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Research Article

ANTI DIABETIC ACTIVITY OF POLYHERBAL EXTRACT ON STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

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Abstract: *Aim of Study***:** The present study was an attempt to investigate the effect of ethanolic of polyherbal extract on streptozotocin induced diabetes in Wistar rats. The polyherbal extract contains the plant parts are whole plant of *Adiantium capillus*, seeds of *Astercantha longifolia*, fruits of *Callicarpa macrophylla*, bark of *Ficus benghalensis*, aerial parts of *Melia azedarach*. *Material and methods:* The streptozotocin-induced diabetic rats were orally treated with glibenclamide (10mg/kg) and Polyherbal extract (100 and 200 mg/kg) to the respective treatment groups. The blood glucose level, body weight, Glycosylated hemoglobin, liver glycogen, lipid profile, Determination of antioxidant status were measured at the end of the study 21days of treatment and compared to the control. *Results:* Polyherbal extract and glibenclamide were found to significantly ($p < 0.001$) reduce the blood glucose level, Glycosylated hemoglobin, lipid profile, whereas they increased body weight, liver glycogen content and antioxidant status when compared to the diabetic control. *Conclusion:* It has been concluded that Polyherbal extract, in addition to the antidiabetic activity, also possess antihyperlipidemic and antioxidant activities in the streptozotocin-induced diabetic model.

Key words: Antidiabetic, antihyperlipidemic, antioxidant, polyherbal extract and streptozotocin

Introduction:

Diabetes mellitus consists of a group of disorders characterized by hyperglycemia altered metabolism of lipids, carbohydrates, and proteins and an increased risk of complications from vascular disease. Most patients can be classified clinically as having either type 1 or type 2 Diabetes mellitus. [1]

Diabetes mellitus complications are mainly due to the imbalance in the antioxidative mechanism Endogenous reactive oxygen species (ROS) help to maintain homeostasis. However, when ROS accumulate in excess for prolonged periods of time, they cause chronic oxidative stress and adverse effects. This is particularly relevant and dangerous for the islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses. Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of vascular, retinal, and renal tissues. Considerably less work has been performed using islet tissue**.** At least six pathways are emphasized in the literature as being major contributors of ROS. There are Six biochemical pathways along which glucose metabolism can form ROS Glyceraldehyde autoxidation, Protein kinase C (PKC) Activation, Glycation, Sorbitol metabolism, Hexosamine Pathway, Oxidative Phosphorylation [2]

According to World Health Organization (WHO) estimates, the urban population in developing regions will increase from 1.9 billion in 2000 to 3.9 billion in 2030.It is estimated that, by 2030, nearly 46% of India's population will be living in urban areas, chronic diseases such as diabetes and cardiovascular disease (CVD), pose a primary challenge for the health care system. India has 41 million diabetics and this number is expected to increase to 70 million by 2025. WHO projects that diabetes death will double between 2005 and 2030.[3]

Many traditional plants were used for treatment of diabetes. The active compounds of medicinal plants play an important role in the management of diabetes mellitus especially in developing countries. Moreover, during the past few years some of the new bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy.[4]

Researchers conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic property. There are many plants and their products (active, natural principles and crude extracts) that have been mentioned or used in the Indian

traditional system of medicine and have shown experimental or clinical anti-diabetic activity.[5]

The polyherbal extract contains the equal proporation of plant parts were whole plant of *Adiantium capillus*, seeds of *Astercantha longifolia*, fruits of *Callicarpa macrophylla*, bark of *Ficus benghalensis*, aerial parts of *Melia azedarach*.

Materials and methods

Collection of plant materials

Coarsely powdered material of the plants were procured as gift sample obtained from SKM Siddha and Ayurveda Company (India) Limited, Erode,Tamilnadu.

Chemicals and reagents

Streptozotocin (Aldrich co., USA) Blood glucose(Merck lab.,USA), Triglycerides(Merck lab.,USA), Cholesterol(Merck lab.,USA), HDL-cholesterol(Merck lab.,USA), LDL(Merck lab.,USA), Ascorbic acid, Nitro blue tetrazolium (NBT), sodium nitroprusside, dimethyl sulphoxide, gallic acid, potassium chloride and sodium chloride from Ranbaxy Laboratories Ltd., India. All other chemicals used in the studies were analytical laboratory grades procured from the following manufactures, Loba chemie, ACROS Organics, Merck lab, S.D.Fine chemicals, Fluke.

