



EVALUATION OF ANTIDIABETIC ACTIVITY OF POLYHERBRAL METHANOLIC EXTRACTION

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ABSTRACT

Polyherbal therapy is said to be a current pharmacological principle having the advantage of producing maximum therapeutic efficacy with minimum side effects. The antidiabetic activity of a polyherbal formulation in alloxan induced diabetic rats was assessed using Alloxan β - cytotoxin induced chemical diabetes in a wide variety of wister albino rats. Methanolic extract of the poly herbal formulation, prepared from powder of plants Fruits of , *Momordica charantia*, stem and root of *Tinospora cordifolia* , hole plant of *Andrographis peniculeta* and wood of *Pterocarpus marsupium* and leaves of *Gymnema sylvestre* was subjected to phytochemical test and pharmacological screening of Antidiabetic activity at different dose level 300 mg/kg and 600 mg/kg. The formulation showed significant activity when compared to respective standard drug glipizide 5mg/kg.

Keywords: Antidiabetic activity, Glipizide, Poly herbal formulation.

INTRODUCTION

Diabetes mellitus is a serious endocrine syndrome and complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin [1]. The number of people suffering from diabetes all over the world has soared to 246 million and the disease now kills more people than AIDS. Diabetes leads to major complication such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases. Type2 diabetes, also known as adult onset or non insulin-dependent diabetes mellitus (NIDDM), accounts for 90% of all cases of diabetes [2]. In type 2 diabetes, hyperglycemia occurs owing to insulin resistance in skeletal muscle, liver and fat cells and a relative failure of pancreatic β -cell function. There are many factors that might contribute to the development of type 2 diabetes, and readers are referred to several previous excellent review articles summarizing the advances in our understanding of this disease based on animal models, such as those aimed at strategically manipulating the components of the insulin

signaling pathway [3–6].

Diabetes mellitus is considered to be a serious in many countries it is traditional to use medicinal plants to control diabetes [7]. The synthetic hypoglycaemic agent can produce serious side effect and are not suitable in pregnancy whereas drug derived from plants are frequently consider to be less toxic with fewer side effects & affordable in price[8] therefore the search for effective and safer hypoglycaemic agent has become area of research.

During the last two decades, traditional systems of medicine and medicinal plants research have become topic of global interest and importance in many developing nations of the world, large no. of people still heavily on traditional healers and medicinal plant to meet their daily primary health care needs. Because of their perceived effectiveness, minimal side effective ness, minimal side effects in clinical experience and relatively low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown in Africa, hundreds of plants are used traditionally for the management of diabetes mellitus [9].

MATERIALS AND METHODS

Plant Materials

Fruits of *Momordica charantia*, stem and root of *Tinospora Cordifolia*, whole plant of *Andrographis peniculeta* and wood of *Pterocarpus marsupium* and leaves of *Gymnema sylvestre* were collected from Thirupathi in Andhra Pradesh and authenticated from Dr. Madhava shetti, Department of botany, S.V University, Thirupathi.

Preparation of Poly Herbal Extract

A wide range of solvents with increasing polarity were chosen.

Step.1: In a 250ml round bottomed flask, weighed quantity of powdered drug were macerated with the respective solvents in the ratio of 1:2 (i.e. 50gm in 100ml) and kept with occasional shaking for a period of 72 hrs. After the maceration process, the active ingredients present in the supernatant solvent were collected in petridishes and concentrated under reduced pressure.

Step.2: These extracts were labelled and its chemical constituents were identified, among the different solvent extracts, the extract possessing more number of active compounds were selected and prepared for bulk extraction similar as step 1.

Phytochemical Screening of Poly Herbal Extract

The poly herbal extract were subjected to qualitative test for the identification of various active constituents viz. alkaloids, carbohydrates & glycosides, cardiac glycosides, steroids, saponins, tannins and flavonoids using standard test procedures results were showed in Table 1 .

