DIURETIC ACTIVITY OF METHANOLIC AND ETHANOLIC EXTRACTS OF CENTELLA ASIATICA LEAVES IN RATS

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ABSTRACT
Diuretics increase the rate of urine flow and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis. Traditionally, Centella asiatica has been used as antileprotic, anxiolytic, nootropic, antioxidant, antibacterial, antiviral and antiinflammatory agent. The present study was undertaken to investigate the diuretic effect of methanolic and ethanolic extracts of Centella asiatica in wistar rats. The extract was given orally at a dose of 500mg/kg p.o.). Relatively the ethanolic extract showed potent diuretic activity than the methanolic extract.

KEY WORDS: Centella asiatica, Ethanolic extract, Methanolic extract, Diuretic, Urine volume, Furosemide.

INTRODUCTION
Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world’s population still rely on plant-derived medicines, usually obtained from traditional healers for basic health-care needs. The study of plant species with diuretic effects is still a fruitful research in search of new diuretics. Some naturally used diuretic plants are Allium sativum, Allium sepa, Acacia nilotica, Aloe barbadensis, Cinnamonum verum, etc. Diuresis is the increase in the production of urine by the kidneys which typically results in a corresponding increase in urine expelled by the body. Drugs that induce diuresis are known as diuretics. Diuretics relieve pulmonary congestion and peripheral edema. This decreases cardiac work load, oxygen demand and plasma volume thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients in situations of fluid over load like acute and chronic renal failure, hypercalciuria, cirrhosis of liver and also as an antihypertensive agent. A number of diuretics like mannitol, thiazides, furosemide and ethacrynic acid are used in practice. Centella asiatica is a small herbaceous annual plant. The stems are slender, creeping stolons, green to reddish green in color, interconnecting one plant to another. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are born on pericladal petioles. The rootstock consists of rhizomes, growing vertically down. They are creamish in color and covered with root hairs. The flowers are pinkish to red in color, born in small, rounded bunches (umbels) near the surface of the soil. Despite the popular use of this species as a medicinal plant, no previous pharmacological or clinical study was carried out to test the diuretic activity of this plant. Since the diuretic effect of Centella asiatica has never been experimentally confirmed, the main aim of the present investigation was to evaluate the claimed diuretic activity in rats.

MATERIALS AND METHODS

Plant collection
The fresh leaves of Centella asiatica belonging to the family Mackinlayaceae were collected in month of January 2011 from the local areas of Anantapur district, Andhra Pradesh, India. They were identified and authenticated. The voucher specimen riper-14/11 was deposited at the college for future reference.

PREPARATION OF EXTRACTS

Preparation of methanolic extract of Centella asiatica leaves (MECA)
The leaves were dried under shade, powdered by a mechanical grinder, sieved through 40mesh. The methanolic extract was obtained by macerating the leaf powder with methanol for 3 days. The extract was filtered and concentrated under reduced pressure.

Preparation of ethanolic extract of Centella asiatica leaves (EECA)
The leaf powder was extracted with ethanol using soxhlet apparatus. The extraction was carried out until the extractive became colorless. The extract was then concentrated and dried under reduced pressure. Solvent free semisolid mass was obtained.

PRELIMINARY PHYTOCHEMICAL ANALYSIS
The preliminary phytochemical analysis was carried out to find out the phytoconstituents present in the crude extracts.

DRUGS AND CHEMICALS
Methanol, Ethanol (Merck chemicals)

ANIMALS
Wistar albino rats weighing between 150-200gm each were used for this experiment. They were procured from bioneeds, Bangalore. The animals were kept under standard conditions in the animal house. They were housed in polypropylene cages and maintained at 27±2°C. The animals were given standard diet.

ETHICAL APPROVAL
The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/015/2011) of Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India, approved the animal experimental protocol. All experiments were conducted according to the norms of committee for the purpose of control and supervision on experiments on animals (CPCSEA).

ACUTE TOXICITY STUDY
Acute toxicity of Centella asiatica was determined by acute toxic class method of OECD guidelines.

EVALUATION OF DIURETIC ACTIVITY
The method of Lipschitz et al10 was employed for the assessment of diuretic activity. According to this method, the animals should be deprived of food and water for 18 hours prior to the experiment. The animals were randomly divided into four groups of six animals each as follows:
Group 1 (Normal) - received distilled water 25ml/kg p.o.
Group 2 (Standard) - received furosemide 20mg/kg p.o.
Group 3 (Experimental) - received methanolic extract 500mg/kg p.o.
Group 4 (Experimental) - received ethanolic extract 500mg/kg p.o.
Group 3 (Test) - received methanolic extract of Centella asiatica 500 mg/kg p.o.

Group 4 (Test) - received ethanolic extract of Centella asiatica 500mg/kg p.o.

Immediately after administration, animals were placed in metabolic cages specially designed to separate urine and faecal matter. During the period of study no food, water was made available to the animals. The total volume of urine was collected and measured from control, standard and extract treated groups up to 5 hours of administration. The parameters monitored for individual rat were total urine volume and urine concentrations of Na⁺, K⁺ and Cl⁻.

