DOI: https://doi.org/10.53350/pjmhs211571569

ORIGINAL ARTICLE

Anti-diabetic effect of *Momordicacharantia* plant extract in Streptozotocin induced diabetic mice

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ABSTRACT

This study describes anti-hyperglycemic effect of *Momordicacharantia* extract commonly known as bitter melon. Random blood glucose levels were observed before and after plant extract administration. Powdered form of plant extract was used as an oral treatment. Diabetes was induced in animal (mice) models by using streptozotcin which is an artificial diabetes inducer. Maximum anti-hyperglycemic effect and blood glucose level reduction was observed in individual treatment of *Momordicacharantia*extract (500mg/kg) which was 75% ± 1.3. This treatment was more effective as compared to Amaryl (3mg/kg) which shows effectiveness of 52% ± 2.4 and Glucophage (500mg/kg) which shows effectiveness of 29% ± 2.1. Results indicate that bitter melon contain anti-hyperglycemic proteins which are helpful in diabetes treatment without any toxic side effects. Still more research, experiments and testing needs to be perform.

Keywords: Amaryl; Anti-hyperglycemic effect; Blood glucose levels; Body weight; Diabetes mellitus

INTRODUCTION

*Momordicacharantia*is a herb which is widely cultivated through-out different regions of the world. It is used as vegetable. It is not only used as food but also contain pharmaceutical properties. Its fruit contain different medicinal effects as anti-hyperglycemic[21], anti-diabetic, anti-fungal[3], anti-oxidant, cytotoxic activity[32] and inhibition against tyrosine[31]. It is reported that *Momordicacharantia* extract had anti-hyperglycemic effect in diabetic mice[30].

Diabetes mellitus is considered as most serious disorder having severe impact on health and quality of life expectancy within patients and on health associated system. It is a chronic disorder mainly grouped as type 1 diabetes mellitus and type 2 diabetes mellitus. Type 1 is due to insulin deficiency and type 2 is due to insulin resistance. For the treatment of this disease, different oral synthetic drugs and insulin are available in market.

Insulin shows low side effects as compared to synthetic drugs which show serious effects and toxicity. Insulin is not available in its oral form. Synthetic and artificial drugs are available for oral administration. So, we are in a strict need for effective and safe agents in order to continue our important portion of an active research. We must grow our interest towards identifying anti-diabetic and natural products in order to treat diabetes.

MATERIALS AND METHODS

Preparation of Momordicacharantia extract: *Momordicacharantia* plant was taken in its raw form and dried its distributed portion in drying oven. It was placed for about one week at 60°C. After obtaining dried form of the required plant, it was converted into powdered form by crushing mechanically or by grinding in a grinder. Greenish yellow powder was obtained and used for practical. Remaining amount was stored in eppendorf tubes at 25°C for further experimental procedures.

Received on 23-02-2021
Accepted on 17-06-2021

Experimental animals: Male albino mice were used having age of about three months. Their weight was in a range of 20-25 g. One group was selected as a control group and other group was selected as an experimental group. They were kept under observation for few days in order to make them suitable towards new environement. During observation, their behavioral changes were also noticd. In summer, room temperature was maintained at 35-37°C and during winter, temperature was maintained at 42-43°C. Day light and night exposure was also maintained. Commercial feed was provided throughout whole research.

Diabetes induction in mouse

0.05M sodium citrate buffer preparation (pH 4.5): Sodium citrate stock solution was prepared by adding 1.2 g sodium citrate and 1.1 g citic acid in some amount of distilled water. Raise the volume up to 100 ml by adding distilled water. This is 0.1M concentration of stock solution. In order to prepare 0.05M concentartion, mix 50 ml of stock solution with 50 ml of distilled water. Adjust pH to 4.5. This solution must be prepared fresh and placed in ice before inducing diabetes.

Streptozotocin-sodium citrate buffer preparation: Streptozotocin was dissolved in buffer. Three low doses (40 mg/kg) were administered for three days and two low doses (50 mg/kg) were provided intraperitoneally for two days. Restainers can be used for mice handling. Scruffing is a technique which can also be used for handling mice. Streptozotocin and buffer were mixed immediately before injecting because buffer degrades STZ within minures. Blood glucose levels were measured after 72 h of buffer injection.

