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Antidiabetic, Hypolipidemic and Antioxidant Activities of Polyherbal Formulations

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ABSTRACT

To consider the Antidiabetic, hypolipidemic and antioxidant potential of the polyherbal formulations I and II were investigated in alloxan-induced diabetic rats. To induce diabetes, alloxan was administered intraperitoneal (150mg/kg; i.p.). Effect of polyherbal formulations on blood glucose levels of diabetic rats was determined at various time intervals. The biochemical parameters studied were serum glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and low-density lipoprotein. All these were compared with metformin as a reference Antidiabetic drug. In order to determine the antioxidant activity of extract, liver tissue was homogenized in ice-cold saline buffer and assay of *lipid peroxidase* (LPO), *superoxide dismutase* (SOD), reduced glutathione (GSH) and catalase (CAT) was performed for *in vivo* activity. Oral administration of polyherbal formulations for 21 days resulted in a significant reduction in blood glucose level. And there is a significant increase in LPO, SOD, GSH, and CAT in liver tissues of alloxan-induced diabetic rats when compared with untreated diabetic rats. Thus, the polyherbal formulation I showed significant Antidiabetic, hypolipidemic and antioxidant effects than polyherbal formulation II in alloxan-induced diabetic rats.

1. INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. It is most common of the serious metabolic disease. The most common form of diabetes is diabetes mellitus, a chronic progressive, the systemic condition of impaired carbohydrate metabolism. Its major manifestations include disorder metabolism and inappropriate hyperglycemia. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and sharp reduction of antioxidant defense. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Treatment imposes an economic burden and the documented incidence is quite ambiguous.

1.1 Aim:

To determine the Antidiabetic, hypolipidemic and antioxidant potential of the polyherbal formulations I and II to investigate in alloxan-induced diabetic rats.

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1.2 Objective:

1. To perform the anti-diabetic activity of polyherbal formulation.

2. To evaluate the hypolipidemic activity of polyherbal formulation.

3. To investigate the antioxidant potential of the polyherbal formulations.

2. MATERIALS AND METHODS

2.1 Polyherbal materials

Both polyherbal formulations I and II were obtained.

P.H.F-1 consists of *Emblica Officinalis, Momordica quarantine, Azadirachta indica, Syzygium cumini, Curcuma longa, Coscinium fenestratum, Terminalia belerica, Terminalia chebula, Pterocarpus marsupium, Sesbania grandiflora, Gymn-esea sylvestere.*

P.H.F-2 consists of *Gymnema sylvestre, Momordica charantia, Trigonella foenum gracum, Emblica Officinalis, Oscimum sanctum, Syzygium cumini, Tinospora cordi-folia, Asphaltum, Andrographis paniculata, Berberis aristata.*

2.2 Preparation of Extract

The suspensions of polyherbal formulations were prepared by using 0.5% SCMC in distilled water as a solvent for the experiment.

2.3. Animals

Male Albino Wistar rats (150-200 g) were obtained from Mahaveer enterprises, Hyderabad, before and after the experiment the animals were fed with the standard diet. After randomization into various groups and before initiation of the experiment, rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 had libitium. Animals were injected intraperitoneally (i.p.) with freshly prepared Alloxan monohydrate in normal saline in a dose of 150mg/kg. Rats after 48 h with blood glucose level 200mg/dl were considered to be diabetic and were used in the experiment.

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2.4. Sample collection

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using a spectrophotometric GOD-POD method. Using a commercial kit (Span Diagnostics, India).

2.5. Experimental Design

All the animals were randomly divided into the seven groups with six animals in each group. Group A, B, and C were served with saline, diabetic and standard drug (metformin 50mg/kg per day p.o.) control, respectively. Group D and E were served with polyherbal formulation I with a dose of 180mg/kg and 360mg/kg. Group F and G with polyherbal formulation II with a dose of 720 mg/kg and 1440 mg/kg, respectively.

