EVALUATION OF ANTIDIABETIC ACTIVITY OF POLYHERBAL FORMULATION IN STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

Snehal Dukare, Dr. R. H. Kale, P.N.Folane,Dr. K.R. Biyani ,

Email: Snehaldukare368@gmail.com

Anuradha College of Pharmacy, Chikhali Dist.- Buldana (M.S) India 443201

ABSTRACT

The present work was executed to evaluate the anti-diabetic potency of a polyherbal preparation. The objective of this study is to induce experimental diabetes mellitus using Streptozocin - in normal wistar rats and study the antidiabetic activity of polyherbal formulation by comparison of changes in body weight and levels of glucose between normal and diabetic rats. Hypoglycaemic agents from natural and synthetic sources are available for treatment of diabetes. Indian medicinal plants have been found to be useful to successfully manage diabetes.

Diabetes is one of the most common and deadly diseases of recent years. Therefore, ways to diagnose, pre vent or delay the disease and its complications have been discussed for a long time. Today, people with dia betes, especially type 2 diabetes, are advised to change their diet and physical activity according to their sy mptoms, then gradually switch to monotherapy, dual therapy, polytherapy and insulin therapy. Despite the progress made, the search for the "perfect" diabetes drug continues. The complex nature of diabetes and its effect on hemodynamics are the most important reasons why these drugs are not currently available. In ad dition, the molecular mechanisms of diabetes are still controversial. Due to the risks, disadvantages, side e ffects and mechanisms of action of different drugs, choosing the most appropriate treatment requires caref ul research.

KEYWORDS: Antidiabetics, Hypoglycaemic agents, cell metabolism, diabetes mellitus, drug therapy, insulin resistance.

INFORMATION

Diabetes mellitus is a group of metabolic disorders characterized by elevated blood glucose levels over a prolonged period. The condition results from defects in insulin production, insulin action, or both. As of 2024, diabetes has become a major global health issue, with millions of people affected worldwide [1].

There are two primary types of diabetes:

- **Type 1 Diabetes (T1D)**: An autoimmune condition where the body attacks its own pancreatic beta cells, which produce insulin.
- **Type 2 Diabetes (T2D)**: A condition characterized by insulin resistance and eventual pancreatic beta-cell dysfunction.

Other forms of diabetes include gestational diabetes, which occurs during pregnancy, and rare forms of monogenic diabetes caused by genetic mutations [2].

Pathophysiology of Diabetes

The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and glucagon. The islets of Langerhans secrete insulin and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels [1,2].

Glucagon is a hormone that opposes the action of insulin. It is secreted when blood glucose level falls. It increases blood glucose concentration partly by breaking down stored glycogen in the liver by a pathway known as glycogenolysis.

Gluconeogenesis is the production of glucose in the liver from non-carbohydrate precursors such as glycogenic amino acids.[3]

Criteria in order to diagnose Pre-diabetes and Diabetes

Sr. no .	Normal	Prediabetic	Diabetic
Alc	≤5.6 %	5.7-6.4 %	≥6.5 %
FPG	399 mg / dL	100-125 mg / dL (6.9 mmol / L)	5.6-126 mg / dL (7.0 mmol / L)
OGTT	1313 139 mg / dL	00-140-199 mg / dL (7.8-11.0 mmol / L)	≥200 mg / dL (11.1) mmol / L) *
RPG			≥200 mg / dL (11.1 mmol / L) **

MATERIALS AND METHOD

Animals

Animals were housed under standard laboratory conditions of temperature 25 ± 1°C with free access to food and water. The experiments were performed during the light cycle (12 12 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee. Proposal no.

GROUP NO	STUDY GROUPS	SPECIES WITH GENDER	NO. OF ANIMALS REQUIRED
1	Healthy Control (10 mg/kg distilled water)	Male Wistar Rats	06
2	Disease Control (Streptozotocin 60 mg/kg)	Male Wistar Rats	06
3	Standard drug	Male Wistar Rats	06

	(Streptozotocin 60 mg/kg + Glibenclamide 0.5mg/kg)		
4	Treatment group (200 mg/kg Polyherbal formulation + Streptozotocin 60 mg/kg)	Male Wistar Rats	06
5	Treatment group (400 mg/kg Polyherbal formulation + Streptozotocin 60 mg/kg)	Male Wistar Rats	06
TOTAL NO. OF ANIMALS REQUIRED			30

Plant material

- The *Gomphrena Celosioides* and *Passiflora incarnata* plant was collected from the available source and authenticated by botanist of an institute's botany department.

