Evaluation of Antidiabetic Activity of a Novel Polyherbal Preparationagainst Streptozotocin Induced Diabetes Rat Model

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Diabetes Mellitus is a chronic disorder characterised by abnormally elevated glucose levels in the blood. Diabetes is caused by one of two mechanisms: insufficient insulin synthesis (which is produced by the pancreas and reduces blood glucose) or insufficient response of cells to insulin action. The current aim of this research project was to formulate and evaluate the Polyherbal preparation (PHP) of the plants constituted with Cinnamonum zeylanicium (CJ) bark, *Eugenia jambolana* (EJ) seeds, *Vinca rosea* (VR) whole plant, *Gymnema sylvestre* (GS) leaves and determination of the anti-diabetic potential of the formulation in the animal model induced by Streptozotocin.
Methods: Plant components in the current study used were Cinnamonum zeylanicium (CJ) bark, *Eugenia jambolana* (EJ) seeds, *Vinca rosea* (VR) whole plant, *Gymnema sylvestre* (GS) leaves were collected.Using a hydroalcoholic solvent, physico-chemical parameters and active chemical constituents were evaluated. The active components present in the extracts were identified by Preliminary phytochemical screening. The PHP acute toxicity analysis was conducted in compliance with OECD Guideline 423, with 200 mg/kg and 4000 mg/kg administered orally to rats over 28 days.

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Results: Diabetes was induced by STZ and treated with PHF did not show any alterations in behavior and no mortality was observed up to the 2000 mg/kg dose level during the interventional period. By oral administration of PHP with a dosage of 200 and 400 mg/kg, OGTT resulted in a steady decrease in blood glucose levels of 68.74±4.63 mg/dl and 63.83±1.74 mg/dl at 180min after the trial which proves that PHP possess anti-diabetic activity. By mixing each extract in varying proportions, PHP was developed and evaluated. PHP (200 and 400mg/kg) antidiabetic activity wasdetermined for streptozotocin (STZ)-induced diabetes in rats and glibenclamide (5.0mg/kg body weight) was used as a standard drug.The investigational drug was administered for 28 days and the blood glucose level effect of the PHP was analysed on the 28th day after the intervention time.
Conclusion: The experimental study showed that a persistent and substantial decrease in the average blood glucose level of diabetic rats was observed with repeated administration of PHP and glibenclamide for 28 days. PHP demonstrated substantial antidiabetic and antihyperlipidemic activity similar to the standard drug. The formulation will emerge as a possible mixture that may challenge the synthetic drug.

1. INTRODUCTION

Type – II diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to insulin resistance or insufficient secretion of insulin by βcells of Islet of Langerhans of the pancreas [1-3]. It was estimated that by 2025 there will be more than 300 Million diagnosed with type II Diabetes by World Health Organization [4-5]. The major risk of facing mortality and morbidity rates in DM is due to the complications associated with cardiovascular problems like micro and macro vascular diseases, which was found as one of the top 5 causes of death in the world [6-7]. Therefore apart from drugs therapy preventive and prophylactic measures were needed for DM patients to counteract the development of various complications associated with Heart, nephropathy, retinopathy and neurological issues [8].

Changing the predominantly day to day standards of living of our ancestors to unhealthy eating habits, physical inactivity and other aspects of lifestyle in developing countries play an important role in the growing incidence of Type II diabetes, which accounts for about 90% of all cases at a young age [9-10]. With a current figure of 40.9 million, India is one of the leading countries with the highest population of diabetics, led by China, the USA, Russia, Germany, Japan, Pakistan, Brazil, Mexico and Egypt [11].

The epidemiology of diabetes in India is multifactorial and involves paired genetic factors associated with increased living standards, continuous urban migration and lifestyle changes with environmental influences such as obesity [12]. It is estimated that diabetes mellitus could affect up to 79.4 million individuals in India by 2030 [13-15]. With ever rising incidence and mortality rates, diabetes has become a severe health concern [16]. It is one of the refractory diseases listed by the Indian Council of Medical Research for which alternative therapy is required for the treatment [17-18].

