Research Article Yadav *et al.*, 7(12): Dec., 2016:5348-5353]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Antidiabetic Activity of Polyherbal Formulation in Streptozotocin Induced Type II Diabetic Rats

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Abstract

The study emphases on polyherbal antidiabetic formulations of different plants used in the treatment of diabetes mixed in different concentrations. In the current study four medicinal plants with proven antidiabetic were selected for the preparation of formulation.The efficacy of prepared mixture has been tested on streptozotocin- (STZ-) induced diabetic rats and compared with a commercially existing drug glibenclamide. The polyherbal formulation was formulated using the ethanol extracts ofleaves of Gymnema sylvestre bark of Pterocarpus marsupium, stem of Tinospora cordifolia and seeds of Trigonella foenum-garecumin the ratio of 3:3:2:2.The investigational drug was administered for 21 consecutive days, and the effect of the polyherbal formulation on blood glucose levels was studied at regular intervals. The elevated level of SGPT, SGOT, and ALP in the diabetic controlled group reflected the significant alteration of liver function by STZ induction and was found to be equipotent to glibenclamide in restoration of the elevated enzyme levels to normal. The elevated lipid levels (triglyceride and total cholesterol) were restored to near normal by these mixtures for all the estimated parameters. The results of the mixtures on treated group were found to restore the glycemic level to the near normal level thereby indicating antihyperglycemic activity of the formulated mixtures.

Key words: Polyherbal, Antidiabetic, Formulations

Introduction

Diabetes is a complex disorder characterized by hyperglycemiaresulting from defective insulin secretion, resistance to insulinaction or both (Gavin JR 1997). Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (Bohannon, 2002). A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems (Scartezzini and Sproni, 2000).Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al*., 2003; Devaki *et al*., 2011).Gymnema Sylvestre, Pterocarpus marsupium, Tinospora cordifolia and Trigonella foenum-graecum are the plants profusely used in Ayurveda and supplementary traditional system of medicines to cure both infectious and degenerative diseases.

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A new polyherbal formulation of antidiabetic activities offered sufficient scope to undertake this research work. Selection of this polyherbal formulation which shows antidiabetic activities is of commercial importance of health benefits. In vitro study is the initial stage of any study and in vivo study involving experimental animals is the next stage by which one can be sure of proving the activity of the plant extracts. This natural scientific imagination was keenly attended by undertaking the in vivo investigation of the polyherbal formulation in animal models.

Material and Methods

Collection of the plant

Taxonomically identified leaves of *Gymnema sylvestre* (Apocynaceae) bark of *Pterocarpus marsupium* (Fabaceae), stem of *Tinospora cordifolia* (Menispermaceae)and seeds of *Trigonella foenum-garecum* (Fabaceae), were collected from the local market in Bhopal. The collected plants were authenticated at the Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.).

Animals

Adult Wistar rats (175 ± 10 g) of either sex were obtained from Jawaharlal Nehru Cancer Hospital and Research centre, Idgah Hills, Bhopal (MP). The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle. Rats had free access to water and rodent pellets diet (Hindustan Lever Ltd, Bangalore, India). The study was approved by the Institute Animal Ethics Committee (IAEC) of the Vedica College of B. Pharmacy, Bhopal (M.P.) and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forests, Government of India.

Acute oral toxicity

Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/ dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the plant extracts and polyherbal formulation in increasing dose levels of 5, 50, 300, and 2000 mg/kg body weight, respectively. The animals were observed for their behavioral (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality (Parasuraman, 2011).

Antidiabetic effect of polyherbal formulation in streptozotocin- induced diabetic rats

The male Wistar rats were divided into five different groups of six animals each as follows.

- Group I: Normal control
- Group II: Diabetic control
- Group III: Diabetic rats treated with polyherbal preparation (250 mg/kg)
- Group IV: Diabetic rats treated with polyherbal preparation (500 mg/kg)
- Group V: Diabetic rats treated with glibenclamide (0.25 mg/kg).

