



DIURETIC POTENTIAL OF ETHANOLIC EXTRACT OF SEEDS OF PLANT CAESALPINIA CRISTA LINN

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Abstract

Background: Diuretic compounds are much helpful to relieve congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension and hypertension associated with oedema.

Aims: Diuretic activity of ethanolic seed extract of *Caesalpinia crista* linn was determined by using various diuretic models in albino rats.

Material & Methods: Two screening models were selected to determine diuretic activity of ethanolic seed extract of plant *Caesalpinia crista* linn at a dose (100mg/kg, 200mg/kg & 400mg/kg).

a. Diuretic activity of seed of *Caesalpinia crista* linn in albino rats was studied by Lipschitz Test

Male albino rats were divided into 5 groups of 6 animals in each. Group I serve as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt). Group II received Furosemide (10 mg/kg, p.o) in vehicle; other groups III, IV, V were treated with low (100mg/kg), medium ((200mg/kg)), and high doses (400mg/kg) of ethanolic extract.

b. Diuretic activity of seed of *Caesalpinia crista* linn in albino rats was studied by using hydrochlorothiazide.

Male albino rats were divided into 5 groups of 6 animals in each. Group I serve as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt). Group II received Hydrochlorothiazide (10 mg/kg, p.o) in vehicle; other groups III, IV, V were treated with low (100mg/kg), medium ((200mg/kg)), and high doses (400mg/kg) of ethanolic extract.

Immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hr and 24 hr respectively was measured at the end. During this period no food and water were made available to animals. Urinary concentrations of sodium, potassium and chloride ions were determined by using Ion Selective Electrode analyzer. Serum concentrations of creatinine, urea, glucose and albumin were also determined.

Statistical Analysis

Experimental results were express as mean ±SEM (n=6). Statistical analysis was performed with one-way-ANOVA followed by Dunnetts t-test.

Result: When control group was compared with Furosemide (10mg/kg), Hydrochlorothiazide and extract treated groups (100mg/kg, 200mg/kg & 400mg/kg) it showed increase in the urine volume, enhanced the excretion of sodium, potassium and chloride ions in urine. Serum concentrations of creatinine, urea, glucose and albumin were also increased when compared to control group.

Conclusion: Results showed that single dose administration of standard Furosemide, Hydrochlorothiazide and ethanolic extract of seeds of *Caesalpinia crista* linn in various treated groups significantly increased the urine

output, enhance elimination of sodium, potassium, chloride ions and also increase in serum concentration of glucose, creatinine, albumin and urea.

Keywords: Diuretic, Furosemide, Hydrochlorothiazide, *Caesalpinia crista* Linn

Introduction

The overuse in automedication of phytotherapeutic preparations is the main means to cure about 80% population who is unable to get access to manufactured drugs.¹ Medicinal plants have been widely used as a source for the treatment of human disorders since the ancient times to this date.² Medicinal plants are the Nature's gift to human beings to help them pursue a disease-free healthy life.³ Herbal drugs are vital for the treatment of some renal problems and few of the herbs shows diuretic activity. Diuretics are the agents that increase the rate of urine out flow and are very much useful to maintain the volume and composition of body fluids in different clinical conditions. These drugs produces so much beneficial effect in most of the renal problems like congestive heart failure, cirrhosis, nephritis, kidney failure, increased blood pressure and pregnancy toxemia.⁴ The presently available diuretics such as thiazides and loop diuretics exhibit various adverse effects such as electrolyte imbalance and metabolic alterations etc. Some of the diuretics are derived from medicinal plants and a vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties.⁵ Plants belonging to the genus *Caesalpinia* have been utilized as crude drugs in folk medicine throughout the regions where they occur botanically.⁶ *Caesalpinia crista* linn is a woody vine that is scandent and straggling. It is also a prickly shrub growing up to 5-10 m tall, which is widely distributed across India growing mainly in marshy and plain terrains, and the habitat of tropical and subtropical regions of Southeast Asia. It is one of the major herbal plants found in Western Ghats region of Karnataka.⁷ Latakaranja has been botanically identified as *Caesalpinia crista* linn belongs to family Caesalpinaceae. *Caesalpinia crista* linn exerted nootropic, anticonvulsant, antioxidant, antianxiety, adaptogenic, antimicrobial, antiprotozoal, anthelmintic, insecticidal, antiproliferative, antidiabetic, hypotensive, hepatoprotective, antioxidant, cardioprotective, anticancer and many other effects.⁸ The decoction of the root has been used for its significant health benefits such as in the treatment of rheumatism, backache and as a tonic. The root has diuretic properties and is useful in bladder stone. A decoction of the root is prescribed in a fever. A decoction of the root has been used for its important health benefits such as in the treatment of rheumatism, backache and as a tonic.⁹ The plant contained flavonoids, tannins, proteins, alkaloids, carbohydrates reducing sugars, phytosterols, triterpenoids, saponins, coumatins, furano-cassanediterpenes, nor cassanediterpenes, neocassanediterpenes and many other bioactive compounds.⁸ There is no scientific information on diuretic potential of ethanolic seed extract of plant *Caesalpinia crista* Linn so The aim of present study is to evaluate scientifically diuretic potential of ethanolic seed extract of the plant *Caesalpinia crista* Linn to justify the traditional use of this plant.

