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EVALUATION OF ANTIDIABETIC, AND ANTIOXIDANT ACTIVITIES OF MADHUMARDAN CHURNA- A POLYHERBAL AYURVEDIC FORMULATION

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ABSTRACT

Madhumardan Churna was tested for its anti-diabetic and anti-oxidant activity using alloxan induced diabetic rats and compared with standard Gliclazide (10 mg/kg). The results expressed that Madhumardan Churna had shown significant protection and maximum reduction in blood glucose as well as other biochemical parameters like SGOT and SGPT were observed in alloxan induced diabetic rats. The results of this comprehensive study reveal that Madhumardan Churna shown statistically significant Anti-diabetic activity in comparison to standard Gliclazide.

KEYWORDS : *Anti - diabetic, Anti – oxidant, Madhumardan Churna, Gliclazide, Alloxan monohydrate*

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INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia related with impairment in insulin secretion and/or insulin action with change in intermediary metabolism of carbohydrates, lipids and proteins. It is a disease in which the pancreas either does not release insulin or insulin sensitive tissues such as liver, muscle and fat do not actually respond to this hormone. Impaired glucose metabolism in diabetes is associated with increase free radical generation which further leads to increased levels of triglycerides and lipoprotein – leading to cardiovascular complications of diabetes, which includes atherosclerosis, ischemic heart diseases etc. So, combination of antihyperglycaemic, antihyperlipidaemic and antioxidant can be advantageous in the prevention of diabetes mellitus and its complications. Oxygen free radical can begin peroxidation of lipids, which in turn stimulates glycation of proteins, inactivation of antioxidant enzymes and play a role in long term complication of diabetes. Regardless of the presence of well-known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease possibly because they are believe to be less toxic and free from side effects compared to synthetic one.

Madhumardan Churna contains 32 different ingredients with purified pearls and purified bitumen which is manufactured by Sri Jain Ayurvedic Pharmacy, Hyderabad, India. These plants are known to possess antidiabetic, antioxidant, antihyperlipidaemic activity. Composition of Madhumardan Churna is given in Table No. 1. According to ayurveda, combinations

of these substances are helpful in curing the disorder and eliminate side effects related to disorder. In view of above information, the present study undertaken to evaluate antidiabetic and antioxidant activity of Madhumardan Churna.

Table No. 1: Composition of Madhumardan Churna

Sr. No.	Common name	Botanical name	Part used	Family	Each 5 gm contain	Activity
1	Jambul	Syzigum cumini	Seed	Myrtaceae	250 mg	Antihyperglycemic
2	Chiragata	Swertia chirata	Flower	Gentianaceae	250 mg	Antihyperglycemic
3	Bitter guard	Momordica charantia	Fruit	Cucurbitaceae	250 mg	Antihyperglycemic
4	Tulsi	Ocimum sanctum	Seed	Lamiaceae	40 mg	Antihyperglycemic, antioxidant
5	Indian sandalwood	Santalum album	Bark	Santalaceae	40 mg	Diuretic
6	Harada	Terminalia chebula	Fruit	Combretaceae	160 mg	Liver stimulant, Mild Laxative
7	Aamla	Emblica officinalis	Fruit	Phyllanthaceae	160 mg	Antihyperglycemic, Antioxidant
8	Bayberry	Myrica esculenta	Fruit	Myricaceae	40 mg	Antioxidant
9	Neem	Melia azadirachta	Leaf, Fruit	Meliaceae	160 mg	Antihyperglycemic, Antihyperlipidaemic
10	Kalijiri	Centratherum antihelminthicum	Fruit	Asteraceae	250 mg	Antihyperglycemic
11	haldi	Curcuma longa	Root	Zingiberaceae	160 mg	Antioxidant, Antihyperlipidemic, antihyperglycaemic
12	Baheda	Terminalia belerica	Fruit	Combretaceae	160 mg	Antihyperglycemic, Antioxidant
13	Vasaka	Adhatoda vasica	Leaf	Acanthaceae	250 mg	Antihyperglycemic
14	Liquorice	Glycerrhiza glabra	Bark, Rhizome	Fabaceae	80 mg	Antioxidant, Antihyperlipidemic
15	Pushkaramoola	Inula racemosa	Flower	Asteraceae	80 mg	Antihyperglycemic
16	Ginger	Zingiber officinale	Rhizome	Zingiberaceae	40 mg	Antihyperglycemic
17	Black paper	Piper nigrum	Fruit	Piperaceae	40 mg	Antioxidant
18	Nut grass	Cyperus rotundus	Leaf	Cyperaceae	160 mg	Antihyperglycemic, Antioxidant
19	Spearmint	Mentha Spicata	Leaf	Lamiaceae	40 mg	Antioxidant
20	Rhubarb	Rhem emodi	Bark	Polygonaceae	80 mg	Antioxidant
21	Dhawai	Woodfordia fruticosa	Flower	Lythraceae	160 mg	Hepatoprotective
22	Senna	cassia auticulata	Leaf	Caesal piniaceae	250 mg	Antihyperglycemic, Antioxidant
23	Gurmar	Gymnema sulvestre	Leaf	Asclepiadaceae	250 mg	Antihyperglycemic

