Austin Journal of Endocrinology and Diabetes

Open Access

Austin Publishing Group

Research Article

Evaluation of the Safety and Efficacy of a Polyherbal Ayurvedic Formulation (PHAF) in Streptozotocin-Induced Diabetes in Wistar Rats

Raja Chakraverty[1,2], Anuradha De[3], Ranjita Santra[2]*, Arunava Biswas[2] and Santanu K Tripathi[2]

[1]Department of Pharmacology, Gupta College of Technological Sciences, India

[2]Department of Clinical & Experimental Pharmacology, Calcutta School of Tropical Medicine, India

[3]Department of Pathology, Calcutta School of Tropical Medicine, India

***Corresponding author:** Ranjita Santra, Department of Clinical & Experimental Pharmacology, Calcutta School of Tropical Medicine Kolkata-700073, India

Received: June 01, 2018; **Accepted:** June 18, 2018; **Published:** July 04, 2018

Abstract

The present study evaluates the safety and efficacy of a Polyherbal Ayurvedic Formulation (PHAF), *DIABIND* alone and in combination with glibenclamide in streptozotocin- induced diabetes in wistar rats. A total of 30 adult rats (130-170g) 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 (60mg/kg). All 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 (10mg/kg), PHAF (200mg/kg) and PHAF (200mg/kg) *plus* glibenclamide (5mg/kg), respectively. Blood glucose, body weight and biochemical parameters such as SGPT, SGOT, serum creatinine and albumin levels were measured on days 5, 10 and 15. Histopathological examinations were performed on the pancreas, liver and kidney at the end of the study. A 15-day treatment with PHAF significantly reversed hyperglycemia in streptozotocin induced diabetes that was comparable to glibenclamide ($p<0.05$). 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. Histopathological observations did not reveal any significant untoward effect on pancreas, liver or kidney. *DIABIND*, the PHAF, possesses significant blood sugar lowering effect with little safety concerns.

Keywords: *DIABIND*; Ayurvedic proprietary medicine; Glibenclamide; Streptozotocin; Synergism

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder, resulting from insulin deficiency, characterized by high blood glucose levels, which result from defects in insulin secretion. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts a lifetime [1].

The insulin deficiency may be absolute relative, and the metabolic abnormalities lead to the classical symptoms of polyurea, polydypsia, polyphagia, and fatigue. Diabetes may be insulin dependent (IDDM) or Non-Insulin Dependent (NIDDM). In insulin dependent diabetes mellitus also known as Type1 diabetes mellitus, the beta cells of Islets of Langerhans of pancreas are either malfunctioning or destroyed. The NIDDM or Type 2 diabetes patient exhibit insulin resistance and ultimately develop concomitant insulin secretory defect.

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. These herbal medicines play an important role in the management of diabetes mellitus [2,3].

In recent years, herbal medicinal formulations have started to gain importance as a potential therapy for diabetes mellitus and its associated complications. Marles and Farnsworth estimated that more than 1000 plant species are being used as herbal medicine for diabetes. However, both the safety as well efficacy aspect of marketed ayurvedic formulations should be assessed experimentally to ensure safe, reliable and rational therapeutics [4,5]. Herbal products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which have been reported to show anti diabetic activity. The use of such herbal products is prevalent in many societies around the world since time immemorial [6-12].

The present study is an attempt towards assessment of the efficacy and safety of a polyherbal proprietary medicine *DIABIND*® in streptozotocin-induced experimental diabetes in wistar albino rats. The study attempts to investigate the possible antidiabetic potential of the polyherbal proprietary medicine *DIABIND*® (Manufactured by India National Drug Co Pvt Ltd) containing extracts of *Gymnema sylvestre, Holarrhena antidysenterica, Trigonella foenum* graecum, *Azadirachta indica, Syzgium cuminii* and *Momordica chirantia* against the pancreatic damage in streptozotocin induced diabetes in wistar albino rats. 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 through the use of biochemical tests and histopathological studies. Thus present study

Citation: Chakraverty R, Anuradha De, Santra R, Biswas A and Tripathi SK. Evaluation of the Safety and Efficacy of a Polyherbal Ayurvedic Formulation (PHAF) in Streptozotocin- Induced Diabetes in Wistar Rats. Austin J Endocrinol Diabetes. 2018; 5(1): 1062.

