

DIURETIC POTENTIAL OF OLEANOLIC ACID ISOLATED FROM *LANTANA CAMARA*

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## ABSTRACT

The present investigation deals with the evaluation of diuretic potential of ethanolic extract of roots and Oleanolic acid isolated from roots of *Lantana camara*. The diuretic potential of different concentration of Oleanolic acid (O.A. 60 mg/kg, O.A. 80 mg/kg, O.A. 100 mg/kg) and ethanolic extracts of roots of *L. camara* (ELC) was assessed in albino rats using *in-vivo* method described by Lipschitz et al. (1943). The volumes of urine, urinary concentration of sodium and potassium ions were the parameters of the study. Furosemide was used as standard. The results indicate that in comparison to the control group, the urine output in OA (60 mg/kg/p.o.; 80 mg/kg/p.o. and 100 mg/kg/p.o.) treated group was found to be increased as evident in 5th and 24th hour readings. The increase in urine output was statistically significant at all the treatment levels.

**Keywords:** Oleanolic acid, Roots, *Lantana camara*, Diuretic activity, Ethanolic extract.

## INTRODUCTION

*Lantana 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, Vanachchedi and in Hindi it is commonly known as Raimuniya.

*L. camara* is an invasive species and has covered large areas in India, Australia and much of Africa. The plant contains various pentacyclic triterpenoids. New Compounds are lantanoside, lantanone, Methyl ursoxylate, Lancamaric acid, Ursoxy acid, Ursangilic acid, Ursethoxy acid, Camangelogyl acid, Linaroside, Camarinic acid, Oleanolic acid acetate, Oleanolic acid, Octadecanoic acid, Lantanilic acid, Ursonic acid. The essential oil was characterized by a high percentage of sesquiterpenes. The major components were: (E)-nerolidol (43.4%),  $\gamma$ -cadinene (7.6%),  $\alpha$ -humulene (4.9%) and  $\beta$ -caryophyllene (4.8%). The major constituents in the fruits oil were palmitic acid (22.8%), stearic acid (12.8%) and germacrene-D (7.1%), while the major constituents in the stem oil were palmitic acid (32.7%) and stearic acid (23.9%). (Sharma et al. 1988) *L. camara* has several uses, mainly as an herbal medicine, extracts from the leaves exhibit antimicrobial, fungicidal, insecticidal and nematocidal activity. Lantana oil is sometimes used for the treatment of skin itches, as antiseptic for wounds, and for leprosy and scabies. In folk medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors and high blood pressure.<sup>1,2</sup>

## MATERIAL AND METHODS

## Procurement and authentication of Crude drugs

The roots of *Lantana camara* were procured from local areas of Bhopal (M.P.) 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 for extraction and isolation. The isolated Oleanolic acid was compared for purity with standard pure Oleanolic acid.

## Plant extraction

The dried and powdered roots of *L. camara* were extracted with ethanol using maceration method. The powdered roots were macerated with 100ml of ethanol in a closed flask and were occasionally shaken with 6hr time period and were allowed to stand for 18hr. After filtration, the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. The extract was then dried in and well stored.<sup>3</sup>

## 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 CHCl<sub>3</sub> 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. The yield of Oleanolic acid was found to be 0.9 % w/w.<sup>4</sup>

## Diuretic activity

## Animal care and handling

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 100 to 150 gm. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44-56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water ad libitum during experiment.<sup>5</sup>

## Oral diuretic activity

The method of Lipschitz *et. al.*, (1943) with some modification was employed for the assessment of oral diuretic activity.<sup>5</sup>

