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Original Article

Antiurolithiasis, Antioxidant, Anti-inflammatory, Analgesic, and Diuretic Activity of Ethanolic Extract of Seeds of *Caesalpinia bonducella*

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ABSTRACT

Background: Urolithiasis, commonly known as nephrolithiasis, kidney stone, or renal stone, is a disorder caused by a decrease in anti-oxidant capability, damaged renal epithelium or consistently supersaturated urine, which leads to increased crystallisation. The aim of this study was to evaluate antiurolithiatic, antioxidant, analgesic, anti-inflammatory and diuretic property of ethanolic extract of *C. bonducella* seeds (EECB). **Methods:** For the probable molecules in EECB, GC-MS analysis was performed. Antioxidant property was investigated using DPPH and FRAP assays. Nucleation and aggregation assays were used for *in vitro* urolithiatic study. The diuretic, anti-inflammatory and analgesic activity were performed in animal model. **Results:** GC–MS analysis of EECB identified the presence of Vitamin E, steroidal ring containing compounds and hydrocarbons. EECB was found to be effective in scavenging free radicals. EECB exhibited significant inhibition of nucleation and aggregation of calcium oxalate crystals. The anti-inflammatory and analgesic activity of EECB were found to be significant effective at dose of 400 mg/kg. Further, EECB exhibited a significant increase in urinary output and electrolytes Na^+, Cl^- excretion at dose of 400 mg/kg. **Conclusion:** The finding of this study suggested that EECB possess significant pharmacological activities like antioxidant, anti-inflammatory, analgesic, diuretic, and antiurolithiatic activities. Antioxidants property of EECB could be used in preventing oxidative stress; the anti-inflammatory and analgesic properties could be used in the prevention of inflammation and pain of damaged epithelial cells of nephrons, which are caused by intra-papillary calcifications; the diuretic activity could be used in reducing supersaturation of calcium oxalate.

Key word: Urolithiasis, Calcium oxalate crystal, Nucleation, Aggregation, GC-MS.

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INTRODUCTION

Urolithiasis also termed as nephrolithiasis, kidney stone, or renal stone and is one of the most prevalent urologic diseases in Asia. There are two types of stone found in urolithiasis. The first one is calcareous stone including calcium oxalate and calcium phosphate; and the second is non-calcareous stone including uric acid, struvite (infection stone by protease bacteria), cystine, uric acid stones. In kidney stones, over 80% cases of calcium oxalate, 5-10 % of uric acid stone, 6%-13% of calcium phosphate, 2%-15% of struvite (infectious stone), 1% of apatite, and 0.5%-1% of cystine are found in the world.[1-3]

In Ayurveda, many herbal plants have been used as antiurolithiasis drug.[2,4] *Caesalpinia bonducella* L (Family: Fabaceae), commonly known as Nata Karanja, Karanj, Kala Karanja (in Hindi), is abundant in the tropical and subtropical regions. The *C. bonducella* is extensively used in folk medicines[5] for the treatment of filarial infection, common cold, tumor, fever, asthma, dysentery, and diabetes in India and China.[5-7] The extract of leaves, seeds and seeds kernel of *C. bonducella* have been studied for antitumor activity,[8] anti-inflammatory effects,[7] an increase of contractile force,[9] contractile activity of uterine smooth muscle,[10] antioxidants,[6,8] and antihyperglycemic.[6] No study has been reported antiurolithiatic effect of *C. bonducella* seed. The purpose of this study is to scientifically justify antiurolithiatic property of ethanolic extract of seed of *C. bonducella*.

MATERIALS AND METHODS

Plant material and extraction

The seeds of *C. bonducella* were collected from Varanasi, India and identified by Dr. Ashwani K. Kushwaha, Department of Dravya Guna, Faculty of Ayurveda, IMS, BHU, Varanasi. The air-dried, powdered seeds of *C. bonducella* (100 g) were soaked with 1 L of 95% ethanol for 7 days. The crude extract was obtained by filtration through filter paper. The filtrate was evaporated by rotatory evaporator to give a viscous brownish dark mass.[11]

GC-MS analysis

GC-MS analysis of EECB was performed at Advance CIF, JNU, Delhi, India, on an instrument Shimadzu QP-2010 Plus TDS TD 20.

