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Antidiabetic activity of *Madhumega Churanam* (*Siddha* formulation) in alloxan induced diabetic rats

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ABSTRACT

The present study was performed to evaluate Siddha formulation for its antidiabetic activity in alloxan (120 mg/kg, i.p.) induced diabetic rats. The effects of Siddha formulation on fasting blood glucose level, body weight, fluid intake cholesterol and triglyceride were studied. Siddha formulation (100 mg/kg/day and 200 mg/kg/day p.o.) was administered to a group of diabetic rats (n=6) for 14 consecutive days and the observed data was compared with glibenclamide (10 mg/ kg/ day p.o.). Administration of churanam causes a significant reduction of blood glucose level (p< 0.001), body weight (p<0.001), fluid intake levels (p<0.001), cholesterol (p<0.001) and triglycerides (p<0.001), vs diabetic control. It can be concluded from the present study that oral administration of Siddha formulation (Madhumega churanam) ameliorated the plasma glucose and lipid levels in alloxan- induced diabetic rats.

Keywords: Antidiabetic, *Siddha* formulation, blood glucose, cholesterol, triglycerides.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia. It is characterized by elevated levels of blood glucose or sugar. It occurs when the body glucose produces little or no insulin, when the cells don't respond to the insulin that is produced. Hyperglycemia as a common end point for all types of diabetes mellitus is followed by micro and macro vascular complications leading to cardiovascular disease, nephropathy, neuropathy and retinopathy. [1] In

modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus there is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. [2–4]

Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists, and molecular biologists to combine their efforts in a search for natural agents that can limit diabetes mellitus and its complications. [5]

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Botanicals constitute of major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the health care.[6] One such product is *Madhumega churanam* which is being marketed all over India for the treatment of diabetes. The product contains*:* Each 10 gms contains *Terminalia chebula-2gms,Murraya koenigi-2 gms Emblica officinalis-2 gms,Eugenia jambolana-1 gm,Tinospora cordifolia-1 gm,Phyllanthus amaras-1 gm,Cyperus rotundus-1 gm* .The present study is an attempt to investigate the antidiabetic activity of *Madhumega churanam* in alloxan induced diabetes in rats.

MATERIALS AND METHODS

Animals

Healthy, adult Wistar rats of both sexes (150-220g) were obtained from the central animal house facility J.S.S College of Pharmacy, Ootacamund, Tamilnadu.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. All the experiments were performed after obtaining prior approval from IAEC Approval [JSSCP/IAEC/M.Pharm/Pharmacology/04/2010-2011]

Collection of Siddha formulation

The Siddha formulation *Madhumega Churanam* was procured from Kannan pharmacy Coimbatore, Tamilnadu**.**

Acute toxicity studies

The study was carried out according to the OECD guidelines 423. [7] Female Wistar rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and *Siddha* formulation was administered orally at a dose of 2000mg/kg in distill water. Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked at 6 hr after dosing. The animals were observed twice daily for 14 days and body weight was taken. The same experiment will be repeated once again on 3 rats (preferably female) if there is no observable clinical toxicity for the animals on the acute toxicity study.

Induction of diabetes in experimental animals by inducing alloxan

Diabetes mellitus was induced in the rats by single intraperitoneal injection of 120 mg/kg b.w. of freshly prepared alloxan monohydrate in normal saline. [8] In order to prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h followed by 5% glucose solution bottles in their cages for a period of 24 h. After one wk, the animals showing blood glucose level >250 mg/dl were considered diabetic and used for the study.

Experimental Design.

Five groups of rats, six in each received the following treatment schedule.

Group I: Normal control (saline).
Group II: Alloxan treated control (120 mg/kg,ip).
Group III: Alloxan (120 mg/kg.ip) + Siddha formulation (100 mg/kg, p.o),
Group IV: Alloxan (120 mg/kg.ip) + Siddha formulation (200 mg/kg, p.o),
Group V: Alloxan (120 mg/kg.ip) + Standard drug, Glibenclamide (10 mg/kg, p.o).

Collection of Blood Sample and Blood Glucose Determination.

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation is done on day 1, 3, 7, and 14 of the study. Glucose estimation in serum was analyzed by using Ecoline diagnostic kit. Body weight and fluid intake were also measured On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated [9]. Serum was separated and analyzed for serum cholesterol [10] and serum triglycerides [11] using Ecoline Diagnostic Kit by auto analyzer

Statistical Analysis.

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Bonferroni multiple comparison tests using graphpad prism software 5.0

RESULTS

Acute oral toxicity study of Siddha Formulation

Acute Toxicity studies on female rats showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days. The behavioral, neurological, autonomic responses were studied and during the study no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

Effect of Siddha formulation on body weight and fluid intake

Table 1 shows that effect of *Siddha* formulation changes in body weight and fluid intake in diabetic rats. In diabetic rats, there was significant ($P< 0.001$) decrease in body weight and an increase in fluid intake was observed as compared to normal rats. Oral administration of *Siddha* formulation significantly increased the body weight ($P< 0.001$) and significantly decreased the fluid intake as compared to diabetic control ($P< 0.001$).