Concentration of Na⁺ and K⁺ were determined using flame photometer¹¹ while Cl⁻ concentration was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as an indicator.¹² Appearance of brick red precipitate was taken as the end point. The mean urine volumes were determined and diuretic potency was assessed by comparison of urine excretion due to extracts with respect to the standard drug furosemide¹³.

Statistical analysis
The data was expressed as mean ± SEM and statistically analyzed using one way ANOVA followed by Bonferroni’s Multiple comparison test.

RESULTS
Physical properties of MECA And EECA
Maceration with methanol yielded a semisolid greenish black residue. The percentage yield of MECA was found to be 17.2% w/w (Table 1).

Extraction with ethanol using soxhlet apparatus yielded a semisolid greenish black residue. The percentage yield of EECA was found to be 8.3% w/w (Table 1).

Phytochemical analysis of MECA And EECA
Both the methanolic and ethanolic extracts of Centella asiatica revealed the presence of alkaloids, carbohydrates, phytosterols, tannins and lignin (Table 2).

Acute toxicity study
In acute oral toxicity study, mortality was not observed up to 5000mg/kg body weight.

Effect of MECA and EECA on urine output in rats
Urine volume in rats of normal group was 2.40ml (Graph 1).

Urine volume in rats treated with methanolic extract of Centella asiatica was 3.23ml (Graph 1).

Urine volume in rats treated with ethanolic extract of Centella asiatica was 4.56ml (Graph 1).

Effect of MECA And EECA on electrolyte excretion in rats
The concentrations of Na⁺, K⁺ and Cl⁻ in rats of normal group were 92.66 mEq/L, 66.50 mEq/L, 103.43 mEq/L respectively (Table 3).

The concentrations of Na⁺, K⁺ and Cl⁻ in rats treated with MECA were 128.46 mEq/L, 75.30 mEq/L, 137.63 mEq/L respectively. (Table 3).

The concentrations of Na⁺, K⁺ and Cl⁻ in rats treated with EECA were 153.23 mEq/L, 87.63 mEq/L, 178.16 mEq/L respectively (Table 3).

DISCUSSION
Diuretics are medicines that help to reduce the amount of water in the body. They are used to treat the buildup of excess fluid in the body that occurs with some medical conditions. An attempt has been made to extrapolate the diuretic action of plant extract from rats to man using the activity of furosemide. The phytochemical tests revealed the presence of alkaloids, carbohydrates, phytosterols, tannins and lignin. These natural products might be acting synergistically or individually to produce diuresis. MECA and EECA were administered to rats at a dose of 500mg/kg.

Diuretics have two separate connotations; increase urinary par se and net loss of solute and water. These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence, promote the formation of urine. The results clearly show that MECA and EECA at a dose of 500 mg/kg produced significant increase in urine output and excretion of urinary sodium, potassium and chloride ions with respect to control and standard drug treated groups. Relatively ethanolic extract produced more significant diuresis than the methanolic extract. The data demonstrates that the extracts have diuretic effect. This indicates the use of MECA and EECA as a diuretic agent based on a sound mechanistic background.

CONCLUSION
From the above results, it was concluded that Centella asiatica showed significant diuretic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as diuretic.

ACKNOWLEDGMENT
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REFERENCES

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<tr>
<th>Plant part</th>
<th>Type of Extract</th>
<th>Yield (% w/w)</th>
<th>Texture</th>
<th>Colour</th>
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<tr>
<td>Leaves of Centella asiatica</td>
<td>Methanolic extract</td>
<td>17.2</td>
<td>Semisolid</td>
<td>Greenish black</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>8.3</td>
<td>Semisolid</td>
<td>Greenish black</td>
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<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Centella asiatica</th>
<th>Methanolic extract</th>
<th>Ethanol extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Carbohydrates</td>
<td>+</td>
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<td>Phytosterols</td>
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<td>Tannins</td>
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<td>Lignin</td>
<td>+</td>
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### TABLE 3: EFFECT OF MECA AND EECA ON ELECTROLYTE EXCRETION IN RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route</th>
<th>Electrolyte levels</th>
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<tr>
<td></td>
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<td>Sodium (m.Eq/L)</td>
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<tr>
<td>Normal</td>
<td>25 ml/kg p.o</td>
<td>92.67±3.524</td>
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<td>Furosemide</td>
<td>20 mg/kg p.o</td>
<td>168.30±2.700***</td>
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<td>Methanolic extract</td>
<td>500 mg/kg p.o</td>
<td>128.50±3.437**</td>
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<td>Ethanollic extract</td>
<td>500 mg/kg p.o</td>
<td>153.20±2.431***</td>
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<td>Potassium (m.Eq/L)</td>
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<td>66.50±2.042</td>
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<td>91.47±1.097***</td>
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<td>75.30±1.845*</td>
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<td>87.63±0.864**</td>
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<td>103.40±2.961</td>
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<td>137.60±2.872***</td>
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<td>178.20±0.887***</td>
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*\( p<0.1 \), **\( p<0.001 \), ***\( p<0.001 \) when compared to normal

Each value represents the mean ± SEM of six rats

![Fig 1: Effect of MECA and EECA on urine output in rats](image)

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