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In vivo Approach: Potential Diuretic Activity of *M. charantia* Linn. on Alloxan Induced Albino Wistar Rats in Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Author UW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AK and LK managed the analyses of the study. Author AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The screening of perfect diuretics for non –clinical utility in Diabetes mellitus is a relatively novel approach which gain insight into underlying the pathophysiological processes.
This study aim to evaluate the diuretic effect of crude aqueous & alcoholic extract of *M. charantia* Linn. using Albino Wistar Rat model. The methodology of animal study includes the spectral analysis of Na^+, K^+, Cl^-concentration against the body weight, this was done by spectrophotometry. In this study, the comparative observation of Diuretic activity with standard and extracted compound has shown that the estimated 24-hour urine contains the Na+-3.82 g, 3.82 g, 3.92 g and K+-1.35 g, 1.39 g, 1.48 gwt. For Vehicle control, Standard drug, and Extracted compound respectively. Which possess the favouring result means from the spot urine were 10.7±7.0 g/24 hand 3.9±2.1 g/24 h, respectively. Coefficients were 0.035, 0.022, 0.046 at (d±2SD=7.07 g,

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4.42 gand 8.92 g) for sodium chloride and 0.068, 0.031, 0.046 at (d ± 2SD = 4.92 g, 2.31 g and 3.34 g) for potassium chloride. The present study guide formulation of non-clinical trials with statistical study to further measuring the claimed efficacy of *M. charantia* as a natural remedy for diabetes mellitus.

Keywords: M. charantia Linn. furosemide; normalsaline; ANOVA; p-valuesetc.

1. INTRODUCTION

In WHO Technical report series of Diabetes mellitus 727 (1985), 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 [1]. Diabetes mellitus is reported to be increased in patients by an increase in Oxidative stress [2]. Thus the role of oxidative stress in the development of complications accumulates the evidence, which suggests that oxidative cellular injury caused by free radicals contribute to the development of diabetes mellitus [3,4].

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, the present study was designed to investigate the protective effect of *Momordica charantia* Linn. on Diuresis. Moreover, the urine Na^+, K^+, Cl^- and Creatinine level estimated by using spectral analysis and clinical signs were investigated [7].

1.1 Objective

The objective of the study was to evaluate the diuretic effects of extracted and eluted samples of seeds of *M. charantia* Linn. in Albino Wistar Rats.

The details of the method mentioned in the subsequent section of the study plan were as per the Appropriate Guideline of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2. MATERIALS AND METHODS

2.1 Chemicals

a) Extracted Test Compound (1000 Da): Biotech/UW/01, 02
b) Normal Saline
c) AlloxanTetrahydrate-10 gm
d) Diuretic Std. Drug- Furosemide-40, 80 mg/d
e) Sodium Bicarbonate Saline Solution – 110 mM NaCl and 30 mM $NaHCO_3$)

2.2 Methods

The method of study is divided into two different phases.

2.2.1 Collection of the plant material, preparation of the extracts and SDS Page

The seed of *M. charantia* was procured from a commercial herbal seed company. Seeds of *M. charantia* were cleaned and cut into small pieces and shade dried for 2-3 days at approx. 30-350°C. The dried seeds were crushed in an electric grinder and then powdered. Extracts of powder were prepared by using different solvents. Extracts were prepared by overnight maceration and continuous hot extraction using the Soxhlet apparatus. Solvents used for the extraction purpose are in Table 1.

2.2.2 SDS Page

After the fractionating, eluted sample from gel filtration chromatography along with crude extract and precipitated sample run on 15% SDS-PAGE gel (Laemmmli, 1970) [8,9]. The separating gel was placed in coomassie blue staining solution on a shaker for 1-1.5 hrs. Excess dye were removed by destaining with Methanol: Acetic Acid: Water (30:60:10 v/v). Finally, the protein concentration in the sample was estimated by Bradford method [10] using Bovine Serum Albumin (BSA) as a standard, and the remaining sample was taken for the animal study.

2.3 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.

Table 1. Solvents used for the extraction purpose

Solvents	Density	Boilingpoint	Polarity
Ethanol+Water	0.789 g/cm^3	78.24°C	Polar/NP
P.Ether+IPA	0.653 g/ml	42-62°C	Non-Polar
Deionized Water	0.998 g/ml	100°C	S.Polar

Acclimatization: 22 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 were randomly allocated to control and different treatment groups. They were divided as6 rats/group. At the commencement of the study, the weight variation of the animals used was minimal and not exceeded ± 20% of the group means body weight.

