

IN VIVO EVALUATION OF HYPOGLYCEMIC ACTIVITY OF AN AYURVEDIC POLYHERBAL COMBINATION (PDBT)

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ABSTRACT

Although many advances have taken place in modern medicine, yet the treatment of hyperglycemia remains unsatisfactory for want of multi-targeted drugs which can reduce the blood sugar with lesser adverse effects. In Ayurvedic Texts and in practice, we find an extensive use of many medicinal plants for treatment of *Diabetes mellitus* popularly known by the name Prameha in Ayurvedic literature. The present paper discusses *in vivo* evaluation of Polyherbal capsules (PDBT) containing extracts of five medicinal plants for its hypoglycemic activity in alloxan induced diabetic rats.

Keywords: *Prameha*, Diabetes mellitus, alloxan induced diabetic rats.

INTRODUCTION

According to the World Health Organisation estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030 (1). The International Diabetes Federation (IDF) also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025 (2). It is quite evident from the above observations that diabetes has become a major health problem in India.

Currently, in the conventional modern system of medicine, oral hypoglycaemic drugs and/or insulin are used for the management of diabetes. Oral hypoglycemic agents although are effective in reducing the blood sugar levels, a significant percentage of patients do report several side effects like hypoglycaemia, weight gain, development of insulin resistance and cardiovascular complications. There is a new understanding of involvement of multiple factors in pathogenesis of diabetes mellitus that has high-

lighted the need of drugs which are multi targeted, safe, easily available, and are cheaper. Ayurvedic system of medicine offers great future in the treatment of diabetes mellitus. Ayurveda mentioned several plants for the management of Diabetes mellitus and PDBT capsules is made from a few of such medicinal plants. PDBT capsules contains water extracts of five medicinal plants in equal quantity such *Zingiber officinale*, *Tinospora cordifolia*, *Pterocarpus marsupium*, *Gymnemma sylvestre* and *Momordica charantia*. Previous studies prove their individual actions for glucose lowering activity as well as safety studies^{5,6,7}. Hence this study was undertaken for *in vivo* evaluation of hypoglycemic activity of a combination of such herbs which was named as PDBT capsules. Hypoglycemic activity was evaluated on alloxan induced diabetic rat model.

MATERIAL AND METHODS

Plant material *Zingiber officinale* (Rhizome), *Tinospora cordifolia* (stem), *Momordica Charantia* (fruit), *Gymnema Sylvestre* (leaves), *Pterocarpus marsupium* (bark) which are indigenous to India were collected from local market and identified by Dravyaguna Department of R.A.Podar Ayurved College, Worli, Mumbai. These herbs were shadow dried & sent to Amruta Herbals, Indore for Water extraction. The extraction was done with Soxhlet apparatus after pulverisation. The extract was evaporated to dryness under vacuum desiccators (15.5% w/w). After the standardisation of extracts as per API guidelines, PDBT capsules of 500mg each containing equal amount of each extract were prepared.

Animals- Wistar albino rats (8–10 weeks) of both sexes having weight range 180gm-210gm were obtained from the animal house of C.U.Shah College of pharmacy SNTD Campus, Juhu Tara road Santacruz (W) Mumbai. Before and during the experiment

rats were fed with standard diet (Gold Moher, Lipton India Ltd. After randomization in to 4 groups before initiation of experiment the rats were acclimatized for period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours *ad libitum*. Some animals were used for induction of diabetes while some were kept aside as a normal control.

- Induction of Diabetes- Diabetes was induced by single *i.p.* of alloxan monohydrate (120 mg/kg).
- Non diabetic control rats received saline solution
- After 72 hours, the blood glucose levels were checked.
- Diabetic rats were considered with the blood glucose levels 200 - 260 mg/dl.

Grouping of animals with 6 animals in each group was done as follows-

	Treatment
I	<u>Non Diabetic control</u> - Normal rats receiving 0.05% Sodium CMC solution.
II	<u>Diabetic control</u> - Diabetic rats receiving vehicle (0.05% Sodium CMC).
III	<u>Standard</u> - Diabetic rats receiving Glibenclamide (10 mg/kg)
IV	<u>Treatment group</u> - Diabetic rats receiving PDBT capsules (100 mg/kg)

EVALUATION

Blood samples were collected at 0th, 7th and 14th day.

Fasting blood glucose levels were estimated.

After the treatment period biochemical parameters like cholesterol, triglyceride, HDL,

LDL, urea, creatinine, AST and ALT were also estimated on **day 15**. . The blood glucose levels and body weight were estimated on the 0th day, 7th day and 14th day of the treatment. On the 15th day blood samples were collected from the overnight fasted animals from the retro orbital plexus for es-

timination of various biochemical parameters. The animals were sacrificed to obtain the vital organs (liver, kidney and pancreas) for histopathology examination.

