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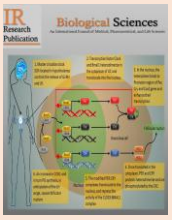

Development and Evaluation of Polyherbal Formulation for Anti Diabetic Potential in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes is the most common metabolic disorder that involves various pathogenic mechanisms and is characterized by high blood sugar levels. Genetic and environmental factors cause insufficient insulin secretion, increased glucose production, abnormal fat, or protein metabolism. Major sources of diabetes are chronic complications that started with prolonged hyperglycemia. According to ayurvedic scriptures such as Charak Samhita, polyherbal formulations have been mentioned to have anti-diabetic potentials. Thus, the present study was aimed to find the anti-diabetic effect of polyherbal formulation in rat models. Diabetes was induced by a single dose of Streptozotocin (60 mg/kg) for 72 hours. The effect of the polyherbal formulation was studied in diabetic rats for 21 days. The biochemical and anti-oxidant parameters such as fasting blood glucose, cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Urea, Uric acid, LPO, SOD, and GSH were measured during the study. The polyherbal formulation produced a significant reduction of fasting blood glucose and lipid profile. Kidney function tests, liver function tests, and anti-oxidant parameters were also improved. Overall, after 21 days of study, the results show that the polyherbal formulation is much more effective for type 1 diabetes in rats.

Introduction

Diabetes is a metabolic disorder of numerous etiology characterized by hyperglycemia, abnormal carbohydrate, fat, and protein metabolism leading to insulin dysfunction. It includes multiple organ failure and chronic illness. Diabetes shows symptoms like thirst, polyuria, blurred vision, weight loss in its most dangerous form, ketoacidosis, or non-ketotic hyperosmolar in severe stage led to death. In a chronic period, it affects our eye retina, kidney, and neurons with the risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction. Diabetic people generally have a high risk of cardio, peripheral, and cerebrovascular disease [1,2,3]. Its treatment is known from the middle ages, but the pathogenic fact was revealed mainly in the 20th century. In 1889

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Joseph Von Mering and Oskar Minkowski discovered the pancreas' role, while in 1910, sir Edward Albert Sharpey Schafer of Edinburgh talked about single chemical produce by the pancreas. Himsworth 1936 proposed it to be insulin. In 1935 Himsworth identify type 1 and type 2 diabetes [4,5]. Globally diabetes has become a major concern, especially in developing nations, which restricts urbanization and has a poor lifestyle. As per the international diabetic federation, it directs various acknowledging modulus and projecting to sort out issue diabetes globally on this issue. Approximately 463 million adults who were diabetes in 2019 will increase by 700 million by 2045. The prevalence and progress of diabetes are increasing rapidly. The increase in burden in developing countries follows the trend of urbanization and lifestyle change, less physical activity, and high-fat diet intake [6]. The formulation containing two or more herbs in a specific ratio is called polyherbal formulation. This concept was taken from ayurvedic literature Samhita, illustrating the concept of synergies behind polyherbal formulation since sometimes single herbs do not produce desirable therapeutic effects. Although single herbs have produced well established therapeutic effect, scientific studies discovered that the herbs have varying potency when used in combination. Thus, producing a more significant result in contrast to the single herb. The new FDA and EMEA guidelines provide acceptance towards synergistic combinations of plant-derived bioactive products [7,8]. In accordance with the aforementioned pieces of literature, in the current study, we aimed to develop and evaluate polyherbal formulation for anti-diabetic activity in Streptozotocin-induced diabetic rats.

Materials and methods

Collection of the Plant Materials and Authentication

The plants were selected based on their anti-diabetic activities and their medicinal uses as reported in the Literature. The plant material of polyherbal formulation was purchased from the local area and authenticated by the Botanical Survey of India Dehradun.

Extraction of Plant Materials

The powdered crude drug of *Allium cepa* bulb, *Allium sativum* bulb, *Trigonella foenum-graecum* seed, and *Curcuma longa* rhizome was extracted through the soxhlet method using ethanol as a solvent. The extraction procedure was carried till a sufficient quantity of extract was obtained. The solvent was removed by the distillation method [9].

Preparation of Polyherbal Formulation

The dried powder of extract of *Allium cepa*, *Allium sativum, Trigonella foenum-graecum,* and *Curcuma longa* was mixed in the optimized ratio of (1:1:1) to from the polyherbal formulation [10].