Material and Methods

Material

Plant Material

Plant part (seeds) were collected from local market of Maharashtra and authenticated by Research Director & Assistant Professor at R. R. Government College, Alwar. Herbarium sample was also preserved at R. R. College, Alwar.

Animals

Healthy Wistar albino male rats weighing between 150-200 gm were taken for the study. They were housed under controlled conditions of temperature ($22 \pm 30^\circ\text{C}$), the relative humidity should be at least 30% but not exceed 70% (other than during room cleaning) It was $55 \pm 5\%$. Lighting was artificial it was 12 h light and 12 h dark cycles according to OECD Guideline 423. Standard pellet diet and water given to all animals. The present work was carried out with a prior permission by IAEC of Alwar Pharmacy College with CSPSEA registration number 963/c/06/ CPCSEA.

Chemicals

All the reagents, chemicals and drugs are of standard quality and purchased by local market of Jaipur.

Methods

Ethanolic extract preparation of authenticated seed of *Caesalpinia crista* linn were washed with clean water before drying in oven at a temperature of 40°C until the moisture content was below 14%. This reduces the chances of fungus infection in samples. The dried seeds were grinded and stored in airtight container. Ethanolic

extraction was carried out by soxhlet extraction. Quantity of powdered material used for ethanolic extraction was 200 gm. These powdered materials of plant were defatted with Petroleum Ether for 72 hours in a Soxhlet apparatus. Then after 72 hours these defatted material is subjected to extraction with ethanol (99.99%) in a Soxhlet apparatus for 48 hours. Make the extract dry under reduced pressure and controlled temperature (40-50°C) using flash evaporator. The ethanolic extract obtained was concentrated under reduced pressure.¹⁰

Preliminary phytochemical screening:

The ethanolic extract was tested for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycoside, phenolic compound, tannins, saponins, flavonoids, fixed oils and fat test. All phytochemical tests were done as per the procedure given in the standard book.¹¹⁻¹⁵

Diuretic Activity

a) Diuretic activity of seed of *Caesalpinia crista* (Linn.) in albino rats will study by the Lipschitz Test.

The diuretic activity of ethanolic extract of seeds of *Caesalpinia crista* linn in albino rats were studied by the Lipschitz Test. Male Albino rats were divided into 5 groups of 6 animals in each.

The group I serve as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt) The group II received Furosemide (10 mg/kg, p.o) in vehicle; Other groups III, IV, V were treated with low (100mg/kg), medium ((200mg/kg)), and high doses (400mg/kg) of ethanolic extract of seeds of *Caesalpinia crista* linn in vehicle and immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hr was measured at the end. During this period no food and water was made available to animals.⁵

b) Diuretic activity of seed of *Caesalpinia crista* linn in albino rats was studied by using hydrochlorothiazide.

The rats were randomly divided into five groups of each with six animals to assess diuretic activity of ethanolic seed extract of *Caesalpinia Crista* linn. Ist group was assigned as control group and received the vehicle only. The IInd group was assigned as standard group and received hydrochlorothiazide 10mg/kg orally. The IIIrd, IVth and Vth groups were assigned as treated groups received as 100mg/kg, 200mg/kg and 400mg/kg ethanolic extract of plant.¹⁶ Urinary concentrations of sodium, potassium and chloride ions were determined by using Ion Selective Electrode (ISE) analyzer (Roche, Germany).⁵ Concentrations of creatinine, urea, glucose and albumin were also determines.