Antioxidant activity

***DPPH assay*:** 7.89 mg of DPPH was dissolved in 100 ml 99.5% ethanol to make 0.2 mmol/L DPPH solution and incubated in dark for 2 hr. The EECB at various concentrations (25, 50, 100, and 200 µg/ml) were diluted with ethanol to get testing sample solution. 200µl of EECB, 1ml of DPPH solution and 800 µl of Tris-HCl buffer (pH 7.4) were mixed in test tube and kept at 25°C for 45 min. The absorbance was recorded at 517 nm by UV-Visible spectrophotometric (Systronics Double Beam UV-VIS Spectrophotometer: 2202). The blank was prepared by mixing

1.2 ml of ethanol and 0.8 µl of Tris-HCl buffer (pH 7.4). The DPPH reagent without sample was used as control and ascorbic acid (2 to 20 µg/ml) as standard.[12-15]

$$\% \text{ scavenging of DPPH free radicals} = [1-(A_t/A_c)] \times 100$$

where A_c is the absorbance of control and A_t is the absorbance of test sample.

***Ferric Reducing Antioxidant Power (FRAP) assay*:** EECB was analysed at concentrations of 25, 50, 100, and 200 µg/ml. 100 ml of $FeCl_3$ solution (0.02 mol/L), 10 ml of pH 3.6 Acetic acid buffer (0.3 mol/L), and 10 ml of TPTZ solution (0.01 mol/L) were mixed to prepare fresh FRAP reagent. 0.2 ml of EECB was added in test tubes and mixed with 18 µl of FRAP reagent. The test tube was incubated at 40°C for 1h. The OD at 593 nm was measured. The FRAP reagent without sample was used as control and ascorbic acid (2 to 20 µg/ml) as standard. The antioxidant power calculated by the following formula:[12-14,16]

$$\% \text{ FRAP} = [1-(A_t/A_c)] \times 100$$

where A_c is the absorbance of control and A_t is the absorbance of test sample.

In vitro Urolithiatic activity

***Nucleation assay*:** Buffer (pH 6.5) containing Tris 50 mmol/L and sodium chloride 150 mmol/L were used as solvent to prepare $CaCl_2$ (5 mmol/L) and $Na_2C_2O_4$ (7.5 mmol/L) and the temperature was maintained at 37°C during the entire experiment. A calcium chloride solution of 9.5 ml was mixed with 2 mL of EECB (at concentrations of 10-1000 µg/ml). Nucleation was initiated by adding 9.5 mL $Na_2C_2O_4$ (7.5 mmol/L). Final solutions were incubated for 60 min at 37°C. The distilled water was used as blank and cystone as a standard. The optical density (OD) was taken at 620 nm by a UV-Visible spectrophotometer.[17-19]

$$\% \text{ inhibition of nucleation (\% IN)} = [1 - (OD_{sample}/OD_{control})] \times 100$$

Where OD_{sample} is the optical density of EECB or cystone, $OD_{control}$ is optical density without EECB or cystone

***Aggregation assay*:** The crystals of calcium oxalate were prepared by adding equal volume of $CaCl_2$ and $Na_2C_2O_4$ at 0.1 mol/L; heated up to 60°C for 3 hr and allowed to stand overnight at 37°C. The calcium oxalate crystals were separated by centrifugation at 2000 rpm and dried at room temperature. Buffer (pH 6.5) containing Tris 50 mmol/L and sodium chloride 150 mmol/L were used as solvent to prepare 0.75 mg/ml calcium oxalate crystals. 9 ml of calcium oxalate crystals solution was mixed with 1 ml of EECB (at concentrations of 10-1000 µg/ml) and incubated for 60 min at 37°C. The distilled water was used as blank and cystone as a standard. The OD was taken at 620 nm by a UV-Visible spectrophotometer.[17-19]

$$\% \text{ AI (aggregation inhibition)} = [1 - (OD_{sample}/OD_{control})] \times 100$$

Where OD_{sample} is optical density with EECB or cystone, $OD_{control}$ is optical density without EECB or cystone

Analgesic Activity

***Animals*:** Healthy Wistar albino rats (120–160 g) of either sex and approximately 8-12 weeks old, were used in the study. The animals were divided into 4 groups (n = 5). All experiments were conducted according to the guideline and approved by the ethics committee of IMS, BHU, Varanasi, India (2211, 07.11.2020).

