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Research Article

Evaluation of diuretic, anti-urolithiatic activities of ethanolic flower extract of *Michelia champaca* (yellow variety)

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Abstract: The aim of present study was to investigate the diuretic and anti-urolithiatic activities of ethanolic flower extract of *Michelia champaca* (yellow variety). Rats were used as an experimental animal model and treated orally with ethanolic flower extract dose of 250mg/kg and 500mg/kg, furosemide (5mg/kg) was used as standard drug in diuretic. The rats treated with ethanolic flower extract of *Michelia champaca* showed higher urine output and increased excretion of electrolytes when compared to control. By administering ethylene glycol (0.75% v/v) in drinking water for 28days urolithiasis was induced in male rats and parameters like phosphate, oxalate and calcium were estimated in urine and creatinine, calcium and uric acid were estimated in serum. The flower extract 250mg/kg and 500mg/kg was assessed for anti-urolithiatic activity for its curative regimen. Cystone (750mg/kg) was used as standard in anti-urolithiatic activity. The results obtained were significant.

Key Words: Diuretic, Anti-urolithiatic, *Michelia champaca*, Saluretic, Natriuretic effect.

Introduction

Diuretics are the substances that increase the excretion of water and electrolytes from the body (k. Sri Devi *et al.,* 2014). Diuretics are used in many pathological conditions like heart diseases, hypertension, liver cirrhosis, water poisoning and certain kidney diseases.

Urolithiasis is referred to the stone formation, it is also known as nephrolithiasis. It can be produced in rats by stimulation of acute and chronic hyperxaluria by wide variety of agents like ethylene glycol, sodium oxalate, ammonium oxalate and glycolic acid (Khan 1997). Renal calculi are formed when urine is supersaturated with salts and minerals like calcium, uric acid, oxalate, cysteine and phosphate (Worcester EM, Coe FL 2010). 60-80% of calculi contain calcium (Nice CKS, 2009).

Michelia champaca (yellow variety) commonly known as sampangi or champaka. It has been reported to possess antipyretic, anti-inflammatory, (Vimal R *et al.,* 1997) insecticidal, antimicrobial and leishmanicidal activity. Flower oil is used in cephalalgoa, opthalmia and gout. Leaves have been reported for anti-hyperglycimic activity (Sumeet Gupta *et al.,* 2011). Diuretic activities have been reported on leaves and stem bark (Ahmad *et al.,* 2011). The work on flower was not reported, hence this research study is taken up to evaluate.

Materials and Methods

Plant materials & Extraction:

The flowers of *Michelia champaca* (sampangi) were collected from the village kadiyam. The plant was identified and authenticated by T. Raghu ram, taxonomist, Maharani College, Pedhapuram. Flowers were dried under shade and then coarsely powdered. The powder was macerated in ethanol for a period of 72 hours and then subjected to hot percolation and distillation. The obtained solution was filtered and dried.

Experimental animals

Healthy male albino rats weighing 200-250gms were selected and housed in a temperature and light/dark controlled room (25^0c; 14 hrs. /10hrs light and dark cycles) with free access to food and water prior to the studies animals were acclimatized to the laboratory conditions for period of 1 week.

Diuretic activity

The diuretic activity was determined by Lipschitz method (Lipschitz *et al., 1943).* 24 rats were fasted for 18 hours and deprived of water prior to the experiment. The primary dose of 25ml/kg of normal saline was given to all the rats. The rats were divided into 4 groups each containing 6 rats.

Immediately after administration rats were placed in metabolic cages, one rat per cage. The metabolic cages were provided with a funnel for urine collection and mesh to separate faeces from urine. The bladder was emptied by pulling the base of tail of each rat

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(Vogel GH1997). The urine was collected in beaker and covered with aluminium foil to avoid evaporation. The volume of urine collected after 5 hrs was recorded and subjected to analysis for determination of sodium, potassium ions by flame photometry (Jeffery GH1998). Chloride and bicarbonate ions by titrimetric analysis (Beckette AH & stenlake, 1997) after 24hrs. The saluretic, natriuretic and diuretic indexes were calculated.