Effect of Siddha formulation on Plasma glucose

The initial blood glucose levels of the diabetic rats selected for the study where in the range of 240-300mg/dL. In the diabetic control rats the blood glucose level increase to 367 mg/dL on the 3^{trd} day the glucose levels on the 7^{h} and 14^{h} day of the animals which survived where 410 mg/dL respectively. In the *Siddha* formulation treated rats the blood glucose level suddenly decreased (P<0.001, P<0.01) thus the *Siddha* formulation treatment restore the serum glucose levels almost nearer to normal value and comparable to that of positive control (P<0.001). The changes in plasma glucose estimation in all groups of animal were given in **Table 2**

Effect of Siddha formulation on serum lipid profile

Alloxan induced diabetic rats group where found to have significantly increase triglycerides and total cholesterol levels as compared to control group (P<0.001). Positive control was significantly preventing the increasing the serum triglycerides and total cholesterol levels as compared to diabetic group. In the *Siddha* formulation treated rats the cholesterol and triglycerides level decreased significantly (P<0.001) Thus the *Siddha* formulation treatment restores all these changes near to normal value. The change in serum lipid profile were tabulated in **Table 3**

Table 1: Effect of *Siddha* formulation on body weight and fluid intake in alloxan induced diabetic rats

S. No	GROUP	Body Weight (g)		Fluid intake g/animal/day
		Before treatment	After treatment	
1.	Untreated control	195±1.88	221.5±1.83	23.047±0.247
2.	Diabetic control	204.66±2.33	169.5±2.51$^{###}$	75.288±0.23$^{###}$
3.	Diabetic+Glibenclamide (10mg/kg)	208.66±1.74	224.33±1.96***	54.610±0.37***
4.	Diabetic+*Siddha* Formulation (100mg/kg)	209±1.93	221.16±1.07***	58.436±0.16***
5.	Diabetic+*Siddha* Formulation (200mg/kg)	195±2.17	214.6±1.47***	60.43±0.35***

All values are expressed as mean ±S.E.M (n=6).
****P<0.001 as compared to diabetic control, $^{###}$P<0.001 as compared to untreated control.*
One-way ANOVA followed by Bonferroni multiple comparison tests.

Table 6.2: Effect of *Siddha* formulation on serum glucose estimation in alloxan induced diabetic rats

S. No	GROUP	Serum glucose (mg/dL)			
		0 day	3rd day	7th day	14th day
1.	Untreated control	84.83±5.41	85.33±5.87	84.66±5.77	84.83±5.09
2.	Diabetic control	298.16±17.20	367.33±4.70$^{##}$	413.83±16.61$^{###}$	410±2.045$^{###}$
3.	Diabetic+Glibenclamide (10mg/kg)	280.33±2.44	205.33±1.14**	165±1.29***	114.83±1.30***
4.	Diabetic+*Siddha* Formulation (100mg/kg)	277±7.69	223±4.19**	164.66±1.40***	116.5±1.23***
5.	Diabetic+*Siddha* Formulation (200mg/kg)	282.66±4.49	223.33±1.94**	164.16±1.40***	115±0.96***

All value are expressed as mean ±SEM (n=6).
****P<0.001, **P<0.01 as compared to diabetic control. $^{##}$P<0.01, $^{###}$P<0.001 as compared to untreated control.*
One-way ANOVA followed by Bonferroni multiple comparison test.

Table 6.3: Effect of *Siddha* formulation on serum lipid profile in alloxan induced diabetic rats

S.No	GROUP	Total cholesterol (mg/dL)	Triglycerides (mg/dL)
1.	Untreated control	96.5±0.76	64.83±1.13
2.	Diabetic control	166.68±0.72$^{###}$	193.5±11.59$^{###}$
3.	Diabetic+Glibenclamide (10mg/kg)	79.33±2.81***	126.5±5.21***
4.	Diabetic+*Siddha* Formulation (100mg/kg)	136.88±0.25***	74.16±1.53***
5.	Diabetic+*Siddha* Formulation (200mg/kg)	136.95±0.22***	79.66±4.63***

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

DISCUSSION

The present study is the preliminary assessment of the anti diabetic activity of the *Siddha* formulation in alloxan induced diabetic rats. To check the safety profile of the *Siddha* formulation 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 extract can be classified in to the safe drug category according to the "Global harmonized Classification System" quoted in the OECD guidelines 1996. [7]

Generally there is a decrease in the body weight of diabetic untreated animals due to the under utilization of glucose. Decrease in body weight of diabetic rats is due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control .The final weight of untreated control group was significantly increased than at the beginning of the experiment. In contrast there was a decrease in body weight in diabetic untreated animals.[12]

Similarly, the treated groups with *Siddha* formulation increased the body weight of the animals in a significant manner. Similarly there was a decreased consumption of fluid in case of treated groups when compared with that of the diabetic untreated groups

The goal of blood glucose tests is to find out whether there is the availability of large amounts of glucose in the blood. The combination of increased hepatic glucose production and reduced metabolism in peripheral tissues leads to elevated plasma glucose levels.[13] The treatment with *Siddha* formulation in alloxan induced diabetic rats significantly decreased the elevated serum glucose levels.

Serum lipid profile is usually raised during diabetes and presents a risk for the coronary heart disease. [14] Serum triglycerides and cholesterol were increased in alloxan diabetic rats. The treatment with Siddha formulation decreased the raise of lipids in serum and improved the lipid level to a normal condition which may be attributed to its potent antidiabetic activity.

From the results it can be stated that *Siddha* formulation increased the body weight, decreased the fluid intake and decreased the levels of serum glucose and lipids

CONCLUSION

The present study reveals the beneficial effect of *Siddha* formulation on lipid profile, body weight, and plasma glucose levels in alloxan induced diabetic rats. It can be concluded from the present study that oral administration of *Siddha* formulation (*Madhumega churanam*) ameliorated the plasma glucose and lipid levels in alloxan- induced diabetic rats. Thus, *Madhumega churanam* may find clinical application in treating dyslipidemia in diabetic patients. Further works are in progress to identify its mechanism of action and effects in diabetes associated complications.

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