Potential antidiabetic activity of *M. charantia* Linn. extract on alloxan induced Albino Wistar Rats in Diabetes mellitus: An in vivo approach

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Abstract: The study of diabetes is not only limited to particular symptoms, but it is consequently affects the pathological and functional changes in the metabolic pathways of human body system. In those symptomatic diseases various drugs are used to treat the diabetes such as biosimilar therapy including use of insulin and insulin analogues, oral hypoglycaemic agents and various other complementary medicines.

In understanding of suggested potential antidiabetic, effect of *M. charaantia* Linn. on fasting blood sugar levels and its biochemical analysis in alloxan- induced diabetic rats were investigated. The extracts of *M. charaantia* Linn. Produced a significant antidiabetic activity at normal dose levels of their lethal doses. A comparison between the action of reduction in blood glucose level in different dose forms of *M. charantia* extract and Std. drug were seen. An oral glucose tolerance or oral tolerance test were performed with the use of glucose strip Accu-check meter.

The different extract viz. ethanol extract + water, petroleum ether + Isopropyl alcohol extract were used for further dosing purpose. The ethanol + water extract were showed significant ($P<0.001$) antidiabetic activity. In alloxan induced rat model blood glucose level were as, 214.5±5 mg/dLfor std.drug and 216.5±5 mg/dL in comparison with diabetic control 225.5±5 mg/dL.

An ANOVA was used for the statistical analysis and p-values less than 0.01 compared to normal group and 0.05 compared to diabetic control group were considered statistically significant. The extract of *M.charantia* Linn. from seed at the dose of 250 mg/kg, significantly shows the better result in reduction of blood glucose level as compared to the concentration of 500 mg/kg.

The increased level of glucose due to the damage of pancreas showed regeneration of pancreatic enzymes by extract of *M. charaantia* Linn. Which were damaged by alloxan treatment. These solvent extract also balance the body weight loss in diabetic rat, hence the present extract shows the potential to act as antidiabetic drug.

Keywords: Alloxan, *M. charaantia* Linn. Lethal Dose, Pancreas, Regeneration, Necrosis, ANOVA, p-Values etc.

I. INTRODUCTION

In depth of Global prevalence of diabetes Report 2017, Diabetes mellitus remains a major health problem, its prevention still lies in the realm of future and until then tens of millions will continue to suffer from this disease. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [1]. Thus the role of oxidative stress in development of complications accumulates the evidence, which suggests that oxidative cellular injury caused by free radicals contribute to the development of diabetes mellitus [2]. More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control [3].

Providers caring for patients with diabetes recognize that the patients are very interested in alternative supplementation and may choose to supplement their pharmacological regimen with supplementation popularly from natural products, i.e., herbal or botanical sources[3]. From the patient perspective, it is considered very acceptable to include herbal or botanical extracts as part of the medical intervention based on the recognition that the herbal intervention is considered to be natural and that the practice may have been part of the culture for many generations [4].

However, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes [5]. Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for Madhumeha and confident claims of cure are on record [6].

In view of the above considerations these present study was designed to investigate the protective effect of *Momordica charantia* Linn. on pancreatic enzymes. Moreover, the blood glucose level was estimated by using glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics GmbH, D-68298, Germany) and clinical signs checked serially.

Fig. 2: The morphology of Bitter Gourd/Flower.

II. OBJECTIVE

The objective of the study was to evaluate the hypoglycaemic effects of extracted and eluted sample of seeds of *M. charantia* Linn. on oral glucose tolerance in normal rats (Albino Wistar Rats).

The details of the method mentioned in the subsequent section of the study plan was as per the Appropriate Guideline of CPCSEA. The animal study design was approved by IAEC having Ethics Approval No. CPCSEA/IAEC/P'cology-59/2017-18/141.

