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RESEARCH ARTICLE

# Diuretic Activity of *Celosia Argentea* Linn

**Kotresh Y*, Ramana MV, Madhulatha B**

*GBN Institute of Pharmacy, Hyderabad, Telangana, India.*

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## ABSTRACT

*Celosia argentea* Linn. is a annual shrub, commonly known as silvercock's comb which belongs to family Amarantheceae was evaluated for its diuretic activity. The plant was extracted with 70% ethanol to obtain hydro-alcoholic extract (70:30) and concentrated to obtain residue. The toxicity study reveals that the animals show no mortality rate at highest dose level i.e; 8 gm /kg body weight which proves that *C.argenta* plant is non toxic. The hydro-alcoholic extract (HECA) at a dose of 400mg/kg b.w, and 600mg/kg b.w by oral route were tested for their diuretic property in *swiss albino* mice of either sex weighing between 18gms-30gms. A significant (*p<0.001) diuretic activity had been observed in the HECA (600mg/kg b.w) followed by HECA (400mg/kg b.w) when compared to control group. Phytochemical screening reveals the presence of alkaloids, steroids, flavonoids, tannins, triterpenoids, carbohydrates and saponins in the hydro-alcoholic extract of *Celosia argentea* Linn.

## KEYWORDS

Celosia Argentea Linn, Hydro-Alcoholic Extract, Diuretic, *Swiss Albino* Mice

## INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis[1]. Abnormalities in fluid volume and electrolyte compositions are common and important clinical problems. Drugs that block the transport functions of the renal tubules are valuable clinical tools in the treatment of these disorders. Although various agents that increase urine flow have been described since antiquity, it was not until 1957 that a practical and powerful diuretic agent (chlorothiazide) became available for widespread use. Technically, the term "diuresis" signifies an increase in urine volume, while "natriuresis" denotes an increase in renal sodium excretion[2].

***Address for Correspondence:**
**Y. Kotresh**
Assistant Professor,
GBN Institute of Pharmacy,
Edulabad (V), Ghatkesar (M), R.R (D),
Hyderabad, Telangana-501301, India.
**E-Mail Id**: kotresh907pharma@gmail.com

*Calotropis argentea* commonly known as silver cock's comb, plumed cock's comb belongs to family Amaranthaceae is a plant of topical origin and is known for its very bright colours[3]. Various chemical constituents have been reported from different parts of plant. Phenolic-glycoside,4-O-β-D-apifuranosyl-(1→2)-β-D-glucopyranosyl-2-hydroxy 6-methoxyacetophenone (2) and 11 known compounds were isolated from the MeOH extract of the plant *C.*argentea[4]. The acidic polysaccharide celosian isolated from the seeds was found to be a potent antihepatotoxic agent for chemical and immunological liver injury models in animals. The antimitotic bicyclic peptides celogentins A–C and moroidin have been isolated from the seeds, and an antiviral protein has been isolated from the leaves. *C.argentea* contains red betacyanins and yellow betaxanthins which are being tested as food colorants. Several glycopyranosyls have been isolated from celosia, including citrusin C which has skin depigmentation properties. The seed

of *Celosia argentea* contains a fatty oil known as 'celosia oil' in India. They were stigmasterol, β-sitosterol, Celosin A, Celosin B, β-daucosterol, n-hexacosnic acid, palmitic acid, stearic acid[5].

According to ayurveda *C.argentea* is used in traditional medicine for sores, ulcers, skin eruptions; as diuretic and astringent. It is used internally for haematological and gynocological disorders and externally to treat inflammation. The whole plant is used to treat dysentery and dysurea and as disinfectant. It is used as poultices externally for broken bones[6].

The present study deals with the preliminary phytochemical studies and pharmacological evaluation of whole plant of *Celosia argentea* Linn. for their diuretic activity using *swiss albino* mice.

## MATERIAL AND METHODS

The plant of *C.argentea* was collected from in and around andhra pradesh region. The plant was identified and authenticated by Dr. Satyanarayana, retd. Professor, botanist, hyderabad, Andhra pradesh. The collected plant material is washed thoroughly with water, dried under shade at room temperature and powdered using hand mill to make a coarse powder and they are stored in well-closed light resistant container until further use.