Toxicity Studies

Albino rats (200-250gm) of either sex were selected and segregated into 8 groups of 6 animals each. Single dose of methanolic extract of polyherbal formulation, starting from the minimal dose of 50mg/kg up to 3000mg/kg administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. From the maximum dose, 1/5th and 1/10th of the concentration was considered as therapeutic dose for further study.

Experimental Animals and Induction of Diabetes

Male albino rats (200-250gm) were selected animal experiments were conducted by the guidelines of CPCSEA and IAEC. (IAEC No: P12/VCP/IAEC/2012/3/VVR/AE1). Animals were fasted for 16 hours before the induction of diabetes with Streptozotocin (STZ), a valuable agent for induction of Type-1 Diabetes mellitus.¹¹ Animals (n=45) were injected intraperitoneally with 0.22 - 0.25ml of freshly prepared solution of STZ (60 mg / ml in 0.01 M citrate buffer, pH 4.5) at a final dose of 60mg / kg body wt. The diabetic state was assessed in STZ - treated rats by measuring the non-

fasting serum glucose concentration after 48 hours. Only rats with serum glucose levels greater than 300 mg / dl were selected and used in this experiment.

Experimental Design

In the experiment six rats were used in each group.

Group 1 Served as a normal control receiving 0.5ml of saline.

Group 2 Served as diabetic control

Group 3 & 4 Served as test groups, PHME I (Polyherbal methanolic extraction I) & PHME II (Polyherbal methanolic extraction II) of 300 & 600 mg /kg respectively, as doses fixed after performing the toxicity study.

Group 5 Treated with standard drug – glipizide (5mg/kg)

Blood Sugar Estimation

Fasting serum glucose concentrations were determined in mg/dl, from rat tail vein by using one touch glucometer (Ultra Co.) device. The rats were dosed daily by gavage with saline, standard drug & polyherbal extract I & II for 15 days for respective groups. On day 14, blood samples were collected from retro orbital vein and serum was separated by centrifuging the blood samples at 3000rpm for 15mins and biochemical parameters like total cholesterol and triglyceride levels were estimated. The periodical body weight difference of the individual animals was also measured.

RESULTS AND DISCUSSION

Based on the results obtained from the chemical tests for various active constituents of different solvent extracts it was confirmed that the methanolic polyherbal extract possessing maximum number of active principles. The results are shown in (Table 1). From the acute toxicity study the maximum therapeutic dose level of polyherbal methanolic extract was studied as 300mg/kg. The double multiple of this dose, of 600mg/kg also considered for comparing the effectiveness of the maximum therapeutic dose (300mg/kg), in the anti diabetic of STZ induced Type-1 Diabetic rats. The experimental data showed that, among 45 no. of animals, 40 nos. was found to have blood glucose levels greater than 400mg/dl. This suggests that streptozotocin (60mg/kg) induction destroyed the pancreatic β -cells and by this it proved that STZ is a valuable agent for induction of experimental type-1 diabetes in rats. The Poly Herbal Methanolic Extracts (PHME I & II), have shown significant ($P < 0.05$) increase in glucose tolerance. The results are given in Table 2. The blood glucose levels were reduced considerably within 60 minutes of the drug administration. The PHME I & II of dose 300 and 600mg/kg respectively, reduced the glucose levels to normal. Maximum effect was observed for PHME I. (Table.3) it was observed that the body weight of control animals gradually decreased, the PHME I (300mg/kg) treated group showed a balanced maintenance of body weight as compared to that of normal control ($^aP < 0.001$)

(Table 3). PHME I and PHME II were found to show significant reduction in the blood glucose level as compared with normal and control group. The control of sugar level in test drug 300 mg /kg dose (P <0.001) is highly effective as compared to 600 mg/ kg dose level, but both dose effects are highly comparable with standard drug. The serum cholesterol and triglycerides of the diabetic control group animals was significantly elevated. But it is found that in

the PHME-I at the dose level of 300mg/kg treated animals the Total Cholesterol and Triglyceride level was effectively reduced to normal condition and their results were remarkable with standard group. In the results of insulin treated group, there is a slight increase in these parameters when compared with standard and normal group of animals (Table-4).