Herbs and phytochemicals play an important role in the development of new therapeutic agents, and sources of antioxidants, hypoglycemic, and antihyperlipidemic agents have gained such importance [19]. There are numerous traditional plants mentioned in Siddha is the Ayurvedic system of medicine used as antidiabetic agents. Sharangdhar Samhita, an Ayurvedic literature of 1300 AD stressed the importance of Poly herbals [20-22]. The therapeutic action of polyherbal preparations (PHP) is strengthened and herbal concentrations are reduced, thereby minimizing adverse events. Compared to a single plant, PHP has greater potential for therapeutic properties [23].

In the Ayurvedic system of medicine, various plants have been claimed for their hypoglycemic effects and still are practice. In the current research work, four plants (mentioned below) hasbeen selected for preparation of polyherbal preparations. The plants and their medicinal parts were: bark of *Cinnamonum zeylanicium,* [24-27] Seeds of *Eugenia jambolana*, [28-32] the whole plant of *Vinca rosea*, [33-35] and leaves of *Gymnema sylvestra* [36-40]. Fig. 1 shows the photographs of plant and plant part used in the current resarch.

Several studies have reported the ant diabetic activity of the above plants in different models of anti-diabetic activity. *Cinnamonum zeylanicium* (CZ) extract lowered blood glucose levels, serum lipid profiles, bodyweight to normal levels in streptozotocin (STZ) and alloxan induced diabetic rats [24]. Dried seeds of *Eugenia jambolana* (EJ) produced a time-dependent decrease in blood glucose level significantly compared to standard drugs like glibenclamide and metformin in STZ induced diabetic rats [29-31]. Administration of ethanolic extract of *Vinca rosea* (VR) decreased the levels of fasting and postprandial blood glucose levels with a concomitant increase in body weight, reduction in lipid profile, and improved hepatic and renal activity in STZ induced diabetics in rats [33]. The dried leaves extract of *Gymnema sylvestre* (GS) showed significant reduction of blood glucose, glycosylated hemoglobin, reduced serum lipid levels and improved insulin activity and glycosylated plasma proteins in diabetic animals [36-38]. Considering the past effectiveness of herbs for diabetics the current research work aims at formulation of polyherbal preparation with the aforementioned herbal parts evaluate the anti- diabetic potential of the PHP mixtures.

2. MATERIALS AND METHODS

2.1 Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (USA). AR grade Ethanol was procured from Merck, Mumbai, India. Glibenclamide (GLB) was kindly gifted by Torrent Pharmaceutical Pvt. Ltd., Ahmedabad, India. All other chemicals used in this study were of analytical grade.

2.2 Plant Material

Candidate plants were collected from their natural habitats in and around the Nallamala forest area near Srisailam, Kurnool Dist. and Seshachalam forest area near Tirupathi, Andhra Pradesh, India. Dr K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, authenticated the plants. Plant parts were dried and defatted with petroleum ether in the shade. Using a soxhlet unit, the defatted material was extracted with 70 percent ethanol and then vacuum dried.

2.3 Preparation of Extracts

The extraction of *Cinnamonum zeylanicium* bark, *Eugenia jambolana* seeds, whole plant of *Vinca rosea* and *Gymnema sylvestre* leaves was carried out by continuous Soxhlet extraction with Ethanol. In a separate mixer, all plant materials (1 kg) were air-dried and coarsely powdered. At a temperature between 60°C and 70°C for 10 hours, 500 g of each crude drug powder was carefully weighed and kept in a soxhlet system and extracted with 70 percent ethanol.The extraction was maintained until the solvent was cleared. Dark brown to black extracts were obtained. These extracts were cooled to remove the residue and filtered. The extracts were concentrated at decreased pressure on a rotary evaporator and then dried to obtain a powder.After calculating their percentage extracts, the extracts were stored in amber glass containers (refrigerated) for further processing. Preliminary Phytochemical tests were performed for the identification of various phytoconstituents in the extracts. The dry powder was diluted with 0.5% carboxy methylcellulose (CMC) in the proportion required for the study. The physical characteristics and % yield of extracts were given in Table 1.

2.4 Animals

From SV animal house, Bangalore, adult healthy Albino Wistar rats of either sex weighing between 180–200 gm of either sex were obtained. For acute toxicity and ant diabetic function, these animals were used. The animals were stabilized for 1 week; they were maintained in polypropylene cages at room temp; 60 ± 5% relative humidity and 12 h light-dark cycle.Throughout the analysis, they were given a normal pellet diet and water ad-libitum. To avoid giving them too much discomfort, the animals were treated gently, which could result in increased adrenal production.