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

Group G1: Vehicle control (10 ml/kg)
Group G2: Standard Diuretic (Furosemide - 40, 80 mg/kg)
Group G3: Crude Extract Sample (Seeds)-40,80 mg/kg

2.4 Induction of Diabetes

Fasting rats for 24 hours were subjected to a 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 48 hrs. of Alloxan injection were considered as diabetic and selected for the proposed study [11,12].

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

2.5 Overall Experimental Procedure

Animals of all the groups have fasted for 16-18 hours before experimentation and fasting were continued till the end of experimentation. However, the animals allowed to have free access of Reverse Osmosis water treated with UV light, and standard sterilized extruded rodent diet were provided throughout the period of experimentation. A 12 hours light and 12 hours dark cycle is maintained with a relative humidity of 45-65% with the maintained ambient temperature throughout period of the experiment. The metabolic cages were provided with 3 rats per cage housed together. Cage changing did once weekly as well as the urine sample was withdrawn with the interval of 4-6 hrs.

Precautions: To avoid death and hypoglycemic shocks for 48 hrs. After injection, glucose was added in drinking water at 5% (In house).

2.6 Dose Administration

Diuretic Study: Overnight fasted and deprived of water rats from standard and test groups G2 to G3 administered with test drug once orally. Animals from the control group (G1) received the vehicle i.e. Normal saline only and handled similarly as of treatment group animals. The dose-volume for each rat calculated based on the recent body weight and the maximum dose volume not exceeded more than 0.5 mL/kg.

Threats Fasted for 18h with free access to normal saline, and then orally administered 30 ml/kg of bicarbonate saline solution (containing 110 mM NaCl and 30 mM $NaHCO_3$).

Thirty Minutes Later, animals were randomized and divided into different groups (n=3). The positive control group received Furosemide (viz.40 mg/kg and 80 mg/kg) in normal saline .The dose formulation of drugs were given in Table 2. The test drugs given In different doses; animals were places in metabolic cages. Urine Samples Were Collected Using Metabolic Cages at hourly intervals of 4h-6h, the urine volume measured.

2.7 Spectral Analysis Used in Experimentation

After the administration of different group viz. vehicle/Furosemide/Sample extract, urine sampleswere collected from the animals by 4-6 hours of intervals and analyzed Spectroscopic study for Na^+, K^+, Cl^- and protein creatinine ratio.

2.8 Statistical Analysis

The Quantitative variables were expressed as mean ± standard deviation (SD). Whereas qualitative variables were expressed in frequency. Estimated 24-hour urine Na+, K+, Cl-

from the spot samples were calculated using formulae described in results and discussion.

The ANOVA analysis for the diuretic study was determined with reference to the coefficient of variation under the heading of results and discussion.

3. RESULTS

3.1 Overall Experimental Results

Animals of all the groups were fasted for 16-18 hours before experimentation and fasting was continued till the end of 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 is maintained with relative humidity of 45-65% with the maintained ambient temperature throughout period of experiment.

3.2 Observations for Diuretic Activity

The following observations were made for all the animals.

3.3 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 found dead and all animal rehabilated after complete study.

Body Weights: All the animals weighed before dose administration. Additionally, body weights on day of receipt and during randomization of animals also recorded in Table 3 consisting Body weight of Diuretic Study Rat (Mean ± SD).

Diuretic Activity: To find out the diuretic effects of the extracted sample, urine samples were collected from each rat at time intervals of 4-6 hrs. After the oral administration of the standard drug and extracted samples. The urine volume was measured for all the animals and the specimen was assayed for Na+, K+, Cl- and Protein creatinine ratio using an atomic emission spectrometer.

The present range of Na+, K+, Cl- (mmol/Ltr) and protein creatinine were computed and plotted along with Mean ± Standard Deviation.

Data Analysis and Report Preparation: All the observations were systematically recorded and individual records were maintained for each animal. All the individual animal data were summarized in terms of groups to get mean and standard deviation. All the parameters were analyzed by using an appropriate statistical method. All analysis and comparisons were also evaluated at 5% ($P<0.05$) level i.e. $p \leq 0.05$, considered significant in all evaluations. The range of Bodyweight, salt concentration, Potassium Concentration, Chloride Concentration and Protein creatinine ratio reported in Tables 3 to 7.