### Preparation of the Extracts

#### *Cold Maceration*

240gms of powdered plant material is subjected for cold maceration for 5 days with 1.5 lts of alcohol (ethanol 70%). The solvent was then separated by filtration and the marc was air dried. The yield of macerated extract is found to be 8%.

#### *Extraction of Plant Materials*

The air dried marc was subjected for extraction with alcohol using soxhlet apparatus at $50^0$C. Materials were extracted until liquid in the side arm of soxhlet apparatus became colourless. Mecilla were collected and combined with the macerates and subjected for solvent recovery using rotator evaporator. Then the extract was dried under reduced pressure. The dried extract was then stored at low temperature ($4^0$C) for further use. The yield of hydro-alcoholic extract was found to be 10%. Alcohol is the moderately polar solvent utilized to extract various groups of compounds present in the crude drug. In this process, alcohol (70%) was used to obtain hydro-alcoholic extract of *Celosia argentea* L. (HECA).

### Preliminary Phytochemical Investigation[7-8]

The preliminary phytochemical investigation of the HECA revealed the presence of alkaloids, saponin glycosides, steroids, flavonoids, triterpenoids and carbohydrate.

### Pharmacological Studies

#### *Experimental Animals*

*Swiss albino* mice of either sex weighing between 18-30gms were used in experimental work. Institutional Animal Ethical Committee approved the experimental protocol. Animals were maintained under standard conditions husbandry, room temperature 24+2$^o$c, relative humidity of 45-55%, 12 hours dark light cycle, in an animal house approved by the committee for the purpose of control and supervision of experiments on animals (Reg.no: 1450/PO/a/11/CPCSEA). Animals were obtained from the central animal house, G.B.N. Institute of pharmacy, edulabad, hyderabad. The animals had free access to standard diet and water and housed in poly propylene cages. All the animals were kept for fasting 24 hours prior to the experiment.

#### *Toxicity Studies[9]*

The acute toxicity study was performed according to the OPPTS (Health Effect Test Guideline 2004, office of prevention, pesticide and toxic substance) by up and down procedure using *Swiss albino* mice. It is observed that there is no change in body weight, food and water consumption by animals from all dose groups (2g /kg body weight to 8gm/kg body weight). There is no mortality rate at highest dose level i.e; 8gm /kg body weight which proves that *C.argenta* plant is non toxic.

#### *Evaluation of Diuretic Activity[10-12]*

Diuretic activity was evolved in-vivo using hydro-alcoholic extract of *C.argentea*. The animals were divided into four groups. Group-I

was received with saline solution and urea 1g/kg b.w; i.e., Normal control. Group-II was received hydrochlorothiazide at a dose of 25mg/kg b.w; and it was considered as standard group. Group-III & Group-IV received HECA at doses of 400mg/kg b.w and 600mg/kg b.w respectively. Twenty–four hours prior to the experiment, the test animals were placed into metabolic cages with total withdrawal of food and water. After oral administration of HECA, the urinary output of each group was recorded at different time intervals from the graduated urine chamber at metabolic cage. Urine samples were analyzed for $Na^+$ and $K^+$ concentration by flame photometric method while $Cl^-$ concentration was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator.

**Experimental Design**

Animals were deprived of food and water 24h before the experiment. They were hydrated with 5ml/kg of water prior to drug/extract administration. Immediately after dosing, animals were placed in metabolic cages (3 in one cage), specially designed to separate urine and faeces. The urine was collected in measuring cylinder up to 24h after dosing. During this period, animals were deprived of food and water. The parameters measured were total urine volume, urine concentration of $Na^+$, $K^+$ and $Cl^-$. Concentration of $Na^+$ and $K^+$ were determined using flame photometer while $Cl^-$ concentration was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator. Appearance of brick red precipitate was taken as the end point.

**Statistical Analysis**

Results were subjected to statistical analysis and expressed as Mean ± S.E. ANNOVA was used to establish significance of difference between the mean of tests and control ($p<0.001$) were considered to be significant.

