



NEPHROPROTECTIVE ACTIVITY OF THE WHOLE PLANT OF OLDENLANDIA CORYMBOSA IN GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

The nephroprotective activity of the methanolic extract of the whole plant of *Oldenlandia corymbosa* were evaluated in albino rats. The plant extract at both the doses (250 and 500 mg/kg) reduced the elevated blood urea, serum creatinine and normalized the histopathological changes which indicate the nephroprotective effect in the curative regimen.

KEYWORDS: OLDENLANDIA CORYMBOSA, GENTAMICIN, NEPHROTOXICITY.

Introduction:

Plant based medicines have been in use against various diseases and disorders since time immemorial. The primitive man used herbs as therapeutic agents and medicaments, which they were able to collect easily. Nature has provided abundant resource of plants, which possess medicinal virtues for all living creatures. The essential values of some plants have long been published but a large number of them remain unexplored as yet¹.

Oldenlandia corymbosa Linn. from Rubiaceae family is a weedy annual herb, found throughout India. It is commonly known as 'Parpatakapullu' in traditional medicine of Kerala. A scrutiny of literature revealed some notable pharmacological activities of the plant such as hepatoprotective, anti-oxidant, cytotoxic and antimalarial activity². The plant is widely used in treating various diseases associated with kidney³. This was the rationale in selecting the particular species of the medicinal plant for evaluating the nephroprotective activity.

Materials and methods:

Source of plant

Whole plants of *Oldenlandia corymbosa* were collected from the campus of Pariyaram medical college, Kannur, Kerala. It was then shade dried and its botanical identity was confirmed by Dr. Abdussalam A K, Assistant Professor, Department of post graduate Studies and Research in Botany, Sir Syed College, Thaliparamba, Kannur, Kerala and the specimen of bearing voucher (No. OC/WP/PG.01) has been deposited in the Department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur District, Kerala.



FIG 1 OLDENLANDIA CORYMBOSA

Preparation of methanolic extract

The whole plant of dried and powdered *Oldenlandia corymbosa* (1kg) was exhaustively extracted by soxhlet apparatus (6 hours) with 95% methanol. The total methanolic extract was then concentrated in vacuo to a syrupy consistency and dried in vacuum desiccator.

Nephroprotective studies

Animals

Healthy adult male albino rats of Wistar strain weighing between 150 – 250 g aged 60 - 90 days were used for the study. Two rats were housed in a cage where humidity and temperature are controlled followed by normal diet. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee

of Academy of Pharmaceutical Sciences, Pariyaram No CPCSEA/IAEC17/18-11. Acute toxicity studies has been conducted as per the OECD guidelines 423.⁴

Drugs and chemicals

Gentamicin (Biochem Pharmaceutical industries, Mumbai), Urea estimation kit (Agappe diagnostics, Maharashtra), Creatinine estimation kit (Agappe diagnostics, Maharashtra).

Treatment schedule for gentamicin induced renal damage

6 groups of 6 rats were used in the evaluation. The first group was administered with equivalent volumes of 2% gum acacia for 23 days orally. The second group was given a higher dose of the plant extract for 23 days orally. The remaining groups were administered with gentamicin 40mg/kg body weight for 13 days subcutaneously. Blood samples were withdrawn on 14th day in third group and on 24th day in the fourth group.

Curative group: The fifth groups of animals were treated with alcoholic extract at lower dose from 14th day for 10 days orally. Blood samples were withdrawn on 24th day to assess renal function. The sixth groups of animals were treated with alcoholic extract at higher dose from 14th day for 10 days orally. Blood samples were withdrawn on 24th day to assess renal function⁸⁵.

