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Biochemical Estimation of Allaxon Induced Hyperglycemia in Rats Treated with Polyherbal Preparation of Two of Indian Medicinal Plants *Cordia Myxa* and *Canscora Diffusa*

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Abstract

A systematic study was conducted on extracts of Cordia myxa and Canscora diffusa to evaluate their qualitative chemical composition, phytochemical content, and antidiabetic activity. Then the Poly herbal extract is prepared with the selected plant powders, subjected to acute-toxicity studies for dose fixation. The herbal extracts formulation was prepared taking three selected dosages. The preparations are evaluated for anti-diabetic activity using Steptozotocine and Alloxan induced diabetic models in rats. The polyherbal formulation was found to include flavonoids, phenolic compounds, glycosides, sterols, phenolic steroids, and carbohydrates. Toxicity tests, conducted following OECD guidelines, showed no harmful effects at a dose of 2000 mg/kg. Additionally, the formulation demonstrated a dose-dependent antidiabetic effect in a model of Alloxan-induced hyperglycemia.

Keywords: *Cordia myxa*, *Canscora diffusa*, hyperglycemia, diabetes, polyherbal formulation

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is marked by a gradual decline in insulin sensitivity and impaired function of β-cells. Despite genetic predisposition, factors like aging, obesity, and sedentary lifestyle contribute significantly to the disease. Current animal models often fail to closely replicate human T2DM, especially in capturing insulin resistance and β-cell dysfunction, the hallmarks of T2DM. This study explores the potential antidiabetic properties of a polyherbal formulation derived from *Cordia myxa* and *Canscora diffusa* using an Alloxan-induced diabetic rat model.

Under normal conditions, beta cells respond to insulin resistance by producing more insulin to maintain glucose balance. However, over time, this compensatory mechanism fails as beta cell function deteriorates, leading to impaired glucose regulation and eventually the progression from glucose intolerance to overt diabetes [1, 2]. In type 2 diabetes, there is typically a relative deficiency in insulin, as circulating insulin levels throughout the day are often comparable to or slightly higher than those of healthy individuals. While genetic predisposition plays a role, factors like aging, obesity, poor diet, and a sedentary lifestyle are the primary contributors to the onset of type 2 diabetes, with obesity being especially prevalent among those diagnosed [3, 4].

Although numerous animal models, both spontaneous and induced, are available for studying type 2 diabetes, most fail to fully mimic the disease's initiation and progression as

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observed in humans. Some genetic models, such as the Zucker diabetic fatty (ZDF) rat and the db/db mouse, develop diabetes spontaneously in a way that resembles human type 2 diabetes. However, diabetes in these models is predominantly driven by genetic factors, which are not entirely representative of the multifactorial nature of the disease in humans [5, 6]. Furthermore, findings from these inbred strains may not be universally applicable to humans due to the significant genetic and lifestyle diversity within the population. These models also have limitations, as they are costly and less accessible for routine research and screening.

In induced diabetic models, animals typically require high doses of streptozotocin (STZ; >50 mg/kg) to develop hyperglycemia [6]. This hyperglycemia is primarily caused by the destruction of pancreatic beta cells, resulting in insulin deficiency, rather than insulin resistance, which aligns more closely with human type 1 diabetes than type 2 diabetes [6, 7]. Consequently, these models often fail to respond effectively to medications that stimulate insulin secretion (e.g., glipizide, tolbutamide) or enhance insulin sensitivity (e.g., pioglitazone, rosiglitazone) [8].

In this research work antidiabetic activity was evaluated, by Allaxon Induced Hyperglycemia in Rats which was treated by polyherbal preparation at various dose intervals.

MATERIAL AND METHODS

Collection of Plant Material

The Leaves of selected plants namely *Cordia myxa* and *Canscora diffusa* was collected from Moolchand Phoolchand Herbal store, Bhopal (M.P.) in the month of Oct. 2019. Leaves of *Cordia myxa* and *Canscora diffusa* were identified by Dr. Saba Naaz, HOD, Department of Botany, Saifia Science College, Bhopal. Voucher specimens were preserved (Voucher Nos. 414/Saif/Sci/Clg/Bpl and 415/Saif/Sci/Clg/Bpl).

