.00 ± 0.32	1.40 ± 0.32	2.00 ± 0.45	1.80 ± 0.37
BASOPHIL	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Data has been expressed as Mean ± SEM.

Table 5
Biochemical parameters in male rats.

Group	Days				
	G1- Control	G2- High dose	G3- Intermediate dose	G4- Low dose	G5- Satellite
SGPT (IU/L)	56.20 ± 2.82	46.20 ± 1.69	53.00 ± 3.00	51.80 ± 4.35	47.80 ± 2.82
SGOT (IU/L)	91.44 ± 3.40	91.96 ± 2.97	98.40 ± 8.60	99.00 ± 4.72	90.40 ± 4.55
ALP (IU/L)	168.90 ± 28.43	132.39 ± 13.67	134.04 ± 9.50	150.30 ± 21.13	138.38 ± 8.59
BBN- D (mg/dl)	0.20 ± 0.03	0.24 ± 0.02	0.26 ± 0.02	0.24 ± 0.01	0.24 ± 0.02
BBN- ID (mg/dl)	0.16 ± 0.02	0.07 ± 0.00	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.02
BBN-T (mg/dl)	0.36 ± 0.04	0.31 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.32 ± 0.04
Urea (mg/dl)	55.74 ± 4.34	56.90 ± 5.27	59.34 ± 1.79	54.44 ± 1.63	57.08 ± 4.95
Creatinine (mg/dl)	0.76 ± 0.02	0.78 ± 0.04	0.76 ± 0.02	0.78 ± 0.02	0.76 ± 0.03
Glucose (mg/dl)	98.00 ± 4.34	101.40 ± 2.42	100.00 ± 4.57	103.80 ± 5.35	101.44 ± 1.82
Triglyceride (mg/dl)	95.20 ± 7.96	76.80 ± 3.60	81.60 ± 9.44	88.00 ± 4.62	78.60 ± 3.56
Cholesterol (mg/dl)	68.20 ± 3.84	62.60 ± 6.18	64.20 ± 5.30	62.60 ± 2.40	63.20 ± 3.27
Albumin (g/dl)	3.58 ± 0.08	3.56 ± 0.15	3.54 ± 0.06	3.45 ± 0.12	3.52 ± 0.10
Total Protein (g/dl)	6.80 ± 0.20	6.76 ± 0.26	6.58 ± 0.34	6.58 ± 0.18	7.00 ± 0.23
HDL Cholesterol (mg/dl)	23.44 ± 1.26	23.18 ± 0.81	21.36 ± 1.23	27.44 ± 0.85	24.08 ± 0.69

Data has been expressed as Mean ± SEM.

Table 6
Biochemical parameters in female rats.

Group	Days				
	G1- Control	G2- High dose	G3- Intermediate dose	G4- Low dose	G5- Satellite
SGPT (IU/L)	62.20 ± 2.24	53.00 ± 2.21	55.20 ± 7.31	58.80 ± 2.66	53.20 ± 2.27
SGOT (IU/L)	98.86 ± 6.11	89.20 ± 5.75	83.66 ± 20.25	85.04 ± 6.10	87.46 ± 6.97
ALP (IU/L)	127.94 ± 21.51	122.20 ± 14.60	134.22 ± 20.86	124.84 ± 27.32	125.80 ± 11.43
BBN- D (mg/dl)	0.22 ± 0.03	0.21 ± 0.01	0.26 ± 0.02	0.23 ± 0.03	0.26 ± 0.02
BBN- ID (mg/dl)	0.09 ± 0.01	0.11 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
BBN-T (mg/dl)	0.31 ± 0.04	0.32 ± 0.03	0.32 ± 0.03	0.30 ± 0.02	0.33 ± 0.02
Urea (mg/dl)	60.14 ± 2.09	58.86 ± 3.24	65.54 ± 4.02	62.88 ± 3.04	61.88 ± 4.21
Creatinine (mg/dl)	0.84 ± 0.05	0.82 ± 0.02	0.88 ± 0.11	0.88 ± 0.05	0.80 ± 0.04
Glucose (mg/dl)	90.40 ± 11.57	98.20 ± 8.40	97.00 ± 6.00	99.00 ± 10.18	93.60 ± 4.32
Triglyceride (mg/dl)	96.80 ± 4.34	89.00 ± 4.45	88.80 ± 17.88	99.40 ± 12.24	93.40 ± 9.32
Cholesterol (mg/dl)	86.40 ± 5.72	84.20 ± 5.06	86.80 ± 5.40	88.20 ± 7.66	87.60 ± 5.56
Albumin (g/dl)	3.99 ± 0.21	3.97 ± 0.11	3.76 ± 0.14	3.84 ± 0.21	3.62 ± 0.20
Total Protein (g/dl)	6.88 ± 0.23	6.66 ± 0.10	6.74 ± 0.52	6.50 ± 0.38	6.54 ± 0.20
HDL Cholesterol (mg/dl)	28.36 ± 1.43	27.66 ± 1.45	25.30 ± 1.76	39.92 ± 2.93	29.40 ± 2.41

