



## EVALUATION OF DIURETIC EFFECT OF ETHANOLIC SEED EXTRACTS OF *MACROTYLOMA UNIFLORUM* AND *CUCUMIS MELO* IN RATS

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### ABSTRACT

The present study was undertaken to investigate the diuretic effect of ethanolic seed extracts of *Macrotyloma uniflorum* and *Cucumis melo* in Albino rats. Ethanolic extracts of the seeds were administered to experimental rats orally at doses of 200mg/kg and 400mg/kg each p.o. Furosemide (5mg/kg) was used as a standard. The diuretic effects of the extracts were evaluated by measuring the Urine volume, Sodium, Potassium, Chloride and Bicarbonate contents. A significant Diuretic effect was observed from the experimental animals treated with extracts of *Macrotyloma uniflorum* and *Cucumis melo* individually compared to the control. Further, extract of *Cucumis melo* (400mg/kg) showed more diuretic effect than standard. This might be the first formal report on diuretic effects of both extracts individually. The results, to some extent explains the use of *Macrotyloma uniflorum* and *Cucumis melo* as a cure for renal diseases in Indian traditional medical practice.<sup>1</sup>

**KEYWORDS:** Diuretic activity, *Macrotyloma uniflorum*, *Cucumis melo*, Albino rats, natriuretic, Saluretic effect.



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## 1. INTRODUCTION

Diuretics are the agents which cause an increase in the urinary output. These drugs are generally used in the treatment of hypertension, pulmonary and systemic edema.<sup>2</sup> The importance of medicinal plants and traditional health systems in solving the health care problems is gaining increasing attention and because of this resurgence of interest, the research on plants of medicinal importance is rapidly increasing at the international level. Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but they were not properly investigated<sup>3</sup>. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on the plant-derived medicines, usually obtained from the traditional healers for basic health-care needs. In Indian indigenous system of medicine *Macrotyloma uniflorum* (Fabaceae) commonly known as horse-gram, is reported to be useful in the treatment of urinary stones. It is an herbaceous plant with annual branches, sub erect or twining, leaflets 2.5-5 cm, seeds are ovoid, 5-8 per pod, brown or black with scattered spots and widely distributed in Africa, Asia and Australia. It is also famous for its other medicinal uses because different parts of the plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones. Literature survey showed that Dolichin A and B, pyroglutaminylglutamine along with some flavonoids were isolated from this plant.<sup>4</sup> *Cucumis melo* (Cucurbitaceae), commonly known as cantaloupe or musk-melon is one of the most important cultivated cucurbits, which is native to India and Africa. It is spreading, annual, more or less hairy vine. Its sprawling branches produce broad green leaves, bright yellow flowers and tendrils. Seeds are whitish of buff, flat, smooth, 5-15 mm long. Seeds have a unique aroma composed of fatty acids, carotenoids, amino acids and terpenes, steroidal glycosides, flavonoids. Musk melon is

recommended for treatment of cardiovascular diseases, anti-tussive, stomachic, as a vermifuge, as analgesic and anti-inflammatory.<sup>5</sup>

## 2. MATERIALS AND METHODS

### 2.1. RAW MATERIALS and EXTRACTION:

The dry seeds of horse gram were obtained from Jeevan Ayurvedic and Medicinal Stores, Main road, Kakinada. The seeds were washed off from dust before extraction. The seeds of musk melon were collected from the fruit and dried under shade. The dried seeds of both the plants were individually macerated in ethanol for a period of 7 days and later subjected to hot-percolation for 8 hours. The extracts obtained were subjected to solvent evaporation for complete drying. The positive control was Furosemide B.P (Batch number F-0908076, manufactured in September 2010).

**2.2 EXPERIMENTAL ANIMALS:** Healthy male albino rats weighing 125-175gm were selected. The animals were housed in a temperature- and light-controlled room (25 °C; 14h/10h light/dark cycle) with free access to food and drinking water. Prior to the animal studies, the animals were acclimatized in the laboratory for a period of at least one week.

**2.3 DIURETIC ACTIVITY :** Diuretic activity was determined, following the Lipschitz method, but with a minor modification. The rats (48) were fasted for 18h and deprived of water prior to the experiment. A priming dose of 25 ml/kg of normal saline was given to all the rats. The rats were grouped into 8 groups (6 rats in each).