Group I	Control	Treated with vehicle-0.5% acacia orally.
Group II	Standard	Treated with furosemide 5mg/kg P.o
Group III	Test (low dose)	Treated with ethanolic flower extract of *Michelia champaca* 250mg/kg orally
Group IV	Test (high dose)	Treated with ethanolic flower extract of *Michelia champaca* 500mg/kg orally

Anti-urolithiatic Activity

Ethylene glycol-induced hyperoxaluria method was used to assess the Anti-urolithiatic activity in male albino rats. Animals were divided into 5 groups, 6 animals in each. Ethylene glycol (0.75% v/v) in drinking water was given to all the groups for induction of renal calculi for 28 days (Mitra SK *et al.,* 1998). The ethanolic extract was assessed for Anti-urolithiatic activity for its curative action in urolithiasis.

Group I	Normal control	Fed regular fat food & drinking water.
Group II	Positive control	Received ethylene glycol (0.75% v/v b.w) in drinking water for induction of renal caliculi-28 days
Group III	Standard	Received ethylene glycol & std. Antiurolithiatic drug Cystone (750mg/kg) from 15^{th}-18^{th} day.
Group IV	Test low dose	Received 250mg/kg of flower extract from 15^{th}-28^{th} day.
Group V	Test high dose	Received 500mg/kg of flower extract from 15^{th} -28^{th} day.

Assessment of Anti-urolithiatic activity

Collection and Analysis of urine: Rats were kept separately in metabolic cages and urine samples of 24hrs were collected on 28^{th} day. To the urine, a drop of Conc. Hydrochloric acid was added before being stored at 4^0c. Calcium, phosphate and oxalate content were analysed in the urine sample.

Serum analysis: At the end of experiment, blood samples were collected from the tail vein and analysed for creatinine, calcium, and uric acid using biochemistry analyzer.

Statistical analysis:

The results were expressed as the mean±SEM and analysed using one-way ANOVA followed by Dunnet's multiple comparison tests. Data was computed for statistical analysis using Graph Pad Prism software and P<0.001 was considered to be statistically significant.

Results

The results obtained in diuretic activity are summarized in Table 1& 2. Table 1 contain urine volume (ml/5hrs) and excretion of electrolytes ($Na^{+,}$ K^+, Cl^-&HCO_3^-) in urine. Table 2 include saluretic, natriuretic and diuretic indexes of ethanolic flower extract of *Michelia champaca.* From the results it was observed that ethanolic flower extract exhibits significant diuretic activity by improving the urine output and excretion of sodium, potassium, chloride and bicarbonate levels when compared to that of control. Table 3 include urine constituents (calcium, oxalate &phosphate) and serum constituents (calcium, creatinine & uric acid) of control, standard and Ethanolic flower extract of *Michelia champaca.*

Table 1: Comparison of diuretic effect of *Michelia champaca* to that of control

Group	Volume of urine (mL) after 5hrs	Na+ µmoles/Kg	K+ µmoles/Kg	Cl— µmoles/Kg	HCO3— µmoles/Kg
Control	0.15±0.04	173.3±0.35	121.48±0.48	98.69±0.59	9.97±0.17
Standard	0.74±0.01	232.14±0.65	144±0.2	152±0.39	25±0.33
MC-250mg/kg	o.24±0.01	183±0.29	125±0.14	137±0.14	13.2±0.02
MC-500mg/kg	0.57±0.03	197±0.23	132±0.11	145±0.44	20±0.17

Values are expressed as Mean± SEM; n=6 (number of animals in each group); p<0.001. All comparisons are made with that of control.

Table 2: Comparison of saluretic, natriuretic and diuretic indexes of *Michelia champaca* to that of control.

