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Protective effects of *Alpinia Purpurata* (*Vieill*) against gentamicin-induced nephrotoxicity in albino rats

Vadivel Subramanian* and Suja. S

Department of Biochemistry, Dr.N.G.P Arts and Science College, Kalapatti road, Coimbatore-641 048, Tamilnadu, India.

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ABSTRACT

The aim of this study was focused on investigating the possible protective effect of *Alpinia Purpurata* rhizome against Gentamicin -induced nephrotoxicity. Nephrotoxicity was measured by various methods like creatinine, cholesterol, blood urea nitrogen, lipid peroxidation (LPO) and suppressed superoxide dismutase (SOD) and catalase activities in renal tissues. Activity of serum creatinine and urea levels significantly increased as a result of nephrotoxicity in the Gentamicin group. Also, creatinine and urea levels significantly decreased in *Alpinia Purpurata* + Gentamicin groups. In the Gentamicin group, increased significantly ($p<0.05$) and SOD and GSH-Px activities decreased significantly ($p<0.05$) when compared with control group. *Alpinia Purpurata* administration with Gentamicin injection result significantly increased SOD and GSH-Px activities when compared with GS group. mononuclear cell infiltration, glomerular and basement membrane alterations were histopathologically detected in the kidneys of the Gentamicin group. Co-treatments with *Alpinia Purpurata* considerably decreased the renal damage when compared with the Gentamicin group. In conclusion *Alpinia Purpurata* rhizome acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of Gentamicin both in the biochemical and histopathological parameters.

Introduction

A number of environmental contaminants, chemicals and drugs including antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine [1, 2]. Gentamicin is effective against Gram negative bacterial infection in human and animals [3]. However, a major complication of therapeutic doses of gentamicin is nephrotoxicity. This is known as one of the most common causes of acute renal failure, which occurs in about 10–30% of patients receiving the drug [4, 5]. Gentamicin is known to generate reactive oxygen species (ROS) associated with an increase in lipid peroxidation (LPO) and decrease in antioxidant enzymes in the intestine and kidney [6, 7]. This is considered as one of the important mechanisms for Gentamicin induced nephrotoxicity and other deleterious effects [8]. The toxicity of amino glycosides, including gentamicin is believed to be related to the generation of reactive oxygen species (ROS) in the kidney [9, 10]. The cellular antioxidant status determines the susceptibility to oxidative damage and usually alters in response to oxidative stress [11]. Several studies have reported that oxygen-free radicals are important mediators of gentamicin mediated nephrotoxicity [12, 13]. The aim of the present study was to evaluate the role of LPO in gentamicin-mediated nephrotoxicity and to highlight the protective effect of *Alpinia Purpurata rhizome,* which has antioxidant action, in gentamicin-induced renal damage in rats.

Materials and methods

Plant Material:

Collection:

Fresh plant material was collected from Kovaipudhur, Coimbatore District, and Tamil Nadu State, India. Efforts were made to collect the plant in rhizomes and flowering conditions for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of Agriculture university of Coimbatore, Tamil Nadu State.

Preparation of Extract:

About 300g of the coarse dried powder of the rhizome of *Alpinia purpurata* was taken in soxhlet apparatus and extracted using 95% ethanol. The extraction was carried out for about 72 hours. The extract was collected by the filtrate was pooled and the solvents were evaporated in a rotator evaporator at temperature below 50°C and the extracts were freeze-dried. The residue was used to analyse the various *in vivo* Nephroprotective activity.

Chemicals

Chemicals used in the study were of analytical grade were procured. All biochemical assay kits were purchased from Sigma, SD fine-chemicals limited and Himedia, India.

Experimental Animals:

The male albino rats of Wistar strain weighing 180g-230g were obtained from Kovai Medical Centre of Research and Hospital (KMCH) Pharmacy College, Coimbatore. The animals were housed in polypropylene cages at controlled temperature (27 ± 2° C), relative humidity (60 ± 5%) and light conditions(12 -12 hours day night cycle). The rats were fed with standard laboratory diet and drinking water was given through a drinking bottle, throughout the experiment. They were given a week's time to get acclimatized to the laboratory conditions. All animal experiments were conducted with the permission from Institutional Ethical Committee (KMCRET/Ph.D/07/2011).