Sr. No.	Common name	Botanical name	Part used	Family	Each 5 gm contain	Activity
24	Velvet bean	Mucuna prurita	Seed	Fabaceae	80 mg	Laxative
25	Puncture wine	Tribulus terrestris	Fruit	Zygophyllaceae	80 mg	Antihyperglycemic
26	Fenugreek	Trigonelia foenum graecum	Fruit	Fabaceae	600 mg	Antihyperglycemic, Antioxidant
27	Four leave cassia	Cassia absus	Flower	Fabaceae	250 mg	Antihyperglycemic, Diuretic
28	Hybathus	Ionidium enncapsermin	Leaf	Violaceae	80 mg	Antihyperlipidemic
29	Guar	Cymopsis tetragonoloba	Fruit	Leguminosae	250 mg	Antihyperlipidemic
30	Nagarjuni	Euphorbia thyme folia	Leaf, Fruit	Euphorbiaceae	150 mg	Antiplasmodic, Antihyperglycemic

MATERIALS AND METHODS

Materials:

Madhumardan churna procure from local market in Nasik district which was manufactured by Sri Jain Ayruvedic pharmacy (Batch No. 421, Mfg. Lic. No. T/1633/Ayur, Mfg. date: November 2011. Mfg by: Sri Jain Ayurvedic Pharmacy, Hyderabad). Alloxan monohydrate was procured from Loba Chemie Private Limited, India. Glucose, SGOT, SGPT kits were purchased from Transasia bio-medical limited, Solan, Himachal Pradesh, India. Other chemicals used were analytical grade.

Preliminary Phytochemical Screening-

Preliminary phytochemical screening were performed for all extracts for the presence of phytochemical like alkaloids, glycosides, flavones, tannis, terpenes, sterols, saponins, fats and sugars, using standard qualitative assays[7,8,9]. Moisture content, Ash values, extractives were determined by using standard procedure [10].

Experimental animals:

Wistar albino rats of either sex weighing between 160 to 200 gm were selected for this study. These animals were kept in animal house of SPTM, Shirpur under 12 hr light and dark cycle. Experiment was performed in optimal environment condition set at 20 °C (± 2 °C) temperature and relative humidity of 25- 40 %. During the experimental procedure animals were fed standard chew diet (Amrut food pallets) and RO treated water.

All experiments and protocols described in present study were approved by Institutions Animal Ethics Committee (IAEC) and are in accordance with guidelines as per " Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Preparation of alloxan solution:

Alloxan monohydrate was dissolved in normal saline solution at room temperature to result 125 mg/ml stock solution. Diabetes was induced by administering alloxan monohydrate IP.

Preparation of standard drug solution:

A dose of 10 mg/kg of gliclazide was administered orally by making stock solution of 3.0 mg/ml considering the maximum weight of the animal and from the stock solution further dose was calculated and administered to other animals.