## RESULTS

The HECA were found to have alkoloids, saponin glycosides, flavonoids, triterpenoids and carbohydrates. (Table 1)

The HECA at a dose of 400mg/kg and 600mg/kg b.w were selected for the evaluation of diuretic activity. Hydrochlorothiazide treated mice showed a significant increase in volume of urine and excretion of sodium, potassium and chloride ($p<0.001$) as compared to control, while HECA were found to increase urine volume significantly ($p<0.001$) in a dose dependent manner. Higher electrolyte excretion ($p<0.001$) was observed in higher dose (600mg/kg b.w) of HECA followed by lower dose (400mg/kg b.w). (Table 2), (Figure 2), (Figure 3). Also HECA showed significant ($p<0.001$) increase in chloride ion excretion. (Table 2), (Figure 4)

Table 1: Preliminary Phytochemical constituents present in HECA

| S. No | Chemical constituent | Test | HECA |
|---|---|---|---|
| 1. | Alkaloids | Mayer's test | + |
| | | Wagner's test | + |
| | | Dragendorff's test | + |
| | | Hager's test | + |
| 2. | Glycosides | Chrysorbin test | + |
| | | Legal test | + |
| 3. | Carbohydra-tes | Molisch test | + |
| | | Fehling test | + |
| | | Benedict's test | + |
| | | Barfoed's test | + |
| 4. | Proteins | Biurett's test | _ |
| | | Xanthoproteic test | _ |
| 5. | Steroids | Libermann buchard test | + |
| | | Salkowski test | + |

| | | | |
|---|---|---|---|
| | | Sulphur test | + |
| | | Acetic anhydride Plus $H_2SO_4$ test | – |
| 6. | Tannins | Ferric chloride test | - |
| 7. | Triterpenes | Salkowski test | - |
| | | Libermann storch morawski test | + |
| | | Hirschorn test | + |
| | | Tschujawes test | + |
| 8. | Flavanoids | Ferric chloride test | + |
| | | Shinoda test | + |
| | | 10% NaOH | + |
| | | 10% Lead acetate | + |
| | | Mineral acid test | + |
| | | Zinc dust test | + |
| 9. | Saponins | Liberman Buchard Sterol Reaction | + |
| | | Salkowski Reaction | + |

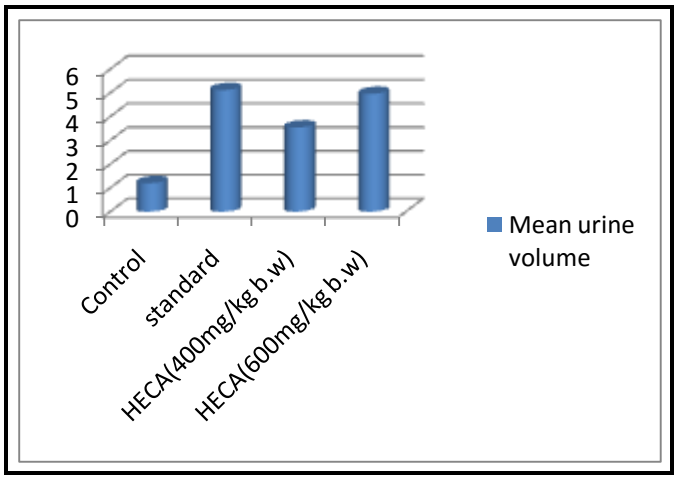


Figure 1: Histograph of effect of *Celosia argentea* L. on mean urine volume

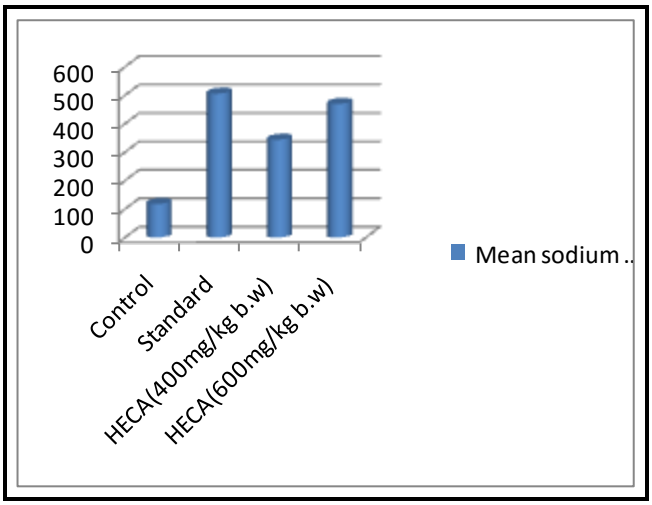


Figure 2: Histograph of effect of Celosia argentea L on mean sodium ion concentration

