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Original Research Article.

The Effect of Aqueous Extract of Momordica Charantia on Blood Glucose Level in Alloxan Induced Diabetic Rats

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ABSTRACT

Introduction: Diabetes mellitus is one of the five leading causes of death in the world.

Objective: Our main goal is to estimate the effect of aqueous extract of Momordica charantia on blood glucose level in alloxan induced diabetic rats.

Methodology: This experimental study was carried out at Department of pharmacology, Dhaka medical college, Dhaka from July 2014 to June 2015 where the study was divided into two parts, Experiment-1 and Experiment-2. In Experiment-1 include group A and group B. Experiment-2 include group C, group D and group E. Blood was collected from group A and group B on day 1 and day 15 and from group C,D and E on day 1,4 and 15 of experiment.

Results: During the result in group-A (served as control and received normal rat diet for 14 days) the blood glucose levels (mean ± SD) were 4.90±0.50 mmol/L and 5.3±0.38mmol/L on day 1 and day 15 respectively. Percent inhibition was 4.8 and in group-B the blood glucose levels (mean ± SD) were 5.00±0.36mmol/L and 5.05±0.32 mmol/L on day 1 and 15 respectively. Also for in group C, the blood glucose levels (mean ± SD) were 5.31± 0.45 and 14.24±.51 on day 1 and day 15 respectively.

Conclusion: From the analysis we can conclude that that aqueous extract of Momordica charantia has hypoglycaemic effect, thus provide a rational for its use in development of new drug require for treatment and prevention of complications of diabetes mellitus.

Keywords: Diabetes Mellitus, Momordica Charantia, Hypoglycaemic.

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INTRODUCTION

Diabetes mellitus is the world largest endocrine disease with deranged carbohydrate, fat and protein metabolism. 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. It is a major global health problem with a projected rise in prevalence from 171 million in 2000 AD to 366 million in 2030 AD with majority still remaining undiagnosed. In Bangladesh age standardized prevalence of DM increased significantly ($p<0.001$) from 1999 to 2009. The term diabetes mellitus describes several diseases of abnormal carbohydrate metabolism that are characterized by hyperglycemia. It is associated with a relative or absolute impairment in insulin secretion, along with varying degrees of peripheral resistance to the action of insulin. Every few years, the diabetes community re-evaluates the current recommendations for the classification, diagnosis, and screening of diabetes, reflecting new information from research and clinical practice.

The 2006 World Health Organization (WHO) criteria define diabetes as an FBG ≥126 mg/dL (7.0 mmol/L) or a two-hour post glucose challenge value ≥200 mg/dL (11.1 mmol/L). In 2011, the WHO concluded that an A1C value of ≥6.5 percent (48 mmol/mol) can be used as a diagnostic test for diabetes A

value of <6.5 percent does not exclude diabetes diagnosed using plasma glucose levels. Impaired glucose tolerance (IGT) is defined as a fasting glucose <126 (7.0 mmol/L), and a two-hour glucose ≥140 mg/dL (7.8 mmol/L) but<200 mg/dL (11.05 mmol/L). Impaired fasting glucose (IFG) is defined as a fasting glucose of 110 to 125 mg/dL (6.1 to 6.9 mmol/L).

In Bangladesh the magnitude of Diabetes mellitus is increasing; the current prevalence rate of diabetes among the people of 20 to 79 years of age is 4.8%.It is supposed to rise to 6.1% in 2025.Diabetes mellitus is a metabolic disorder and in Bangladesh, the prevalence of DM mainly NIDDM (non-insulin dependent diabetes mellitus) which comprises 90 to 95% of all diabetes, has been rising alarmingly specially in our country and reaching the epidemic status.[1-3]