2.5 Development of Polyherbal Preparation

The PHP of CZ, EJ, VR and GS was developed by combining the dried extracts of the plant materials in various ratios. Table 2 shows the composition of PHP.
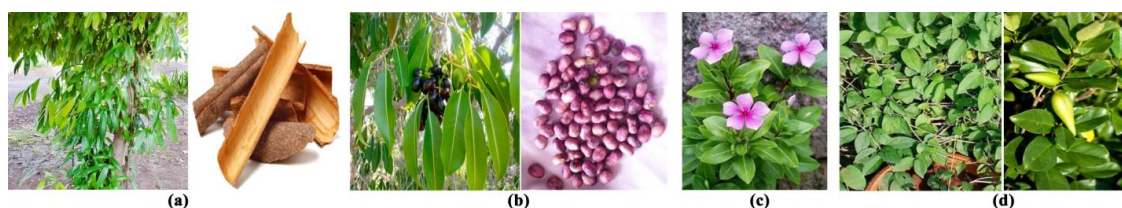
Fig. 1. The plants and their medicinal parts (a) Whole plant and bark of *Cinnamonum zeylanicium*; (b) Whole Plant and seeds of *Eugenia jambolana*; (c)Whole plant of *Vinca rosea*; (d) Whole Plant and leaves of *Gymnema sylvestra*

Table 1. Physical characteristics and % yield of extracts

Plant	% Yield (w/w)	Moisture content (%w/w)	Ash Value (%w/w)			Foaming index	Swelling index (ml)
			Total	Acid insoluble	Water insoluble		
CZ Bark	12.50	2.38±0.13	2.77±0.21	0.13±0.04	0.11±0.02	<100	3.73±0.34
*EJ*seeds	10.78	2.18±0.90	3.52±1.01	0.25±0.12	0.24±0.10	<100	2.77±0.73
VR Whole plant	11.34	2.11±0.89	4.38±1.33	0.67±0.03	0.31±0.22	<100	4.88±0.34
GS Leaves	10.86	1.83±0.22	3.71±0.44	0.30±0.14	0.21±0.08	<100	3.90±0.89

All values are expressed in Mean(n)± SD, n=3

Table 2. Composition of polyherbal preparation

S. No	Code	Formulation	Ratio
1	PHP 1	CZ : EJ : VR : GS	2 : 2 : 2 : 1
2	PHP 2	CZ : EJ : VR : GS	2 : 2 : 1 : 2
3	PHP 3	CZ : EJ : VR : GS	2 : 1 : 2 : 2
4	PHP 4	CZ : EJ : VR : GS	1 : 2 : 2 : 2

2.6 Acute Toxicity Study

The acute toxicity study was carried out in adult female albino rats by "fixed" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. The fixed-dose method: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. Animals fasted overnight and polyherbal preparation (suspended in 0.5% w/v sodium CMC) was administered by orally at a dose level of 2000 mg/kg the following day. Then, for general behavioral, neurological, autonomic profiles, the animals were continuously monitored for three hours and then every 30 minutes for the next three hours and eventually for mortality after 24 hours to 14 days [41].

2.7 Selection and Preparation of Doses

For the evaluation of antidiabetic activity, the level of two doses was selected in such a way that, during the acute toxicity trials, one dose was approximately one tenth of the maximum dose,2000 mg/kg and a high dose was twice the

dose of one tenth (200mg/kg& 400mg/kg) [42]. The dose of 200 and 400 mg/kg of polyherbal preparation was made by dissolving an appropriate quantity of extracts in normal saline.

2.8 Grouping of Animals

The animals were divided into 11 groups of 6 animals in each category (n=6). Regular control, diabetic control, Glibenclamide treated group and polyherbal preparations (1-4) were named as treated groups at doses of 200 mg/kg and 400 mg/kg. The treatment was continuously given for 28 days. The detailed description and function of the animal groupings is given in Table 3.

2.9 Determination of Oral Glucose Tolerance Test (OGTT) Activity

The chief source of energy in our body is glucose, and the oral glucose tolerance test (OGTT) assesses the body's capacity to use glucose efficiently. The study used albino rats of either sex weighing 130-150 g. The rats were fasted overnight and allowed unrestricted access

to water. During the tests, the animals were grouped into four classes, each of six animals. The blood samples were taken by tail vein pricking method. An oro-gastric tube was used to administer the formulation.