III. MATERIALS AND METHODS

1.1. MATERIALS:

1.1.1. CHEMICALS

a) Extracted Test Compound (10 KDa): Biotech/UW/01, 02

b) Normal Sodium Saline (NS)

c) Alloxan Tetrahydrate -10gm

d) Wistar Albino Rats: 22 (8-10 Weeks at initiation)

e) Antidiabetic Std. Drug (Procured from Medical Outlet)

f) Glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, Germany)

Table: 1. Test Item Details:

Test Compound Code	**: Biotech/UW/01, 02**
Description	: Crystalline, Brownish, and Bitter in taste
Lot/Batch number	: NA
Molecular weight	: 10kDa
Storage conditions	: Room Temperature
Handling Precautions	: Standard laboratory precautions

Table: 2. Vehicle Details:

Vehicle	**: Normal Saline**
Storage Conditions	: 4-8^{0}C
Handling Precautions	: Standard laboratory precautions
Supplier	: In-House
Justification	: This is the most commonly used vehicles

Table: 3. Test System Details:

Species and Strain	**: Albino Wistar Rats**
Sex	: Male or Female
Age	: 8-10 weeks at initiation
Body weight	: 220-300 gm
Source	: Y.B Chavan College of Pharmacy, Dr. Zakaria Campus Aurangabad.

Table: 4. Standard Antidiabetic Drug:

Metformin	: 15 or 30 mg/d
Gliclazide	: 15 or 30 mg/d
Sitagliptin + Metformin	: 15 or 30 mg/d
Pioglitazone + Metformin	: 15 or 30 mg/d

1.2. SOURCES OF THE DATA:

Whole work was planned to generate data from laboratory based animal experimental studies as described in both the National and International journals. Development in the research area updated by conducting literature survey through e-publishing and Helinet provided by RGUHS, Bangalore [7] and animal experimentation guideline by Y.B Chavan College of Pharmacy, Dr. Zakaria Campus Aurangabad.

1.3. METHOD:

The method of study is divided into three different phases.

PHASE-I

1.3.1. COLLECTION OF THE PLANT MATERIAL, PREPARATION OF THE EXTRACTS AND SDS PAGE:

The seed and leaves of *M.charantia* Linn. was procured from commercial herbal seed company i.e. Nath Bio-Gene India Ltd. Seeds and leaves of *M.charantia* Linn. was cleaned and cut in to small pieces and shade dried for 2-3 days at approx. 30-35^0 C. crushed it in an electric grinder and then powdered. Extracts of powder was prepared by using different solvents. Extracts prepared by overnight maceration and continuous hot extraction using Soxhlet apparatus. Solvents used for the extraction purpose given in table 5.

Table 5. Differential solvent system and their properties used for the extraction:

Solvent /Parameter	Density	Boiling Point	Polarity
Ethanol + Water	0.7893 g/cm3	78.24 ± 0.09 °C	Polar as well as Non-Polar
Petroleum Ether + Isopropyl Alcohol	0.653 g/ml	42–62 °C	Non-Polar
Deionized Water	0.998926 g/ml	100 °C	Strongly Polar

1.3.2. SDS PAGE:

After the fractionating eluted sample along with crude extract and the eluted sample run on 15% SDS-PAGE gel (Laemmmli, 1970) [8]. As the sample run at appropriate level to be achieved the separating gel was placed in coomassie blue Staining solution on a shaker for 1-1.5 hrs. Excess dye was removed by destaining with Methanol: Acetic Acid: Water (30:60:10 v/v). The analysis of absorbance spectrum was plotted against the eluted sample fraction. Finally the protein concentration in sample was estimated by Bradford method using Bovine Serum Albumin (BSA) as a standard [9], and the remained sample was taken for the animal (Albino Wistar Rat) study.

1.3.3. RECOVERY OF PROTEINS FROM SDS POLY-GEL BY ELECTROPHORETIC ELUTION:

The electrophoretically separated protein in gel was recovered by extraction or solubilisation of the excised gel, and later by electrophoretic elution. Regarding the electrophoretic recovery of proteins, a number of methods reported, which can be divided into two categories: (1) “electroelution”, which recovers the protein of interest from excised gel electrophoretically, and (2) “continuous elution” of applied proteins from a preparative-scale gel during electrophoresis, whereas the electrophoretically separated proteins that migrate through the gel into a buffer stream at the gel bottom was fractionated consecutively. Characteristic features of the electroelution method resided in its simple requirements for the elution apparatus and that microgram amounts of protein was quantitatively recovered in the concentrated form [10].

PHASE-II

1.3.4. EXPERIMENTAL PRE-ACTIVITY:

Animal Welfare:

The present study was carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [11] Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study.

Acclimatization:

20 male rats allowed to acclimatize at least for three to five days prior to dosing. During that period, animals observed daily for clinical signs. Animals with any abnormalities or ill health or poor physical condition were discarded.

Randomization:

After completion of the acclimatization period 18 healthy rats randomly allocated to control and different treatment groups. There was 6 rats/group. At the commencement of the study the weight variation of the animals used were minimal and not exceeded ± 20 % of the group means body weight.

