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PREPARATION AND EVALUATION OF POLYHERBAL FORMULATION FOR THE TREATMENT OF DIABETES IN RATS

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ABSTRACT

Diabetes Mellitus (DM) is a chronic disorder characterized by hyperglycemia, with its worldwide prevalence predicted to rise significantly. This study aimed to formulate and evaluate a polyherbal preparation (PHP) containing extracts of four medicinal plants for anti-diabetic activity and to prepare a Fast-Disintegrating Tablet (FDT) dosage form. Hydroalcoholic extracts were prepared from the bark of Cinnamomum zeylanicum (CZ), seeds of Eugenia jambolana (EJ), the whole plant of Vinca rosea (VR), and leaves of Gymnema sylvestre (GS). The extracts were subjected to pharmacognostic, physicochemical, and preliminary phytochemical screening, which confirmed the presence of various constituents like glycosides and alkaloids in all four extracts. Five polyherbal preparations (PHP 1 to PHP 5) were formulated with varying ratios. Acute toxicity studies demonstrated that all PHP formulations were safe at a dose of 2000 mg/kg. Pharmacological evaluation in Streptozotocin (STZ)-induced diabetic Wistar rats showed that PHP-H (PHP-3 at 400 mg/kg) was the most effective in reducing blood glucose levels over 28 days. The optimized PHP-H was then used to formulate Fast Dissolving Tablets (FDTs) using direct compression, with F-8 emerging as the best FDT formulation based on pre-formulation (good flow properties) and tablet evaluation (disintegration time of 291±3 sec and friability of 0.11±0.01%). Stability studies of F-8 showed the formulation remained stable for six months under accelerated conditions.

KEY WORDS: *Diabetes Mellitus (DM), Polyherbal formulation (PHP), Cinnamomum zeylanicum, Eugenia jambolana, Vinca rosea, Gymnema sylvestre, Streptozotocin (STZ), Fast Disintegrating Tablets (FDT).*

1. INTRODUCTION

Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycemia, polyurea, polydipsia, polyphagia, emaciation [1] and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and / or insulin action [2]. DM is a condition in which the sugar level is above the normal sugar level 80-120 mg/dl of the whole blood [3].

In India, the prevalence rate of diabetes is estimated to be 1 - 5 % [4]. The worldwide figurers of people with diabetes are set to raise from 150 million in the year 2000 to 300 million in 2025 [5]. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes [6]. Hyperglycemia and metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessel [7]. The prevalence of DM increases with age in both sexes and is consistently higher in men than in women of 20 - 49 year of age [8].

1.1. AN ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS

Although all forms of DM share hyperglycemia a common feature, the pathogenic processes involved in the development of hyperglycemia vary widely [9]. The previous classification schemes of DM were based on the age of onset or on the mode of therapy; in contrast, the recently revised classification reflects our greater understanding of the pathogenesis of each variant [10].

I. Type 1 diabetes (β-cell destruction, usually leading to insulin deficiency) [11]

A. Immune-mediated
B. Idiopathic

II. Type 2 diabetes (may range from insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance) [12]

1.2 DRUG TREATMENT OF DIABETES

A brief overview of drugs commonly used in clinic to treat or control DM is the following:

- Insulin: There are many kinds of preparations. Figure 1.5 shows the insulin synthesis and secretion.

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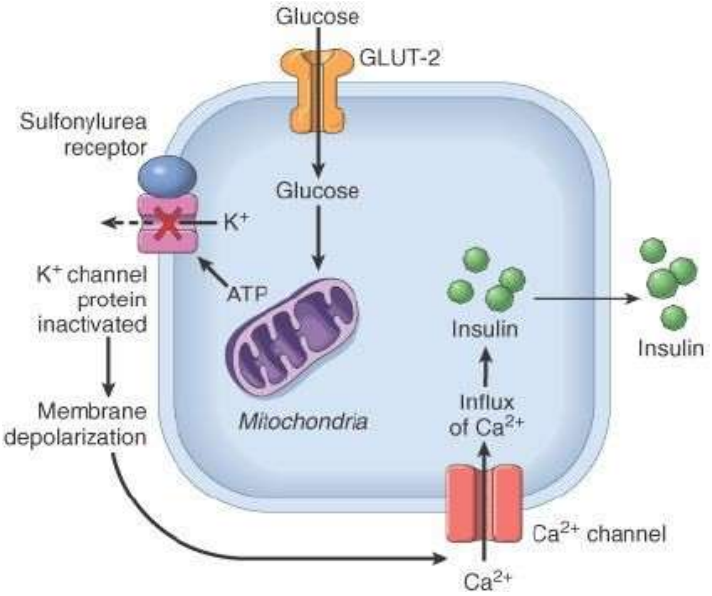