Statistical Analysis

Experimental results was express as mean ±SEM (n=6). Statistical analysis was performed with one-way-ANOVA followed by Dunnetts t-test.

Result

Phytochemical screening of ethanolic seed extract of plant *Caesalpinia crista* linn showed presence of various phytoconstituents like carbohydrates, proteins, amino acids, flavonoids, alkaloids, glycosides, saponins and tannins.

Table 1: Effect of ethanolic extract of seed of *Caesalpinia crista* linn in albino rats on urine volume and electrolyte concentration in Furosemide induced model

Sr. No	Groups	Total Urine volume (ml/kg b.wt./5hr)	Na ⁺ mmol/L	K ⁺ mmol/L	Cl ⁻ mmol/L
1.	Control	12.45±0.02	112.03 + 2.16	49.09 + 1.51	80.95 + 1.42
2.	Furosemide (10 mg/kg)	20.23±0.01***	189.05+2.09***	85.81+1.60***	127.06+1.67***
3.	EECC (100mg/kg)	14.20±0.02***	127.40+2.80***	62.13+1.82***	92.42 + 1.73***
4.	EECC (200mg/kg)	16.41±0.02***	162.99+2.00***	75.93+2.67***	109.44+1.20***
5.	EECC (400mg/kg)	20.46±0.02***	182.53+2.32***	83.11+1.79***	119.39+2.00***

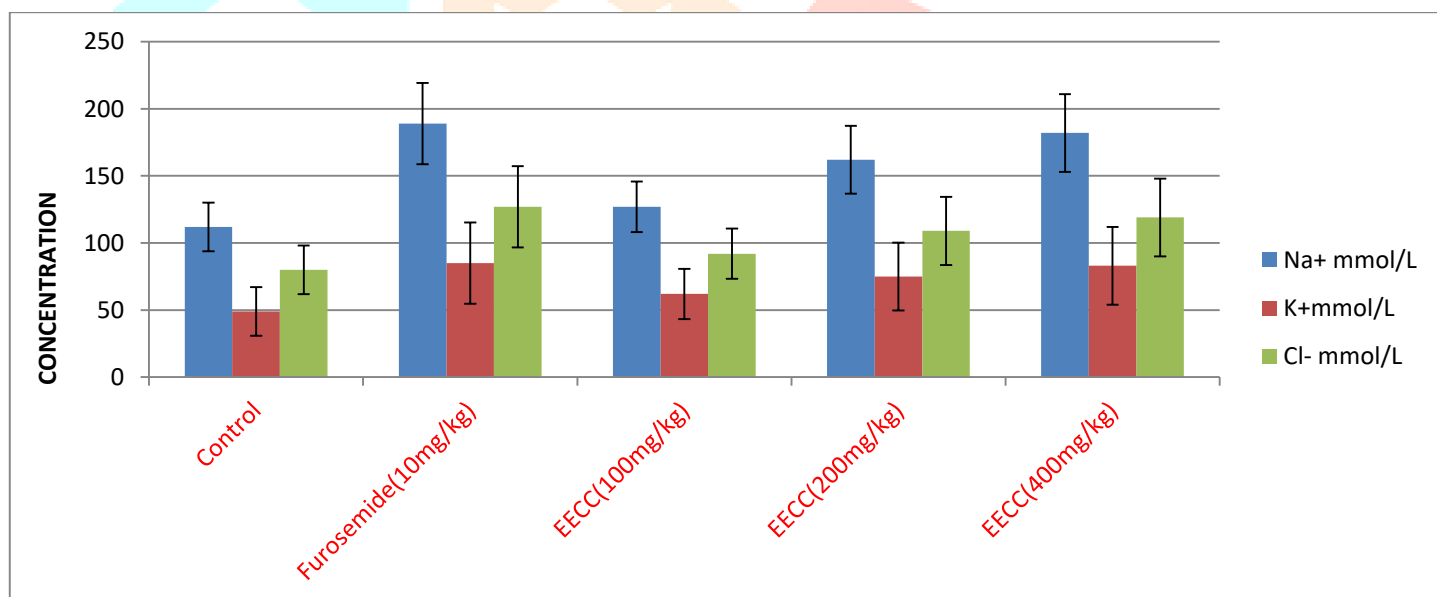


Fig. 1: Effect of ethanolic extract of seed of *Caesalpinia crista* linn on urinary sodium, potassium and chloride (mmol/L) ions concentration
EECC – Ethanolic extract of *Caesalpinia crista* linn