Group I : Negative control treated with normal saline
Group II : Positive control – treated with Glibenclamide (5mg/kg)
Group III : Test – Treated with Polyherbal preparation (200 mg/kg of CZ : EJ : VR : GS 2 : 2 : 2 : 2)
Group IV : Test – Treated with Polyherbal preparation (400 mg/kg of CZ : EJ : VR : GS 2 : 2 : 2 : 2)

All of the animals were given 3 g/kg body weight of glucose diluted in sterile water 30 minutes before administrating normal saline, glibenclamide, or the test drug in their respective groups. At 30, 60, 120, and 180 minutes after glucose administration, blood glucose levels were assessed using a glucometer (glucose oxidase method) [42].

2.10 Induction of Diabetes

A single intraperitoneal injection of freshly prepared streptozotocin (STZ) (60 mg/kg dissolved in normal saline) was then injected into the animals for 24 hours. After that, the animals were left for 4 hours and then, for 24 hours, a 10 percent glucose solution was kept in the cages. By estimating the amount of blood glucose (BGL) on the 3rd day, diabetes was confirmed.

2.11 Determination of Antidiabetic Activity

Test samples were given orally using oral gastric gavages to the animals once before food daily was given. The blood glucose concentrations of the animals were measured using a glucometer (glucose oxidase method)at the beginning of the study and the measurements were repeated on $7^{th}14^{th}$ $21^{st}$and 28th day after the initial of the experiment.

2.12 Determination of Biochemical Parameters

Blood from the orbital plexus was collected. For different biochemical parameters such as triglycerides, cholesterol, VLDL, LDL and HDL levels calculated using lipid testing kits (Span diagnostic Ltd, Surat, India) respectively, serum was isolated and estimated.All the biochemical parameters were measured at the beginning of the study and the measurements were repeated on 7th,14th,21st and 28^{th}day of the experimentation.

2.13 Histopathology

The pancreas was isolated and conserved in 10 percent formalin. Histopathological experiments were performed on the stained paraffin portion of 5-micron thick Hematoxylin and Eosin (H&E) in albino rats at X400 [43-46].

2.14 Statistical Analysis

The data was expressed as ±SEM mean. Antidiabetic behaviour, OGTT and other data were analysed using a one-way variance analysis (ANOVA) followed by a Dunnett test.A P value <0.05 was considered statistically significant [47-48].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Acute oral toxicity of polyherbal preparation in rats

In female rats, acute toxicity studies showed no mortality at a dose of 2000 mg/kg over 14 days Table 4. For a period of 3 hours of toxicity testing, the behavioural, neurological, autonomic responses were examined. No notable responses were seen in the rats during the study. This helps to predict that it contains no toxicity of any sort and is safe.

3.1.2 Effect of PHP on glucose-loaded rats (OGTT model)

Oral glucose tolerance test result shows a dose dependant blood glucose reducing nature of PHP on glucose loaded non-diabetic rats Table 5. More significant reduction was shown at dose of 400 mg/kg.The positive group,and PHP-treated groups at 200 and 400 mg/kg body weight showed an increase in serum glucose level at 95.50±2.00, 98.83±3.75 and 93.70±1.56 respectively after 30 min of glucose administration.Gradually the blood glucose level significantlyreduced for GLB, PHP groups at 200 and 400 mg/kg body weight respectivelystarting 120 min after glucose administration ($P<$).The study found that both 200 mg/kg and 400 mg/kg of PHP possess significant hypoglycemic activity in normal rats. The reduction of blood glucose level at a dose of 400 mg/kg was similar tothe

standard anti-diabetic drug, 5 mg/kg of gliben-clamide. Therefore, the dose of PHP400 mg/kg was selected for further studies in the STZ-induced diabetic rat model.However, all groups of animals nearly normalized serum glucose levels within five hours, indicating that the animals' pancreas was healthy to remove the glucose load from the body.