Data has been expressed as Mean ± SEM.

3.5. Post-prandial blood glucose (PPBG)

Blood glucose post-prandial showed significant reduction ($p < 0.001$) from 276.8 ± 59.7 mg/dL to 191.9 ± 49.3 mg/dL in test drug group as compared to placebo group where post-prandial blood glucose reduced from 294.9 ± 56.3 mg/dL to 262.6 ± 52.9 mg/dL (Table 8).

3.5.1. Glycosylated haemoglobin

Glycosylated haemoglobin significantly ($p = 0.001$) decreased from 9.56 ± 1.15 to 7.58 ± 0.99 in the test drug group. In the placebo group there was relatively a lesser reduction in the glycosylated haemoglobin level from 9.91 ± 1.05 to 8.86 ± 1.30 during the 16 week study period (Table 9).

3.6. Adverse events

Test drug effect was well tolerated by all patients during the course of study. Further, no adverse hematological or biochemical abnormalities was experienced by any patient.

4. Discussion

The major goals of toxicological studies in the present studies was to determine the safe dose level of test drug for clinical trial. The acute toxicity studies revealed that 2000 mg/kg bw of test drug

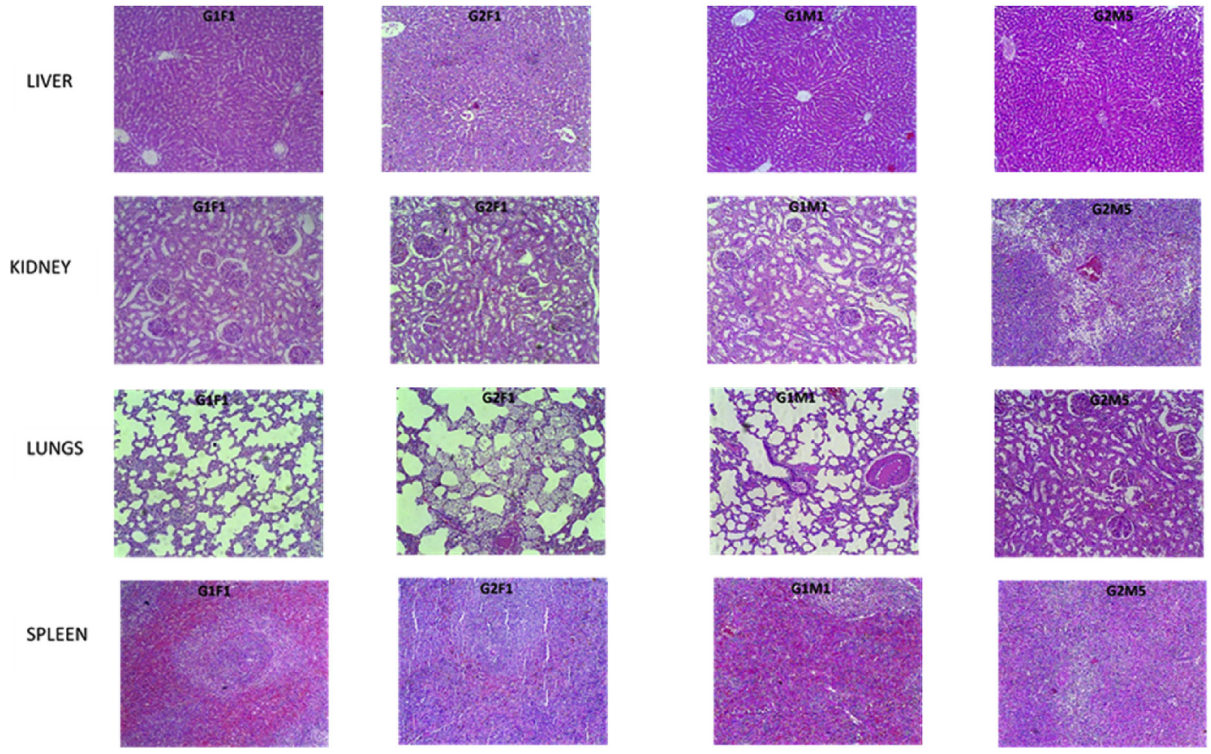

Fig. 4. Histopathological analysis of the vital organs from control and *BGR-34* treated rats at 2000 mg/kg body weight. (A–D) indicate control liver, kidney lung, and spleen, respectively, while (E–H) indicate treated control liver, kidney lung, and spleen, respectively of female rat; (I–L) indicate control liver, kidney lung, and spleen, respectively, while (M–P) indicate treated control liver, kidney lung, and spleen, respectively of male rat.

Table 7
Effect of BGR-34 and Placebo on Fasting Blood Glucose (FBG) mg/dL at baseline and after completion of study.

Variables	Drug Group (n = 28) (mean ± sd)	Placebo (n = 28) (mean ± sd)	Difference (95% CI)	p value[a]
FBG (mg/dL)				
Baseline	196.0 ± 32.7	187.2 ± 43.3	8.8 (−11.7 to 29.3)	0.3939
