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Research Article

The efficacy of a marketed polyherbal ayurvedic formulation in streptozotocin-nicotinamide model in type 2 diabetic rats

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Abstract

The objective of the present study is the thorough evaluation of the efficacy of a polyherbal ayurvedic formulation (PHAF), in streptozotocin-nicotinamide induced diabetes in wistar rats. A total of 30 adult rats (130-180 g) were employed. They were divided into five groups with 6 animals in each. Experimental diabetes was induced in 24 rats by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight) after dissolving in citrate buffer at pH; 4.5 proceeded by nicotinamide 120 mg/kg body weight within an interval of 10 minutes. The animals with blood glucose level above 200 mg/dl were chosen for the study. All experimental animals received 15-day treatment as follows: the non-diabetic control and diabetic control groups both received vehicle - 2 % CMC; the other three groups received glibenclamide (10 mg/ kg), PHAF (200 mg/kg) and PHAF (200 mg/kg) plus glibenclamide (5 mg/kg), respectively. Thus, it may be hypothesized that the PHAF, possesses significant blood sugar lowering effect with little safety concerns as evident from histological studies on kidney, pancreas and liver.

Keywords: Proprietary medicine, sulphonylurea, streptozotocin- nicotinamide, organ weight evaluation

Introduction

Presently there is growing interest in herbal remedies due to the side effect considerations associated with the synthetic oral hypoglycemic agents in the treatment of diabetes mellitus. Hence the traditional herbal medicines are mainly used which are obtained from plants [1-4]. The present study is an attempt towards assessment of the efficacy and safety of a polyherbal proprietary medicine in streptozotocin-nicotinamide induced experimental diabetes in wistar albino rats. The study attempts to investigate the possible antidiabetic potential of the polyherbal proprietary medicine (Table 1) against the pancreatic damage in streptozotocin induced diabetes in wistar rats [5-9].

Although the individual constituents of this formulation has been studied previously but the proprietary medicine, as a whole, has not been studied for its efficacy in treating diabetes in established animal models as well as to assess the extent of impact of the medicine on major body organs such as liver, kidney, pancreas though the use of biochemical tests and histopathological studies [10-12]. Thus present study is aimed at an overall assessment of the safety and efficacy of this polyherbal formulation [13-15].

Table 1: Composition of the polyherbal ayurvedic formulation (PHAF) in capsule dosage form

Ingredients	Amount
Meshashingi (Gymnema sylvestre)	1.5 gm
Indrayav (Holarrhena antidysenterica)	950 mg
Methi (Trigonella foenum graecum)	1 gm
Neem (Azadirachta indica)	500 mg
Jambeez (Syzgium cuminii)	500 mg
Karala (Momordica chirantia)	500 mg
Swarna Bhasma, Roupya Bhasma	4 mg each
Abha, Probal, Mukta	8 mg each

Materials and Methods

Chemicals and reagents

Streptozotocin (Batch No: T-6737644, Manufacturer: Sisco Research Laboratories Pvt. Ltd.) Glibenclamide tablets (Manufacturer : Sanofi-Aventis, Batch no: 11T-0332), PHAF capsules (Batch no : DA-2354) was received as a gift sample from the manufacturers , Nicotinamide (Mf. Himedia) Trisodium citrate, Carboxymethylcellulose starch (CMC) (Manufacturer: Hi Media Lab Pvt. Ltd)

Experimental Animals

Healthy male Wistar rats (130-180 gm) maintained at standard laboratory conditions and fed standard pellet diet and given water ad libitum [16]. Permission was obtained from the Institutional Animal Ethics Committee prior to conducting the experiments (IAEC/2013-Feb/03). Experimental groups and their treatment schedules are given in Table 2.

Table 2: Experimental groups and their treatment schedules

Group	Treatment
GROUP 1- Vehicle Control	2% CMC solution.
GROUP 2- Positive Control (STZ) plus nicotinamide 120 mg/kg b.w	STZ 60 mg/kg dose + 120 mg/ kg nicotinamide +2% CMC solution.
GROUP 3- Standard Group (Glibenclamide treated)	Glibenclamide 10 mg/kg body weight along oral route
GROUP 4- DIABIND treated	200 mg/kg along oral route
GROUP 5- Glibenclamide + DIABIND treated	Glibenclamide 5 mg/kg body weight along oral route + DIABIND 200 mg/kg along oral route

Study in normoglycemic animals

Healthy rats were divided into three groups (n=6). After overnight fasting with free access to water, fasting blood glucose (FBG) levels of each animal was determined at the beginning of the experiment (at 0 h). Animals in control group (group I) received only the vehicle and the test group animals (group II) were treated with DIABIND (200 mg/kg b.w) orally. The animals (group III) received DIABIND (200 mg/kg) plus glibenclamide (5 mg/kg). Blood glucose levels were determined again at 1/2 h, 1 h and 2 h after oral administration of test samples to assess the effect of test samples on normoglycemic rats [15, 16].

