Evaluation of the Antidiabetic and antioxidant potential of a Polyherbal Extract in streptozotocin-Induced Diabetes Rats

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Abstract:

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels. This research project aimed to investigate the anti-diabetic potential of a Polyherbal Preparation (PHP) composed of dried leaves of *Tinospora cordifolia*, dried bark of *Cinnamomum zylanicum*, dried seeds of *Trigonella foenum*, and dried seeds of *Nigella sativa* in streptozotocin-induced diabetes in Albino Wistar rats. The individual plants were extracted using n-hexane, petroleum ether, and ethanol, and prepared 15 different PHPs in varying proportions. The PHPs were then evaluated for their in vitro antioxidant activity, and the ethanol extract exhibited superior scavenging activity against DPPH and hydrogen peroxide radicals compared to the other extracts. Further investigations were conducted to assess the antidiabetic potential of the PHPs. In non-diabetic rats, the PHPs exhibited a dose-dependent glucose-reducing effect, with the most significant reduction observed at a dose of 400 mg/kg body weight. Glucose levels gradually and significantly decreased in the PHP-treated groups, reaching near-normal levels by the 28th day. The glucose-lowering effect at the 400 mg/kg dose was comparable to that of the standard antidiabetic drug, glibenclamide. In diabetic rats, treatment with the PHPs led to a significant reduction in glucose levels compared to the diabetic control group. Notably, the ethanol extract of PHP 1 (EPHP 1) demonstrated potent anti-diabetic activity, similar to that of glibenclamide. These findings provide scientific evidence supporting the anti-diabetic potential of the PHPs. EPHP 1 emerged as a promising candidate for further development as a natural alternative for diabetes management.
Keywords: Diabetes mellitus, *Tinospora cordifolia, Cinnamomum zylanicum, Trigonella foenum, Nigella sativa*, glibenclamide.

Introduction:

Diabetes mellitus is a severe disorder that causes a serious, complex, and chronic condition all over the world[1]. Diabetes mellitus is a significant public health concern worldwide, with its prevalence steadily increasing over the past few decades. According to the International Diabetes Federation (IDF), as of 2021, an estimated 463 million adults (age 20-79) were living with diabetes globally. This number is projected to rise to 700 million by 2045 if current trends continue[2]. Diabetes mellitus, characterized by hyperglycaemia, is linked to changes in glucose, protein, and lipid metabolism[3]. Diabetes mellitus patients have higher oxidative stress and reduced antioxidant defence systems, which appear to contribute to the onset and progression of diabetes-related problems[4]. The increased glucose levels cause direct cell damage and lipid peroxidation[5].

The use of drugs that selectively damage pancreatic cells to induce experimental diabetes in rats is very straightforward and simple. Alloxan and streptozotocin (STZ) are the most used drugs to produce diabetes in rats[6]. Understanding the alterations in pancreatic -cells as well as the entire organism following alloxan or streptozotocin treatment is critical for employing these drugs as diabetogenic agents[7,8].Diet, exercise, and insulin replacement treatment are the primary methods of treating diabetes. Hypoglycaemic medications such as insulin, biguanides, sulfonylureas, and glucosidase inhibitors have unpleasant side effects such as acute hypoglycaemia, lactic acidosis, peripheral edema, and gastrointestinal discomfort[9]. Synthetic hypoglycaemic medications have severe side effects, but bioactive molecules obtained from natural resources are thought to be both safe and cost effective.

Herbs and phytochemicals play a significant role in the development of new therapeutic agents. Phytochemicals are naturally occurring compounds found in plants, and they have been used for centuries in traditional medicine to treat various ailments. Over time, scientific research has identified and isolated specific phytochemicals from herbs, leading to the

development of new therapeutic agents. Several plants have long been advised for the treatment of diabetes, and the role of these plants in diabetes management has been determined by numerous research[10]. More than 400 plant species with hypoglycaemic activity have been identified in the literature; yet, the prospect of discovering new antidiabetic, antihyperlipidemic, and antioxidant medications from natural plants remains appealing[11]. The Ayurvedic medical system, and Siddha, incorporates various traditional plants for the treatment of diabetes. The Sharangdhar Samhita, an Ayurvedic text dating back to 1300 AD, highlights the importance of polyherbal, which are a combination of multiple herbs, in this context[12–14]. By utilizing polyherbal preparations (PHP), the concentrations of individual herbs can be reduced, while simultaneously enhancing the overall medicinal effect. This approach minimizes the risk of undesirable side effects. The utilization of PHP holds greater promise for therapeutic benefits compared to using a single plant alone.

The Ayurvedic system of medicine has long recognized the potential hypoglycaemic effects of various plants, which continue to be practiced to this day. In the present research, a selection of four plants has been made to create polyherbal preparations. These plants, along with their specific medicinal parts, include thedried leaves of *Tinospora cordifolia,* dried bark of *Cinnamomum zylanicum*, dried seeds of *Trigonella foenum and d*ried seeds of *Nigella sativa*. In the rural villages of Rayalaseema districts in Andhra Pradesh, India, a polyherbal extract derived from the aforementioned plant parts has been traditionally used as a remedy for diabetes mellitus, despite lacking scientific validation. To address this gap in knowledge and ascertain the antidiabetic potential of the polyherbal preparation, a comprehensive study was conducted with the aim of providing scientific evidence supporting its efficacy in diabetes management.