Figure 1: Shows Insulin synthesis and secretion

Intracellular transport of glucose is mediated by GLUT-2, an insulin-independent glucose transporter in β cells. Glucose undergoes oxidative metabolism in the β cell to yield ATP. ATP inhibits an inward-rectifying potassium channel receptor on the β-cell surface; the receptor itself is a dimeric complex of the sulfonylurea receptor and a K+-channel protein. Inhibition of this receptor leads to membrane depolarisation, influx of Ca^{2+} ions, and release of stored insulin from β cells [13].

1.3 FAST DISINTEGRATING TABLETS (FDT)

There are many different drug delivery systems in the market and many surer to be developed. Among all the dosage forms, solid dosage forms (especially - Tablets) are more convenient and among them conventional Tablets like chewable Tablets, Lozenges, dispersible Tablets, effervescent Tablets, seems to be most popular because of greater advantages over others.

Most of medicaments are prescribed to geriatrics and pediatric patients only, as they fell ill very frequently when compared to adults. It was found that these patients have certain difficulty in swallowing Tablets. (Slowson.M and Slowson.M, 1985) [14].

2. MATERIALS USED

The list of chemicals and reagents used is shown in tables, which include the list of assay kits and equipment used in the research. Borosilicate glassware was used.

Table 1: List of chemicals/Reagents

S. No	Chemicals/Regents	Grade	Purchased from
1.	Water	-	Spectrochem
2.	Distilled water	-	in-house
3.	Sulphuric acid	AR	Merck India Ltd, Mumbai
4.	Potassium dihydrogen orthophosphate	AR	SD Fine chem, Mumbai
5.	Glibenclamide	API	Dr. Reddy's labs, Hyd.
6.	Streptozotocin	AR	Sigma-Aldrich, USA
7.	Ferric chloride	AR	SD Fine chem, Mumbai
8.	Ortho-phosphoric acid	AR	SD Fine chem, Mumbai
9.	Sodium hydroxide	AR	Loba chemi, Mumbai
10.	Ethanol	AR	Merck India Ltd, Mumbai
11.	Methanol	AR	Merck India Ltd, Mumbai
12.	Petroleum ether	AR	Merck India Ltd, Mumbai
13.	Iodine	-	SD Fine chem, Mumbai
14.	Chloroform	AR	Merck India Ltd, Mumbai
15.	Diethyl ether	AR	Merck India Ltd, Mumbai
16.	Cyclohexane	AR	Merck India Ltd, Mumbai
17.	Ninhydrin solution	SR	Merck India Ltd, Mumbai
18.	Isopropyl alcohol	SR	Merck India Ltd, Mumbai
19.	Acetone	SR	Merck India Ltd, Mumbai

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20.	Xylol	--	Merck India Ltd, Mumbai
21.	Hematoxylin	--	Merck India Ltd, Mumbai
22.	Eosin	--	Merck India Ltd, Mumbai

Table 2: List of assay kits

S. No	Name of the equipment	Manufacturer
1.	Blood glucose kit (GOD-POD Method)	
2.	Serum glutamate oxaloacetic transaminase (SGOT)	Oscare Medicare, India.
3.	Serum glutamate pyruvate transaminase (SGPT)	
4.	Alkaline phosphate	
5.	Bilirubin	
6.	Superoxide dismutase	
7.	Catalase	
8.	Glutathione peroxidase	