2.6. Assessment of Polyherbal formulations on alloxan-induced diabetic animals

Blood samples were drawn at weekly intervals till the end of the study (i.e. 3 weeks). Fasting blood glucose estimation was measured on day 1, 7, 14 and 21 of the study. On day 21, blood was collected by retro-orbital bleeding under mild anesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for serum TC was estimated by the CHOD-PAP method, TG was estimated by GPO-POD method, HDL was analyzed by kits (Span Diagnostics, Mumbai). LDL and VLDL cholesterol using Friedevald's equation.

2.7. *In vivo* antioxidant studies

2.7.1. Preparation of liver post-mitochondrial supernatant (Liver-PMS)

At the end of the study, animals were decapitated and cut open to excise the liver. The excised livers were immediately and thoroughly washed with ice-cold physiological saline. The tissue of 100mg was homogenized in 1ml of 0.1M cold Tris-HCl buffer (pH 7.4) in a potterElvehjam homogenizer fitted with a Teflon plunger at 600rpm for 30 min. The homogenate was centrifuged at 10,000g for 20 min at 40^0Cand the supernatant with firmly packed pellets were resuspended by homogenization in 100mM Tri-HCl buffer containing 20% w/v glycerol and 0.1ml of 10mM EDTA, pH 7.4. The post-mitochondrial supernatant was used to assay Lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity.

2.7.2. Estimation of Lipid peroxidation (LPO) from liver PMS

LPO was induced and assayed in rat hepatic-PMS (Wright et al., 1981). In 1ml of the reaction muddle, 0.58ml phosphate buffer (0.1 M, pH 7.4), 0.2ml of hepatic PMS (10%W/V), 0.2ml ascorbic acid (100mM) and 0.02ml ferric chloride (100mM) and was incubated at 37^0 C in a shaking water bath for 1 h. The reaction was clogged by the addition of 1ml TCA (10% w/v), subsequently, 1ml TBA (0.67%w/v) was added and all the tubes were kept in a boiling water bath for 20 min. The tubes were shifted to ice-bath and centrifuged at 2500×g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535nm allied with the reagent blank without tissue homogenate. The molar extinction coefficient for

MDA was taken to be $1.56\times105M^{-1}cm^{-1}$

Calculation= 3 × absorbance of sample/50.156× (mg of tissue taken) = µM/mg tissue.

2.7.3 Estimation of Reduced glutathione from Liver PMS

Glutathione was assayed by the method of (Jollow et al., 1974.). An aliquot of 1ml of hepatic PMS (105w/v) was mixed with 1ml of sulphosalicylic acid (4%w/v) and centrifuged at 1200g for 5 min and filtered. From the filtrate, 0.1ml filtered aliquot, 2.7ml phosphate buffer (0.1M, pH 7.4) and 0.2 ml DTNB (40mg/10ml of phosphate buffer 0.1M, pH7.4) in a total volume of 3ml. The yellow color developed was comprehended at 412 nm on a spectrophotometer.

2.7.4. Estimation of Superoxide dismutase (SOD) from liver PMS

Superoxide dismutase activity was estimated by Fridovich et al., 1971 method. The reaction mixture consisted of 0.5ml of hepatic PMS, 1ml 50mM sodium carbonate, 0.4ml of 25µM NBT (Nitro blue tetrazolium) and 0.2ml, 0.1mM EDTA. The reaction was initiated by addition of 0.4ml of 1mM hydroxylamine hydrochloride. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required inhibiting the reduction of NBT by 50%.

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2.7.5. Estimation of catalase (CAT) from liver PMS

CAT activity was assayed by the (Claiborne et al., 1985). The assay mixture consisting of 1.95ml phosphate buffer (0.05M, pH 7), 1ml H_2O_2 (0.019M), 0.05ml of hepatic PMS (10%w/v). Changes in absorbance were recorded at 240nm for 2min with 60 seconds interval using a spectrophotometer (Model 106).

