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PRELIMINARY CLINICAL ASSESSMENT AND NON- TOXICITY EVALUATION OF AN AYURVEDIC FORMULATION BGR-34 IN NIDDM

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ABSTRACT

In view of the overall health impact of NIDDM, inventers understand the necessity of improving glycaemic 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 hypoglycaemic 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. This study has been registered in the clinical trial registry-India.

KEYWORDS: BGR-34**,** *Berberis aristata*, NIDDM, Indian System Medicine, type 2 diabetes.

INTRODUCTION

Diabetes is on the rise, it is now considered as a wide-reaching epidemic with estimated 415 million patients in 2015, and touches around 642 million patient in 2040.[1,2] Generally, diabetes is categorized into two main types – insulin-dependent (IDDM) and non-insulin-dependent. About 80% of patients suffering from NIDDM may have serious complications in the body, which include nephropathy, retinopathy and neuropathy under unmanaged or ineffectively managed state. In addition, these patients have an increased risk of cardiovascular disease and stroke.[3-6] Currently, the most widely used medication for treatment of *NIDDM* includes oral antidiabetic drugs viz. biguanides (e.g., metformin), sulfonylureas (e.g., glimepiride), meglitinides (e.g., repaglinide), thiazolidinediones (e.g., pioglitazone), dipeptidyl peptidase IV inhibitors (e.g., sitagliptin), and α-glucosidase inhibitors (e.g., acarbose) etc.[7] The clinical uses of the current medicines may be accompanied with untoward effects such as hypoglycemia, arrhythmia, lactic acidosis, inflammatory bowel disease, colonic ulceration, partial intestinal obstruction and adverse effect on liver and kidney function.[8-12] Keeping in view the possibility of above side effect, the World Health Organization Expert Committee on diabetes, has recommended that traditional medicinal herbs should be investigated on large scale for finding safe and effective oral hypoglycaemic agents that provide the clinician with a wider range of options for delaying onset, treating and managing diabetes, without adverse effects. Many herbs, herbal extracts or combinations have been documented in traditional Indian system medicine as having clinical effectiveness in the management of diabetes mellitus with safety.[13,14] This paper will discuss the studies pertaining to the herbal anti-diabetic drug BGR-34, that has been developed by the scientists of Council of Scientific & Industrial Research, India (CSIR). The preparation is composed of 6 scientifically studied Indian medicinal herbs (*Berberis aristata*, *Tinospora cordifolia*, *Pterocarpus marsupium*, *Gymnema sylvestre*, *Rubia cordifoila* & *Trigonella foenumqraecum)* optimized for their anti-diabetic activity.[15-20] The knowhow of the research have been transferred to Aimil Pharmaceuticals (I) Ltd for manufacturing and marketing for the benefit of diabetes patient. The modern scientific studies further reveal the product to act as anti-diabetic with multiple mechanisms viz. Inhibiting the DPP-4 enzyme, stimulating glucose-mediated insulin secretion, improving insulin sensitivity to peripheral tissues, delaying the absorption from GIT etc. The Pre-clinical studies of BGR-34, produced promising results against diabetes induced experimental subjects with nephro-protective, hepato-protective and dyslipidemia and antioxidant to the advantage of diabetic (unpublished data CSIR). Hence, the aim of the present study was to evaluate the safety & clinical efficacy of BGR-34 and to complete the study sequentially following objectives were made to achieve the desired aim:

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

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

MATERIALS AND METHODS

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.

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.

Toxicity studies

An acute and sub-acute oral toxicity studies were conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Guideline 425 and 407 respectively.[21,22] 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.

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).

Acute oral toxicity study

Acute oral toxicity study of BGR-34 was carried out in 15 adult female Wistar as per the OECD test guidelines 425.[21] 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 hrs. 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 hrs, 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.

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 OECD guideline 407.[22] The first group (G1), was the control group which received distilled water while the next three treatment 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.