Table 1: Effect on Body Weight in Rats.

Day/ 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
Effect %	1.12	-21.34	-11.24	-16.67	-12.5

Table 2: Comparison of SGPT across study groups.

Day / Group	Group 1	Group 2	Group 3	Group 4	Group 5
Day-0	47±0.5	52±0.6	44±1.1	55±1.0	49.5±0.6
Day-15	44.83±0.4	97.50±0.9	64.76±1.3	62.33±1.3	55.5±1.3
Change %	-4.61	87.5	47.18	13.33	12.12

Table 3: Changes in SGOT levels across study groups on Days 0 and 15 respectively.

Day/ Group	Group 1	Group 2	Group 3	Group 4	Group 5
Day-0	41.5±1.3	46±1.4	51±1.2	48.6±1.3	44.33±0.9
Day-15	39±1.9	84.5±1.2	63±1.4	49.5±1.1	47.5±1.0
% Change	-6.02	82.7	23.52	1.81	7.22

Table 4: Comparison of changes in serum creatinine levels in study groups (n=6).

Day/ Group	Group 1	Group 2	Group 3	Group 4	Group 5
Day-0	0.32±0.01	0.27±0.02	0.31±0.02	0.37±0.02	0.39±0.01
Day-15	0.33±0.02	0.36±0.01	0.37±	0.41±0.01	0.42±0.01
% Change	3.13	33.3	19.35	10.81	7.69

Mean ± Standard Error of Mean (SEM) (n=6)

is aimed at an overall assessment of the safety and efficacy of this polyherbal formulation [13-15].

Materials and Methods

Chemicals and reagents

Streptozotocin (Batch No: T-4535896, Manufacturer: Sisco Research Laboratories Pvt. Ltd.) Glibenclamide tablets (Brand name: DAONIL, Manufacturer : Sanofi-Aventis, Batch no: 11T-0332), *DIABIND*® capsules (Batch no: DA-2354; Manufacturer: India National Drugs Co. Pvt. Ltd), Trisodium citrate, Carboxymethylcellulose starch (CMC) (Manufacturer: Hi Media Lab Pvt. Ltd)

Experimental animals

Healthy male wistar albino rats (130-180gm) maintained at standard laboratory conditions and fed standard diet and given water *ad libitum* [16]. The animals were acclimatized to laboratory conditions for a week before commencement of experiment. Permission was obtained from the Institutional Animal Ethics Committee prior to conducting the experiments (GCTS/IAEC/2013-Feb/02).

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 hr). Animals in control group (group I) received only the vehicle and the test group animals (group II) were treated with *DIABIND* (200mg/kg b.w) orally. The animals (group III) received *DIABIND* (200mg/kg) plus glibenclamide (5mg/kg). Blood glucose levels were determined again at 1/2 hr, 1 hr and 2 hr 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). The following treatments were provided for 14 days: Group I- Vehicle (2% CMC), Group II diabetic control group (2% CMC plus STZ 60mg/kg b.w; i.p), Group III -Glibenclamide (10mg/kg), Group IV-*DIABIND* (200mg/kg) and Group V- *DIABIND* (200mg/kg) plus Glibenclamide (5mg/kg).

Testing of Fasting blood glucose (FBG), body weight

FBG level of each animal were monitored on day 0, 5, 10 and 15th. Blood samples were collected from lateral tail vein maintaining asepsis and the blood glucose levels were estimated by using glucometer (One Touch Ultra of Life scan, Johnson & Johnson).