STZ per mouse

STZ (40mg/kg): $\frac{20}{1000} \times 40 = 0.0008$ g (Table 1)

STZ (50mg/kg): $\frac{20}{1000} \times 50 = 0.001$ g (Table 1)

Table 1: STZ-Na citrate dose

Streptozotocin dose (mg/kg)	Mouse weight (g)	STZ-Na citrate dose
40mg/kg	20g	0.0008g STZ + 0.3ml buffer
50mg/kg	20g	0.001g STZ + 0.3ml buffer

Table 2: Experimental design

Groups	Treatment
Negative control	Non-diabetic and untreated group
Positive control	Diabetic and untreated group
Group 1	Administration of *Momordicacharantia*extract in diabetic mice
Group 2	Administration of Amaryl in diabetic mice
Group 3	Administration of Glucophage in diabetic mice

Administration of treatments

Administration of *Momordicacharantia*extract (Table 2): Plant extract was prepared by adding *Momordicacharantia* powder (500 mg/kg dose) in 0.2 ml of distilled water. Assure homogenous mixing by using a vortex mixer. This prepared solution was administered orally for one week. Mice can be handled by using restrainer technique or by scruffing. Blood glucose level was monitored before and after treatment (Table 3).

Dose calculation formula = $\frac{\text{weight (g)}}{1000} \times \frac{500\text{ mg}}{1000}$

Administration of Amaryl: Amaryl is a synthetic drug used by diabetic patients. Medicinal extract was prepared by adding Amaryl powder (3 mg/kg dose) in 0.1 ml of distilled water. Assure homogenous mixing by using a vortex mixer. This prepared solution was administered orally for one week. Mice can be handled by using restrainer technique or by scruffing. Blood glucose level was monitored before and after treatment (Table 4).

Dose calculation formula = $\frac{\text{weight (g)}}{1000} \times \frac{3\text{ mg}}{1000}$

Administration of Glucophage: Glucophage is a synthetic drug used to treat diabetes. Medicinal extract was prepared by adding Glucophage powder (500 mg/kg dose) in 0.2 ml of distilled water. Assure homogenous mixing by using a vortex mixer. This prepared solution was administered orally for one week. Mice can be handled by using restrainer technique or by scruffing. Blood glucose level was monitored before and after treatment (Table 5).

Dose calculation formula = $\frac{\text{weight (g)}}{1000} \times \frac{500\text{ mg}}{1000}$

Estimation of glucose levels: Testing glucometer was used for the estimation of blood glucose levels of each mouse before and after treatments. Glucose level crossing barrier of 150 mg/dl is considered diabetic.

Statistical analysis: SPSS was used to perform the statistical analysis of different conditions. Paired t-test was conducted in order to calculate the significance of each result obtained. ANOVA (Analysis of Variance) was also applied and data was statistically analyzed. P-value is obtained in this case. This is the value of significance showing the quality of results obtained. It is denoted by alpha (α). If the value of alpha is less than 0.05, then the results are considered significant.

$$\text{Reduced BGLs} = (\text{BGLs after STZ} - \text{Control}) - (\text{BGLs after treatment} - \text{Control})$$

$$\%\text{ reduction in BGLs} = \frac{\text{Reduced BGLs}}{\text{BGLs after STZ} - \text{Control}} \times 100$$

RESULTS

Table 3: Effect of *Momordicacharantia*plant extract (500mg/kg) in diabetic mice

(Group 1)	Glucose level (mg/dl)				% Decrease in BGLs	Treatment effect remain for days
Mice No.	-Ve Control	+Ve Control	After STZ	After MC extract treatment		
1	97	290	300	150	74%	18
2	**90**	**328**	**353**	**120**	**89%**	**21**
3	72	300	312	160	63%	15
4	89	250	306	122	85%	11
5	91	300	340	180	64%	20

Standard mean of percentage reduction in blood glucose levels came out to be 75% ± 1.3.

