PREPARATION OF INDEGENOUS HERBAL FORMULATION AND ITS EVALAUTION FOR ANTIDIABETIC ACTIVITY

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Summary

The objective of the study is to prepare and investigate the herbal formulation of *Tinospora cordifolia, Trigonella foenum* and *Emblica officinalis* for antidiabetic effects. Herbal formulations PD1, PD2 and PD3 were prepared using *Tinospora cordifolia, Trigonella foenum* and *Emblica officinalis* extracts. Herbal formulations were evaluated for hypoglycemic effects and Oral Glucose Tolerance Test (OGTT) in normal and Alloxan induced diabetic rats. In hypoglycemic study and OGTT, there was a significant decrease in Blood Glucose Level (BGL) in normal rats with formulation PD3, marginal decrease in formulation PD2 and very less decrease in formulation PD1. In diabetic rats PD3 showed significant decrease in Fasting Blood Glucose Level (FBGL) which was comparable to Glibenclamide while the effects of formulation PD2 and PD1 was not significant after treatment with prepared herbal formulations. These results were also supported by serum lipid profile and histological studies of liver and kidney.

Keywords: *Tinospora cordifolia, Trigonella foenum, Emblica officinalis* Hypoglycemic activity**,** Anti-diabetic activity**,** Alloxan.

Introduction

It is a general belief that a synergism between two or more plant extracts enhances the physiological potential of the bio-organic substances. Therefore, a combination of different plant extracts is very often preferred over single extracts. Although several reports are available on the effects of different formulations on the regulation of various disorders, only few investigations have been done to study the combined effects of three plant extracts and practically no literature is available with respect to the regulation of hyperglycemia [**1**].

Considering the information gap in this area of research and based on the fact that *Trigonella foenum* graecum, *Embelica officinalis, Tinospora cordifolia* extracts were able to ameliorate hyperglycemia in rats individually, a study was made to evaluate possible synergistic effects of these three extracts in single formulation.

Material and methods

Collection of standardized extracts

Standardized extracts of *Tinospora cordifolia* was obtained from Himalaya Drug Company and *Trigonella foenum* and *Emblica officinalis* were obtained from SAMI Labs, Bangalore (India)

Drugs/ chemicals

Tween-80 (Rankem Ranbaxy Fine Chemicals Ltd, New Delhi, India), Glibenclamide Tab. (Aventis Pharma Ltd., Mumbai, India), Alloxan (Spectrochem Pvt. Ltd., Mumbai, India), Glucon D (Heinz India Pvt. Ltd., Mumbai, India), Glucose estimation kit. (Span Diagnostic Ltd., Surat, India). All the other solvents and chemicals used, were of analytical grade and were purchased from S.D. Fine Chemicals Pvt. Ltd. Mumbai, India

Preparation of herbal formulation

All the standardized extracts were sieved through 80 mesh sieve separately. Powder of standardized extract were weighed accurately for different formulation according to description given below and mixed well together. Thus the Herbal formulation Churna was prepared. Formulations were kept in air tight containers. (Agrawal and Paridhavi, 2007)

PD1 : *Tinospora cordifolia* : *Trigonella foenum : Emblica officinalis* **(1:1:2)**

PD2 : *Tinospora cordifolia* : *Trigonella foenum : Emblica officinalis* **(1:2:1)**

PD3 : *Tinospora cordifolia* : *Trigonella foenum : Emblica officinalis* **(2:1:1)**

Dose fixation 100, 200, 300 and 400 mg/kg doses were tried at first, among of these 100 and 200 mg/kg doses did not show significant reduction in blood glucose level in alloxan induced diabetic rats, 300 mg/kg showed a moderate antidiabetic effect while 400 mg/kg showed significant reduction in blood glucose level. So all our study was carried out with 400 mg/kg dose level.

Animals

Albino Wistar rats (150-200 g) were used for the study. The animals had free access to standard rat pellet (Pranav Agro Industries Ltd, Bangalore, India), with water supplied *ad libitum* and kept under strict hygienic standard conditions. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of drugs:

Standardized extracts of *Trigonella foenum, Tinospora cordifolia* and *Emblica officinalis* suspended in water in presence of 3% v/v Tween-80 solution. All the drugs were administered orally for experimental purpose. Each time fresh preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10 ml/kg for each animal.