Albino rats were divided into six groups (Six in each) and were fasted and deprived of water for 10 hours.<sup>6</sup> Individual animals in all the study groups received 5 ml/100 g (body weight) Saline p.o. The negative control animals were orally administered 0.5% of CMC dissolve in saline at dose of 5 ml/100 g (body weight). The positive control group (Standard group) was given Frusemide 3 mg/kg body weight<sup>7</sup> administered in volume of 5 ml/100 g of body weight p. o.<sup>8</sup> The test groups received ELC (Ethanolic extract of *Lantana camara* roots at a dose of 200mg/kg) and Oleanolic acid at three different doses of 60 mg/kg (OA 60), 80 mg/kg (OA 80) and 100 mg/kg (OA 100) dissolved in 0.5% of CMC and given in volume of 5 ml/100 g of body weight p.o.<sup>9</sup>

The parameters taken for each individual rat were body weight before and after test period, urine volume, concentration of Na<sup>+</sup> and K<sup>+</sup> in urine.<sup>10,11</sup>

## RESULTS

In comparison to the control group, the urine output in OA (60 mg/kg/p.o.; 80 mg/kg/p.o. and 100 mg/kg/p.o.) and ELC treated group was found to be increased as evident in 5<sup>th</sup> and 24<sup>th</sup> hour readings. The increase in urine output was statistically significant at all the treatment levels.