Table 3: List of Equipment

S. No	Name of the equipment	Manufacturer
1.	UV/Visible spectrophotometer (Double beam)	Shimadzu UV 1800, Japan
2.	FTIR	Shimadzu FTIR – 8400S
6.	Electronic Weighing Balance	LC-GC India
7.	pH Meter	Elico, Model LI 612
8.	Centrifuge	Remi Motors
9.	Ultrasonic bath	Enertech
10.	Thermostatic oven	Thermolab
11.	Distillation unit	Borosil glass unit
12.	Micropipettes	Genie
13.	Vacuum pump	Millipore
14.	Refrigerator	Samsung
15.	Vortexer	Orion
16.	Nitrogen evaporator	Zymark turbovap, Caliper

2.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The bark of *Cinnamomum zeylanicum,* the seeds of *Eugenia jambolana,* the whole plant of *Vinca rosea*, and the leaves of *Gymnema sylvestre* (Figure 4.4) were collected from Tirupati, Andhra Pradesh, India, from November to January 2018. The plant was identified and authenticated by Dr K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. Voucher specimens were prepared and deposited in the Dept. of Pharmacognosy herbarium (CZ: S-PCOG- 2018-01; EJ: S-PCOG-2018-02; VR: S-PCOG-2018-003 and GS: S-PCOG-2018-04), St. Johns College of Pharmaceutical Sciences, Yemmiganur, Kurnool Dist. Andhra Pradesh, AP, India for future reference.

2.2. PHARMACOGNOSTIC AND PHYSIOCHEMICAL EVALUATION

The organoleptic properties of crude extracts were assessed by their color, texture, and odor. These organoleptic characters were determined using the senses of sight (eyes), touch (skin) and smell (nose) [15].

3. EXTRACTION

Cinnamaldehyde from *Cinnamomum zeylanicium;* Gallic Acid from *Eugenia jambolana* Seeds*;* Vincristine and Vinblastine from *Vinca rosea* whole plant; Gymnemic Acid *Gymnema sylvestre* leaves.

Powdered and dried plant parts (200 g each) was extracted with 1L of 95% ethanol at room temperature for 72 h in separate soxhlet apparatus and filtered using Whatman No. 1 filter paper. The crude extracts were concentrated *in vacuo* at 40°C by using rotary flash evaporator to about 1/4th of the original volume. The ethanolic extracts were evaporated under reduced pressure to a dark green semisolid i.e., ethanolic extracts of bark of CZ, the seeds of EJ, the whole plant of VR, and the leaves of GS and they were lyophilized to further use [16-18]

The concentrated crude extract was successively extracted with water, n- hexane (3×400 mL) and n-hexane fraction was removed, the water fraction is further extracted with 70% ethanol (3×800 mL). The extracts were separately concentrated to dryness *in vacuo* by using a rotary flash evaporator to give four hydro alcoholic extracts.

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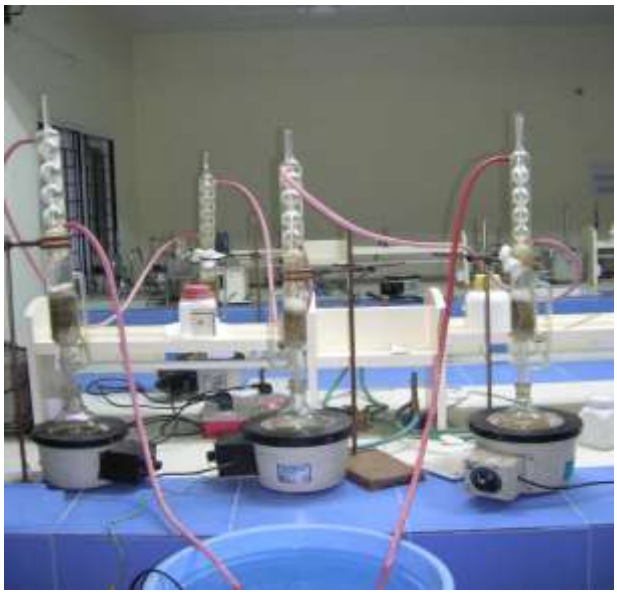


Figure 2: Experimental setup for the extraction

3.1. QUALITATIVE CHEMICAL EVALUATION

The preliminary phytochemical (qualitative) screenings of crude extracts were conducted using standard procedures. Each crude extract (final concentration of 1 mg/mL) was assayed for the presence of phytochemical constituents such as carbohydrates, alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins and terpenoids [19-21].