Animals Selection

Wistar albino rats of either sex (Body Weight 200 to 250 gm) were used for the study. First of all, permission was taken from the Institutional Animal Ethics Committee (Approval No. CPCSEA/IAEC/SBS/2019-20/005) for conducting the experimental animal studies. The animals were placed in standard polypropylene cages and maintained under controlled room temperature (22±2°C) and humidity (55±5 %) with 12:12 hours light and dark cycle. A normal pellet diet and water were provided to the animals throughout the study.

Induction of Diabetes

Streptozotocin (60 mg/kg) was used to induce experimentally diabetes mellitus. Diabetes was induced by a single dose of Streptozotocin for 72 hours. After 72 hours, blood was collected from animals, and serum was separated. Animals that showed an increase in fasting blood glucose levels more than 200 mg/dl were considered diabetic and included in the study [11].

Experiment design

The animals were divided into 6 groups with 6 animals in each group and received the following treatment for 21 days.

Group 1: Normal Control (NC): Rats received normal saline orally (1ml/kg) for 21 days.

Group 2: Diabetic Control (DC): Diabetic rats received a vehicle (1ml/kg) orally for 21 days.

Group 3: Standard Group (STD): Diabetic rats received Glibenclamide (10 mg/kg) for 21days.

Group 4: Treatment 1 (TRT1): Diabetic rats received polyherbal formulation (100 mg/kg) for 21 days.

Group 5: Treatment 2 (TRT2): Diabetic rats received polyherbal formulation (200 mg/kg) for 21 days.

Group 6: Treatment 3 (TRT3): Diabetic rats received polyherbal formulation (400 mg/kg) for 21 days.

Selection of Dose

The dose selection of polyherbal formulation (100 mg/kg, 200 mg/kg, 400mg/kg) was made based on the previous anti-diabetic activity of polyherbal formulation determined in Streptozotocin-induced diabetes [12]. Similarly, the dose (10 mg/kg) of Glibenclamide was selected based on previous studies [13].

Estimation of different biochemical parameters

The blood samples were collected from the retroorbital plexus of the rats, and the serum was separated by centrifugation. The biochemical parameters, such as fasting glucose level, triglyceride, total cholesterol, HDL, LDL, VLDL, were measured on 0, 7, 14, and 21 days post-treatment. Parameters of diabetes complications such as SGOT, SGPT, Urea, Uric acid, LPO, SOD, and GSH were measured on day 21.

Statistical analysis

The statistical analysis was carried out using Graph Pad Prism 5.0 software. All values were presented as Mean ± SEM and statistically assessed by One way analysis of variance (ANOVA) followed by Tukey's test. The values were considered to be significant when $p < 0.05$

Results and discussion

In the present study, we evaluated the anti-diabetic effect of polyherbal formulation on Streptozotocin-induced diabetic rats and its complications. Animals which having fasting glucose level more than 200 mg/dL were selected for the study. Animals were divided into 6 groups, and each group containing 6 animals. One group served as normal control. Polyherbal formulation extract was given for 21 days. Biochemical parameters such as fasting glucose level, triglyceride, total cholesterol, HDL, LDL, and VLDL were measured on 0, 7, 14, and 21 days. Parameters of diabetes complications such as SGOT, SGPT, Urea, Uric acid, LPO, SOD, and GSH were measured on day 21.

Effect of treatment on fasting blood glucose level (hypoglycemic activities)

The blood glucose level was measured on 0, 7, 14, and 21 days of the treatment. There was an increase in the blood glucose level after the injection of Streptozotocin. After 21 days of the treatment, it was found that there was a more significant ($p<0.001$) decrease in blood glucose level in animals treated with the polyherbal formulation and standard drug Glibenclamide as compared to the diabetic control group (**Table 1**).

Effect of treatment on lipid profile (hypolipidemic activity)

A lipid profile is one of the most important cardiovascular parameters, which was measured on 0 and 21 days of the study. It was increased after the injection of Streptozotocin. After 21 days of study, it was found that there was a significant ($p<0.001$) reduction in lipid profile in rats when we treat with the polyherbal formulation. Findings are tabulated in **Table 2** to **6**.

Effect of treatment on liver function test

Liver function tests such as SGOT & SGPT were measured on 21 days after the treatment. There was a significant ($P<0.001$) reduction in SGOT & SGPT upon treatment. The TRT1, TRT2, TRT3, and STD (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg). groups were significant when it's compared with DC (**Table 7**).

