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ORIGINAL RESEARCH ARTICLE

Antidiabetic Activity of Hydroalcholic Extract of Herbal Marketed Product *Madhuhari Churna* in Alloxan - Induced Diabetic Rats

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ABSTRACT

Hypoglycemic and antidiabetic activity of hydroalcoholic extract of Madhuhari churna was evaluated in alloxan induced diabetic rats. Effect on normal blood glucose level (hypoglycemic), Oral glucose tolerance test (OGTT), hyperglycemia (antidiabetic) and glycogen level in liver was evaluated. Hydroalcoholic extract was administered orally at two dose levels 200 mg/kg and 400 mg/kg body weight, for consecutive 10 days in diabetic rats, and glibenclamide was kept as standard. Blood glucose (BG) and OGTT in normal healthy rats produced significant fall in BG and improved glucose tolerance. The present study concludes that the Madhuhari churna is effective in reducing the blood glucose level of diabetic animals. The extract did not exhibit a dose dependent effect.

Key Words: OGTT, Hypoglycemic, Antidiabetic, Madhuhari Churna.

INTRODUCTION

Diabetes is possibly the world's fastest growing metabolic disease [1]. Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by defective regulation of carbohydrate, fat and protein or in other words it is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometime ketonemia[2].

The use of traditional plant for treatment of diabetes mellitus is widely practiced in world. Many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of diabetes mellitus[3]. The World Health Organization has recommended that use of herbs should be encouraged[4].

In India; herbal medicines are widely used to treat this condition. Several marketed poly herbal products are available in the market for the treatment of diabetes. One such product is Madhuhari churna which is being marketed all over India for the treatment of diabetes. The product contains following ingredients; Gudmar (30%), Karela beej (10%), Jamun guthli (10%), Babul ki chhal (10%), Amba Haldi (10%), Gudvel (10%), Bilva Patra (10%), Neem Patra (5%), Shilajit (2.5%) and Trivanga Bhasma (2.5%).

This study has been planned to investigate the hypoglycaemic effect of hydroalcholic extracts of Madhuhari churna on normal and alloxan-diabetic rats and to determine the oral acute toxicity.

MATERIALS AND METHODS

Collection of herbal product

The herbal marketed product Madhuhari churna was purchased from Attar Drug House at Mandsaur.

Extraction

Cold Maceration technique was used for extraction. The solvent used was hydroalcoholic (70% ethanol: 30% water). Churan was kept for four days with occasional shaking and was filtered. The filtrate was evaporated and residue was obtained. The % yield was calculated after drying and qualitative chemical examination was carried out[5].

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 hr light dark cycle. They were given standard pellet diet and water *ad-libitum*

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throughout the course of the study.

Acute toxicity studies of the extracts

The acute toxicity study was carried out in adult female albino rats by "fixed dose" method of OECD Guideline No.420. The animals were fasted overnight and next day extract of the Madhuhari 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[6].

Selection of doses

For the assessment of hypoglycemic and antidiabetic activity, two dose levels were chosen in such a way that, one dose was approximately one tenth of the maximum dose during acute toxicity studies and a high dose, which was twice that of one tenth dose (200mg/kg and 400mg/kg).

Assessment of hypoglycemic activity of extract.

Four groups of fasted rats, six rats in each, were used. Group first (N1) was treated with normal saline and was considered as a normal control group and group second (N2) was treated with glibenclamide 0.5 mg/kg and considered as standard group. Group third and fourth (N3 and N4) were given orally *churna* extract 200mg/kg and 400mg/kg of body weight respectively. Blood samples were collected at 30, 60, 120, 180 and 240 min after administration of extract[2].

OGTT (Oral glucose tolerance test) in normoglycaemic rats

Four groups of fasted rats, six rats in each, were used. Group O1 was treated with normal saline, 02 (standard) was treated with glibenclamide 5 mg/kg. While the third group (O3) and fourth group (O4) were given orally Churna extract 200mg/kg and 400mg/kg of body weight respectively. Glucose solution 2 gm/kg was given orally to all the groups after 30 min of extract administration. Blood samples were collected at 30, 60, 120 and 180 min after administration of extract[7].

Antidiabetic studies

Rats of either sex with body weight (150-200 g) were made diabetic with single injection of alloxan monohydrate (150 mg/ kg) i.p. dissolved in normal saline. Fasting blood glucose levels were estimated by commercially available glucose kit based on glucose oxidase method[8]. Rats with blood glucose more than 150 mg/dl were included in the study.