3.2. PREPARATION OF POLYHERBAL PREPARATION (PHP)

Using the extracts of CZ, EJ, VR, and GS, five polyherbal preparations (PHP 1 to PHP 5) were made with varying proportions.

Table 4: Composition of polyherbal preparations

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

4. PHARMACOLOGICAL STUDIES

4.1. Procurement and selection of animals

Wistar albino rats weighing between 130–180 gm of either sex was obtained from SV animal house, Bengaluru, India. These animals were used for the acute toxicity and antidiabetic activity. The animals were stabilized for 10 days; they were maintained in standard condition at room temperature, 60±5% relative humidity and 12 H light-dark cycle. They had been given a standard pellet diet and water *ad-libitum* throughout the course of the study. The animals were managed gently to avoid giving them too much stress, which could result in an increased adrenal output. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of St. Johns college of pharmaceutical sciences, Yemmiganur (Reg No.- 1561/PO/Re/S/11/ CPCSEA)

Table 5: The animal groups and treatment schedules for the evaluation of the anti- diabetic activity of PHP in STZ-induced diabetes in Albino Wistar rats.

S. No	Groups	Treatment	Purpose
1	Normal control	Vehicle (normal saline)	Serves to study normal parameters of rat
2	Diabetic control	STZ 60mg/Kg, i.p.	Serve as diabetic control
3	Glibenclamide (5mg/Kg, po)	STZ60mg/Kg, i.p+ Glibenclamide at 5mg/Kg, p.o.	To study the effect of glibenclamide at 5mg/Kg in disease condition
4	PHP – A	STZ 60mg/Kg, i.p + PHP – 1 200mg/Kg, p.o.	To study the effect of PHP– 1 at 200 mg/Kg in diabetic condition

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5	PHP – B	STZ 60mg/Kg, i.p + PHP – 2 200mg/Kg, p.o.	To study the effect of PHP - 2 at 200 mg/Kg in diabetic condition
6	PHP – C	STZ 60mg/Kg, i.p + PHP – 3 200mg/Kg, p.o.	To study the effect of PHP - 3 at 200 mg/Kg in diabetic condition
7	PHP – D	STZ 60mg/Kg, i.p + PHP – 4 200mg/Kg, p.o.	To study the effect of PHP - 4 at 200 mg/Kg in diabetic condition
8	PHP – E	STZ 60mg/Kg, i.p + PHP – 5 200mg/Kg, p.o.	To study the effect PHP - 5 at 200 mg/Kg in diabetic condition
9	PHP – F	STZ 60mg/Kg, i.p + PHP – 1 400mg/Kg, p.o.	To study the effect of PHP – 1 at 400 mg/Kg in diabetic condition
10	PHP – G	STZ 60mg/Kg, i.p + PHP – 2 400mg/Kg, p.o.	To study the effect of PHP - 2 at 400 mg/Kg in diabetic condition
11	PHP – H	STZ 60mg/Kg, i.p + PHP – 3 400mg/Kg, p.o.	To study the effect of PHP - 3 at 400 mg/Kg in diabetic condition
12	PHP – I	STZ 60mg/Kg, i.p + PHP – 4 400mg/Kg, p.o.	To study the effect of PHP - 4 at 400 mg/Kg in diabetic condition
13	PHP – J	STZ 60mg/Kg, i.p + PHP – 5 400mg/Kg, p.o.	To study the effect of PHP - 5 at 400 mg/Kg in diabetic condition

S. No	Groups	Treatment	Purpose
1	Normal control	Vehicle (normal saline)	Serves to study normal parameters of rat
2	Diabetic control	STZ 60mg/Kg, i.p.	Serve as diabetic control
3	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
4	PHP – A	STZ 60mg/Kg, i.p + PHP – 1 200mg/Kg, p.o.	To study the effect of PHP– 1 at 200 mg/Kg in diabetic condition
5	PHP – B	STZ 60mg/Kg, i.p + PHP – 2 200mg/Kg, p.o.	To study the effect of PHP - 2 at 200 mg/Kg in diabetic condition
6	PHP – C	STZ 60mg/Kg, i.p + PHP – 3 200mg/Kg, p.o.	To study the effect of PHP - 3 at 200 mg/Kg in diabetic condition