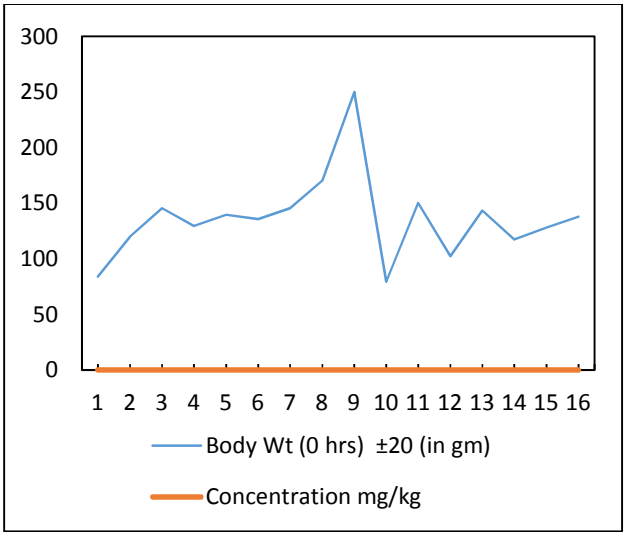

Fig. 1. Body weight of animal at 0 hrs. interval

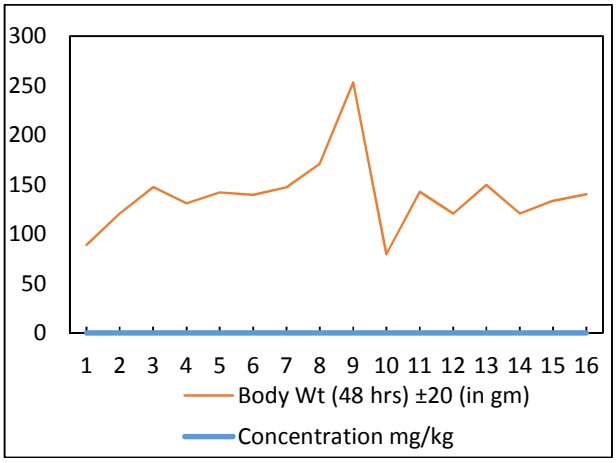

Fig. 2. Body weight of animal at 48 hrs. interval

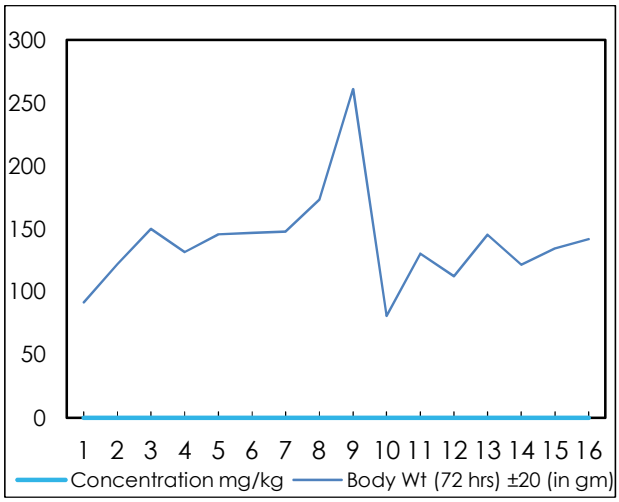

Fig. 3. Body weight of animal at 72 hrs. interval

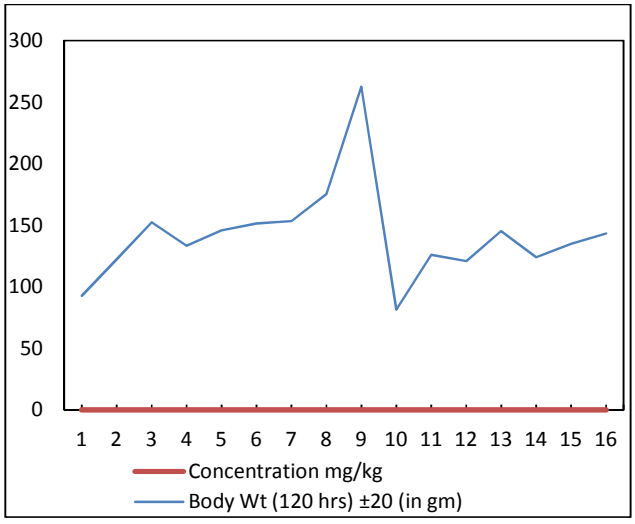

Fig. 4. Body weight of animal at 120 hrs. interval

Table 2. Dose formulation (Diuretic Activity)