Treatment protocol

Group I was kept as normal control (non-diabetic) received normal saline

Group II was kept as diabetic control received normal saline

Group III was kept as standard (Glibenclamide 5 mg/kg)

Group IV & V were treated with 200 mg/kg & 400 mg/kg of extract.

Test samples were given orally using micro suction canula to the animals once daily. The blood glucose concentrations of the animals were measured at the beginning of the study (0^{th} day) and the measurements were repeated on 3^{rd}, 7^{th} and 10^{th} day [9,10].

Collection of blood and determination of blood glucose

Blood samples from rats were collected from the tail vein under light anaesthesia. Blood glucose levels were determined by Accu-chek active glucometer using Accu-chek active strips and levels were expressed in mg/100 ml of blood.

Determination of glycogen level in Liver.

On the 11th day of diabetic study all groups of animals were sacrificed. Livers were taken and washed with saline and stored in chilled condition until used. Liver tissues were homogenized in hot ethanol (10 ml) at tissue concentration of 100 mg /ml. Centrifuged at a rate 8000 X g for 20 min. 20 µl of it was used for initial glucose level estimation. The residue was collected and allowed to dry over water bath. Then 5 ml of KOH (10%) and 6 ml perchloric acid (52%) were added and left aside for 20 min at 0°C. The collected material was then centrifuged at 8000 X g for 15 min. From collected supernatant 20 µl of supernatant was removed for final estimation of glucose level. The glucose was estimated by glucose estimation kit at 500 nm[11].

The Glucose conc. was calculated as

Glucose conc. (mg/dl) = AT / AS X 100

Where; **AT** means Absorbance of test

AS means absorbance of standard.

Statistical analysis

The data were expressed as mean ± SEM. The data was analyzed by one way analysis of variance (ANOVA) followed by "Dunnett's t test". p value less than 0.05 was considered as statistically significant.

RESULTS and DISCUSSION

The present investigation reports the hypoglycaemic and antidiabetic effect of hydroalcholic extract of churna.

Acute Toxicity studies

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 extract on blood glucose levels of normal rats

Churna extract at doses, 200 mg/kg and 400 mg/kg, decreased glucose level till the 4th h. The effect of extract was dose dependent (**Table 1**). The maximum reduction in fasting serum glucose levels in multi dose study was observed to be 7.65 % on eleventh day (**Table 1**). In order to find out whether the extract interfered with glucose determination, a known quantity of the serum from extract treated animals were mixed with assay mixture. It was found that the serum from animals treated with the extract did not inhibit the glucose determination indicating that reduction in blood glucose was not due to an artifact of glucose estimation.

Table 1. Effect of extract on blood glucose level of normal rats

Group	Blood glucose level (mg/dl)					
	0 min	30 min	60 min	120 min	180 min	240 min
N 1	89.40 ±1.03	90.60± 0.68	90.00 ±1.00	91.20± 0.86	89.80± 1.07	89.20± 0.58
N 2	87.00± 2.91	82.80 ±3.38*	78.40 ±1.96*	61.00 ±2.30*	56.20 ±1.99 *	50.00 ±2.74*
N 3	89.00± 1.51	96.20 ±1.08*	90.20 ±1.77*	71.40 ±1.78*	62.00 ±2.00*	78.40± 3.79*
N 4	99.00± 1.70	95.80 ±1.16*	91.40 ±1.03*	78.20 ±1.39*	60.40 ±1.57*	73.80 ±1.66*

*N 1- normal control; N 2 standard & N 3 & 4 – Churna extract 200mg/kg and 400mg/kg respectively, n=6, *p < 0.01 vs normal control, ANOVA followed by Dunnett's test, Values are expressed in mean ± SEM*

Effect of extract in glucose – hyperglycaemic animals (Oral glucose tolerance test, OGTT)

The groups treated with churna extract showed initially increase in glucose level upto 30 min then it gradually decreased, the level was maintained at 120 min i.e. the glucose level reached the normal level and maintained upto 180 min. Where as glibenclamide maintained glucose level at normal range through out the study period (**Table 2**).