Parameters assessed for renal function

1. Body weight: the weights (in grams) of the animals were noted on the first and last day of treatment and the percentage change in body weight was calculated.
2. Blood urea level: The estimation has been conducted by modified Berthelot method. Urea concentration in the blood was estimated by enzymatic method using Urease enzyme kit⁵.
3. Serum creatinine level: Creatinine level in serum was estimated by Alkaline picrate method using creatinine kit⁶.
4. Histopathological examination

Animals were sacrificed (2 from each group) on the day of blood withdrawal and their kidneys were isolated. It was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The sections were stained with Hematoxylin and Eosin and observed under light microscope after processing and embedding in paraffin wax.

Results

Acute toxicity studies

There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of alcoholic extract of *Oldenlandia corymbosa* up to the dose level of 2000mg/kg weight in rats.

Percentage change in Body weight

Gentamicin treated group on 14th day had a significant

reduction in body weight when compared with the control group. The reduction in body weight increased up to 24th day following gentamicin administration significantly. The alcoholic extract of the whole plant of *Oldenlandia corymbosa* at both the doses (250 and 500mg/kg) prevented the reduction in the body weight of albino rats when compared with gentamicin treated group on 24th day.

Blood urea

Gentamicin treated rats had a significant increase in blood urea levels on 14th day and the same was persisted up to 24th day following gentamicin administration. The extract of *Oldenlandia corymbosa* at the dose level of 250 and 500mg/kg of body weight showed reduction in the blood urea levels when compared with the gentamicin treated group on 24th day. The reduction was more significant with 500mg/kg body weight.

Serum Creatinine

Gentamicin treated rats on 14th and 24th day had a significant increase in serum creatinine levels when compared with the control group. The drug extract at the dose level of 250 and 500mg/kg of body weight decrease the creatinine concentration when compared to 24th day following gentamicin treatment. The reduction was more significant with 500mg/kg body weight.

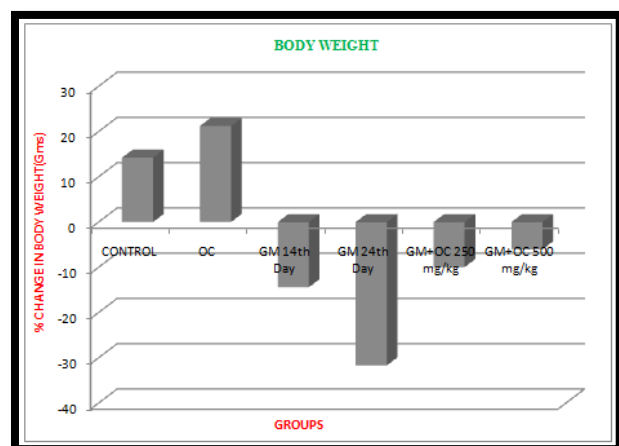
Histopathological examination: On the 24th day gentamicin treated rat kidney sections showed glomerular congestion along with infiltration of the inflammatory cells. These features were suggestive of acute tubular necrosis. Sections of rat kidney, treated with 500 mg/kg alcoholic extract of the whole plant of *Oldenlandia corymbosa* showed a normalization of kidney. However, mild glomerular and peritubular congestion were also noticed in the 250 mg/kg drug treated group following gentamicin administration.

TABLE 1 CURATIVE EFFECT OF THE ALCOHOLIC EXTRACT OF THE WHOLE PLANT OF *OLDENLANDIA CORYMBOSA*

Group N=6/group	Treatment Regimen	Mean±SE		
		%change in body weight	Blood urea mg/dl	Serum Creatinine Mg/dl
1	Control	14.21±3.25	33.61±4.62	0.97±0.06
2	Alc.Ext.500mg/kg	21.21±5.86	27.02±3.36	0.82±0.07
3	Gentamicin 14 th day	-14.40±2.44 ^a	72.54±5.52 ^a	2.62±0.05 ^a
4	Gentamicin 24 th day	-31.69±4.41 ^a	91.97±8.15 ^a	2.82±0.09 ^a
5	Gentamicin + Alc.Ext.250mg/kg	-9.94±3.87 ^b	49.95±2.82 ^b	2.4±0.09
6	Gentamicin + Alc.Ext.500mg/kg	-6.0±2.74 ^b	46.09±4.88 ^b	1.89±0.04 ^b

a p< 0.05 vs control (group 1), b p< 0.05 vs gentamicin 24th day (group 4), One way ANOVA followed by Sheffe's test