Numerous studies have documented the anti-diabetic properties of the aforementioned plants using different anti-diabetic models. *Tinospora cordifolia* (TC) extract, for instance, has demonstrated its ability to normalize blood glucose levels, serum lipid profiles, and body weight in diabetic rats induced by streptozotocin (STZ) and alloxan[15–18]. Dried bark of *Cinnamomum zylanicum* (CZ) exhibited a time-dependent reduction in blood glucose levels, surpassing the effects of standard drugs like glibenclamide and metformin in STZ-induced diabetic rats[19–21]. The administration of an ethanolic extract of dried seeds of *Trigonella foenum* (TF) lowered fasting and postprandial blood glucose levels, while concurrently improving body weight, reducing lipid profiles, and enhancing hepatic and renal function in STZ-induced diabetic rats[22,23]. Additionally, the *d*ried seeds of *Nigella sativa* (NS) significantly reduced blood glucose levels, glycosylated hemoglobin, serum lipid levels, and improved insulin activity and glycosylated plasma proteins in diabetic animals[24,25]. All the four plants mentioned has been proved to be a potent anti diabetic agent individually. A comprehensive literature survey was undertaken to explore the existing scientific literature pertaining to the efficacy of the polyherbal preparation consisting of the mentioned plants in managing diabetes mellitus. However, the survey revealed a notable absence of scientific literature addressing the specific anti-diabetic potential of this particular mixture of plants as a polyherbal preparation. Based on the established efficacy of these herbs in diabetic treatment, the present research aims to formulate a polyherbal preparation using the mentioned herbal parts and evaluate the anti-diabetic potential of the resulting PHP mixtures.

Materials and Methods

Chemicals:

Streptozotocin (STZ) was purchased from Merck, Mumbai, Inda. AR grade ethanol, n-hexane and petroleum ether was purchased from SD fine Chem, Mumbai, India. Glibeclamide (GLB) was kindly gifted by Dr. Reddy's laboratories, Hyderabad, India. All other chemicals used in this study were of analytical grade.

Plant material:

Dried bark of *Cinnamomum zylanicum*, dried seeds of *Trigonella foenum and* dried seeds of *Nigella sativa* were purchased from local market of Kurnool, Andhra Pradesh, India. The dried leaves of *Tinospora cordifolia* was collected from its natural habitat in and around the Nallamala Forest area near Srisailam, Kurnool Dist. Andhra Pradesh, India. Dr. K. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India authenticated the plants. Plant parts were dried in shades.

Preparation of extracts:

The dried leaves of TC, dried bark of CZ, dried seeds of TF, and dried seeds of NS were subjected to continuous soxhlet extraction using n-hexane, petroleum ether, and ethanol as solvents. To begin, all plant materials (2.5 kg) were air-dried and coarsely powdered separately. Subsequently, 1 kg of each crude drug powder was carefully weighed and placed in a soxhlet system, where it was extracted with the aforementioned solvents at a temperature range of 70-70 °C for 48 hours. The extraction process was carried out until the solvent became clear, resulting in the production of dark brown to black extracts. After cooling and removing any residue, the extracts were filtered. To obtain a powder form, the extracts were concentrated under reduced pressure using a rotary evaporator and then dried. The percentage of extracts was calculated, and the extracts were stored in amber glass containers under refrigeration for further processing. Preliminary phytochemical tests were conducted to identify various phytoconstituents present in the extracts. For the study, the dry powder was diluted with 0.5% carboxymethyl cellulose (CMC) in the required proportion. Table 1 provides information on the physical characteristics and percentage yield of the extracts.

Table 1: Physical characteristics and percentage yield of the extracts.

Plant extracts	% yield (w/w)	Moisture content (% w/w)	Ash value (% w/w)			Foaming index	Swelling index (mL)
			Total	Acid insoluble	Water insoluble		
n-hexane extract							
TC	6.84	1.48±0.04	2.87±0.93	0.10±0.01	0.12±0.01	<100	2.63±0.03
CZ	4.23	2.56±0.16	3.74±0.65	0.10±0.02	0.10±0.02	<100	4.82±0.02
TF	8.90	2.55±0.11	4.15±1.02	0.12±0.04	0.14±0.04	<100	3.91±0.05
NS	7.99	1.49±0.13	4.98±0.75	0.20±0.01	0.09±0.02	<100	2.99±0.04
Petroleum ether extract							
TC	8.45	1.53±0.23	2.93±0.58	0.23±0.01	0.13±0.01	<100	2.77±0.59
CZ	6.33	2.33±0.22	4.27±1.13	0.37±0.02	0.08±0.05	<100	4.12±0.84
TF	5.39	2.49±0.12	3.65±0.87	0.45±0.02	0.17±0.02	<100	3.25±0.91
NS	8.64	0.98±0.01	3.22±0.72	0.63±0.03	0.15±0.04	<100	2.64±1.02
Ethanol extract							
TC	10.55	1.81±0.04	2.79±0.95	0.13±0.03	0.41±0.05	<100	2.95±0.42

CZ	11.48	1.25±0.02	2.56±0.87	0.07±0.05	0.53±0.01	<100	4.51±1.03
TF	9.56	1.98±0.07	4.23±0.41	0.17±0.02	0.60±0.04	<100	3.78±0.92
NS	12.56	2.36±1.02	3.63±0.66	0.16±0.04	0.84±0.02	<100	2.56±0.76

TC: The dried leaves of *Tinospora cordifolia*, CZ*:* dried bark of *Cinnamomum zylanicum,* TF*:* dried seeds of *Trigonella foenum,* and NS: dried seeds of *Nigella sativa*.All the values are expressed in mean (n) ± Standard error mean (SEM), n=3

Animals:

Albino Wistar rats of both sexes, weighing between 160-200 grams, were obtained from SV Animal House and Enterprises located in Bangalore, India. These rats were utilized for both acute toxicity studies and anti-diabetic activity evaluations. After acquisition, the animals underwent a stabilization period of 10 days. They were housed in polypropylene cages under specific conditions, including room temperature, 60±5% relative humidity, and a 12-hour light-dark cycle. Throughout the experiment, the rats were provided with a normal pellet diet and had access to water ad libitum. Care was taken to handle the animals gently, minimizing any potential discomfort that could otherwise result in increased adrenal production.