Table 1. Phytochemical Screening of Polyherbal Extract

Test	PHME
Alkaloids	+
Carbohydrates& Glycosides	+
Specific Glycosides	+
Cardiac Glycoside	+
Reducing sugars	-
Steroids	-
Saponins	+
Tannins	+
Condensed tannins	+
Pseudo tannins	-
Flavonoids	+

Table 2. Oral glucose tolerance test

Treatment (dose / kg body weight)	Blood glucose (mg/dl)		
	Fasting	30 min	90 min
Glucose; 2g.	79.0 ± 3.0	180.0 ± 1.0	263.0 ± 11.0
PHME-I (300mg/kg)+Glucose	81.0 ± 3.0	90.5 ± 5.5 ^a	84.5 ± 3.5 ^a
PHME-II (600mg/kg)+Glucose	78.5 ± 2.5	85.8 ± 1.13 ^a	84.5 ± 2.5 ^a
Glipizide (5 mg/kg)	82.5 ± 4.5	98.0 ± 9.0 ^a	88.0 ± 6.0 ^a

Values are expressed as mean ± S.E.M.; n = 6

^aP<0.05 VS Normal control

Table 3. Fasting serum Glucose concentration is normal and Streptozotocin (STZ)-induced diabetic rats

Treatment	Fasting serum Glucose concentration (mg/dl) measured at regular intervals			
	Day I	Day IV	Day VIII	Day XIV
Normal Group	68.33 ± 2.40	71.33 ± 3.13	71.83 ± 2.81	73.0 ± 2.39
Diabetic Control	449.66±16.64 ^a	457.0 ± 14.6 ^a	464.83 ±12.17 ^a	469.66 ± 6.76 ^a
PHME –I (300mg/kg)	71.33 ± 3.09 ^b	66.0 ± 2.20 ^b	64.16 ± 1.24 ^b	66.66 ± 2.07 ^b
PHME –II (600mg/kg)	70.66 ± 2.69 ^b	72.33 ± 2.39 ^b	72.16 ± 2.83 ^b	77.33 ± 2.69 ^c
Glipizide (5 mg/kg)	91.96 ± 2.65 ^b	100.16±3.30 ^{c,b}	95.5 ± 4.24 ^b	101.16 ± 6.10 ^b

n=6; Values are expressed as mean ±S.E.M

^aP <0.001; ^cP <0.05 Vs Normal ^bP <0.001 Vs Diabetic Control

Table 4. Total Cholesterol and Triglyceride levels in normal and Streptozotocin (STZ) induced diabetic rats.

Treatment	Dose	Parameters (mg/dl)	
		Total Cholesterol	Triglycerides
Normal control	10mg/kg of vehicle	83.0 ± 1.0	87.0 ± 2.82
Diabetic control	-	117.5 ± 3.5 ^a	123.5 ± 6.36 ^a
PHME-I	(300mg/kg)	73.0 ± 1.0 ^{b,d}	66.5 ± 2.12 ^{b,d}
PHME-II	(600mg/kg)	74.0 ± 1.1 ^{c,d}	70.5 ± 2.12 ^{c,d}
glipizide	(5 mg/kg)	79.0 ± 1.2 ^d	86.5 ± 3.53 ^d

n=6; Values are expressed as mean ±S.E.M

^aP <0.001; ^bP<0.01; ^cP<0.05 Vs Normal ^dP <0.001 Vs Diabetic Control

CONCLUSION

The phyto chemical study reveals the presence of flavonoids and free phenolic and triterpenoids. Streptozotocin produced significant loss of body weight as compared with non-diabetic control rats. Treatment with polyherbal methanolic extract at two different dose levels significantly alters the body weight. Test drug PHME treatment significantly decreased the fasting blood glucose level and significantly lowered the total cholesterol as well

as triglycerides. Based on the above results, we conclude that the polyherbal methanolic extract was effective in decreasing the blood glucose level in STZ – induced diabetic rats. This effect could be due to its antioxidant property. Further the precise mechanism and the active constituents of PHME are responsible for its anti diabetic and related pharmacological activities are still to be determined and further toxicological studies are to be established.

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