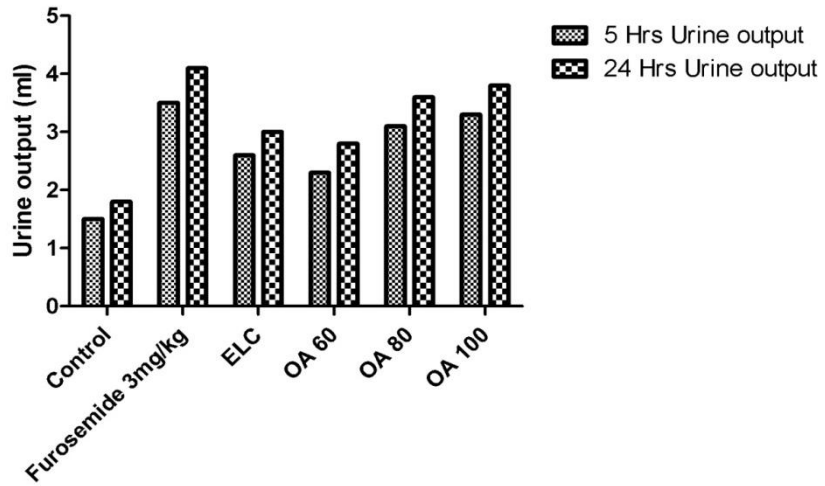


Figure- 1: Graph showing effect of Oleanolic Acid & ELC on urine output

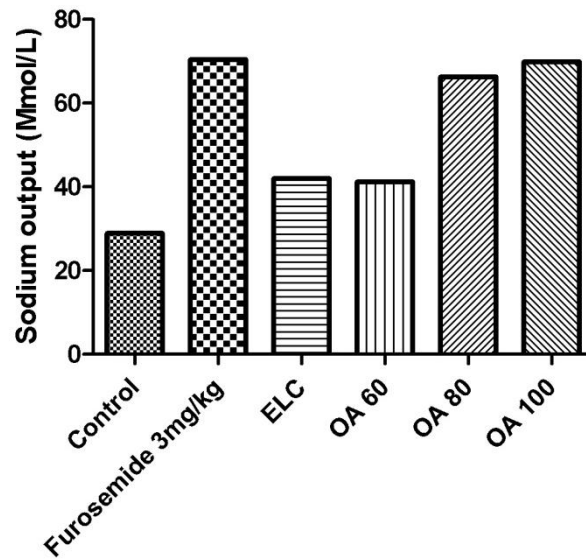


Figure- 2: Graph showing effect of Oleanolic Acid & ELC on sodium output

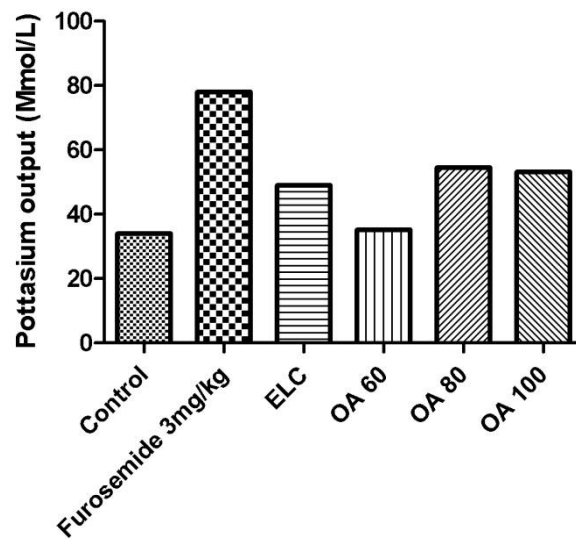


Figure- 3: Graph showing effect of Oleanolic Acid & ELC on potassium output

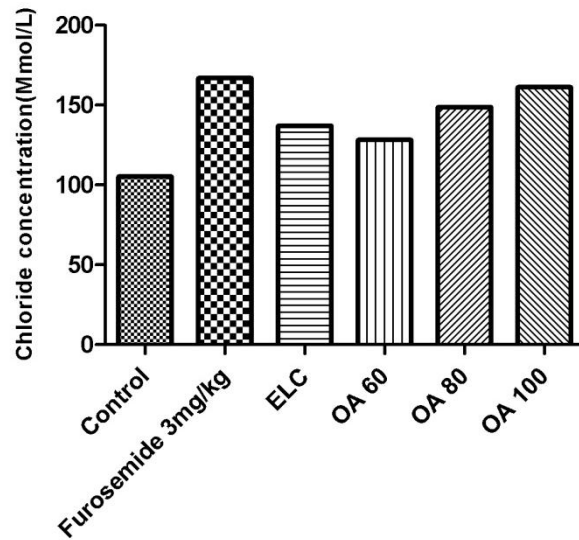


Figure- 4: Graph showing effect of Oleanolic Acid & ELC on chloride output

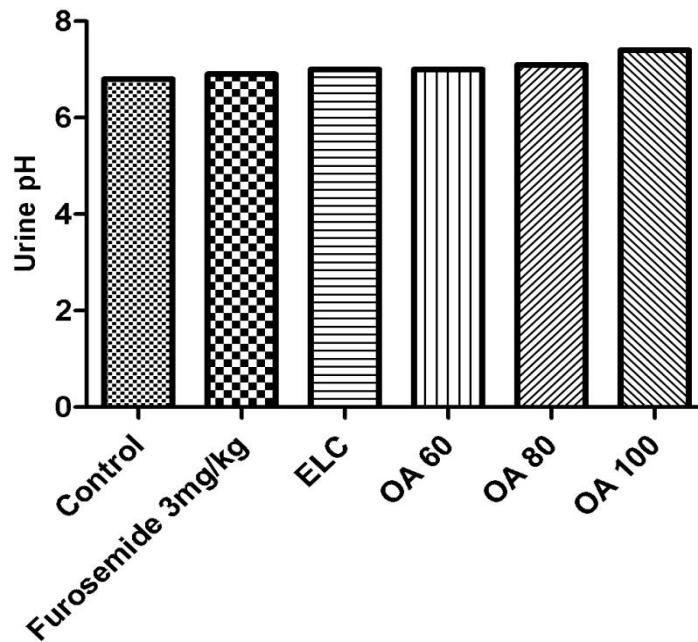


Figure- 5: Graph showing effect of Oleanolic Acid & ELC on urine pH.