According to World Health Organization (WHO), medicinal plants are an accessible, affordable and culturally appropriate source of primary health care for more than 80% of Asia's population. Despite all the progress in synthetic chemistry and biotechnology, plants are still an indispensable source of medicinal preparations, both preventive and curative. Hundreds of species are recognized as having medicinal values and many of those are commonly used to treat and prevent specific ailments and diseases. Herbal medicine have several advantages such as fewer side effects, better patient tolerance, relatively less expensive and well accepted due to long history of use. The more important cause is that herbal medicine provide rational means for the treatment of many diseases that are obstinate and incurable in other system of medicine. *Momordica charantia* (bitter melon or bitter gourd) is a flowering vine in the family Cucurbitaceae. It is a tropical plant that is widely cultivated in Asia, India, East Africa, and South America for its intensely bitter fruits that are commonly used in cooking and as a natural remedy for treating diabetes. It is a climbing perennial that usually grows up to 5 m, and bears elongated fruits with a knobbly surface. It is a useful medicinal and vegetable plant for human health and one of the most promising plants for diabetes. *Momordica charantia* may have hypoglycemic effects, but data are not sufficient to recommend its use in the absence of careful supervision and monitoring.

Till now no scientific body has issued definite guidelines and clear recommendation for the use of *Momordica charantia* in diabetes mellitus. As of now, it has only been the matter of personal choice rather than a conventional antidiabetic. However, it could be a good anti-diabetic to the remote underprivileged ethnic minorities and those who take only natural treatments. Alloxan has been chosen to induce diabetes mellitus in rats. Blood glucose level has been estimated and the histological feature of pancreas was studied.[4,5]

In this study our main objective is to find out the effect of aqueous extract of Momordica *charantia* on blood glucose level in alloxan induced diabetic rats.

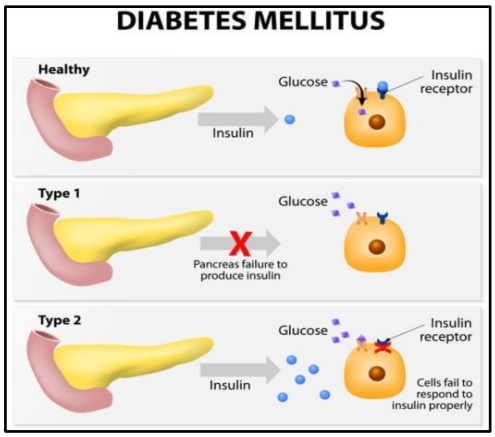

Figure-1a and 1b: shows diabetes mellitus and *Momordica charantia*in

OBJECTIVE

General Objective

- To estimate the effect of aqueous extract of *Momordica charantia* on blood glucose level in alloxan induced diabetic rats

Specific Objective

- To detect the effect of aqueous extract of *Momordica charantia* on blood glucose level in nondiabetic rats.
- To detect the effect of aqueous extract of *Momordicacharantia* on blood glucose level in alloxan induced diabetic rats.
- To compare the effect of aqueous extract of *Momordica charantia* on blood glucose level in alloxan induced diabetic rats with that of Glibenclamide.

METHODOLOGY

Study Type

- This study was an experimental study.

Place and Period of the Study

- This study was conducted at Department of pharmacology, Dhaka medical college, Dhaka from July 2014 to June 2015 where the study was divided into two parts, Experiment-1 and Experiment-2. In Experiment-1 include group A and group B. Experiment-2 include group C, group D and group E. Blood was collected from group A and group B on day 1 and day 15 and from group C,D and E on day 1,4 and 15 of experiment.

Materials

The following materials were used to see the effect of aqueous extract of *Momordica charantia*on blood glucose in experimentally induced diabetic rats:

- **Collection and Preparation of Plant Material:** *Momordica charantia* was bought from Dhaka Newmarket vegetable shop and identified and authenticated by National Herbarium, Dhaka and procured. DACB accession number is

41166. Aqueous extract was prepared in Drug research Laboratory of Centre for Advanced Research of Sciences (CARS) of Dhaka University.1 kg *Momordica charantia*was cleaned and crushed through a shell and morter. This usually yield 250 ml, which was obtained by crushing the outer shells with a pestle and morter, after removal of the seeds and collecting the juice through fine cotton cloths to remove debris. The dose of *Momordica charantia* was2ml/100gm body weight for 14 days in normal rats and 10 days in diabetes induced.