Preparation of test solution:

1 gm of churna was dissolved in 0.25% Sodium carboxy methyl cellulose at room temperature to result 100 mg/mL stock solution

Induction of diabetes:

Alloxan monohydrate was injected intra peritoneal at a dose of 125 mg/kg body weight in the normal saline. After 1h of alloxan administration the animals were fed on standard pellets & water ad libitum and to overcome fatal hypoglycemia, rats were treated with 20% glucose solution (5-10 ml) orally after 1 h & next 24 hrs provided with 5% glucose solution bottles in their cage. Diabetes was

confirmed by measuring the fasting blood glucose concentration, 72 hrs after alloxanisation. Animals with blood glucose level above 150 mg/dl were considered to be diabetic & were used in the study. Blood samples (0.5 ml) were obtained for glucose estimation by retro orbital plexus using capillary tubes, before and 72 hrs after Alloxanisation.

Administration of drugs:

A predetermined dose of the standard and the test drug was given by using curved blunt needle and 1ml capacity syringe. The dosing was done from 0th day to 21th day of treatment. Blood was collected at 0, 7, 14 and 21 days of treatment. Blood glucose level was determined using Erba auto analyzer, at respective days.

Experimental design:

Blood sugar levels of animals were measured after 72 hrs of administration of alloxan. Animals showing blood serum level above 250 mg/dl were selected and divided in 5 groups of animals. One more group of animals was selected to which Alloxan was not administered. These animals served as negative control.

Group 1	_	Served as control and did not receive any treatment.
Group 2	_	Served as diabetic control and received alloxan monohydrate and vehicle (Saline).
Group 3	_	Gliclazide (10 mg/kg, p.o.) and served as standard
Group 4	_	Administered Madhumardan Churna (125 mg/kg, p.o.)
Group 5	_	Administered Madhumardan Churna (250 mg/kg, p.o.)
Group 6	_	Administered Madhumardan Churna (500 mg/kg, p.o.)

DPPH radical scavenging assay:

DPPH quenching ability of MC was measured according to Hanato et al. (1988). A methanol DPPH solution was mixed with serial dilutions (100–500 µg/ml) of the Madhumardan churna and after 30 min, the absorbance was read at 517 nm. The antiradical activity was expressed as IC50 (µg/ml), (the antiradical dose required to cause a 50% inhibition). Ascorbic acid was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = ($A_0 – A_1$)/ A0 * 100

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

Assessment of oral glucose tolerance test (OGTT) of Madhumardan Churna:

Overnight fasted normal rats were divided into six groups of six rats each. They were orally administered with vehicle, Madhumardan churna (125, 250 and 500 mg/kg) and gliclazide (10 µg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extract. Blood was withdrawn through the tail vein at 0, 30, 60 and 120 min of glucose administration and glucose levels were estimated within 1 h, by glucose oxidase – peroxidase method.

Biochemical analysis

The blood glucose was estimated by glucose oxidase – peroxidase method. SGOT and SGPT activities were determined.

Statistical analysis

The results were presented as mean ± SEM. Statistical analysis of all the data obtained was evaluated using one-way ANOVA followed by Dunnet's test (GraphPad prism; Version 6.02). The differences were considered as significance set at P ≤ 0.05.Results:

RESULTS:

Preliminary Phytochemical Investigation:

Phytochemical screening of Madhumardan Churna was carried out to evaluate qualitative presence of phytochemicals. It was found that the drug is a mixture of various ingredients. As shown in Table No. 2, MC was found to contain, alkaloids, glycosides, amino acids, flavonoids. MC doesn't showed presence of any steroids in it.

Table No. 2: Results of preliminary phytochemical analysis

S. No.	Test	Observation	Inference
1	**Test for Alkaloids**		
	a) Dragendorff's test	Brown ppt.	Pass
	b) Wagner's test	Brown ppt.	Pass
	c) Hager's test	yellow ppt.	Pass
2	**Test for amino acids**		
	a) Millon's test	White ppt.	Pass
	b) Ninhydrine test	Purple colour	Pass
3	**Test for Carbohydrate**		
	a) Molisch's test	Violet ring develops at the junction of two liquids	Pass
	b) Fehling's test	Brick red ppt.	Pass
	c) Benedicts test	Yellow colour	Pass
	d) Barfoed's test	Red ppt.	Pass
	e) Selwinoff's test	Red colour	Pass
4	**Test for flavonoids**		
	a) Alkaline reagent test	Yellow ppt.	Pass
5	**Test for Glycosides**		
	a) Legal test	Red colour	Pass
	b) Borntrager's test	Pink – red colour	Pass
6	**Test for Saponine glycoside**		
	a) Froth formation test	Foam form	Pass
7	**Test for Steroids**		
	a) Salkowski test	Blue colour formed	Fails
8	**Test for tannins and phenolic compounds**		
	a) Fecl3 solution	Blue colour	Pass
	b) Lead acetate solution	White ppt.	Pass
	c) Bromine water	Decoloration of Br_2 H_2O	Pass
	d) Acetic acid	Red colour	Pass
	e) Potassium di-chromate	Red ppt.	Pass
	f) Dilute iodine solution	Transient red colour	Pass

