Research Article

Evaluation of Antidiabetic Effect of Polyherbal Formulations in STZ-Induced Mice

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ABSTRACT

The aim of the study was to evaluate the antidiabetic activity of polyherbal formulation I and II in streptozotocin (STZ) induced type 2 diabetic mice. Swiss albino female mice were used for the present study. Acute toxicity study was done on screened mice for 14 days. The mice were divided into five groups like normal, diabetic, formulation I and II treated and standard (diabetes treated with metformin). STZ 20 mg/kg body weight (bw) was administered intra-peritoneally to mice for the induction of type 2 diabetes. After the confirmation for diabetes, the mice were subjected to further *in-vivo* studies. The poly herbal formulations I and II (100 mg/kg bw) were administered orally to diabetic mice for 28 days. The poly herbal formulation I contains *Eclipta prostrata* (leaves), *Syzygium cumini* (seeds), *Phyllanthus niruri* (leaves) and formulation II contains *Eclipta prostrate* (leaves), *Syzygium cumini* (seeds), in the ratio of 1:2:1 and 1:2 respectively. Body weight and fasted blood glucose levels were monitored and recorded every week. The fasted mice were examined for the period of 28 days after which they were sacrificed. The blood and tissue samples were collected for analyses of biochemical parameters and for other related tests. Biochemical parameters like blood glucose level, lipid profile test and kidney profile test were done to confirm the efficacy of the formulations. Formulation I and II did not show any toxicity at the level upto 250 mg/kg bw. Based on the toxicity studies, dosage level was fixed to 100 mg/kg bw. Formulation I showed significant antidiabetic effect than formulation II in STZ-induced diabetic mice. This might conclude that the polyherbal formulation I possess most efficient in antidiabetic activity when compared to formulation II and metformin.

Keywords: FBG, HDL, LDL, polyherbal formulations, STZ.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease which is characterized by insulin resistance and lack of insulin[1]. The prevalence of diabetes is rapidly increasing worldwide and it is the 16th leading cause of mortality[2]. India has the largest number of diabetic subjects earning the uncertain distinction of being termed the "diabetes capital of the world"[1]. According to the Diabetes Atlas 2013, International Diabetes Federation most recent estimates indicate that 8.3% of adults, the majority of the 382 million people with diabetes are aged between 40 and 59, and 80% of them live in low- and middle-income countries. All the three types of diabetes are on the increasing stage, on which type 2 diabetes in particular may increase upto 55% by 2035[3]. Many synthetic antidiabetic drugs are available for treating type 2 diabetes including biguanides but they have significant adverse effects like decrease in the elevation level of liver and serum enzymes, which makes them less efficient to be used[4].

As antidiabetic therapies developed (synthetic drugs) are often limited in efficacy, expensive and also carries the risk of adverse effects, there is an increasing demand by patients to use the natural products with antidiabetic activity[5]. In India, many herbal plants have been mentioned in the ancient literature for the treatment of diabetes[6]. Plant polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse effects[7].

Herbal medicines were considered to be less toxic with fewer side effects than synthetic drugs[8]. Plant formulation and combined extracts of plants have been used as a drug rather than individual[5]. Exploring an effective drug either single or in combination against diabetes is challenging still[4].

Many researchers have explored the different biological activities of *Eclipta prostrata, Syzygium cumini* and *Phyllanthus niruri* but no one has tried those plants in combinations (formulation I and formulation II). Few works were reported on antiproliferative[9], antitumor[10], antivenom[11], antihypolipidemic[12], antidermatophytic[13] and antidiabetic[14] activities of *Eclipta prostrata.* Whereas, anti-inflammatory[15], antidiabetic[16], antioxidant[17], anti-hyperalgesic[18], anti-infection, anti-asthmatic, anti-diuretic, anti-soresis, hepatoprotective[19,20] effects of *Phyllanthus niruri* have been explored well. Glucose transport activator[21] and its antidiabetic activity[22] of *Syzygium cumini* were proved earlier. The reduction of glycemia was supported by researcher[22] in diabetic mice. Many Ayurveda-based formulations are used in the form of decoctions, tinctures, infusions and powders[23].