Pharmacognostical Studies

- *Macroscopic characteristics:* The leaves of *Cordia myxa* and *Canscora diffusa* were examined macroscopically to assess their shape, size, surface features, texture, color, consistency, odor, and taste [9].
- *Microscopic characteristics:* The leaves of *Cordia myxa* and *Canscora diffusa* were collected, and the sections were cleared with chloral hydrate, stained with safranin, and mounted in glycerin. The same procedure was applied to examine the microscopic characteristics of the powdered material from both plants [9].

Extract Preparation

The leaves were air-dried, powdered, and extracted with methanol using a Soxhlet apparatus. The extract was then concentrated using a rotary evaporator and kept in a desiccator. After the solvent had completely evaporated, the dried crude extract was kept in an airtight bottle at 4°C. The percentage yield was calculated for the hydroalcoholic extracts prepared in a 60:40 ratio. The hydroalcoholic extract of *Cordia myxa* and *Canscora diffusa* was used for the whole study [10].

Acute Toxicity Studies

Acute toxicity was evaluated in Swiss albino mice and Wistar rats following OECD guideline 423. No deaths or toxic effects were observed at doses up to 2000 mg/kg.

Phytochemical Screening

Phytochemical analysis showed that the plants contained carbohydrates, flavonoids, proteins, phenols, diterpenes, saponins, and steroids. Alkaloids were absent.

Preparation of Hydroalcoholic Extracts [11]

Powdered material of hydroalcoholic extract of *Cordia myxa* and *Canscora diffusa* leaves was subjected to solvent extraction using appropriate methods.

Preparation of Extract (Hydroalcohol Methanol and Water 60:40)

About 500 gm powdered material was subjected to extraction (by maceration method) using approx. 5.0 L purified water for 7 days. After 7 days, the menstruum was separated by filtration through muslin cloth. Marc was washed with approx. 500 ml fresh solvent and pressed to take off the solvent completely. The washings were filtered through muslin cloth and added to the menstruum. The solvent was removed using a rotary flash evaporator at approximately 45ºC ± 5ºC, and the extract was concentrated to a dry residue in a desiccator containing anhydrous sodium sulfate. The percentage yield of the extract was calculated relative to the air-dried powdered material, and its color, consistency, and yield were analyzed.

PHYTOCHEMICAL ANALYSIS

Qualitative Phytochemical Analysis

Preliminary chemical tests were carried out for hydroalcoholic extract of *Cordia myxa* and *Canscora diffusa* to identify different phytoconstituents like alkaloids, flavonoids, protein, amino acids, terpenoids, glycosides etc. [12, 13]

ACUTE TOXICITY STUDIES

The acute toxicity studies for two poly herbal preparations were conducted as per OECD 423 guideline.

Principle of the Test

Animals of the same sex are tested in a stepwise fashion using fixed doses of 5, 50, 300, and 2000 mg/kg, with a 5000 mg/kg dose considered only in exceptional cases. The initial dose is chosen based on a preliminary sighting study, aiming for a level that causes mild toxicity without severe adverse effects or mortality. Clinical signs, pain, suffering, and signs of impending death are detailed in a separate OECD Guidance Document. Based on the observed toxicity or mortality, further groups may receive higher or lower doses. This process continues until a dose that results in clear toxicity, no more than one death, or no effects at the highest dose is found, or if deaths occur at the lowest dose.

Selection of Animals

Swiss albino mice weighing 20–25 g and Albino Wistar rats weighing 150–250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and kept under standard conditions, including a 12-hour light/dark cycle, a temperature of 25 ± 3ºC, and humidity levels ranging from 35 to 60%. They had free access to standard pelletized feed and tap water.

After five days, the chosen animals were divided into four groups, each consisting of three animals. Each group was administered with a fixed single dose of the polyherbal preparation. The animals were monitored individually after doing exercise, with checks done at least once within the first 30 minutes, periodically over the first 24 hours, and with particular attention during the first 4 hours. Daily observations continued for 14 days to detect any signs of toxicity or mortality.

EVALUATION OF ANTI DIABETIC ACTIVITY

Oral Glucose Tolerance Test (OGTT)

The rats were separated into four groups ($n = 6$). Blood glucose levels were measured at various intervals after administering the test drug, the standard drug (Glibenclamide), or distilled water.

