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ORIGINAL RESEARCH ARTICLE

Antidiabetic Activity of a Herbal Marketed Product *Madhumeha Churna* in Alloxan - Induced Diabetic Rats

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ABSTRACT

The present study was performed to evaluate Madhumeha Churna for its antidiabetic activity in alloxan (100 mg/kg, i.p.) induced diabetic rats. The effects of Churna on fasting blood glucose level (BGL), oxidative stress parameters such as malondialdehyde (MDA) levels, and glutathione (GSH) in liver homogenates, were observed. Churna (200 mg/kg/day and 400 mg/kg/day p.o.) was administered to a group of diabetic rats (n=6) for 10 consecutive days and the observed data was compared with glibenclamide (n=6, 10 mg/ kg/ day p.o.). Administration of Churna causes a significant reduction of BGL ($p< 0.01$), MDA ($p<0.001$), GSH levels ($p<0.05$) vs diabetic control. Based on our data it can be concluded that Madhumeha Churna possess moderate antihyperglycemic effect and may effect GSH and MDA levels and has mixed effect on body & various organ weight.

Keywords: Antidiabetic, Madhumeha Churna, MDA, GSH.

INTRODUCTION

Diabetes mellitus is a serious complex chronic condition characterized by hyperglycaemia and disturbance in metabolism. The incidence of Diabetes mellitus is on the rise worldwide. Based on the World Health Organization (WHO report), the number of diabetes patients is expected to increase continuously [1]. 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 [2].

In India herbal medicines are widely used to treat diabetic condition. Several marketed herbal products are available in the market for the treatment of diabetes. One such product is Madhumeha churna which is being marketed all over India for the treatment of diabetes. The product contains; *Syzigium cumini* (60gm), *Cassia auriculata* (60gm), *Salacia oblonga* (60gm), *Ficus bengalensis* (60gm), *Smilex china* (60gm), *Acacia catechu* (60gm), *Hemidesmus indicus* (60gm), *Zizyphus mauritiana* (40gm), *Michelachampaca* (40gm), *Piper longum* (40gm), *Terminalia chebula* (40gm), *Myristica fragrance* (40gm), *Eugenia caryophyllus* (40gm), *Embellica officinallis* (40gm), *santalum album* (40gm), *Enicostemma axillare* (40gm), *Sida rhambifolin* (40gm), *Saplum insighe* (100gm), *Trikatu* (100gm), *Aegle marmelos* (100gm), *Phyllanthus amarus* (100gm), *Centela asiatica* (100gm), *Ocimum centcum* (100gm), *Murreya koenigii* (100gm), *Psidium guava* (100gm), *Melochia corchorifolia* (100gm), *Hybanthus enneaspermus* (100gm), *Trigonella foenum graecum* (250gm).

MATERIALS AND METHODS

Animals

Wistar albino rats of either sex weighing between 100 – 200 gm were obtained from B.R.N.C.P. Mandsaur animal house, protocol was approved by IAEC, BRNCP, Mandsaur. These animals were used for the acute toxicity and antidiabetic activity. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 60 ± 5% relative humidity and 12 hrs light dark cycle. They were given standard pellet diet and water *ad-libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put.

Collection of herbal product churna

The herbal marketed product Madhumeha churna was collected from Dr. G. Elias,

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Leelavilasam Siddha Varma Vaidyasalai, Karungal, Tamil Nadu as gift sample.

Acute toxicity studies of the churna

The acute toxicity study was carried out in adult female albino rats by "fixed dose" method of OECD Guideline No.420, test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and next day Madhumeha churna (suspended in 5% v/v Tween 80 solution) was administered orally at a dose level of 2000 mg/kg to 5 female animals. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days[3]. From the results of toxicity study two dose levels were chosen in such a way that, the doses were approximately one fifth & one tenth of the maximum dose during acute toxicity studies.

Antidiabetic studies

Animals were fasted for 24 hours and then a single i.p. injection of freshly prepared alloxan (100 mg/kg dissolved in 0.9% saline) was injected except group I. After that the animals were left aside for 2 hrs and then 10% glucose solution was placed in the cages for 24 hrs. The diabetes was confirmed by estimation of blood glucose level (BGL) on 3rd day. Rats having BGL more than 250 mg/dl were used for the study. Animals were divided into five groups (n=5). Group I was kept as normal control (non-diabetic), Group II was kept as diabetic control, Group III was treated with standard Glibenclamide 5 mg/kg and Group IV & V were treated with 200 mg/kg & 400 mg/kg of Churna. Test samples were given orally using micro suction canula to the animals once daily. The fasting blood glucose concentrations of the animals were measured at the 0th , 3rd, 7th and 10th day by commercially available glucose kit based on glucose oxidase method (Accu check active glucometer & Accu check active strips) on 11th day the animals were euthanized by over dose of anesthetic ether and the liver were collected [4-7].

Tissue preparation

Livers from each group were finely minced and homogenized in 50 mM phosphate buffer, pH 7 and centrifuged at 10,000 x g for 15 min at 4°C (REMI, RM-12 MicroCentrifuge.). The supernatant was used for measurement of all oxidative stress parameters [8]

Measurement of oxidative stress parameters

Determination of Glutathione

Glutathione (GSH) was measured using DTNB (2, 2´-dinitro-5, 5´-dithiodibenzoic acid) as the reagent. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. For its measurement 0.5 ml supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of DTNB, in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, pH 8). The absorbance was read at 412 nm. Total groups assay was expressed as mg/100 g tissue [9].

