

Nephroprotective effect of ethanolic extract of roots and oleanolic acid isolated from roots of *Lantana camara*

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ABSTRACT

Background: It is estimated that about 12% of men and 55% of women have at least one episode of kidney stone during their life time. The incident in general population is about 1 in 1000 adults per year.

Objectives: The present study was done to evaluate the nephroprotective effect of ethanolic extract of roots and oleanolic acid (OA) isolated from roots of *Lantana camara* (*L. camara*)

Materials and methods: The nephroprotective effect of different concentrations of oleanolic acid (60 mg/kg, 80 mg/kg and 100 mg/kg) and ethanolic extracts of roots of *L. camara* (ELC) at a dose of 200 mg/kg was assessed in albino Wistar male rats using gentamicin induced nephrotoxicity model. The parameters studied were urine volume, serum creatinine, urine creatinine, serum albumin, urine albumin, serum blood urea nitrogen, serum urea, urine blood urea nitrogen, urine urea, weight of kidney, body weight and glomerular filtration rate (GFR). Statistical analysis was performed by Dunnett's Multiple Comparison test. All values were represented as Mean \pm SEM. A p value < 0.05 was considered as statistically significant.

Results: Urine creatinine, serum creatinine, blood urea, blood urea nitrogen, serum albumin, urine albumin and weight of rat kidneys were found to be increased in rats treated with gentamicin, but these indicators of nephrotoxicity were normalized in OA (p < 0.001) and ELC (p < 0.05) treated rats in a dose dependent manner. Reduced level of GFR in gentamicin treated group was also normalized by OA and ELC treated groups in a dose dependent manner.

Conclusion: The result of the above study concludes that the OA showed promising nephroprotective activity in dose dependent manner. These results suggest the therapeutic utility of OA in renal injury.

Key words: *Lantana camara*, nephroprotective activity, oleanolic acid, gentamicin.

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INTRODUCTION

Nephrotoxic effects may develop in glomerular and tubular epithelial cells as a result of mechanisms that disrupt normal cellular functions of mitochondria and/or membrane integrity, induce renal injury through intratubular obstruction such as crystal disposition, and promote cellular swelling and tubular luminal occlusion so called osmotic effects. Medications can also cause chronic renal failure leading to chronic interstitial injury and papillary necrosis.^[1]

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.^[2]

The plant contains various pentacyclic triterpenoids. These are lantanoid, lantanone, methyl ursoxylate, lancamaric acid, ursoxy acid, ursangilic acid, ursethoxy acid, camangelogly acid, linaroid, camarinic acid, oleanolic acid

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acetate, oleanolic acid, octadecanoic acid, lantanilic acid and ursonic acid. The essential oil was characterized by a high percentage of sesquiterpenes. The major components were: (E)-nerolidol (43.4%), γ -cadinene (7.6%), α -humulene (4.9%) and β -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%).^[3-5] *L. camara* leaves extract had showed antimicrobial, fungicidal, insecticidal and nematocidal activity.^[6] Lantana oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds, and for leprosy and scabies. In folk medicine *L. camara* is used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors and high blood pressure.^[7]

The roots of *L. camara* are recommended for kidney stone disorders in the Indian traditional system of medicine.^[8] Oleanolic acid, a pentacyclic triterpenoids that has structural similarities with lupeol and betulinic acid and has strong diuretic action.^[9] Lupeol (Lupa-21, 20 29. dien, 3-ol) and betulin (Lupa-20 (29) ene-3, 28 diol) are known to possess antilithiatic and protective effect in nephrotoxicity induced by oxidative damage in animal models.^[10]

The present study was done to evaluate the protective effect of oleanolic acid isolated from roots of *L. camara* and ethanolic extract of roots of *L. camara*.

MATERIALS AND METHODS

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 $(25 \pm 3) ^\circ\text{C}$ and 35-60% humidity). Standard palletized (Golden feed, New Delhi, India) 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).