***Writhing Test*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug diclofenac sodium (10 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 30 min of dosing, 0.1 ml of acetic acid (0.6% v/v) as irritant was injected intraperitoneal and the number of writhes (stretching of the abdomen, twisting of the trunk, contractive movements of abdomen, stretching of one or two hind limb, extension of body) was counted from 5 to 30 min.[20-22]

***Formalin Test*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug morphine (5 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 30 min of dosing, 0.05 ml formalin (10% v/v) as irritant was injected into the dorsal portion of the front paw. The total time spent in licking and biting of the irritant injected paw were recorded on stopwatch in early phase (1-5 min) considered as neurogenic phase and in late phase (15-30 min) considered as inflammatory phase.[20,21,23]

***Hot Plate Test*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug morphine (5 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. The surface of analgesio-meter was maintained at temperature 55±1°C and 15 seconds cut-off time. After 30, 60, and 90 min of dosing, the animals were placed on analgesio-meter (Techno Eddy's hot plate) and the time was recorded between placement and licking, bitting, or withdrawal of the hind paws.[21,23]

***Tail Flick Test*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug morphine (5 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 5, 15, 30, 60, and 90 min of dosing, the tip of the rat's tail (last 3 cm) was placed on the radiant heat source (Techno analgesio-meter) and the time was recorded between placement and removal of tail with the cut-off time of 15 seconds (to avoid tail injury).[21,23]

Anti-inflammatory activity

***Carrageenan model*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug indomethacin (20 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 60 min of dosing, 0.1 ml of 1 % w/v carrageenan was given subcutaneously (s.c.) into the plantar surface of the hind paw of rats. Paw edema was measured by plethysmometer at 0 min, 1h, 2hr, 3hr, and 4hr.[20,21]

***Formalin model*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug indomethacin (20 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 60 min of dosing, 0.1 ml of 1% formalin was given s.c. into the plantar surface of the hind paw of rats. Paw edema was measured by plethysmometer at 0 min, 1hr, 2hr, 3hr, and 4hr.[21]

***Egg albumin model*:** Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug indomethacin (20 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After 60 min of dosing, 0.1 ml of egg albumin was given s.c. into the plantar surface of the hind paw of rats. Paw edema was measured by plethysmometer at 0 min, 1hr, 2hr, 3hr, and 4hr.[21]

Diuretic activity in rats

Twenty-five (25) healthy Wistar albino rats were divided into five groups (n=5). All rats received 0.9 % Sodium chloride (25 ml/kg) to maintain uniform water and salt load. Normal control group (Group I) received p.o. vehicle (0.5% CMC). Standard drug furosemide (10 mg/kg) was administered p.o in Group II, while 200 and 400 mg/kg of EECB were administered p.o. in groups III and IV, respectively. After dosing, the rats

were placed in metabolic cage for the urine collection at every hour up to 5hr. Total urine volume and urinary concentration of Na^+, K^+, and Cl^- were measured.[24,25]

Statistical analysis

The data were expressed as mean ± SEM and analysed by GraphPad Prism version 8.0.2 software. Ordinary one-way and two-way ANOVA with Dunnett's multiple comparisons test was applied.

RESULTS

The identified constituents of EECB by GC-MS was given in Table 1. The results were revealed that the scavenging of the free radicals was found to be effective and concentration-dependent for EECB in DPPH and FRAP assays. The IC_{50} of ascorbic acid was 7.36 and 7.85 µg/ml while that of EECB was 95.32 and 153.13 µg/ml in DPPH and FRAP assays, respectively. The ascorbic acid equivalent mg/g dry weight extract was 77.24 and 51.23 AA E mg/g DW extract in DPPH and FRAP assays, respectively.

The effect of EECB on nucleation and aggregation at different concentrations of EECB is presented in Figure 1A and 1B. Figure 2B, 4C, 4D and 4E showed that minimum number and the smallest size of crystals were seen with both EECB and cystone as compared with control (Figure 4A).

Pre-treatment with EECB (200 and 400 mg/kg) showed a significant analgesic potential in the tail-flick model (Figure 3A). EECB at dose of 400 mg/kg increased significantly (P<0.05 to P<0.0001) an anti-nociceptive effect as compared with the normal control group. The Eddy's hot plate results in Figure 3B showed that EECB (200 and 400 mg/kg) significantly (P<0.05 to P<0.0001) enhanced response time (latency) as compared with normal control group. Anti-nociceptive effects of the EECB using acetic acid-induced writhing model are showed in Figure 3C. EECB decreased pain significantly (P<0.01 to P<0.0001) as comparison with the normal control group. In formalin model, the analgesic potential of EECB is presented in Figure 3D. In phase I and phase II, EECB reduced pain significantly (P < 0.01) at 400 mg/kg dose in both phase as compared with the normal control group.

As shown in Figure 4A, 4B, and 4C, EECB (400 mg/kg) showed a significant (P<0.05 to P<0.0001) inhibition of paw edema in carrageenan, formalin, and egg albumin model as compared with normal control group. In carrageenan and formalin-induced edema, EECB at dose of 200 mg/kg showed a significant (P<0.05 to P<0.0001) inhibition of edema at 2hr and 3h while, in egg albumin, 200 mg/kg caused a significant (P<0.01) inhibition of edema at 2hr in comparison to normal control group.