Group code	Treatment	No. of Animals	Dose (ml/kg)
G1	Vehicle control	01-04	NA
G2	Furosemide- 40 mg/kg Furosemide-80 mg/kg	05-11	10 mL/kg
G3	Extracted (Seeds)- 40 mg/kg Extracted (Seeds)- 80 mg/kg	12-18	

Table 3. Body weight of experimental albino wistar rats (Mean±SD)

ANOVA Analysis									
Diuretic 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)**	**Body Wt. (120 hrs) ±20 (in gm)**	**Mean**	**Standard deviation**	**Coefficient of Variation *(p)***
Vehicle Control (NS) (G1) No. of Animals: 6	0.5 mL/kg	I	83.95	89.05	91.56	92.70	89.31	3.89	0.043
		II	120.00	120.50	122.00	122.50	121.25	1.19	0.009
		III	145.50	147.50	150.00	152.50	148.87	3.03	0.020
		IIII	129.50	131.00	131.60	133.50	131.40	1.65	0.012
Standard Diuretic Drug (Furosemide) (G2) No. of Animals: 6	40 mg/kg	I	139.50	142.09	145.67	146.00	143.31	3.09	0.021
		II	135.76	139.50	147.04	151.45	143.43	7.10	0.049
	80 mg/kg	III	145.56	147.05	148.00	153.35	148.49	3.39	0.022
		IIII	170.45	170.80	173.34	175.50	172.52	2.36	0.013
		IIIII	250.34	253.07	261.12	262.50	256.75	5.96	0.023
		IIIIII	79.50	79.50	81.00	81.50	80.37	1.03	0.012
Compound Extract of seeds (G3) No. of Animals: 6	BIOTECH/UW/0140 mg/kg	I	150.34	142.65	130.50	126.00	137.37	11.14	0.081
		II	102.45	120.65	112.50	121.00	114.15	8.73	0.076
		III	143.34	149.65	145.50	145.50	145.99	2.63	0.018
	BIOTECH/UW/0280 mg/kg	IIII	117.50	120.75	121.60	124.00	120.96	2.68	0.022
		IIIII	128.23	133.50	134.50	135.00	132.80	3.11	0.023
		IIIIII	138.00	140.00	142.00	143.50	140.87	2.39	0.016

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

Table 4. Effect of *M. charantia* Linn. Extract on Na^+ conc. in alloxan induced diabetic albino wistar rats (Mean±SD)

ANOVA Analysis							
Normal Range of Na^+: 20-250 mmol/L							
Group treatment	**Concentration mg/kg**	**Numbering of Animals**	**Urine Collection (4-6 hrs)in mL**	**NA+(mmol/Ltr)**	**Mean**	**Standard deviation**	**Coefficient of variation**
Vehicle Control (NS) Orally(G4)	0.5 mL/kg	I	1.5	201	199	7.07	0.035
		II	2.5	192			
		III	1.5	195			
		IIII	3.5	208			
Standard Diuretic Drug-Furosemide (G5)	Std.40 mg/kg	I	1.5	201	200	4.42	0.022
		II	1.5	196			
	Std.80 mg/kg	III	1.5	206			
		IIII	4.5	198			
		IIIII	3.0	195			
		IIIIII	2.0	204			
Compound Extract of Seeds(G6)	BIOTECH/UW/01-40 mg/kg	I	1.5	199	190	8.92	0.046
		II	1.5	180			
		III	1.7	178			
	BIOTECH/UW/02-80 mg/kg	IIII	3.0	196			
		IIIII	3.0	196			
		IIIIII	3.0	191			

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

Table 5. Effect of *M. charantia* Linn. Extract on K^+ conc. in alloxan induced diabetic albino wistar rats (Mean±SD)