Alloxan-Induced Hyperglycemia

Diabetes was induced in the rats using Alloxan monohydrate (80 mg/kg). Those with fasting blood glucose levels greater than 200 mg/dL were assigned to six groups and treated with different doses of the polyherbal formulation or Glibenclamide for 14 days. Blood samples were collected and analyzed for glucose, cholesterol, triglycerides, HDL, LDL, serum urea, and creatinine levels.

Oral Glucose Tolerance Test (OGTT)

The rats were fasted overnight and then separated into four groups, with 6 animals in each group. Group I was given distilled water as the control. Group II was treated with Glibenclamide (0.5 mg/kg) as the standard. Group III received PHF-I (500 mg/kg body weight). All groups administered their respective treatments 30 minutes before a glucose load (2.5 g/kg). Blood samples were collected at 15-, 30-, 45-, 60-, 75-, 90-, and 120-minutes following glucose administration. The serum was separated, and glucose levels were immediately measured.

ALLAXON INDUCED HYPERGLYCEMIA IN RATS

A single dose of Alloxan monohydrate (80 mg/kg body weight, s.c.), dissolved in ice-cold normal saline, was used to induce diabetes in rats after an overnight fast. One hour after Alloxan administration, the animals were given standard pellets and allowed free access to water. To prevent hypoglycemia, they were provided with a 20% glucose solution in their cages for the following 24 hours. After 72 hours, fasting blood glucose levels were measured using the GOD-POD method. Rats with blood glucose levels above 200 mg/dL were selected for the study. The rats were then separated into six groups, each containing six animals, and treated for 14 days.

- Group I: Normal control (saline).
- Group II: Alloxan treated control (150 mg/kg.ip).
- Group III: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o).
- Group IV: Alloxan (150 mg/kg.ip) + Poly herbal preparation (100 mg/kg, p.o).
- Group V: Alloxan (150 mg/kg.ip) + Poly herbal preparation (200 mg/kg, p.o).
- Group VI: Alloxan (150 mg/kg.ip) + Poly herbal preparation (400 mg/kg, p.o).

Blood samples were collected from the tail tip of the rats weekly throughout the 2-week study. Fasting blood glucose levels and body weight were measured on days 0, 7, and 14. Blood glucose was measured using a One Touch electronic glucometer. On day 14, blood was collected from the retro-orbital plexus of overnight-fasted rats under mild ether anesthesia to assess fasting blood glucose. The serum was separated and analyzed for cholesterol, triglycerides (using the enzymatic DHBS colorimetric method), HDL, LDL, creatinine, urea, and alkaline phosphatase (using the hydrolyzed phenol amino antipyrine method). After the animals were euthanized, their entire pancreas was removed, fixed in 10% formalin, and processed using the paraffin method. Thin (5 μ) sections were cut and stained with hematoxylin and eosin (H&E) for histological analysis.

Body weight was calculated for all the animals in all groups on Day 0, 7 and 14 days to assess the toxicity induced.

RESULTS AND DISCUSSION

Yield of Plant Extracts

Total yield of plant extracts is varying based on the solvents used in this study. The alcoholic extracts of *Cordia myxa* and *Canscora diffusa* produced higher yield (8.67 and 9.11 mg/g). In general, extractability of a particular component appeared to depend on extraction medium polarity and the ratio of solute to solvent (Table 1).

Table 1. Yield of plant extracts.

Solvent Extracts	***Cordia Myxa* (g)**	***Canscora Diffusa* (g)**
Petroleum extract.	2.66	2.9
Ethyl acetate extract.	4.6	4.13
Hydroalcoholic extract.	8.76	9.11

Phytochemical Screening of the Plant Extract

Phytochemical screening of extracts of *Cordia myxa* and *Canscora diffusa*. Extracts showed significant levels of flavonoids, phenolics, glycosides, sterols, and carbohydrates, indicating potential antidiabetic activity (Table 2).

Table 2. Preliminary phytochemical screening of *Cordia myxa*.

S. N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1.	Alkaloids.	Wagner's Test	–(ve)
2.	Carbohydrates.	Fehling's Test	+(ve)
3.	Flavonoids.	Lead acetate	+(ve)
		Alkaline reagent test	+(ve)
4.	Proteins & Amino acids.	Precipitation test	+(ve)
5.	Phenols.	Ferric chloride test	+(ve)
6.	Diterpenes.	Copper acetate test	+(ve)
7.	Saponins.	Foam test	+(ve)

Table 3. Preliminary phytochemical screening of *Canscora diffusa*.