Diabetes was induced in overnight-fasted rats by administering single intraperitoneal (i.p.) injection of freshly ready streptozotocin (STZ) 50 mg/kg b.w. (Annadurai, *et al.* 2012). Diabetes was confirmed in the STZ treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of STZ injection, the rats were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and they were divided randomly into four different groups. The standard (glibenclamide) and herbal formulation were suspended in 1% w/v carboxymethyl cellulose (CMC) and administered once daily through oral gavages' for 21 consecutive days. The blood samples were collected on 1st, 7th, 14th, and 21st days of the treatment, through the tail vein of rats by pricking and were immediately used for the estimation of blood glucose with a glucometer. Weekly body weight variations were monitored for all the experimental animals.

At the end of the experiment, the blood sample was withdrawn from all the experimental animals through retro-orbital plexus puncture/posterior vena cava in plain and sodium ethylenediaminetetraacetic acid (EDTA) tubes for biochemical analysis (Parasuraman, *et al.* 2010). Finally the animals were sacrificed by diethyl ether anesthesia, and liver and pancreatic tissues were excised and used for biochemical and pathological examination. Part of the tissue sample was preserved in an ice-cold container for biochemical analysis and the remaining was stored in 10% formalin solution for histopathologic analysis.

Biochemical analysis

Biochemical determinations

After 15 days of treatment, overnight fasted rats were sacrificed and blood was collected. Glycosylated hemoglobin (HbA1c) was determined in heparinized whole blood by ion exchange method (Kumar, 2013, Lohar, *et al.* 2008) using commercial kit. The serum was separated and analyzed for lysosomal enzymes such as transaminases (serum glutamate oxaloacetate transaminase, SGOT and serum glutamate pyruvate transaminase, SGPT) and alkaline phosphatase (ALP), by colorimetric method.

The liver, diaphragm and pancreas were dissected out and washed with ice-cold saline immediately. Liver glycogen was estimated by the method of Caroll *et al.* 1956. A portion of pancreatic tissue was homogenized and the extract was used for the estimation of enzymatic antioxidants (catalase, CAT and glutathione peroxidase, GPx) activities including also lipid peroxidation process to see the effect of 15 days treatment with DRF/AY/5001.

Effect on glucose uptake by hemidiaphragm (in vitro) isolated diaphragm was divided intoapproximately 2 equal halves. The hemidiaphragms were rinsed with cold Tyrode solution, to remove blood clots. The hemidiaphragms were placed in 2 small

tubes containing 2 ml of Tyrode solution with 2% glucose and incubated for 30 min at 37° ± 0.2°C with appropriate aeration to enable stirring and also to provide oxygen. Following 30 min of incubation, the hemidiaphragms were taken out, dried at 60°C till constant weight was obtained. The glucose content of the incubated medium was measured (Parasuraman, *et al.* 2010). Glucose uptake by the hemidiaphragm was calculated as the difference between the initial and final glucose content in the incubation medium (James, *et al.* 2011).

Histopathological study of pancreas Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissues was carried out at the Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal, India.

Histopathologic analysis

Part of the liver and pancreas tissue were preserved in 10% formalin for 2 days. The liver and pancreas were dehydrated with alcohol (subsequently with 70, 80, 90%, and absolute alcohol) for 12 h each. Again the tissues were cleaned by using xylene for 15-20 min and they were subjected to paraffin infiltration in automatic tissue processing unit. The tissue blocks were prepared and the blocks were cut using microtome to get sections of thickness 5 µm. The sections were taken on a microscopic slide on which egg albumin (sticky substance) was applied and allowed for drying. Finally, the sections were stained with eosin (acidic stain) and

Statistical analysis

All the data were expressed as mean ± SEM. Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test post-hoc test. A P less than 0.5 were considered significant.

Results and Discussion

Toxicity study of polyherbal formulation

Acute toxicity studies did not show any mortality up to 2000 mg/kg given as single oral administration. Hence, the study was carried out at the dose levels of 250 and 500 mg/kg.

Antidiabetic activity of the polyherbal formulation

Diabetic control animals showed severe hyperglycemia compared to normal animals. The mean blood glucose level in the diabetic control group on day 0 was 248.63 ± 2.11 mg/dl and on day 21 was 328.92 ± 1.20 mg/dl. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly, bringing it back to near normal level, whereas the polyherbal capsule at 250 mg/kg and 500 mg/kg significantly ($P < 0.001$) decreased the fasting blood serum glucose level in the diabetic rats on 7^{th}, 14^{th}, and 21^{st} days, as compared to the diabetic control group. The results are presented in Table 1.