Estimation of biochemical parameters

On day 15, blood samples were collected from the sacrificed rats. The samples were incubated at 37˚C followed by centrifugation for separation of the plasma at 2000 rpm for 15 minutes for biochemical estimation of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), serum creatinine and albumin. All analyses were performed by using commercially available kit from Accurex Ltd [17,18].

Evaluation of organ weight and histology

After sacrificing the rats, parts of their pancreas, liver and kidney tissues were carefully weighed and subsequently collected for histologic studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a minimum period of 24 hrs, dehydrated with alcohol, and embedded in paraffin blocks, cut into 3 to 4-µm thick sections and stained with hematoxylin-eosin dye for photo microscopic observation [19,20].

Statistical analysis

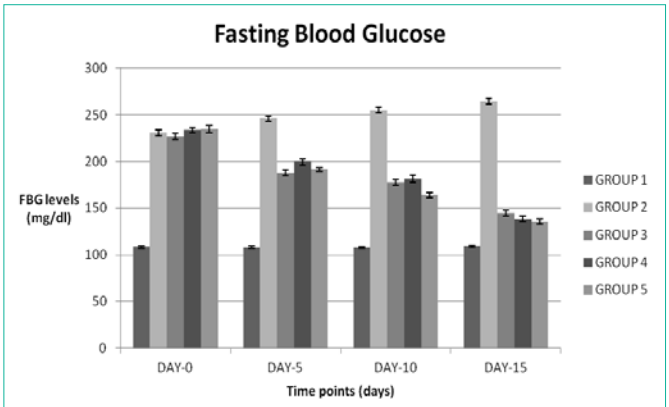
Values were expressed as Mean ± Standard Error Mean. Between groups comparison was done using Analysis of Variance (ANOVA) followed by post-hoc Tukey's multiple comparison test. Analysis of data was achieved with the help of standard statistical software, namely Microsoft Excel, Primer of Biostatistics version 5.1 and SPSS version 17. $P < 0.05$ was considered statistically significant.

Results

The study was focused to assess the safety and efficacy of the

Table 5: represents comparison of changes in serum albumin levels in study groups (n=6).

Day/ Group	Group 1	Group 2	Group 3	Group 4	Group 5
Day-0	3.30±0.3	3.8±0.4	3.7±0.6	3.91±0.9	3.78±0.8
Day-15	3.21±0.8	5.1±0.4	4.69±0.9	4.57±1.1	4.23±0.9
% Change	-2.72	34.21	26.75	16.87	11.90



Graph 1: Comparison of fasting blood glucose across groups.

PHAF. The data are represented as Mean ± SEM (n=6).

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 as follows (Table 1).

All values represent Mean ± Standard Error of Mean (SEM); (n=6). Diabetic control group *vs.* normal control group, P<0.05, P<0.05 treated group *vs.* diabetic control group, the level of significance was assessed by one-way ANOVA followed by post hoc Tukey's test.

Fasting blood glucose

Observation: All values represent Mean ± Standard Error of Mean (SEM); (n=6). Diabetic control group *vs.* normal control group, P<0.05, P<0.05 treated group *vs.* diabetic control group, the level of significance was assessed by one-way ANOVA followed by post hoc Tukey's test.

The changes in fasting blood glucose (mg/dl) across the study groups (n=6) expressed on Day 0 and on Day 15 are given as follows (Graph 1).

Biochemical parameters

Serum Glutamate Pyruvate Transaminase (SGPT): The changes in SGPT across different study groups expressed on Day 0 and on Day 15 are given as follows (Table 2).

All values represent Mean ± Standard Error of Mean (SEM); (n=6). Diabetic control group *vs.* normal control group, P<0.05, P<0.05 treated group *vs.* diabetic control group, the level of significance was assessed by one-way ANOVA followed by post hoc Tukey's test.