Glibenclamide Solution: Glibenclamide tablet was dissolved in 1ml of distilled water to give 500 µg/ml solution. This solution was administered at a dose of 10mg/ kg body weight using clean and dry oral feeding needle for 21days.

Experimental Procedure

Assessment of hypoglycemic activity in normal rats [2]

Fasted rats were divided in to five groups consisting of 8 animals in each group. First group received vehicle (3% v/v tween 80 in distilled water, orally in a volume of 10 ml/kg, which served as control). Group II received Glibenclamide (5 mg/kg, p.o.) as standard drug suspended in vehicle. PD I, PD II, PD III formulations suspended in vehicle were administered at the doses of 400 m/kg, p.o. in a volume of 10 ml/kg to the rats of group III, IV and V respectively. Blood samples were collected from the tail vein or by retro-orbital puncture method on 0 day and after 7 days of treatment after 16 h of overnight fasting for glucose level estimation. Oral glucose tolerance test was performed on 8^{th} day.

Induction of diabetes

Alloxan induced diabetes

Diabetes was induced by single injection of alloxan monohydrate (120 mg/kg, i.v.) in ice cold normal saline. Five days after the injection of alloxan, the animals were fasted for 16 h and blood sugar was determined of the surviving animals. Rats with blood glucose level >200 mg/dl were considered diabetic and were used in the experiment.

Acute and sub-acute effect of ALEBF in alloxan diabetic rats

Diabetic rats were divided into 5 groups consisting of 8 animals in each group. Group I received vehicle (3 %v/v tween 80, p.o.) which served as control. Group II received glibenclamide as reference drug (5 mg/kg, p.o.) suspended in vehicle. PD I, PD II, PD III formulations suspended in vehicle were administered at the doses of 400 m/kg, p.o. in a volume of 10 ml/kg to the rats of group III, IV and V respectively. Blood samples were collected from the tail vein after 1 h of extract administration on 12^{th} and 21^{st} day. OGTT was performed at the end of 22 day. The blood samples were analyzed for glucose and lipid profile. At the end of the study period rats were sacrificed and histology of liver and kidney were performed.

Statistical Analysis

The values were expressed as mean ± SEM. Statistical comparisons between all groups were performed by using ANOVA-1.

Results

Hypoglycemic activity in normal rats

Among herbal formulations PD1, PD2 and PD3 only PD3 significantly decreased the blood glucose level on 7^{th} day after treatment. Glibenclamide (10 mg/kg) significantly reduced blood glucose level (BGL) on 7^{th} day as compare to vehicle and normal groups. (Table No: 1)

Table No: 1 Hypoglycemic activity in normal rats after 7 days treatment with herbal formulations.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)	
		0 Day	7th Day
Vehicle Control	10	93.79 ± 2.58	93.82 ± 2.35
Glibenclamide	10	97.43 ± 3.66	48.28^{++**} ± 4.27
PD1	400	98.74 ± 2.09	90.06 ± 2.32
PD2	400	98.07 ± 5.29	81.85 ± 6.65
PD3	400	94.38 ± 3.82	71.27^{++**} ± 3.18

Oral glucose tolerance test (OGTT) on 8th day:
Administration of glucose (2g/kg) to 7 days pretreated rats significantly suppress the rise in BGL with PD3 at 1 hour and 2 hour with 400 mg/kg as compare with vehicle control. While in PD1 (400 mg/kg) did not produce significant reduction in BGL. Glibenclamide (10 mg/kg) showed significant suppress in BGL rise at 1 & 2 hour. (Table No: 2)

Table No: 2 Oral Glucose Tolerance Test in normal rats on 8th day, after treatment with herbal formulations.

Treatment	Dose (mg/kg)	Percentage change in Blood glucose level (%)			
		1st hour	2nd hour	3rd hour	6th hour
Vehicle Control	10	52.30 ± 3.76	19.54 ± 2.19	1.64 ± 0.45	1.41 ± 0.56
Glibenclamide	10	25.51^{++**} ± 3.47	3.39^{++**} ± 1.14	1.41 ± 1.00	0.19 ± 0.79
PD1	400	41.84 ± 1.12	14.03 ± 2.51	3.56 ± 1.82	1.86 ± 0.54
PD2	400	37.22^{+*} ± 4.64	11.24 ± 2.19	4.25 ± 0.66	2.19 ± 0.90
PD3	400	36.79^{++**} ± 1.46	9.47^{++**} ± 1.87	0.32 ± 0.97	0.58 ± 0.52

Values are expressed as mean ± S.E.M. n = 8. $^{+}$ P <0.05, $^{++}$ P <0.01 Normal Control Vs all groups. * P <0.05, ** P <0.01 Vehicle Control Vs all groups.