- **Animals:** A total number of 35 Norwegian rats of either sex, weighting 150-200g and age between 8-10 weeks were selected for the study. They were kept in animal house of the Department of Pharmacology, Dhaka medical college and were allowed to feed on standard laboratory diet and drink ad libitum. These rats were acclimatized for five days at room temperature and humidity before commencement of the study.
- **Alloxan:** Alloxan was supplied by department of pharmacology, Dhaka Medical College Hospital, Dhaka. The drug was dissolved in normal saline and was administered intraperitonially in a dose of 120mg/kg body weight.
- **Glibenclamide:** Which was purchased from a pharmacy, the trade name was Dibenol (5mg) made by Square Pharmaceutical Pvt Ltd. The dose of glibenclamide was 1.5mg/kg body weight.
- **Estimation of Blood Glucose Level:** Blood glucose levels were estimated by Blood Glucose Meter, 'One Touch Ultra' manufactured by Life Scan Inc. a Jhonson & Jhonson company, U. S. A. Result range is 1.1 to 33.3 m.mol/L. Test time was 10 second. Each strip was inserted into the instrument. A drop of blood was collected from each of the animals by aseptically cutting the tail at the tip with a sharp sterile blade. The drop of the blood was applied to the test area of the strip making sure that both the test zones were completely covered. The instrument was kept in 'on' position and wait for 10 seconds. Then the result was displayed on the monitor.

Data Collection and Statistical Analysis

- All the results were appropriately recorded in the computer in a tabulated form. Statistical analysis was done by SPSS 11 software. The variables were expressed as mean±SD. 'Unpaired student's t test' was done for statistical analysis of comparison of means.

Table-I: Effect of aqueous extract of *Momordica charantia*on blood glucose level in nondiabetic rats

Group (n=7)	FBG(mmol/L) on day 1 (mean±SD)	FBG(mmol/L) on day 15 (mean±SD)	t- test for equality of means on day 15 between Gr-A(control) & Gr-B		
			Percent inhibition	t	significance p value
A	4.9±0.50	5.14±0.38	4.80		
B	5.0±0.36	5.2+0.31^{ns}	4.00	0.45	0.66

Table –II: Showing the effect of alloxan on blood glucose level of group C, D and E rats on day 4

Group (n=7)	FBG(mmol/L) on day 1 (mean±SD)	FBG(mmol/L) on day 4 (mean±SD)	t- test for equality of means on day 4 between Gr-C& Gr-D& Gr-E		
			Percent inhibition	t	significance P value
C	5.31±0.45	14.18±0.45	167.04		
D	4.87±0.46	14.17±0.46^{ns}	190.96	0.32	0 .786
E	5.30±0.42	14.18±0.45^{ns}	167.54	0 .27	0. 748

Table-III: Effect aqueous extract of *Momordica charantia* on blood glucose level in alloxan induced diabetic rats:

Group (n=7)	FBG(mmol/L) on day 1 (mean±SD)	FBG(mmol/L) on day 15 (mean±SD)	t- test for equality of means on day 15 between Gr-C & Gr-B& Gr-E		
			Percent inhibition	t	significance P value
C	5.31± 0.45	14.24±.51	168.17		
D	4.87±0.46	7.17±0.56 ^{s}	47.22	24.45	< 0.001^{s}
E	5.30±0.42	7.07±0.52^{s}	33.39	25.77	< 0.001^{s}

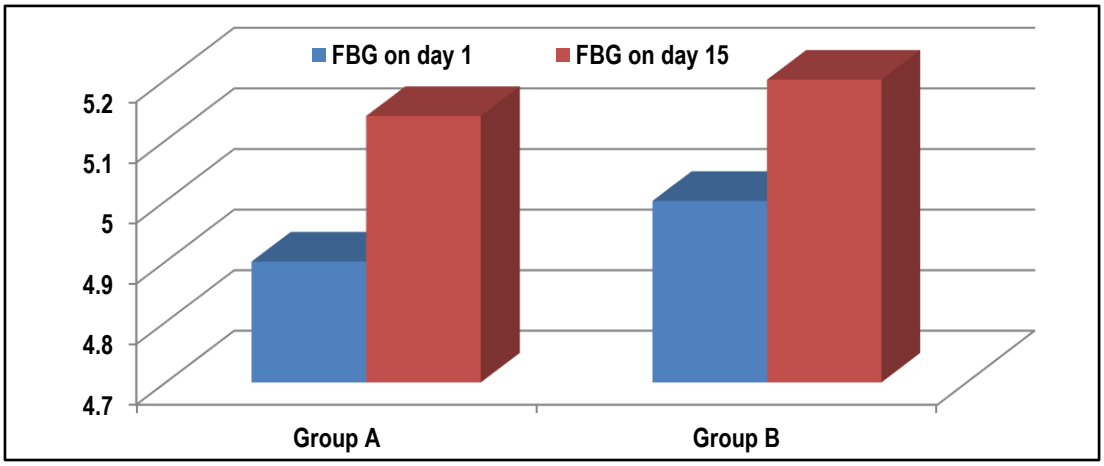

Fig-2: Outcome of aqueous extract of *Momordica charantia* on blood glucose level in non diabetic rats.