S. N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1.	Alkaloids.	Wagner's test.	–(ve)
2.	Carbohydrates.	Fehling's test.	+(ve)
3.	Flavonoids.	Lead acetate.	+(ve)
		Alkaline reagent test.	+(ve)
4.	Proteins & Amino acids.	Precipitation test.	+(ve)
5.	Phenols.	Ferric chloride test.	+(ve)
6.	Diterpenes.	Copper acetate test.	+(ve)
7.	Saponins.	Foam test.	+(ve)

The phytochemical analysis revealed the presence of carbohydrates, flavonoids, proteins and amino acids, phenols, diterpenes, and saponins in the extracts of *Cordia myxa* and *Canscora diffusa*. However, alkaloids were not detected. The results also showed that all polar, methanolic, and aqueous soluble compounds were present in the leaf extracts of both plants (Table 3).

ACUTE TOXICITY STUDIES

Acute Toxicity

No adverse effects or mortality were observed at a dose of 2000 mg/kg, confirming the safety of the formulation.

Antidiabetic Activity

- *OGTT results:* Polyherbal formulation improved glucose tolerance significantly ($P < 0.05$).
- *Alloxan-induced hyperglycemia:* Significant reductions in fasting blood glucose levels, lipid profile improvement, and enhanced renal markers were observed with dose-dependent effects. Histological analysis of the pancreas indicated potential β-cell regeneration.

Hence 1/5th, 1/10th and 1/20th of the dose of 2000 mg/kg, i.e., 100 mg/kg, 200 mg/kg and 400 mg/kg has been fixed as ED50 for the evaluation of Poly herbal formulation.

EFFECT OF POLYHERBAL FORMULATION IN ALLOXAN INDUCED DIABETES IN RATS

Glucose Tolerance

The effects of PHF (100 mg/kg, 200 mg/kg and 400 mg/kg) on glucose tolerance tests. The supplementation of PHF improved the glucose tolerance in fasted normal rats. Serum glucose levels decreased significantly (P < 0.05) to 90 minutes and continued to decrease significantly (P < 0.01) to 150 minutes. Both preparations also demonstrated a significant hypoglycemic effect 90 minutes after treatment (Table 4) (Figure 1).

Table 4. Effect of various groups of Poly herbal formulation on glucose level in diabetic rats.

Group/Treatment	Blood Glucose Levels (mg/dl)			
	0 min	**30 min**	**90 min**	**150 min**
Control	64.78 ± 1.1	67.43 ± 1.7	64.22 ± 2.8	69.25 ±14
Glucose treated	70.44 ± 6.9*	156 ± 6.6*	134 ± 11.4*	125.4 ± 6.2*
Glibenclamide (5 mg/kg) + glucose (2 g/kg).	70.87 ± 2.47#	68.54 ± 1.5#	66.25 ± 41#	60.38 ± 0.4#
PHF (100 mg/kg) + glucose (2 g/kg).	71.25 ± 5.4	139.54 ± 3.5	115.47 ± 2.5	97.14 ± 0.8
PHF (200 mg/kg) + glucose (2 g/kg).	69.74 ± 6.6**	118.94 ± 3.8**	100.4 ± 7.9**	80.14 ± 0.55**
PHF (400 mg/kg) + glucose (2 g/kg).	67.58 ± 4.7**	80.96 ± 6.5**	77.5 ± 7.7**	65.34 ± 8.8**