3.1.3 Effect of PHP on serum glucose level in diabetic rats

Diabetic control rats showed a steady and gradual increase in glucose levels during the studycompared with the negative group ($P<$ 0.05). Rats treated with GLB (5 mg / kg body weight),PHP – A, PHP – B, PHP – C, PHP – D, PHP – E, PHP – F, PHP – G, PHP – H showed asignificant decrease of glucose level starting from 7th day after exposure to drug ($P<$ 0.05) Table 6. The effect was found to be time dependent up to day 28 of the study. The decrease in glucose level was more significant (P <0.05) on day 28 than with the standard drug.

There was also a significant reduction in body weight in diabetic animals particularly, animals treated with 400 mg of PHP (PHP-G) and GLB showed significant control of body weight loss on days 21 and 28 compared to start day of the study(P <0.001). This effect can be attributed to the increase in insulin secretion and food consumption. These results imply that the PHP – G (PHP-3 composition: CZ: EJ : VR : GS 2 : 1 : 2 : 2) developed may reduce body weight complications and associated cardiovascular risk factors during diabetes.

3.1.4 Effect of the PHP on the lipid profile

The serum lipid profile (and in the diabetic control group revealed an significant increase in serum triglycerides (TG), total cholesterol (TC), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL)while a decrease of high-density lipoprotein (HDLC)compared to the normal controlrats ($P<$ 0.01 Table 7. However, serum TG, TC, and LDLC levels in the GLB and PHF-treated groups were significantly reduced) while HDL was increased compared with those of the diabetic rat groups ($P<0.001$).

3.1.5 Histopathological studies

In the pancreatic islets of Langerhans, normal acini and normal cell population were seen in the photomicrograph of vehicle-treated normal rats Fig. 2(a). Extensive necrotic changes accompanied by fibrosis and atrophy with shrinkage of the islets of Langerhans have been seen in the islets of STZ diabetic rats Fig. 2(b). The pancreas of GLB treated rats shows a much improved pancreatic islet of Langerhans Fig. 2(c). The PHP-A to PHP-D treated rats showeda limited to moderate degree of necrotic and fibrotic changes and atrophy of the Langerhans islets. (Illustration 2(d to g). The fibrotic and necrotic changes observed were mild for the PHP-E to PHP-H group of animals treated Fig. 2 (h to k). However, in the pancreatic islets of PHP-G, the necrotic changes were found to be minimal Fig. 2 (j).

4. DISCUSSION

The pancreas is the chief organ involved in detecting the body's energy and dietary states through the concentration of glucose in the blood and, in response to high blood glucose, insulin will be secreted. However, Streptozotocin is currently the most commonly used agent in laboratory animals to induce insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus. STZ causes rat islets of Langerhans beta cells to distinctly degenerate.STZ causes the pancreas to swell within three days, which subsequently causes the islet of Langerhans beta cells to degenerate and trigger experimental diabetes. Compared to normal rats, it also alters normal metabolism in diabetic rats.Compared to normal rats, food and water intake, urine volume, improved serum glucose in diabetic animals but reduced serum insulin, C-peptide and bodyweight levels. Increased muscle wasting in diabetes induces the normal loss of body weight.

Herbs play an important role in the treatment than allopathic drugs, because of fewer side effects, ease and simple accessibility. The examination of the work carried out on this premise and the chosen plants for the study has been demonstrated for the therapeutic use of antidiabetic purpose. The Polyherbal Preparation (PHP) seems to be safe up to 2 g/Kg because at this high dose also no harmful or deleterious effects were seen immediately or up to 3 days of the observation period.

In the present research work, the PHP at 400 g/kg and PHP-Gat 400mg/kg,similar toGLB, showed significant anti-diabetic effect both in normal as well as glucose loaded normal fasted rats respectively. Currently, the work is in progress to establish the molecular