Development of Polyherbal preparation

The polyherbal preparation (PHP) of the dried leaves of TC, dried bark of CZ, dried seeds of TF, and dried seeds of NS was developed by combining the dried extracts of the plant materials in various ratios. Table 2 shows the composition of PHPs.

Table 2: Composition of polyherbal preparations

Extracts	PHP Code	PHP formulation	Ratio
n-hexane extract	HPHP 1	TC: CZ: TF: NS	1: 1: 1: 1
	HPHP 2	TC: CZ: TF: NS	2: 2: 2: 1
	HPHP 3	TC: CZ: TF: NS	2: 2: 1: 2
	HPHP 4	TC: CZ: TF: NS	2: 1: 2: 2
	HPHP 5	TC: CZ: TF: NS	1: 2: 2: 2
Petroleum ether extract	PPHP 1	TC: CZ: TF: NS	1: 1: 1: 1
	PPHP 2	TC: CZ: TF: NS	2: 2: 2: 1
	PPHP 3	TC: CZ: TF: NS	2: 2: 1: 2
	PPHP 4	TC: CZ: TF: NS	2: 1: 2: 2
	PPHP 5	TC: CZ: TF: NS	1: 2: 2: 2
Ethanol extract	EPHP 1	TC: CZ: TF: NS	1: 1: 1: 1
	EPHP 2	TC: CZ: TF: NS	2: 2: 2: 1
	EPHP 3	TC: CZ: TF: NS	2: 2: 1: 2
	EPHP 4	TC: CZ: TF: NS	2: 1: 2: 2
	EPHP 5	TC: CZ: TF: NS	1: 2: 2: 2

In vitro Antioxidant assay

The in vitro antioxidant activity of the polyherbal preparations (PHP) was assessed using the DPPH free radical scavenging assay and hydrogen peroxide scavenging activity. The different extracts were dissolved in ethanol at concentrations ranging from 50 to 200 mg/mL. All assays were performed in triplicate, and average values were considered.

DPPH Scavenging activity:

To evaluate the free radical scavenging activity, the DPPH radical scavenging assay was employed, following the method described by Blois[26] and Desmarchelier *et al*.[27] The hydrogen atom donating ability of the plant extracts was determined by observing the decolorization of a methanol solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces a violet/purple color in methanol solution, which fades to shades of yellow in the presence of antioxidants.For the assay, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of the extract in methanol at various concentrations ranging from 12.5 to 150 µg/mL. The reaction mixture was thoroughly vortexed and left in the dark for 30 minutes. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Butylated hydroxytoluene was used as a reference compound. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\%\, DPPH\ Radical\ Scavening\ activity = \frac{(Absorbance\ of\ the\ control - Absorbance\ of\ the\ PHPs)}{Absorbance\ of\ the\ control} X100$$

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration.

Hydrogen peroxide scavenging activity

The in vitro antioxidant activity assay using hydrogen peroxide (H2O2) scavenging activity is a widely used method to determine the ability of a compound or sample to neutralize hydrogen peroxide, which is a reactive oxygen species (ROS) and a major contributor to oxidative stress[28]. Hydrogen peroxide generates free radical (OH^-) by which acts as an oxidising agent. 40 mM solution of hydrogen peroxide was prepared in phosphate buffer of pH 7.4. Various concentrations of 20, 40, 60, 80, 120µg/ml of extracts were prepared and hydrogen peroxide solution was added (0.6 ml) absorbance was measured at 230 nm after 10 min. Ascorbic acid used as a control and plain phosphate buffer with hydrogen peroxide as blank.

$$\%\, of\ Hydrogen\ peroxide\ Scavening\ activity = \frac{(Absorbance\ of\ the\ control - Absorbance\ of\ the\ PHPs)}{Absorbance\ of\ the\ control} X100$$

By measuring the ability of a compound or sample to scavenge the DPPH free radical and hydrogen peroxide, these assays provide valuable information about its antioxidant capacity and PHPs potential to protect against oxidative stress-related damage. It helps in evaluating the effectiveness of PHPs as potential antioxidants in assessing antidiabetic activity.

Acute toxicity study:

An acute toxicity study was conducted following the fixed method outlined in OECD[29] guidelines no. 420. The study utilized adult female Albino rats. The fixed dose method was employed, starting with a dose of 2000 mg/kg body weight. Prior to dosing, the animals underwent an overnight fast. The polyherbal preparation, suspended in a 0.5% w/v sodium CMC solution, was administered orally at the dose level of 2000 mg/kg on the following day. To assess general behavioral, neurological, and autonomic profiles, the animals were continuously monitored for the first 3 hours post-dosing, and subsequently, every 30 minutes

for the next three days. Mortality was recorded from 24 hours up to 14 days following administration of the test substance.

Selection and preparation of doses:

To assess the antidiabetic activity, two doses were chosen based on the acute toxicity trials. One dose was approximately one-tenth of the maximum dose of 2000 mg/kg and another dose was twice of it, resulting in doses of 200 mg/kg and 400 mg/kg body weight. The 200 mg/kg and 400 mg/kg doses of the polyherbal preparation (PHP) were prepared by dissolving the appropriate number of extracts in a 0.5% sodium CMC solution.

Grouping of animals:

The animals were divided into 3 batches 6 animals in each category (n=6). Regular control, diabetic control, Glibenclamide treated groups and polyherbal preparation treated groups (1-5) at doses of 200 mg/Kg and 400 mg/Kg. The treatment was continuously given for 28 days.

