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.

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 (±2SD=7.07 g,

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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 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-values etc.

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 NaHCO₃)

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. 30-35°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 (Laemmllii, 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/cm³ | 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 as 6 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 NaHCO₃).

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 samples were 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 rehabilitated 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≤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](Image)
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      | 05-11          | 10 mL/kg     |
| G2         | Furosemide-80 mg/kg       |                |              |
| G3         | Extracted (Seeds)- 40 mg/kg | 12-18         |              |
| G3         | Extracted (Seeds)- 80 mg/kg |              |              |

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

| 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                     |
|                                          |                     | 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                     |
|                                          |                     | 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                     |
|                                          |                     | IIIII                | 79.50                         | 79.50                         | 81.00                         | 81.50                         | 80.37        | 1.03              | 0.012                     |
| Standard Diuretic Drug (Furosemide) (G2) No. of Animals: 6 | 40 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                     |
|                                          |                     | 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                     |
| 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                     |
|                                          |                     | 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. - intraperitoneal, 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
| 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)                    | 0.5 mL/kg           | I                    | 1.5                             | 201                 | 199                | 7.07                    | 0.035                |
| Orally (G4)                             |                     | 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                 |                    |                         |                      |
|                                         |                     | IIIIIII              | 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                 |                    |                         |                      |
|                                         |                     | IIIIIII              | 3.0                             | 191                 |                    |                         |                      |

i.p. - intraperitoneal, 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)

| 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          |        |                   |                          |
|                                        |                     | III                   | 1.5                             | 73.6          |        |                   |                          |
|                                        |                     | IIII                  | 4.5                             | 70.4          |        |                   |                          |
|                                        |                     | IIIII                 | 3                               | 69.2          |        |                   |                          |
|                                        |                     | IIIIII                | 2                               | 74.2          |        |                   |                          |
|                                        | Std.80 mg/kg        | I                     | 1.5                             | 74.1          |        |                   |                          |
|                                        |                     | II                    | 1.5                             | 76.2          |        |                   |                          |
|                                        |                     | III                   | 1.7                             | 73.8          |        |                   |                          |
|                                        |                     | IIII                  | 3                               | 70.1          |        |                   |                          |
|                                        |                     | IIIII                 | 3                               | 68.2          |        |                   |                          |
|                                        |                     | IIIIII                | 3                               | 68.4          |        |                   |                          |

*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)

| Group treatment | Concentration mg/kg | Numbering of Animals | Urine Collection (4-6 hrs) in mL | Cl⁻ (mmol/Ltr) | Mean (mmol/Ltr) | 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 | 306.50 | 2.20 | 0.007 |
|                  |         | III | 1.5 | 309 | 306.50 | 2.20 | 0.007 |
|                  |         | IV | 3.5 | 302 | 305.25 | 3.30 | 0.010 |
| Standard Diuretic Drug- Furosemide (G5) | Std.40 mg/kg | I | 1.5 | 308 | 303.16 | 4.44 | 0.014 |
|                  |         | II | 1.5 | 298 | 302.50 | 2.20 | 0.007 |
|                  |         | III | 3.5 | 302 | 305.25 | 3.30 | 0.010 |
|                  | Std.80 mg/kg | I | 1.5 | 301 | 303.16 | 4.44 | 0.014 |
|                  |         | II | 1.5 | 298 | 302.50 | 2.20 | 0.007 |
|                  |         | III | 4.5 | 303 | 306.50 | 3.30 | 0.010 |
|                  |         | IV | 3 | 300 | 303.16 | 4.44 | 0.014 |
|                  |         | V | 2 | 309 | 306.50 | 3.30 | 0.010 |
| Compound Extract of Seeds(G6) | BIOTECH/UW/01-40 mg/kg | I | 1.5 | 306 | 301.66 | 5.13 | 0.017 |
|                  |         | II | 1.5 | 293 | 301.66 | 5.13 | 0.017 |
|                  |         | III | 1.7 | 308 | 301.66 | 5.13 | 0.017 |
|                  | BIOTECH/UW/02-80 mg/kg | I | 3 | 301 | 301.66 | 5.13 | 0.017 |
|                  |         | II | 3 | 307 | 301.66 | 5.13 | 0.017 |
|                  |         | III | 3 | 295 | 301.66 | 5.13 | 0.017 |