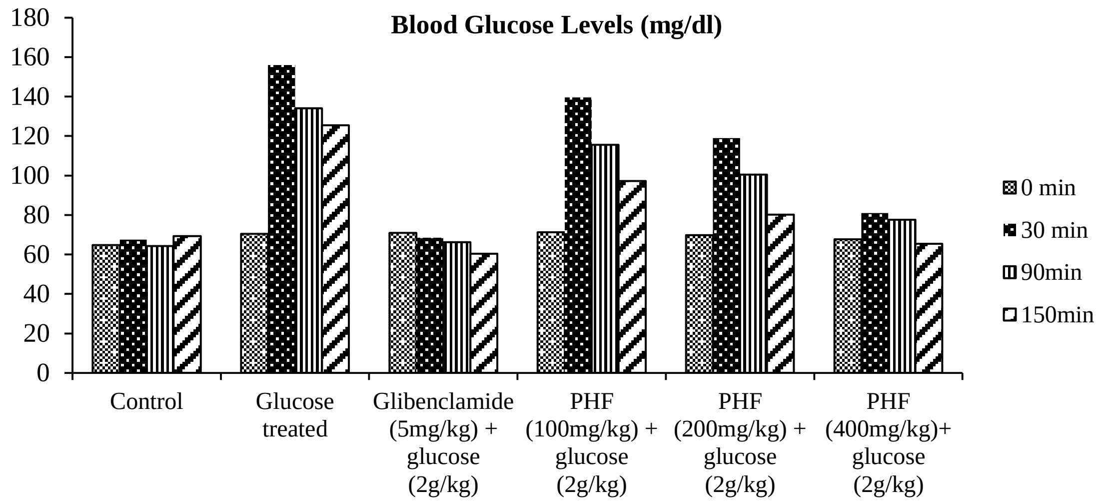

Figure 1. Effect of various groups of Poly herbal formulation on glucose level in diabetic rats.

The values are expressed as mean ± SEM for groups of six animals each. *P < 0.01 indicates that the diabetic control group was compared with the vehicle control. #P < 0.001 shows a comparison with the Alloxan group, and **P < 0.001 represents a comparison with the Alloxan group, all analyzed using one-way ANOVA followed by Dunnett's multiple comparison test (Table 5) (Figure 2).

Table 5. Effect of various groups of Poly herbal formulation on blood glucose level in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

Group	Treatment	0th day	7th day	14th day
Group-I.	Control 1 % gum acacia.	64.66 ± 2.2	64.31 ± 1.1	66.57 ± 8.7
Group-II.	Diabetic control 1 % gum acacia.	182.59 ± 4.4*	190.25 ± 9.8*	100.8 ± 7.4*
Group-III.	Glibenclamide 5 mg/kg.	188.47 ± 6.5#	144.7 ± 2.5#	98.7 ± 2.4#
Group-IV.	PHF 100 mg/kg.	181.47 ± 11.7	162.5 ±1.5	112.54 ± 3.5
Group-V.	PHF 200 mg/kg.	180.33 ± 6.88**	157.25 ± 2.8**	97.14 ± 2.58**
Group-VI.	PHF 400 mg/kg.	179.22 ± 8.8**	154.14 ± 6.8**	88.47 ± 1.4**

Group-VII PHF-II 100 mg/kg 179.56 ± 12.5 159.27 ± 1.4 138.35 ± 2.5 Group-VIII PHF-II 200 mg/kg 169.54 ± 5.69** 148.96 ± 5.6** 104.63 ± 5.25** Group-IX PHF-II 400 mg/kg 175.51 ± 7.6** 146.65 ± 5.6** 82.63 ± 2.2**.

The values are presented as mean ± SEM for groups of six animals each. *P < 0.01 indicates a comparison between diabetic control and vehicle control. #P < 0.001 represents a comparison with the Alloxan group, and **P < 0.001 denotes a comparison with the Alloxan group, all assessed using one-way ANOVA followed by Dunnett's multiple comparison test (Tables 6 and 7) (Figures 3 and 4).