Table 1: The effect of polyherbal formulation on fasting blood glucose levels (mg/dl) in STZ induced diabetic rats

Treatment	Blood glucose level in mg/dl			
	0^{th} Day	7^{th} Day	14^{th} Day	21^{st} Day
Normal Control	90.26±40	94.16±1.32	92.20±1.24	91.42±1.92
Diabetic Control	248.12±1.64	279.94±2.26	321.06±2.41	329.12±1.63
PHF 250 mg/kg	243.34±2.45***	199.12±3.14***	148.23±2.65***	120.21±1.67***
PHF 500 mg/kg	238.25±1.34***	192.15±2.04***	141.11±1.94***	116.30±2.16***
Glibenclamide 0.25 mg/kg	236.41±2.31***	180.64±2.68***	129.21±2.40***	112.18±1.98***

[PHF: Polyherbal formulation, Value are expressed as mean ±SEM (n=6). ***<0.001 compared to diabetic control (one way ANOVA followed by a dunnette's test) STZ: streptozotocin.]

Diabetic animals showed significant decrease in plasma insulin, hemoglobin, and HbA_{1c} levels when compared with control animals. Herbal formulation and glibenclamide reversed the insulin depletion in diabetic condition and also brought back the hemoglobin and HbA_{1c} levels to normal.

Table 2: Effect of polyherbal formulation on plasma insulin level in STZ induced diabetic rats

Treatment	Plasma insulin level (µIU/ml)				Hb (mg/dl)	HbA1C (mg/g of Hb%)
	0th Day	7th Day	14th Day	21st Day	21st Day	21st Day
Normal Control	21.01±0.02	21.03±0.46	21.51±0.54	21.39±0.13	13.7±1.86	0.34±0.02
Diabetic Control	6.79±0.01	5.14±2.01	4.46±0.62	4.17±0.43	5.64±0.34	2.01±0.62
PHF 250 mg/kg	7.01±1.57	13.14±0.65**	16.84±0.34***	20.16±0.42***	12.10±0.35**	0.62±0.06**
PHF 500 mg/kg	7.1±1.62	13.9±0.37**	17.93±0.68***	20.84±0.61***	13.21±0.54***	0.46±0.12***
Glibenclamide 0.25 mg/kg	7.42±1.23	14.10±0.71**	19.14±0.38***	21.32±0.25***	13.85±0.84***	0.41±0.04***

[PHF: Polyherbal formulation, Hb: Hemoglobin; HbA1C: Gycosylated hemoglobin. Value are expressed as mean ±SEM (n=6). **<0.01, ***<0.001 compared to diabetic control (one way ANOVA followed by a dunnette's test) STZ: streptozotocin.]

Diabetic animals showed significant reduction in liver glycogen and total protein levels when compared to the control animals, whereas herbal formulation and glibenclamide treated animals showed normal liver glycogen and total protein levels. The prevention of depletion of glycogen in the liver tissue was possibly due to stimulation of insulin release from the β cells that activates the glycogen synthase system. Effects of herbal formulation and glibenclamide on the liver and renal markers of diabetic animals are presented in Table 3.

Table 3: Effect of polyherbal formulation on serum creatinine, protein, urea, and liver glycogen levels in STZ-induced diabetic rats

Treatment	Liver glycogen (g/100 mg wet tissue)	Total protein (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	SGOT (IU/I)	SGPT (IU/I)
Normal control	5.26±1.06	9.59±0.24	21.86±0.21	0.43±0.12	101.52±2.64	81.23±0.94
Diabetic control	2.14±0.12	4.26±0.1	50.45±0.27	1.16±0.07	154.11±1.64	150.24±2.18
PHF 250 mg/kg	5.06±1.06***	8.26±0.16*	23.9±0.38***	0.49±0.82*	112.21±2.64***	88.34±1.45***
PHF 500 mg/kg	5.42±0.54***	8.48±0.68**	23±0.56***	0.47±0.06**	106.25±2.42***	85.64±2.02***
Glibenclamide 0.25 mg/kg	5.58±0.52***	8.86±0.52**	22.8±0.86***	0.41±0.89**	104.32±2.6***	83.42±1.52***

[PHF: Polyherbal formulation. Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 campared to diabetic control animal (one-way ANOVA followed by a Dunnett's *t*-test) STZ: Streptozotocin.]