**Group I** was control group and treated with the vehicle, 0.5% acacia orally,

**Group II** was treated with Furosemide (5mg/kg p.o.) dissolved in the vehicle,

**Group III and IV** were treated with *Macrotyloma uniflorum* (200mg/kg and 400 mg/kg p.o. respectively),

**Group V and VI** were treated with *Cucumis melo* (200mg/kg and 400mg/kg p.o. respectively),

**Group VII** were treated with combination of both the extract at doses 500mg/kg (100 mg/kg of *Macrotyloma uniflorum* + 400 mg/kg of *Cucumis melo* p.o.)

**Group VIII** were treated with combination of both the extract at doses 500 mg/kg (400 mg/kg of *Macrotyloma uniflorum* + 100 mg/kg of *Cucumis melo* p.o.) respectively.

Immediately after the administration, the rats were placed in the metabolic cages, one rat per

cage. The metabolic cages were provided with a funnel for urine collection and a mesh to separate the faeces from the urine. The volume of urine collected was recorded after 5h and urine was subjected to analysis for sodium, potassium ions by flame photometry and chloride, bicarbonate by titrimetric analysis (against standardized 0.01M AgNO<sub>3</sub> and 0.1M H<sub>2</sub>SO<sub>4</sub> respectively) after 24h. The Saluretic, natriuretic and diuretic indices were also calculated. The results were analyzed statistically using student's t-test.



(a) Group IV



(b) Group VI



(c) Group VII

Images showing albino rats under the study of diuretic activity in the metabolic cages

**Table 1**

**Comparison of diuretic effect of *Macrotyloma uniflorum* to that of standard drug Furosemide**

Group	Volume of urine (ml) After 5h	Na <sup>+</sup> μmoles/kg	K <sup>+</sup> μmoles/kg	Cl <sup>-</sup> μmoles/kg	HCO <sub>3</sub> <sup>-</sup> μmoles/kg
Control	0.15±0.04	173.33±0.35	121.48±0.48	98.66±0.59	09.97±0.17
Furosemide (5mg/kg)	0.59±0.06 <sup>***</sup>	232.14±0.65 <sup>***</sup>	134.34±0.53 <sup>***</sup>	131.52±0.62 <sup>***</sup>	15.36±0.32 <sup>***</sup>
<i>Macrotyloma uniflorum</i> (200mg/kg)	0.27±0.03 <sup>*</sup>	191.2±0.26 <sup>***</sup>	126.56±0.66 <sup>***</sup>	146.01±0.54 <sup>***</sup>	19.83±0.50 <sup>***</sup>
<i>Macrotyloma uniflorum</i> (400mg/kg)	0.33±0.01 <sup>**</sup>	200.01±0.45 <sup>***</sup>	129.93±0.46 <sup>***</sup>	152.98±0.52 <sup>***</sup>	24.24±0.29 <sup>***</sup>

Values are expressed as Mean±SEM; n=6(number of animals in each group). \* p<0.05, \*\* p < 0.01, \*\*\* p < 0.001

**Table 2**  
**Comparison of diuretic effect of *Cucumis melo* to that of standard drug Furosemide**

Group	Volume of urine (ml)After 5h	Na <sup>+</sup> μmoles/kg	K <sup>+</sup> μmoles/kg	Cl <sup>-</sup> μmoles/kg	HCO <sub>3</sub> <sup>-</sup> μmoles/kg
Control	0.15±0.04	173.33±0.35	121.48±0.48	98.66±0.59	09.97±0.17 <sup>***</sup>
Furosemide (5mg/kg)	0.59±0.06 <sup>***</sup>	232.14±0.65 <sup>***</sup>	134.34±0.53 <sup>***</sup>	131.52±0.62 <sup>***</sup>	15.36±0.32 <sup>***</sup>
<i>Cucumis melo</i> (200mg/kg)	0.36±0.03 <sup>**</sup>	243.49±0.62 <sup>***</sup>	132.61±0.45 <sup>***</sup>	165.83±0.50 <sup>***</sup>	20.84±0.35 <sup>***</sup>
<i>Cucumis melo</i> (400mg/kg)	0.63±0.05 <sup>***</sup>	333.91±0.66 <sup>***</sup>	142.66±0.43 <sup>***</sup>	171.88±0.56 <sup>***</sup>	22.10±0.38 <sup>***</sup>

Values are expressed as mean±SEM; n=6(number of animals in each group). \*\*\* p < 0.001

