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Research article

ANTI DIABETIC EFFECT OF MOMORDICA CHARANTIA (BITTER MELONE) ON ALLOXAN INDUCED DIABETIC RABBITS.

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ABSTRACT

Objective: to investigate the anti diabetic effect of the bitter melon on Alloxan induced diabetes in experimental animals (rabbits). **Materials and Methods:** the alcohol extract of whole fruit was tested for its efficacy in Alloxan (150mg/kg) induced diabetic rabbit. The diabetic rabbits were divided into 5groups. Group I (control) received 2% gumacasia, groupie (positive control) received standard drug Metformin (62.5mg+2%GA), group III, IV, V (T_1 T_2 T_3) were treated orally with a daily dose of 0.5(gm) 1gm, 1.5gm respectively for 35 days, for all diabetic rabbits after giving TEST,NC,PC preparations, the blood samples were collected and determined the blood glucose level 0,1,3,24hrs intervals. 0hr reading is before drug giving and remaining 3 readings after drugs giving. 24th her reading is considered as 0hr reading for the next day. **Results**: administration of alcohol of an extract of bitter melon produced a dose dependent decrease in blood glucose levels in Alloxan induced rabbits. There was a significant fall in blood sugar level in High dose (1.5GM/kg) in comparison to low dose (0.5gm/kg) and median dose (1gm/kg) shown by LSD test. This is comparable to the effect of Metformin. **Conclusion**: the results of this study show that chronic oral administration of an extract of Momordica charantia fruit at an appropriate dosage may be good alternative anti diabetic agent.

Keywords : Hyperglycemia, Metformin, Alloxan, *Momordica charantia*

INTRODUCTION

Diabetes mellitus is the world largest endocrine disease with deranged carbohydrate, fat and protein metabolism. The diabetes mellitus is mainly classified into two major groups, Type-1 (insulin dependent diabetes mellitus), Type-2 (non-insulin dependent diabetes mellitus. As per WHO report, approximately 150 million people have Diabetes mellitus worldwide, and this number may well double by the year 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 57 million in 2025 in India. This number

is making India the country with the highest number of diabetics in the world.[1] Long-term complications of diabetes are micro vascular (neuropathy, retinopathy, nephropathy) and macro vascular (heart complications)[2] diseases. The anti diabetic drugs are mainly used for to replace the insulin deficiency or to enhance the action of insulin and/or decrease the insulin resistance. Although many drugs and interventions are available to manage diabetes, these are expensive for the large diabetic population of developing countries like India, apart from their inherent adverse effects.[3] So it is necessary to look for new cheep alternatives to manage this major health problem. Different indigenous drugs have been used in this subcontinent for several centuries for the treatment of Diabetes mellitus with conflicting reports of their efficacy because of lack of scientific investigation in a laboratory setting. One such plant, *Momordica charantia* (Karela) whose fruit has long been used traditionally in the treatment of Diabetes mellitus in South Asian countries and has rich Ayurvedic reference to select for the study. In this study, the anti diabetic potential of this unripe fruit extract of *Momordica charantia* (Karela) was screened on laboratory animal model.[4,5]

MATERIALS AND METHODS

Institutional Animal Ethics Committee (IAEC) permission was obtained before starting the study. The study was conducted strictly in accordance with the protocol.

Plant material: Fresh green *fruits* of bitter gourd popularly known as karela was obtained in sufficient quantity from a local market in Nandyal, A.P. in November 2012. They were carefully washed to remove dust particles and other foreign materials and dried in shaded areas. The completely dried fruits were powdered with electric grinder and stored in well closed bottles.

Alcohol extract preparation: The extract is a concentrated preparation of vegetables or animal source Extract: The extract is the process or act of pulling or drawing out the active principle of a particular material like plants or animal organs. In the present study the percolation method was selected to extract the active principle of bitter gourd plant material.[6] **Cold percolation method:** This is a traditional method of extraction used by the herbalists throughout the world. This is the original extraction method, and it's continuing to be the backbone of the present extracting technology. The distillation devices are "modified Soxhlet extractions" made by Eden Labs [7,8] **Extraction procedure:** The dried fine powder of the bitter gourd was weighed in balance 25g and taken into the sac like cloth material and placed in the Soxhlet basket. 250ml of ethyl alcohol was taken as solvent into the Soxhlet flask. The extract laden solvent falling from the Soxhlet basket is dark in color and it becomes clearer, that indicates the extraction process is finished[9.] At the end of the extraction process the solvent is then evaporated and the remaining mass is measured. The percentage yields are calculated as mg per gm dried powder in 250ml of alcohol, 25gms powder was suspended. 5gms (20%) of extract was obtained. The extract was suspended in 5ml of 2% Gum acacia and used for the oral administration in diabetic rabbits.