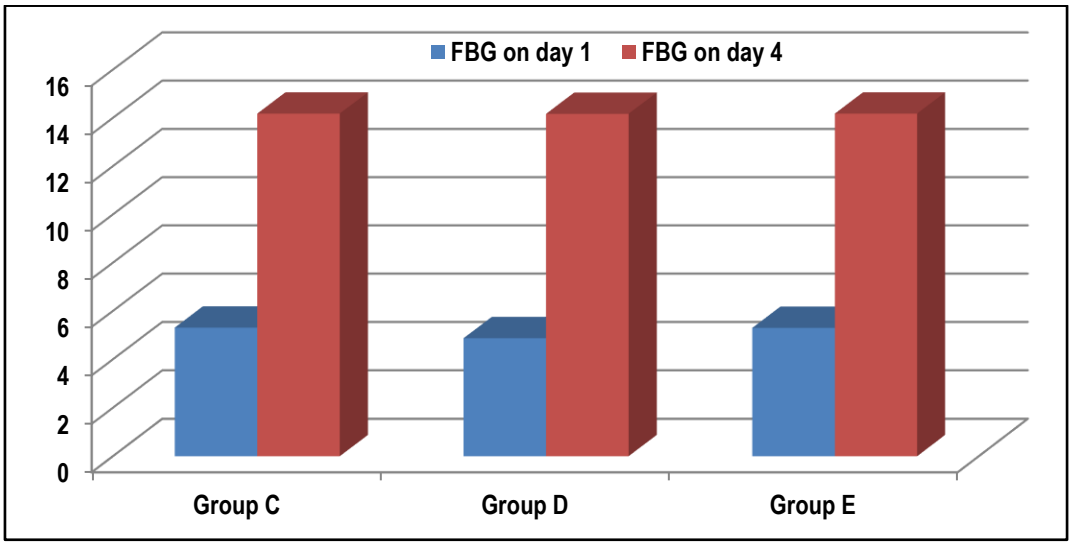

Fig-3: On day-4 outcome of alloxan on blood glucose level of group C, D and E rats

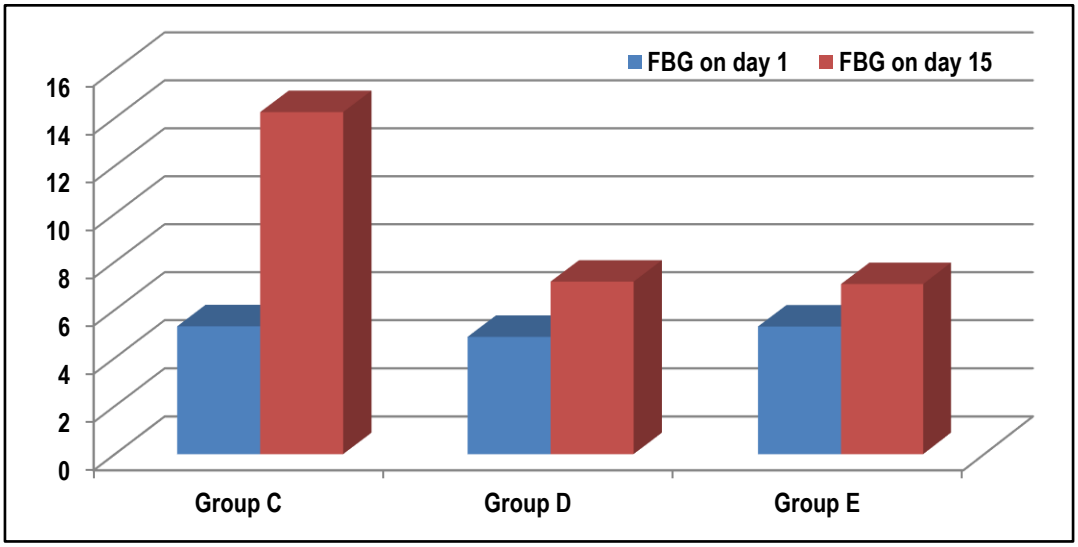

Fig-4: In diabetic rats outcome of aqueous extract of *Momordica charantia* on blood glucose level