Chemicals

Gentamicin (Hi Media, Mumbai), carboxymethyl cellulose (Amar Cellulose Industries, Ahmedabad, Gujarat), creatinine kit (Accurex Biomedical Pvt.Ltd., India), urea/BUN Kit (Ranbaxy Fine Chemicals Limited, India) and albumin kit (Beacon Diagnostics Pvt.Ltd., India) were obtained.

Plant material

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

Preparation of plant extract

The powdered roots of *L. camara* were extracted with ethanol using maceration method.^[11] The extract was then dried and stored. Phytochemical screening^[12, 13] of the extract was done and results showed the presence of tannins, protein, reducing sugars, triterpenoids etc. in ethanolic extract of *L. camara* roots.

Isolation of oleanolic acid (OA)^[14]

The powdered crude drug was defatted thrice with cold petroleum ether, kept overnight and then extracted exhaustively with ethanol four times over night at room temperature. The solvent was removed under vacuum at $40 ^\circ\text{C}$ 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.

Study design

Six groups ($n = 6$) were used for this study. The naïve control group received only 0.4 ml of saline i.p. instead of gentamicin along with 3 ml of carboxymethyl cellulose (CMC) p.o. for

7 Days. The second gentamicin treated group received only gentamicin 100 mg/kg i.p.^[15] and 3ml of CMC p.o. for 7 Days. The remaining four groups were administered gentamicin 100 mg/kg i.p. and oleanolic acid at different doses of 40 mg/kg, 60 mg/kg, 80 mg/kg and ethanolic extract of *L. camara* roots (LC) at a dose of 200 mg/kg p.o. respectively dissolved in 0.5% of CMC.^[16]

Biochemical estimation

On the seventh day of the experiment, immediately after dosing, individual animals were placed in the metabolic cages for collection of 24 hour urine samples for determination of urine biochemical parameters and urine volume. 1 ml blood was withdrawn from each animal through retro-orbital puncture at the end of the urine collection period. Serum and urine samples were processed for creatinine, blood urea nitrogen and albumin concentration determination.^[17]

Histopathological examination

Three animals from each group were sacrificed^[18] after completion of 24 hours of urine collection. Kidney were removed and were weighed.^[19] Then kidneys were processed for histopathological examination.^[20] The kidneys were kept in 10 % neutral formalin solution. Both kidneys were processed and embedded in paraffin wax.^[21] The sections were stained with hematoxylin and eosin and were observed under light microscopy.

Statistical Analysis

Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison test. All values were represented as Mean \pm SEM. A p value of < 0.05 is considered as statistically significant.

RESULTS

Table 1 shows levels of biochemical parameters in various rat groups. The urine volume was found to be decreased in the gentamicin treated rats but significantly increased statistically in groups which received gentamicin along with OA ($p < 0.001$) and gentamicin along with ELC ($p < 0.05$). In all OA treated

groups, the rise was statistically significant ($p < 0.001$) as compared with gentamicin treated group.

Elevated level of creatinine, urea, and albumin in urine as well as in serum are indicator of impaired renal function. Urine creatinine, serum creatinine, blood urea, blood urea nitrogen, serum albumin, urine albumin and weight of rat kidneys were found to be increased in rats treated with gentamicin only. Marked elevation in serum creatinine and blood urea nitrogen was observed in gentamicin treated group, which is a marker of nephrotoxicity. These indicators of nephrotoxicity were normalized in OA and ELC treated rats in a dose dependent manner. Serum and urine albumin was found to be increased in the gentamicin treated group as compared to control group, and was found to be normalized by the OA treatment. Treatment with gentamicin reduced the GFR but treatment with OA increased the level of GFR in a dose dependent manner (Table 1).

Control rats showed normal glomerular and tubular histology whereas gentamicin was found to cause glomerular, peritubular, blood vessel congestion and inflammation in kidney cells. Concurrent treatment with OA was found to reduce such changes in kidney histology induced by gentamicin (Figures 1 to 6).