Dose Formulation:

Dose formulations of Seeds extracted drug were prepared by dissolving them in normal saline having concentration mention in the table 6. The formulations was prepared fresh before dosing. The details of the formulation preparation also captured in the table 6.

Induction of Diabetes:

Fasting rats for 24 hours were subjected to single intra-peritoneal route injection of alloxan at the dose of 100 mg/kg weight of rat. Rats that exhibit blood glucose concentration more than 100 mg/dl after 06 hrs. of Alloxan injection was considered as diabetic and selected for the proposed study.

The blood glucose concentration before and after respective treatment at above specified time intervals were determined.

PRECAUTIONS:

To avoid death and hypoglycemic shocks during 48 hrs. After injection, added glucose in drinking water at 5% concentration.

Dose Administration:

Antidiabetic Study:

Overnight fasted rats from standard and test groups G2 to G3 administered with test drug once by orally. Animals from control group (G1) received the vehicle only and handled in similar way as of treatment group animals. After 30 minutes of dose administration all the animals received oral dose of 5% glucose. The dose volume for each rat was calculated based on the recent body weight and the maximum dose volume which was not exceeded 10 mL/kg.

Antidiabetic activity was determined, following the methods of Kawashima et al 2000 [12], but with a minor modification.

PHASE-III

Overall Experimental Procedure:

Animals of all the groups will be fasted for 16-18 hours before experimentation. However, the animals allowed to have free access of Reverse Osmosis water treated with UV light, and Standard sterilized extruded rodent diet was provided throughout the period of experimentation. A 12 hours light and 12 hours dark cycle were maintained with relative humidity of 45-65% with the maintained ambient temperature throughout period of experiment. The cage was provided with 6 rats per cage and housed together in the autoclaved polypropylene cages. Cage changing was done once three days interval as well as the Autoclaved rice husk were used as bedding material for better safety of animal.
Before administration of different group vehicle viz. vehicle /Metformin/ sample extract, thereafter blood samples was collected for fasted animals from the end of rat tail at 0, 1, and 4 hours intervals and analyzed for glucose concentration using *Glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, Germany).*

Hypoglycemic Activity:

This was conducted for all the extracts proposed in the study. However, here the common procedure involved in the determination of hypoglycemic activity due to any extract were explained, which was common for all other extracts.

Albino rats of either sex weighing between 150-200 gms was categorized into 3 groups, each group consisting of 6 animals except the vehicle.

Group G1: Vehicle control (Vehicle control was received the Normal Saline only)

Group G2: Standard Antidiabetic (Metformin -250, 500 mg/kg)

Group G3: Extracted Seed Sample (250, 500 mg/kg)

IV. RESULT AND DISCUSSION:

Overall Experimental Results

Experimental Design for Antidiabetic Activity:

Table 6: Drug Formulation (Antidiabetic Activity)

Group Code	Treatment	No. of Animals	Dose (ml/kg)
G1	Vehicle control	01-04	NA
G2	Standard Antidiabetic Drug *(Metformin)*	05-11	10 mL/kg
G3	Extracted Seed Sample (Seeds)	12-18	

OBSERVATIONS FOR ANTIDIABETIC ACTIVITY:

All the following observations was made for all the animals.

Mortality and Clinical Sign Observations:

After dose administration all the rats observed carefully to find out treatment related clinical signs and mortality and it was observed that no animals were found dead and all animal rehabilated after complete study.

Body Weights:

All the animals weighed prior to dose administration. Additionally, body weights on day of receipt and during randomization was also recorded, however data were included in study report.

Hypoglycaemic Effects:

To find out the hypoglycemic effects of extracted sample, blood samples were withdrawn from each rat at predetermined time intervals of 60 min. and 4 hrs. The blood glucose level was estimated by using glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, Germany).

Histological Studies of Pancreas:

A Biopsy of pancreas of normal rat showed islets cells with normal cavity & abundant of cytoplasm surrounding it, as shown in the Fig.2 Pancreas of untreated diabetic control rats showed degeneration & necrosis of pancreatic tissues & invasion of connective tissues in pancreatic islets as shown in Fig. 3. The pancreas of *M. charantia* Linn. Extract treated diabetic rat showed slight regeneration of pancreatic cells with normal islets cells as shown in Fig.4 (250 mg) and Fig.5 (500 mg).