2.8 Statistical Analysis

Data for various parameters were analyzed using ANOVA and the group means were compared by Tukey-Kramer test (Graph Pad Version 3.06, La Jolla, CA, USA). Values were considered statistically significant when at $P<0.05$.

3. RESULTS

The anti-hyperglycemic effect of the extracts on fasting blood sugar levels of diabetic rats is shown in Figs. 1 and the administration of alloxan led (150mg/kg, i.p.) led to the elevation of

fasting blood sugar levels which were maintained over a period of 3 weeks. Three weeks of daily treatment of various Polyherbal formulations I and II lead to fall in blood sugar levels.

Serum total cholesterol, serum triglycerides,(Table-1)serum LDL and serum VLDL levels (Table-2) were decreased significantly by metformin and all polyherbal formulations both I and II due to 21 days of treatment. HDL levels were increased by metformin and Polyherbal formulations I and II (Table-2).

In the present study, the polyherbal formulations I and II treated groups showed a statistically significant decrease in LPO and a statistically significant increase in GSH, SOD, and CAT similar to standard Metformin (Table-3).

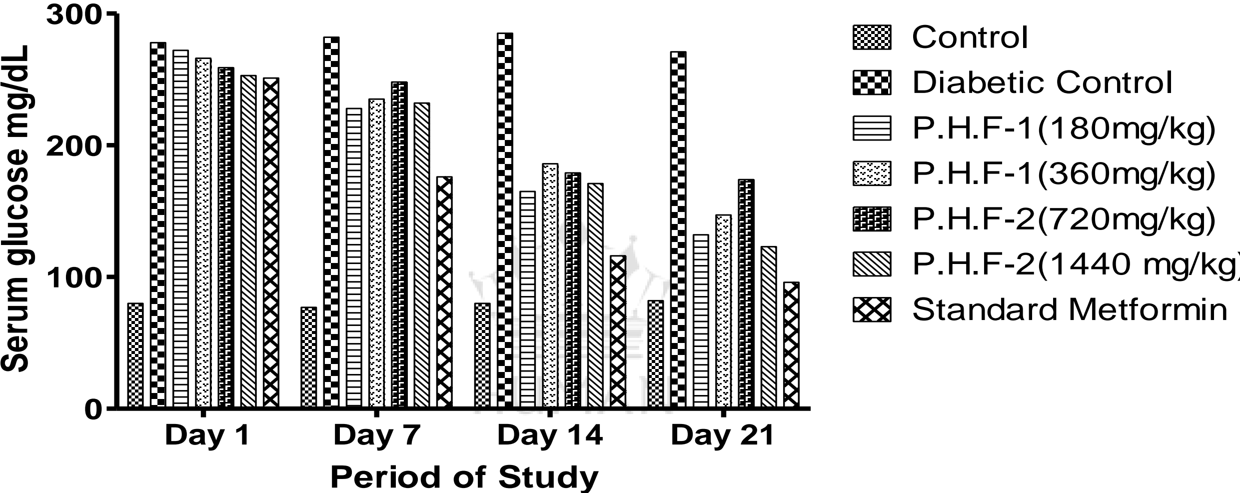

Fig no. 1: Diagrammatic representation of the Results of Polyherbal formulations on Serum Glucose levels.

Table-1: Determination of Results of Serum Glucose, Total Cholesterol and Triglycerides.