Animals used: 25 Rabbits of either sex, adult, healthy albino rabbits of local strain weighing between 1 to 4 kg were used in this experiment. All the animals were kept in an air-conditioned animal house in the Pharmacology Department at the Santhiram Medical College, Nandyal, Kurnool Dist. AP. The animals, rabbits were offered a natural food like grass and leaves and allowed a tap water to drink.[10]

Preparation of diabetic rabbits: the 25 rabbits weighing between 1 to 4 kg were made diabetic by injecting intravenously 150mg/kg body weight of Alloxan monohydrate[11,12] Before giving Alloxan, the normal blood glucose levels of all rabbits were estimated. After 2hours of Alloxan injection the Dextrose (5gm) mixed with water fed to the all-diabetic rabbits orally to prevent a hypoglycemic condition of rabbits with

Alloxan.[7] After 72hours of Alloxan injection, the blood glucose levels of all surviving rabbits were determined by the glucose oxidase method. The rabbits with blood glucose levels of 220 to 500mg/dl were considered as diabetic and were employed to further study.[13]

Table.1: Grouping of animals

Groups	Animals	Drug	Remarks
I	Control	(2% gum acasia) 5ml	Placebo
II	Positive control	(Metformin 62.5mg+2% ga) 5ml	Positive
III	Test (low dose **0.5gm**)	Alcohol extract+2%ga 5ml	ExL
IV	Medium dose (**1gm)**	Alcohol extract+2%ga 5ml	ExM
V	High dose (**1.5gm)**	Alcohol extract+2%ga 5ml	ExH

All Alloxan diabetic rabbits were randomly divided into five groups (n=5).

For all the diabetic rabbits after giving test, negative control and positive control preparations, the blood samples were collected and determined the blood glucose 0, 1 & 3hrs intervals. **'0'** hour reading is before drug giving.'1 & 3' hours reading is after drug giving. After administration of drugs to the diabetic rabbits the blood was collected 1,3 and 24-hour interval daily up to 35 days and blood glucose level was determined by the glucose oxidase method by using Glucometer for 15 days and then weakly for 3 weeks. The glucose oxidase method is more accurate, rapid and time saving method. It requires only a small amount of blood. So this method is popularly used in India people suffering from diabetes for self-monitoring of blood glucose levels.[14]

RESULTS

In the present study, alcohol extract of the unripe fruit of the *Momordica charantia* (Bitter gourd) was assessed for its anti diabetic activity in Alloxan-induced diabetic rabbits. The results obtained were recorded (Table 2).

Table.2: Average Blood Glucose levels (mg/dl) of groups I to V before and after treatment up to 35th day.

S.No.	Group – I			Group – II			Group – III			Group – IV			Group - V		
Before Alloxan	**78.2**			**75**			**86**			**88**			89		
After Alloxan (72 hrs.)	**311**			**293**			**310**			**300**			305		
After Treatment	**0 Hr**	**1 Hr**	**3 Hr**	**0 Hr**	**1 Hr**	**3 Hr**	**0 Hr**	**1 Hr**	**3 Hr**	**0 Hr**	**1 Hr**	**3 Hr**	**0 Hr**	**1 Hr**	3 Hr
Day 1	311	308	307	293	283	279	301	307	308	300	292	292	314	319	312
1st week	312	287	278	254	238	244	277	274	283	260	275	279	251	244	247
2nd week	307	298	298	184	173	171	228	224	225	198	204	203	145	158	157
3rd Week	289	286	299	158	149	153	227	232	228	169	175	145	147	141	146
4th Week	293	276	295	150	144	153	202	194	191	166	161	165	145	143	148
5th Week	**272**	**278**	**274**	**109**	**112**	**130**	**184**	**194**	**199**	**149**	**151**	**146**	**120**	**127**	**131**

After 35 days of treatment, there is a significant decrease in blood glucose levels was seen with the standard drug Metformin and Ethanolic extract of *M-Charantia* but there is no significant reduction in the control group treated with gum acacia.

Table 3: Mean blood sugar level of different groups:

Time	Group I	Group II	Group III	Group IV	Group V
0 Hour	272±16.1	109.2±3.3**	184±13.3*	149.6±5.7**	120±7.8***
1st Hour	278.4±8.3	112.2±2.6***	194±13.4**	151.8±14.2***	127.6±5.2***
3rd Hour	274.8±11	130.2±16.1***	199.6±15*	146±4.6***	131±10.2***

*P<0.05, **P<0.01, ***P<0.001 compared to the control.

Table.4: Mean Blood Glucose levels (Mean±SEM) at 35th day.