ANOVAAnalysis							
Normal Range of K^+: 03-100 mmol/L							
Group treatment	**Concentration mg/kg**	**Numbering of Animals**	**Urine Collection (4-6 hrs)in mL**	**K^+ (mmol/Ltr)**	**Mean**	**Standard deviation**	**Coefficient of variation**
Vehicle Control (NS) Orally(G4)	0.5 mL/kg	I	1.5	72.6	72.10	4.924	0.068
		II	2.5	71.4			
		III	1.5	66.2			
		IIII	3.5	78.2			
Standard Diuretic Drug-Furosemide (G5)	Std.40 mg/kg	I	1.5	72.1	72.45	2.316	0.031
		II	1.5	75.2			
	Std.80 mg/kg	III	1.5	73.6			
		IIII	4.5	70.4			
		IIIII	3	69.2			
		IIIIII	2	74.2			
Compound Extract of Seeds(G6)	BIOTECH/UW/01-40 mg/kg	I	1.5	74.1	71.80	3.348	0.046
		II	1.5	76.2			
		III	1.7	73.8			
	BIOTECH/UW/02-80 mg/kg	IIII	3	70.1			
		IIIII	3	68.2			
		IIIIII	3	68.4			

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

Table 6. Effect of *M. charantia* Linn. Extract on Cl^- conc. in alloxan induced diabetic albino wistar rats (Mean±SD)

ANOVA Analysis							
Normal Range of Cl^- : 20-300 mmol/L							
Group treatment	**Concentration mg/kg**	**Numbering of Animals**	**Urine Collection (4-6 hrs) in mL**	**Cl^-(mmol/Ltr)**	**Mean**	**Standard deviation**	**Coefficient of variation**
Vehicle Control (NS)Orally(G4)	0.5 mL/kg	I	1.5	303	305.25	3.30	0.010
		II	2.5	307			
		III	1.5	309			
		IIII	3.5	302			
Standard Diuretic Drug-Furosemide (G5)	Std.40 mg/kg	I	1.5	308	303.16	4.44	0.014
		II	1.5	298			
	Std.80 mg/kg	III	1.5	301			
		IIII	4.5	303			
		IIIII	3	300			
		IIIIII	2	309			
Compound Extract of Seeds(G6)	BIOTECH/UW/01-40 mg/kg	I	1.5	306	301.667	6.43	0.021
		II	1.5	293			
		III	1.7	308			
	BIOTECH/UW/02-80 mg/kg	IIII	3	301			
		IIIII	3	307			
		IIIIII	3	295			

*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.*

Table 7. Effect of *M. charantia* Linn. Extract on protein creatinine ratio in alloxan induced diabetic albino wistar rats (Mean±SD)

ANOVAAnalysis							
Diuretic Study (Protein Creatinine)							
GroupTreatment	**Concentration mg/kg**	**Numbering of Animals**	**Urine Collection (4-6 hrs.) in mL**	**Protein: Creatinine Ratio mg/dL**	**Mean**	**StandardDeviation**	**Coefficientofvariation**
VehicleControl(NS)Orally(G4)	0.5 mL/kg	I	1.5	0.65	0.73	0.056	0.077
		II	2.5	0.73			
		III	1.5	0.77			
		IIII	3.5	0.77			
StandardDiureticDrug-Furosemide(G5)	Std.40 mg/kg	I	1.5	0.72	0.72	0.034	0.047
		II	1.5	0.68			
	Std.80 mg/kg	III	1.5	0.69			
		IIII	4.5	0.73			
		IIIII	3	0.75			
		IIIIII	2	0.77			
CompoundExtractofSeeds(G6)	BIOTECH/UW/01-40 mg/kg	I	1.5	0.75	0.69	0.061	0.088
		II	1.5	0.69			
		III	1.7	0.63			
	BIOTECH/UW/02-80 mg/kg	IIII	3	0.72			
		IIIII	3	0.77			
		IIIIII	3	0.62			