Study in STZ induced diabetic rats

Healthy male wistar albino rats were divided into five groups (n=6). 24 animals were induced with STZ at 60 mg/kg body weight followed by nicotinamide 120 mg/kg b.w. The animals were checked after 72 h (after the triphasic reaction on glycemic levels) and only those animals with random blood glucose levels greater than 200 mg/dl were selected for inclusion in the study thereafter. The streprozotocin dose of 60 mg/dl with nicotinamide 120 mg/kg replicates a model of Type 2 diabetes mellitus in Wistar rats.

All experimental animals received 15-day treatment as follows: the non-diabetic control and diabetic control groups both received vehicle - 2 % CMC; the other three groups received glibenclamide (10 mg/ kg), PHAF (200 mg/kg) and PHAF (200 mg/kg) plus glibenclamide (5 mg/kg), respectively. Post prandial blood glucose measures was taken 2 h post food being accorded to these animals with carbohydrates. Blood was withdrawn from the dorsal tail vein. The results were checked in a semi-autoanalyser and correlated with the glucometer readings (Figures 1, 2, 3; Table 3)

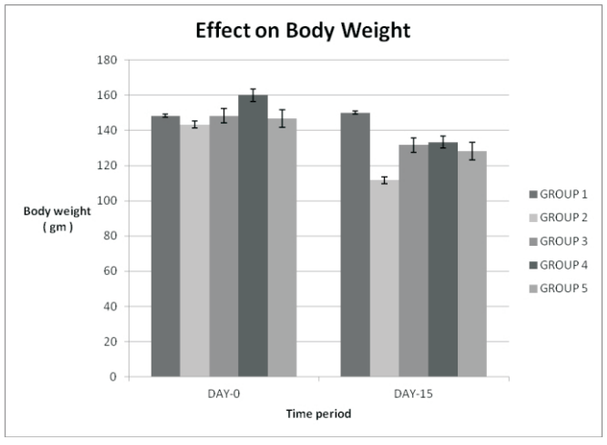

Figure 1: Comparison of body weights of study groups at the beginning and end of the study. (n=6). All values represent Mean and SEM values

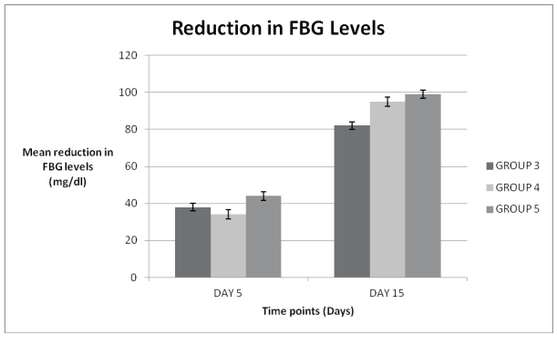

Figure 2: Comparison of reduction in Fasting Blood Glucose across study groups. All values represent Mean and SEM values

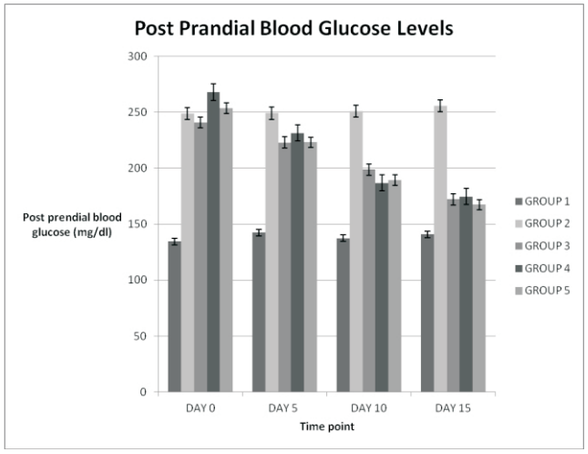

Figure 3: Comparison of reduction in post prandial blood glucose across the treatment groups. Intervals of 0, 5, 10 and 15th day. (n=6)

Table 3: Findings of the organ weight post study period. Liver, kidney, heart and spleen was isolated from an experimental animal after sacrifice and compared to the standard weights. (n=6). Pancreas weight was also incorporated. Wistar albino rat. Body weight of the animal 160-180 gm

Group	Liver	Heart	Kidney	Spleen	Pancreas
Group 1	7.178gm	0.512 gm	1.141 gm	1.163 gm	0.88 gm
Group 2	5.901 gm	0.5156 gm	1.39 gm	1.211 gm	0.64 gm
Group 3	7.29 gm	0.5423 gm	1.010 gm	1.151 gm	0.81 gm
Group 4	7.44 gm	0.522 gm	1.133 gm	1.166 gm	0.73 gm
Group 5	7.361 gm	0. 5343 gm	1.226 gm	1.213 gm	0.67 gm

Statistical Analysis

Values were expressed as Mean ± Standard Error Mean. Between groups comparison was done using Analysis of Variance (ANOVA) followed by post-hoc Tukey's multiple comparison test. Analysis of data was achieved with the help of standard statistical software, namely Microsoft Excel, Primer of Biostatistics version 5.1 and SPSS version 17. $P < 0.05$ was considered statistically significant [17-20]. ANOVA using SPSS was used to find out the difference in the groups at 95 % C.I. Treatment and the Disease Control groups were compared. Since more than 2 groups were used. t tests were not liable for being used.