Table 1. Blood glucose level (mg/dL) in normal and treated groups measured on days 0, 7, 14, and 21 of study

Fasting Blood Glucose (mg/dL)					
	Before Induction of Diabetes	**0th Day**	**7th Day**	**14th Day**	**21st Day**
NC	85.51±1.47	87.80± 2.42	84.79± 0.68	87.50± 1.64	91.93± 0.64
DC	79.13±2.46	276.30±4.05	293.29± 2.58	304.34± 3.00	317.45± 3.66
STD	89.21±3.59	275.40±3.59	210.94± 9.34	175.36±8.41***	99.19±2.39***
TRT1	91.13±4.87	250.19±8.63	206.92± 5.30	158.73± 4.1***	105.89±4.1***
TRT2	85.94±2.41	270.94±5.18	200.74±3.73**	146.89±7.33***	104.95±3.0***
TRT3	84.79±1.68	287.60±6.68	231.37± 3.24	180.53±2.90**	99.47±0.95***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n= 6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Table 2. Cholesterol (mg/dl) recorded in normal, diabetic, and treated rats on days 0 and 21 of the study

	0th Day	**21st Day**
NC	74.19± 1.50	76.44± 2.06
DC	142.77± 1.24	145.76± 1.57
STD	140.78±1.67	77.89± 1.26***
TRT1	134.11± 1.37	82.53± 1.90**
TRT2	139.45± 1.61	80.42± 1.3**
TRT3	141.84± 1.11	78.92±1.30***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF

Table 3. Triglyceride Level (mg/dl) was recorded in normal, diabetic, and treated rats on days 0 and 21 days of studies.

	0th Day	**21st Day**
NC	79.85± 1.29	82.08± 0.72
DC	149.97±2.22	168.48± 1.13
STD	141.26±0.81	88.93± 1.81***
TRT1	138.33±2.93	97.33± 1.86**
TRT2	143.05± 2.57	90.36± 2.04 ***
TRT3	143.57± 1.68	89.35± 0.82***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Table 4. HDL Cholesterol (mg/dl) was recorded in normal, diabetic, and treated rats on days 0 and 21 of the study.

	0th Day	**21st Day**
NC	31.01± 0.49	34.08± 1.50
DC	14.72± 1.19	13.82± 0.68
STD	17.23± 0.56	33.45± 0.73***
TRT1	14.82± 0.43	28.32± 0.50**
TRT2	12.98± 0.52	32.82± 0.59***
TRT3	11.80± 2.38	35.47± 0.76***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Effect of treatment on kidney function

Kidney function parameters such as urea and uric acid were measured on 21 days of the study. After 21 days of study, it was found significant (P<0.001) reduction in urea and uric acid in rats when treated with polyherbal formulation extracts (**Table 8**).

Effect of treatment on oxidative stress parameters

Anti-oxidant tests such as LPO, SOD, GSH were performed on 21 days after the injection of

Streptozotocin. On 21 days of study, we found a significant alteration in LPO, SOD, and GSH levels in DC group. However, The TRT1, TRT2, TRT3, and STD groups demonstrated significantly improved levels when compared with the DC animals (**Figure 1** to **3**).

Table 5. LDL (mg/dl) recorded in normal, diabetic, and treated rats on days 0 and 21 of the study.

	0th Day	21st Day
NC	27.20± 1.99	26.28± 3.04
DC	98.98± 1.47	98.23± 2.23
STD	97.54± 2.36	27.36± 1.15***
TRT1	94.01± 2.71	35.78±1.97**
TRT2	96.00± 1.69	28.04± 1.69***
TRT3	100.14± 1.47	25.65± 1.71***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Table 6. VLDL (mg/dl) was recorded in normal, diabetic, and treated rats on days 0 and 21 of the study.

	0th Day	21st Day
NC	15.96± 0.25	16.41± 0.14
DC	29.99± 0.44	33.69± 0.28
STD	27.28± 0.32	17.78± 0.36***
TRT1	28.15± 0.21	19.46± 0.37**
TRT2	28.59± 0.51	18.07± 0.40**
TRT3	28.71± 0.33	17.86± 0.20***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Table 7. SGOT & SGPT level (IU/L) in normal, diabetic and treated groups measured on day 21 of study.

Groups	SGOT(IU/L)	SGPT(IU/L)
NC	51.46± 1.47	29.30± 0.92
DC	80.96± 0.98	53.10± 1.32
STD	68.47± 1.80*	32.48± 0.96**
TRT1	65.68± 1.60*	34. 18± 1.38**
TRT2	62.28± 2.90**	32.00 ± 0.64**
TRT3	47.50± 1.16***	28.33± 1.02***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Table 8. Urea, Uric acid (mg/dl) level in normal, diabetic and treated groups measured on day 21 of study.