The diabetic rats showed significant (P < 0.001) increase in serum lipid profiles except HDL when compared to the control animals, whereas the levels in the treatment group remained within normal limits at the end of the study. Effects of herbal formulation and glibenclamide on the lipid profile of diabetic animals are presented in Table 4.

Table 4: Effect of ethanolic extracts of the polyherbal formulation on serum lipids

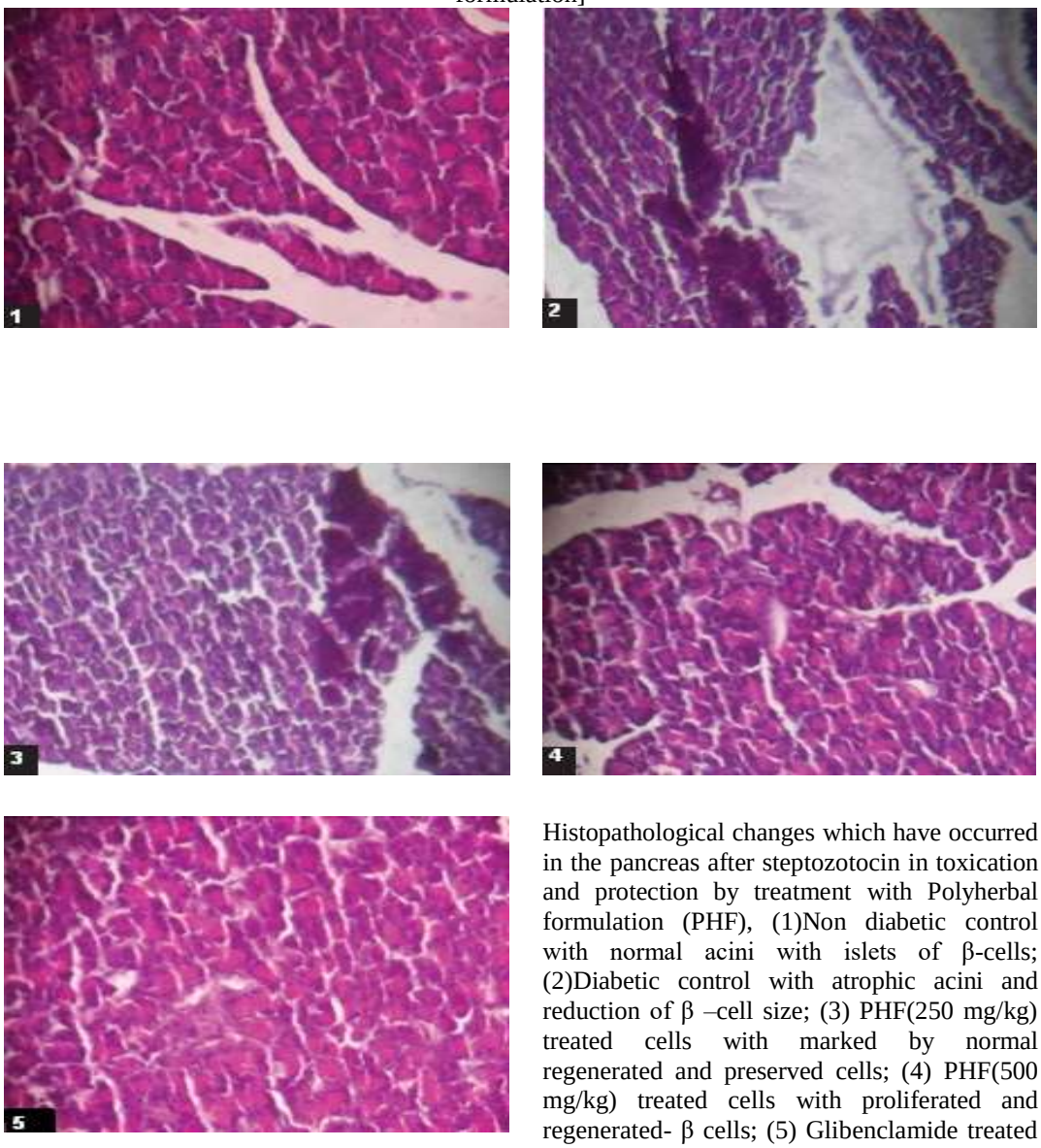
Group	Treatment	Lipid profile (mg/dl)				
		Triglyceride	Total cholesterol	HDL	LDL	VLDL
I	Normal control	68.86±0.42	75.65±2.36	30.98±1.06	38.42±1.68	13.28±0.84
II	Diabetic control	162.06±0.82	142.12±1.20	13.28±0.42	93..25±0.16	41.34±0.34
III	PHF 250 mg/kg	75.84±1.02***	81.26±0.34***	28.66±1.02***	43.26±1.42***	15.68±0.65***
IV	PHF 500 mg/kg	74.26±0.54***	79.42±1.26***	30.21±0.94***	40.02±0.82***	14.85±1.02***
V	Glibenclamide 0.25 mg/kg	71.24±0.43***	77.14±0.38***	31.34±0.71***	39.86±1.08***	14.02±0.22***