*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*

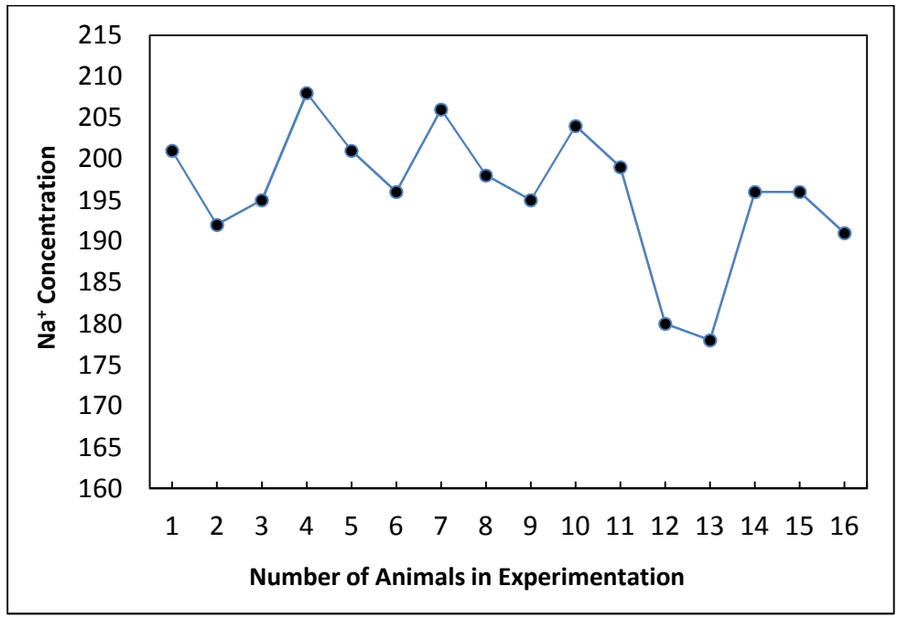

Fig. 5. No. of animals in Na^+ Conc.determination

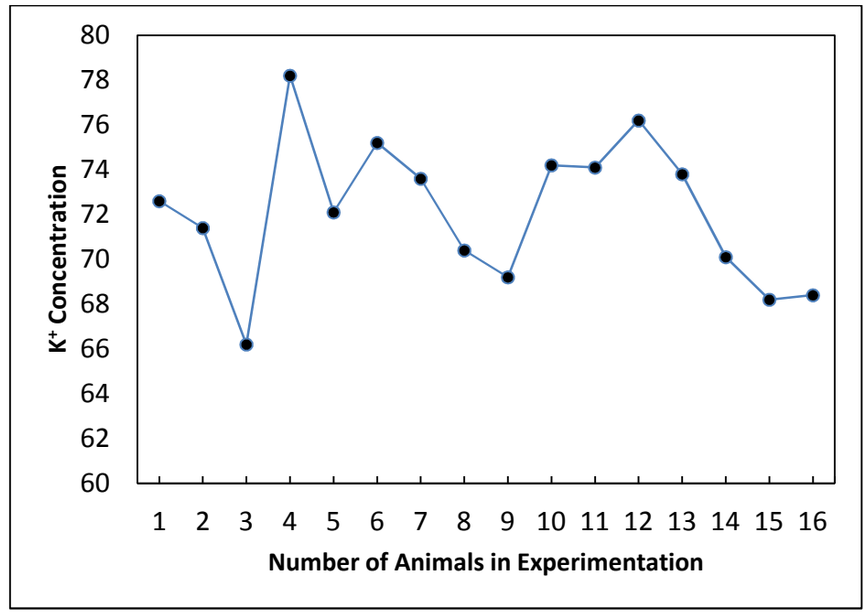

Fig. 6. No.of animals in K^+ Conc.determination

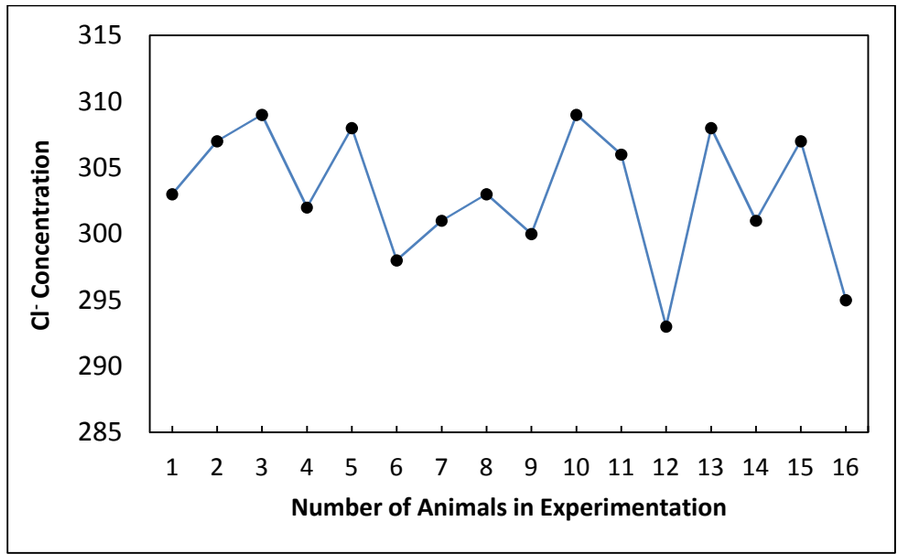

Fig. 7. No. of animals in Cl^- Conc.determination

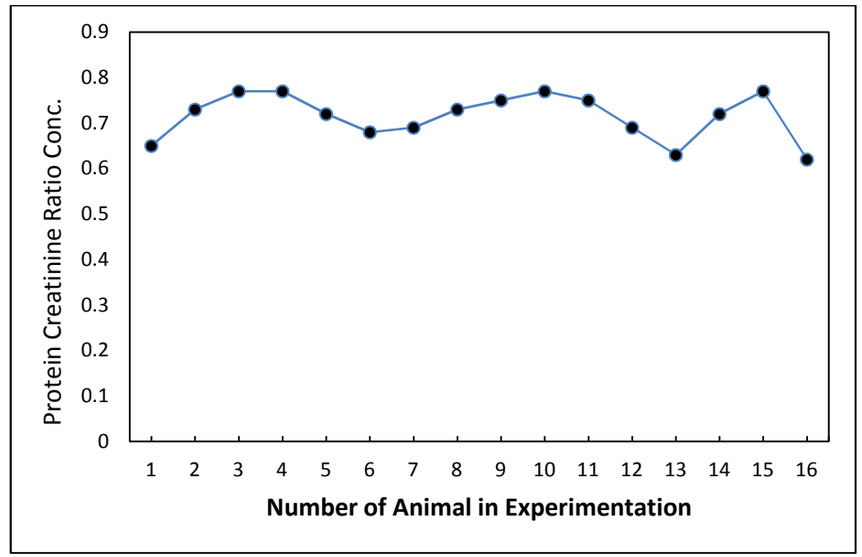

Fig. 8. No. of animals in protein creatinine ratio determination

Table 8. The Quantitative and qualitative variables Mean ± SD

Estimated _24 HUNa^{+} for Vehicle control (g) $=\frac{\text{Na+}}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUNa+- for Vehicle Control (g) $=\frac{199}{7.21\text{ (g)}\times 24\text{ HU }7.21\text{ (g)}} = 3.82\text{ g at }24\text{ HUNa} -+$
Estimated _24 HUNa^{+-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{\text{Na+}}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUNa^{+} for Std. Diuretic Drug (Furosemide) (g) $=\frac{200}{7.23\text{ (g)}\times 24\text{ HU }7.23\text{ (g)}} = 3.82\text{ g at }24\text{ HUNa} +$
Estimated _24 HUNa+- for Extracted Seeds sample (g) $= \frac{\text{Na}-}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}=$	Estimated _24 HUNa^{+-} for Extracted Seeds and Leaves sample (g) $=\frac{190}{6.96\text{ (g)}\times 24\text{ HU }6.96\text{ (g)}} = 3.92\text{ g at }24\text{ HUNa} +$
Estimated _24 HUK+- for Vehicle control (g) $=\frac{\text{K+}-}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUNa^{+-} for Extracted Seeds and Leaves sample (g) $=\frac{190}{6.96\text{ (g)}\times 24\text{ HU }6.96\text{ (g)}}$= 3.92 g at 24 HUNa^{+}