Serum Glutamate Oxaloacetate Transaminase (SGOT): The changes in SGOT across different study groups expressed on Day 0 and on Day 15 are given as follows. Values are Mean ± Standard Error of Mean (SEM) (n=6) (Table 3).

Serum creatinine

The changes in serum creatinine across different study groups expressed on Day 0 and on Day 15 are given as follows (Table 4).

Serum albumin

The changes in serum creatinine across different study groups expressed on Day 0 and on Day 15 are given as follows. Values are Mean ± SEM (n=6). Findings of histological studies and their interpretations (Table 5) (Figures 1-3).

Discussion

DIABIND® is a polyherbal drug containing six herbal drug combinations like *Gymnema sylvestre, Holarrhena antidysenterica,*

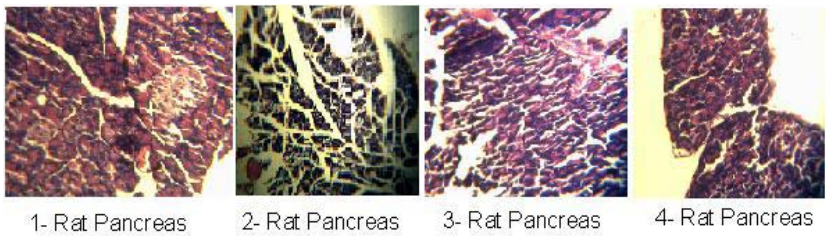


Figure 1: Findings of the histological study of rat pancreas sections under (40 X magnification) across groups (1-4) showed the destruction of beta cells in the disease controlled group. The treatment groups (3 and 4) showed signs of regaining of normal architecture of the cells of the pancreas indicating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

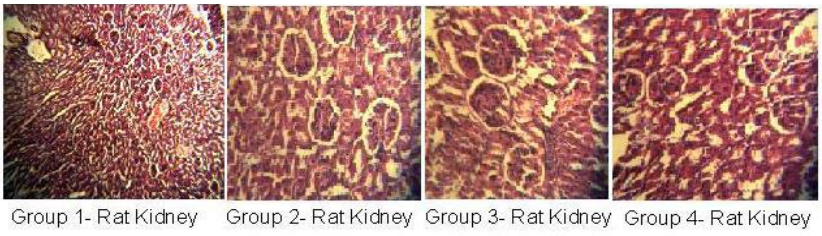


Figure 2: Findings of the histological study of rat kidney sections under (10 x and 40 x magnification) did not show any remarkable changes across groups (1-4).

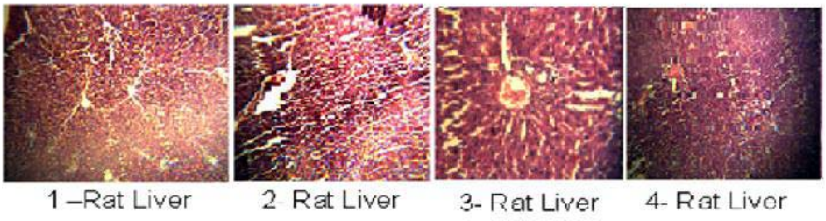


Figure 3: Findings of the histological study of rat liver sections under 40 x magnification did not reveal any remarkable changes across the study groups (1-4).

Trigonella foenum graecum, Azadirachta indica, Syzgium cuminii, Momordica chirantia and traditional ayurvedic excipients such as bhasma. The study revealed that this PHAF reduces blood glucose level significantly *(P< 0.05) as compare to glibenclamide. It demonstrated a significant reduction (p <0.05) in fasting blood glucose level by 40.7% compared to 36.25% in the glibenclamide treated group. Organ weight and histological evaluations also did not reveal any significant untoward effect of *DIABIND*® 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.

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.

Histopathological observations did not reveal any significant untoward effect on pancreas, liver or kidney. *DIABIND*, the PHAF, possesses significant blood sugar lowering effect with little safety concerns. However, future studies are warranted to corroborate the present findings.