Phytochemical Evaluation:

Test for saponins

A. Foam test - Drug sample or dry powder was wobbled vigorously with water. If persistent foam observed, shows presence of saponins**.**

B. Haemolytic test -Added drug sample or dry powder to one drop of blood placed on glassslide. If hemolytic zone is found indicates presence of saponins.

Test for alkaloids

1) **Dragendorff's test: -** The drug sample was treated with Dragendorff's reagent (potassium bismuth iodine solution), formation of reddish brown precipitate indicates the presence of alkaloids.

2) **Mayer's test: -** The drug sample was treated with Mayer's reagent (potassium mercuric iodide solution) formation of creamy colour precipitate, indicate the presence of alkaloids**.**

3) **Wagner's test: -** The drug sample was treated with Wagner's reagent (iodine potassium iodide solution) reddish brown precipitate indicated the presence of alkaloids.

4) **Hager's test**: - The drug sample was treated with Hager's reagent (saturated solution of picric acid) formation of yellow precipitate indicated the presence of alkaloids[4].

Test for flavonoids

1) **Shinoda test: -** to the drug sample was add few magnesium turnings and concentrated Hcl drop wise , pink scarlet ,crimson red, or occasionally green to blue appears after few minutes.

2) **Alkaline reagent test: -** to the drug sample was add few drops of NaOH solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicated the presence of flavonoids[5]

3) **Zinchydrochloride test: -** to the drug sample add a mixture of zinc dust and concentrated Hcl. It gives red colour after few minutes**.**

Experimental Design for Anti-Diabetic Activity

For Anti-diabetic study, the fasted diabetic rats were divided in to 5 groups of 6 animals each.

Table 08. Experimental Design for Anti-Diabetic Study

Group No	STUDY GROUPS	Received
1	Healthy Control	0.5 ml of NS daily
2	Disease Control	0.5 ml of NS daily
3	Standard Control	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily
4	Treatment group 200 mg/kg	200 mg/kg of Polyherbal formulation
5	Treatment group 400 mg/kg	400 mg/kg of Polyherbal formulation

STREPTOZOTOCIN INDUCED DIABETES MODEL

Streptozotocin induced diabetes in wistar rats model will be used by us. Diabetes will be induced by injecting streptozotocin at a reference dose of 60mg/kg body weight intra peritoneally. After 1 hour of streptozotocin administration, the animals will be given feed ad libitum, and 5% dextrose solution in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals will be kept under observation and after 48 hour blood glucose is measured. The diabetic rats with glucose level >300 mg/dl will be separated and divided into six different groups for experimental study, with each group containing six animals[7].

The animals will then be administered with the polyherbal formulation to evaluate the antidiabetic activity. Polyherbal tablet will be given by dissolving in NS orally to rats, For 200 mg/kg one tablet and for 400 mg/kg two tablets.

Readings will be taken at 1, 7th, 14th and 21th day.

Evaluation parameters:

- Blood glucose levels- glucometer
- Cholesterol (HDL & LDL)
- Body weight
- Water intake
- Food intake
- Haematological parameters

RESULTS

Preformulation Studies:

Table 09 . Preformulation Studies result.

Sr No.	Parameters	Observation
1	Angle of repose	28.7 ± 1.53
2	Bulk Density	0.427 ± 0.003 g/cc
3	Tapped Density	0.515 ± 0.005 g/cc
4	Loss on Drying	5.5 ± 0.05 % w/w

Evaluation of polyherbal tablet formulation:

Table 10. Evaluation of polyherbal tablet formulation:

Sr no.	Post compression parameters	Result
1	Colour	Light Brown
2	Odour	Characteristic
3	Shape	Circle
4	Texture	Smooth
5	Average Weight	499 ± 0.71mg
6	Thickness	4.18 ± 0.03 mm
7	Hardness Test	5.8 ± 0.15 kg/cm^2

8	Friability test	0.72 ± 0.37%
9	Disintegration time	4.13 ± 0.26 min
10	pH	6.3

The phytochemical investigation for various chemical constituents in polyherbal formulation is given below.