4.2. Preparation of Fast Dissolving Tablets (FDT)

All the ingredients were passed through sieve no 40 to remove any aggregates present in powder. Optimized PHP, Sodium starch glycolate and Lactose were mixed for 3 min. To this blend Magnesium stearate and talc (screened #60) were added and mixed in the poly bag for 3 min. The tablets are prepared by direct compression method. 200mg of well-mixed powder are compressed using a ten-station rotary tablet compression machine (M/c Rimek Minipress Machinery Co. Pvt. Ltd.), at the hardness of nearly 5 Kg/cm^2.

Table 6: Ingredients used for the preparation of PHP fast-dissolving tablets

S. No	Ingredients	Formulations Quantity in mg								
		F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
1.	PHP (Optimized)	66	66	66	66	66	66	66	66	66
2.	Sodium starch glycolate	5	10	15	20	25	30	35	40	45
3.	Lactose	115	110	105	100	95	90	85	80	75
4.	Magnesium stearate	10	10	10	10	10	10	10	10	10
5.	Purified Talc	4	4	4	4	4	4	4	4	4
6.	Total tablet weight	200	200	200	200	200	200	200	200	200

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4.3. Evaluation of Tablets: The tablets were evaluated as per the criteria

Stability study

Table 7: ICH Conditions for the stability studies

Study protocol	Storage conditions		Minimum time to be stored
	Temperature (°C)	Relative humidity (%)	
Long term	25°C±2°C	60%±5%	12 Months
Intermediate	30°C±2°C	65%±5%	6 Months
Accelerated	40°C±2°C	75%±5%	6 Months

5. RESULTS

5.1. MORPHOLOGICAL FEATURES

Table 8: Morphological features of selected plants

S. No	Name of the plant	Plant part used	Size	Shape	Colour	Odour	Taste
1.	*Cinnamomum zeylanicum*	Bark	1-6 cm	Multiple-layered, curls inward from both edges.	Tan brown	Exotic aroma	Mild sweet
2.	*Eugenia jambolana*	Seeds	1-2 cm diameter	Oval or round	Cream	Characteristics	Bitter and Astringent
3.	*Vinca rosea*	Whole plant	The plant grows 1-2 feet	Fruit is follicle with numerous black seeds. Leaf: Petiolate, entire margin, ovate or oblong, glossy appearance and with acute apex.	Leaf - Green. Flowers - violet, pinkish, white, or carmine red Roots – pale grey	Characteristics	Very bitter
4.	*Gymnema sylvestre*	Leaves	2-6 cm length 1-4 cm width	Simple, petiolate, rounded to cordate base	Green	Characteristics	Bitter and Astringent

5.2. PHYSIOCHEMICAL EVALUATION

Table 9: Physicochemical evaluation of extracts

S. No	Parameters	Result (% w/w)			
		CZ	EJ	VR	GS
1.	Foreign organic matter	2.12	0.56	1.62	1.38
2.	Total ash value	8.33	7.65	8.84	7.96
	Acid insoluble ash	1.26	2.58	0.36	1.93
4.	Water soluble ash	2.36	1.20	1.85	2.55
5.	Moisture content	2.56	1.36	3.54	1.32
6.	Swelling index	1.25	3.55	0.85	1.75
7.	Foaming index	0.66	2.20	1.03	0.96

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5.3. EXTRACTION OF PLANT MATERIAL

Table 10: Percentage yield of hydro alcoholic extracts of selected medicinal plants

S. No	Plant & Part	% Yield(w/w)	Characteristics	pH
1	*Cinnamonum zeylanicum* Bark	12.56	Semi-solid, brown with yellowish shade in colour, characteristics odour	7.03
2	*Eugenia jambolana* Seeds	10.78	Semi-solid, dark brown with yellowish shade in colour, characteristics odour	7.04
3	*Vinca rosea* Whole plant	11.34	Semi-solid, dark green in colour, characteristics odour	6.98
4	*Gymnema sylvestra*	10.86	Semi-solid, dark green in colour, characteristics odour	7.03