Group	Treatment	Serum Glucose (mg/dl)				Serum Total Cholesterol (mg/dl)				Serum Triglycerides (mg/dl)			
		1 Day	7 Day	14 Day	21 Day	1 Day	7 Day	14 Day	21Day	1 Day	7 Day	14 Day	21 Day
1.	Normal saline (10ml/kg p.o.)	80.5± 2.527	76.66± 1.358	79.83± 2.007	82.5± 2.012	61.16± 1.138	61.16 ± 0.609	60.83± 0.542	60.5± 1.478	58.66± 1.229	60.33± 0.714	59.66± 0.760	59.16± 1.014
2.	Diabetic control(Alloxan 150mg/kg i.p)	278 ± 4.726^{+++}	282.16± 3.26^{+++}	285.16± 5.350^{+++}	270.66± 7.338^{+++}	82.33± 1.202^{+++}	83.83± 1.195^{+++}	84.5± 1.258^{+++}	87± 2.793^{+++}	82.5± 1.384^{+++}	83± 1.770^{+++}	83.66± 1.333^{+++}	84.5± 2.754^{+++}
3.	P.H.F-1 (180 mg/ Kg p.o.) + Alloxan	272.33± 5.602^{ns}	227.83± 6.091***	165.33± 11.644***	131.66± 3.095***	81.16± 0.862^{ns}	80± 1.826^{ns}	72.66± 1.358***	65.3± 1.706***	80.83± 1.046^{ns}	78.33± 2.591^{ns}	74.33± 1.202***	70.66± 1.476***
4.	P.H.F-1 (360mg/ Kg p.o.) + Alloxan	265.33± 3.792^{ns}	235.33± 9.552***	186.16± 6.052***	147.5± 2.778***	82.16± 1.014^{ns}	82.66± 1.382^{ns}	74.16± 1.014**	70.33± 1.542***	82.41± 1.186^{ns}	81.5± 1.765^{ns}	77.16± 0.703**	73.83± 1.493**
5.	P.H.F-2 (720 mg/ Kg p.o.) + Alloxan	259.16± 4.564*	248.16± 6.253**	179.16± 5.016***	174.66± 1.851***	83.5± 1.408^{ns}	80.83± 0.945^{ns}	75.33± 1.382***	71.16± 1.701***	83.16± 1.447^{ns}	80.83± 1.470^{ns}	75.83± 1.078***	73.08± 0.986**
6.	P.H.F-2 (1440 mg/ Kg p.o.) + Alloxan	253± 2.221*	231.83± 6.096***	171.5 ± 4.522***	122.83± 3.240***	80.33± 1.022^{ns}	76.5± 0.763***	73.5± 1.232***	68.16± 0.60***	81.25± 1.289^{ns}	76.16± 1.046^{ns}	72.83± 1.078***	68.66± 2.246***
7.	Std Metformin (50 mg/kg p.o)+Alloxan	251.33± 3.721***	176± 3.642***	116.33 ± 4.580***	96.16± 2.845***	81.33± 1.054^{ns}	78.1± 0.6009*	67.33± 0.802***	62.33± 1.256***	82.08± 1.003^{ns}	78.5± 1.384^{ns}	71.16± 1.352***	70.33± 2.15***

The values are expressed Mean ± S.E.M (n =6 animals in a group). The values are analyzed for Statistical significance by ANOVA; the comparison was done by Tukey's t-test.

+++P<0.001; ++P<0.01; +P<0.05; P>0.05 when compared to Normal control group.

***P<0.001; **P<0.01; *P<0.05; P>0.05 when compared to Diabetic control group.

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Table-2 Determination of Results of Serum HDL, VLDL, and LDL