The Pancreas of metformin treated diabetic rat revealed partial regeneration of islet cells with presence of numerous beta cells in pancreatic islets as shown in Fig.6.

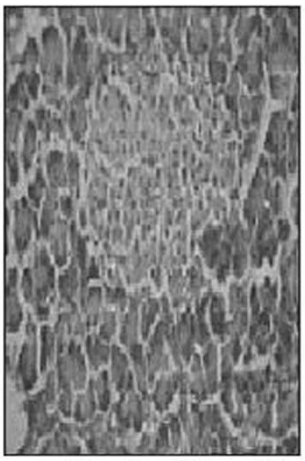

Fig.2. Histology of pancreatic section of normal healthy rats

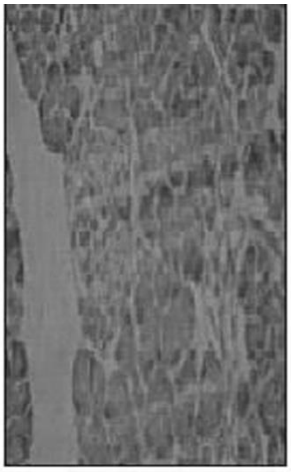

Fig.3. Histology of pancreatic section of diabetic control rat

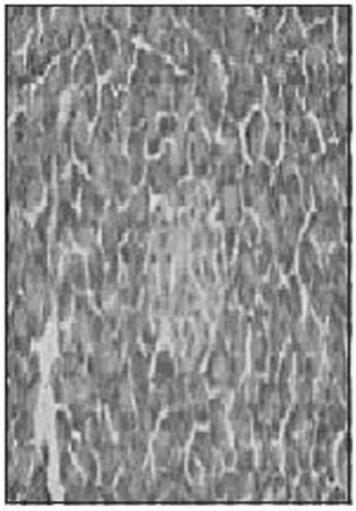

Fig.4. Histology of pancreatic section of diabetic rat with *M. charantia* Linn. seed extract (250 mg/kg)

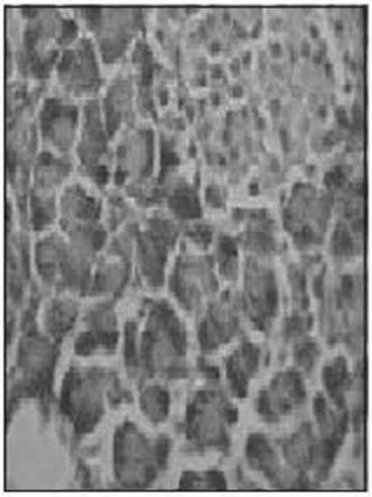

Fig.5. Histology of pancreatic section of diabetic rat with *M. charantia* Linn. seed extract (500 mg/kg)

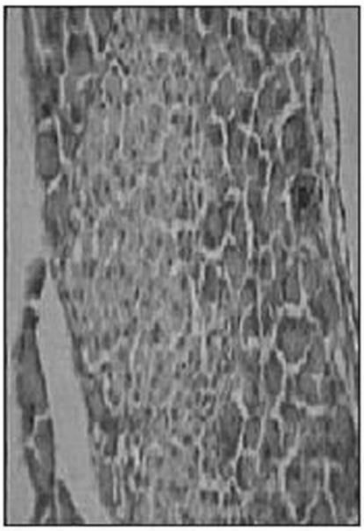

Fig. 6. Histology of pancreatic section of diabetic rat treated with Metformin (250, 500 mg/kg)

STATISTICAL ANALYSIS FOR ANTIDIABETIC STUDY:

The hypoglycaemic activity of Metformin/Extracted sample at any given time, "t" in rats was calculated as the percent blood glucose reduction at that time with respect to initial blood glucose level according to the formula given below.

Percentage of blood glucose reduction at time "t" = [(a − b)/a] ×100,

The percentage reduction in blood glucose levels was computed and plotted against time in fig. 8 & 9.

The % reduction of blood glucose level has summarized in table 7 & 8.

Where, a is the initial blood glucose level and b is the blood glucose level at time "t".