* i.p -intraperitonal, 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.
| Group                      | Treatment                | 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                              | 0.77                           |      |                   |
|                           |                          | III                 | 1.5                  | 0.77                              | 0.77                           |      |                   |
|                           |                          | IV                  | 3.5                  | 0.77                              | 0.77                           |      |                   |
| StandardDiureticDrug-      | Std.40 mg/kg             | I                   | 1.5                  | 0.72                              | 0.72                           | 0.034| 0.047            |
| Furosemide(G5)             |                          | II                  | 1.5                  | 0.72                              | 0.72                           |      |                   |
|                           |                          | III                 | 1.5                  | 0.68                              | 0.68                           |      |                   |
|                           |                          | IV                  | 1.5                  | 0.69                              | 0.69                           |      |                   |
|                           |                          | V                   | 4.5                  | 0.73                              | 0.73                           |      |                   |
|                           |                          | VI                  | 3                    | 0.75                              | 0.75                           |      |                   |
|                           |                          | VII                 | 2                    | 0.77                              | 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                              | 0.69                           |      |                   |
|                           |                          | III                 | 1.7                  | 0.69                              | 0.69                           |      |                   |
|                           |                          | IV                  | 3                    | 0.72                              | 0.72                           |      |                   |
|                           |                          | V                   | 3                    | 0.77                              | 0.77                           |      |                   |
|                           |                          | VI                  | 3                    | 0.62                              | 0.62                           |      |                   |

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.
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

| Variable Description | Formula | Calculation |
|----------------------|---------|-------------|
| Estimated \(24\) HUNa\(\) for Vehicle control (g) | \[
\text{Estimated } \_24 \text{ HUNa}^+ \text{ for Vehicle control (g)} = \frac{\text{Na}^+}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{7.21 (g)}{7.21 (g)} = 3.82 \text{ g at } 24 \text{ HUNa}^+
\] |
| Estimated \(24\) HUNa\(\) for Std. Diuretic Drug (Furosemide) (g) | \[
\text{Estimated } \_24 \text{ HUNa}^+ \text{ for Std. Diuretic Drug (Furosemide) (g)} = \frac{\text{Na}^+}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{7.23 (g)}{7.23 (g)} = 3.82 \text{ g at } 24 \text{ HUNa}^+
\] |
| Estimated \(24\) HUNa\(\) for Extracted Seeds sample (g) | \[
\text{Estimated } \_24 \text{ HUNa}^+ \text{ for Extracted Seeds sample (g)} = \frac{\text{K}^+}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{6.96 (g)}{6.96 (g)} = 3.92 \text{ g at } 24 \text{ HUNa}^+
\] |
| Estimated \(24\) HUK\(\) for Vehicle control (g) | \[
\text{Estimated } \_24 \text{ HUK}^+ \text{ for Vehicle control (g)} = \frac{\text{K}^+}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{7.23 (g)}{7.23 (g)} = 1.39 \text{ g at } 24 \text{ HUK}^+
\] |
| Estimated \(24\) HUK\(\) for Std. Diuretic Drug (Furosemide) (g) | \[
\text{Estimated } \_24 \text{ HUK}^+ \text{ for Std. Diuretic Drug (Furosemide) (g)} = \frac{\text{K}^+}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{6.96 (g)}{6.96 (g)} = 1.48 \text{ g at } 24 \text{ HUK}^+
\] |
| Estimated \(24\) HUCl\(\) for Vehicle control (g) | \[
\text{Estimated } \_24 \text{ HUCl}^− \text{ for Vehicle control (g)} = \frac{\text{Cl}^−}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{7.3 (g)}{7.3 (g)} = 5.72 \text{ g at } 24 \text{ HUCl}^−
\] |
| Estimated \(24\) HUCl\(\) for Std. Diuretic Drug (Furosemide) (g) | \[
\text{Estimated } \_24 \text{ HUCl}^− \text{ for Std. Diuretic Drug (Furosemide) (g)} = \frac{\text{Cl}^−}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{7.3 (g)}{7.3 (g)} = 5.80 \text{ g at } 24 \text{ HUCl}^−
\] |
| Estimated \(24\) HUCl\(\) for Extracted Seeds and Leaves sample (g) | \[
\text{Estimated } \_24 \text{ HUCl}^− \text{ for Extracted Seeds and Leaves sample (g)} = \frac{\text{Cl}^−}{\text{Creatinine SpotU (g)}} \times 24 \text{ HU Creatinine (g)}
\] | \[
\frac{6.96 (g)}{6.96 (g)} = 6.22 \text{ g at } 24 \text{ HUCl}^−
\] |
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 (Instituional Animal Ethics Committee) having Ethics Approval No. CPCSEA/IAEC/ Pharmacology-59/2017-18/141.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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