Post intervention	129.3 ± 33.3	162.9 ± 41.59	−33.5 (−53.7 to −13.3)	0.0016
Change (reduction)	66.7 ± 23.2	24.4 ± 14.3	42.3 (31.9–52.6)	<0.001
% Change (% reduction)	34.3 ± 10.7	13.2 ± 7.7	21.2 (6.1–26.2)	<0.001

[a] Student's *t*-test, FBG; fasting blood glucose.

Table 8
Effect of BGR-34 and Placebo on Post Prandial Blood Glucose (PPBG) mg/dL at baseline and after completion of the study.

Variables	Drug Group (n = 28) (mean ± sd)	Placebo (n = 28) (mean ± sd)	Difference (95% CI)	p value
PPBG (mg/dL)				
Baseline	276.8 ± 59.7	294.9 56.3	−18.1 (−49.2 to 12.9)	0.2482
Post intervention	191.9 ± 49.3	262.6 ± 52.9	−70.7 (−98.1 to −43.3)	<0.001
Change (reduction)	84.8 ± 36.3	32.2 ± 18.4	52.6 (37.2–68.0)	<0.001
% Change (% reduction)	30.5 ± 10.6	10.9 ± 5.9	19.6 (14.9–24.2)	<0.001

was safe dose level, where the mortalities, clinical signs of toxicity, changes in general behaviour, or changes in the physical activity were entirely absent. Therefore test drug was found to be safe according to the Guidance Document on Acute Oral Toxicity Testing, 2001 based on oral LD50. The result of sub-acute toxicity studies of test drug at the doses of 1500,750 and 375 mg/kg/day for 28 days, on both female and male, had no mortality, signs of behaviour changes and toxic signs during the period of experimentation. Test drug administration showed significant increase in body weight in test rats when compared with the control group. Body weight gain of test animal is an integral part of the conventional safety evaluation of a test drug.[26] The decrease in body weight as an indicator of adverse effects of a test material.[27] The significant body weight loss was considered as one of the most sensitive indicators of an

Table 9
Effect of BGR-34 and Placebo on Glycosylated Haemoglobin (HbA1c) at baseline and after completion of the study.