According to formula,

"t" = [(a − b)/a] ×100,

Four major glucose reduction percentage of *M.charantia Linn.* Extract on fasting rat at 1 hrs. Interval (Mean ± SD):

1. **For Std. Antidiabetic Drug (Metformin) - 250 mg/kg,**

"t" = [(224 − 220)/224] ×100 = ***1.78 %***

2. **For Std. Antidiabetic Drug (Metformin) - 500 mg/kg,**

"t" = [(229 − 221)/229] ×100 = ***3.78 %***

3. **For Compound Extract - 250 mg/kg,**

"t" = [(215 − 208)/215 ×100 = ***3.25 %***

4. **For Compound Extract - 500 mg/kg,**

"t" = [(220 − 214)/220] ×100 = ***2.72 %***

Four Major glucose reduction percentage of *M.charantia Linn.* Extract on fasting rat at 4 hrs. Interval (Mean ± SD):

1. **For Std. Antidiabetic Drug (Metformin) - 250 mg/kg,**

"t" = [(225 − 220)/225] ×100 = ***2.22 %***

2. **For Std. Antidiabetic Drug (Metformin) - 500 mg/kg,**

"t" = [(226 − 218)/226] ×100 = ***3.53 %***

3. **For Compound Extract - 250 mg/kg,**

"t" = [(218 − 212)/218 ×100 = ***2.75 %***

4. **For Compound Extract - 500 mg/kg,**

"t" = [(220 − 214)/220] ×100 = ***1.81 %***

ANOVA ANALYSIS FOR ANTIDIABETIC STUDY:

Analysis and Report Preparation:

All the observations was systematically recorded and individual records were maintained for each animal. All the individual animal data was summarized in terms of groups to get mean ± standard deviation. All the parameters was analyzed by using appropriate statistical method. All analysis and comparisons were evaluated at 5% ($P<0.05$) level i.e. $p \leq 0.05$ and considered significant in all evaluations.

However the four major coefficient of variation compared to normal group, Std. antidiabetic group, and Extracted compound was about to $P<0.01$ wisely the coefficient variation compared to diabetic control group was $P<0.05$.

The coefficient of variation of body weight for normal group was 0.033. Coefficient of variation for std. antidiabetic drug metformin was 0.034 at the concentration 500 mg/kg, and coefficient of variation for extracted compound was 0.043 at the concentration 500 mg/kg.

The coefficient of variation for normal group at glucose level fasting at 1 hrs. Interval was 0.017, coefficient of variation for std. antidiabetic drug metformin was 0.019, and coefficient of variation for extracted compound was 0.019.

The coefficient of variation for normal group at glucose level fasting at 4 hrs. Interval was 0.015, coefficient of variation for std. antidiabetic drug metformin was 0.017, and coefficient of variation for extracted compound was 0.020.

Table 6 – Effect of *M. charantia* Linn. Extract on body weight of experimental rats (Mean ± SD):

ANOVA Analysis								
Antidiabetic Study (Body Weight)								
Group Treatment	**Concentration mg/kg**	**Numbering of Animals**	**Body Wt (0 hrs) ±20 (in gm)**	**Body Wt (48 hrs) ±20 (in gm)**	**Body Wt (72 hrs) ±20 (in gm)**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
Vehicle Control (NS) – (G1)	5 mL/kg	I	92.09	97.09	98.08	95.75	3.210	0.033
Std. Antidiabetic drug (Metformin) (G2)	250 mg/kg	I	135.01	137.05	142.03	138.03	3.611	0.026
		II	233.06	237.01	241.01	237.02	3.975	0.016
		III	242.04	250.02	252.02	248.02	5.280	0.021
	500 mg/kg	IIII	138.00	140.00	143.00	140.33	2.516	0.017
		IIIII	126.05	130.15	135.15	130.45	4.557	0.034
		IIIIII	120.25	125.14	128.12	124.50	3.973	0.031
Compound Extract of Seeds (G3)	BIOTECH/UW/01- 250 mg/kg	I	73.00	76.00	78.50	75.83	2.753	0.036
		II	102.05	104.09	108.08	104.74	3.067	0.029
		III	106.97	108.06	109.25	108.09	1.140	0.010
	BIOTECH/UW/02- 500 mg/kg	IIII	95.56	99.06	102.00	98.87	3.224	0.032
		IIIII	110.00	112.00	116.00	112.66	3.055	0.027
		IIIIII	123.67	128.67	135.00	129.11	5.677	0.043

-intraperitonial, M.C - M.Charantia, NS-Normal Saline,Valuses are expressed as mean± SD, n=5, P values:*P<0.01 compared to normal group.*P<0.05 compared to diabetic control group