Anti-diabetic activity in alloxan induced diabetic rats.
Treatment with alloxan (120 mg/kg, i.p.) increased the BGL to a range of 260-325 mg/dl after 7 days. Treatment with herbal formulation PD3 (400 mg/kg) had significantly reduced the BGL on 12th and 21st day in alloxan induced diabetic rats. PD2 (400 mg/kg) significantly reduced BGL on 21st day in alloxan induced diabetic rats, indicating the formulation PD2 possess anti-diabetic activity after 3 weeks. Whereas, anti-diabetic activity is significant on 12th and 21st day for glibenclamide treated groups as compare with diabetic control groups. (Table No 3)

Table No 3. Antidiabetic activity in alloxan induced diabetic rats after 21 days treatment with herbal formulations.

Treatment	Dose (mg/kg)	Blood Glucose Level (mg/dl)		
		0 Day	12th Day	21st Day
Normal Control	---	83.16 ± 1.95	84.02 ± 1.17	82.33 ± 2.17
Diabetic Control	10	322.33 ± 11.16	268.32 ± 10.02	272.83 ± 14.04
STD	10	260.33 ± 3.88	112.14^{++**} ± 1.48	100.83^{++**} ± 4.86
PD1	400	270.33 ± 6.80	224.01 ± 1.60	190.67 ± 8.21
PD2	400	266.33 ± 8.67	193.00 ± 1.84	146.00^{++**} ± 2.30
PD3	400	269.5 ± 8.80	168.21^{++**} ± 1.23	108.83^{++**} ± 3.12

Values are expressed as mean ± S.E.M. n = 8.
$^{+}$ P <0.05 and $^{++}$ P <0.01 Normal Control Vs all groups. * P <0.05 and ** P <0.01 Diabetic Control Vs all group

Oral Glucose Tolerance Test (OGTT) in alloxan induced diabetic rats on 22nd day:
Repeated administration of herbal formulation PD3 and PD2 (400 mg/kg), and Glibenclamide (10 mg/kg) significantly inhibited the increase in BGL at 1st, 2nd and 3rd hour after glucose loading (2 g/kg) in alloxan induced diabetic rats. (Table No 4.)

Table No 4. Oral Glucose Tolerance Test in alloxan induced diabetic rats on 22nd day, after treatment with herbal formulations.

Treatment	Dose (mg/kg)	Percentage change in Blood glucose level (%)			
		1st hour	2nd hour	3rd hour	6th hour
Normal Control	---	40.22 ± 2.29	17.03 ± 1.38	2.29 ± 0.51	0.89 ± 0.36
Diabetic Control	10	47.72 ± 3.06	24.55 ± 1.28	4.90 ± 1.20	1.06 ± 0.61
Glibenclamide	10	28.96^{++**} ± 1.90	11.89^{++**} ± 1.41	2.32^{**} ± 0.54	1.09^{*} ± 0.46
PD1	400	40.23 ± 1.52	20.34 ± 1.18	3.81 ± 0.42	1.49 ± 0.42
PD2	400	37.14^{+**} ± 3.76	15.32^{++**} ± 1.08	2.61^{**} ± 0.75	1.20^{*} ± 0.38
PD3	400	32.34^{++**} ± 1.30	13.61^{+**} ± 1.75	2.50^{+**} ± 0.24	1.12^{*} ± 0.44

Values are expressed as mean ± S.E.M. n = 8.
$^{+}$ P <0.05, $^{++}$ P <0.01, Control Vs all groups. * P <0.05, ** P <0.01, Diabetic Control Vs all groups.