FIG 2 CURATIVE EFFECT OF *OLDENLANDIA CORYMBOA* ON PERCENTAGE CHANGE IN BODY WEIGHT



a p < 0.05 vs control, b p < 0.05 vs gentamicin 24th day

a p < 0.05 vs control, b p < 0.05 vs gentamicin 24th day

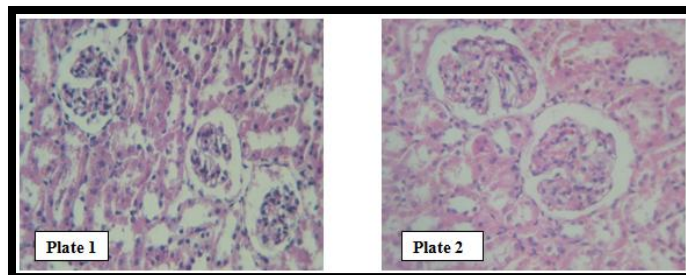


Plate 1: Photomicrograph of the section of the rat kidney showing normal glomeruli and normal tubule of the control group

Plate 2: Photomicrograph of the section of rat kidney showing congested glomeruli after administration of Gentamicin

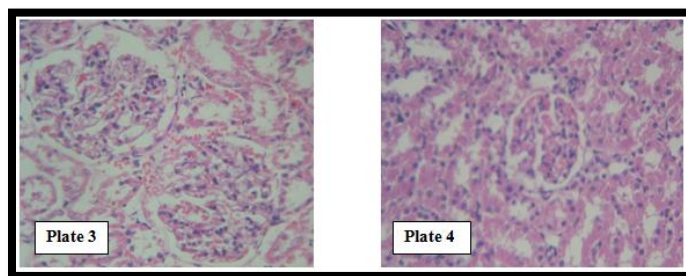
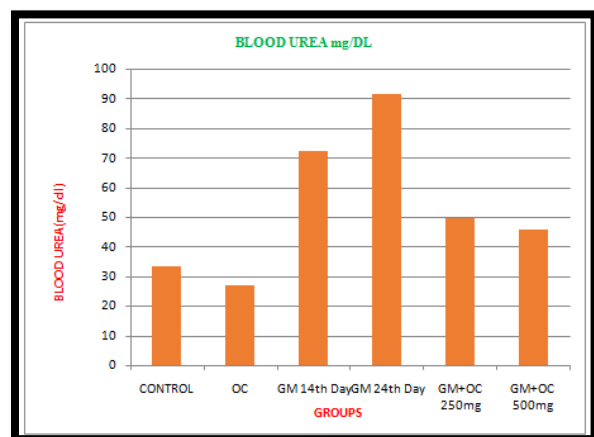


Plate 3: Photomicrograph of the section of rat kidney treated with 250 mg/kg alcoholic extract of *Oldenlandia corymbosa* showing mild glomerular congestion

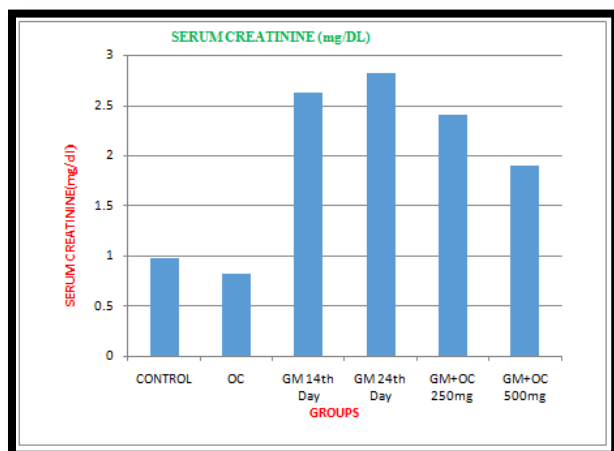
Plate 4: Photomicrograph of the section of rat kidney treated with 500 mg/kg alcoholic extract of *Oldenlandia corymbosa* showing nearly normalization of kidney.