As there was no evidence on either two or three of these poly herbal combinations on antidiabetic effect, the present study was focused on polyherbal combinations I and II, *Eclipta prostrata, Syzygium cumini, Phyllanthus niruri* (1:2:1) and *Eclipta prostrata, Syzygium cumini* (1:2) respectively. These herbs have antidiabetic activity individually and have been used in the traditional medicine system[9]. Poly herbal formulations of three

different medicinal plants, *Eclipta prostrata, Syzygium cumin* and *Phyllanthus niruri* with different ratios, for the treatment of type 2 diabetes in STZ induced diabetic mice will be studied.

MATERIALS AND METHODS

Metformin – Standard drug for type 2 diabetes

Formulation I – *Eclipta prostrata, Syzygium cumini, Phyllanthus niruri* (1:2:1)

Formulation II - *Eclipta prostrata, Syzygium cumini* (1:2)

Plant materials and Poly herbal formulation

The leaves of *Eclipta prostrate, Phyllanthus niruri* and seeds of *Syzygium cumini* were procured from in and around local area of Vellore. It was then subjected to shade dried and finely ground using mixer grinder and was collected in sterile polythene bags. The poly herbal formulations I and II contains powdered plant parts of *Eclipta prostrata, Syzygium cumini, Phyllanthus niruri* (1:2:1) and *prostrata, Syzygium cumini* (1:2) and were prepared respectively. The formulations were dissolved in distilled water and kept in airtight container for further *in-vivo* studies.

Animals

Swiss albino female mice weighing about 25-30g were used for the study. The animals were approved by institutional animal ethical committee (IAEC) at VIT University and the study was conducted in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animals were maintained on 12:12hr dark-light cycle in cages, fed with sterile food pellets and water available *ad-libitium.* Before the experiment, the animals were screened for blood glucose level and body weight for the estimation of diabetic condition of mice.

Acute treatment of non diabetic mice

Five different doses of two formulations (I and II) with 50, 100, 150, 200, 250 mg/kg bw for 14 days were administered and glycemic condition was monitored 3h after the first and last dose.

Induction of diabetes and experimental design

Streptozotocin was induced into mice as per IACUC guidelines[4]. Streptozotocin was purchased from Himedia Laboratories, Mumbai and dissolved in 100mM citrate buffer (pH 4.5) and calculated amount of the dose 20mg per kg bw was administered intraperitoneally to overnight fasted mice. Control mice were injected with citrate buffer to standardize the hormonal changes. Blood glucose level was measured after 48h of incubation and animals fed with regular food pellets and water *ad-libitium.* The blood glucose level >250 – 300 mg/dl were treated as diabetics and were subjected to further antidiabetic and antilipidemic studies.

The mice were divided into 5 groups

Group I : Normal
Group II : Diabetes
Group III: Diabetes treated with formulation I
Group IV: Diabetes treated with formulation II
Group V : Diabetes treated with metformin

Blood glucose level monitoring

The blood samples were collected from a tail vein of mice in EDTA tubes. The fasted and random blood glucose levels were monitored regularly in the intervals of 0, 7, 14, 21, 28 days using One Touch glucometer (glucose oxidase- peroxidase method). At the end of the examination period, the mice given with anesthesia and the blood samples collected by heart puncture method and the animals were sacrificed as per the guidelines given in CPCSEA.

Biochemical parameter analysis

The blood samples were centrifuged at 5000 rpm for 5 mins and the serum sample was collected in sterile test tubes. The biochemical parameters were analysed using semi auto analyzer with the help of respective kits. The biochemical parameters like blood glucose level, cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), serum creatinine, urea, total proteins, alkaline phosphatase, SGPT and SGOT and whole blood was used to estimate the diabetic condition of mice.

Determination of body weight and food consumption

Daily food consumption of the whole experimental group and body weight of each animal treated with the extracts were determined daily for 7 days.

Statistical analysis

All data were statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests using Prism Graph Pad version 5.0, the P value was <0.05. The data were expressed as mean±SEM.

RESULTS

Acute toxicity studies

The mice were administered with formulation I and II at different concentration like 50, 100, 150, 200, 250 mg/kg bw and was observed for the period of 14 days. No mortality changes were observed with any of these doses during the entire study period. Hence can proceed with 100 mg/kg bw of formulation I and II to evaluate the antidiabetic effect *in-vivo* model.

Hypoglycemic activity of formulation I and formulation II

The treatment effect of formulation I and formulation II were compared with metformin and the blood glucose level in normal fasted and diabetic mice were shown in table 1. Metformin is commonly prescribed antidiabetic agent, plays an important role in the reduction of gluconeogenesis and increasing AMPK signaling[4].