EECB at dose of 200 mg/kg showed a significant (P<0.01) increase in urine output in 3h to 5h and 400 mg/kg dose of EECB showed a significant (P<0.001 to P<0.0001) increase in urine output in 2hr to 5hr

Table 1: List of major identified constituents of ethanolic *C. bonducella* extract in GC-MS analysis.

S.N.	Peak	R. Time	Area	% Area	Chemical name
1	1	6.958	7831055	1.05	5-Hydroxymethylfurfural
2	4	11.835	60214169	8.09	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)
3	6	15.678	172473960	23.18	4-O-Methylmannose
4	8	16.389	23159181	3.11	n-Hexadecanoic acid
5	12	18.081	73027827	9.82	10(E),12(Z)-Conjugated linoleic acid
6	13	18.123	22544898	3.03	cis-Vaccenic acid
7	31	21.228	8382836	1.13	Octacosane
8	42	22.947	11384976	1.53	16,17-Epoxy--pregna-5,7-diene-3,20-dione
9	44	23.155	5911270	0.79	Lycopene
10	47	23.741	944846	0.13	Nandrolone
11	48	23.826	7781038	1.05	Pregnenolone
12	53	24.813	4571908	0.61	Retinol, acetate
13	54	25.194	5044584	0.68	Retinol, acetate
14	55	25.309	19261965	2.59	3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid
15	56	25.663	41680714	5.6	Retinol, acetate
16	59	26.618	12752988	1.71	Ergosterol, acetate
17	62	27.504	26255142	3.53	15,17,19,21-Hexatriacontatetrayne
18	63	27.751	14701154	1.98	7,11-Dihydroxyprogesterone
19	64	28.07	4145630	0.56	Simvastatin
20	65	28.599	10449890	1.4	Simvastatin
21	67	29.573	37326707	5.02	Tanshinon II
22	68	29.882	11316491	1.52	Pregna-4,16-diene-3,20-dione
23	69	30.026	10834880	1.46	Corticosterone 21-acetate
24	70	30.118	6277371	0.84	Anthraegostatrine
25	71	30.249	5590219	0.75	Phorbol 12,13,20-triacetate
26	72	30.525	4545773	0.61	Retinol
27	73	31.089	15040232	2.02	Retinol
28	74	31.325	23239899	3.12	Ergosterol, acetate

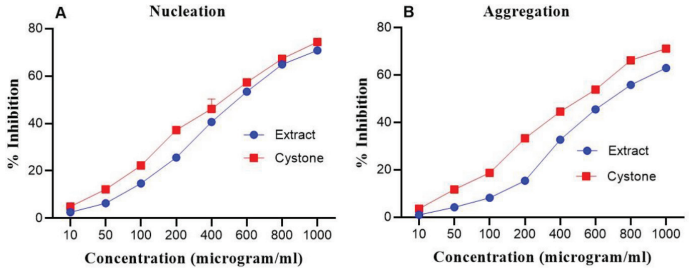

Figure 1: (A) nucleation assay and (B) aggregation assay.

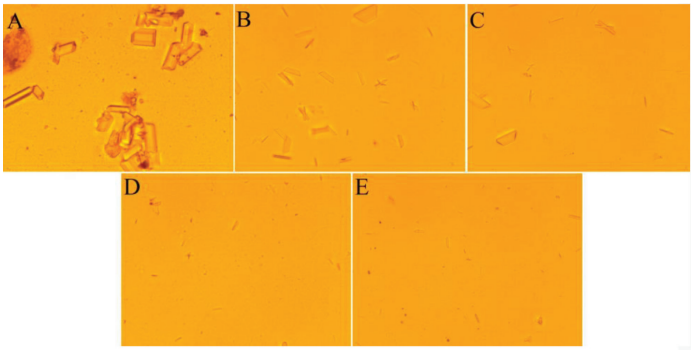

Figure 2: Light microscopic image of (A) control (without inhibitor), (B) EECB at 400 µg/ml, (C) Cystone at 400 µg/ml, (D) EECB at 1000 µg/ml, (E) Cystone at 1000 µg/ml.