References

1. Ali KM, Chatterjee K, De D, Bera TK, Ghosh D. Efficacy of aqueous extract of seed of *Holarrhena antidysenterica* for the management of diabetes in experimental model rat: A correlative study with antihyperlipidemic activity. Int J Appl Res Nat Prod. 2009; 2: 13–21.
2. Mohamed M, Mohamed E, Perumal S. Effect of *Pongamia pinnata* to lipid peroxidation and antioxidants in hyperammoneomic rats: With references to circadian variation. Ir J Pharmacol Therap. 2007; 6: 119–123.
3. Trivedi NA, Mazumder B, Bhtt JD, Hemavathi KG. Effect of Silajit on blood glucose and lipid profiles in alloxan-induced diabetic rat. Ind J Pharmacol. 2004; 36: 373–376.
4. Sathishekar D, Subramanian S. Antioxidant properties of *Momordica charantia*(bitter gourd) seeds on streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr. 2005; 14: 153–158.
5. Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin induced diabetic rats. J Ethnopharmacol. 2004; 92: 87–93.
6. Szkudelski T. The mechanism of alloxan and streptozotocin action on ß-cells of rat pancreas. Physiol Res. 2001; 50: 536–546.
7. Chandalia HB, Sadikot S, Bhargava DK, Krisnaswami PR. Estimation of glycosylated haemoglobin by a simple chemical method and its use in monitoring control of diabetes mellitus. J Assoc Phys Ind. 1980; 28: 285–286.
8. Chou AC, Wilson JE. Carbohydrate metabolism. In: Wood WA, editor. Methods in Enzymol. New York: Academic Press. 1975; 20–21.
9. Sadasivam S, Manickam A. Carbohydrate. In: Sadasivam S, Manickam A, editors. Methods in Biochemistry. New Delhi: New Age International Pvt Ltd. 1996; 11–12.
10. Henry RJ, Chiamori M, Gonub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamate oxaloacetic transaminase, glutamic pyruvate transaminase and lactic acid dehydrogenase. Am J Clin Pathol.1960; 34: 381–398.
11. Oberley LW. Free radical and diabetes. Free Rad Biol Med. 1988; 5: 113–124.
12. Carter AC, Broder L, Friedman M. Streptozotocin and metastatic insulinoma. Ann Intern Med. 1971; 74: 445–446.
13. Barthel A, Schmoll D. Novel concepts in insulin regulation of hepatic gluconeogenesis. Am J Physiol Endocrinol Metab. 2003; 285: 685–692.
14. Maiti R, Jana D, Das UK, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biol Pharm Bull. 2005; 28: 1172–1176.
15. Slave A, Carrupt PA, Tillement JP, Testa B. Structural damage to protein caused by free radicals: Assessment, protection of antioxidant influence of protein binding. Biochem Pharmacol. 2001; 61: 1237–1242.
16. Prakasam A, Subramaniam S, Pugalendi KV. Effect of *Caseria esculenta* on blood glucose and plasma antioxidant status in Stretozotocin diabetic rats. Polish J Pharmacol. 2003; 55: 43–49.
17. Mandal S, Barik B, Mallick C, De D, Ghosh D. Therapeutic effect of ferulic acid, an ethereal fraction of ethanolic extract of seed of *Syzygium cumini* against streptozotocin induced diabetes in male rats. Methods Find Exp Clin Pharmacol. 2008; 30: 121–128.
18. Saravanan S, Pari L. Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. BMC Com Alt Med. 2005; 5: 1–8.
19. Heidi KO. *In-vivo* insulin regulation of skeletal muscle glycogen synthase in calorie-restricted and in ad *libitum*-fed rhesus monkeys. J Nutr. 2001; 131: 907–913.
20. Ruiter JD. Overview of the antidiabetic agent. Endocrine Pharmacotherapy Module spring. 2003: 1-33.