NEPHROPROTECTIVE ACTIVITY POTENTIAL OF ETHANOLIC HERBAL EXTRACT OF MELOTHRIA MADERASPATNA ON UNILATERAL URETERAL OBSTRUCTION INDUCED RAT MODEL.

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ABSTRACT
Melothria maderapatna is also called as Mukia maderapatna belongs to genus family Cucurbitaceae. It is a well known drug of siddha system of medicine. Recent research explored its antihyperglycemic and hypolipidemic potential. In present study ethanolic extract of Melothria maderapatna was screened for Nephroprotective activity using Unilateral Ureteral Obstruction rat model. Parameters like change in body weight, cholesterol, creatinine, haemoglobin, levels of RBC, post operative survival rate, potassium, sodium, triglyceride, urea, and uric acid were estimated in experimental rats. The results showed that post operative survival rate of rats in the investigated novel method to be 100 percentages. Change in the body weight of ethanolic extract of Melothria maderapatna (EMME) treated groups-I and II was found to be 41gms and 36 gms respectively. The above values suggested that EMME treatment normalized the elevated body weight level in experimental rats. Furthermore EMME treatment normalized the decreased RBC level and also normalized the elevated hemoglobin, triglyceride, cholesterol, creatinine, urea, uric acid, Sodium and Potassium levels in experimental rats. The results indicate that Melothria maderapatna has strong nephroprotective activity. But, further scrutiny is essential for isolation and characterization of the active components, which can be employed to allay various human maladies.

KEY WORDS: Melothria maderapatna, EMME, UUO.

1. INTRODUCTION
Melothria maderaspatana (Syn. Mukia maderaspatana L.) belongs to the family Cucurbitaceae. The plant is a tendril climber/prostate herb. The plant was reported to have activities such as hepatoprotective[1], antirheumatic[2], diuretic, stomachic (a digestive tonic), gentle aperient, antispetic and antiflatulent, antiastmatic, anti-inflammatory, antidiabetic antibronchitis and is used for toothaches besides its use in vertigo and biliousness.[3-4] According to Ayurveda the plant is reported to possess expectorant, refrigerant, carminative, anodyne, sudorific and offer relief from cough, asthma, sensation, colic, flatulance, dyspepsia, neuralgia, constipation, ulcers, nostalgia, vertigo.[5-7] The fruits are reported for the treatment of piles, polyuria, T.B. and dysuria.[8-9] It is also used in siddha system for treatment of fever, cough, vomiting, fever, abdominal disorders,[10] nasobronchial diseases.[11] In naturopathy plant parts are used in wheezing, allergy, dry cough, sneezing, lethargy, asthma and Tuberculosis.[12-16]

Although Melothria maderapatna has been used appreciably in the folk medicine, its nephroprotective activity remains un-evaluated Hence, in the present study an effort has been made to explore its nephroprotective activity.

2. MATERIALS AND METHODS
2.1. PREPARATION OF MELOTHRIA MADERASPATNA EXTRACT
Authenticated whole Plant of Melothria maderapatna by Dr. Rajan and the herbarium sheet was deposited in Arya College of Pharmacy, MM/ACOP/2013 and was collected and its leaves were collected and shade dried for 15 days. Dried leaves were subjected to soxhalation using ethanol for 48hours. Extract was collected and concentrated on water bath. Ethanolic extract of Melothria maderapatna was stored in well closed container for further study.

2.2. CHEMICALS
Bupreonorphine, ethanol, hydralazine, Ketamine, tetracycline were obtained from Apollo pharmacy,
Hyderabad and ethanolic extract of Melothria Maderaspatna. (EMME).

2.3. PREPARATION OF STANDARD EMME DOSE
Accurately weighed quantity of EMME was suspended in 0.3% w/v carboxy methyl cellulose (CMC) suspension. 2 dose levels of 100 and 200 mg/kg body weight (b.wt.) of EMME were administered orally to animals at a dose volume of 0.1ml/100g of rat body weight. The volume administered was 0.1ml/100gm of rat body weight.