Group	Treatment	Serum HDL (mg/dl)				Serum VLDL (mg/dl)				Serum LDL (mg/dl)			
		1 Day	7 Days	14 Days	21 Days	1 Day	7 Days	14 Days	21 Days	1 Day	7 Days	14 Days	21Days
1.	Normal saline (10ml/kg p.o.)	22.16± 0.307	23.83± 0.477	23.3± 0.714	24.16± 0.703	11.73± 0.245	12.06± 0.143	11.93± 0.152	11.83± 0.202	27.26± 1.543	25.26± 1.062	24.96± 1.089	25.33± 1.261
2.	Diabetic control (Al-loxan 150mg/kg i.p)	19.66± 0.802^{+++}	19± 0.577^{+++}	18.83± 0.307^{+++}	16.83± 0.477^{+++}	16.5± 0.276^{+++}	16.6± 0.354^{+++}	16.73± 0.266^{+++}	16.9± 0.550^{+++}	46.16 ± 0.938^{+++}	48.36± 1.142^{+++}	50.93± 1.328^{+++}	53.26± 2.546^{+++}
3.	P.H.F-1 (180 mg/ Kg p.o.) + Alloxan	18.5± 0.223^{ns}	20.33± 0.714^{ns}	24.5± 0.846***	25.33± 0.614***	16.16± 0.209^{ns}	15.66± 0.518^{ns}	14.86± 0.240***	14.13± 0.295***	46.5± 0.842^{ns}	44± 2.492^{ns}	32.46± 1.892***	26.7± 2.266***
4.	P.H.F-1 (360mg/ Kg p.o.) + Alloxan	19.16± 0.307^{ns}	20.5± 0.670^{ns}	21.83± 0.60***	23.16± 0.654***	16.48± 0.237^{ns}	16.3± 0.353^{ns}	15.43± 0.140**	14.76± 0.298**	46.51± 1.127^{ns}	45.86± 1.858^{ns}	35.56± 0.770***	33.73± 1.166***
5.	P.H.F-2 (720 mg/ Kg p.o.) + Alloxan	19.83± 0.401^{ns}	20.83± 0.945^{ns}	22.33± 0.428***	24.33± 0.614***	16.63± 0.289^{ns}	16.16± 0.294^{ns}	15.16± 0.215***	14.61± 0.197**	47.03± 2.014^{ns}	43.83± 0.844^{ns}	35.83± 2.109***	34.05± 1.577***
6.	P.H.F-2 (1440 mg/ Kg p.o.) + Alloxan	18.33± 0.210^{ns}	21.5± 0.763^{ns}	23.16± 0.494***	25.5± 0.885***	16.25± 0.257^{ns}	15.23± 0.209^{ns}	14.56± 0.215***	13.73± 0.449***	45.75± 1.061^{ns}	39.76± 1.035**	33.43± 1.288***	31.1± 0.913***
7.	Std Metformin (50 mg/kgp.o.)+Alloxan	19.08± 0.271^{ns}	20.66± 0.666^{ns}	23.83± 0.749***	25.16± 0.60***	16.4± 0.193^{ns}	15.7± 0.276^{ns}	14.23± 0.270***	14.06± 0.430***	44.18± 1.511^{ns}	41.8± 0.973*	29.76± 1.080***	24.43± 1.624***

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The values are expressed Mean ± S.E.M (n =6 animals in a group). The values are analyzed for Statistical significance by ANOVA; comparison was done by Tukey's t test.

+++P<0.001; ++P<0.01; +P<0.05; P>0.05 when compared to Normal control group.

***P<0.001; **P<0.01; *P<0.05; P>0.05 when compared to Diabetic control group.

Table-3 Determination of Results of LPO, GSH, SOD &CAT

Group	Treatment	LPO (µM/mg tissue)	GSH (µM/mg tissue)	SOD (U/mg tissue)	CAT (µM/mg tissue)
1.	Normal Control (saline 10ml/kg p.o.)	0.0491± 0.0002	0.184± 0.0026	0.0334± 0.0012	0.0550± 0.0017
2.	Diabetic Control (Alloxan 150mg/kg i.p.)	0.4763± 0.0033+++	0.0423± 0.0007+++	0.4346± 0.0219+++	0.0041± 0.0003+++
3.	P.H.F-1 (180mg/kg p.o.) + Alloxan (150 mg/kg i.p.)	0.0629± 0.0014***	0.194± 0.0049***	0.0278± 0.0013***	0.0398± 0.0024***
4.	P.H.F-1 (360 mg/kg p.o.) + Alloxan (150 mg/kg i.p.)	0.0579± 0.0010***	0.205± 0.0063***	0.0357± 0.0011***	0.0617± 0.0033***
5.	P.H.F-2 (720 mg/kg p.o. + Alloxan (150 mg/kg i.p.)	0.0607± 0.0011***	0.195± 0.0105***	0.0351± 0.0014***	0.0636± 0.0027***
6.	P.H.F-2 (1440 mg/kg p.o.) + Alloxan (150 mg/kg i.p.)	0.0641± 0.0013***	0. 187± 0.0034***	0.0264± 0.001***	0.0466± 0.0017***
7.	Standard Metformin (50mg/kg p.o.) + Alloxan (150 mg/kg i.p.)	0.0665± 0.0001***	0.1856± 0.0019***	0.0245± 0.0009***	0.0456± 0.0020***