Table 3. Animal groupings and the treatment given

Groups	Treatment	Purpose
Normal control	Vehicle (normal saline)	Serves to study normal parameters of rat
Diabetic control	STZ 60mg/kg, i.p.	Serve as diabetic control
Glibenclamide (5mg/kg, po)	STZ 60mg/kg, i.p + Glibenclamide at 5mg/kg, p.o.	To study the effect of glibenclamide at 5mg/kg in disease condition
PHP – A	STZ 60mg/kg, i.p + PHP – 1 200mg/kg, p.o.	To study the effect of polyherbal preparation - 1 at 200 mg/kg in diabetic condition
PHP – B	STZ 60mg/kg, i.p + PHP – 2 200mg/kg, p.o.	To study the effect of polyherbal preparation - 2 at 200 mg/kg in diabetic condition
PHP – C	STZ 60mg/kg, i.p + PHP – 3 200mg/kg, p.o.	To study the effect of polyherbal preparation - 3 at 200 mg/kg in diabetic condition
PHP – D	STZ 60mg/kg, i.p + PHP – 4 200mg/kg, p.o.	To study the effect of polyherbal preparation - 4 at 200 mg/kg in diabetic condition
PHP – E	STZ 60mg/kg, i.p + PHP – 1 400mg/kg, p.o.	To study the effect of polyherbal preparation - 1 at 400 mg/kg in diabetic condition
PHP – F	STZ 60mg/kg, i.p + PHP – 2 400mg/kg, p.o.	To study the effect of polyherbal preparation - 2 at 400 mg/kg in diabetic condition
PHP – G	STZ 60mg/kg, i.p + PHP – 3 400mg/kg, p.o.	To study the effect of polyherbal preparation - 3 at 400 mg/kg in diabetic condition
PHP – H	STZ 60mg/kg, i.p + PHP – 4 400mg/kg, p.o.	To study the effect of polyherbal preparation - 4 at 400 mg/kg in diabetic condition

Table 4. Results of acute oral toxicity studies

Treatment	Body weight (gm)	Dose (mg/kg)	Mortality (Animal dead)			Toxicity profile
	Rat (N=6)		After 24 hrs	After 7 days	After 14 days	
PHP 1	140 ± 10.46	2000	0	0	0	Safe
PHP 2	132 ± 9.67	2000	0	0	0	Safe
PHP 3	142 ± 9.39	2000	0	0	0	Safe
PHP 4	133 ± 10.26	2000	0	0	0	Safe

Table 5. Results of oral glucose tolerance test

Group	Blood Glucose Level (mg/dl)*				
	0 min	30 min	60 min	120 min	180 min
Negative control	89.00±1.46	88.00±1.60	87.30±2.18	90.70±1.92	89.50±1.38
Positive control (5mg/kg of Glibenclamide)	89.33±2.61	95.50±2.00[a]	48.33±1.14[a]	55.67±0.71[a]	60.17±1.13[a]
Polyherbal preparation (200 mg/kg)	90.64±4.73	98.83±3.75b	88.24±7.34[b]	76.55±4.84[b]	70.74±4.63[b]
Polyherbal preparation (400 mg/kg)	90.47±1.83	93.70±1.56b	81.50±2.48[b]	70.50±2.10[b]	62.83±1.74[b]

All data presented in Mean±SD (n=6) a- P <0.05; b-P<0.01 as compared to negative control animals (ANNOVA followed by Dunnet's test)

Table 6. Anti diabetic activity of PHP on STZ induced diabetes in rats

Group	Treatment	Blood Glucose (mg/dl) levels on*					% Reduced
		0 day	7th day	14th day	21st day	28th day	
Normal control	Vehicle (normal saline)	94.2±1.35	95.99±1.34	93.48±0.35	94.87±0.37	96.67±0.36	2.62⇧
Diabetic control	STZ 60mg/kg, i.p.	285.98±0.23[a]	310.67±2.62[a]	308.45±1.32[a]	314.73±5.94[a]	319.43±3.25[a]	2.62⇧
GLB	STZ 60mg/kg, i.p + Glibenclamide at 5mg/kg, p.o.	287.84±2.93[b]	219.98±4.34[b]	178.34±3.88[b]	142.36±3.92[b]	115.02±2.90[b]	60.04⇩
PHP – A	STZ 60mg/kg, i.p + PHP – 1 200mg/kg	292.88±2.01[b]	255.83±3.97[b]	228.73±4.82[b]	203.28±0.38[b]	184.82±2.54[b]	36.90⇩
PHP – B	STZ 60mg/kg, i.p + PHP – 2 200mg/kg	288.98±4.92[b]	261.83±3.92[b]	232.87±3.83[b]	200.67±9.73[b]	189.37±3.92[b]	34.47⇩
PHP – C	STZ 60mg/kg, i.p + PHP – 3 200mg/kg	290.74±3.85[b]	258.38±3.98[b]	234.73±4.82[b]	195.83±3.52[b]	178.78±3.83[b]	38.51⇩
PHP – D	STZ 60mg/kg, i.p + PHP – 4 200mg/kg	287.48±4.83[b]	260.37±4.32[b]	229.83±5.85[b]	205.83±7.45[b]	188.23±0.34[b]	34.52⇩
PHP – E	STZ 60mg/kg, i.p + PHP – 1 400mg/kg	290.43±3.24[b]	235.44±4.86[b]	194.67±4.28[b]	163.67±3.72[b]	142.42±2.55[b]	50.96⇩
PHP – F	STZ 60mg/kg, i.p + PHP – 2 400mg/kg	289.66±4.69[b]	228.84±4.63[b]	184.88±3.54[b]	153.75±3.56[b]	133.25±4.24[b]	54.00⇩
PHP – G	STZ 60mg/kg, i.p + PHP – 3 400mg/kg	291.78±4.82[b]	214.45±3.54[b]	180.34±7.34[b]	143.62±3.55[b]	125.73±3.21[b]	58.62⇩
PHP – H	STZ 60mg/kg, i.p + PHP – 4 400mg/kg	288.74±3.43[b]	224.74±4.56[b]	182.47±4.89[b]	150.45±5.33[b]	130.42±3.65[b]	55.52⇩