Acute oral toxicity study

Acute oral toxicity of *Alpinia purpurata* extract was performed on Swiss albino rat, according to OECD guideline

Tele:
E-mail addresses: vasanth205@gmail.com

423. Two groups of six rats each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of ethanolic extract of *Alpinia purpurata* rhizome (2000 mg/kg). The animals were observed for gross behavioural, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 15 days. Food consumption was monitored daily and body weights were recorded weekly. On 15th day, animals were sacrificed and all the organs were removed for gross pathological examination.

Experimental Design:

Albino rats 4-6 weeks, 180-230g, were divided into five groups of six animals each. Gentamicin (100mg/kg /bw/i.p) was administered to all groups of animals except for group I. Group-I controls rats fed with 0.3% carboxymethyl cellulose (1ml/kg p.o) once a day for 14 days. Group II- rats administered with gentamicin (100mg/kg/b.w,ip) in alternate days. Group III, IV and V- rats fed with *Alpinia purpurata* extracts of 200mg, 400mg and 600/mgkg/bw,p.o respectively for 14 days and gentamicin in alternate days.

All animals were sacrificed at the end of 15th day after gentamicin administration, blood was drawn from the carotid artery and serum was separated and used for the biochemical parameters and kidney was removed, preserved for biochemical parameters like serum Creatinine,[14] Urea,[15] Creatinine clearance,[16] and antioxidants enzymes in the kidney tissues like Catalase,[17] superoxide dismutase,[18] glutathione peroxidise,[19] reduced glutathione,[20] and LPO,[21] Urinary glucose,[22] Urinary sodium,[23] Urinary potassium,[24] were analysed in all the five group of rats. The part of the kidney was fixed in 10% formalin and stained with eosin for histopathological examination.

Induction of experimental nephrotoxicity:

Nephrotoxicity was induced by injecting gentamicin orally at a dose of 100 mg/kg body weight on the 15 consecutive days and treated with extracts 15 consecutive days.

Measurement of Biochemical Parameters:

All animals were sacrificed at the end of 15th day after gentamicin administration, blood was drawn from the carotid artery and serum was separated and used for the biochemical parameters and kidney was removed, preserved for biochemical parameters like serum Creatinine, Urea and antioxidants enzymes in the kidney tissues like catalase, superoxide dismutase, glutathione peroxidise and LPO were analysed in all the five group of rats.

Histopathological Examination:

The part of the kidney was fixed in 10% formalin and stained with eosin for histopathological examination and later the Microscopic slides of the kidney cells were photographed at a magnification of x100.

Statistical Analysis:

Values were represented as mean±SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test using statistical package for social sciences (SPSS) version 10.0. *P*<0.05 was considered significant. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group.

Results and discussion:

Acute Lethal Dosage Study:

When the rats were observed for the behavioural changes after orally administration of a single dose of the extract, none of the rats exhibited any abnormal behaviour responses at doses of 2000 mg/kg. Administration of repeated daily doses of 2000 for 15 days did not influence the body weight of the rats. The weights of liver, kidney, and spleen were also not altered by the treatment. Haematological parameters like haemoglobin and RBC count remained unaltered at the dose of 2000 mg/kg. Thus, it was concluded that ethanolic extract of *Alpinia purpurata* rhizome extract was safe at 2000 mg/kg.

Biochemical Assessment:

Table1 Effect of ethanolic extract of Alpinia purpurata on serum creatinine, blood urea and Creatinine clearance in the gentamicin induced toxicity in rats.

Gentamicin induced nephrotoxicity can be monitored by determining the blood urea and serum Creatinine level and Creatinine clearance were estimated and the results obtained are presented in table - 1. Gentamicin causes an elevation of plasma Creatinine, urea level and decreased Creatinine clearance (group II) when compared to control group (group I).

The ethanolic extract of *Alpinia purpurata* inhibit the increase of Creatinine and Urea level and increased the Creatinine clearance rate (group III, IV and V), significantly in the gentamicin treated group. The increase of Creatinine level (group II) is due to impaired Creatinine clearance and glomerular filtration, which indicate renal damage.