Determination of oral glucose tolerance test (OGTT) activity:

Glucose serves as the primary source of energy in our bodies, and the oral glucose tolerance test (OGTT) is a method to evaluate the body's ability to utilize glucose effectively. In this study, albino rats weighing between 150-180 g and of either sex were employed. The rats underwent an overnight fasting period while having access to water. During the experiment, the animals were divided into 8 groups, each containing six individuals. Blood samples were collected using a tail vein pricking method. The formulation was administered to the rats using an oro-gastric tube.

Group I: Negative control treated with 0.5% Sodium CMC

Group II: Positive control treated with standard drug, Glibenclamide (5 mg/Kg)

Group III: Test – treated with HPHP (200 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Group IV: Test – treated with PPHP (200 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Group V: Test – treated with EPHP (200 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Group VI: Test – treated with HPHP (400 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Group VII: Test – treated with PPHP (400 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Group VIII: Test – treated with EPHP (400 mg/Kg of TC: CZ: TF: NS 1: 1: 1: 1)

Prior to administering 0.5% sodium CMC, glibenclamide, or the test drugs in their respective groups, all the animals received a glucose solution of 3g/kg body weight, which was diluted in sterile water. The glucose solution was given to the animals 30 minutes before the administration of the substances. Blood glucose levels were assessed at 30, 60, 120, and 180 minutes after the glucose administration using a glucometer with the glucose oxidase method.

Induction of Diabetes:

To induce diabetes, a single intraperitoneal injection of freshly prepared Streptozotocin (STZ) was administered to the animals at a dosage of 60 mg/kg. The STZ was dissolved in normal saline before injection. Following the STZ injection, the animals were observed for a 24-hour period. After this initial period, a 10% glucose solution was provided in the cages for 24 hours. On the third day, the amount of blood glucose (BGL) was estimated to confirm the

presence of diabetes. This measurement served as an indicator to determine if the STZ injection successfully induced diabetes in the animals.

Determination of antidiabetic activity:

The test samples were administered orally to the animals using oral gastric gavages once daily, prior to their daily food intake. Blood glucose concentrations of the animals were measured using a glucometer at the beginning of the study. These measurements were then repeated on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days following the start of the experiment. This allowed for the evaluation of the potential effects of the test samples on blood glucose levels over a period of 28 days.

Statistical analysis

The data obtained from the study were expressed as mean ± Standard error mean (SEM). The analysis of antidiabetic behaviour, oral glucose tolerance test (OGTT), and other relevant data was performed using a one-way analysis of variance (ANOVA) followed by Dunnet's Multiple Comparison Test. A significance level of $P < 0.05$ was used to determine statistical significance. Any results with a P value below this threshold were considered statistically significant, indicating a notable difference or effect.

Results and discussion:

Invitro antioxidant activity

The in vitro antioxidant activity of various PHPs was assessed using two methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and hydrogen peroxide scavenging activity. Comparing the different PHPs, it was observed that the ethanol extract exhibited the highest antioxidant potential, surpassing the n-hexane and pet ether extracts. Detailed results for the in vitro antioxidant activity of PHPs using the DPPH and hydrogen peroxide scavenging assays are presented in Tables 3 and 4, respectively.

Table 3: DPPH scavenging activity of PHPs

Conc. (µg/ml)	**Polyherbal preparations**			**Ascorbic acid**
	n-hexane extract (HPHP 1)	**Pet. Ether extracts (P**PHP 1)	**Ethanol extracts (**EPHP 1)	
20	04.7±0.27	27.2±0.59	33.1 ± 0.69	30.5 ± 0.15
40	13.8± 0.39	34.8±0.37	42.3 ± 0.68	53.9 ± 0.92
60	26.4±0.68	47.9±0.52	56.4 ± 0.56	62.8 ± 0.38
80	39.5± 0.17	58.2±0.18	65.7 ± 0.47	71.2 ± 0.72
100	53.6±0.49	62.7±0.69	70.6 ± 0.98	82.3 ± 0.69
120	56.2±0.52	65.8±0.35	76.8 ± 0.69	90.9 ± 0.45
IC_{50}	90.5	73.6	50.3	28.3

Table 4 Hydrogen Peroxide scavenging activity of PHPs

Conc. (µg/ml)	**Polyherbal preparations**			**Ascorbic acid**
	n-hexane extract (HPHP 1)	**Pet. Ether extracts (P**PHP 1)	**Ethanol extracts (**EPHP 1)	
20	12.52±0.49	21.81±0.39	26.79 ± 0.36	18.21 ± 0.32
40	25.38±0.25	29.32±0.57	31.39 ± 0.93	35.6 ± 0.10
60	38.56±1.09	47.25±1.07	53.29 ± 0.46	43.82 ± 1.26
80	46.39±0.47	53.12±0.96	60.38 ± 0.91	63.13 ± 0.51
100	51.71±0.71	55.63±0.27	68.21 ± 0.52	82.3 ± 0.69
120	53.83±1.09	58.49±0.57	71.32 ± 0.59	88.9 ± 0.25
IC_{50}	93.4	72.8	53.8	32.3

Acute oral toxicity of polyherbal preparations

In acute toxicity studies conducted on female rats, it was observed that there were no instances of mortality over a 14-day period at a dose of 2000 mg/kg. During the 3-hour toxicity testing period, the behavioral, neurological, and autonomic responses of the rats were examined, and no significant abnormalities were observed. These findings provide evidence to suggest that the polyherbal preparations (PHPs) used in the study do not exhibit any form of toxicity and can be considered safe for use.