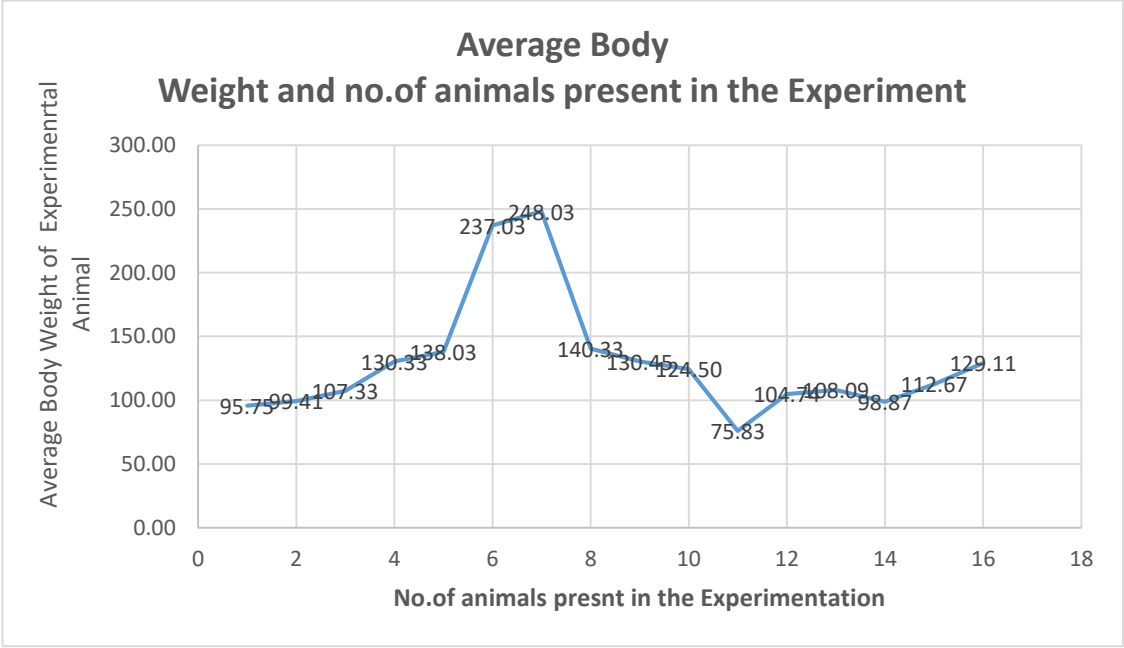

Fig 7: The average body weight of animals used in experimentation

Table 7 – Effect of *M.charantia* Linn. Extract on Glucose level by fasting at 1 hrs. Interval (Mean ± SD):

ANOVA Analysis								
Antidiabetic Study - (% of Reduction in Blood Glucose In Between Fasting and Posting Range of 1 hr. Interval)								
Group Treatment	**Concentration mg/kg**	**Numbering of Animal**	**Glucose (0 hrs.) Fasting Level (mg/dL)**	**Glucose (1 hrs.) Posting Level (mg/dL)**	**Mean**	**Standard Deviation**	**Coefficient of Variation**	**% of Reduction in Blood Glucose Level "t" = [(a − b)/a] ×100**
Vehicle Control (NS) - (G1)	5 mL/kg	I	220	220	225.5	3.87	0.017	0
		II	229	229				0
		III	226	227				0.44
		IIII	225	226				0.44
Std. Antidiabetic Drug (Metformin)- (G2)	250 mg/kg	I	221	222	219.0	2.82	0.012	0.45
		II	224	220				1.78
		III	220	220				0
	500 mg/kg	IIII	217	216				0.46
		IIIII	218	215				1.37
		IIIIII	229	221				3.78
Compound Extract of Seeds- (G3)	BIOTECH/UW/01- 250 mg/kg	I	215	208	214.8	4.02	0.018	3.25
		II	225	220				2.22
		III	220	216				1.81
	BIOTECH/UW/02- 500 mg/kg	IIII	220	214				2.72
		IIIII	218	217				1.05
		IIIIII	220	214				2.72

i.p -intraperitonial, M.C - M.Charantia, NS-Normal Saline,Valuses are expressed as mean± SD, n=5, P values:*P<0.01 compared to normal group.*P<0.05 compared to diabetic control group