Chemical constituents	**Name of the test**	**Procedure**	**Observation**	**Inference**
Flavonoids	Shinoda test	2-3 ml of Extract + few drops of cone. HCI+0.5 gm magnesium turnings	Slight pink color	Flavonoids present
Flavonoids	Lead acetate test	2-3 ml of Extract lead acetate	Yellow color ppt	Flavonoids present
Flavonoids	NaOH test	2-3 ml of Extract + increasing amt. of NaOH	Yellow color which decolourized on addition of acid	Flavonoids present
Tannins & phenolic comp.	$FeCl_3$ test	2-3 ml of extract+5% $FeCl_3$ solution	Deep blue color	Tannins & phenolic comp. present
Tannins & phenolic comp.	HNO_3TEST	2-3 ml of Extract +Dil. HNO_3	Reddish yellow color	Tannins & phenolic comp. present
Tannins & phenolic comp.	Acetic acid test	2-3 ml of Extract+ Acetic acid solution	Red color	Tannins & phenolic comp. present
Tannins & phenolic comp.	$KMnO_4$	2-3 ml of Extract+ $KMnO_4$ solution	Decolourisation $KMnO_4$	Tannins & phenolic comp. present
Alkaloids	Mayer's test	2-3 ml of Extract+ Mayer's solution	Cream color ppt	Alkaloids present
Alkaloids	Dragondroff's test	2-3 ml of Extract+	Orange color ppt	Alkaloids present

Water Intake:

While water consumption increased in the untreated diabetic rats (Group 2), the administration of Polyherbal formulation significantly reduced the quantity of water intake in diabetic animals [8].

Effect of oral administration of Polyherbal formulation on water intake. (n = 6, mean ± SEM).

Group	Treatment	Water intake (ml/day)
Group I [Healthy group]	0.5ml of NS daily for 21 days.	20.09 ± 1.08
Group II [Diabetic control group]	0.5ml of NS daily for 21 days.	92.90 ± 2.12
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 21 days.	48.05 ± 1.48
Group IV [Diabetic test group I]	200mg/kg of polyherbal formulation daily for 21 days.	75.55 ± 1.53**
Group V [Diabetic test group II]	400mg/kg of Polyherbal formulation daily for 21 days	45.94 ± 1.49*

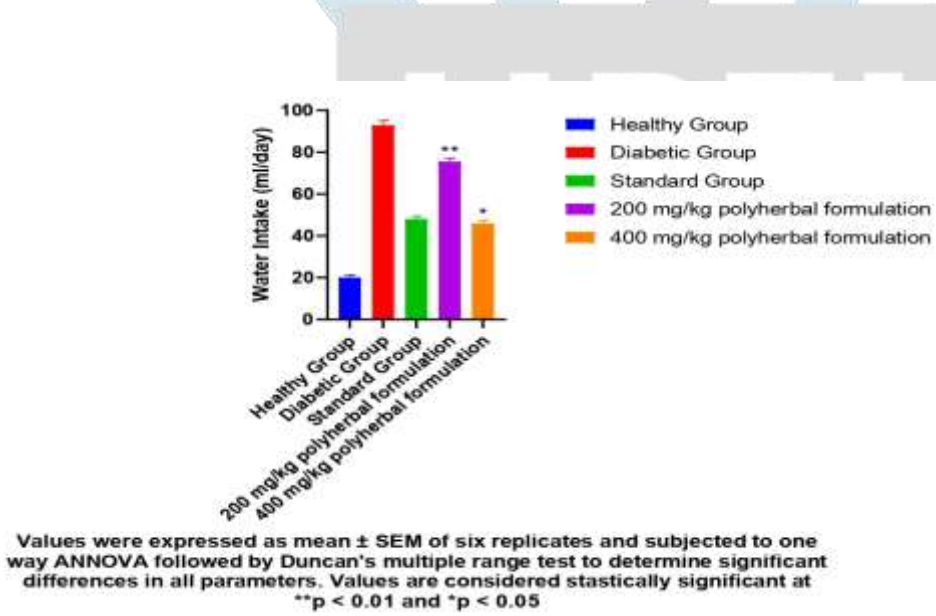


Fig 01. Effect of oral administration of Polyherbal formulation on water intake

Feed Intake:

While food consumption increased in the untreated diabetic rats (Group 2), the administration of Polyherbal formulation significantly reduced the quantity of feed intake in diabetic animals. Similarly, the untreated diabetic rats showed polyphagic condition and consumed higher quantity of feed compared to the control and treatment groups[10].

Effect of oral administration Polyherbal formulation on feed intake.

(n = 6, mean ± SEM).

Group	Treatment	Feed intake (g/day)
Group I [Healthy group]	0.5ml of NS daily for 21 days.	14.10 ± 1.48
Group II [Diabetic control group]	0.5ml of NS daily for 21 days.	32.92 ± 3.13
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 21 days.	21.98 ± 1.02
Group IV [Diabetic test group I]	200mg/kg of Polyherbal formulation daily for 21days.	22.24 ± 1.06**
Group V [Diabetic test group II]	400mg/kg of Polyherbal formulation daily for 21 days	20.05 ± 1.18*

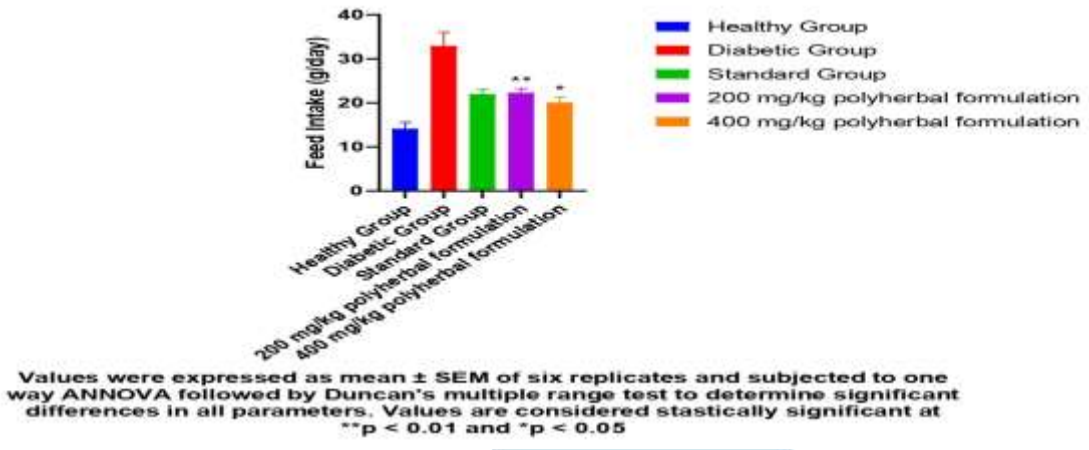


Fig 2 Effect of oral administration of Polyherbal formulation on feed intake

Body Weight:

The Polyherbal formulation significantly increased the body weight of diabetic animal at higher dose. Generally, body weights are reduced in diabetic animals, but in this study, the decrease in body weight was diminished by the drug treatment [11].

Effect of oral administration of Polyherbal formulation on Body Weight. (n = 6, mean ± SEM).