Estimated _24 HUK^{+-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{\text{K+}}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUK^{-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{72.45}{7.2\text{ (g)}\times 24\text{ HU }7.23\text{ (g)}}$= 1.39 g at 24 HUK^{+}
Estimated _24 HUK^{+} for Extracted Seeds Sample (g) $=\frac{\text{K+}}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUK^{-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{71.80}{6.96\text{ (g)}\times 24\text{ HU }6.96\text{ (g)}} = 1.48\text{ g at }24\text{ HUK} +$
Estimated _24 HUCl^{-} for Vehicle control (g) $=\frac{\text{Cl}-}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUCl^{-} for Vehicle Control (g) $=\frac{305.25}{7.3\text{ (g)}\times 24\text{ HU }7.3\text{ (g)}} = 5.72\text{ g at }24\text{ HUCl} -$
Estimated _24 HUCl^{-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{\text{Cl}-}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUCl^{-} for Std. Diuretic Drug (Furosemide) (g) $=\frac{303.16}{7.2\text{ (g)}\times 24\text{ HU }7.23\text{ (g)}}$= 5.80 g at 24 HUCl^{-}
Estimated _24 HUCl^{-} for Extracted Seeds and Leaves sample (g) $=\frac{\text{Cl}-}{\text{Creatinine SpotU (g)}\times 24\text{ HU Creatinine (g)}}$	Estimated _24 HUCl^{-} for Extracted Seeds and Leaves sample (g) $=\frac{301.66}{6.96\text{ (g)}\times 24\text{ HU }6.96\text{ (g)}} = 6.22\text{ g at }24\text{ HUCl} -$