Animals	Control	Metformin	ExL	ExM	ExH
R1	-18.14	-80.67	-55.02	-80.67	-142.74
R2	-36.81	-77.19	-41.79	-54.24	-69.29
R3	-14.45	-69.67	-90.66	-67.21	-94.03
R4	-52.09	-106.60	-4.10	-68.98	-160.09
R5	7.83	-50.78	-77.74	-67.98	-70.59
Variation	ns	sig	sig	less sig	sig

DISCUSSION

The present study, we have evaluated the alcohol extract of the unripe fruit of the *Momordica charantia* (Bitter gourd) was assessed for its anti diabetic activity in Alloxan-induced diabetic rabbits. The results obtained were recorded. A placebo-controlled sub-acute study was conducted on 5 groups of 5-diabetic rabbit models to show the hypoglycemic effect of 3 increasing doses (0.5 gm / kg, 1.0 gm / kg and 1.5 gm / kg body weight) of the alcohol extract of *M. Charantia* suspended in gum acacia. The result was compared with the established anti-diabetic drug Metformin in the dose of 150 mg per Kg body weight. Gum acacia was taken as the placebo in this study. The blood sugar level was recorded daily by Glucometer for 15 days and then once weekly for another 3 weeks but in the above table only weekly report is given. The decrease in the blood sugar level was recorded daily from the initial value and was shown in the above table.

The blood sugar levels were highly decreased of a treatment with high dose of extract. The blood sugar levels are almost comes to the Normal levels. The high dose effect of the extract is almost similar to Metformin effect after 35 days of treatment. There was significant variation in the decrease of blood sugar among the diabetic rabbit models in each group except in dose of 1 gm / Kg (ExM) body weight (Table 3).

Overall comparison between different groups of rabbits

The fall in the blood sugar was compared among the groups of animals with ANOVA. It was found that there was significant variation ($P < 0.01$) among the groups. Multiple comparison tests were performed to find out the differences between the groups.

Comparison with Control: The Dunnett's test was conducted between the control group and the groups that were given Metformin and the extracts in 3 increasing doses. As expected the fall in the blood sugar level was significant ($P < 0.05$) in the Metformin group. There was no significant difference in the fall in the blood sugar levels with ExL, ExM in comparison with the control but there was significant ($P < 0.05$) fall in blood sugar level in ExH group (Table 4).

Comparison with Metformin: The effect of Extract in all the 3 doses in lowering blood sugar level showed no statistically significant

difference with that of Metformin in the doses used in this study. This result was checked by two post-ANOVA multiple comparison tests like LSD test of Fisher accommodates a lot of Type I error) and FSD test of Scheffe (accommodates a lot of Type II error). Both the tests gave an identical result. It gave a strong hint that the Extracts of *M. charantia* were as efficient as Metformin in lowering the blood sugar in diabetic rabbits and that was achieved in a broad range of doses ranging from 0.5 gm / Kg to 1.5 gm / Kg, so it might be a much safer alternative to the established drugs.

Comparison between different doses of the Extract : There was significant fall in blood sugar level in ExH dose in comparison to ExL and ExH dose in comparison with ExM as shown by LSD test. But such difference was not found in with Scheffe's test.

The present study, the hypoglycemic effect of *M. Charantia* fruit extract was compared with metformin. Similar studies by Akhtar MS et al, in 1981 and Biyani MK et al (2003) the acute hypoglycemic effect was compared with sulphonylureas and concluded positive effect.

So the present study showed the hypoglycaemic effect of the alcoholic extract of the unripe fruit of *M. charantia* in the dose ranging from 0.5 gm / Kg to 1.5 gm / Kg body weight of diabetic rabbits given orally. The hypoglycemic effect was comparable to that of the standard anti-diabetic drug Metformin in the dose of 62.5 mg / Kg body weight of rabbits. The broad dose range of hypoglycemic effect of *M. charantia* may be an interesting finding which may prove it safer in comparison to the established hypoglycemic drugs.

CONCLUSION

M. charantia or Karela was taken traditionally for control of diabetics in India and in other countries for long time. Three doses of alcoholic extract of the powder of unripe fruit of *M. charantia* were taken to study the hypoglycemic effect of in 5 groups of alloxan-induced diabetic in Rabbits. It was a placebo-controlled open study where blood sugar levels were recorded daily for 5 weeks. The study showed hypoglycemic effect of the extract in the oral dose range of 0.5 to 1.5 gm / kg body weight of rabbits. The hypoglycemic effect was comparable to that of established anti-diabetic drug Metformin in the dose of 62.5 mg / Kg. The broad dose range of the extract producing a hypoglycaemic effect in diabetic rabbit was an interesting observation, we believe that extract of *M-Charantia* has the potential to be used as an adjuvant in the treatment of Diabetes but which requires further study.

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