2.4. ACUTE TOXICITY OF MELOTHRIA MADERASPATNA EXTRACT
As per Organization for Economic Co-operation and Development (OECD) guidelines number: 423 Quantity of animals used: 10 wistar rats Dosage levels employed as per OECD 423: 2000 mg/kg & 5000 mg/kg Parameter assessed: Death of animal within one or two day The study was carried out according to OECD guideline 423 (OECD, 1992).

Adult male wistar rats were taken for Investigation. Out of them 6 healthy wistar rats of weight (120-160 g) were separated and kept for overnight fasting. Next day, body weight was measured for all rats. A dose of 2000 mg/kg in 0.3% w/v CMC, EMME was administered orally to animals. Animals were observed for mortality and morbidity at interval of 0, 1/2, 1, 2, 4, 6, 8, 12 and 24 hours. After 4h of the dosing Feed was given to the animals and the body weight was checked at 6h after dosing. Morbidity like convulsions, lethargy, grip strength, ptosis, tremors, pupil dilation were observed and noted. The animals were observed twice daily for 14 days and body weight was measured. The experiment was repeated once again on 6 mice (20-30g) as there was no observable clinical toxicity for the animals on the phase I study.

2.5. Unilateral Ureteral Obstruction rat model
The animals (adult male wistar rats 130-150g) were procured from the Arya College of Pharmacy, central animal house for investigations. They were kept in polypropylene cages and housed in a comfortable ambient adhering to a temperature of 20-25°C, humidity of 60% and other standardized conditions (12 hour light-dark cycle) and furnished with standard laboratory diet and water. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the committee for the purpose of control and supervision of experiments on animals (1376/ac/2010/CPCSEA).

2.5.1. GROUPING OF ANIMALS
The total numbers of animals used for nephroprotective study were: 30. These 30 animals were further divided into 5 groups of six animals each.
Group I: Control adult male wistar rats of 130-150g
Group II: Control ‘–ve’ using saline as a solvent.
Group III: UUO adult male wistar rats treated with furosemide
Group IV: UUO adult male wistar rats treatment 1(EMME 100 mg/kg)
Group V: UUO adult male wistar rats treatment 2 (EMME 200 mg/kg)

The standard and test drugs were administered daily for 30 and 60 consecutive days by oral route. The drugs were administered to all the groups twice daily. They were given the treatment at 9.00 am and 5.00 pm At the end of 60th day study, the rats were sacrificed with excess dose of anaesthetic ether. The blood was collected by sino orbital puncture and subjected to determination of hematological parameters like cholesterol[18] creatinine[19], haemoglobin[17], Potassium[20], RBC[17], Sodium[20], triglyceride[18], urea[20] uric acid[20].

2.5.2. UNILATERAL URETERAL OBSTRUCTION TECHNIQUE
2.5.2.1. Surgical Preparation: Healthy adult male wistar rat was anesthetized with 2.5-3.0% Halothane (Fluothane) and clipped over the abdominal region. Sterile eye lubricant was placed in both eyes, and the clipped skin area over the abdomen was surgically prepared. The animal was placed in dorsal recumbency on a surgery board fitted with a sterile board drape. Analgesic Buprenorphine was administered sub cutaneously with a duration of 8-12 hrs at a dose of 0.01-0.05 mg/Kg.

2.5.2.2. Surgical Description: The surgical site was draped with a sterile drape. To expose the ureter, a ventral, midline, longitudinal incision was made to the skin and abdominal wall from approximately the symphysis pubis to the midpoint of the abdomen. Padded retractors may be used to increase visibility. The left (or right) ureter was located and isolated using blunt dissection. Suture was permanently ligated in two locations (approximately 5-15mm apart) around the ureter. The ureter between the ligated sutures was cut away and removed.

2.5.2.3 Surgical Closing: The abdominal incisions are closed with approved closing materials (suture or stainless steel clips), and the surgery sites were treated with an approved disinfectant followed by an alcohol swabbing.