Values expressed as mean ± S. E. M., n=6, Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One way ANOVA followed by Dunnett's 't' Test)

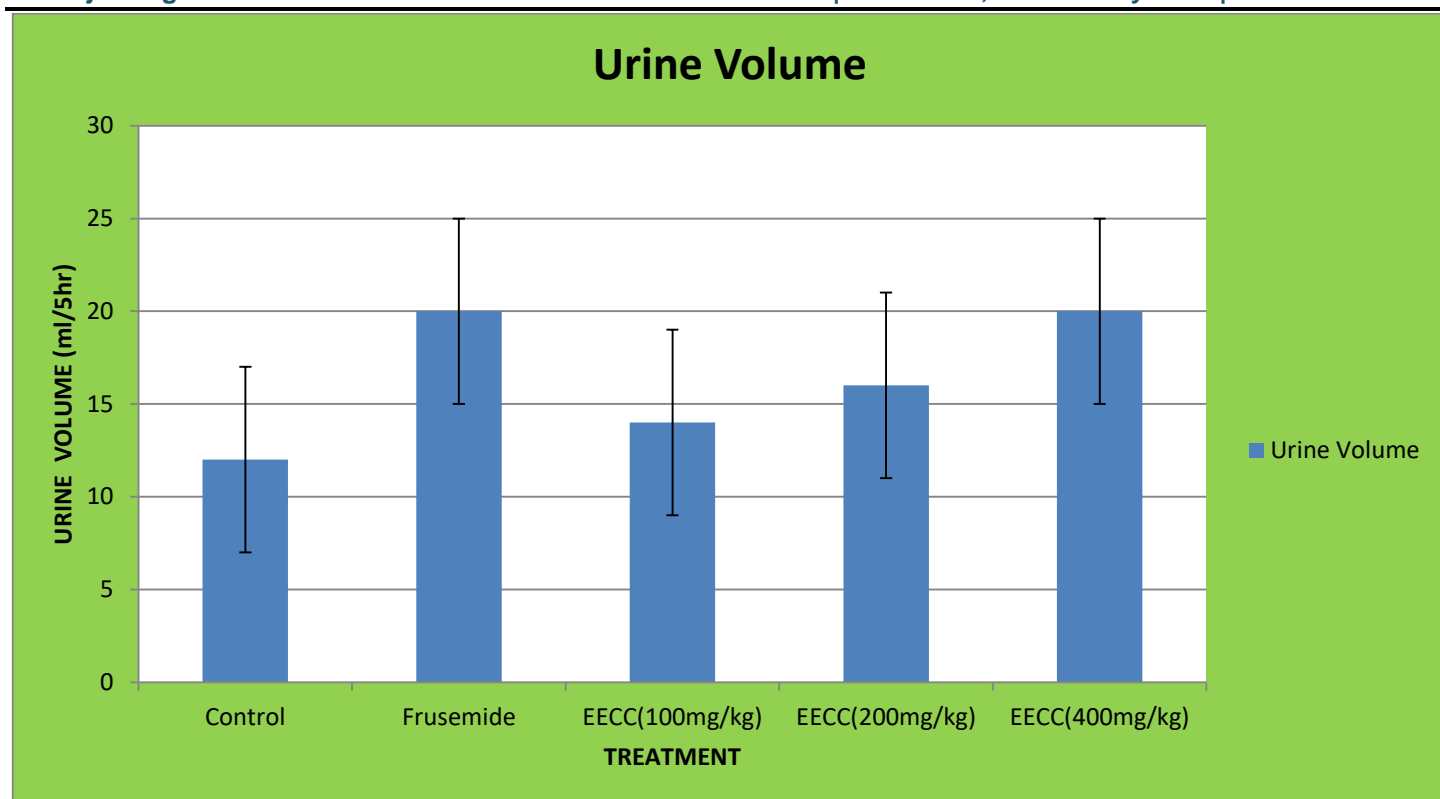


Fig. 2: Effect of Furosemide and ethanolic extract on urine volume in albino rats
EECC – Ethanolic extract of *Caesalpinia crista* linn