Animals

Healthy, adult Wistar rats of both sexes (150-220g) were obtained from the Sipra Labs. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3ºC.The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution.

Plant Extraction

Equal amount of the weighed coarse powders were mixed and blended. The coarse powder was used for the extraction by successive solvent extraction by Soxhlet apparatus using solvents like petroleum either, chloroform, acetone, ethanol for about 48 hours. The extracts were concentrated under reduced pressure using rotary vacuum flash evaporator to get a constant volume. Then the marc extracted by cold maceration. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were stored in dessicator.

Acute toxicity study

Acute toxicity study was carried out for the polyherbal extract following OECD guidelines [6]. The extract was suspended in water and selected 3 female rats, giving dose 2000mg/kg .body wt and Behavioural parameters was assessed for1/2 ,1,2,4,8,12,24 hours. No mortality was seen at that dose during 14 days observation period.

Induction of experimental diabetes mellitus and treatment protocol

The animals were divided into six groups of six animals each as follows:

Group I—vehicle control, p.o. (nondiabetic)
Group II—diabetic control
Group III—diabetic standard treated, 10mg/kg of glibenclamide, p.o.
Group IV—diabetic treated with ployherbal extract 100 mg/kg, p.o.
Group V—diabetic treated with ployherbal extract 200 mg/kg, p.o.
Group VI—normal rats treated with ployherbal extract 200 mg/kg, p.o.

Diabetes was induced in all groups except vehicle control and normal control rats following overnight fasting (deprived of food for 16 h allowed free access to water) by a single intraperitoneal injection of 65 mg/kg of streptozotocin (STZ) dissolved in a freshly prepared 0.1M citrate buffer (pH 4.5). The animals of vehicle control (Group I) were injected with buffer alone. Streptozotocin-injected animals were given 5% glucose solution (2 ml/kg body weight) for 24 h following streptozotocin injection to prevent initial drug-induced hypoglycemic mortality. After 72 h, blood was withdrawn by retro orbital puncture under light ether anesthesia and the blood glucose level was estimated. After 1 week of induction, blood glucose level was estimated again and a fasting blood glucose level of more than 200 mg/dL was considered as diabetic [7]. The treatment was orally given to the respective groups once a day for 21 days

Estimation of plasma glucose, body weight and lipid profile

Every week, following overnight fasting (16 h fasting with free access to water), the blood samples were withdrawn from the animals by retroorbital puncture under light ether anesthesia.
The plasma glucose estimation was done by the glucose oxidase/ peroxidase (GOD/POD) method using a standard kit obtained from Span Diagnostics, India. Body weight of all experimental animals was recorded using a digital weighing scale. The serum triglycerides (TG), total cholesterol (TC) Serum lowdensity lipoproteins (LDL) and highdensity lipoproteins (HDL) levels were estimated using standard kits obtained from Span Diagnostics, India [8].

Estimation of in vitro glucose uptake by rat hemi diaphragm

Glucose uptake by rat hemi-diaphragm was estimated by a standard method. Albino rats of either sex weighing between 180-220 gm were selected .The animals were sacrificed by decaptitation and diaphragms were dissected out quickly with minimal trauma and divided into two halves. The hemi-diaphragms were rinsed in cold tyrode solution (without glucose) to remove any blood clots and were placed in small culture tubes containing 2ml tyrode solution with 2% glucose and incubate for 30 minutes at 37ºC in an atmosphere of 100% O_2 with shaking. Four sets containing six numbers of graduated test tubes were taken as follows.

Group I- 2ml of tyrode solution with 2% glucose solution.
Group II - 2ml of tyrode solution with 2% glucose and regular insulin (0.25IU/ml)
Group III - 2 ml of tyrode solution with 2% glucose + Polyherbal extract
Group IV - 2ml of tyrode solution with 2% glucose + Polyherbal extract + Insulin

Two diaphragms from the same animal were not used for the same set of experiment. Following incubation, the hemi diaphragms were taken out and weighed. The glucose content of the incubated medium was measured by GOD- POD method. The uptake of glucose was calculated in mg/dl. Glucose uptake in mg/dl of tissue was calculated as the difference between the initial and final glucose content in the incubated medium [9,10].

Estimation of glycogen content

Liver glycogen content was estimated by 1 gm of the liver is homogenised with phosphate buffer and 6ml of 0.6N hydrochloric acid was introduced into a test tube provided with an air-cooled condenser or covered with a glass bulb, and the sample was hydrolysed by heating for 2–2.5 h in boiling water bath. The solution was cooled, neutralized with 0.5N sodium hydroxide with phenol red as an indicator, transferred to a volumetric flask, and diluted to volume, and thus obtained glucose in the solution is estimated by the Trinder's method. A conversion factor of 0.93 was taken by calculating the amount of glycogen from the glucose determined in the hydrolysed glycogen sample [8],.