Collection of Blood sample and Blood Glucose Determination-

Blood samples were drawn from tail tip of rat on 0, 7 and 14 day. Blood sugar estimation done by “one touch electronic glucometer” using glucose test strips. Body weight was measured by electronic weighing machine. On day 14, blood was collected from retro-orbital plexus. From overnight fasted rats serum was separated and analysed for serum cholesterol ⁽¹⁰⁾, triglycerides by enzymatic DHBS colorimetric method. ⁽¹¹⁾ Serum HDL ⁽¹²⁾, Serum LDL ⁽¹³⁾, Serum Creatinine ⁽¹⁴⁾ Serum Urea ⁽¹⁵⁾, and serum alkaline phosphatase were estimated by hy-

drolyzed phenol amino antipyrine method ⁽¹⁶⁾. The whole pancreas, liver and kidneys were removed after sacrificing the animal and were collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5µ thickness were cut and stained haematoxyllin and eosin (H&E) for histo-pathological examination. Histo-pathological examination was done at Excel Pathological Laboratory, Mumbai, India.

Statistical Analysis- All the values of body weight, Fasting Blood Sugar and biochemical estimations were expressed as Mean ± standard error of mean (S.E.M) and analysed for ANOVA and post hoc Dunnet’s t-test. Differences between groups were considered significant at p<0.01 levels.

RESULTS

1) Effect on Blood Sugar Level

Groups	Blood glucose levels (mg/dl)			% Activity
	0 th Day	7 th Day	14 th Day	
Non Diabetic Control	74.83 ± 0.70	74.33 ± 0.50	73.00 ± 0.25	-
Diabetic Control	232.67 ± 3.11	248.67 ± 5.15	263.00 ± 1.52	-
Diabetic Standard Control	235.66 ± 2.40	133.50 ± 2.19	96.00 ± 1.34	60.33 %
PDBT capsules (100 mg/kg in divided doses)	233.83 ± 3.4	171.17 ± 4.97	118.17 ± 5.04	49.46 %

Values are expressed as mean ± SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

2. Effect on Liver markers

Groups	Parameters (U/L)	
	AST	ALT
Non Diabetic Control	48.65 ± 0.12	32.2 ± 0.19
Diabetic Control	121.52 ± 0.14	187.92 ± 0.24

Diabetic Standard Control	83.38 ± 0.12	77.93 ± 0.22
PDBT capsules (100mg/kg in divided doses)	95.92 ± 0.11	86.25 ± 0.21

Values are expressed as mean ± SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

3. Effect on Renal markers-

The levels of Blood Urea and Serum Creatinine on the 14th day were compared in between the groups. The pretreatment levels were not taken because of the ethical issues.

Groups	Parameters (U/L)	
	Urea	Creatinine
Non Diabetic Control	16.14 ± 0.01	0.7 ± 0.0054
Diabetic Control	35.53 ± 0.15	1.69 ± 0.007
Diabetic Standard Control	21.17 ± 0.12	0.63 ± 0.006
PDBT capsules (100mg/kg in divided doses)	23.64 ± 0.12	0.76± 0.008

Values are expressed as mean ± SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

4. Effect on lipid profile

The levels of different constituents of lipid profile on the 14th day were compared in between the groups. The pretreatment levels were not taken because of the ethical issues.

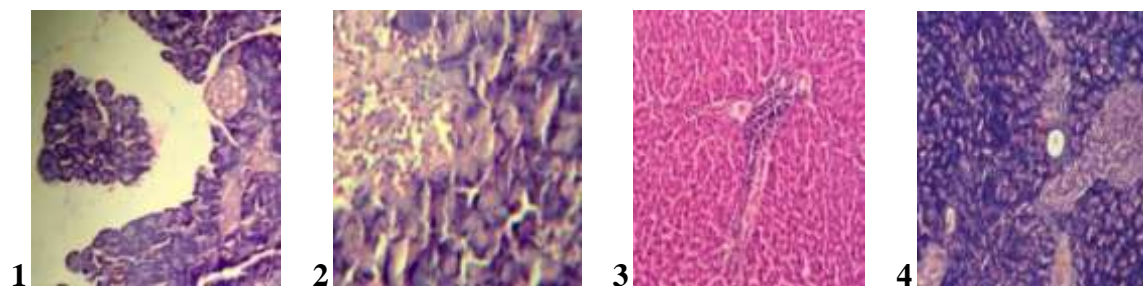
Groups	Parameters (mg/dl)			
	Cholesterol	Triglyceride	HDL	LDL
Non Diabetic Control	69.16 ± 0.79	71.00 ± 0.85	51.00 ± 0.73	3.96 ± 0.45
Diabetic Control	141.83 ± 1.07	124.33 ± 0.88	21.66 ± 0.71	95.30 ± 0.71
Diabetic Standard Control	80.167 ± 0.9	81.00 ± 0.85	42.83 ± 0.70	21.13 ± 0.33
PDBT capsules (100mg/kg in divided doses)	89.5 ± 0.88	93.00 ± 1.00	37.33 ± 0.66	32.53 ± 0.82

Values are expressed as mean ± SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