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The values are expressed Mean ± S.E.M (n =6 animals in a group). The values are analyzed for Statistical significance by ANOVA; comparison was done by Tukey's t test.

+++P<0.001; ++P<0.01; +P<0.05; P>0.05 when compared to Normal control group.

***P<0.001; **P<0.01; *P<0.05; P>0.05 when compared to Diabetic control group.

4. DISCUSSION

The present study was undertaken to demonstrate the effect of polyherbal formulations on Serum glucose, Lipid parameters and Antioxidant activity in alloxan-induced diabetic rats. Alloxan causes diabetes through its ability to destroy the insulin-producing β-cells which reduce the synthesis and the release of insulin. While affecting organs such as liver, kidney. Decreased anti-oxidant enzyme levels and enhanced lipid peroxidation have been well documented in alloxan-induced diabetes.

Administration of alloxan increased serum glucose levels when compared to normal animals and also induced persistent diabetes mellitus in rats. Our investigation reveals that the polyherbal formulations decrease the serum glucose levels in alloxan-induced diabetic rats. The concentration of lipids such as cholesterol, triglycerides, LDL-cholesterol was significantly increased and whereas HDL-cholesterol was decreased in the diabetic rats than control rats.

The impairment of the insulin secretion results in the enhanced metabolism of lipids from adipose tissue to plasma. A variety of arrangements in metabolic and regulatory mechanisms, due to insulin deficiency is responsible for the observed accumulation of lipids. Further, it has been reported that diabetic rats treated with insulin shows normalized lipid levels.

Diabetic rats treated with Polyherbal formulations and Metformin also shown normalized lipid levels. Thus the results indicate that the polyherbal formulations also may possess insulin-like actions by virtue of the ability to lower the lipid levels. Lipid peroxidation is one of the characteristic features of chronic diabetes and lipid peroxidation mediated tissue damage has been observed in diabetic conditionsHyperglycemia generates reactive oxygen species (ROS) which in turn cause lipid peroxidation and membrane damage. Increased concentrations of lipid peroxides in the liver are reported to decrease cytochrome P450 and cytochrome b5 activities, which affect the drugs may metabolize activity in chronic diabetesGlutathione (GSH), a tripeptide normally present in high concentrations intracellularly, constitutes the major reducing capacity of the cytoplasm. And protects the cellular system against the toxic effects of lipid peroxidation.

The diabetic animals in the present study registered lowered levels of GSH reflecting its increased utilization owing to oxidative stress while a significant elevation of GSH in Polyherbal formulations administered to diabetic rats coincided with the significant decline in lipid peroxidation. It appears that the effect of Polyherbal formulations 1 & 2 on GSH could be at low levels by increasing the biosynthesis of GSH or by inhibiting its utilization by reducing oxidative stress.

The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen while CAT brings about the reduction of H_2O_2 and protects higher tissues from the highly reactive hydroxyl radicals. In the present investigation, both these enzymes registered low levels of

activity in diabetic control indicating diabetes-induced stress. Such decline in these enzyme activities has been reported earlier. When polyherbal formulations 1 &2 administered to the diabetic rats improved both SOD & CAT activities substantially, reflecting the antioxidant potency of Polyherbal formulations. The results were compared with standard metformin.

5. CONCLUSION

Polyherbal formulations I and II exhibited significant Antihyperglycemic and antioxidant activities in alloxan-induced diabetic rats. These showed improvement in parameters like body weight and lipid profile as well as in antioxidant parameters.

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