To assess more phytochemical value of drug as per compendial requirements, loss on drying for the formulation was determined. It was found that the LOD value of the drug was 5.68 % indicating a little moisture that will prevent the growth of moulds and other microorganisms.

Ash values and extractives of the drug were evaluated using compendial methods. Table No. 3 represents the ash values of the drug.

Table no. 3: Physicochemical test

Sr. No.	Test	Results
1	Total ash	8.75 %
2	Acid insoluble ash	13.98 %
3	Sulphated ash	6.93 %
4	Water soluble ash	54.05 %
5	Alcohol soluble extractive	9.84 %
6	Water soluble extractive	61.58 %

DPPH radical scavenging assay:

MC shows reductive capabilities significantly compare with standard ascorbic acid (figure 1). The reducing power of MC increased with increasing quantity of the sample. MC exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC50) at a concentration of 380.54 ± 1.74 g/ml.

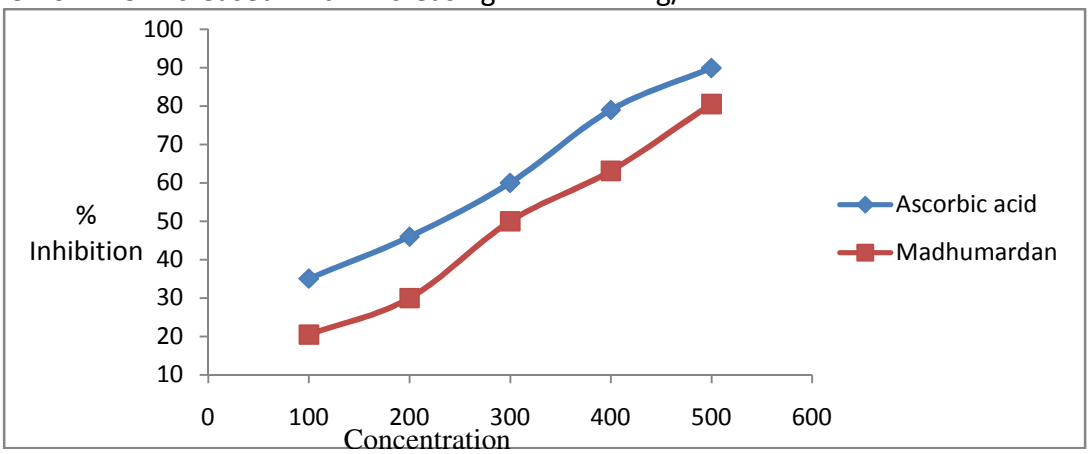

Figure no. 1: DPPH radical scavenging effect of Madhumardan churna and Ascorbic acid.

Oral Glucose tolerance test:

Glucose tolerance test is performed to evaluate body's capacity to handle glucose. Being one of the parameters responsible for determination of metabolic capacity of the body in presence or absence of drug, this test is carried out. As depicted in Table No. 4 it was found that drug in the dose of 250 mg/Kg, showed comparable effects in handling glucose when compared to standard.

Table No. 4: Effect of Madhumardan churna on oral glucose tolerance.