3.4 ANOVA Analysis for Diuretic Study

Estimated sodium chloride and potassium chloride means from the spot urine were 10.7±7.0 g/24 h and 3.9±2.1 g/24 h, respectively. Coefficients were 0.035, 0.022, 0.046 at (d±2SD= 7.07 g, 4.42 g and 8.92 g) for sodium chloride and 0.068, 0.031, 0.046 at (d±2SD=4.92 g, 2.31 g, and 3.34 g) for potassium chloride.

The respected ANOVA analysis for Body weight, Sodium chloride concentration, Potassium chloride concentration, and creatinine percentage was calculated as per Tables 4, 5, 6 and 7.

3.5 Statistical Analysis for Diuretic Study

The estimated 24-hour urine Na^+ and K^+ from the spot samples were calculated using the following formulae of Table 8.

Na^+ Spot U and K^+ Spot U = Na^+ and K^+ measured from spot urine sample and compared with the quantitative method. Creatinine Spot U = Urinary creatinine measured and the protein creatinine ration given in Table 2-4. HU creatinine = Urinary creatinine measured from 24-hour urine collection.

Results are expressed in grams of sodium chloride (Nacl) and potassium chloride (KCl), as this is the form in which Na^+ and K^+ are excreted in urine. The Na^+ and K^+ results can be deduced by conversion (1 g Nacl=0.4 g Na^+, 1 g KCl≈0.5 g K^+).

4. DISCUSSION

The concept of food as medicine is a central theme in diabetic and nutritional sciences. *M. charantia* was used as dietary supplements [13] 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 [14]. It was described as a versatile plant worthy of treating almost any disease inflicted on mankind. This may be because the plant possesses over 225 different medicinal constituents [15].

The Mechanism of Action of Furosemide is like other loop diuretics, acts by inhibiting the luminal Na^+, K^+, Cl^- cotransporter in the thick ascending limb of the loop of Henle in nephron of kidney, by binding to the chloride transport channel, thus causing sodium, chloride, and potassium loss in urine.

5. CONCLUSION

This article initiate the comparative observation of Diuretic study with standard and extracted compound were shown that the estimated 24-hour urine contains the Na^+ - 3.82 g, 3.82 g, 3.92 g and K^+ - 1.35 g, 1.39 g, 1.48 g wt. for Vehicle control, Standard drug, and Extracted compound respectively. Which possess the favouring result means from the spot urine were 10.7±7.0 g/24 h and 3.9±2.1 g/24 h, respectively. Coefficients were 0.035, 0.022, 0.046 at (d±2SD = 7.07 g, 4.42 g and 8.92 g) for sodium chloride and 0.068, 0.031, 0.046 at (d±2SD =4.92 g, 2.31 g, and 3.34 g) for potassium chloride. The Na^+ and K^+ results can be deduced by conversion (1 g NaCl=0.4 g Na^+, 1 g KCl≈0.5 g K^+).The present study guide formulation of clinical trials with sufficient sample size and statistical study to further measuring the claimed efficacy of *M. charantia* as a natural remedy for diabetes mellitus. In particular, *M. charantia* may be a feasible option who have a high prevalence of diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal study design was approved by IAEC (Instituonal Animal Ethics Committee) having Ethics Approval No. CPCSEA/IAEC/ Pharmacology-59/2017-18/141.

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COMPETINGINTERESTS

Authors have declared that no competing interests exist.

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