Group	Treatment	Body Weight (g)
Group I [Healthy group]	0.5ml of NS daily for 21 days.	207.4 ± 0.8
Group II [Diabetic control group]	0.5ml of NS daily for 21 days.	191.4 ± 0.02
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 21 days.	197.9 ± 1.8

Group IV [Diabetic test group I]	200mg/kg of Polyherbal formulation for 21 days.	193.6 ± 0.8**
Group V [Diabetic test group II]	400mg/kg of Polyherbal formulation for 21 days	206.32 ± 1.6**

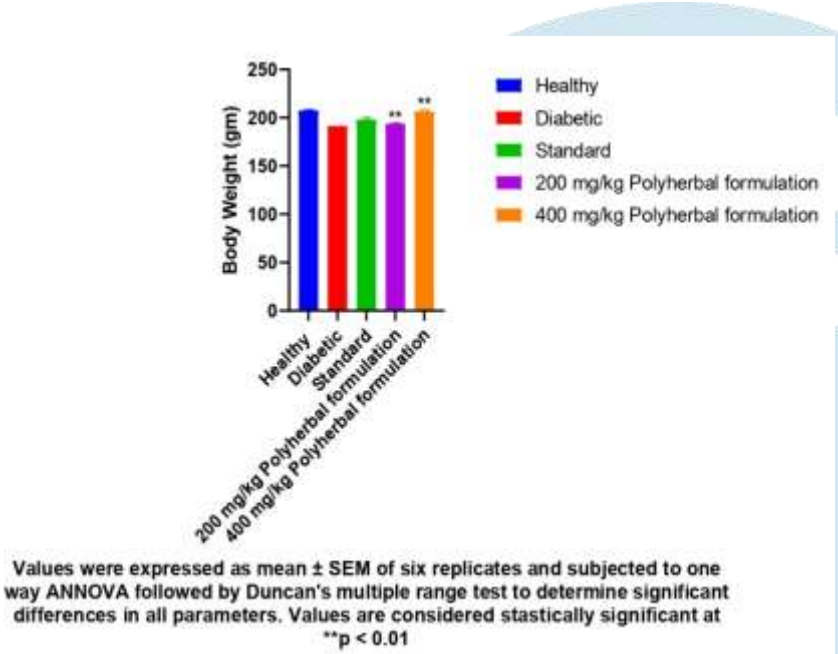


Fig 03. Effect of oral administration of Polyherbal formulation on Body Weight

CHOLESTEROL:

There was a significant elevation in the levels of serum cholesterol concentrations in diabetic rats when compared with the control group. The polyherbal formulation and glibenclamide significantly reduced the levels of serum cholesterol, to near normalcy as observed in the control after 21 days of treatment[12]

. Effect of Polyherbal formulation on Cholesterol In Streptozotocin Induced Diabetic Wistar Rats. (n =6, mean ± SEM).

Group	**Treatment**	**Cholesterol (mg/dl)**
Group I [Healthy group]	0.5ml of NS daily for 21 days.	51.40 ± 2.80
Group II [Diabetic control group]	0.5ml of NS daily for 21 days.	77.63 ± 1.21
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 21 days.	54.96 ± 2.90
Group IV [Diabetic test group I]	200mg/kg of Polyherbal formulation daily for 21 days.	57.86 ± 2.41**
Group V [Diabetic test group II]	400mg/kg of Polyherbal formulation daily for 21 days	55.95 ± 2.80*