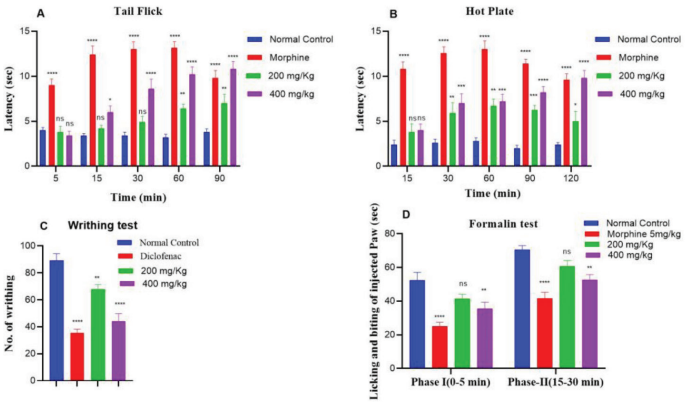

Figure 3: Effect of EECB on Tail flick test (A), Eddy's hot plate (B), writhing test (C), formalin test (D). *n* = 5; mean ± SEM; ns *P*>0.05; * *P*<0.05; ** *P*<0.01; *** *P*<0.001; **** *P*<0.0001 compared with normal control.

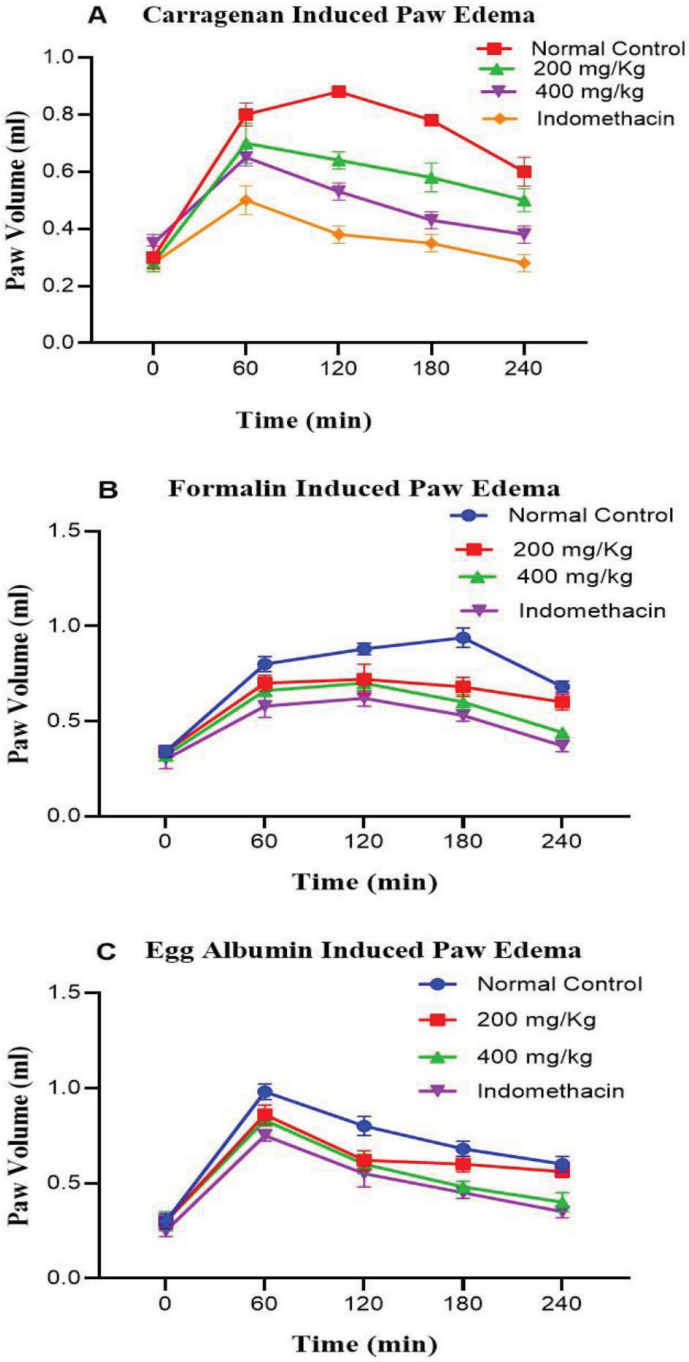

Figure 4: Effect of EECB on carrageenan model (A), formalin model (B), egg albumin model (C). n = 5; mean ± SEM.

as compared to the control group. EECB at dose of 400 mg/kg showed a significantly (*P*<0.05 to *P*<0.0001) increase in urinary Na^+, Cl^- excretion, and non-significant (*P*>0.05) in urinary K^+ excretion as compared with the normal control group (Table 2).

DISCUSSION

Extraction of *C. bunducella* seed were performed in ethanol (95%) based on literature studies stating that ethanol (95%) can offer superior extraction results for phenolic like chemicals because it dissolves the most polar and non-polar compounds. GC–MS analysis of EECB identified the presence of Vitamin E, steroidal ring containing compounds and hydrocarbons which may responsible for its pharmacological action.