Groups	Blood glucose levels (mg/dl)			
	0 min	30 min	60 min	120 min
Normal control	94.1 ± 1.94	120.5 ± 2.07	112.3 ± 1.96	99.5 ± 1.87
Gliclazide (10 mg/kg)	92.8 ± 4.16	116.6 ± 5.77	103 ± 5.21	96.3 ± 4.76
Test 1(125 mg/kg)	97.6 ± 3.20	116.7 ± 3.26	107.5 ± 3.61	103 ± 3.40
Test 2 (250 mg/kg)	91.3 ± 3.26	118.1 ± 2.63	111.3 ± 3.18	98.8 ± 2.56
Test 3 (500 mg/kg)	94.8 ± 2.92	122.6 ± 3.38	113.8 ± 4.62	96.5 ± 3.83

Values are given as (mean ± SD), n=6 (number of animals)

Effect of drugs on general status of animals

To evaluate effect of diabetes and administration of drugs on the status of animals, daily parameters were analysed. They included body weight, food and water intake etc. as shown in Table No. 5 it was found that there were no significant deviations from the mean in body weights. Water intake and food intake was increased in the animals being treated with standard and test drugs.

Table No. 5: Effect of changes in body weight, food intake and water intake of normal and diabetic rats after 21 days of treatment with Madhumardan Churna

Parameters	Non diabetic	Diabetic control	Diabetic treated with test
Body weight after treatment (gm)	216 (± 0.37)	155 (± 1.75)	189 (± 1.32)
Food intake (gm)	23 (± 1.59)	43 (± 1.59)	38 (± 1.03)
Water intake (mL)	27 (± 1.08)	70 (± 2.31)	60 (± 3.66)

Values are given as (mean ± SD), n=6 (number of animals)

Biochemical analysis:

Effect of Standard and test on blood glucose level in diabetic rats

Blood glucose level depicted in Table No. 6.

Table No 6: Effect of Madhumardan churna on fasting blood glucose level in diabetic rats

Groups	0 day	4th day	7th day	14th day	21th day
Normal control	72.2 (± 12.01)	78.9 (±11.84)	101.2 (± 12.05)	98.1 (± 10.62)	97.66 (± 9.54)
Diabetic control	525.1 (± 7.29)	490.1 (±10.02)	601.3 (±7.17)	446.8 (±8.47)	431 (± 9.79)
Standard Gliclazide (10mg/kg)	588.6 (±12.56)	560.5 (± 12.07)	361.6(± 10.76)	237.6 (± 19.88)	107.33 (± 10.91)
Test 1 (125 mg/kg)	488.8 (± 13.02)	475.1 (±11.66)	433.6 (± 9.68)	391.1 (±10.53)	220.5 (± 13.73)
Test 2 (250 mg/kg)	517.6 (± 13.06)	495.83 (± 13.71)	417.1 (± 10.38)	330.3 (± 10.87)	183.6 (± 16.95)
Test 3 (500 mg/kg)	570.3 (±11.43)	503.5 (± 13.67)	390.1 (± 12.18)	231.5 (± 12.06)	107.8 (± 12.71)

Values are given as (mean ± SD), n=6 (number of animals)

Values are statistically significant at *P<0.05, ***P<0.0001 compared with control group. n = 6 (no. of animals)

Statistical analysis by using ANOVA test:

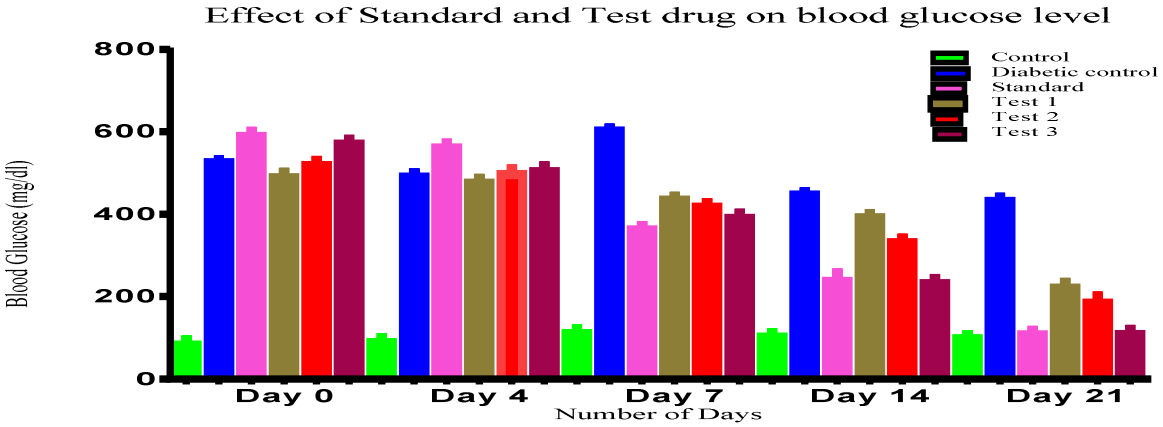

Figure No. 2: Effect of Madhumardan churna on blood glucose level in alloxan induce diabetes rats, where Values are given as mean ± SD (n=6 in each group). Values are statistically significant at *P<0.05, ***P<0.0001 compared with control group. n = 6 (no. of animals)

Effect of Madhumardan churna on SGOT and SGPT levels in diabetic rats

Biochemical parameters like SGOT and SGPT was given in the Table No. 7.

Table No 7: Effect of Madhumardan churna on fasting SGOT and SGPT level in diabetic rats

Groups	SGOT (IU/l)	SGPT(IU/l)
Normal control	29.79 (± 1.43)	30.11 (± 2.98)
Diabetic control	89.15 (± 1.41)	94.2 (± 4.50)
Standard Gliclazide (10 mg/kg)	34.2 (± 1.23)	28.08 (± 1.09)
Test 1 (125 mg/kg)	10.08 (± 0.41)	15.23(± 0.98)
Test 2 (250 mg/kg)	9.89 (± 1.09)	11.98 (± 1.98)
Test 3 (500 mg/kg)	8.84 (± 0.52)	10.85 (± 2.00)

Values are given as mean ± SEM (n=6 in each group).

DISCUSSION:

Phytochemical investigation showed that MC contains different herbs which are a mixture of various chemical with different chemical constituents. It gives a multiprong approach for the treatment of diabetes in which it produces antihyperglycaemic, antioxidant, antihyperlipidaemic, laxative, digestive etc. effects.

The experimental groups were treated with standard (Gliclazide 10 mg/kg b.w.) and test drug i.e. Madhumardan churna. The experiment was conducted in accordance with parallel design where three doses of test drug i.e. 125, 250 and 500 mg/kg was administered to three separate group of animal at a single time. After completion of the study protocol, it was found that with test and standard treatment, the serum level of glucose, improved significantly ($p<0.0001$) as compared to diabetic control while there was increase in the body weight of the rats after administration of formulation.

Results obtained in the present study showed that administration of the fasted diabetic rats with the Madhumardan churna of (125,250 and 500 mg/kg) resulted in a significant dose dependent decrease in blood glucose level. The maximum hypoglycaemic activity of the churna was observed at the dose of 500 mg/kg with a reduced percentage of blood glucose after 21 days of treatment. Results obtained in the study of SGOT and SGPT were significant with the polyherbal formulation.

Oral administration of the Madhumardan churna at doses of 500 mg/kg body weight produced significant ($P<0.0001$) hypoglycaemic effects in fasted animals after 7^{th} day. On day 21^{th} 500 mg/kg body weight dose of Madhumardan churna produced a significant ($P<0.0001$) reduction in blood glucose level in fasted animals. The dose of 250 mg/kg also reduced blood glucose level but the results were found statistically insignificant on 21^{th} day. Gliclazide produced a significant ($P<0.0001$) reduction in blood glucose on 21^{th} day it produced statistically good results i.e. ($P<0.0001$) compare to diabetic control.

CONCLUSION

In conclusion the findings of the study suggest hyperglycemia was induced by the administration of alloxan monohydrate (125 mg/kg i. p.). It was found that the Madhumardan Churna showed antihyperglycaemic and antioxidant activity in alloxan induced diabetes mellitus. Gliclazide (10 mg/kg b.w.) used as a standard reference showed significant reduction in the blood glucose level in fasted diabetic rats. From the results it was found that oral dose used of Madhumardan Churna 125 mg/kg and 250 mg/kg showed significant reduction in blood glucose level in alloxan induced animals but 500 mg/kg showed more reduction in blood glucose level in dose dependent manner.

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