Table 4: Effect of Amaryl (3mg/kg) in diabetic mice

(Group 2)	Glucose level (mg/dl)				% Decrease in BGLs	Treatment effect remain for days
Mice No.	-Ve Control	+Ve Control	After STZ	After Amaryl treatment		
1	92	315	325	209	50%	03
2	71	361	341	230	41%	05
3	102	322	300	200	51%	02
4	97	291	324	190	59%	04
5	**90**	**300**	**320**	**182**	**60%**	**07**

Standard mean of percentage reduction in blood glucose levels came out to be 52% ± 2.4.

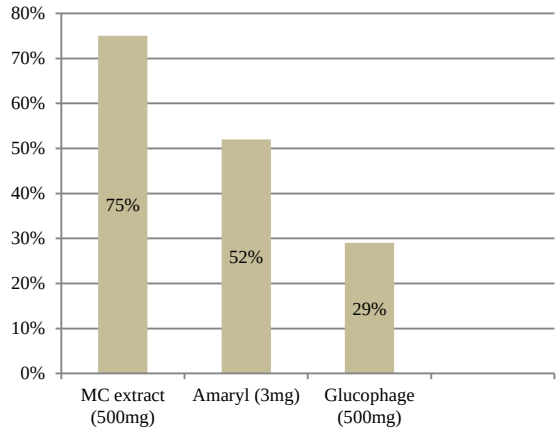
Table 5: Effect of Glucophage (500mg/kg) in diabetic mice

(Group 3)	Glucose level (mg/dl)				% Decrease in BGLs	Treatment effect remain for days
Mice No.	-Ve Control	+Ve Control	After STZ	After Glucophage treatment		
1	84	349	362	300	22%	02
2	**90**	**300**	**320**	**230**	**39%**	**07**
3	93	371	328	248	34%	03
4	106	291	315	270	22%	05
5	73	322	309	240	29%	02

Standard mean of percentage reduction in blood glucose levels came out to be 29% ± 2.1.

Comparing results of *Momordicacharantia*plant extract, Amaryl and Glucophage

Fig. 1: Comparison among best treatments in diabetic mice



DISCUSSION

Diabetes mellitus is linked with different changes in carbohydrates, proteins and lipids profile with increased rate of heart problems[4]. Liver and tissues are involved in metabolic conversion of different components. Most of the plants and herbs contain specific products which are helpful in overcoming diseases and related complications[5]. *Momordicacharantia* plant is one of them showing anti-hyperglycemic properties[17]. It is studied that treatment of streptozotocin induced diabetic mice with *Momordicacharantia* plant extract shows insulin like effect by lowering blood glucose levels and increasing insulin levels in plasma[25].

Plant extract is also involved in partially reversing the hyperglycemic condition in STZ induced diabetic mice.It is helpful in improving the action and secretion of level of insulin in blood plasma. It is involved in the increased output of insulin from the pancreatic beta cells in diabetic animal models[29]. Plant extract not only mimics the activity of insulin but also shows synergistic effect on the performance of insulin. Increased production of beta cells was also reported after treating with karela extract[2].

Maximum anti-hyperglycemic effect and blood glucose level reduction was observed in individual treatment of *Momordicacharantia*extract (500mg/kg) which was 75%±1.3. This treatment was more effective as compared to Amaryl (3mg/kg) which shows effectiveness of 52%±2.4 and Glucophage (500mg/kg) which shows effectiveness of 29%±2.1.

CONCLUSION

Results indicate that bitter melon contain anti-hyperglycemic proteins which are helpful in diabetes treatment without any toxic side effects. Still more research, experiments and testing needs to be perform.

Acknowledgement: We wish to thank Professor Dr Hamid Mukhtar, Institute of Industrial Biotechnology, Government College University, Lahore Pakistan, for their support and encouragement. We are also indebted to M. Atta-ur-Rehman Bin Sultan, for taking care of the experimental animals and for helping us during statistical analysis.

Conflict of interest: Nil

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