Table 2: Effect of ethanolic extract of seed of *Caesalpinia crista* linn in albino rats on urine volume and electrolyte concentration in Hydrochlorothiazide diuretic model

Sr. No	Groups	Total Urine volume (ml/kg b.wt./24hr)	Na ⁺ mmol/L	K ⁺ mmol/L	Cl ⁻ mmol/L
1.	Control	208.22 ± 2.43	23.32 ± 1.11	23.11 ± 0.66	19.33 ± 1.41
2.	HCTZ (10mg/kg)	237.11 ± 4.33**	81.77 ± 1.31***	29.66 ± 1.13**	77.22 ± 2.11***
3.	EECC(100mg/kg)	254.11 ± 3.23***	59.22 ± 2.23***	44.18 ± 1.31***	53.16 ± 1.32***
4.	EECC(200mg/kg)	286.13 ± 2.13***	112.33 ± 1.33***	60.34 ± 1.22***	107.33 ± 1.41***
5.	EECC(400mg/kg)	358.08 ± 3.13***	166.28 ± 1.22***	52.33 ± 2.14***	158.67 ± 1.42***

Values are means ± S.E.M., n = 6, p < 0.05*, p < 0.01**, p < 0.001***, significant difference compared to the control one.

EECC – Ethanolic extract of *Caesalpinia crista* linn

HCTZ – Hydrochlorothiazide

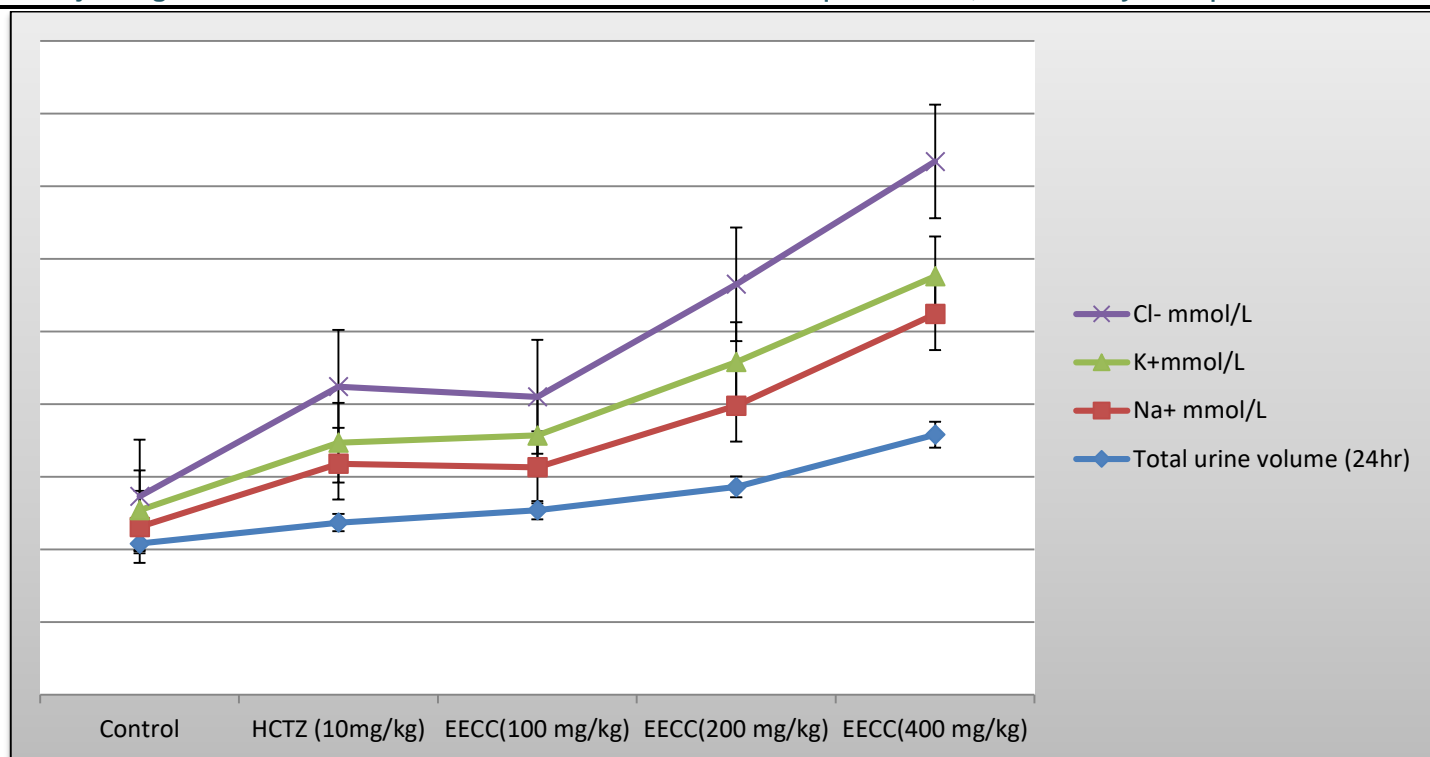


Fig. 3: Effect of ethanolic extract of seed of *Caesalpinia crista* linn in albino rats on urine volume and electrolyte concentration in Hydrochlorothiazide diuretic model
 HCTZ – Hydrochlorothiazide
 EECC – Ethanolic extract of *Caesalpinia crista* linn

Table 3: Effects of the ethanolic extract of *Caesalpinia crista* linn on the serum parameters

Sr. No.	Groups	glucose (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Albumin (g/l)
1.	Control	89.22 ± 2.11	0.55 ± 0.22	21.12 ± 0.38	42.52 ± 1.14
2.	HCTZ (10mg/kg)	94.22 ± 3.55*	0.74 ± 0.61***	26.22 ± 1.13***	43.66 ± 1.32***
3.	EECC(100mg/kg)	90.45 ± 2.34*	0.62 ± 0.45*	22.38 ± 1.11 ^{NS}	43.66 ± 1.22 ^{NS}
4.	EECC(200mg/kg)	91.66 ± 4.33*	0.66 ± 0.57**	23.89 ± 2.44**	44.72 ± 2.34**
5.	EECC(400mg/kg)	95.88 ± 3.41*	0.87 ± 0.29***	26.77 ± 2.33***	44.65 ± 3.67***

Discussion and Conclusion

Diuretics are an invaluable and heterogeneous class of agents commonly used in the treatment of hypertension, heart failure, and electrolyte disorders.¹⁷ Seeds of *Caesalpinia crista* linn having diuretic potential as mentioned in Indian system of medicines but not proved scientifically; Plant seeds were procured from local market of Maharashtra and authenticated by Research Director & Assistant Professor of R. R. Government College, Alwar, Rajasthan. Seeds were subjected to its extraction followed by phytochemical and then pharmacological evaluation for diuretic activity using following screening models.