Treatment with the OA and ELC at all the tested doses (60 mg/kg 80 mg/kg and 100 mg/kg) significantly increased Na<sup>+</sup> excretion and the natriuretic effect at 80 and 100 mg/kg dose was comparable to that of Furosemide (3 mg/kg, Lasix Injection, Aventis) treated group. OA was found to increase the K<sup>+</sup> excretion in all the treated dose levels, however statistically significant effect was observed only at 80 mg/kg. As compared to Furosemide treated group, the K<sup>+</sup> excretion was lesser in OA treated groups. The Cl<sup>-</sup> output increased significantly in Furosemide treated group and also in groups receiving ELC, 200mg/kg, O.A., 80 mg/kg and 100 mg/kg. The urinary pH was not significantly altered in Furosemide treated groups but in OA receiving groups the pH of the urine increased and the rise was statistically significant at 100 mg/kg.

Table 1-2 depicts the results of effects of OA on urine output, urinary electrolyte content and urinary pH in rats.

Table: 1 Effect of Oleanolic Acid & ELC on urine output

S.NO.	GROUPS	5 HRS URINE OUTPUT	24 HRS URINE OUTPUT
1.	Control	1.5 ± 0.1	1.8 ± 0.2
2.	Furosemide 3mg/kg	3.5 ± 0.8***	4.1 ± 0.3***
3.	ELC	2.6 ± 0.3*	3.0 ± 0.9*
4.	OA 60	2.3 ± 0.5	2.8 ± 0.3
5.	OA 80	3.1 ± 0.2***	3.6 ± 0.2***
6.	OA 100	3.3 ± 0.5***	3.8 ± 0.4***

Value represents, Mean ± S.E.M. Statistical analysis was performed by Dunnett's Multiple Comparison test \*p< 0.05, \*\*\* p< 0.001 as compared with Control.

**Table: 2 Effects of Oleanoloc Acid & ELC on urine parameters (concentraion of Na<sup>+</sup>, K<sup>+</sup> & CL<sup>-</sup>)**

S.NO.	GROUPS	SODIUM OUTPUT μ Mol/L	POTASSIUM OUTPUT μ Mol/L	CHLORIDE OUTPUT μ Mol/L	URINE pH
1.	Control	28.9±3.1	34±3	105± 5	6.8
2.	Furosemide 3mg/kg	70.4±4.2***	78±8.5***	166±9***	6.9
3.	ELC	42±3	49±5.1	137±4	7
4.	OA 60	41.2±4.1	35.2±4.3	128±5	7
5.	OA 80	66.3±3.4***	54.5±3.4*	148±8*	7.1
6.	OA 100	69.9±3.8***	53.2±4.3	161±12***	7.4*

Value represents, Mean ± S.E.M. Statistical analysis was performed by Dunnett’s Multiple Comparison test \*p< 0.05, \*\*\* p< 0.001 as compared with Control.

**DISCUSSION & CONCLUSION**

Somova et al., (2003) has reported the cardiovascular, antihyperlipidemic and antioxidant effects of OA. The antihypertensive effect has been attributed mainly to the potent antihyperlipidemic and antioxidant activity, combined with diuretic activity, due to inhibition of Na<sup>+</sup> and K<sup>+</sup> reabsorption in the early portion of the distal tubule. OA isolated from *Lantana camara* has showed significant diuretic potentials. Treatments of rats with ELC and OA increased the urine output both after 5 and 24 hours in comparison to control group suggesting that OA exerts diuresis. All the treatment groups, Furosemide treated and OA treated, showed increase in Na<sup>+</sup> ion excretion as compared to control group. However, the loss of K<sup>+</sup> was significantly less in ELC and OA treated groups as compared to Furosemide treated groups. This indicates that OA selectively spares K<sup>+</sup> ions from being excreted. Chloride output is also increased in Furosemide treated as well as ELC and OA treated groups. There was significant rise in the PH of urine in OA treated groups which negates the possibility of carbonic anhydrase (CA) inhibition as CA inhibitors are known to cause acidification of urine.

Further, the alkalinisation of urine is also helpful in dissolution of CaOx crystals. This effect of OA might have contributed to Antiuro lithiatic activity of OA as evident models of urolithiasis. The low urine pH favors the formation of urinary stone. <sup>12</sup>

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