DISCUSSION

The results of present study indicate the significant effect of polyherbal formulation I on STZ induced type 2 diabetic mice. Type 2 diabetes mellitus (T2DM) is one of the fastest growing metabolic syndromes of multiple aetiologies including dyslipidaemia[24]. Many synthetic drugs are available for treating diabetes but due to adverse side effects, herbal plants play an important role in treating various life threatening diseases including diabetes. Also, Poly herbal formulations were used extensively in Indian traditional medicine system for the management of type 2 diabetes[4]. Consequently the present study focused on the poly herbal formulations for the treatment of type 2 diabetes.

The poly herbal formulations I and formulation II contains extracts of powdered plant parts of *Eclipta prostrata* (leaf), *Syzygium cumini* (seed), *Phyllanthus niruri* (leaf) (1:2:1) and *Eclipta prostrata* (leaf), *Syzygium cumini* (seed) (1:2) respectively were chosen for the study. Researchers proved the biological properties of individual plants of *Eclipta prostrata*, *Syzygium cumini* and *Phyllanthus niruri* and no one has reported those plant parts in combinations.

Healthy swiss albino female mice weighing about 25-30g was chosen for the study and was approved by institutional animal ethical committee (IAEC) at VIT University. The animals were maintained on 12:12hr dark-light cycle in cages and fed with sterile food pellets and water available *ad-libitium.* The oral acute toxicity studies were done with different dose range and there was no behavior changes were observed during the entire study period of 14 days. It indicates the poly herbal formulation does not have toxic effect so the formulations were subjected to further *in-vivo* studies.

Streptozotocin (STZ) is naturally occurring chemical derived from *Streptomyces achromogenes* and was found to be selectively toxic to beta cells of the pancreatic islets. STZ, a chemical found to be selectively toxic to the beta cells of the pancreatic islets[25,26]. The swiss albino mice were administered with streptozotocin (STZ) 20mg/kg bw for the induction of type 2 diabetes. The body weight was measured before and after induction, STZ induce weight loss in mice due to protein wasting in the absence of carbohydrates for utilization as an energy source[27]. The present study shows the increase in body weight of mice after the induction with STZ which is similar with earlier report[28]. The diabetes induced group III and group IV mice were administered orally with formulation I and II respectively and group V mice were administered with standard antidiabetic drug metformin. Metformin and Formulation I, II had similar effect on body weight and fasted blood glucose given in table 1.

The fasted blood glucose (FBG) level was used to monitor the diabetic condition of mice. The fasted blood glucose (FBG) was measured before and after induction with STZ, after 28 days of study period the hyperglycemic condition was well controlled by formulation II in contrast to formulation I which is shown in table 1. The poly herbal formulations I and II treated groups showed the significant decrease in blood glucose level than the diabetic mice.

Among the poly herbal formulations, the *Phyllanthus niruri* has antidiabetic potential as it lowers blood glucose and suppresses a postprandial rise in blood glucose levels[16]. Formulation II had a significant decrease in blood glucose level at the range of 77.29 mg/dl and not exceed than control level which indicates the supportive action of formulations in the glucose consumption.

Serum lipids are elevated in diabetic patients due to lipolytic hormonal actions. Insulin deficiency in the diabetic state leads to hypertriglyceridemia and hypercholesterolemia[8]. In the present study, the formulations I and II showed a significant decrease in both cholesterol and triglycerides which given in table 2. The cholesterol, triglycerides, HDL, LDL, VLDL level was significantly lowers than the diabetic control group (table 2). In the Formulation I and II, the cholesterol level was 152.8 mg/dl and 163.51 mg/dl respectively. Triglycerides level of formulation I and II was 138.5 mg/dl and 144.8 mg/dl respectively hence the Formulation I showed effective than formulation II. The cholesterol transport happens through LDL and HDL. In vascular bed the cholesterol is deposited through LDL, on the other hand HDL functions as reverse transport carrying excess cholesterol from cells in peripheral tissues to the liver[29]. Thus, the formulations promise to have an antidiabetic constituent either of single or in synergism.