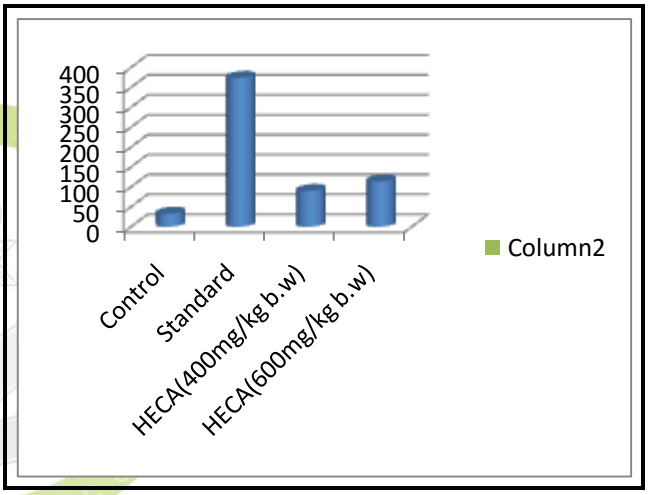


Figure 3: Histograph of effect of Celosia argentea L on mean potassium ion concentration

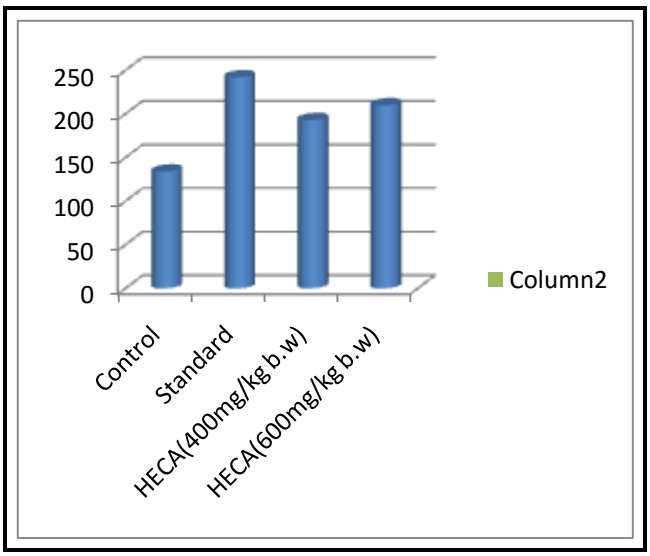


Figure 4: Histograph of effect of *Celosia argentea* L on mean chloride ion concentration

Table 2: Effect of *C.argentea* on urine volume and electrolyte concentration of *Celosia argentea* L. in mice

| Groups | Treatment | Mean urine volume (ml) | Electrolyte $Na^+$ | Concentration $K^+$ | (meq/l) $Cl^-$ | $Na^+/K^+$ | Diuretic index | Lipschitz Index |
|---|---|---|---|---|---|---|---|---|
| I | Normal saline (25ml/kg b.w) + Urea(1gm/kg b.w) | 1.2 ± 0.04 | 118 ± 0.02 | 33 ± 0.05 | 134 ± 0.02 | 3.575 | --- | --- |
| II | Hydrochlorothiazide (25mg/kg b.w) | 5.12 ± 0.03*** | 506 ± 0.06*** | 374 ± 0.03*** | 242 ± 0.03*** | 1.352 | 4.26 | --- |
| III | HECA (400mg/kg b.w) | 3.54 ± 0.025** | 344 ± 0.03** | 90 ± 0.03** | 193 ± 0.04** | 3.878 | 2.95 | 0.69 |
| IV | HECA (600mg/kg b.w) | 4.96 ± 0.04*** | 470 ± 0.04*** | 114 ± 0.02*** | 210 ± 0.02*** | 4.298 | 1.00 | 0.96 |