Group	Saluretic Index [Na++ Cl–]	Natriuretic Index [Na+/ K+]	Diuretic Index
Control	272.02	1.42	-
Standard	384	1.61	4.9
MC-250mg/kg	304	1.33	1.38
MC-500mg/kg	329	1.35	2

Diuretic Index = {volume of urine in test group/volume of urine in control}

Table 3: Comparison of Antiurolithiatic activity of *Michelia champaca* to that of control in rats.

Parameter Unit	Group I (Control)	Group II (calculi Induced)	Group III (Cystone Treated) 750mg/kg	Group IV (CO 250mg/kg)	Group V (CO 500mg/kg)
Calcium	0.61±0.14	3.61±0.13	0.59±0.03	3.15±0.13	2.25±0.17
Phosphorous	1.09±0.08	3.77±0.18	1.58±0.04	5.36±0.26	3.08±0.12
Oxalate	3.32±0.21	6.91±0.21	3.69±0.14	1.25±0.18	0.54±0.02
Serum(mg/dl)					
creatinine	7.98±0.53	14.38±0.45	7.09±0.24	0.80±0.04	0.9±0.017
Uric acid	0.71±0.02	1.01±0.04	0.78±0.26	5.17±0.21	3.18±0.16
calcium	3.91±0.25	6.1±0.2	4.38±0.19	13.1±0.16	10.48±0.13

Values are expressed as Mean±SEM, n=6(number of animals in each group) P<0.001. All comparisons are made with that of control.

Ethylene glycol 0.75% (v/v) by chronic administration induce Hyperoxaluria increases oxalate, calcium and phosphate in urine and serum calcium, creatinine and uric acid (Group2). Ethanolic flower extract decreases the elevated levels of calcium, oxalate and phosphate in urine and serum creatinine, uric acid and calcium (Group4 and Group5).

Discussion

The present study reveals that ethanolic flower extract *of Michelia champaca* exhibit dose dependent diuretic activity. It has been established that improved regional blood flow, vasodilation and inhibition of tubular secretion contributes to improved urinary excretion. They act by decreasing sodium reabsorption at different sites in the nephron, thereby increasing urinary sodium and different electrolytes and water loss (Stanic *et al., 1993*). Diuretics are used in different pathological conditions like oedema and hypertension because of its ability to induce negative fluid balance. Any of this process may be associated with diuretic activity of flower extract.

The principle target for Ethylene glycol induced toxicity was kidney. Its administration to the rats for 18 days resulted in extensive excretion of oxalate and deposition of microcrystals in kidney. When the urine is supersaturated with salt and minerals like calcium, phosphate, uric acid, and cysteine, oxalate leads to renal calculi. The glomerular filtration rate declines due to the damage of renal parenchyma in urolithiasis. The additional factor that leads for stones production is the development of Randall's plaque (Wolf J *et al.,* 2012). In the basement membrane of the loop of Henle, calcium oxalate precipitates; these ultimately accumulate in the sub epithelial space of the renal papillae, leading to Randall's plaque and ultimately a calculus.

Treatment with Ethanolic flower extract depresses the levels of calcium, phosphate and oxalate in urine excretion and serum - creatinine, uric acid and calcium. The decline in serum and urine levels is due to anti-urolithiatic activity of *Michelia champaca.*

The plant *Michelia champaca* also favours anti-urolithiatic activity in addition it has a diuretic activity by quicken the process of dissolving or by cleansing the preformed stones by improving urinary excretion. The probable mode of action of *Michelia champaca* may owe to decrease in the urinary concentration of the salts that avoid super saturation of the crystallizing salts by increasing its urinary output.

Conclusion

Ethanolic extract of *Michelia champaca* flowers possess diuretic activity by increasing urine output and excretion of sodium, potassium, chloride and bicarbonate ions and also have significant anti-urolithiatic activity by lowering increased levels of salts and minerals like creatinine, uric acid, calcium in serum and calcium, oxalate, phosphate in urine. However, further clinical assessment is required to support this proposal.

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