Effect of PHPs on glucose loaded rats (OGTT)

The results of the oral glucose tolerance test demonstrated that the polyherbal preparation (PHP) exhibited a dose-dependent glucose-reducing effect on non-diabetic rats. A more pronounced reduction in glucose levels was observed at a dose of 400 mg/kg body weight. In the Positive group and the PHP-treated groups at 200 mg and 400 mg/kg body weight, there was an initial increase in serum glucose levels, 30 minutes after the administration of glucose. However, over time, the blood glucose levels gradually and significantly decreased to normal levels in the PHP-treated groups at 200 mg and 400 mg/kg body weight.Table 5 shows the results of OGTT.

Table 5: Results of Oral glucose tolerance test

Group	**Treatment**	**Blood glucose level (mg/dL)**				
		0 min	**30 mins**	**60 min**	**120 min**	**180 min**
I.	0.5% Sodium CMC	76.65±1.77	98.11±2.23	101.87±1.57	97.99±1.92	95.08±0.88
II.	Glibenclamide (5 mg/Kg)	77.32±1.89	95.43±2.13	98.11±1.72	87.89±1.57	76.62±2.02
III.	HPHP – 200 mg/Kg	70.62±2.02	95.76±2.01	98.24±2.37	91.58±1.98	86.98±2.03
IV.	PPHP - 200 mg/Kg	69.91±1.68	95.77±1.97	98.63±2.11	92.08±1.77	87.12±2.29
V.	EPHP - 200 mg/Kg	76.63 ± 1.92	95.98 ± 1.48	89.11 ± 2.45	88.05 ± 1.65	81.39 ± 1.92

VI.	HPHP – 400 mg/Kg	70.10 ± 2.14	97.86 ± 1.72	92.33 ± 1.88	85.75 ± 2.21	77.92 ± 1.56
VII.	PPHP - 400 mg/Kg	78.34 ± 3.01	95.62 ± 2.16	89.01 ± 3.79	79.93 ± 3.33	75.78 ± 4.36
VIII.	EPHP - 400 mg/Kg	69.18 ± 1.06	95.92 ± 3.45	85.01 ± 0.87	76.73 ± 2.27	70.59 ± 1.63

Effect of PHP on serum glucose level in diabetic rats

During the study, diabetic control rats exhibited a gradual and steady increase in glucose levels compared to the negative control group, with statistical significance ($P<0.05$). However, when rats were treated with GLB (5mg/kg) and PHPs, there was a significant reduction in glucose levels starting from the 7^{th} day after drug exposure ($P<0.05$). This effect continued until day 28^{th} day of the study, and on day 28^{th} day, the decrease in glucose levels was even more significant ($P<0.05$) with the PHPs compared to the standard drug. Table 6, 7 and 8 shows the anti-diabetic activity of n-hexane extracts (HPHP), petroleum ether extracts (PPHP), and ethanol extracts (EPHP) at 200 and 400 mg/Kg body weight.

Table 6: Anti diabetic activity of n-hexane extracts of PHP on STZ induced diabetes in rats

Group	Treatment	Blood glucose levels (mg/dL) on				
		0 day	**7th day**	**14th day**	**21st day**	**28th day**
Normal control	Vehicle (0.5 % Na CMC)	76.68±2.03	78.36±2.24	82.45±2.02	80.11±1.86	84.77±2.33
Diabetic control	STZ 60 mg/Kg i.p	68.29±3.23[a]	285.16±3.82[a]	292.11±4.12[a]	304.98±4.85[a]	313.73±4.36[a]
Standard	STZ 60 mg/Kg i.p+ GLB 5 mg/Kg	73.28±3.65[b]	287.79±3.94[b]	202.15±3.54[c]	166.62±4.17[b]	121.36±4.21[c]
HPHP 1	STZ 60 mg/Kg i.p+ HPHP 1 200 mg/Kg	61.29±3.23[b]	276.16±3.82[b]	204.11±4.12[c]	181.98±4.85[b]	179.73±4.36[c]
HPHP 2	STZ 60 mg/Kg i.p+ HPHP 2 200 mg/Kg	76.74±4.46[b]	281.28±3.89[c]	215.42±4.12[b]	199.57±4.67[c]	184.73±4.23[b]
HPHP 3	STZ 60 mg/Kg i.p+ HPHP 3 200 mg/Kg	66.74±4.46[b]	292.28±3.86[b]	217.82±4.19[b]	202.36±4.56[c]	187.90±4.27[b]
HPHP 4	STZ 60 mg/Kg i.p+ HPHP 4 200 mg/Kg	71.28±3.68[b]	285.52±3.26[b]	199.85±3.97[b]	193.92±4.17[b]	189.47±4.92[b]
HPHP 5	STZ 60 mg/Kg i.p+ HPHP 5 200 mg/Kg	69.65±3.34[b]	277.92±3.78[b]	202.11±3.59[b]	197.55±3.12[b]	187.68±2.85[b]
HPHP 1	STZ 60 mg/Kg i.p+ HPHP 1 400 mg/Kg	71.12±3.14[b]	282.88±2.79[b]	214.62±2.93[b]	185.28±3.52[b]	162.72±3.99[b]
HPHP 2	STZ 60 mg/Kg i.p+ HPHP 2 400 mg/Kg	77.23±6.29[b]	272.65±4.96[c]	215.74±5.83[c]	198.81±5.51[b]	160.65±6.79[b]
HPHP 3	STZ 60 mg/Kg i.p+ HPHP 3 400 mg/Kg	76.24±3.91[b]	275.59±3.84[c]	228.41±3.57[c]	202.93±4.12[b]	163.08±4.44[c]
HPHP 4	STZ 60 mg/Kg i.p+ HPHP 4 400 mg/Kg	74.59±4.25[c]	282.78±3.64[b]	193.86±4.07[b]	182.42±3.21[c]	160.81±3.89[c]
HPHP 5	STZ 60 mg/Kg i.p+ HPHP 5 400 mg/Kg	81.59±8.11[c]	279.22±6.24[c]	197.81±5.97[c]	178.43±5.42[b]	166.89±5.04[b]

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. a-$p<0.05$ as compared with control group, b-$p<0.01$ and c-$p<0.05$ as compared with STZ group.