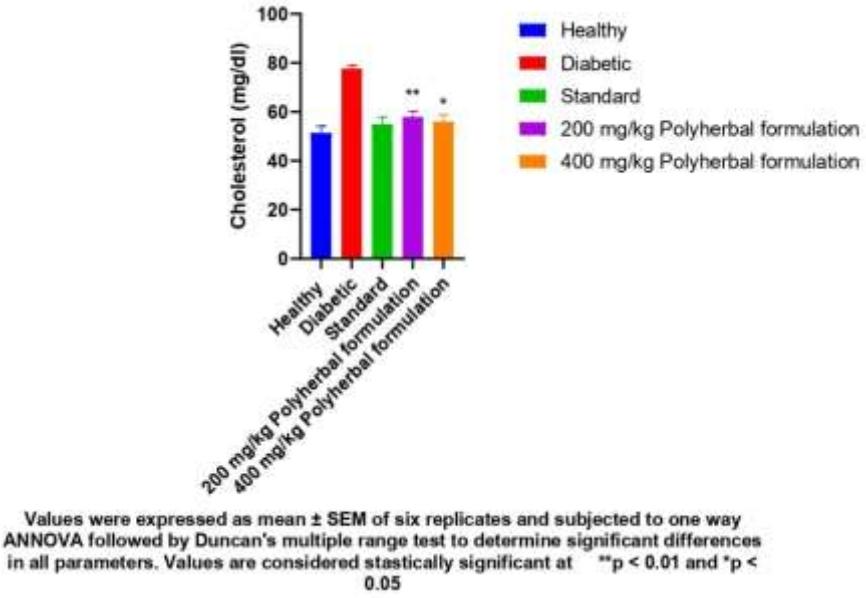


Fig 04. Effect of Polyherbal formulation on Cholesterol levels

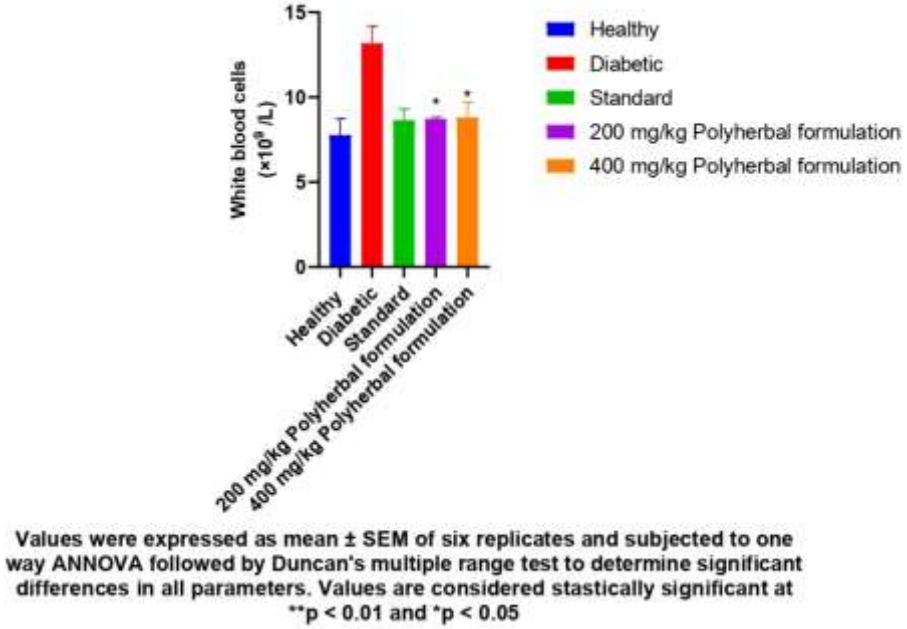
HEMATOLOGICAL PARAMETERS:

In addition, the diabetic rats exhibited significantly reduced levels in all the haematological parameters with the exception of white blood cell count and lymphocytes which were significantly increased. Oral administration of of Polyherbal formulation in diabetic rats for 30 days, however, restored the haematological parameters to normalcy with the exception of platelets and neutrophils which were significantly increased but not to the control levels[13].

Effect of Polyherbal formulation on some haematological parameters of diabetic rats ($n = 6 \pm$ SEM).