RESULTS

In table-1 shows Effect of aqueous extract of Momordica charantia on Fasting blood glucose level in non-diabetic rats where in in group-A(served as control and received normal rat diet for 14 days) the blood glucose levels (mean ± SD) were 4.90±0.50 mmol/L and 5.3±0.38mmol/L on day 1 and day 15 respectively. Percent inhibition was 4.8 and in group-B (received Momordica charantia 2ml/100 gm body weight daily for 14 days) the blood glucose levels (mean ± SD) were 5.00±0.36mmol/L and5.05±0.32 mmol/L on day 1 and 15 respectively. Percent inhibition was 4.

In figure-2 shows blood glucose level in nondiabetic rats where in Group A standard rat diet and water were given for 14 days and in Group B Aquesous extract *of Momordica charantia* at 2ml/100gm body weight/day, standard rat diet and water were given for 14 days.

In Table-2 shows effect of alloxan on blood glucose level of group C, D and E rats on day 4 where in group C (Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given), the blood glucose levels (mean ± SD) were 5.31±00.45 and 14.18±0.45 on day 1 and day 4 respectively.

Percent inhibition was 167.04 and in group D-(Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given for 3 days. The fasting blood glucose levels (mean ± SD) were 4.87±0.46 and 14.17±0.46 on day 1 and day 4 respectively. Percent inhibition was 190.9. Also in group E (Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given for 3 days.

In figure-3 shows on day-4 outcome of alloxan on blood glucose level of group C, D and E rats where group C Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given. In group D: Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given for 3 days. And in group E:Alloxan at a dose of 120mg/kg body weight given on day 1 then standard rat diet and water were given for 3 days.

In table-3 shows effect of aqueous extract of Momordica *charantia* and Glibenclamide on blood glucose level in alloxan induced diabetic rats where in group C, the blood glucose levels (mean ± SD) were 5.31± 0.45 and 14.24±.51 on day 1 and day 15 respectively. Percent inhibition was 168.17. In group D, the blood glucose levels (mean ± SD) were 4.87±0.46 and 7.17±0.56 on

day 1 and day 15 respectively. Percent inhibition was 47.22 and in group E, the blood glucose levels (mean ± SD) were 5.30±0.42 and 7.07±0.52 on day 1 and day 15 respectively. Percent inhibition was 33.39. In figure-4 shows in diabetic rats outcome of aqueous extract of *Momordica charantia* on blood glucose level where in group C: Intraperitoneal administration of alloxan at a dose of 120mg/kg body weight on day 1 then standard rat diet and water were given for 14 days. In group D: Intraperitoneal administration of alloxan at a dose of 120mg/kg body weight on day 1 then standard rat diet and water were given for 3 days after that aqueous extract of *Momordica charantia* at a dose of 2ml/100gm body weight/day were given for 10 days. And in group E: Intraperitoneal administration of alloxan at a dose of 120mg/kg body weight on day 1 then standard rat diet and water were given for 3 days after that Glibenclamide at a dose 1.5mg/kg/day were given for 10 days.

DISCUSSION

In this present study, diabetes was induced by the intraperitoneal injection of alloxanmonohydrate in normal saline to overnight fasted animals at a dose of 120 mg/kg body weight,[6] and the blood glucose levels in animals were measured 72 hours after administration of alloxan.[7]

In this study, intraperitonial administration of single dose of alloxan (120mg)/kg body weight, increased blood glucose level significantly. The mean±SD of fasting blood glucose level (mmol/L) of group C on day 1 was 5.31±0.45 and on day4 was14.18±0.45. Similar observation was reported by number of researchers.[8]

Another study also found that hyperglycaemia occurs by intraperitoneal administration of alloxan at a dose of 120mg/kg body weight in the experimental rats.[9] In this study, the hypoglycaemic effect of *Momordica charantia* were demonstrated in alloxan induced diabetic rats.

