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ANTIUROLITHIATIC EFFECT OF OLEANOLIC ACID ISOLATED FROM *LANTANA CAMARA* ON GLYCOLIC ACID-INDUCED UROLITHIASIS IN RATS

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ABSTRACT

The present study was done to evaluate the antiurolithiatic effect of ethanolic extract of roots (ELC 200mg/kg) and oleanolic acid (O.A. 60 mg/kg, O.A. 80 mg/kg, O.A. 100 mg/kg) isolated from roots of *Lantana camara* on Glycolic Acid-induced Urolithiasis in Rats. Oxalate urolithiasis was produced by the addition of 3% glycolic acid to the diet for a period for 42 days. Glycolic acid treatment resulted in a significant increase in the levels of calcium and oxalate in the kidney as well as in the total kidney weight. Also, the urinary levels of calcium, oxalate and inorganic phosphorus were increased. Oleanolic acid treatment at a dose 60 mg/kg, 80 mg/kg, 100 mg/kg and ethanolic extract of roots at a dose 200mg/kg for 42 days revealed a dose-related effect in the reduction of lithogenic substances, following glycolic acid induced urolithiasis. Results suggest that the Oleanolic acid had significantly reversed the glycolic acid-induced urolithiasis, presumably by preventing the urinary supersaturation of lithogenic substances, especially of oxalate and calcium in a dose dependant manner. These observations indicate that ethanolic extract of roots and oleanolic acid can play an important role in the prevention of such disorders.

Keywords: Antiurolithiatic activity, Glycolic acid, *Lantana camara*, Oleanolic acid.

INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia [1]. The worldwide incidence of urolithiasis is quite high [2]. and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis [3].

It is estimated that about 12% of men and 55 % of women have at least one episode of kidney stone during their life time. Once kidney stone develops the recurrence rate is estimated to be 14% at 1 year, 35% at 5 years, and 52% at 10 years. The incident in general population is about 1 in 1000 adults per year. The main cause of urolithiasis is still unknown but probably positive family history, Overweight, obesity, or increased BMI, Low urine volume < 1500 ml/day, High dietary animal protein intake, Increased urine excretion of calcium oxalate, uric acid, Cystine, Urinary tract structural abnormalities leading to stasis of urine flow [4].

Lantana camara (L. camara), also known as Spanish Flag or West Indian Lantana, is a species of flowering plant in the verbena family, Verbenaceae, which is native to the American tropics. Its Ayurvedic names are Chaturaangi, Vanachchhedi and in Hindi it is commonly known as Raimuniya. *L. camara* has covered large areas in India, Australia and much of Africa [5].

In the present study, antiurolithiatic effect of ethanolic extract of roots (ELC 200mg/kg) and oleanolic

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acid (O.A. 60 mg/kg, O.A. 80 mg/kg, O.A. 100 mg/kg) isolated from roots of *Lantana camara* was evaluated for its effects on experimentally induced urolithiasis in rats.

MATERIALS AND METHODS

Plant material

The roots of *Lantana camara* were procured from local areas of Bhopal (Madhya Pradesh, India) and authenticated from Department of Botany, Safia College Bhopal (Voucher No. 280/bot/saf/11). The roots were then allowed to dry in air and crushed in small pieces and powdered for extraction.

Plant extraction

The powdered roots of L. camara were extracted with ethanol using maceration method. The extract was then dried and stored. Phytochemical screening of the extract was done and results show the presence of tannins, protein, reducing sugars, triterpenoids etc. in ethanolic extract of L. camara roots [6].

Isolation of oleanolic acid (OA)

The powdered crude drug was defatted thrice in cold overnight with petroleum ether and then extracted exhaustively with ethanol four times over night at room temperature. The solvent was removed under vacuum at 40 ° C and the crude extract was dissolved in chloroform and left over night for precipitation. The precipitate so obtained was crystallized with Methanol. Precipitation and crystallization process were repeated 4 times, which gave oleanolic acid crystals [7].

Animals

Healthy male albino Wistar rats of 150-250 g body weight were used for this study. The animals were housed in polypropylene cages and maintained under standard conditions (12 hrs light and dark cycles, at 25±30C and 35-60% humidity). Standard palletized feed and tap water were provided ad libitum. The study was approved by Institutional Animal Ethical Committee of Sapience Bio-analytical Research Laboratory (SBRL), Bhopal, India, registered under CPCSEA, India (Registration No. 1413/a/11/CPCSEA).

Glycolic acid induced Urolithiasis

The rats were divided into seven groups of six each. Rats of group I received the commercial diet and served as control, group II was fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 42 days [8]. Groups III, IV, V, VI and VII received Cystone 500 mg/kg, ethanolic extract of roots (ELC 200mg/kg) and oleanolic acid at three different doses (O.A. 60 mg/kg, O.A. 80 mg/kg, O.A. 100 mg/kg) respectively once a day orally in addition to the CPD for 42 days [9, 10].

Collection and analysis of urine samples

On day 42, immediately after administration of the respective assigned doses, the rats were housed in metabolic cages for 24 h urine collection. A drop of concentrated hydrochloric acid was added to the collected urine and stored at 4°C. Levels of oxalate [11] calcium [12] and inorganic phosphorus [13] were determined spectrophotometrically. Sodium and potassium were estimated using a flame photometer.

