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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 under taken 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. Total urine volume and the concentration of sodium, potassium and chloride ions in the urine were taken into account during the experimental work. The extracts showed a significant diuretic effect with increase in electrolyte concentration in urine when compared with standard drug furosemide (20mg/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.[1] 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 barbidensis, Cinnamomum 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.[2] Drugs that induce diuresis are known as diuretics.[3] 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[4] 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 ethacrinic acid are used in practice.[5]

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 pericladial 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[6,7] was carried out to find out the phytoconsituents present in the crude extracts.

Drugs and chemicals

Methanol, Ethanol (Merck specialities pvt. Ltd.), Furosemide (Aventis pharma Ltd).

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 ± 2^0C. 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.[8]

Evaluation of diuretic activity

The method of Lipschitz et al[9,10] 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 (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 5hours 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 photometer[11] while Cl^- concentration was estimated titrimetrically using 0.02N $AgNO_3$ 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 furosemide[13].

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 92.66mEq/L, 66.50mEq/L, 103.43mEq/L respectively (Table 3).
The concentrations of Na^+, K^+ and Cl^- in rats treated with MECA were 128.46mEq/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 153.23mEq/L, 87.63mEq/L, 178.16mEq/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.

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TABLE 1: %YIELD, TEXTURE AND COLOUR OF MECA AND EECA

Plant part	Type of Extract	Yield (% w/w)	Texture	Colour
Leaves of *Centella asiatica*	Methanolic extract	17.2	Semisolid	Greenish black
	Ethanolic extract	8.3	Semisolid	Greenish black

TABLE 2: PHYTOCHEMICAL ANALYSIS OF MECA AND EECA

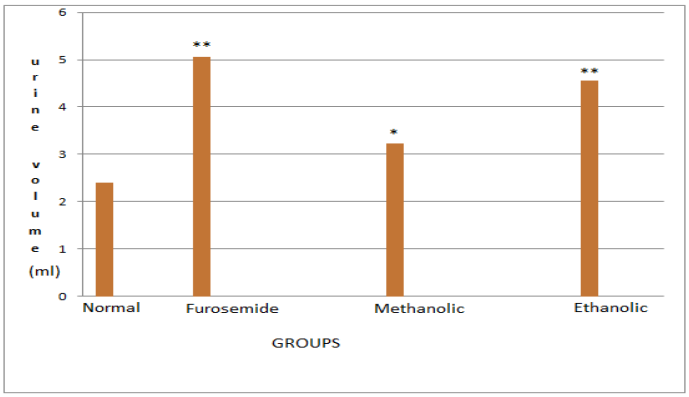
Chemical constituents	*Centella asiatica*	
	Methanolic extract	Ethanolic extract
Alkaloids	+	+
Carbohydrates	+	+
Phytosterols	+	+
Tannins	+	+
Lignin	+	+

+ = Present

TABLE 3: EFFECT OF MECA AND EECA ON ELECTROLYTE EXCRETION IN RATS

Treatment	Dose & Route	Electrolyte levels		
		Sodium (m.Eq/L)	Potassium (m.Eq/L)	Chloride (m.Eq/L)
Normal	25 ml/kg p.o	92.67±3.524	66.50±2.042	103.40±2.961
Furosemide	20 mg/kg p.o	168.30±2.700***	91.47±1.097***	187.40±2.500***
Methanolic extract	500 mg/kg p.o	128.50±3.437**	75.30±1.845*	137.60±2.872***
Ethanolic extract	500 mg/kg p.o	153.20±2.431***	87.63±0.864***	178.20±0.887***

*p<0.1, **p<0.001, ***p<0.001 when compared to normal



Each value represents the mean ± SEM of six rats
*p<0.1, **p<0.001 when compared to normal

Fig 1: Effect of MECA and EECA on urine output in rats

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