- Diuretic activity of seed of *Caesalpinia crista* Linn in albino rats will study by the Lipschitz Test.⁵
- Diuretic activity of seed of *Caesalpinia crista* (Linn) in albino rats will study by using hydrochlorothiazide.¹⁶

Shed dried seeds are converted to powder and extraction was carried out using ethanol and ether. Ethanolic plant extract was subjected to various phytochemical tests showing presence of carbohydrate, proteins, glycosides, alkaloids, tannins and flavonoids etc. Diuretic potential was evaluated by using two screening models. Furosemide (10 mg/kg) and hydrochlorothiazide's (10 mg/kg) were used as standard drugs. Three different doses of ethanolic seed extract 100 mg/kg, 200 mg/kg and 400 mg/kg were used to determine diuretic activity of ethanolic extract. Urine electrolyte and various biochemical parameters were evaluated. In Lipschitz urine was collected for 5 hrs and urinary sodium, potassium and chloride concentration were measured. Total urine volume was increased in all groups when compared to control. Sodium, potassium and chloride excretion were increased for all treated group when compared to control groups. When diuretic activity was evaluated by using hydrochlorothiazides, Urine was collected for 24 hrs, Urinary concentration of sodium, potassium and chloride were measured. Total urine volume) was increased in all treated groups and sodium, potassium and chloride excretion was increased for all treated groups when compared to control group. Serum concentration of glucose, creatinine, urea and albumin were increased in all groups when compared to control group. From the above study it was concluded that ethanolic seed extract of *Caesalpinia crista* linn having active constituents like carbohydrate, proteins, glycosides, alkaloids, tannins and flavonoids etc. Results shows that plant showed diuretic activity and it may be possible that phytoconstituents present in plant seeds were responsible for diuretic activity. To isolate bioactive compounds from ethanolic extract; which are responsible for pharmacological activity further analytical measures should be adopted.

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