Effect on Body Weight in Rats

The changes in body weight across different study groups expressed on Day 0 and on Day 15 are given in Table 4.

Table 4: Changes in body weight in Wistar albino rats

Group	Group 1	Group 2	Group 3	Group 4	Group 5
Day-0	148.33 ± 3.02	143.33 ± 2.96	148.33 ± 2.11	160 ± 1.87	146.67± 1.91
Day-15	150 ± 1.91	111.67± 2.36	131.67 ± 1.44	133.3 ± 1.67	128.33± 1.79

Findings of histological studies is given in Figures 4, 5 and 6.

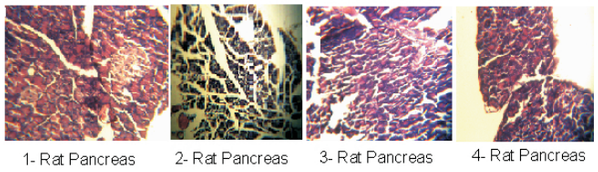

Figure 4: Findings of the histological study of rat pancreas sections under (40 X magnification) across groups (1-4) showed the destruction of beta cells in the disease controlled group. The treatment groups (3 and 4) showed signs of regaining of normal architecture of the cells of the pancreas indicating the efficacy of the PHAF in therapy.

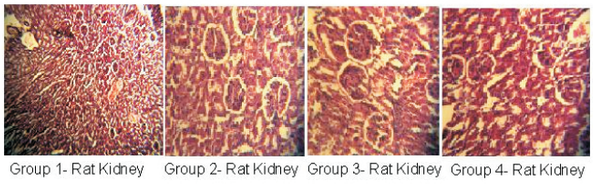

Figure 5: Findings of the histological study of rat kidney sections under (10 x and 40 x magnification) did not show any remarkable changes across groups (1-4)

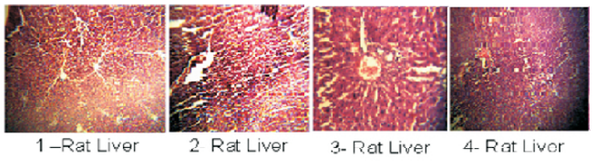

Figure 6: Findings of the histological study of rat liver sections under 40 x magnification did not reveal any remarkable changes across the study groups (1-4)

Discussion

The polyherbal drug being studied contains six herbal drug combinations like Gymnema sylvestre, *Holarrhena antidysenterica, Trigonella foenum graecum, Azadirachta indica, Syzgium cuminii, Momordica chirantia* and traditional ayurvedic excipients such as bhasma. The study revealed that this PHAF reduces fasting blood glucose and post prandial blood glucose level significantly * ($P < 0.05$) as compare to glibenclamide. It demonstrated a significant reduction ($p < 0.05$) in reduction of median fasting blood glucose level by 40.7 % compared to 36.25 % in the glibenclamide treated group. Organ weight also did not reveal any significant untoward effect of the polyherbal marketed formulation as capsules on the major organs such as pancreas, kidney and the liver of wistar rats. Follow up studies using larger samples need to be performed to substantiate and corroborate this present hypothesis about this polyherbal product. From the findings we hypothesize that the polyherbal formulation was found to be safe and effective, having an add-on effect when co administered with glibenclamide. Histological studies revealed normal architecture of the tissues in the treatment as well as standard groups at 10 x and 40 x magnification levels. The tissue sections showed no untoward effects in the treatment groups but deleterious changes were visible in the negative control and diabetic control groups.

Conclusion

A 15-day treatment with PHAF significantly reversed hyperglycemia in streptozotocin induced diabetes that was comparable to glibenclamide ($p<0.05$). The said PHAF in combination with glibenclamide produced a synergistic effect ($p<0.05$). Routine biochemical parameters, body weight and vital organ histological studies, indicated no significant changes between different groups. This leads us to hypothesize that the marketed PHAF actually possesses significant blood sugar lowering property with little safety concerns. However, future studies in clinical settings are warranted to corroborate the present findings conclusively.

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Conflicts of interest

The authors declare no competing interest.

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