Estimation of glycosylated hemoglobin content

Glycosylated peptides are elevated several folds in diabetics. The use of the glycosylated hemoglobin (HbA1c) assay for long-term diabetic monitoring of diabetic control is gaining much wider use and acceptance. The hematological parameter glycosylated hemoglobin was determined by standard laboratory techniques [7].

Determination of reduced glutathione.

One ml of post mitochondrial supernatant (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4 °C for at least 1 h and then subjected to centrifugation at 1200 ×g for 15 min at 4 °C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm [8].

Estimation of superoxide dismutase.

Cytosolic superoxide dismutase activity assay system consist of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture was taken and to it 0.05 ml of post mitochondrial supernatant and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH) were added. The auto-oxidation of hydroxylamine was observed by measuring the change in optical density at 560 nm for 2 min at 30/60 s intervals [8].

Estimation of catalase.

Catalase activity assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml post mitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k min^{-1} and expressed as mean ± S.E.M [8].

Statistical analysis

Experimental results were expressed as mean ± SEM of six animals [7]. Analysis of variance was performed by ANOVA followed by Tukey's post hoctest. P Values less than 0.05 were regarded as significant.

Results

1.1Preliminary phytochemical screening

The Polyherbal extract was subjected to chemical tests as per the standard methods for the identification of the various constituents. The results of phytochemical analysis were given in **Table 1.1**

1.2 Glucose uptake assay from rat hemi-diaphragm

The estimation of glucose content in rat hemi diaphragm was employed for *in vitro* study of peripheral uptake of glucose. The effect of the Polyherbal extract on glucose uptake by isolated rat hemi diaphragm was shown in **Table 1.2.** The Polyherbal extract enhances the uptake of glucose by isolated rat hemi diaphragm significantly ($P<0.05$) and was not found to be more effective than insulin. Administration of Polyherbal extract and insulin

together was found to be effective than insulin treated group (P< 0.001).

1.3 Effect of polyherbal extract on serum glucose levels

Streptozotocin treatment produced significant increase in serum glucose level 0.001) with respect to normal control group. The administration<(P of polyherbal extract 100mg/kg, 200mg/kg and glibenclamide 0.001) significantly reversed the increase in serum<10mg/kg (P glucose concentration in Streptozotocin induced rats. Polyherbal extract *per se* did not show significant change in serum glucose level. The changes in serum glucose estimation in all groups of animal were given in **Table 1.3**

Table 1.1 Qualitative phytochemical screening of polyherbal extract

Plant constituent	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
Alkaloids	-	+	+	+	+
Carbohydrates	+	+	+	+	+
Glycosides	-	+	-	+	-
Saponins	-	-	-	-	-
Protein and amino acids	-	-	-	+	-
Phytosterol	-	+	+	+	+
Phenols and tannins	-	-	+	+	-
Flavonoids	-	-	+	+	+
Fixed oils and fat	+	+	+	+	+

"+" Present, "-"Absent.

Table 1.2. Glucose uptake assay from rat hemi-diaphragm

Group	Incubation medium	Glucose uptake (mg/dL/30min
1	Tyrode solution + glucose (2%)	6.131 ± 0.24
2	Tyrode solution + glucose + Insulin	12.972 ± 0.81***
3	Tyrode solution + glucose + PE(100 mg/ml)	10.341±0.59 ***
4	Tyrode solution + glucose + PE +Insulin	15.249±0.77***

All values are expressed as mean ± SEM (n=6), The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's post hoctest. ***P<0.001as compared to group 1

1.4 Effect of polyherbal extract on body weight

There was gradual increase in body weight in normal control while the diabetic control continue to lose the weight However treated diabetic group gained 8.65%,10.78% as compared to diabetic control 0.001).<and body weight of diabetic treated towards normal range(P Polyherbal extract *per se* did not show significant change in body weight. The change in body weight were tabulated in **Table 1.4**