DISCUSSION

The mechanism of gentamicin induced nephrotoxicity is not completely known. However, reactive oxygen species particularly superoxide anion radicals involve in the pathophysiology of gentamicin nephrotoxicity. Reactive oxygen species including hydroxyl radical have been implicated in the gentamicin induced nephrotoxicity. It has been demonstrated that gentamicin administration increased renal cortical lipoperoxidation, renal nitric oxide generation and mitochondria H_2O_2 generation. Gentamicin also decreases the activities of catalase (CAT), glutathione peroxidase (GSHPx) enzymes and reduces the level of reduced glutathione (GSH)^[22, 23] whereas OA is known to preserve GSH level and also had been reported to possess antioxidant activities.

Thus, it is proposed that OA induced protection of gentamicin nephrotoxicity involves its antioxidant potentials.^[24]

Gentamicin is known to get actively transported into proximal tubules after glomerular filtration in a small proportion. It causes proximal tubular injury and abnormalities in renal circulation that leads to reduction of GFR, increased in creatinine, albumin and urea in serum as well as in urine.^[25-28]

Creatinine is excretory product of muscles and is mainly filtered by kidney and impaired kidney function causes increase in level of serum creatinine.^[29] Treatment with gentamicin increased the level creatinine. However, OA treated groups showed fortification from such changes.

Most proteins are too large to pass through the kidney filters into the urine unless the kidney is damaged. The main protein that is most likely to appear in urine is albumin.^[28] Gentamicin treated group showed increased in urine albumin, which indicates nephrotoxicity of gentamicin. Group received OA along with gentamicin showed protection from such changes.

Results of this study confirmed that gentamicin produces nephrotoxicity as evident by the reduction in GFR and increase in serum creatinine. This impairment in glomerular function was accompanied by an increase in BUN (Table 1).

Table 1: Effect of oleanolic acid and extracts of roots of *Lantana camara* on gentamicin Induced nephrotoxicity

Parameters	Control	Gentamicin treated	ELC	OA 60	OA 80	OA 100
Urine Volume (ml)	2.8 ± 0.15	2.2 ± 0.14	3.8 ± 0.9*	3.4 ± 0.16 [‡]	4.5 ± 0.17 [‡]	5.2 ± 0.11 [‡]
Serum creatinine (mg/dl)	1 ± 0.1 [†]	2 ± 0.2	1.9 ± 0.3	1.6 ± 0.1	1.3 ± 0.3	1 ± 0.4 [†]
Urine creatinine (mg/dl)	9.3 ± 1.5 [‡]	37.2 ± 4	25 ± 1.5 [‡]	14 ± 3 [‡]	12 ± 1.6 [‡]	10 ± 1.5 [‡]
Serum albumin (g/dl)	3.2 ± 0.1 [†]	3.7 ± 0.1	3.3 ± 0.1*	3.2 ± 0.1*	3.1 ± 0.1*	2.9 ± 0.1 [‡]
Urine albumin (g/dl)	0.1 ± 0.01 [†]	0.4 ± 0.04	0.3 ± 0.1	0.24 ± 0.1	0.21 ± 0.1	0.11 ± 0.01 [†]
Serum BUN (mg/dl)	19 ± 0.25 [‡]	51 ± 0.32	26 ± 0.33 [‡]	24 ± 0.26 [†]	21 ± 0.42 [‡]	20 ± 0.47 [‡]
Serum urea (mg/dl)	41 ± 0.32 [‡]	160 ± 0.72	82 ± 0.41 [†]	64 ± 0.43 [†]	55 ± 0.31 [‡]	42 ± 0.56 [‡]
Urine BUN (mg/dl)	132 ± 27 [‡]	250 ± 10	208 ± 8.2	172 ± 09 [†]	154 ± 16 [†]	90 ± 16 [‡]
Urine urea (mg/dl)	282 ± 48 [‡]	536 ± 18	445 ± 15	329 ± 29	271 ± 17 [†]	193 ± 30 [‡]
Weight of kidney (g)	0.51 ± 0.02	0.89 ± 0.02*	0.61 ± 0.02	0.59 ± 0.02	0.55 ± 0.01	0.52 ± 0.01
Body weight (g)	180 ± 2.02	151 ± 1.07 [#]	170 ± 1.6	172 ± 1.3	175 ± 1.1	178 ± 1.2
Glomerular Filtration Rate	13.4 ± 1.8	6.1 ± 0.09 [#]	10.8 ± 3.5	13.6 ± 2.2	15.6 ± 4.1	18.6 ± 4.1