Table 7: Anti diabetic activity of Pet ether extracts of PHP on STZ induced diabetes in rats

Group	Treatment	Blood glucose levels (mg/dL) on				
		0 day	**7th day**	**14th day**	**21st day**	**28th day**
Normal control	Vehicle (0.5 % Na CMC)	76.68 ± 2.03	78.36 ± 2.24	82.45 ± 2.02	80.11 ± 1.86	84.77 ± 2.33
Diabetic control	STZ 60 mg/Kg i.p	68.29 ± 3.23[a]	285.16 ± 3.82 [a]	292.11 ± 4.12 [a]	304.98 ± 4.85 [a]	313.73 ± 4.36 [a]
Standard	STZ 60 mg/Kg i.p+ GLB 5 mg/Kg	73.28 ± 3.65 [b]	287.79 ± 3.94[c]	202.15 ± 3.54 [b]	166.62 ± 4.17[c]	121.36 ± 4.21 [b]
PPHP 1	STZ 60 mg/Kg i.p+ PPHP 1 200 mg/Kg	74.84 ± 5.38[c]	284.59 ± 3.97 [b]	219.12 ± 3.73[c]	205.92 ± 4.22[c]	180.45 ± 3.88[c]
PPHP 2	STZ 60 mg/Kg i.p+ PPHP 2 200 mg/Kg	81.83 ± 4.96 [b]	282.04 ± 4.21[c]	207.49 ± 3.38 [b]	198.10 ± 2.89[c]	183.72 ± 3.11[c]
PPHP 3	STZ 60 mg/Kg i.p+ PPHP 3 200 mg/Kg	78.07 ± 6.18 [c]	277.62 ± 5.91 [b]	198.73 ± 5.09[c]	188.14 ± 3.72 [b]	182.36 ± 3.33 [b]
PPHP 4	STZ 60 mg/Kg i.p+ PPHP 4 200 mg/Kg	79.18 ± 4.54[c]	278.46 ± 5.17 [b]	207.89 ± 4.08 [c]	191.32 ± 5.09 [b]	184.87 ± 3.98[c]
PPHP 5	STZ 60 mg/Kg i.p+ PPHP 5 200 mg/Kg	80.12 ± 5.21 [b]	286.88 ± 4.36 [b]	203.45 ± 3.79[c]	195.72 ± 5.08 [b]	185.16 ± 3.92 [b]
PPHP 1	STZ 60 mg/Kg i.p+ PPHP 1 400 mg/Kg	80.36 ± 5.24[c]	272.94 ± 4.92 [b]	206.78 ± 4.61 [b]	185.63 ± 4.07 [b]	167.25 ± 4.32[c]
PPHP 2	STZ 60 mg/Kg i.p+ PPHP 2 400 mg/Kg	81.73 ± 3.82 [b]	277.59 ± 5.04[c]	219.14 ± 4.31 [b]	186.95 ± 3.67 [c]	164.29 ± 3.93 [b]
PPHP 3	STZ 60 mg/Kg i.p+ PPHP 3 400 mg/Kg	88.43 ± 6.77 [b]	285.98 ± 5.91 [b]	212.31 ± 5.16 [b]	184.92 ± 5.84 [b]	166.20 ± 3.92[c]
PPHP 4	STZ 60 mg/Kg i.p+ PPHP 4 400 mg/Kg	81.37 ± 3.82 [b]	284.09 ± 4.26 [b]	204.62 ± 3.58 [b]	189.74 ± 3.42 [c]	161.85 ± 2.91 [b]
PPHP 5	STZ 60 mg/Kg i.p+ PPHP 5 400 mg/Kg	82.56 ± 6.11 [b]	288.28 ± 5.03 [b]	214.02 ± 6.55[c]	185.46 ± 4.80[c]	170.81 ± 3.99[c]

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. a-$p<0.05$ as compared with control group, b-$p<0.01$ and c-$p<0.05$ as compared with STZ group.

Table 8: Anti diabetic activity of Ethanol extracts of PHP on STZ induced diabetes in rats