All data presented in Mean±SD (n=6); a-P <0.05 as compared to negative control; b- P <0.05 as compared to Diabetic control animals. ⇧-% increased in blood glucose level on 28th day compared to 0th day; ⇩-%reduced in blood glucose levels on 28th day compared to 0th day

Table 7. Effect of PHP on lipid profile

Group	Lipid Profile (mg/dl)*				
	TC	TG	VLDL	LDL	HDL
Normal control	80.46±3.78	53.82±5.54	15.33±0.45	45.38±5.68	38.77±4.80
Diabetic control	210.63±8.78[a]	137.98±3.90 [a]	64.89±1.89 [a]	110.44±0.24 [a]	8.33±0.89 [a]
GLB	110.73±1.09[b]	88.63±2.67 [b]	22.87±4.89 [b]	51.22±4.89 [b]	29.83±2.43 [b]
PHP – A	134.84±3.76 [b]	81.77±3.89 [b]	31.28±4.82 [b]	63.83±2.90 [b]	18.36±7.93 [b]
PHP – B	129.73±2.43 [b]	75.98±3.98 [b]	25.66±1.09 [b]	50.67±7.89 [b]	24.37±4.58 [b]
PHP – C	120.43±3.45 [b]	70.53±3.86 [b]	20.88±3.49 [b]	49.33±2.78 [b]	30.56±2.01 [b]
PHP – D	125.73±3.45 [b]	73.67±4.98 [b]	22.73±2.65 [b]	52.63±3.78 [b]	32.67±5.98 [b]
PHP – E	118.64±2.78 [b]	70.67±3.89 [b]	28.56±3.23 [b]	61.33±5.42 [b]	20.33±6.32 [b]
PHP – F	115.78±2.89 [b]	68.66±6.90 [b]	22.73±5.93 [b]	58.83±4.34 [b]	29.66±1.94 [b]
PHP – G	101.73±6.89 [b]	63.76±5.89 [b]	16.63±8.33 [b]	46.87±5.98 [b]	34.76±2.54 [b]
PHP – H	113.73±2.89 [b]	66.86±3.01 [b]	18.67±6.98 [b]	55.34±4.76 [b]	31.57±7.34 [b]