Result and Discussion

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. In this context, the changes in urinary oxalate levels are relatively much more important than those of calcium. In the present study, feeding 3% glycolic acid resulted in hyperoxaluria, which is known to be due to the ready conversion of glycolic acid to oxalate by the oxalate synthesizing liver enzyme glycolate oxidase [14]. Hyperoxaluria is usually the initiating factor of oxalate urolithiasis. Glyolic acid, the precursor of oxalic acid, is known to increase significantly the incidence of oxalate lithiasis [15]. Our results are in agreement with these studies, as shown by the significant increase in kidney weight. The increase in urinary calcium and oxalate levels were also found to be highly significant. Oleanolic acid at a dose 60 mg/kg, 80 mg/kg, 100 mg/kg revealed a dose related response. Cystone treatment at dose 500 mg/kg showed a better protective effect. Oleanolic acid treated groups showed decrease in oxalate values ($p<0.01$) probably by its inhibitory action on glycolate oxidase. Cystone treatment also significantly lowered the oxalate values. The reduction in the urinary oxalate level will be beneficial in preventing the urinary supersaturation with respect to oxalate. Urinary sodium and urinary potassium excretion was significantly elevated in the Oleanolic acid and ELC treated animals. Calcium and phosphorus play a vital role in renal calculogenesis. Calcium and inorganic phosphorus levels were also elevated in the rats receiving a calculi-producing diet. The increase in calcium excretion may be due to defective tubular reabsorption in the kidneys [16]. Cystone treatment markedly reduced the levels of calcium and phosphorus ($p<0.01$) in urine. Similarly Oleanolic acid and ELC treated animals also showed reduced the levels of calcium and phosphorus in a dose dependant manner (Table 1).

There was a significant increase in the kidney weight of animals receiving 3% glycolic acid which was almost normalized in the Oleanolic acid treated animals in a dose dependant manner. ELC treated animals also showed preventive effect against increase in kidney weight. Increase in the kidney weight was also normalized in the Cystone treated animals. (Table 2)

Table 1. Effect of oleanolic acid and ELC on various parameters in Glycolic acid induced urolithiasis model

Groups	Oxalate (mg/24h)	Calcium (mg/24h)	Inorganic Phosphorus (mg/24h)	Sodium (mEq/24h)	Pottasium (mEq/24h)
Control	12.28±0.82**	4.20±0.29***	0.919±0.118**	10.22±0.96***	11. 67±0.51***
Negative Control	24.10±2.80	7.15±0.72	1.586±0.180	4.22±0.72	6.51±0.48
Cystone (500mg/kg)	12.42±2.81**	3.67±0.46***	0.802±0.121**	10.42±1.32***	10.12±2.24
OA (60mg/kg)	15.11±2.10**	5.16±0.42	1.189±0.121	7.24±1.34**	7.32±1.69
OA (80mg/kg)	14.52±1.98**	4.30±0.30**	1.115±0.113	8.26±1.19**	8. 89±1.56
OA (100mg/kg)	13.10±2.95**	3.81±0.31***	0.888±0.136**	10.14±1.11***	10.78±1.92
ELC (200mg/kg)	18.89±3.37	5.82±0.89	1.211±0.112	6.51±0.89*	8.44±1.51

Value represents, Mean ± S.E.M. (n=6): Statistical analysis was performed by Dunnett's Multiple Comparison test. *p< 0.05, **p< 0.01, ***p< 0.001 as compared with group II

Table 2. Effect of Oleanolic acid and ELC on kidney weight in Glycolic acid induced urolithiasis model

Groups	**Wet weight** (g/100g b.wt.)	**Dry weight** (g/100g b.wt.)
Control	0.341±0.0052***	0.087±0.0016***
Negative Control	0.429±0.0086	0.119±0.0018
Cystone (500mg/kg)	0.352±0.008***	0.092±0.0018***
OA (60mg/kg)	0.406±0.016**	0.112±0.0012**
OA (80mg/kg)	0.400±0.018**	0.103±0.0020**
OA (100mg/kg)	0.355±0.014***	0.094±0.0022***
ELC (200mg/kg)	0.413±0.0018**	0.119±0.0018**

Value represents, Mean ± S.E.M. (n=6): Statistical analysis was performed by Dunnett's Multiple Comparison test. *p< 0.05, **p< 0.01, ***p< 0.001 as compared with group II

CONCLUSION

Glycolic acid feeding for 42 days resulted in renal tissue deposition of calcium and oxalate. The increased deposition of calcium and oxalate in the renal tissue is known to lead to papillary calcification and eventual calculi formation [17]. A similar elevation in renal stone forming constituents in rats fed with CPD has been reported earlier [18]. Oleanolic acid and ELC administration significantly reduced both calcium and oxalate levels in kidneys, which is known to prove beneficial in preventing calculi formation due to supersaturation of these lithogenic substances. These effects suggest the antilithic and lithotriptic property of this Oleanolic acid and ELC.

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