ELC - Ethanolic extract of roots of *L. camara* at a dose of 200 mg/kg

OA60 - oleanolic acid at a dose of 60mg/kg

OA80 - oleanolic acid at a dose of 80mg/kg

OA100 - oleanolic acid at a dose of 100mg/kg

BUN - Blood urea nitrogen

Value represents Mean ± SEM

*P < 0.05, [†]P < 0.01, [‡]P < 0.001 vs. gentamicin treated group

[#] P < 0.05 vs. control group

Figure 1: Control group showing normal histology of kidney (normal glomeruli and tubules).

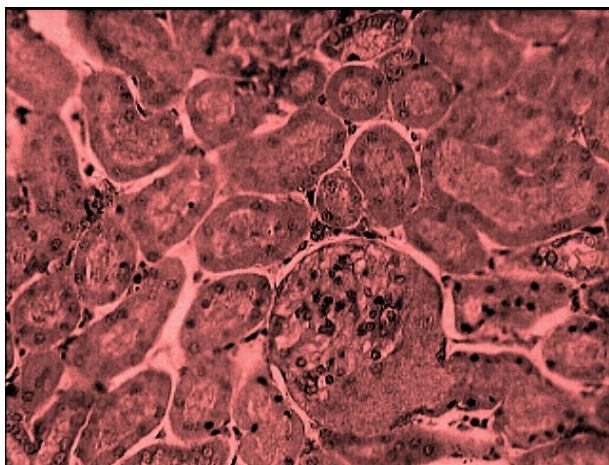


Figure 2: Gentamicin treated group showing tubular necrosis.

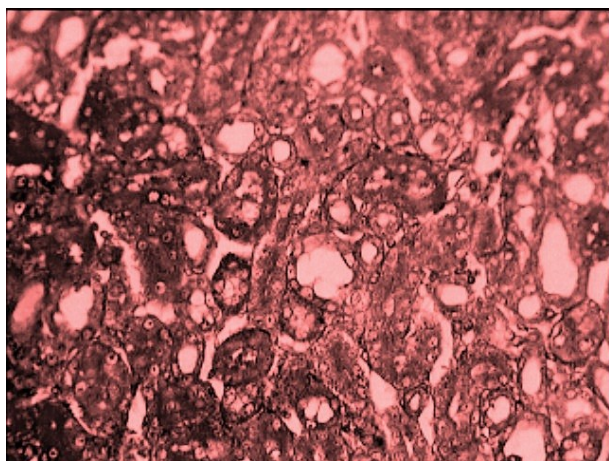
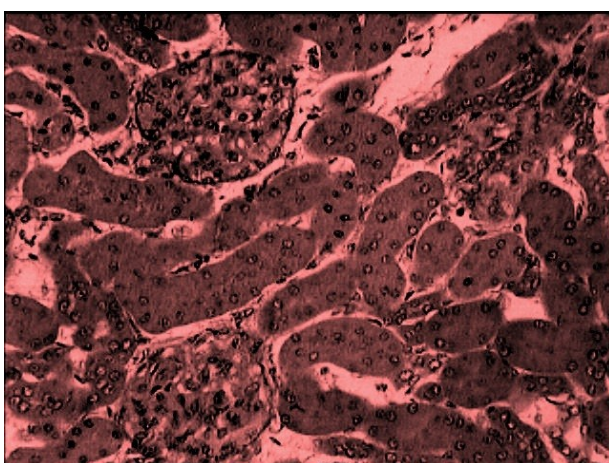


Figure 3: Gentamicin treated + 60 mg/kg OA treated group showing minimal congestion.