1.5 Effect of polyherbal extract on liver glycogen and Glycosylated hemoglobin

The 0.01) with<liver glycogen content was significantly decreased (P experimentally induced diabetes mellitus and there was a significant 0.001) with polyherbal<increase in liver

glycogen content (P extract and Glibenclamide treatment as compared to diabetic control. 0.001)<There was a significant increase in glycosylated hemoglobin (P a level was observed in diabetic rats. The level of glycosylated 0.001) significantly in Polyherbal<hemoglobin was decreased (P extract and glibenclamide treated rats as compared to diabetic control rats. Polyherbal extract *per se* did not show significant change in Glycosylated hemoglobin content and liver glycogen content and the results were tabulated in **Table 1.5**

Table 1.3 Effect of Polyherbal extract on serum glucose levels in normal control and STZ induced diabetic rats

		Serum glucose level (mg/dL)	
S. No	**TREATMENT**	**Initial**	**Final**
1	Normal control	82.2 ±0.85	84.01±0.8
2	Diabetic control	293.7±0.93	393.29±0.82*** (-33.91)
3	Diabetic + Glibenclamide (10mg/kg)	286.9±0.77	120.61±1.94*** (57.96)
4	Diabetic + PE (100mg/kg)	292.7±1.15	135.89±2.07*** (53.57)
5	Diabetic + PE (200mg/kg)	283.9±2.26	124.3±1.05*** (56.21)
6	Normal + PE (200mg/kg)	89.3±1.42	88.01±1.10 (1.45)

±All values are expressed as mean SEM (n=6), 0.001, as compared to<***P diabetic control ###0.001, as compared to untreated<P control. Figure in parenthesis indicate % fall in glucose level as compared to initial value .One-way ANOVA followed by Tukey's post hoc multiple comparison tests.

Table 1.4 Effect of Polyherbal extract on body weight in normal control and STZ induced diabetic rats

		Body weight (gm)	
S. No	**TREATMENT**	**Initial**	**Final**
1	Normal control	251.4± 2.93	281.41± 1.22
2	Diabetic control	257.1± 1.64	201.59±1.00*** (-27.52)
3	Diabetic + Glibenclamide (10mg/kg)	255.3± 1.62	282.6±1.06***(9.66)
4	Diabetic + PE (100mg/kg)	256.5±2.51	280.79±2.76*** (8.65)
5	Diabetic + PE (200mg/kg)	264.7±3.23	296.72±2.82***(10.78)
6	Normal + PE (200mg/kg)	251.6±3.94	271.01±1.53(7.15)

±All values are expressed as mean SEM (n=6). 0.001, as compared to<***P diabetic control ###0.001, as compared to Normal<P control. Figure in parenthesis indicate % fall in body weight as compared to initial value One-way ANOVA followed by Tukey's post hoc multiple comparison tests.

1.6 Effect of polyherbal extract on serum lipid and lipoprotein profile

STZ diabetic rats group were found to have significantly increased VLDL, LDL, TG, TC, levels and markedly decreased HDL levels as compared to 0.001). Treatment with Polyherbal<normal control group(P extract 100 mg/kg and 200mg/kg reduced significantly VLDL,

LDL, TG, TC, levels and markedly increased HDL levels as compared to 0.001). Positive control was<diabetic control group(P significantly preventing the increasing the serum TC, TG, LDL, VLDL and decreasing the HDL level as compared to diabetic group. Thus the Polyherbal extract treatment restored all these changes near to normal value. Polyherbal extract *per se* did not show significant change in serum lipid and lipoprotein profile .The change in serum lipid and lipoprotein profile were tabulated in **Table 1.6**

1.7 Effect of polyherbal extract on in –vivo anti oxidant parameter from pancreas homogenate

The STZ-induced diabetic rats exhibited a significant decrease in SOD, CAT and GSH levels (P<0.001) when compared with the normal control rats. Administration of Polyherbal extract for 21 days produced a marked increase (P<0.001) in antioxidant parameters (SOD, CAT, and GSH). Siddha formulation per se did not show significant change in antioxidant enzyme activity and were tabulated in **Table 1.7**

Table 1.5 Effect of Polyherbal extract on liver glycogen and Glycosylated hemoglobin in normal control and STZ induced diabetic rats

S. No.	TREATMENT	Liver glycogen (mg/gm)	Glycosylated hemoglobin (%)
1	Normal control	18.06±0.52	6.21±0.38
2	Diabetic control	6.69±0.35$^{###}$	15.03±0.81$^{###}$
2	Diabetic+Glibenclamide(10mg/kg)	16.53±0.53***	6.16±0.43***
4	Diabetic + PE (100mg/kg)	11.91±0.73***	11.06±0.40**
5	Diabetic + PE (200mg/kg)	14.91±0.73***	9.71±0.962***

±All values are expressed as mean SEM (n=6), 0.001, as compared to<***P diabetic control $^{###}$0.001, as compared to Normal<P control. ,0.01, as compared to<**P diabetic control. One-way ANOVA followed by Tukey's post hoc multiple comparison tests.