5.4. QUALITATIVE CHEMICAL EVALUATION (Preliminary phytochemical screening)

Table 11: Preliminary phytochemical screening of selected plants

S. No	Chemical Constituent	Observation			
		CZ	EJ	VR	GS
1.	Carbohydrates	-	+	-	-
2.	Starch	-	-	+	+
3.	Steroids	-	+	+	-
4.	Glycosides	+	+	+	+
5.	Proteins	+	-	+	+
6.	Flavanoids	++	++	+	++
7.	Tannins	+	-	+	-
8.	Alkaloids	+	+	+	+
9.	Saponins	+	+	-	+
10.	Triterpenoids	-	++	+	-

+: Present; -: absent

5.5. CHROMATOGRAPHIC EVALUATION

Table 12: Rf values of TLC samples

S. No	Name of the sample	Mobile phase	No. of spots	Rf value	Inference
1.	Fraction – 3	Hexane: Ethyl Acetate 7:3 v/v	1	STD: 0.93 Test: 0.93	Gymnemic acid
2.	Fraction – 10	Ethyl Acetate: Methanol 5:5 v/v	1	STD: 0.87 Test: 0.87	Vincristine
3.	Fraction – 11	Ethyl Acetate: Methanol 3:7 v/v	1	STD: 0.94 Test: 0.94	Vinblastine
4.	Fraction – 15	Methanol: Water 7:3 v/v	1	STD: 0.96 Test: 0.96	Cinnamaldehyde
5.	Fraction – 16	Methanol: Water 5:5 v/v	1	STD: 0.88 Test:0.88	Gallic Acid

5.6 ANALYTICAL CHARACTERIZATION OF POLYHERBAL PREPARATION

Table 13: Rf values observed in EJ seeds extract

Sample	UV at 287 nm		Compound	Amount of analyte (% w/v)
	Start	End		
Standard	0.05	0.08	Gallic acid	18.56
EJ seeds extract (Test)	0.05	0.08	Gallic acid	11.63
	0.08	0.14	Unidentified	1.26

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5.6. Pharmacological evaluation of polyherbal preparations

Table 14: Results of acute toxicity study

Treatment	Body weight (gm) Rat (N=6)	Dose (mg/kg)	Mortality (Animal dead) After 24 hrs	After 7 days	After 14 days	Toxicity profile
PHP 1	140 ± 10.50	2000	0	0	0	**Safe**
PHP 2	132 ± 11.89	2000	0	0	0	**Safe**
PHP 3	142 ± 13.10	2000	0	0	0	**Safe**
PHP 4	133 ± 13.28	2000	0	0	0	**Safe**
PHP 5	145 ± 9.30	2000	0	0	0	**Safe**

5.7.Oral glucose tolerance test (OGTT)

Table 15: Results of Oral glucose tolerance test

Group	Blood Glucose Level (mg/dl) 0 min	30 min	60 min	120 min	180 min
Normal	89.00□1.46	129.00□1.60	117.30□2.18	102.70□1.92	94.50□1.38
Positive control Glibenclamide (5mg/kg)	89.33□2.61	95.50□2.00[a]	78.33□1.14[b]	65.67□0.71[b]	58.17□1.13[b]
Polyherbal preparation (200 mg/kg)	90.64□2.73	98.83□3.75[a]	78.24□2.34[b]	70.55□3.84[b]	68.74□2.63[b]
Polyherbal preparation (400 mg/kg)	90.47□1.83	93.70□1.56[a]	81.50□2.48[b]	70.50□2.10[b]	60.83□1.74[b]

*All data presented in Mean±SD (n=6); a-P <0.05; b-P<0.01 as compared to Normal animals (ANOVA followed by Dunnett's test)