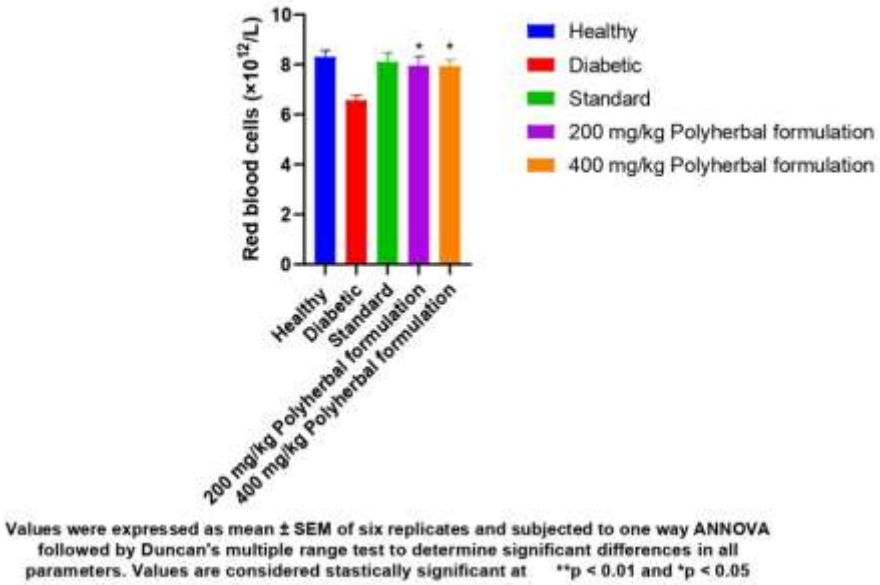
Group	**Group I**	**Group II**	**Group III**	**Polyherbal formulation**	
	Healthy group	**Diabetic group**	**Standard Control**	**Group IV 200mg/kg**	**Group V 400mg/kg**
White blood cells ($\times 10^9$ /L)	7.77 ± 0.96	13.17 ± 1.03	8.64 ± 0.66	8.72 ± 0.1*	8.80 ± 0.90*
Red blood cells ($\times 10^{12}$/L)	8.30 ± 0.27	6.57 ± 0.20	8.10 ± 0.37	7.95 ± 0.37*	7.93 ± 0.26*
Haemoglobin (g/dL)	15.44 ± 0.30	12.56 ± 0.35	15.37 ± 0.47	15.31 ± 0.42*	15.08 ± 0.46*
Platelets ($\times 10^9$ /L)	926 ± 11.36	637 ±12.85	766.2 ± 14.12	746.34 ± 15.8**	746.02 ± 13.16**
Neutrophils (%)	13.21 ± 0.27	4.56 ± 0.23	8.12 ± 0.42	7.85 ± 0.35*	7.90 ± 0.31*

Lymphocytes (%)	60.24 ± 1.94	68.70 ± 1.08	61.34 ± 1.44	62.35 ± 1.47*	62.42 ± 1.25*
Eosinophils (%)	3.04 ± 0.50	1.17 ± 0.17	2.67 ± 0.48	2.52 ± 0.18*	2.48 ± 0.32*



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Fig 05. Effect of of Polyherbal formulation on WBC



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

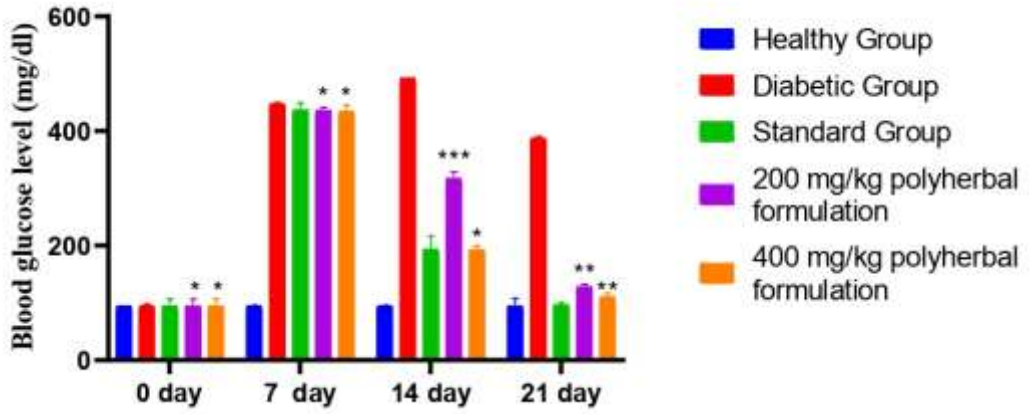
Fig 06. Effect of Polyherbal formulation on RBC

BLOOD GLUCOSE LEVELS:

The continuous administration of Polyherbal formulation was found to significantly reduce the blood glucose level in diabetic rats at the end of the experiment. Again, the effect was more pronounced in the rats treated with 400mg/kg of the extract and it compared favorably well with Glibenclamide treated rats.

Effect of Polyherbal formulation on blood glucose levels In Streptozotocin Induced Diabetic Wistar Rats. (n = 6, mean ± SEM).