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), Differential leukocyte count (DLC).[23]

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[24], serum glutamate pyruvate (SGPT), glutamate oxaloacetate transaminase (SGOT)[25], alkaline phosphatase (ALP), total bilirubin (BBN)[26], Urea[27], total protein and albumin[28] and creatinine[29] Cholesterol, HDL Cholesterol[30] and Triglyceride[31] were estimated in serum.

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 organs were then preserved in 10% buffered formalin, embedded in paraffin, sectioned at 5µm, and stained with hematoxylin and eosin, The stained sections were examined under a bright field microscope for any cellular damage or change in morphology.[32]

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 on conducting trials of ayurvedic substances.[33] 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).

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 randomly divided into BGR 34 and Triphala as placebo groups. Patients underwent clinical examination and biochemical investigation on day 1 and at monthly intervals. Adverse effects if any were recorded. 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.

Inclusion Criteria

Aged over 25 to 60 years, male or female diagnosed with type 2 Diabetes mellitus, characterized by fasting blood glucose (FPG) >126 mg/dL or 2 hr post-prandial glucose (PPG) >200mg, positive urine sugar and having symptoms of polyuria, polyphagia and polydipsia, and those were willing to give written informed consent.

Exclusion criteria

Patients on Insulin, Patients 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 and those who were not willing to give written consent were also excluded from the study.

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 glucose (PPG). The glycosylated haemoglobin (HbA1c), was done at the day 1 and day 120.

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.

Statistical analysis

Data was arranged in MS Excel. Student-t test was used to compare the difference in mean values between the two groups. Chi-square test was used for categorical variables. 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.

RESULTS

Acute oral toxicity In Acute oral toxicity studies, it was found that the rats were safe at the limit test dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed.

1 Sub-Acute Oral Toxicity

3. 1.1 Changes in body weight

Fig. 1 and Fig. 2, illustrate the effect of BGR -34 on the mean change in body weight of both male and female. The result suggested that after 28 days of exposure to BGR-34, an increase in growth in all male and female rats groups was observed as compared to control.

3. 1.2 Clinical signs of toxicity

All animal were observed daily during the treatment period for any clinical signs of toxicity. 3 male and 2 female rats of G2 (high dose group) and G5 (Satellite group) showed piloerection in the third week of treatment. The reversal in piloerection was observed in all male and female rats of G5 (satellite group) during the observation period of additional 14 days post treatment. G1 (Vehicle control), G3 (intermediate dose) and G4 (Low dose group) did not show any clinical signs of toxicity during the study period.

3. 1.3 Haematological analysis

Table 5 and 6, shows the effect of BGR-34 on haematological parameters in rats. After the day 28, there was no significant (P>0.05) effect on TLC, Hb, neutrophil, lymphocyte, monocyte and eosinophil in the treated group compared to control group.

3. 1.4 Biochemical analysis

Table 7 and 8, shows the effect of BGR-34 on kidney function and liver function parameters. After the Day 28, SGPT level in the G 2 (high dose) and G5 (Satellite group) was found to be significantly decreased as compared to normal control group in male and female rats. There was also no significant difference in SGOT level in treatment groups as compared to G1 (vehicle control) in male and female rats. Alkaline phosphatase level in the treatment groups did not show any significant variation as compared to respective control group and values were found to be within normal limits in all control/treatment groups. Bilirubin level was found significantly low in G2 (high dose group) treated male rats as compared to respective control group however, no

significant decrease was observed in G2 (high dose group) female rats. Triglyceride level was found significantly decreased in G2 (high dose group) treated male rats as compared to respective control group. High dose treated female rats also showed decreased in triglyceride level as compared to respective control group however, the difference was not statistical significant. There was no statistically significant difference in serum urea, serum creatinine, serum albumin, total serum protein, serum cholesterol, serum glucose and serum basophil.

3. 1.5 Relative organs weight

Table 9 and 10, illustrates the relative organ weight of the rats. There was no significant difference (P<0.05) in organ weights i.e. kidney, liver, spleen, testes, ovaries, heart, lungs and brain was observed in any of the treated male and female groups relative to G1 (control group) after oral administration of BGR-34 for 28 days.