Table 1.6 Effect of Polyherbal extract on serum lipid and lipoprotein profile in normal control and STZ induced diabetic rats

S.No	TREATMENT	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dl)
1.	Normal control	92.91±2.45	66.05±1.39	44.1±1.48	95.79±1.67	21.35±1.18
2.	Diabetic control	238.59±7.52***	138.769±4.02***	23.2±0.81***	136.01±3.29***	54.55±2.92***
3.	Diabetic + Glibenclamide (10mg/kg)	145.9±5.47***	109.59±3.92***	50.9±1.44***	106.42±2.07***	38.84±1.78***
4.	Diabetic + PE (100mg/kg)	146.89±4.16***	120.01±2.47**	57.9±2.65***	113.01±2.69***	43.71±1.90*
5.	Diabetic + PE (200mg/kg)	140.39±3.97***	110.69±3.10***	52.11±1.34***	105.31±2.05***	42.89±2.02***
6.	Normal + PE (200mg/kg)	93.41±2.68	58.61±3.47	41.7±1.52	100.6±1.49	23.71±2.256

±All values are expressed as mean SEM (n=6).
0.01, as compared to<** P diabetic control
0.001, as compared to<*** P diabetic control
$^{###}$ 0.001, as<P compared to untreated control
One-way ANOVA followed by Tukey's post hoc multiple comparison tests.

Table 1.7 Effect of Polyherbal extract on in –vivo anti oxidant parameter from pancreas homogenate in normal control and STZ induced diabetic rats

S.No	TREATMENT	GSH (µg/mg of protein)	SOD (unit/min/gm tissue)	CAT (µmol of H_2O_2/ min / gm tissue)
1.	Normal control	52.31±1.88	14.8±0.71	7.21±0.30
2.	Diabetic control	34.1±1.00***	4.5±0.55***	3.1±0.37***
3.	Diabetic + Glibenclamide (10mg/kg)	56.31±2.22***	12.29±0.72***	8.79±0.45***
4.	Diabetic + PE (100mg/kg)	42.21±1.28*	9.59±0.62***	7.51±0.35***
5.	Diabetic + PE (200mg/kg)	46.01±1.13***	11.41±0.64***	8.31±.263***
6.	Normal + PE (200mg/kg)	53.59±1.71	13.81±0.91	s 9.01±0.36

±All values are expressed as mean 0.001, as compared to diabetic control<SEM (n=6).*** P
0.1, as compared to<* P diabetic control ### 0.001, as compared to untreated<P control
One-way ANOVA followed by Tukey's post hoc multiple comparison tests

Discussion

Diabetes is a common chronic ailment for which the patient has to take insulin to maintain the blood sugar level. It is very interesting to see how Polyherbal extract tackles this problem. It corrects the function of pancreas, stimulating it to produce insulin in the natural way, which in turn maintains the blood sugar level. Polyherbal extract revitalizes and rejuvenates the organs, the dysfunction of which is causing the disease. This brings back normal functioning of the organs. It also maintaining the healthy state of the body. Since no artificial chemicals are involved, it doesn't cause any side effects.

Qualitative phytochemical screening and ethno botanical survey on the polyherbal extract revealed the presence of certain phyto constituents such as alkaloids, tannins, carbohydrates, glycosides, protein and amino acids, phytosterol, high amount of phenolic content, terpenoids and flavonoids. These Phytoconstituents have diverse biological activities.

Phytochemical constituents such as glycoside, tannins, triterpenoids, flavonoids, alkaloids may be linked to the antidiabetic activity.

To check the safety profile of the polyherbal extract it was subjected to acute toxicity study which confirmed the absence of any toxicity or mortality at a higher dose of 2000mg/kg. Thus the polyherbal extract can be classified in to the safe drug category according to the "Global harmonised Classification System" quoted in the OECD guidelines 1996.6

Based on the acute toxicity studies two dose levels were selected for the evaluation of various pharmacological properties (100 mg/kg, 200 mg/kg).