The study was divided into two parts, Experiment-1 and Experiment-2. Experiment-1 include group A and group B. Experiment-2 include group C, group D and group E. Blood was collected from group A and group B on day 1 and day 15 and from group C,D and E on day 1,4 and 15 of experiment.

In Experiment-1 of this study, the mean±SD of fasting blood glucose level in Group A was on day 15 was5.14±0.38 mmol/L which received normal rat diet for14 days. Whereas the mean±SD of fasting blood glucose level of Group B was 5.2±0.31 mmol/L, which was treated with aqueous extract of *Momordica charantia* at a dose of 2 ml/100gm body weight. There was no statistically significant changes (P>0.05) in the mean value of fasting blood glucose level of non-diabetic rats treated with aqueous extract of *Momordica charantia* at a dose of 2ml/100gm body weight as compared with normal control. It may be concluded that aqueous extract of *Momordica charantia* has no effect on lowering the blood glucose level of normal rats. This result complieswith other reports.[10,11]

In experiment-2, the effect of aqueous extract of *Momordica charantia* was observed in alloxan induced diabetic rats and compered with a standard antidiabetic drug Glibenclamide. The mean ±SD of fasting blood glucose level of Group C (diabetic control group) on day 15 was 14.24±.51mmol/L and Group D (*M.charantia* treated group) was 7.17±0.56mmol/L. There was statistically significant changes (P<0.001) in the mean value of fasting blood glucose level of aqueous extract of *Momordica charantia* treated Group(Gr-D) compared with diabetic control group(Group-C). The mean ±SD of fasting blood glucose level of Group E (Glibenclamide at a dose of 1.5mg/kg body weight) on day 15 was7.07±0.52 mmol/L. There was statistically significant changes (P<0.001)in the mean value of fasting blood glucose level of Glibenclamide treated Group(Gr-E) compared with diabetic control group(Group-C). So there is reduction in the mean blood glucose level was observed in the experimentally hyperglycaemic group when treated with aqueous extract of *Momordica charantia* 2ml/100gm body weight (group D) and compared with Glibenclamide 1.5 mg/kg body weight (group E) on 15 day.

In one study described that extract of *Momordica charantia* fruit have antidiabetic activity and beneficial in improving complications associated with diabetes. They had used hydroalcoholic fruit extract of the plant for 21 days in alloxan induced diabetic rats.[12]

Another study observed that ethanolic extract of *Momordica charantia* suppressed gluconeogenesis in normal and streptozotocin (STZ) induced diabetic rats by depressing the hepatic gluconeogenic enzymes fructose-1, 6-bisphosphatase and glucose-6-phosphatse. The herbal extract had also enhanced the activity of glucose-6-phosphate dehydrogenase, the rate limiting enzyme of hexose monophosphate shunt (a pathway for the oxidation of glucose.[13]

Another study investigated that the anti-diabetic effect of the bitter melon on Alloxan induced diabetes in experimental animals. The study concluded that administration of alcoholic 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 was comparable to the effect of Metformin.[14]

In histopathological study in normal control group there was normal distribution of cells of islets, acini, blood capillaries, pancreatic ducts were observed. In case of *Momordica charantia* treated normal rats all the histological features were similar with normal control group. Marked inflammatory change in blood vessels and ruptured blood capillaries and degranulation and necrosis of the β cells occurred in alloxan treated diabetic control but these features were considerably absent rather normal features were present in *Momordica charantia* treated and glibenclamide treated diabetic rats.

In this study it was observed that *Momordica charantia* have hypoglycaemic effect in alloxan induced diabetic rats but not in normal rats. The result suggests that *Momordica charantia*may be an useful agent in the treatment of diabetes mellitus. However to validate this claim and to evaluate the mechanism of action more studies would be necessary. It is suggested to measure plasma insulin level, glucose tolerance test, tissue glucose level, liver glycogen level after treatment with *Momordica charantia.*

CONCLUSION

After many examination and analysis we can say that maqueous extract of Momordica charantia has hypoglycaemic effect, thus provide a rational for its use in development of new drug require for treatment and prevention of complications of diabetes mellitus.However further study are needed including well design human trial to understand its effect and use it rationally.

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