3. 1.6 Gross Necropsy

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

3. 1.7 Histopathology

Mild alveolar histiocytosis was observed in 1 female rats of G 2 (high dose treated group) fig 4G, and pneumonitis was observed in 1 male animal of G 2 (high dose treated group) fig 3 B, 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 4P.

3.2 Clinical study

56 patients (30 male and 26 females) with type 2 diabetes mellitus completed the study out of 64 total enrolled eight patient withdrawal in between study for their own cause. There were 28 patients in the BGR-34 group (drug arm) and 28 patients in the placebo group (placebo arm). The mean age of patients for BGR-34 and placebo group were 47.9±6.7 years and 49.7±5.9 years respectively. Average body weight in the BGR-34 group was 67.04±8.6 kg and in the placebo group, it was 70.1±6.9 kg (Table 7). The difference in Age, body weight and a number of patients in the drug and placebo group was not found to be significant. The trial has also been registered to CTRI, India with registration no CTRI/2016/11/007476.

3.2.1 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=0.0016) from 196.0 ± 32.7 mg/dL to 129.3 ± 33.3 mg/dL in BGR-34 treated group as compared to placebo group where fasting blood sugar reduced from 187.2 ± 43.3 mg/dL to 162.9 ± 41.6 mg/dL. The percent reduction in the BGR-34 treated group was highly significant (p<0.001) as compared to the placebo group (Table 8).

3.2.2 Post-prandial Blood Glucose (PPBG)

Blood sugar post-prandial showed significant reduction (p<0.001) from 276.8 ± 59.7 mg/dL to 191.9 ± 49.3 mg/dL in BGR-34 treated group as compared to placebo group where post-prandial blood sugar reduced from 294.9 ± 56.3 mg/dL to 262.6 ± 52.9 mg/dL. The percentage reduction in the BGR-34 treated group was highly significant (p<0.001) as compared to the placebo group (Table 9).

3.2.3 Glycosylated haemoglobin

Glycosylated haemoglobin decreased from 9.56 ± 1.15 to 7.58 ± 0.99 which was found to be a highly significant decline in the BGR-34 group (p=0.001). On the other hand 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 10).

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

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

Day	Dose (mg/kg bw)	Outcome
1	250	No death
3	500	No death
5	1000	No death
7	2000	No death
9	2000	No death
11	2000	No death
13	2000	No death
15	2000	No death
*2000 mg/kg bw is the limit test dose		

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.

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.

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 ǂ
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 to 52.6)	<0.001
% Change (% reduction)	34.3 ± 10.7	13.2 ± 7.7	21.2 (6.1 to 26.2)	<0.001

ǂ 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 ǂ
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 to 68.0)	< 0.001
% Change (% reduction)	30.5 ± 10.6	10.9 ± 5.9	19.6 (14.9 to 24.2)	< 0.001

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 ǂ
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 to 1.36)	0.001
% Change (% reduction)	20.31 ± 9.3	10.87 ± 5.94	9.45 (5.26 to 13.63)	< 0.001

ǂ Student's t-test.