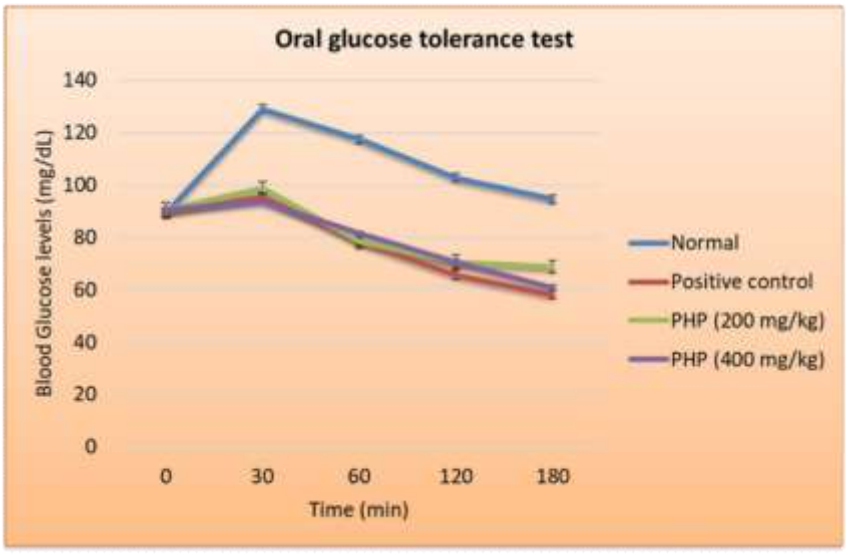


Figure 3: Graphical representation of OGTT

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5.8. Anti - diabetic activity

Table 16: Effect of PHPs on Blood Glucose levels

Group	Treatment	Blood Glucose (mg/dl) levels on				
		0 day	7th day	14th day	21st day	28th day
Normal control	Vehicle (normal saline)	94.22±1.35	95.99±1.34	93.48±0.35	94.87±0.37	96.67±0.36
Diabetic control	STZ 60mg/kg, i.p.	285.98±0.23 a	310.67±2.62 a	308.45±1.32 a	314.73±2.94 a	310.43±3.25 a
Glibenclamide (5mg/kg, po)	STZ 60mg/kg, i.p + Glibenclamide at 5mg/kg	287.84±2.93 a	219.98±2.34 b	178.34±3.88 b	142.36±3.92 b	115.02±2.90 b
PHP – A	STZ 60mg/kg, i.p + PHP – 1 200mg/kg	292.88±2.01 a	255.83±3.97 b	228.73±2.82 b	203.28±0.38 b	184.82±2.54 b
PHP – B	STZ 60mg/kg, i.p + PHP – 2 200mg/kg	288.98±3.92 a	261.83±3.92 b	232.87±3.83 b	200.67±3.73 b	189.37±3.92 b
PHP – C	STZ 60mg/kg, i.p + PHP – 3 200mg/kg	290.74±3.85 a	258.38±3.98 b	234.73±4.82 b	195.83±3.52 b	178.78±3.83 b
PHP – D	STZ 60mg/kg, i.p + PHP – 4 200mg/kg	287.48±2.83 a	260.37±2.32 b	229.83±2.85 b	205.83±2.45 b	188.23±0.34 b
PHP – E	STZ 60mg/kg, i.p + PHP – 5 200mg/kg	292.38±2.84 a	279.28±2.31 b	239.55±2.90 b	211.74±2.98 b	201.74±3.89 b
PHP – F	STZ 60mg/kg, i.p + PHP – 1 400mg/kg	290.43±3.24 a	235.44±2.86 b	194.67±2.28 b	163.67±3.72 b	142.42±2.55 b
PHP – G	STZ 60mg/kg, i.p + PHP – 2 400mg/kg	289.66±2.69 a	228.84±2.63 b	184.88±3.54 b	153.75±3.56 b	133.25±2.24 b
PHP – H	STZ 60mg/kg, i.p + PHP – 3 400mg/kg	291.78±2.82 a	214.45±3.54 b	180.34±1.34 b	143.62±3.55 b	120.73±3.21 b
PHP – I	STZ 60mg/kg, i.p + PHP – 4 400mg/kg	288.74±2.43 a	224.74±2.56 b	182.47±2.89 b	150.45±2.33 b	128.42±3.65 b
PHP – J	STZ 60mg/kg, i.p + PHP – 5 400mg/kg	285.27±1.84 a	247.84±2.92 b	201.82±3.33 b	176.43±2.94 b	143.32±2.66 b