Several researchers have reported that oxidative stress or free radical generation is increased with urinary supersaturation of oxalate which causes damage to epithelial cells. These damaged cells may work as binding surfaces or receptors for oxalate which promote the formation of the nucleus for urolithiasis.[26-28] Antioxidants could be used in preventing the formation of the intrapapillary calcifications which is induced by oxidative stress that lead to papillary calculi formation.[28] By treating renal epithelial cells with antioxidants we showed through *in-vitro* studies that EECB could be used as an agent for prevention of urolithiasis.

After 60 min of incubation, cystone showed the highest nucleation inhibition of 74.35 ± 0.48% at 1000 µg/ml, which was nearly similar to

Table 2: Effect of extract on urine excretion in rats for 5 hr after treatment.

Treatment	Volume of Urine (ml)					Concentration of ions (meq/l)			Saluretic	
	1 hr	2 hr	3 hr	4 hr	5 hr	Na+	K+	Cl-	Cl/Na+K	Na/K
Control 10 ml/kg	0.59 ± 0.1	1.4 ± 0.19	2.3 ± 0.17	3.2 ± 0.22	3.98 ± 0.1	109.2 ± 2.29	60.6 ± 3.91	140.2 ± 3.43	0.83	1.80
Furosemide 10 mg/kg	2.24 ± 0.11 ****	3.94 ± 0.20 ****	5.5 ± 0.24 ****	7.2 ± 0.09 ****	8.64 ± 0.13 ****	153.2 ± 3.98 ****	77.8 ± 2.85 **	165.8 ± 2.65 ****	0.72	1.97
Extract 200 mg/kg	0.74 ± 0.14^{ns}	1.56 ± 0.12^{ns}	2.5 ± 1.2**	3.6 ± 0.11**	4.78 ± 0.12 ****	127.8 ± 3.93 **	64.2 ± 2.92^{ns}	146 ± 3.38^{ns}	0.76	1.99
Extract 400 mg/kg	0.46 ± 0.07^{ns}	1.7 ± 0.17 ***	2.74 ± 0.19 ****	3.8 ± 0.13 ****	4.88 ± 0.32 ****	136.6 ± 2.64 ****	67.4 ± 3.87^{ns}	152.8 ± 4.57*	0.75	2.03

N=5; mean±SEM; $^{ns}P>0.05$; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ vs saline (control 10 ml/kg, p.o).

the effect of EECB (70.86 ± 1.23% at 1000 µg/ml). In aggregation assay, cystone had 71.18 ± 152% of inhibition while the extract only had 62.95 ± 4.11% of inhibition at 60 min. The increasing concentration of EECB had inhibited the calcium oxalate crystal growth in both nucleation and aggregation assay.

The anti-nociceptive effect of EECB was evaluated by chemical (formalin and acetic acid) and thermal models (Eddy's hot plate and tail-flick) of nociception. The formalin test has been divided into two phases. In first phase, substance "P" is released by direct stimulation of the sensorial C-fibers. The second phase is mediated by the increased level of PG, stimulation of COX, and release of nitric oxide. Two phases of pain response can be used to clarify the probable mechanism of anti-nociceptive. Centrally acting drugs prevent both phases of pain (such as opioids), whereas peripheral acting drugs only inhibit the second phase (inhibit COX activity, such as acetylsalicylic acid).[21,23] The tail-flick test measures spinal anti-nociceptive action and the hot plate measures supra-spinal anti-nociceptive action.[21,23] The antinociceptive effect in acetic acid, formalin, hot plate and tail-flick tests were altered by treating with EECB. These findings suggest that the anti-nociceptive activity of EECB is exhibited through central as well as peripheral mechanisms.

After 1h of injection of phlogistic agent (carrageenan, egg-albumin, and formalin), the first phase (early phase) of edema is by the release of histamine and serotonin; and after 2hr to 4hr of injection of phlogistic agent, the second (later phase) is by release of bradykinin, protease and prostaglandin.[21] In this study, EECB showed edema inhibition in both phases.