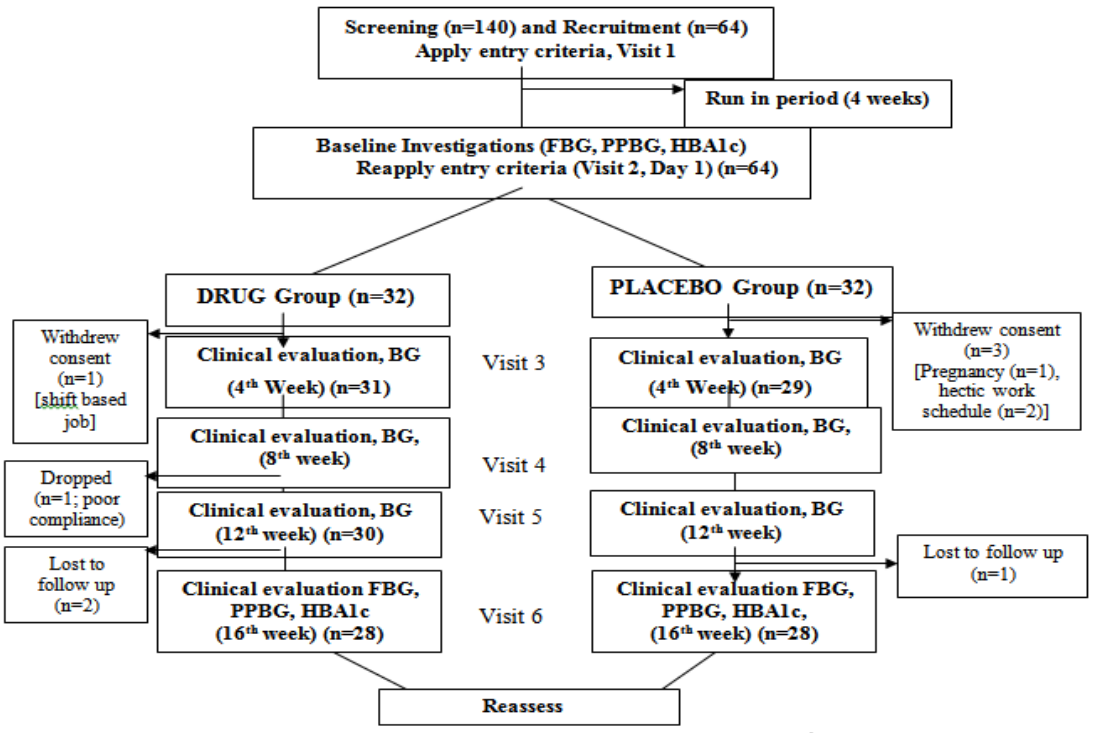

Figure 1: Assessment Method: Study design and patient recruitment process.

BG; Blood glucose, FBG; Fasting blood glucose, PPBG; Post prandial blood glucose, HBA1c; Glycosylated haemoglobin

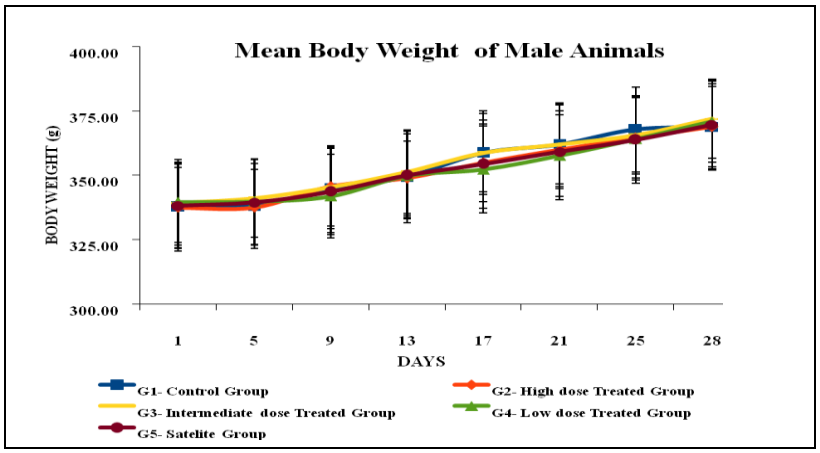

Figure 2: Mean body weight of male rats during the treatment period.