Diabetic mellitus (DM) is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cell or because of inadequate release of insulin from the pancreatic beta cell or insensitivity of target tissue to insulin. The fundamental mechanism underlying hyperglycemia involved over production (excessive hepatic glyconeogenesis and gluconeogenesis) and decrease utilization of glucose by the tissue. In the present study it was observed that whether the polyherbal extract have any effect on lipid profile, antioxidant system or not and in addition to its antihyperglycemic action in STZ induced diabetic rats.[7]

Phenolic compounds or phenolic Phytochemicals are secondary metabolites of plant origin and are important parts of the diet providing potential antioxidant benefits for managing oxidation stress-related chronic diseases such as diabetes.[11]

In the present study the total phenolic content in the polyherbal extract is high and may be responsible for antioxidant activity.

The estimation of glucose content in rat hemi-diaphragm is a commonly employed and reliable

method for in vitro study of peripheral uptake of glucose. In our present study polyherbal extract also enhanced the uptake of glucose by isolated rat hemi-diaphragm and polyherbal extract with insulin was found to be effective than insulin.[12]

Streptozotocin a beta cytotoxin induces chemical diabetes in a wide variety of animal species including rats by selectively damaging the insulin secreting beta cells of pancreas, i.p. injection of STZ produces fragmentation of DNA of beta cells of pancreas which stimulates poly (ADP-ribose and deflects NAD ultimately leading to destructions of beta cells and it is evidenced by clinical symptoms of hyperglycemia.[13]

Dose dependant effects and Glibenclamide showed rapid normalization of blood glucose due to its insulin releasing effects.

In our present study there was a significant weight gain in Polyherbal extract treated diabetic rats compared with normal control rats and this observation shows anabolic effect of Polyherbal extract on body weight on the diabetic rats.

Hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of macrovascular complications. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The hyperglycemia in diabetes might inhibit tissue repair in macrovascular beds.[14] In the present study Poly herbal extract treated groups shows hypoglycemic activity and it confirms the presence of anti diabetic activity.

Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds.[8]

In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. The Polyherbal extract treated group exhibited significant reduction of fasting plasma glucose levels as compared to the diabetic control group. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus.

Increased glycation of protein has been found to be a consequence of diabetic complications. A number of proteins, including haemoglobin, are glycated to a greater degree in diabetes. Glycosylated haemoglobin (HbA1c) is the measurement of the mean blood glucose levels over the previous 6–8 weeks, during the life span of RBC. It has been shown to be an important parameter of chronic glycaemic control in patients with diabetes mellitus (DM), an elevated HbAlc almost always indicate DM. In our present study glycation of protein is significantly lowered by the treatment with Polyherbal extract.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in diabetic control rats. Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia. There was a significant control of the levels of serum lipids in Poly herbal extract treated diabetic rats. In diabetes, LDL carries cholesterol to the peripheral tissues where it is deposited, whereas HDL transports cholesterol from peripheral tissues to the liver and thus aids its excretion. Hence increase in LDL is atherogenic. In our present study, there was a significant decrease in TG, LDL, and total cholesterol levels, whereas there was a significant increase in the HDL level.

Associated with the changes in lipid peroxidation, diabetic animals showed decreased activity of the key antioxidant enzymes viz. SOD, CAT and reduced GSH, which play an important role in scavenging the toxic intermediates of incomplete oxidation. A decrease in the activity of these enzymes can lead to an excess availability of superoxide anion (O_2^-) and hydrogen peroxide in the biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation. The Polyherbal extract treatment increased the activity of enzymes and may thereby help to control free radicals, as polyherbal extract has been reported to be rich in total phenolic content, flavonoids and triterpenoids, well-known antioxidants and also to possess *in vitro* free radical scavenging and antioxidant activity.

Conclusion

The present study is an attempt to investigate the effect of ethanolic of polyherbal extract on streptozotocin induced diabetes in Wistar albino rats.

The Phytochemical screening showed the presence of tannins, flavonoids, alkaloids, phenols, reducing sugar and amino acids which are responsible for the antidiabetic activity and free radical scavenging activity.

The *in vitro* glucose uptake by rat hemi diaphragm showed the significant decrease in hyperglycemic activity along with the insulin.

The serum glucose, lipid profile, Glycosylated hemoglobin content shown to be decreased in polyherbal extract treated diabetic animals.

Body weight, liver glycogen, antioxidant parameters activity shown to be increased in Polyherbal extract treated diabetic animals .

The findings of the present investigation suggest that Polyherbal extract has potential for its evaluation as a protective agent against toxicity induced by Streptozotocin.

Clinical assessment of polyherbal extract determination of underlying mechanism(s) of the protective effects is interesting topics requiring further study.

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