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5.10. Pre-Formulation Results

Table 17: Results of pre-formulation studies of PHP-H

Formulation Code	Flow properties				
	Angle of repose (^{0})	Density		Compressibility Index	Hausner ratio
		Bulk	Tapped		
F-1	28.11±0.02	0.689±0.02	0.726±0.01	5.096±0.03	1.053±0.01
F-2	29.19±0.02	0.785±0.03	0.845±0.02	7.100±0.06	1.076±0.01
F-3	29.38±0.01	0.854±0.04	0.932±0.03	8.369±0.02	1.091±0.02
F-4	28.84±0.02	0.758±0.06	0.798±0.02	5.012±0.08	1.052±0.02
F-5	27.92±0.03	0.689±0.05	0.757±0.05	8.982±0.04	1.098±0.01
F-6	29.21±0.05	0.628±0.05	0.681±0.02	7.782±0.05	1.084±0.02
F-7	28.02±0.01	0.815±0.02	0.882±0.06	7.596±0.07	1.082±0.03
F-8	29.16±0.02	0.761±0.04	0.808±0.03	5.816±0.03	1.061±0.02
F-9	28.88±0.05	0.846±0.06	0.894±0.02	5.369±0.01	1.056±0.02
Values in mean ±SD; trials (n=3)					

5.11. Evaluation of polyherbal tablets

Table 18: Physical Characteristics

Formulation	Physical parameters				
	Uniformity of weight (mg)	Hardness (Kg/cm^2)	Thickness (mm)	Friability (%)	Disintegration (sec)
F-1	199.8±2.36	5.8±0.03	3.01±0.02	0.21±0.01	580±6
F-2	200.2±1.79	6.2±0.02	2.99±0.02	0.44±0.02	515±7
F-3	201.1±1.98	7.1±0.06	3.01±0.01	0.42±0.01	478±5
F-4	200.1±2.07	6.3±0.03	3.02±0.02	0.39±0.02	419±2
F-5	200.4±1.75	5.8±0.02	2.99±0.01	0.37±0.02	394±9
F-6	199.8±1.98	6.2±0.04	3.00±0.02	0.25±0.01	320±5
F-7	199.9±1.08	5.3±0.03	3.01±0.01	0.34±0.01	305±4
F-8	200.1±1.95	5.3±0.01	3.00±0.02	0.11±0.01	291±3
F-9	200.6±1.25	6.4±0.04	2.99±0.01	0.31±0.01	295±5
Values in mean ±SD; trials made (n=3)					

5.12. Stability studies

Table 19: Results of stability studies

Physical parameters	Results after			
	Initial	2 months	4 months	6 months
Color	Grey	Grey	Grey	Grey
Appearance	Smooth and intact	Smooth and intact	Smooth and intact	Smooth and intact
Average weight (mg)	200.1±1.95	200.1±1.98	199.9±1.23	199.0±1.15
Thickness (mm)	3.00±0.02	3.00±0.01	2.99±0.01	3.00±0.01
%Friability	0.11±0.01	0.12±0.03	0.12±0.02	0.12±0.01
Disintegration time (sec)	291±3	291±3	293±2	292±1

6. CONCLUSIONS

The study successfully prepared and evaluated polyherbal preparations (PHPs) using hydroalcoholic extracts of *Cinnamomum zeylanicum*, *Eugenia jambolana*, *Vinca rosea*, and *Gymnema sylvestre*. The PHP-H formulation

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(PHP-3 at 400 mg/kg) demonstrated the most significant anti-diabetic activity in STZ-induced diabetic rats, comparable to the standard drug Glibenclamide, over 28 days. The optimized PHP was successfully formulated into a Fast Dissolving Tablet (FDT) using the direct compression method. The final optimized FDT formulation, F-8, displayed good pre-formulation flow characteristics, acceptable physical parameters (low friability), and a rapid disintegration time of 291±3 seconds. The stability of the FDT formulation was also confirmed. Thus, the formulated polyherbal Fast Dissolving Tablet presents a potential and efficacious dosage form for the treatment of diabetes mellitus.

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