Table 1: Effect of body weight and fasted blood glucose levels in normal and diabetic mice

Groups	Body weight (Before induction)	Body weight (after induction)	FBG (mg/dl) before induction	FBG (mg/dl) day 28
Normal	25.9±1.32	26.90±1.32	75.25±0.91	73.23±0.83
Diabetic	33.0±1.55	28.23±0.92	189.30±1.25	197.5±2.83
D+F-I	28.5±0.95	29.5±0.33*	190.51±2.39**	85.20±0.99
D+F-II	27.2±0.87	31.9±1.22*	192±3.01*	77.29±0.20*
D+Metformin	25.29±1.81	30.21±0.85	195.75±2.53	73.74±2.5

Each value represents the mean ± SEM of five observations. n=5 in each group; D-Diabetic, F- formulation, FBG-Fasted blood glucose. P* <0.05 P** <0.01. Streptozotocin (STZ) induced diabetic mice showed significant reduction in body weight and blood glucose level when compared to normal control mice.

Table 2: Effect of polyherbal formulation I and II on Lipid profile

Groups	Cholesterol	Triglycerides	HDL	LDL	VLDL
Normal	125±2.29	82.5±1.09	36.1±0.98	72.4±1.09	16.5±0.22
Diabetic	288±3.09	243.52±4.23	23.29±1.35	216.01±1.12	48.704±0.62
D + F1	152.8±2.53*	138.5±2.23	42.92±1.44	82.18±0.64	27.7±0.45*
D + F2	163.51±3.29	144.8±3.21*	45.63±1.25	88.92±1.4**	28.96±0.64
D + Metformin	136±1.99	110±0.98	40.29±2.35	73.71±0.56	22±0.20

Each value represents the mean ± SEM of five observations. HDL- High Density Lipoprotein; LDL- Low Density Lipoprotein; VLDL- Very low Density Lipoprotein. $P^{*}<0.05$ $P^{**}<0.01$.

Total cholesterol and triglycerides level was elevated in STZ induced mice and was decreased in formulation treated mice. HDL level was decreased in diabetic mice and increased in formulations treated mice.

Table 3: Effect of polyherbal formulation I and II on Kidney profile

Groups	Creatinine	Urea	Total proteins g/dl	ALP	SGPT	SGOT
Normal	0.6±0.4	22.23±0.5	7.9±1.5	98.2±0.35	22.1±0.14	52.3±0.25
Diabetic	1.9±0.9	81.5±1.25	4.0±1.02	241.8±1.36	51.5±0.55	161.4±0.6
D + F1	0.62±0.21*	31.9±0.62	5.9±0.83	170.9±0.9*	26.7±0.5*	101.6±0.7
D + F2	0.73±0.63	33.7±1.2	5.5±0.85	197.4±0.7*	29.1±0.78	120.2±0.9
D + Metformin	0.52±0.21	29.1±1.5	7.3±0.25	101.5±0.56	31.2±0.74	80.3±0.4

Each value represents the mean ± SEM of five observations. $P^{*}<0.05$, ALP – alkaline phosphatase; SGPT - alanine aminotransferase (ALT or SGPT); SGOT - aspartate aminotransferase (AST or SGOT).

Diabetic patients found to have an elevation of urea and creatinine in plasma which is considered as a significant marker in renal dysfunction[30]. After the treatment with polyherbal formulation I and II, there was a significant drop off the creatinine and urea level in plasma and the values of creatinine were 0.62 mg/dl, 0.73 mg/dl and urea level was 31.9 mg/dl and 33.7 mg/dl after the treatment with formulation I and II respectively.

Many studies reported that the ALP, SGPT and SGOT level were increased in STZ induced diabetic mice[31]. The present study agreed with those researchers which showed the elevation of ALP, SGPT and SGOT (table 3) in diabetic groups and after the treatment of formulation I and II there was significantly decreased in the elevation level. The total protein level was decreased in diabetic groups and after the treatment of formulation I and II there was significantly reduces the protein level.

CONCLUSION

The present study concludes that the poly herbal formulation I showed significant anti diabetic activity compared to formulation II which helps to reduce the diabetic complications without any side effects. The poly herbal formulation I where *Eclipta prostrata*, *Syzygium cumini* and *Phyllanthus niruri* were mixed in the ratio of 1:2:1 and observed the mostly enhanced synergetic activity of the triple combination against the type 2 diabetes. This is the first study on poly herbal formulations of against type 2 diabetes. Future investigations can be carried out towards the isolation and characterization of active constituents present in the tested formulation.

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