OA60 - oleanolic acid 60 mg/kg
OA 80 - oleanolic acid 80 mg/kg

Figure 4: Gentamicin treated + 80 mg/kg OA showing minimal congestion.

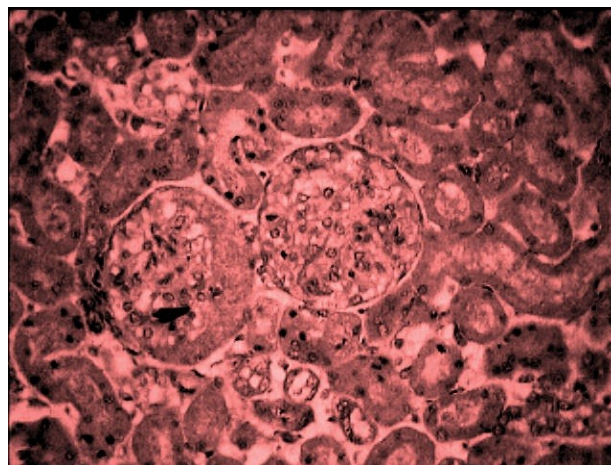


Figure 5: Gentamicin treated + 100 mg/kg OA showing normal histology with less tubular damage.

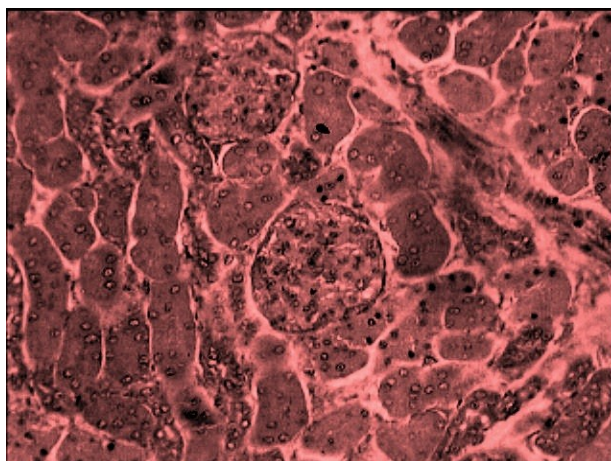
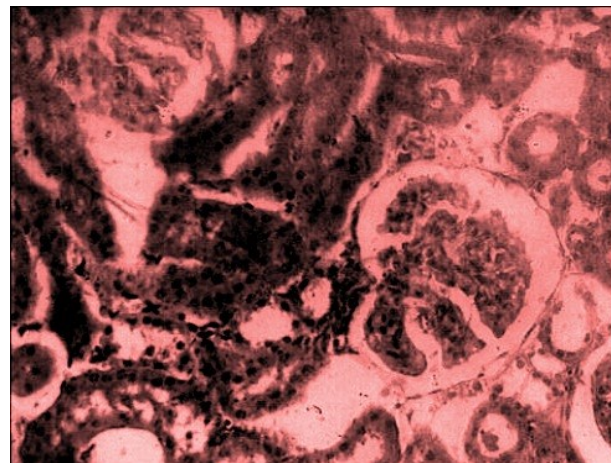


Figure 6: Gentamicin treated + ELC (200 mg/kg) treated group showing minimal tubular necrosis and slightly dilated and thrombosed vessels.



OA 100 - oleanolic acid 100 mg/kg
ELC - Extracts of roots of *Lantana camara* 200 mg/kg

OA acid was found to improve GFR in dose dependent manner even in presence of gentamicin and also decreased the level of BUN in serum. The protection from histological changes was also observed in the higher doses of OA.

Thus from the above results it can be concluded that the OA showed promising nephroprotective activity in dose dependent

manner. These results suggest the therapeutic utility of OA in renal injury.

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