**Table 3**  
**Comparison of diuretic effect of combination of *Macrotyloma uniflorum* and *Cucumis melo* (M.u+C.m) to that of standard drug Furosemide.**

Group	Volume of urine (ml)After 5h	Na + μmoles/kg	K <sup>+</sup> μmoles/kg	Cl <sup>-</sup> μmoles/kg	HCO <sub>3</sub> <sup>-</sup> μmoles/kg
Control	0.15±0.04	173.33±0.3	121.48±0.48	98.66±0.59	09.97±0.17
Furosemide (5mg/kg)	0.59±0.06 <sup>***</sup>	232.14±0.6 <sup>***</sup>	134.34±0.53 <sup>***</sup>	131.52±0.6 <sup>***</sup>	15.36±0.32 <sup>***</sup>
M.u+C.m (100mg/kg+400 mg/kg)	0.73±0.04 <sup>***</sup>	361.74±0.52 <sup>***</sup>	148.43±0.58 <sup>***</sup>	182.44±0.59 <sup>***</sup>	29.52±0.27 <sup>***</sup>
M.u+C.m (400mg/kg+100 mg/kg)	0.33±0.08 <sup>***</sup>	236.52±0.48 <sup>***</sup>	135.93±0.39 <sup>***</sup>	169.66±0.45 <sup>***</sup>	19.46±0.41 <sup>***</sup>

Values are expressed as mean±SEM; n=6(number of animals in each group). \*\*\* p < 0.001

**Table 4**  
**Comparison of Saluretic, natriuretic and diuretic indices of extracts to that of standard drug Furosemide**

GROUP	SALURETIC INDEX [Na <sup>+</sup> +Cl <sup>-</sup> ]	NATRIURETIC INDEX [Na <sup>+</sup> /K <sup>+</sup> ]	DIURETIC INDEX <sup>**</sup>
Control	—	1.42±0.11	
Furosemide (5mg/kg)	363.66±0.54	1.72±0.09	3.93±0.06
<i>Macrotyloma uniflorum</i> (200mg/kg)	337.21±0.48	1.51±0.07	1.80±0.04
<i>Macrotyloma uniflorum</i> (400mg/kg)	352.99±0.38	1.54±0.04	2.20±0.05
<i>Cucumis melo</i> (200mg/kg)	409.32±0.56	1.83±0.08	2.40±0.08
<i>Cucumis melo</i> (400mg/kg)	505.79±0.62	2.34±0.08	4.20±0.07
M.u + C.m (100mg/kg +400mg/kg)	406.18±0.58	1.74±0.05	2.20±0.08
M.u + C.m (400mg/kg +100mg/kg)	544.18±0.49	2.43±0.06	4.87±0.01

<sup>\*\*</sup>Diuretic Index= volume of urine in test group/volume of urine in control group

## RESULTS

*Macrotyloma uniflorum* showed significant ( $p < 0.05$ ) diuretic effect when compared to the control group (Table 1) whereas *Cucumis melo* at a dose 400mg/kg showed an extremely significant ( $p < 0.0001$ ) diuretic effect compared to the positive control Furosemide (Table 2). A combination of both the extracts showed synergistic effect only when a lower dose of horse gram (100mg/kg) and a higher dose of musk melon (400mg/kg) were administered (Table 3). Both the extracts showed greater Sal uretic, natriuretic and diuretic indices (Table 4) but when compared to the control, *Cucumis melo* at 400mg/kg and M.u+C.m (100mg/kg +400mg/kg) showed indices greater than that of standard.

## DISCUSSION

The present study revealed that apart from renal protection, the extracts of horse gram and musk melon possessed significant diuretic

activity. However, musk melon possessed more diuretic activity when compared to the standard (Furosemide). A combination of both the extracts was expected to show synergistic effect. However, combination dose of 400mg/kg of musk melon and 100 mg/kg of horse gram alone showed synergistic effect of diuresis and diuretic indices. A significant increase in the sodium ions in the urine supports that the extracts can be used to treat hypertension. The mechanism involved might be an increase in the Glomerular Filtration Rate and decreased tubular reabsorption.

## CONCLUSION

The Ethanolic seed extracts of both *Macrotyloma uniflorum* and *Cucumis melo* have produced a significant diuretic activity individually, and synergistic activity in their combination and thus can be used to produce diuresis during edema and also to treat hypertension.

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