Standard drug furosemide enhances urine output and urinary excretion of Na^+, K^+, and Cl^- by inhibiting Na^+–K^+–$2Cl^-$ cotransport.[24,29] This could suggest that the mechanism of EECB is not the same as the furosemide. EECB increases urine output and urinary excretion of Na^+, Cl^- which may be due to inhibition Na^+-Cl^- symport like thiazide. Cl^-/ Na^++K^+ ratio was calculated and the ratios between 0.8 and 1.0 suggest that the carbonic anhydrase inhibition (CAI) mechanism is not applicable and as ratio decreases below 0.8 indicate slight to strong CAI.[21,29] The Cl^-/Na^++K^+ ratio for EECB at doses of 200 mg/kg, 400 mg/kg, and furosemide showed a very weak Carbonic anhydrase inhibitory effect with values of 0.76, 0.75, and 0.72, respectively. EECB at 400 mg/kg possesses significant diuretic activity which may be improve the anti-urolithiatic effect by decreasing the supersaturation of stone-forming compound.[30]

CONCLUSION

It was confirmed that EECB possesses significant antiurolithiatic activity. The calcium oxalate crystals induce oxidative stress that leads to papillary calculi formation causing damage to epithelial cells, thus increasing oxalate crystal binding to cells and forming the nidus for urolithiasis. Antioxidants properties of EECB could be used in preventing oxidative stress and the formation of intra-papillary calcifications. The anti-inflammatory and analgesic properties of EECB could be used in the prevention of inflammation and pain of damaged epithelial cells of nephrons, which are caused by intra-papillary calcifications. The diuretic activity of EECB helps in reducing supersaturation of calcium oxalate, which proves that EECB can be used as an effective antiurolithiatic drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H, Zeng G. Epidemiology of urolithiasis in Asia. Asian J Urol. 2018;5(4):205-14. doi: 10.1016/j.ajur.2018.08.007, PMID 30364478.
2. Patel VB, Acharya N. Effect of *Macrotyloma uniflorum* in ethylene glycol induced urolithiasis in rats. Heliyon. 2020;6(6):e04253. doi: 10.1016/j.heliyon.2020.e04253, PMID 32637686.
3. Ashok P, Koti BC, Vishwanathswamy AHM. Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats. Ind J Pharmacol. 2010;42(6):380-3. doi: 10.4103/0253-7613.71925, PMID 21189910.
4. Kieley S, Dwivedi R, Monga M. Ayurvedic medicine and renal calculi. J Endourol. 2008;22(8):1613-6. doi: 10.1089/end.2008.0020, PMID 18620498.
5. Ata A, Gale EM, Samarasekera R. Bioactive chemical constituents of *Caesalpinia bonduc* (Fabaceae). Phytochem Lett. 2009;2(3):106-9. doi: 10.1016/j.phytol.2009.02.002.
6. Jana K, Chatterjee K, Ali KM, De D, Bera TK, Ghosh D. Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of *Caesalpinia bonduc* on streptozotocin-induced diabetes in male albino rats. Pharmacogn Res. 2011;4(1):57-62.
7. Liu T, Wang M, Qi S, Shen X, Wang Y, Jing W, Yang Y, Li X, Gao H. Bioorganic Chemistry New cassane-type diterpenoids from kernels of *Caesalpinia bonduc* (Linn.) Roxb. Bioorg Chem. 2020;96:103573. doi: 10.1016/j.bioorg.2020.103573.
8. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi ML. Antitumor Activity and Antioxidant Status of *Caesalpinia bonducella* Against Ehrlich ascites Carcinoma in Swiss albino Mice. J Pharmacol Sci. 2004;94(2):177-84. doi: 10.1254/jphs.94.177, PMID 14978356.