FIG 3 CURATIVE EFFECT OF *OLDENLANDIA CORYMBOA* (OC) ON BLOOD UREA LEVELS IN GENTAMICIN (GM) NEPHROTOXIC MODEL



a p < 0.05 vs control, b p < 0.05 vs gentamicin 24th day

FIG 4 CURATIVE EFFECT OF *OLDENLANDIA CORYMBOA* (OC) ON SERUM CREATININE LEVELS IN GENTAMICIN (GM) NEPHROTOXIC MODEL



Discussion

The present study was undertaken to evaluate the possible nephroprotective effect of the whole plant of *Oldenlandia corymbosa* in gentamicin nephrotoxic model. In the present study, results showed the evidence of nephrotoxicity with gentamicin on the 24th day as seen by raised blood urea, serum creatinine and congestion of the glomeruli with inflammatory cells in the histopathological examinations. *Oldenlandia corymbosa* at both the doses (250 and 500 mg/kg) reduced the blood urea, serum creatinine and normalized the histopathological changes which indicate the nephroprotective effect in the curative regimen. *Oldenlandia corymbosa* at the dose level of 500 mg/kg body weight showed significant protective effect than that of 250mg/kg body weight which was evidenced from all the parameters analysed including histopathological examination.

In our study, we also observed that *Oldenlandia corymbosa* possessed nitric oxide free radical scavenging effect in *invitro* evaluation⁷. In addition, Mammen D Daniel et.al., 2011 isolated flavonols such as Quercetin, 3-Methoxy quercetin and 3,4-Dimethoxy quercetin from the methanolic extracts of *Oldenlandia corymbosa* ⁸. According to Raj Narayanan K et.al., 2001 flavonoids are

well documented to have potent antioxidant and free radical scavenging activity⁹. Hence the probable mechanism of nephroprotection by *Oldenlandia corymbosa* could be due to its antioxidant and free radical scavenging activity.

In addition, the preliminary phytochemical investigation of *Oldenlandia corymbosa* showed the presence of phenolic compounds in both alcoholic and aqueous extracts. The nephroprotective effects of phenolic compounds in gentamicin induced renal injuries are well established by Kannappan¹⁰, 2010 and Ruby Varghese¹¹, 2011. Hence the significant nephroprotection exhibited by the alcoholic extract of *Oldenlandia corymbosa* may be attributed to its high phenolic content coupled with its antioxidant and free radical scavenging activity.

The nephrotoxicity of gentamicin is well established in man and experimental animals. Gentamicin induces nephrotoxicity by the initiation of lipid peroxidation¹². The nephrotoxicity produced by the gentamicin is evidenced by increase in blood urea and serum creatinine levels accompanied by a significant loss in body weight.

In the histopathological examination, gentamicin treated rat kidney sections exhibited congestion of the glomeruli with inflammatory cells in the lumen indicates acute renal necrosis.

Oldenlandia corymbosa scavenge NO and DPPH radicals and prevent peroxynitrite formation leading to Nephroprotection. Oxygen free radicals are potentially important in glomerular disorders¹³. Gentamicin activates phospholipases and alters lysosomal membrane in addition to oxidative stress. Hence natural antioxidants and free radical scavengers are claimed to provide nephroprotection in gentamicin renal injury.

The Whole plant of *Oldenlandia corymbosa* possess marked nephroprotective activity with minimal toxicity. The plant has promising role in the treatment of acute renal injury induced by nephrotoxin like gentamicin.

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Conflict of interest: Nil

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