Table 2. Effect of extract in glucose induced hyperglycaemic animals

Group	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	120 min	180 min
O 1	92.67 ±1.45	143.30± 4.80	126.71± 4.67	95.37± 2.96	89.67± 1.45
O 2	90.33 ±2.33	95.33 ±1.20*	86.00± 1.53*	83.67 ±1.45*	81.67 ±0.88*
O 3	89.67 ±1.76	137.30 ±2.40	108.70± 2.03*	94.67 ±1.20*	83.67± 1.42 *
O 4	88.39 ±1.51	129.64 ±4.23	99.19± 3.52*	94.81 ±4.92*	82.13± 2.18*

*O 1- normal control; O 2- standard; O 3&4 –Churna extract 200mg/kg and 400mg/kg respectively, n=6, *p < 0.01 vs normal control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM.*

Table 3. Effect of extract blood glucose level and glycogen level in alloxan induced diabetic animals

Groups	Blood glucose level (mg/dl)				Glycogen level (mg/gm wet tissues)
	0th Day	3rd Day	7th Day	10th Day	11th Day
G-I	87.40±1.69	92.40±2.89	92.60±1.47	87.80±5.54	137.18 ±3.24
G-II	87.00±2.47	424.00±23.33	466.00±9.85	494.60±8.06	117.05 ±2.85
G-III	85.80±1.65	394.40±17.82*	191.80±13.31**	144.00±10.78**	210.25 ±2.45*
G-IV	94.40±2.67	387.20±29.83*	193.20±28.26**	145.40±4.06**	198.73 ±2.29*
G-V	91.40±2.27	398.00±17.79*	208.80±15.86**	141.00±3.03**	195.54± 4.62*

*G 1- normal control; G 2- diabetic control; G 3- standard; G 4 & G 5- Churna extract 200mg/kg and 400mg/kg respectively,Value expressed in mean ± SEM (n=6), *p>0.05, **p<0.01 vs negative control, ANOVA followed by Dunnett's t test.*

Experiments with doses of 200 and 400 mg/kg of extract on normal BG and OGTT in normal healthy rats produced significant fall in BG (**Table 1**) and improved glucose tolerance (**Table 2**). Extract at both the doses were found equally effective, hence the dose of 200 mg/kg

may be appropriate dose for diabetic. This study also revealed that the maximum hypoglycaemic effect was produced only 1 h after administration of extract to the fasted animals. This indicates that it takes about 1 h for the active ingredient(s) or its (their) metabolites in the extract to enter into the circulation and reach target tissues to bring about hypoglycaemic effect, which is maintained for at least 2 1/2 h. In the OGTT experiment reduction in blood glucose level was started after 1 h and maintained at least for 2 h.

Antidiabetic activity

Treatment of rats with alloxan induced diabetes for 10 days (**Table 3**) brought down the elevated blood glucose levels, ranging from 150 to 250 mg/dl to nearly normal range. Alloxan not only destroys the pancreatic *β*-cells but causes kidney damage, which is however reversible causing diabetes close to type-II in humans[12]. The elevated blood glucose levels in the diabetic animals used by us were more than 150 mg/dl, which resembles type-II diabetes (150 to about 250 mg/dl) with partially functional pancreas. This shows that the *churna* extract might be useful in type-II diabetes, irrespective of whether the pancreas is partly functional or almost totally destroyed. This is an advantage, keeping in mind that the present-day sulphonylurea drugs act only when there is a functional pancreas [13].

Churna extract at a dose of 400 mg/kg showed a decrease in glucose level highly and brought the blood glucose level at near normal on 7th day of diabetes. Churna extract at a dose of 200 mg/kg also showed significant effect. The activity was found dose dependant (**Table 3**). Body weight of rats showed a decline initially but after 10 days of treatment, the treated Churna extract groups showed increase in body weight.

From the phytochemical analysis it was found that the major chemical constituents of extract were carbohydrate, alkaloid and saponins. Alkaloids and saponins[14,15] are reported to have antidiabetic activity. So the extract of churna may be active due to the presence of these compounds.

Estimation of glycogen level in liver

Insulin is also main regulator of glycogenesis in muscle and liver. The decrease in the glycogen level of the liver was been observed which is due to diabetic condition of experimental rats[16]. The lack in the level may probably due to inactivation of glycogen synthetase system. Rat liver showed an increase in level of glycogen of both glibenclamide treated and leaves extract treated group at both the dose levels. The increase in Glycogen level in liver of animal treated with the leaf extract was lesser than glibenclamide (**Table 3**). The treated group shows an elevation in the glycogen content of the liver this suggest that extract causes stimulation of glycogenesis process, may be Madhuhari churna activate glycogen synthetase system.

The present study concludes that the marketed product, Madhuhari churna is effective in reducing the blood glucose level of diabetic animals. Further works are in progress to identify its effects in diabetes associated complications.

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