Groups	Uric acid (mg/dl)	Urea (mg/dl)
NC	0.53± 0.13	18.26± 0.55
DC	2.71± 0.27	39.87± 1.39
STD	1.05± 0.08**	19.97± 0.36***
TRT1	1.49± 0.16*	23.79± 1.05**
TRT2	1.11± 0.05**	20.34± 0.42**
TRT3	0.7± 0.11***	17.46± 0.69***

*P<0.05, **P<0.01, ***P<0.001 when compared treated groups with diabetic control (One Way ANOVA). Values are given as mean ± SEM (n=6/group). NC-Normal Control, DC- Diabetic Control, STD- Standard, TRT1- PHF (100 mg/kg), TRT2- PHF (200 mg/kg), TRT3-PHF (400 mg/kg).

Conclusion

From the results, it can be concluded that polyherbal formulations have a role in improving hyperglycaemic disorder. The polyherbal formulation at a dose of 400 mg/kg shows a better result than the other doses. Thus, the polyherbal formulation is the safest therapy and easiest method of preventing and control diabetes. Further study can be carried out at different dose levels and other models of diabetes such as genetically induced diabetes animal models and chemical-induced diabetes models for more scientific data.

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Conflict of Interest

No conflict of interests

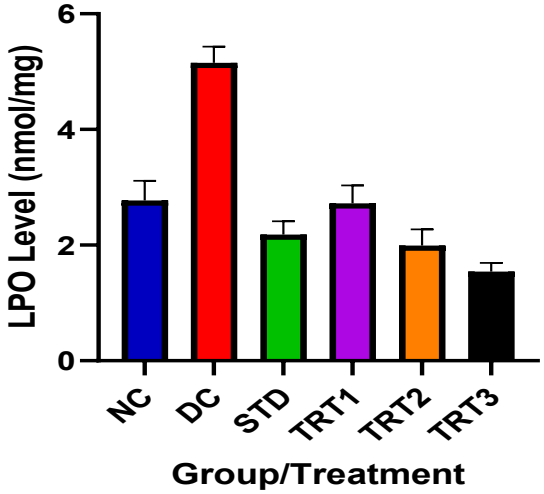

Figure 1. Effect of treatment on LPO level (nmol/mg) in normal, diabetic, and treated rats on 21st day. The column represents the mean± SEM (n=6)

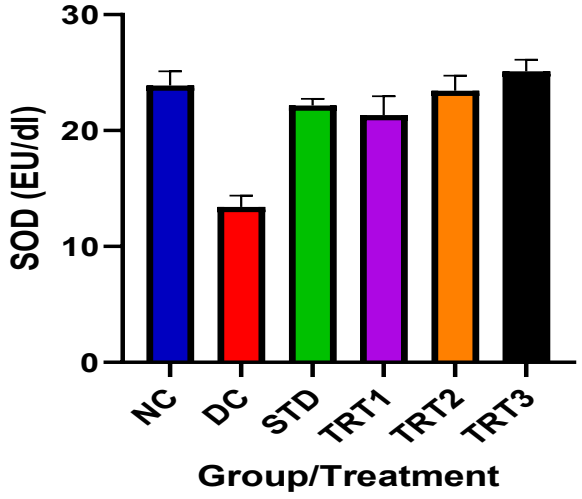

Figure 2. Effect of treatment on SOD level (µg/mg protein) in normal, diabetic, and treated rats on 21st day. The column represents the mean± SEM (n=6)

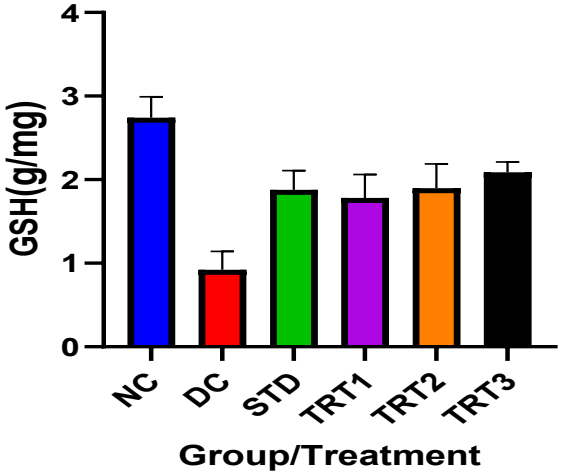

Figure 3. Effect of treatment on GSH level (µg/mg protein) in normal, diabetic, and treated rats on 21st day. The column represents the mean± SEM (n=6)

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