2.5.2.4 Pre-Operative Procedures: Animals were anesthetized, most commonly with an inhalant anaesthetic such as isoflurane and prepared (prepped) for surgery by shaving the appropriate regions, e.g., the abdomen and dorsal cervical (nape) regions were the most commonly shaved areas. After these areas were clipped and vacuumed, sterile ophthalmic ointment was placed in both eyes, and the surgical site was disinfected by the procedure described below:

The surgical sites were scrubbed with an approved surgical scrub, usually povidone iodine, in a circular
pattern starting from the incision area and working out to the periphery of the clipped area. This was followed by an alcohol (isopropyl) rinse by swabbing the surgical area in the same manner as the scrub was done, starting from the surgical site and swabbing out in a circular pattern to the periphery. After the initial prep, the animal was placed on a surgical board that was draped with a sterile drape and repeated disinfection was done by treating the surgical site with an approved disinfectant followed by an alcohol swabbing. The animal was draped with a sterile surgical drape. Appropriate facilities and equipment were available for the post-operative care of animals.

2.5.2.5 Intra and Post-Operative Procedures

The surgical procedures were performed in accordance with the protocol for that procedure. Animals were monitored during surgery for the surgical plane of anaesthesia by checking the reflexes noted above and respiration rate. The heart and mucous membranes (color, refill time) were also monitored. Fluids, antibiotics, analgesics, vascular catheter heparin locks, and other treatments were administered as appropriate for the specific surgical procedure.

Internal sutures for ligation of vessels, ducts or other structures uses 3-0 to 5-0 approved suture materials. If the surgery required opening a major body cavity, the body cavity (e.g., abdominal laparotomy), layer and subcutaneous tissues may be closed separately with 3-0 approved suture material. The skin was closed with approved wound clips or approved suture material. The surgical site was cleansed, and the animal was placed in the heated portion of a paper towel-lined plastic rodent cage, which was partially heated to allow the animal to move to the temperature that was most comfortable.

2.5.3. PHARMACOLOGICAL EVALUATION ON 30th and 60th DAY:

Short term study was carried out for 30 days whereas long term studies were carried out for 60 days. At the end of short term study (30 days) and long term studies (60 days), blood was collected by Sino orbital puncture under light ether anaesthesia; kept in heparinised tubes and subjected to determination of haematological and biochemical parameters like blood urea, creatinine, haemoglobin (Hb), RBC, total lipids, triglycerides and uric acid.

Moreover, the Biochemical parameters were also determined like RBC, haemoglobin, triglyceride, cholesterol, creatinine, urea, uric acid, Sodium and Potassium.

2.5.4. ASSESSMENT OF PARAMETERS

Parameters were estimated at the end of 30 and 60 days interval. Firstly, physical parameter like Body weight was estimated. The difference in the body weight was measured between nephrectomised and non-nephrectomised rats. These values are statistically expressed in mean ± SEM.

ORGAN COLLECTION AND HISTOPATHOLOGICAL STUDIES

At the end of 30th and 60th day study, the animals were sacrificed with excess dose of anaesthetic ether. Animals were then dissected and organs were isolated for their histopathological study. Kidneys, liver, spleen, were collected and weighed. The organs were stored in 10% buffered neutral formalin. Tissues from these organs were scraped and embedded in liquid paraffin; sections of 5-6 μm were cut and stained with haematoxylin and eosin for histopathological findings.

STATISTICAL ANALYSIS

The collected data were subjected to appropriate statistical test like one-way ANOVA (Analysis of variance) followed by an appropriate post hoc test like dunnett’s test. P values of less than 0.01 were considered as significant. The analysis was carried out using Graph pad prism software of version 4.

3. RESULTS AND DISCUSSION

Study into nephroprotective property of leaf extract of Melothria maderapatna in Unilateral Ureteral Obstruction rat model showed substantial (100%) post operative survival rate of rats in the test drug treated groups. The elevated body weights, triglycerides, cholesterol, creatinine, urea and uric acid levels in nephrectomized experimental animals were found to be lowered or normalized on treatment with the investigational plant leaf extract, efficacy of which corresponded positively to increasing order of dose. Further the extract was found to increase or normalize the depleted haemoglobin level and RBC count in the nephrectomized animals, effectiveness of which correlated strongly with increasing dose. But further rigorous study needs to be carried out The post operative survival rate of the rats in test drug group was found to be 100%.(Table 1)