MDA determination

Determination of malondialdehyde (MDA) levels; an index of lipid peroxidation is based on measurement of the purple colour generated by the reaction of thiobarbituric acid (TBA) with MDA. 0.5 ml of supernatent was mixed with 2.5 ml of trichloroacetic acid (10% w/v) in a centrifuge tube which was kept in boiling water bath for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000 x g for 10 min and its 2 ml was transferred to a tube containing 1 ml of TBA solution (0.67 % w/v). Absorbance was read after cooling at 532 nm. MDA concentration was calculated using extinction coefficient of $1.56 \times 10^5 cm^{-1}M^{-1}$ [8].

Statistical analysis

The data were expressed as mean ± SEM. The data of antidiabetic activity was analyzed by one way analysis of variance (ANOVA) followed by "Dunnett's test". p value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

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.

Alloxan-induced diabetes is a well-documented model of experimental diabetes this compound causes severe necrosis of pancreatic β-cells [10-11].

Effect of plant extracts on blood glucose levels in diabetic rats The effect of Churna in alloxan induced diabetic rats is shown in table 1. Administration of alloxan lead to elevation of fasting blood glucose levels from 87.00 to 404.00 on day 3 after alloxan treatment, where as treatment group (churna) shows a decline in blood

glucose level as compared to alloxan treatment (negative control) group and equivalent reduction in glucose level as compared to glibenclamide treated rats; as shown in **Table 1.**

Table 1: Effect of churna on blood glucose level (mg/dl) of diabetic animals.

Groups	Treated	0 DAY	3 DAY	7 DAY	10 DAY
G-I	Normal saline	87.40 ± 1.69	92.40 ± 2.89	92.60 ± 1.47	87.80 ± 5.54
G-II	Alloxan (no treatment)	87.00 ± 2.47	404.00 ± 23.33	486.00 ± 9.85	484.60 ± 8.06
G-III	A+Glibenclamide (10 mg/kg)	85.80 ± 1.65	384.40 ± 17.82	181.80 ± 13.31**	141.00 ± 10.78**
G-IV	A + Herbal drugs (200mg/kg)	89.60 ± 1.56	377.80 ± 12.93	204.80 ± 8.25**	138.60 ± 5.24**
G-V	A + Herbal drugs (400mg/kg)	88.75 ± 4.72	394.52 ± 16.83	200.50 ± 12.54**	134.42 ± 4.89**

*Value expressed in mean ± SEM (n=6), **p<0.01 vs G-II, ANOVA followed by Dunnett's test. A stands for alloxan*

Body weight, liver, renal and pancreatic weight

Table 2 shows the effect of Churna on body, renal and hepatic weight of normal, diabetic and diabetic treated rats. Results showed no significant intra-group variation in the basal body weight. The liver weight of the normal rats was greater as compared to the diabetic control rats and treated diabetic rats. The liver mass was increased in diabetic treatment groups and glibenclamide treatment groups significantly ($p<0.05$) with respect to diabetic control groups. Alloxan administration also caused a decrease in the pancreatic tissue weight. Treatment with the Churna caused a significant increase in pancreatic tissue weight ($p<0.05$) with respect to diabetic control. Churna reduced the elevated kidney weight slightly as comparison to untreated diabetic rats, although this did not reach statistical significance level.

Table 2. Effect of churna on body, liver, pancreatic & kidney weight and GSH & MDA levels.

Groups	Body weight			Liver weight (g/100g b.wt)	Pancreatic weight (g/100g b.wt)	Terminal kidney weight (g/100g b.wt)	GSH (mg/100 g tissue)	MDA (nmole/mg protein)
	0 day	Day 6	Day 11					
Group I	160±5.7	163.3±18.5	156.7±23.3	3.96±0.75	0.6±.003	1.0±0.14	18.0±0.2	1.36±0.02
Group II	176±21.9	140.0±5.7	133.4±12.0	1.15±0.2	0.4±0.05	0.9±0.02	6.4±0.10	22.5±0.14
Group III	183.3±14.5	166.6±23.3	156.7±18.5	3.49±0.15*	1.5±0.09*	0.9±0.0	7.53±0.02*	5.5±0.34***
Group IV	170.8±12.4	160.9±21.7	162.6±18.4	3.47±0.24*	1.8±0.1*	0.9±0.1	8.37±0.28*	5.9±0.82***
Group V	206.7±14.5	213.3±16.6	196.6±17.6	3.43±0.032*	2.3±0.36***	0.9±0.02	10.34±0.27*	6.09±0.02***

*Values are in mean ± SEM; n=6, *p<0.05, **p<0.01, ***p<0.001 values significant with respect to diabetic control (one way ANOVA followed by Dunnett's t test),*

Measurement of oxidative stress parameters

In order to explore the effect Churna on the oxidative stress parameters on hepatic tissue of diabetic rats, reduced glutathione (GSH), lipid peroxidation were evaluated. Table 2 shows the level of GSH, lipid peroxidation marker (MDA), components in liver homogenates of normal and experimental diabetic rats and the effect Churna on these parameters. There was a significant elevation in MDA concentration, while the activity of GSH content decreased in diabetic when compared with corresponding normal group. Treatment of the rats with Churna significantly decreased lipid peroxidation end product, MDA ($p<0.001$) with respect to diabetic control. In addition, the GSH content in hepatic tissue significantly increased as compared to corresponding diabetic group.

In conclusion, the present data revealed that Churna possess antihyperglycaemic effect and antioxidative potential which was shown by significant quenching impact on the extent of lipid peroxidation and along with, enhancement of antioxidant defense systems in rat hepatic tissue. Further works are in progress to identify its mechanism of action and effects in diabetes associated complications.

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