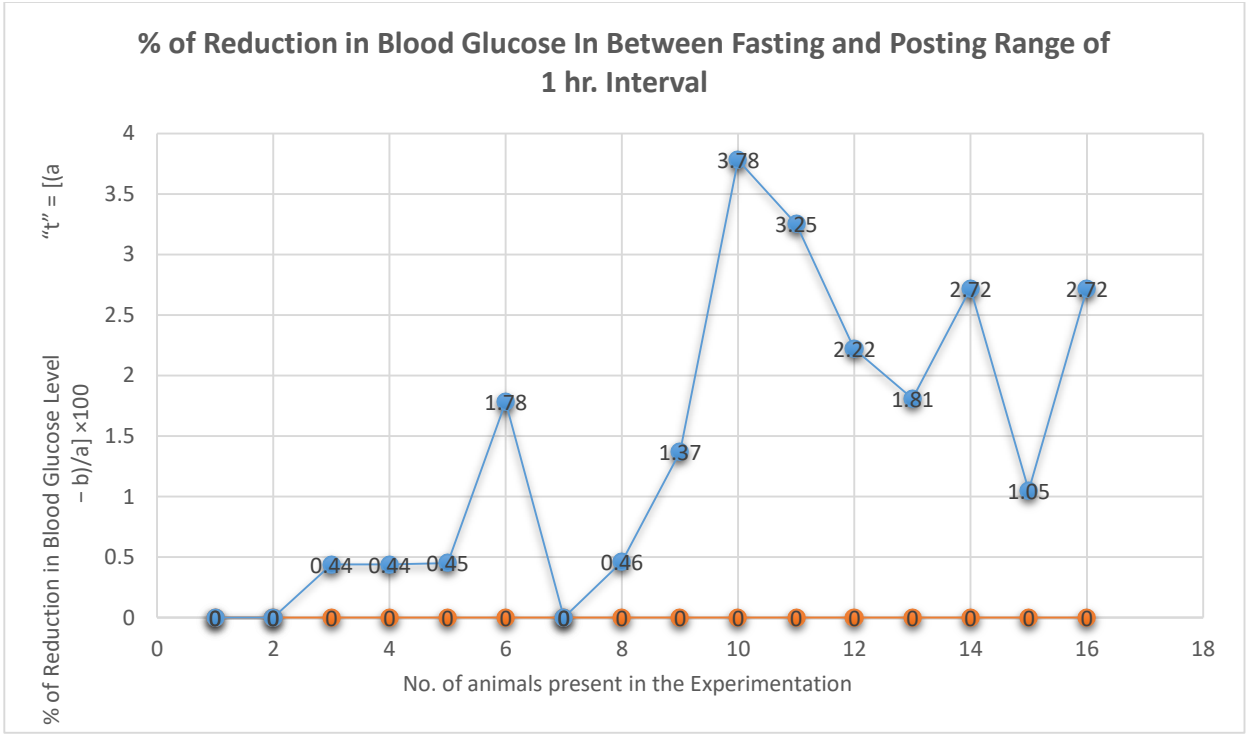

Fig 8: The percentage of reduction in blood glucose in between fasting and posting range of 1 hr. interval

Table 8– Effect of *M.charantia* Linn. Extract on Glucose level by fasting at 4 hrs. Interval (Mean ± SD):

ANOVA Analysis								
Antidiabetic Study - (% of Reduction in Blood Glucose In Between Fasting and Posting Range of 4 hrs. Interval)								
Group Treatment	**Concentration mg/kg**	**Numbering of Animal**	**Glucose (0 hrs) Fasting Level (mg/dL)**	**Glucose (4 hrs) (mg/dL)**	**Mean**	**Standard Deviation**	**Coefficient of Variation**	**% of Reduction in Blood Glucose Level "t" = [(a − b)/a] ×100**
Vehicle Control (NS) - (G1)	5 mL/kg	I	220	220	224.7	3.40	0.015	0
		II	229	228				0.45
		III	226	226				0
		IIII	225	225				0
Std. Antidiabetic Drug (Metformin) - (G2)	250 mg/kg	I	221	220	218.3	2.06	0.009	0.45
		II	225	220				2.22
		III	220	220				0
	500 mg/kg	IIII	217	217				0
		IIIII	218	215				1.37
		IIIIII	226	218				3.53
Compound Extract of Seeds- (G3)	BIOTECH/UW/01- 250 mg/kg	I	218	212	216.0	2.82	0.013	2.75
		II	225	220				2.22
		III	220	216				1.81
	BIOTECH/UW/02- 500 mg/kg	IIII	220	216				1.81
		IIIII	218	218				0
		IIIIII	220	214				2.72

i.p -intraperitonial, M.C - M.Charantia, NS-Normal Saline,Valuses are expressed as mean± SD, n=5, P values:*P<0.01 compared to normal group.*P<0.05 compared to diabetic control group