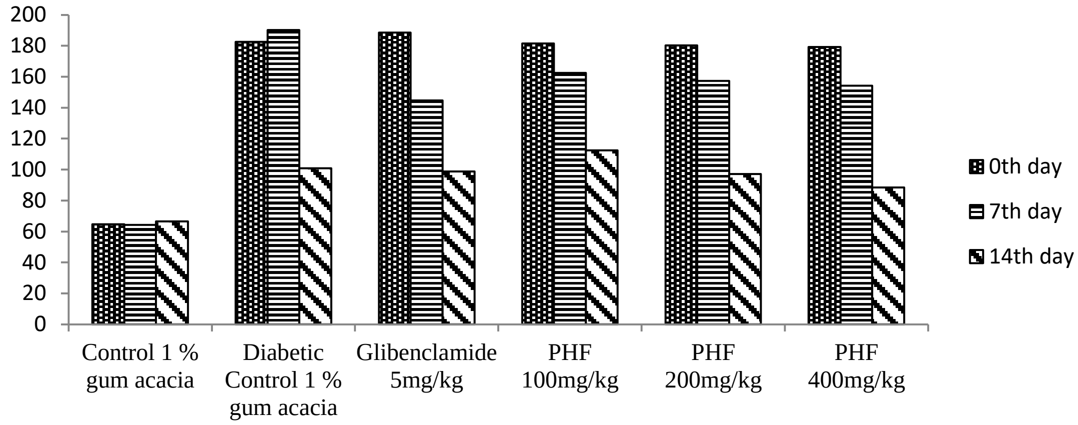

Figure 2. Effect of various groups of Poly herbal formulation on blood glucose level in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

Table 6. Effect of various groups of Poly herbal formulation on body weight in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

Group	**Treatment**	**Body Weight (Gms)**		
		0th Day	**7th Day**	**14th Day**
Group-I.	Control 1 % gum acacia.	168.78 ± 1.9	164.77 ± 3.1	170.48 ± 1.5
Group-II.	Diabetic Control 1 % gum acacia.	181.47 ± 6.6*	170.54 ± 1.4*	159.47 ± 3.2*
Group-III.	Glibenclamide 5 mg/kg.	168.78 ± 2.22#	163.47 ± 4.5#	160.58 ± 1.4#
Group-IV.	PHF 100 mg/kg.	176.85 ± 11.45	171.25 ± 1.7	166.74 ± 4.4
Group-V.	PHF 200 mg/kg.	178.59 ± 6.6**	179.8 ± 4.44**	181.7 ± 5.5**
Group-VI.	PHF 400 mg/kg.	176.8 ± 7.8**	177.7 ± 4.4**	180.11 ± 2.4**

Table 7. Effect of various groups of Poly herbal formulation on serum profile in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

Group	**Treatment**	**Cholesterol (mg/dl)**	**H.D.L (mg/dl)**	**L.D.L (mg/dl)**	**Creatinine (mg/dl)**
Group-I.	Control 1 % gum acacia.	158.7 ± 4.6	37.88 ± 1.5	92.5 ± 1.5	0.49 ± 0.4
Group-II.	Diabetic Control 1 % gum acacia.	294.1 ± 8.9*	31.01 ± 2.2*	190.1 ± 1.11*	2.14 ± 0.2*
Group-III.	Glibenclamide 5 mg/kg.	148.6 ± 6.8#	37.85 ± 2.1#	93.11 ± 4.2#	0.6 ± 0.1#
Group-IV.	PHF 100 mg/kg.	188 ± 2.5	33.57 ± 5.8	152.47 ± 1.5	1.44 ± 0.59
Group-V.	PHF 200 mg/kg.	181.74 ± 6.6**	36.87 ± 3.3**	132.67 ± 8.8**	0.99 ± 0.33**
Group-VI.	PHF 400 mg/kg.	161.52 ± 8.5**	37.88 ± 3.3**	95.25 ± 2.8**	0.71 ± 0.3**

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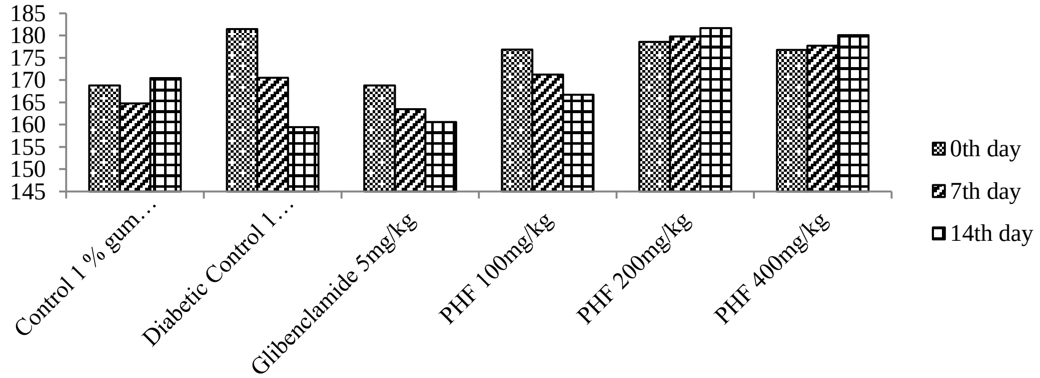