The change in the body weight of EMME treated groups-I and II was found to be 41gms and 36 gms respectively. The above values suggested that EMME treatment normalizes the elevated body weight level in experimental rats. Furthermore EMME treatment decreased RBC level of nephrectomized animals normalizes the elevated or did not reduce haemoglobin level, normalizes the elevated triglyceride level, reduced the elevated cholesterol level in nephrectomized rats, normalizes the elevated creatinine level, reduced the elevated urea level, elevated uric acid level in experimental rats.
Model-I Unilateral Ureteral Obstruction Rat Model Short term study (30 Days treatment)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urea</th>
<th>Uric Acid</th>
<th>Sodium</th>
<th>Potassium</th>
<th>BUN</th>
<th>GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-I</td>
<td>22.7±0.688**</td>
<td>3.71±0.014***</td>
<td>135±0.833***</td>
<td>5.14±0.009***</td>
<td>9.09±0.038***</td>
<td>0.402±0.017***</td>
</tr>
<tr>
<td>Control-II (UOO Control)</td>
<td>65.6±0.705</td>
<td>6.78±0.016</td>
<td>153±1.20</td>
<td>4.07±0.017</td>
<td>27±0.493</td>
<td>1.30±0.010</td>
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<tr>
<td>Positive Control (Furosemide P.O)</td>
<td>45.3±0.709***</td>
<td>4.03±0.040***</td>
<td>134±1.80***</td>
<td>5.23±0.029***</td>
<td>12.3±0.1033***</td>
<td>1.36±0.01*</td>
</tr>
<tr>
<td>Treatment-I (EMME-100mg/kg)</td>
<td>25±0.349***</td>
<td>3.92±0.058***</td>
<td>140±1.91***</td>
<td>6.35±0.063***</td>
<td>14.4±0.090***</td>
<td>1.19±0.010***</td>
</tr>
<tr>
<td>Treatment-II (EMME -200mg/Kg)</td>
<td>23.2±0.405***</td>
<td>4.17±0.021***</td>
<td>141±1.38***</td>
<td>5.3±0.108***</td>
<td>11.2±0.028***</td>
<td>0.733±0.011***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group.

**P<0.001 when compared to control-II.

*P<0.01 & P<0.05 when compared to control-II.

#P≥0.05 when compared to control-II.

Unilateral Ureteral Obstruction Rat Model
Long term study (60 Days treatment)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urea</th>
<th>Uric Acid</th>
<th>Sodium</th>
<th>Potassium</th>
<th>BUN</th>
<th>GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-I</td>
<td>14.33±0.530***</td>
<td>3.81±0.018***</td>
<td>135±0.833***</td>
<td>5.14±0.009***</td>
<td>12.4±0.088***</td>
<td>0.482±0.012***</td>
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<tr>
<td>Control-II (UOO Control)</td>
<td>38.35±0.134</td>
<td>6.90±0.019</td>
<td>153±1.20</td>
<td>4.07±0.017</td>
<td>31.9±0.339</td>
<td>1.36±0.012</td>
</tr>
<tr>
<td>Positive Control (Furosemide P.O)</td>
<td>22.35±0.064***</td>
<td>7.24±0.038***</td>
<td>134±1.80***</td>
<td>5.23±0.029***</td>
<td>16.3±0.029***</td>
<td>1.43±0.014***</td>
</tr>
<tr>
<td>Treatment-I (EMME-100mg/kg)</td>
<td>16.30±0.35***</td>
<td>6.72±0.006***</td>
<td>140±1.91***</td>
<td>6.35±0.063***</td>
<td>22.2±0.021***</td>
<td>1.24±0.025***</td>
</tr>
<tr>
<td>Treatment-II (EMME -200mg/Kg)</td>
<td>15.05±0.46***</td>
<td>5.96±0.015***</td>
<td>141±1.38***</td>
<td>5.3±0.108***</td>
<td>14.6±0.229***</td>
<td>1.01±0.070***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group.

**P<0.001 when compared to control-II.

*P<0.01 & P<0.05 when compared to control-II.

#P≥0.05 when compared to control-II.

Figure 1: Unilateral Ureteral Obstruction technique of Albino Rat.

Figure 2: Ureter Ligation of animal

Figure 3 & 4: Whole plant of Melothria Maderaspatna
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