9. Datté JY, Traoré A, Offoumou AM, Ziegler A. Effects of leaf extract of *Caesalpinia bonduc* (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats. J Ethnopharmacol. 1998;60(2):149-55. doi: 10.1016/s0378-8741(97)00144-x, PMID 9582005.
10. Datté JY, Yapo PA, Kouamé-Koffi GG, Kati-Coulibaly S, Amoikon KE, Offoumou AM. Leaf extract of *Caesalpinia bonduc* Roxb. (Caesalpiniaceae) induces an increase of contractile force in rat skeletal muscle *in situ*. Phytomedicine. 2004;11(2-3):235-41. doi: 10.1078/0944-7113-00292, PMID 15070178.
11. Bashir S, Gilani AH. Antiurolithic effect of *Bergenia ligulata* rhizome: an explanation of the underlying mechanisms. J Ethnopharmacol. 2009;122(1):106-16. doi: 10.1016/j.jep.2008.12.004, PMID 19118615.
12. Abu F, Mat Taib CN, Mohd Moklas MA, Mohd Akhir S. Antioxidant properties of crude extract, partition extract, and fermented medium of *Dendrobium sabin* Flower. Evid Based Complement Alternat Med. 2017;2017:2907219. doi: 10.1155/2017/2907219.
13. Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z. *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Suef Univ J Bas Appl Sci. 2017;6(1):10-4.
14. Khan MA, Rahman AA, Islam S, Khandokhar P, Parvin S, Islam MB, Hossain M, Rashid M, Sadik G, Nasrin S, Mollah MN, Alam AH. A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba L*. (Moraceae). BMC Res Notes. 2013;6:24. doi: 10.1186/1756-0500-6-24, PMID 23331970.
15. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac J Trop Biomed. 2012;2(12):960-5. doi: 10.1016/S2221-1691(13)60007-6, PMID 23593576.
16. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. Food Front. 2020;1(1):60-9. doi: 10.1002/fft2.10.
17. Bawari S, Negi Sah AN, Tewari D. Antiurolithiatic activity of *Daucus carota*: an *in vitro* study. Pharmacogn J. 2018;10(5):880-4. doi: 10.5530/pj.2018.5.148.
18. Abu Zarin MA, Tan JS, Murugan P, Ahmad R. Investigation of potential anti-urolithiatic activity from different types of Musa pseudo-stem extracts in inhibition of calcium oxalate crystallization. BMC Complement Med Ther. 2020;20(1):317. doi: 10.1186/s12906-020-03113-0, PMID 33076892.
19. Sasikala V, Ramu S, Vijayakumari B. *In vitro* evaluation of *Rotula aquatica* Lour. for antiurolithiatic activity. J Pharm Res. 2013;6(3):378-82.
20. Bukhari IA, Gilani AH, Meo SA, Saeed A. Analgesic, anti-inflammatory and antiplatelet activities of *Buddleja crispa*. BMC Complement Altern Med. 2016;16:79. doi: 10.1186/s12906-016-1021-4, PMID 26911873.
21. Vogel HG, Vogel WH, Schölkens BA, Sandow J, Müller G, Vogel WF. Drug discovery and evaluation: pharmacological assays. 3rd ed. Berlin, Germany: Springer-Verlag; 2007.
22. Zeb A, Ahmad S, Ullah F, Ayaz M, Sadiq A. Anti-nociceptive activity of ethnomedicinally important analgesic plant *Isodon rugosus* Wall. ex Benth: mechanistic study and identifications of bioactive compounds. Front Pharmacol. 2016;7:200. doi: 10.3389/fphar.2016.00200, PMID 27458379.
23. Rezaee-Asl M, Sabour M, Nikoui V, Ostadhadi S, Bakhtiarian A. The study of analgesic effects of *Leonurus cardiaca* L. in mice by formalin, tail flick and hot plate tests. Int Sch Res Not. 2014;2014:1-5. doi: 10.1155/2014/687697, PMID 687697.
24. Gasparotto A Jr, Boffo MA, Lourenço EL, Stefanello ME, Kassuya CA, Marques MC. Natriuretic and diuretic effects of *Tropaeolum majus* (Tropaeolaceae) in rats. J Ethnopharmacol. 2009;122(3):517-22. doi: 10.1016/j.jep.2009.01.021, PMID 19429322.
25. Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of *Acorus calamus* L. rhizome in experimental animal models. J Trad Complement Med. 2016;6(4):431-6. doi: 10.1016/j.jtcme.2015.12.004, PMID 27774431.
26. Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J Med Life. 2016;9(3):259-62. PMID 27974930.
27. Kizivat T, Smolic M, Maric I, Tolušic Levak M, Smolic R, Bilic Curcic I, Kuna L, Mihaljevic I, Vcev A, Tucak-Zoric S. Antioxidant pre-treatment reduces the toxic effects of oxalate on renal epithelial cells in a cell culture model of urolithiasis. Int J Environ Res Public Health. 2017;14(1):109. doi: 10.3390/ijerph14010109, PMID 28125004.
28. Marhoume FZ, Aboufatima R, Zaid Y, Limami Y, Duval RE, Laadraoui J, Belbachir A, Chait A, Bagri A. Antioxidant and polyphenol-Rich ethanolic Extract of *Rubia tinctorum* L. Prevents Urolithiasis in an ethylene glycol Experimental Model in Rats. Molecules. 2021;26(4):1005. doi: 10.3390/molecules26041005, PMID 33672875.
29. Hailu W, Engidawork E. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of *Ajuga remota* Benth (Lamiaceae) leaves in mice. BMC Complement Altern Med. 2014;14:135. doi: 10.1186/1472-6882-14-135, PMID 24720845.
30. Siddiqui WA, Shahzad M, Shabbir A, Ahmad A. Evaluation of anti-urolithiatic and diuretic activities of watermelon (*Citrullus lanatus*) using *in vivo* and *in vitro* experiments. Biomed Pharmacother. 2018;97:1212-21. doi: 10.1016/j.biopha.2017.10.162, PMID 29145146.

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