Figure 3. Effect of various Poly herbal formulations on body weight in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

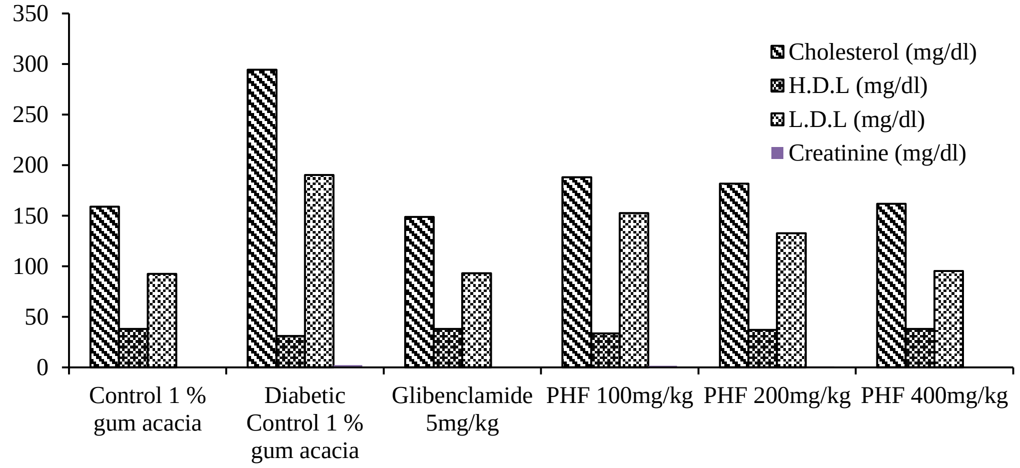

Figure 4. Effect of various groups of Poly herbal formulation on serum profile in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

CONCLUSIONS

The polyherbal formulation of *Cordia myxa* and *Canscora diffusa* demonstrated significant antidiabetic properties in Alloxan-induced hyperglycemia models. The results indicate that these plants have potential as complementary treatments for diabetes management, thanks to their ability to regenerate β-cells and enhance metabolic parameters. Further clinical research is required to validate these findings.

REFERENCES

1. Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes. 1996;45:1661–9.
2. Lebovitz HE, Banerji MA. Treatment of insulin resistance in diabetes mellitus. Eur J Pharmacol. 2004;490:135–46.
3. Ramarao P, Kaul CL. Insulin resistance: Current therapeutic approaches. Drugs Today. 1999;35:895–911.
4. McIntosh CHS, Pederson RA. Non insulin dependent animal models of diabetes mellitus. In: McNeil JH, editor. Experimental models of diabetes. Florida: CRC Press LLC; 1999. p. 337–98.

5. Luo J, Quan J, Tsai J, Hobensack CK, Sullivan C, Hector R, et al. Nongenetic mouse models of non-insulin-dependent diabetes mellitus. Metabolism. 1998;47:663–8.
6. Shafrir E. Diabetes in animals: Contribution to the understanding of diabetes by study of its etiopathology in animal models. In: Porte D, Sherwin RS, Baron A, editors. Diabetes mellitus. NewYork: McGraw-Hill; 2003. p. 231–55.
7. Rerup CC. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev. 1970;22:485–518.
8. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S. Islet secretion in a new experimental model for non insulin dependent diabetes. Diabetes. 1981;30:590–5.
9. Khandelwal, K_R. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008.
10. Martins N, Barros L, Santos-Buelga C, Silva S, Henriques M, Ferreira IC. Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. Food Chem. 2015;167:131–137.
11. Akbaribazm, M., Khazaei, M. R., & Khazaei, M. (2020). Phytochemicals andantioxidant activity of alcoholic/hydroalcoholic extract of Trifolium pratense. Chin Herb Med. 2020;12(3):326–335
12. Harborne, A. J.Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media; 1998.
13. Chanda, S., & Ramachandra, T. V. (2019). A review on some Therapeutic aspects of Phytochemicals present in Medicinal plants. Int J Pharmacy Life Sci. 2019;10(1).