Histological studies: (Fig No. 1)

Liver:

Liver sections of normal rats showed normal liver architecture with well brought out central vein, well-preserved cytoplasm and prominent nucleus and nucleolus. Treatment with alloxan (120 mg/kg, i.p) displayed feathery degeneration, micro and macro cellular fatty changes and inflammatory cells around portal tract after 22 days. Treatment with glibenclamide for 21 days in alloxan intoxicated showed an mild protection from alloxan-induced changes in the liver. Treatment with formulations PD1, PD2 and PD 3 showed various level of liver protection against alloxan intoxication (figure 1). PD 3 showed good protection against alloxan induced toxicity as compared to other formulations.

Kidney:

Liver sections of normal showed normal kidney architecture with well brought glomeruli and tubules well-preserved cytoplasm, prominent nucleus and nucleolus. Treatment with alloxan (120 mg/kg, i.p) , displayed feathery degeneration, thickening of glomeruli, inflammatory cells & severe congestion indicating damaged occurred due to the development of diabetes. Treatment with glibencamide, PD 1, PD2 and PD3 for 21 days in alloxan intoxicated rats showed various level of protection (figure 2).

Discussion

In light of the above reports, an attempt was made to study the synergistic effect in the different combinations of extracts of *Tinospora cordifolia, Trigonella foenum* and *Emblica officinalis* in herbal formulation.The standard drug glibenclamide (10 mg/kg) treated group has shown significant decrease in fasting glucose level [**3**].

Herbal formulation PD3 produced a statistically significant decrease in blood glucose levels for both normoglycaemic and alloxan induced hyperglycaemic rats.Formulation PD2 showed significant reduction in blood glucose level in alloxan induced hyperglycaemic rats, only on 21st day while herbal formulation PD1 did not show significant reduction in blood glucose level. Alloxan selectively destroys insulin secreting β-cells in the islets of Langerhans and the effect is irreversible. Alloxan treated animals receiving the herbal formulation PD3 showed rapid normalization of blood glucose level in comparison to the control and this could be due to the possibility that many β-cells are still surviving and cell regeneration can not be ignored and the reductions in the serum glucose levels may be due to the increase in action of GLUT4 receptors or insulinomimetic action as per previous reports [**4-6**].
Histopathological studies revealed that glibenclamide and herbal formulations treated groups shown hepatoprotective and nephroprotective effect against oxidative stress compared to diabetic control group. Diabetic control group shown the symbol of nephrotoxicity and hepatic-injury due to oxidative stress.
The study of blood glucose levels, lipid profile and histological changes in liver and kidney, in herbal formulations treated rats, support the Herbal formulation PD3 is a potent antidiabetic.