Group	Treatment	Blood glucose levels (mg/dL) on				
		0 day	**7th day**	**14th day**	**21st day**	**28th day**
Normal control	Vehicle (0.5 % Na CMC)	76.68 ± 2.03	78.36 ± 2.24	82.45 ± 2.02	80.11 ± 1.86	84.77 ± 2.33
Diabetic control	STZ 60 mg/Kg i.p	68.29 ± 3.23 [a]	285.16 ± 3.82 [a]	292.11 ± 4.12 [a]	304.98 ± 4.85 [a]	313.73 ± 4.36 [a]
Standard	STZ 60 mg/Kg i.p+ GLB 5 mg/Kg	73.28 ± 3.65 [b]	287.79 ± 3.94 [b]	202.15 ± 3.54 [b]	166.62 ± 4.17 [c]	121.36 ± 4.21 [c]
EPHP 1	STZ 60 mg/Kg i.p+ EPHP 1 200 mg/Kg	70.71 ± 5.32 [c]	277.84 ± 4.59 [b]	213.99 ± 6.13 [c]	179.67 ± 3.80 [c]	167.28 ± 4.01 [c]
EPHP 2	STZ 60 mg/Kg i.p+ EPHP 2 200 mg/Kg	74.38 ± 7.91 [b]	282.29 ± 5.22 [b]	195.74 ± 3.82 [c]	179.61 ± 4.76 [b]	166.12 ± 4.28 [b]
EPHP 3	STZ 60 mg/Kg i.p+ EPHP 3 200 mg/Kg	79.65 ± 7.72 [c]	275.41 ± 5.86 [c]	215.03 ± 5.04 [b]	176.21 ± 5.14 [b]	163.95 ± 5.62 [c]
EPHP 4	STZ 60 mg/Kg i.p+ EPHP 4 200 mg/Kg	68.76 ± 3.19 [b]	286.55 ± 3.45 [c]	212.89 ± 3.58 [b]	174.42 ± 4.02 [b]	162.14 ± 3.97 [c]
EPHP 5	STZ 60 mg/Kg i.p+ EPHP 5 200 mg/Kg	78.78 ± 4.31 [c]	283.62 ± 3.66 [c]	223.40 ± 2.87 [b]	200.94 ± 4.18 [b]	165.15 ± 3.99 [b]
EPHP 1	STZ 60 mg/Kg i.p+ EPHP 1 400 mg/Kg	66.94 ± 6.11 [c]	278.78 ± 4.95 [c]	205.63 ± 4.62 [c]	157.12 ± 4.25 [b]	126.42 ± 4.05 [b]
EPHP 2	STZ 60 mg/Kg i.p+ EPHP 2 400 mg/Kg	67.79 ± 5.17 [c]	283.12 ± 4.32 [b]	201.65 ± 4.66 [c]	164.89 ± 3.87 [c]	133.57 ± 4.08 [b]
EPHP 3	STZ 60 mg/Kg i.p+ EPHP 3 400 mg/Kg	65.16 ± 7.49 [b]	280.02 ± 6.54 [b]	215.28 ± 5.89 [c]	170.11 ± 5.82 [c]	136.49 ± 5.16 [c]
EPHP 4	STZ 60 mg/Kg i.p+ EPHP 4 400 mg/Kg	61.78 ± 6.15 [c]	281.02 ± 4.93 [b]	201.46 ± 4.32 [c]	188.93 ± 4.14 [c]	143.21 ± 3.56 [c]
EPHP 5	STZ 60 mg/Kg i.p+ EPHP 5 400 mg/Kg	66.17 ± 6.28 [b]	282.41 ± 4.78 [c]	206.94 ± 5.02 [b]	185.03 ± 5.41 [b]	136.82 ± 4.68 [b]

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. a-$p<0.05$ as compared with control group, b-$p<0.01$ and c-$p<0.05$ as compared with STZ group.

Discussion:

Diabetes mellitus, a chronic metabolic disorder, has become a global health concern affecting millions of individuals worldwide[30]. Characterized by elevated blood glucose levels, diabetes imposes a significant burden on individuals, healthcare systems, and economies. While conventional pharmaceutical interventions play a crucial role in diabetes management, there is an increasing interest in exploring alternative approaches, particularly the utilization of medicinal plants and herbal remedies[31].Poly herbals play a significant role in the treatment of diabetes mellitus. Their multi-targeted approach, antioxidant and anti-inflammatory properties, potential to enhance insulin secretion, and relatively low side effects make them valuable additions to the diabetes management arsenal[32]. Through further research, standardization, and evidence-based practices, herbals can continue to contribute to the comprehensive and personalized care of individuals living with diabetes[29].

In this current study, an extensive literature survey was conducted, revealing a lack of information regarding the assessment of the anti-diabetic potential of a mixture comprising dried leaves of *Tinospora cordifolia*, dried bark of *Cinnamomum zylanicum*, dried seeds of *Trigonella foenum*, and dried seeds of *Nigella sativa*. To address this gap, individual plants were extracted using n-hexane, petroleum ether, and ethanol solvents. Subsequently, a polyherbal preparation was formulated by combining different compositions, as specified in Table 2, resulting in the creation of 15 distinct PHPs.

The initial evaluation of the PHPs focused on their in vitro antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay and the hydrogen peroxide scavenging activity assay. Since oxidative stress plays a significant role in diabetes mellitus, plants with the potential to reduce oxidative stress could have positive effects in its treatment and management. The IC50 values, representing the concentration required for 50% scavenging activity, were determined for each extract and ascorbic acid as a reference. The IC50 values for DPPH scavenging activity were found to be 90.5%, 73.6%, 50.3%, and 28.3% for n-hexane, petroleum ether, ethanol, and ascorbic acid, respectively. Similarly, for hydrogen peroxide scavenging activity, the IC50 values were 93.4%, 72.8%, 53.8%, and 32.3% for n-hexane, petroleum ether, ethanol, respectively. These results indicate that the ethanol extract exhibited superior scavenging activity compared to the petroleum ether and n-hexane extracts. The findings from this study provide valuable insights into the antioxidant potential of the PHPs derived from the aforementioned plants. The observed superior scavenging activity of the ethanol extract suggests its potential in mitigating oxidative stress, which is closely associated with diabetes mellitus. Following the initial evaluation of the PHPs for their antioxidant activity, further research and exploration were carried out to assess their antidiabetic potential. The objective was to investigate whether these PHPs could exhibit beneficial effects in managing diabetes mellitus.

The acute oral toxicity study conducted on female rats aimed to evaluate the safety profile of the polyherbal preparations (PHPs). The study involved administering a high dose of 2000 mg/kg of the PHPs to the rats and monitoring them for a 14-day period. One of the key findings from the study was that there were no instances of mortality observed during the 14-day observation period. This suggests that the PHPs at the tested dose did not cause any acute lethal effects in the rats. This absence of mortality is an important indicator of the safety of the PHPs at the tested dose.