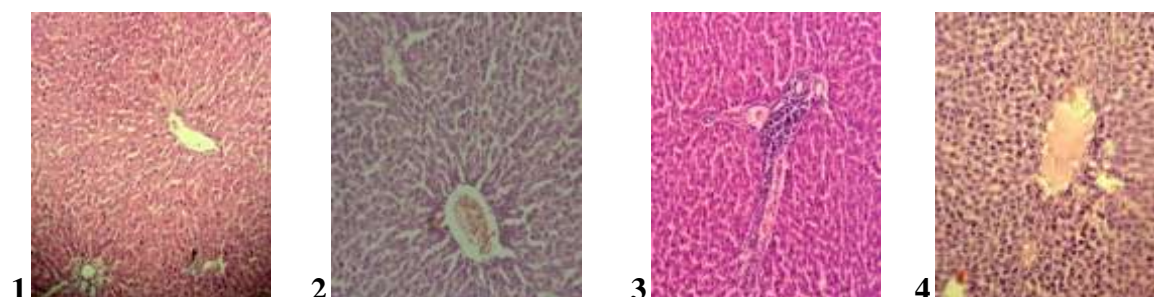
RESULTS OF HISTOPATHOLOGY

Pancreas:



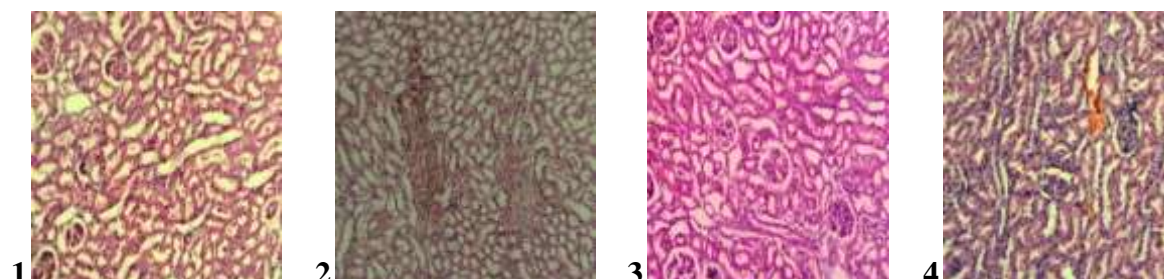
1-Normal control 2-Diabetic control 3-Glibenclamide 4-PDBT capsules.

➤ **Liver:**



1-Normal control 2-Diabetic control 3-Glibenclamide 4-PDBT capsules.

➤ **Kidney:**



1-Normal control 2-Diabetic control 3-Glibenclamide 4-PDBT capsules.

DISCUSSION

Non diabetic control rats receiving saline were considered as normal & healthy, diabetic control rats showed what could have happened without treatment. Diabetic rats treated with Glibenclamide (10mg/kg) decreased BSL 235.66 ± 2.40 mg/dl to 96.00 ± 1.34 mg/dl (60.33%). Diabetic rats treated with PDBT capsules formulation showed decreased blood glucose levels from

233.33 ± 3.4 mg/dl to 118.17 ± 5.03 mg/dl at the end of 14th day of treatment indicating good hypoglycemic activity (49.46%).

PDBT treated group also showed decrease in Sr. Triglyceride & Sr. Cholesterol along with increase in HDL as compared to diabetic control group. Rats treated with PDBT capsules also showed statistically significant decrease ($p < 0.01$) in AST and ALT levels as compared to Diabetic control

indicating hepatoprotective effect. Rats treated with PDBT capsules also showed statistically significant decrease ($p < 0.01$) in Urea and Creatinine levels as compared to Diabetic control indicating nephroprotective effect.

• **Interpretation of Histopathology results-**

Pancreas:

Group I - Histology of the pancreas of Normal control group showed normal pancreatic islets pattern and exocrine pancreatic architecture. In the pancreas of normal control rats, many rounded and elongated islets were evenly distributed throughout the cytoplasm, with their nucleus lightly stained than the surrounding acinar cells.

Group II- Histopathology of pancreas of Diabetic rats showed diffuse degeneration of islet of langerhans

Group III-Only minor pathological features were identified in pancreas of the diabetic rats treated with glibenclamide.

Group IV- Degenerative changes are very few in PDBT Capsule treated rats.

Liver:

Group I - Histopathologically showed normal hepatic structure with hepatic lobule.

Group II - The histopathological examination of diabetic rats showed hepatocellular injury pronounced in loss of the normal architecture of the liver. Histopathology showed that sinusoidal spaces are dilated in diabetic rats with multifocal fatty degeneration.

Group III - Degenerative changes are very few in this group.

Group IV- PDBT Capsule treated rats showed minimal single cell necrosis and kuffer cell infiltration.

Kidney:

Group I -Examination of the kidney of the normal control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space were normal. No major detectable abnormalities were noted in the histopathological studies of kidney.

Group II Histopathology showed that diffuse degeneration of tubular epithelium and multifocal area of hemorrhages in diabetic rats.

Group III- Minimal changes are seen in this group

Group IV -These changes are minimal in PDBT Capsule treated rats.

CONCLUSION

- Polyherbal combination under study (PDBT) showed statistically significant hypoglycemic and hypolipidemic activity in alloxan induced diabetic rats,
- Thus PDBT Capsule is a multi targeted, cheaper, herbal drug which is a promising candidate for consideration for the treatment of diabetes mellitus.

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