Fig. No: 1 Histopathology of Liver after 21 Days of Treatment

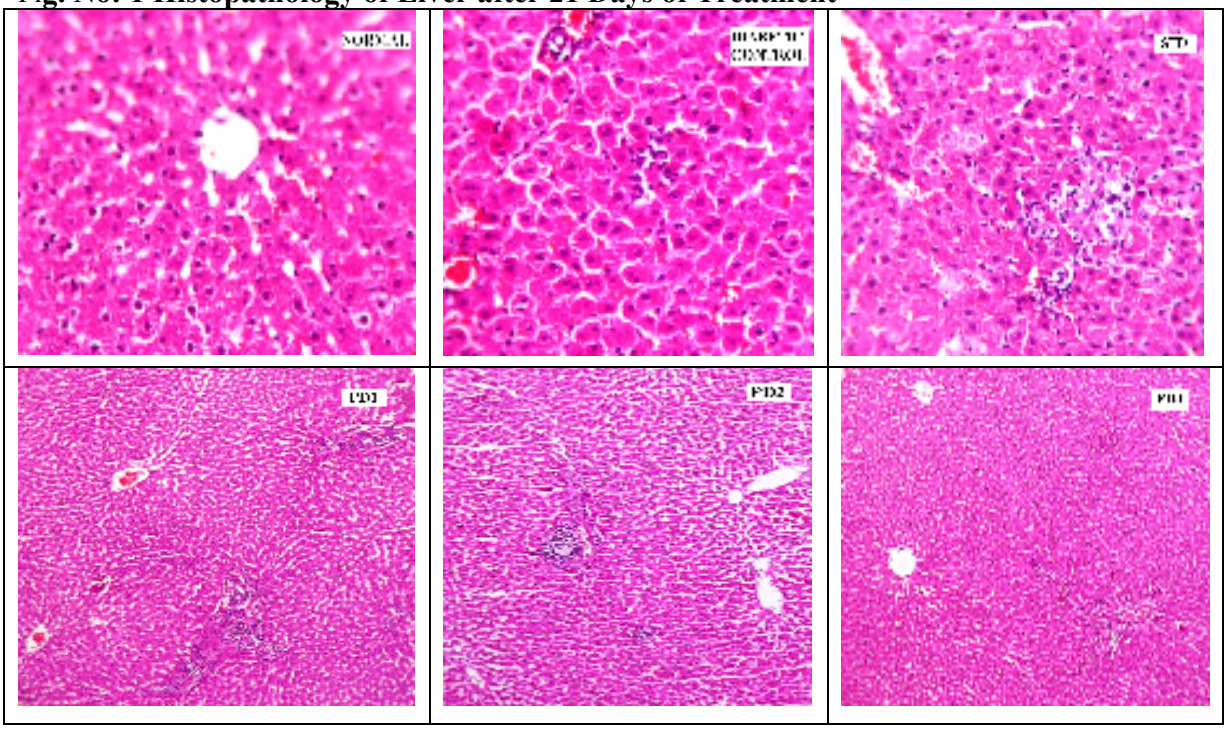
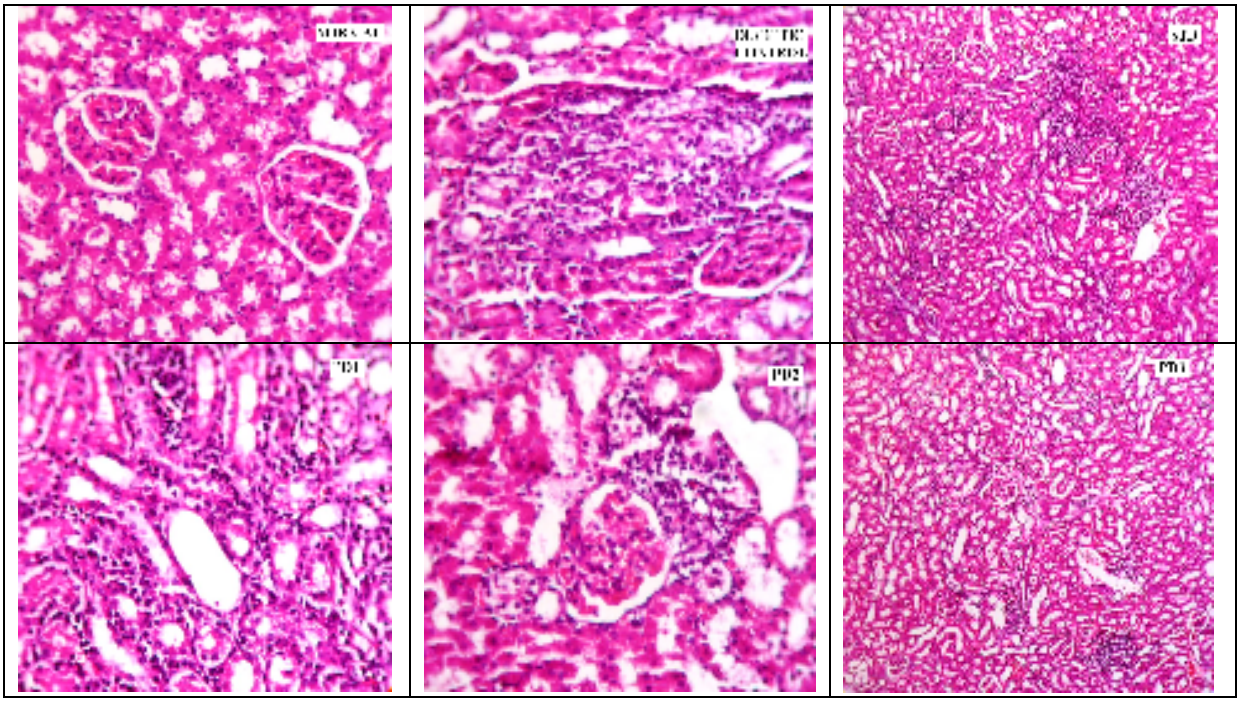


Fig. No: 2 Histopathology of Kidney after 21 Days of Treatment



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