Group	Treatment	0 day (mg/dl)	7 day (mg/dl)	14 day (mg/dl)	21 day (mg/dl)
Group I [Healthy group]	0.5ml of NS daily for 21 days.	94.1 ± 0.5	94.5 ± 1.1	94.3 ± 1.6	94.4 ± 13.5
Group II [Diabetic control group]	0.5ml of NS daily for 21 days.	94.7 ± 3.8	447 ± 2.7	492 ± 2.2	387.1 ± 3.2
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 21 days.	94.5 ± 12.4	436.4 ± 13	193.6 ± 23.4	96.2 ± 4.3
Group IV [Diabetic test group I]	200mg/kg of polyherbal formulation daily for 21 days.	94.3 ± 12.4*	435.6 ± 5.4*	317 ± 11.3***	127.8 ± 4.9**
Group V [Diabetic test group II]	400mg/kg of Polyherbal formulation daily for 21 days	94.5 ± 12.4*	434.2 ± 10.8*	192.7 ± 6.5*	110.1 ± 7**



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001, **p < 0.01 and *p < 0.05

Fig 07. Effect of Polyherbal formulation on blood glucose levels

DISCUSSION:

The diabetic state induced by streptozotocin (STZ) was confirmed by characteristic symptoms, including polydipsia, polyphagia, hyperglycemia, and polyuria. The polyherbal formulation (PHF) of *Gomphrena celosioides* and *Passiflora incarnata* demonstrated significant antidiabetic activity, with efficacy comparable to glibenclamide, a standard hypoglycemic drug. Treatment with PHF effectively mitigated excessive thirst and hunger, key manifestations of uncontrolled diabetes[15,16].

Glycemic Control

STZ-induced diabetes resulted in marked hyperglycemia, a hallmark of the condition. PHF administration significantly reduced blood glucose levels, restoring them toward normal ranges observed in non-diabetic control rats. This suggests enhanced glucose utilization, potentially through:

- Stimulation of pancreatic β-cell insulin secretion
- Improved peripheral glucose uptake
- Inhibition of hepatic gluconeogenesis

Lipid Profile Modulation

Diabetic rats exhibited dyslipidemia, including elevated total cholesterol, LDL, and reduced HDL—key risk factors for cardiovascular complications. PHF treatment:

- Reduced total cholesterol and LDL by inhibiting fatty acid mobilization from adipose tissue
- Increased HDL, thereby lowering the risk of atherosclerosis and myocardial infarction

These effects mirror insulin's role in suppressing hormone-sensitive lipase, highlighting PHF's potential to ameliorate diabetes-associated dyslipidemia.

Hematological Restoration

Diabetes-induced hematological abnormalities—such as decreased RBC, hemoglobin, and platelets alongside elevated WBC and lymphocytes—were reversed by PHF. This normalization indicates:

- Improved erythropoiesis and oxygen transport
- Restored immune function
- Reduced inflammation

Mechanistic Insights

The PHF's antidiabetic action may involve:

1. Pancreatic β-cell protection against STZ toxicity
2. Insulin-mimetic effects enhancing glucose uptake
3. Antioxidant activity countering oxidative stress in diabetes

CONCLUSION:

Our investigation demonstrated that oral administration of a polyherbal formulation containing *Gomphrena celosioides* and *Passiflora incarnata* exerted significant hypoglycemic effects in streptozotocin-induced diabetic Wistar rats. The study further revealed the formulation's capacity to ameliorate diabetes-associated dyslipidemia, indicating its dual therapeutic potential.

Key findings include:

1. **Glucose-lowering activity**: The formulation effectively reduced hyperglycemia in diabetic subjects
2. **Lipid profile modulation**: Treatment restored balance to impaired lipid metabolism parameters
3. **Hematoprotective properties**: The preparation showed protective effects against diabetes-induced blood abnormalities

These results suggest that the *G. celosioides* and *P. incarnata* polyherbal formulation offers comprehensive antidiabetic benefits beyond glycemic control, including protection against metabolic and hematological complications of diabetes mellitus.

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