*Values expressed as Mean ± S.E.M. One way ANOVA: p<0.01 (urine volume, electrolyte concentration) considered extremely significant. Tukey-Kramer's multiple comparison test *p<0.01, **p<0.001; when compared with the control group.*

*Diuretic Index=Mean urine volume of test/Mean urine volume of control.*

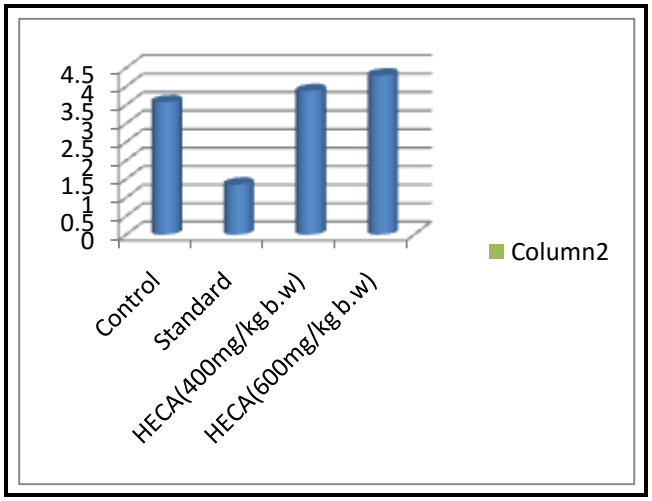


Figure 5: Histograph of effect of *Celosia argentea* L on sodium ion / potassium ion ratio

## DISCUSSION

Diuretics relieve pulmonary and peripheral edema. Application of diuretics to the management of hypertension has out stripped their use in edema by decreasing cardiac workload (preload), oxygen demand and plasma volume. These are also indicated in drug poisoning to promote its excretion. The extract may be useful in these cases for their observed diuretic activity[13-19].

The diuretic activity of HECA at 400mg/kg b.w and 600mg/kg b.w were significant (p<0.001) when as compared to control. The graded doses of HECA in normal saline showed a very significant increase in diuresis, natriuresis,

kaliuresis, GRF. The extract cause increase urine elimination (Table 2), (Figure 1) and increase in $Na^+$, $k^+$ and $cl^-$ excretion as compared to normal control (Table 2), (Figure 2, 3, 4).

The extract possibly act by the synergistic action mechanism of the [$HCO_3^-$ / $Cl^-$], [$HCO_3^+$ /$H^+$] exchangers and the [$N^+/H^+$] antiporter, to cause diuresis. There was an increase in the ratio of concentration of extracted sodium and potassium ions after HECA treatment.

An emphasized, diuretic property of HECA could be due to other active principles such as alkaloids, flavonoids, saponins and triterpinoids. It is also possible that diuretic effect of HECA could be due to other secondary active metabolites.

## CONCLUSION

On the basis of the above results and discussion, we can conclude that HECA treatment produced a marked diuresis when animals were acutely treated. In our study, no lethality was observed at least for the dose and duration used. It remains necessary to study eventual adverse effect of this plant such as alteration of some neural, metabolic, biochemical parameters and hormonal parameters, which are undetermined in this study before its recommendation to clinical use. The precise site and the molecular and cellular mechanisms of HECA action remain to be elucidated in further studies[20].

All treated groups showed increased diuresis possibly by inhibiting tubular reabsorption of water and accompanying anions. Hence increase in GFR. The HECA showed significant ($p<0.001$) activity at 600mg/kg b.w. followed by 400mg/kg b.w respectively.

So the potency of diuretic activity was found to be more with 600mg/kg b.w followed by 400mg/kg b.w. by increased diuresis and with increase in GFR. All studied parameters in the present work clearly substantiate the traditional claim of diuretic property of *C.argentea*. Moreover, the study clearly shows the HECA at 600mg/kg b.w. has got significant diuretic property followed by 400mg/kg b.w. The actual phytoconstituents responsible for diuretic activity is needed to be determined. Hence, there is a further scope in detail phytochemical investigation and activity guided isolation of active constituents from the plant of *C.argentea*.

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