PHF: Polyherbal formulation. Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 campared to diabetic control animal (one-way ANOVA followed by a Dunnett's *t*-test) STZ: Streptozotocin; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

Histopathology results

The histopathologic analysis of pancreas revealed severe congestion, huge decrease in the number of islets of Langerhans and β cells, and fibrosis and inflammatory cell infiltration into the islets of Langerhans in STZ- induced hyperglycemic rats. While the polyherbal formulation at the dose of 250 mg/kg and 500 mg/kg showed mild congestion and mild decrease in the number of islets of Langerhans with normal β cell population, indicating significant amount of recovery. Glibenclamide treatment showed moderate congestion with moderate decrease in the number of islets of Langerhans and β cells and mild lymphocytic infiltration [Fig. 1].

Fig. 1: Histopathology of pancreas in rats [photomicrograph of hematoxylin and eosin (H and E) stained paraffin section from the pancreas (×400); D: Damage, I: Islet cells, N: Nuclei, R: Regeneration, PHF: Polyherbal formulation]



Histopathological changes which have occurred in the pancreas after steptozotocin in toxication and protection by treatment with Polyherbal formulation (PHF), (1)Non diabetic control with normal acini with islets of β-cells; (2)Diabetic control with atrophic acini and reduction of β –cell size; (3) PHF(250 mg/kg) treated cells with marked by normal regenerated and preserved cells; (4) PHF(500 mg/kg) treated cells with proliferated and regenerated- β cells; (5) Glibenclamide treated cells with hyper plastic condition.

Histopathology of the liver in normal control animals showed normal hepatic cells with well-preserved cytoplasm, nucleus, nucleolus, and central vein. In diabetic control animals, the liver sections showed that the lobular architecture was maintained, but there was also a severe fatty change, sinusoidal dilation and congestion, mild portal inflammation, fibrosis, severe feathery degeneration, and necrosis. Diabetic rats

treated with herbal formulation showed hepatocytes with nearly normal appearance and minimal necrosis. The sections of glibenclamide treated animals showed normal hepatic cells and no abnormality.

Conclusion

Therefore, our study conclusion demonstrates the antidiabetic effect of the polyherbal formulation at the dose levels of 250 and 500 mg/kg. The antidiabetic potential of the polyherbal formulation is analogous with that of glibenclamide, which is evidenced by decreased levels of blood glucose, HbA_{1c}, total cholesterol, triglyceride, low density lipoprotein (LDL)-cholesterol, urea, creatinine, SGOT, and SGPT, and increase in plasma insulin, HDL-cholesterol, liver glycogen, and total protein levels.

Acknowledgement

Special thanks to all staff of Sri Sathya Sai Institute of Pharmaceutical Scienses and Vedica College of B. Pharmacy, Bhopal, for their support and technical assistance.

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How to cite this article

Yadav A.P., Kori M.L and Sagar R. (2016). Antidiabetic Activity of Polyherbal Formulation in Streptozotocin Induced Type II Diabetic Rats. *Int. J. Pharm. Life Sci.*, 7(12):5348-5353.

Source of Support: Nil; Conflict of Interest: None declared

Received: 18.11.16; Revised: 29.11.16; Accepted: 18.12.16