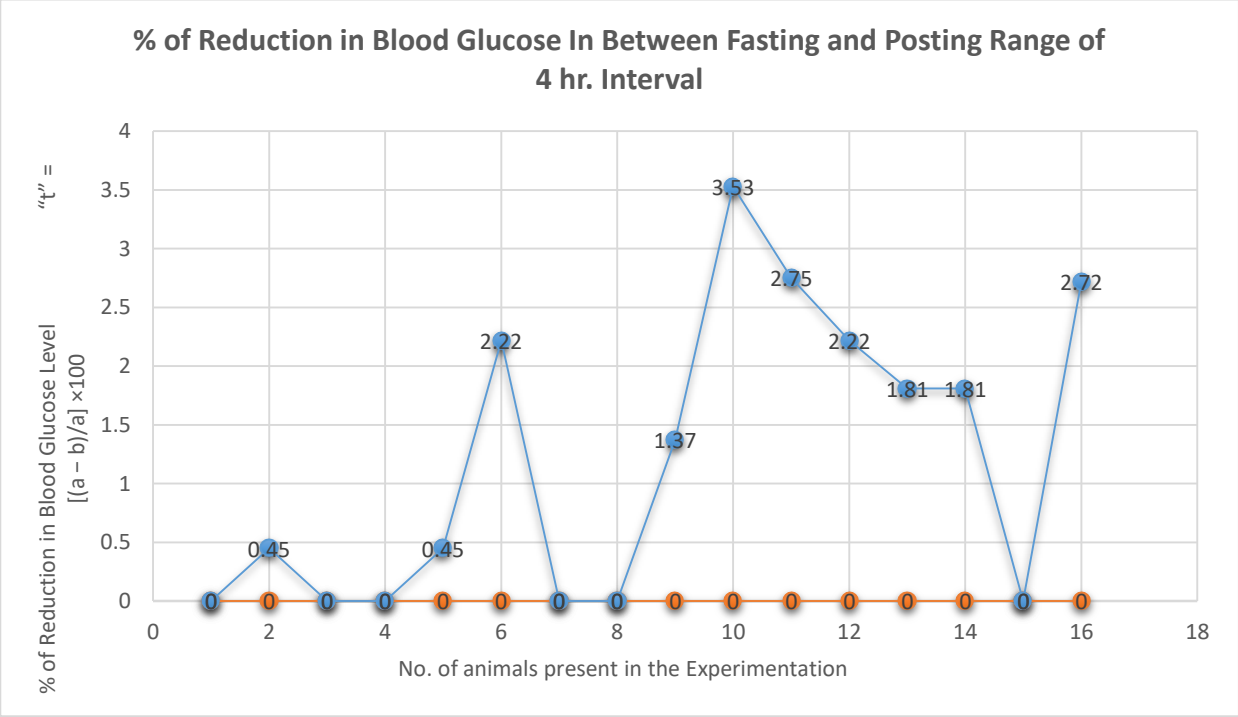

Fig 9: The percentage of reduction in blood glucose in between fasting and posting range of 4 hr. interval

4. Conclusion

The concept of food as medicine is a central theme in diabetic and nutritional sciences. *M. charantia* has been used as dietary supplements and ethnomedicine throughout centuries for relieving symptoms and conditions related to what we know in modern days as diabetes. To date, *M. charantia* has been extensively studied worldwide for its medicinal properties to treat a number of diseases [13]. This may be due to the fact that the plant possesses over 225 different medicinal constituents [14].

Despite the abundant data from biochemical and animal studies, available clinical data as reviewed in the present article are often flawed by small sample size, lack of control and poor study designs. The present research supports the need for better-designed clinical trials with sufficient sample size and statistical power to further indicate the acclaimed efficacy of *M. charantia* as a natural nutritional treatment for diabetes mellitus [15]. In particular, *M. charantia* may be a feasible option for ethnic minorities who have a high prevalence of diabetes but prefer treatment based on natural products according to their cultural beliefs [16].

Declaration of interest

The authors report no conflicts of interest.

Acknowledgements

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