All data presented in Mean±SD (n=6); Values are expressed as Mean±SEM (n=6); a- $p<0.01$compared to normal, b - $p<0.001$ compared to diabetic control (one-way ANOVA followed by a Dunnett's t test)
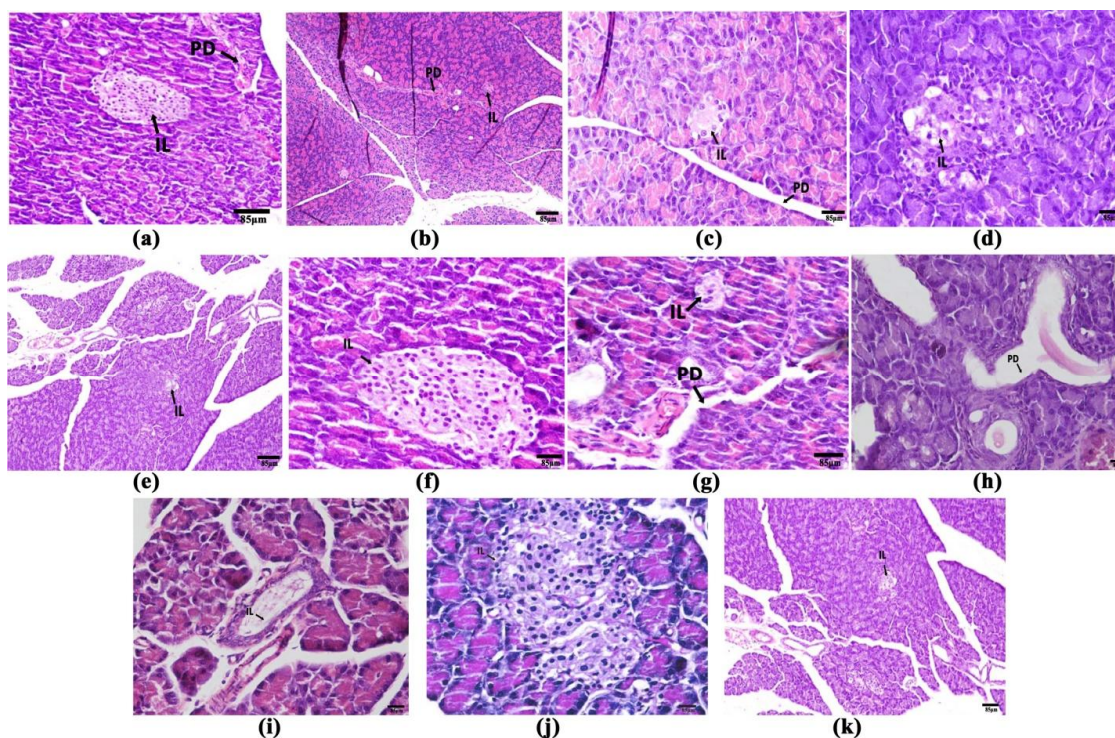
Fig. 2. (a-k): Photomicrographs of a 5 micron thick H&E stained paraffin sections of Albino rats of (a) the Normal control; (b) Diabetic control (STZ); (c) GLB; (d) PHP-A; (e) PHP-B; (f) PHP-C; (g) PHP-D; (h) PHP-E; (i) PHP-F; (j) PHP-G; (k) PHP-H; showing islets of langerhans (IL), pancreatic duct(PD) and lymphocytes infiltration (L)

mechanism behind the antidiabetic effect of PHP-G, whether it has bought about these changes by acting through a pancreatic mechanism similar to GLB or by inhibition of glucose absorption through gastrointestinal tract like other herbs. PHP-G showed a significant reduction of blood glucose levels in a month, which is similar to that of GLB. Both drugs bought about the remarkable reduction in blood glucose.

Increased lipid peroxidation seen in the diabetic condition is attributed to increased oxidative stress in the cells as a result of depletion of antioxidant systems. A significant reducation in triglycerides, cholesterol, VLDL and LDL and prominent rise in HDL demonstrates the antihyperlipidemic activity of PHP. This shows that the PHP has a potential of reversing all the abnormalities either by pancreatic or hepatic mechanisms.

Histopathological findings of the pancreas of the diabetic rats showed necrosis, atrophy, fibrotic changes and shrinkage of Islet of Langerhans. But the pancreas of rats treated with PHP-G and GLB showed improved and minimal necrosis and mild atrophy, fibrotic changes and almost normal islets of Langerhans.

5. CONCLUSION

Polyherbal preparation containing ethanolic extracts of *Cinnamonum zeylanicium* bark, *Eugenia jambolana* seeds, *Vinca rosea* whole plant and *Gymnema sylvestre* leaves were formulated for the treatment of diabetes mellitus. In the view of results, it is concluded that the PHP-G showed a prominent antidiabetic and antihyperlipidemic activity compared to the rest of the preparations. However, the studies are still in progress to establish the molecular mechanism of action and also long term toxicity studies of PHP-G.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and

producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the study SJCP/PCOL/AD2019-10/002 was approved by the institutional animal ethics committee and experiments were conducted according to guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) bearing the registration number: 1519/PO/Re/S/11/CPCSEA.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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