HBA1c	Drug Group (n = 28) (mean ± sd)	Placebo (n = 28) (mean ± sd)	Difference (95% CI)	p value[a]
Baseline	9.56 ± 1.15	9.91 ± 1.05	−0.35 (−0.94 to 0.25)	0.2469
Post intervention	7.58 ± 0.99	8.86 ± 1.30	−1.28 (−1.90 to −0.66)	0.001
Change (reduction)	1.98 ± 1.02	1.05 ± 0.52	0.93 (0.49–1.36)	0.001
% Change (% reduction)	20.31 ± 9.3	10.87 ± 5.94	9.45 (5.26–13.63)	<0.001

[a] Student's t-test.

animal's deteriorating health status after test drug administration.[26] As BGR-34 administration had no adverse effect like body weight loss, in experimental rats, hence this novel drug was confirmed as safe for human use based on our findings. The significant decrease in SGPT, bilirubin, triglyceride post test drug administration suggest no liver toxicity. In our study, activities of creatinine and urea level remains unaffected by repeated oral dosing of the test drug suggesting no renal impairment or kidney toxicity. Serum HDL Cholesterol level notably increased in G4 (Low dose) treated male and female, in contrast to respective control group. An increase in HDL Cholesterol level in serum is positively associated with a decreased risk of coronary heart disorder.[28] In a study significant decrease in HDL Cholesterol level was viewed as one of the most sensitive indicators of a risk of coronary heart disease.[29] The histo-pathological study revealed the normal structure of liver, kidney, spleen and lungs of rats administered with the test drug, except mild alveolar histiocytosis was determined in 1 female rat of G2 (High dose treated group) and pneumonitis was observed in 1 male rat of G2 (High dose treated group). No other abnormality was viewed in any organ of high dose treated group as in contrast to vehicle control.

Clinical studies of test drug is found to be effective and safe in the management of NIDDM as per the Indian council of medical research Guidance Document, 2006[25] on conducting trials of ayurvedic substances. Among fifty six patients treated with test drug (Oral administration, two tablet two times aday) were showed a significant improvement in the feeling of wellbeing due to better control of hyperglycaemia. Achieving near normal glycated hemoglobin (HbA1c) significantly decreases risk of macrovascular and microvascular complications.[30] About 50% of diabetic patients reached their target Glycosylated Haemoglobin (HbA1c) whereas the rest confirmed about 10% reduction (HbA1c). Our test drug also reduces bloodglucose levels in approximately 80% patients whereas about 60% of patients showed a significant reduction in postprandial glucose vs placebo that can be comparable to acarbose which targets postprandial hyperglycaemia.[31] Other diabetic signs and symptoms like polyuria, polyphagia, polydipsia, general fatigue, pain in calf muscles, burning sensation of soles and palms, dryness of mouth, itching genitals, blurred vision, and glucose in urine were absent and overall improvement in feeling of wellbeing was observed among patient. Improvement in appetite and digestion with no gastric discomforts were additionally reported in the test drug group. Inaddition, nearly all patient have shown the beneficial diagnostic effect onthebiochemicalparameterand experienced a reasonable improvement after treatment. The antidiabetic actions of BGR-34 may be attributed to various mechanism already reported i) delays in absorption of glucose from GIT, ii) inhibition of advanced glycation end products (AGEs) accumulation and iii) enhancing insulin release and conversion of pro-insulin to insulin. It is further suggested that test drug should be further used as a mono therapy/adjunctive therapy with OHGAs for the management of blood glucose level. The synergistic approach of test drug with OHGAs shall help reduce the dosage dependence on OHGAs therefore reducing the risk from their long-term usage. Advance studies are under running on a large number of patients with additional clinical biochemical parameters against the test drug BGR-34.

5. Conclusions

The safety study of BGR-34 on rats and the managed clinical study on human beings suggest that BGR-34 is significantly effective and safe drug for the management of NIDDM and can be used frequently in clinical practice to reduce the dependence on synthetic OHGAs thereby reducing their complication.

Conflicts of interest

The authors declare that they have no competing interests.

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