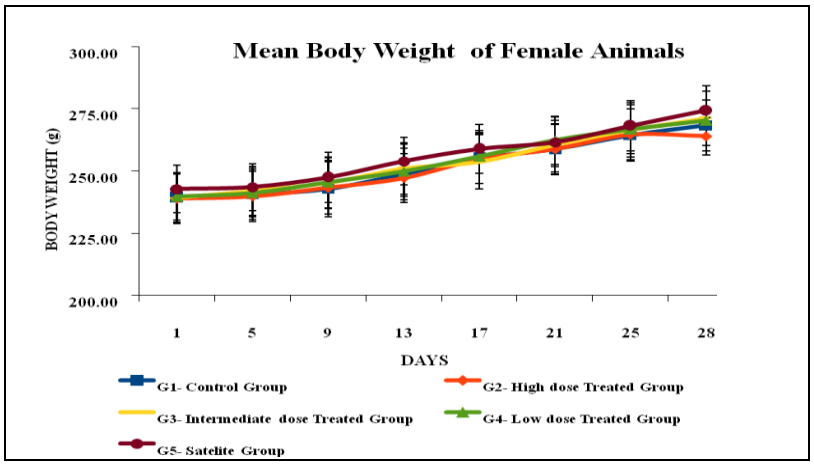

Figure 3: Mean body weight of female rats during the treatment period.

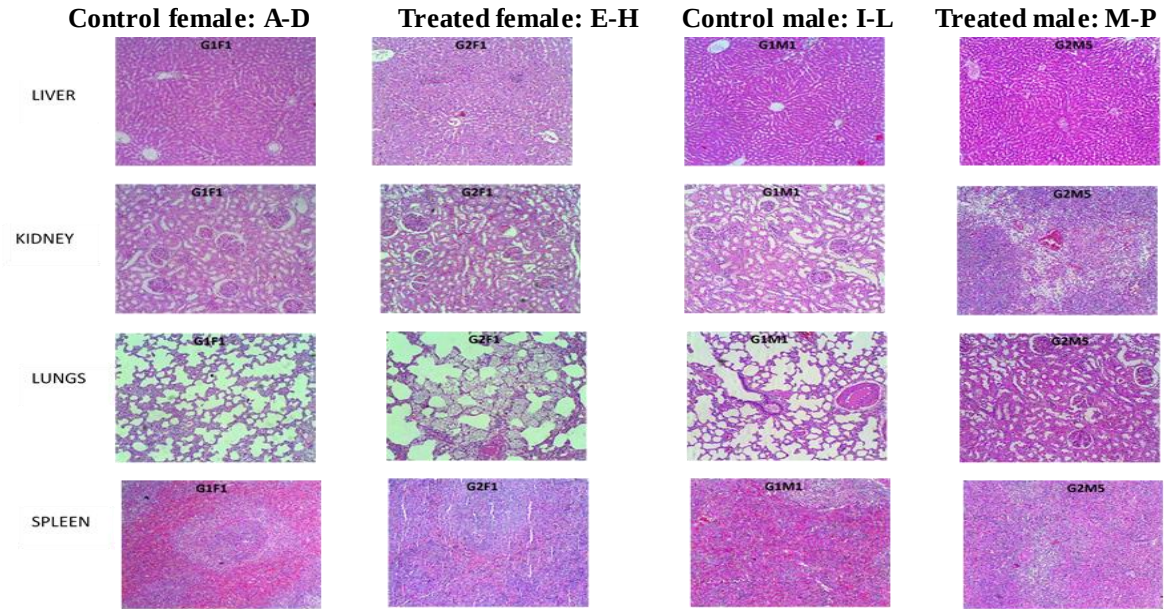

Figure 4: Histopathological analysis of the vital organs from control and *BGR-34* treated rats at 2000 mg/kg body weight. (A-D) indicate control liver, kidney lung, and spleen, respectively, while (E-H) indicate treated control liver, kidney lung, and spleen, respectively of female rat; (I-L) indicate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