The oral glucose tolerance test was conducted to evaluate the effect of the polyherbal preparation (PHP) on glucose metabolism in non-diabetic rats. The results demonstrated a dose-dependent reduction in glucose levels upon PHP administration. The most significant reduction in glucose levels was observed at a dose of 400 mg/kg body weight.

In both the Positive group and the PHP-treated groups at 200 mg and 400 mg/kg body weight, there was an initial increase in serum glucose levels 30 minutes after the administration of glucose. However, as time progressed, the blood glucose levels gradually and significantly decreased to normal levels in the PHP-treated groups at 200 mg and 400 mg/kg body weight.Starting from 120 minutes after the glucose administration, it was observed that PHP at doses of 200 mg and 400 mg/kg body weight, exhibited significant hypoglycemic activity in normal rats. The reduction in blood glucose levels at the dose of 400 mg was comparable to that of the standard antidiabetic drug, glibenclamide, at a dose of 5 mg/kg body weight. This suggests that the PHP at the higher dose exhibited a potent glucose-lowering effect, similar to that of the established antidiabetic drug.

It is noteworthy that in all groups of animals, including the PHP-treated groups, the serum glucose levels nearly normalized within 5 hours. This observation indicates that the pancreas of the animals was functioning properly to remove the glucose load from the body, suggesting a healthy pancreatic function. The PHP treatment did not interfere with the normal physiological response of the pancreas to regulate blood glucose levels.Overall, the results of the oral glucose tolerance test provide evidence of the PHP's dose-dependent glucose-reducing effect in non-diabetic rats. The significant hypoglycemic activity observed at the higher dose, comparable to that of the standard antidiabetic drug, supports the potential of the PHP as an effective agent in managing diabetes mellitus. Further studies using a diabetic rat model would shed light on the PHP's efficacy in diabetic conditions and its potential as an alternative or adjunctive therapy for diabetes management.

Throughout the study, the diabetic control group exhibited a gradual and steady increase in glucose levels compared to the negative control group, indicating the presence of hyperglycemia. This difference in glucose levels between the two groups was statistically significant ($P<0.05$), confirming the development of diabetes in the experimental animals.However, when the rats were treated with the standard antidiabetic drug, glibenclamide (GLB) at a dose of 5 mg/kg, and the polyherbal preparations (PHPs), a significant reduction in glucose levels was observed starting from the 7^{th} day after drug exposure ($P<0.05$). This effect continued until the 28^{th} day of the study, and on the 28^{th} day, the decrease in glucose levels was even more significant ($P<0.05$).In addition to the glucose-lowering effect, the diabetic animals in the study experienced a notable reduction in body weight. However, the animals treated with 400 mg of PHPs and GLB demonstrated significant control over body weight loss on day 21 and day 28, respectively, when compared to the start day of the study. This observation suggests that the PHPs and the standard drug could prevent or attenuate the weight loss commonly associated with diabetes.The positive effect of the PHPs and GLB on body weight could be attributed to increased insulin secretion and improved food consumption, indicating improved metabolic regulation. Maintaining

body weight is important in diabetes management, as excessive weight loss can lead to further complications.

The blood glucose levels approached normal levels by the 28th day of the drug treatment. In particular, the blood glucose level of animals treated with glibenclamide at the end of the study was 121.36±4.21 mg/dL. Among the polyherbal preparations (PHPs), HPHP 2 demonstrated a significant reduction in blood glucose levels, reaching 160.65±6.79 mg/dL, compared to other HPHPs as indicated in Table 6. Similarly, PPHP4 exhibited a significant reduction in blood glucose levels, with a value of 161.85±2.91 mg/dL, surpassing other PPHPs as shown in Table 7. Notably, EPHP 1 effectively reduced the blood glucose level to 126.42±4.05 mg/dL, which is superior to all other PHPs tested (Table 8). Therefore, it can be concluded that the ethanol extract PHP 1 (EPHP 1) possesses potent anti-diabetic properties comparable to the effect of the standard drug, glibenclamide, outperforming the other extracts (n-hexane and pet ether). These findings highlight the antidiabetic potential of EPHP 1 in effectively lowering blood glucose levels in diabetic animals. The observed reduction in glucose levels indicates improved glycemic control and suggests that the EPHP 1 have a similar or even superior effect compared to the standard antidiabetic drug.

Conclusion:

In conclusion, the findings from the various discussions highlight the promising potential of the polyherbal preparations (PHPs) in the treatment of diabetes mellitus. The PHPs exhibited significant antioxidant activity, as demonstrated by their ability to scavenge free radicals in vitro. This indicates their potential in reducing oxidative stress, which is known to play a role in the development and progression of diabetes. Of all the PHPs tested, the ethanol extract of PHP 1 (EPHP 1) emerged as a particularly potent antidiabetic preparation. It showed a significant reduction in blood glucose levels comparable to that of the standard drug, glibenclamide. This suggests that EPHP 1 has the potential to be developed as a natural alternative for diabetes management. Overall, the results from the discussions provide scientific evidence supporting the anti-diabetic potential of the PHPs. The antioxidant activity, safety profile, and glucose-lowering effects observed in non-diabetic and diabetic rat models suggest that these polyherbal preparations hold promise for the treatment and management of diabetes mellitus. However, further research, including clinical trials, is necessary to validate their efficacy, determine optimal dosages, and assess long-term safety and effectiveness in human subjects.

Ethical approval

The institutional animal ethics committee authorised the study procedure SJCP/PCOL/AD2022-10/011. The ethics committee and the experiments were carried out in accordance with the recommendations of the committee for the purpose of control and supervision of animal experiments (CPCSEA) with the registration number: 1519/PO/Re/S/11/CPCSEA.

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Conflict of Interest

Authors herewith disclose that there is no conflict of interest.

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