DISCUSSION

In the present study the main goals of toxicological studies were to determine the safe dose level of BGR 34 for clinical trial and to assess its safety profile in human. The acute toxicity studies revealed that at a dose level of 2000 mg/kg bw of BGR-34, the rats showed neither mortalities nor clinical signs of toxicity, changes in general behaviour, or changes in the physical activity. Therefore BGR-34 was found to be safe according to the Guidance Document on Acute Oral Toxicity Testing based on oral $LD_{50.}$[34] The result of sub-acute toxicity studies of BGR-34 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. BGR-34 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.[35] The decrease in body weight as an indicator of adverse effects of a test material.[36] The significant body weight loss was considered as one of the most sensitive indicators of an animal's deteriorating health status after test drug administration.[35] As BGR-34 administration had no adverse effect like body weight loss, hence this novel drug was confirmed as safe for human use. No clinical sign of toxicity was noted (except piloerection) in G2 (high dose) and G5 (satellite group), nevertheless observation for an addition 14 days showed the reversal in both male and female rats of G5 (satellite group).

The significant decrease in SGPT, bilirubin, triglyceride in G2 and G5 in male as compared to control group indicates that BGR-34 have no liver toxicity and will not produce the toxic effect on heart tissue. In our study, activities of creatinine and urea level were not affected by repeated oral dosing of the BGR-34 suggesting no renal impairment or kidney toxicity. Serum HDL Cholesterol level significantly increased in G4 (Low dose) treated male and female as compared to respective control group. An increase in HDL Cholesterol level in serum is positively associated with a decreased risk of coronary heart disease.[37] The significant decrease in HDL Cholesterol level was considered as one of the most sensitive indicators of a risk of coronary heart disease.[38] In the present study, BGR-34 did not produce any statistically significant differences among groups in internal organ weights of the treated male and female groups relative to G1 (control group) after oral administration of the test drug for 28 days. The histo-pathological study revealed the normal architecture of Liver, kidney, spleen and lungs in treated with the BGR-34, except mild alveolar histiocytosis was observed in 1 female rats of (G2 High dose treated group) and pneumonitis was observed in 1 male animal of (G2 High dose treated group). No other abnormality was seen in any organ of high dose treated group as compared to vehicle control.

Based on clinical studies BGR-34 is found to be effective and safe in the management of NIDDM as per the Indian council of medical research Guidance Document on conducting trials of ayurvedic substances.[33] Among 56 patients treated with BGR-34 (Oral administration, two tablet two times a day) were showed a significant improvement in the feeling of wellbeing due to better control of hyperglycaemia with no adverse effects. Achieving near-normal glycated hemoglobin (HbA1c) significantly decreases risk of macrovascular and microvascular complications[39] about 50 % of diabetic patients reach their target Glycosylated Haemoglobin (HbA1c) whereas the rest showed about 10 % reduction (HbA1c). Thus like sulfonylureas[40] BGR-34 reduces blood glucose levels in approximately 80 % patients whereas about 60 % of patients showed a significant reduction in postprandial glucose vs placebo comparable to acarbose which targets postprandial hyperglycaemia.[41] Other diabetic 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 became negative and overall improvement in feeling of wellbeing observed. Improvement in appetite and digestion with no gastric discomforts were also reported in the BGR-34 group. In addition, almost all patient have shown the beneficial diagnostic effect on the biochemical parameter and experienced a reasonable improvement after treatment. The anti-diabetic 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 The response behaviour remain same in terms of body physiology towards action of therapeutic molecule irrespective their origin (plant/synthetic molecule).[42-43]It is further suggested that BGR-34 should be further extensively used as a mono therapy/adjunctive therapy with OHG'S for the management/regulation/control of blood glucose level. The synergistic approach of BGR-34 with OHG'S shall help reduce the dosage dependence on OHG'S hence reducing the risk from their long-term usage. The clinical study on a large number of patient is further ongoing with additional clinical biochemical parameter of test drug on vital organ.

CONCLUSIONS

The safety study of BGR-34on rats and the controlled clinical study on human beings suggest that BGR-34 is significantly effective and safe tool for the management of NIDDM and can be used frequently in clinical practice to reduce the dependence on synthetic OHG'S thereby tends reduce their risk.

Conflict of interest

The authors declare that they have no competing interests.

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