Table 1 Level of serum creatinine, Blood urea and creatinine clearance in different experimental groups of rats

GROUPS	Serum Creatinine (mg/dl^{-1})	Urea (mg/dl^{-1})	Creatinine clearance (ml/min)
Group I (Control)	0.75 ± 0.04	23.07 ± 5.84	0.42 ± 0.02
Group II (Gentamicin treated)	2.54 ±0.04*	28.80 ± 1.97*	0.63 ± 0.05*
Group III (Gentamicin + 200mg/kg)	1.75 ± 0.08*	26. 02 ± 3.92*	0.48 ± 0.08*
Group IV (Gentamicin + 400mg/kg)	0.65 ± 0.04*	24. 85 ± 3.79*	0.46 ± 0.06*
Group V (Gentamicin + 600mg/kg)	0.72 ± 0.03^{ns}	23. 72 ± 2.25^{ns}	0.42 ± 0.02^{ns}
CD ($p<0.05$)	0.183	6.41	0.07

Values are mean ± SD of six samples in each group
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance : * - Significant at $p<0.05$; ns – Not significant

Table 2 Effect of the ethanolic extract of Alpinia purpurata on urinary glucose, sodium and potassium in gentamicin induced toxicity in rats.

Urinary glucose is significantly high in (group II) and there is also change in the urinary sodium and potassium and excretion level. The elevated level of urinary glucose, sodium and potassium was significantly reduced in the (group III, IV and V) when compared with group II gentamicin induced toxicity. This result revealed that the ethanolic extracts of *Alpinia purpurata* riveted the damage cause by gentamicin to almost complete normalization. In (group IV and V) no significant difference in the level of urinary glucose, sodium and potassium was noticed.

Table 2. Level of urinary glucose, sodium and potassium in experimental groups of rats

GROUPS	Urinary glucose (mg/day)	Urinary sodium (meq/day)	Urinary potassium (meq/day)
Group I (Control)	00.0	140 ± 3.4	5.22 ± 0.33
Group II (Gentamicin treated)	73.2 ± 6.4*	153 ± 2.6*	4.27 ± 0.45*
Group III (Gentamicin + 200mg/kg)	8.45 ± 3.2*	145 ± 2.2*	4.33 ± 0.50*
Group IV (Gentamicin + 400mg/kg)	12.39 ± 2.6*	142 ± 1.7*	4.93 ± 0.45*
Group V (Gentamicin + 600mg/kg)	00.0	140 ± 3.4^{ns}	5.18 .± 0.52^{ns}
CD (p<0.05)	4.79	4.38	0.98

Values are mean ± SD of six samples in each group
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance : * - Significant at p<0.05; ns – Not significant

Table 3. Level of enzymic antioxidants and lipidperoxidation in the kidneys of different experimental groups of rats

GROUPS	CAT	SOD	GPx	GSH	LPO
Group I (Control)	95.04 ± 0.01	6.51 ± 0.12	8.32 ± 0.87	20.75 ± 0.28	1.86 ± 0.22
Group II (Gentamicin treated)	50.30 ± 0.08*	3.55 ± 0.02*	4.28 ± 0.15*	7.22 ± 0.15*	4.74 ± 0.96*
Group III (Gentamicin + 200mg/kg)	76.97 ± 0.01*	4.12 ± 0.01*	5.98 ± 0.17*	17.70 ± 0.44*	3.92 ± 0.07*
Group IV (Gentamicin + 400mg/kg)	84.26 ± 0.12*	5.04 ± 0.02*	7.53 ± 1.12*	16.05 ± 0.47*	2.62 ± 0.06*
Group V (Gentamicin + 600mg/kg)	93.05 ± 0.12*	6.42 ± 0.01^{ns}	8.21 ± 0.95^{ns}	20.63 ± 0.36^{ns}	2.02 ± 0.01^{ns}
CD (p<0.05)	0.35	0.16	1.64	0.85	0.58

Values are mean ± SD of six samples in each group
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance : * - Significant at p<0.05; ns – Not significant

Table 3. Effect of the ethanolic extract of Alpinia purpurata on the enzymic antioxidant and lipid peroxidation in gentamicin induced toxicity in rats.

The decreased SOD activity in the (group II) present study may be because of highly reactive oxygen metabolites (ROMS) production. Over production of O_2 itself or O_2 during oxidative stress causes membrane damage due to over production of free radical possibly cause conformational changes and hence inactivate enzymes such as SOD. In the ethanolic extract treated (Group III, IV and Group V) the level of antioxidant enzymes was significantly increased in the kidney tissues. The decreased CAT activity observed in the present study in (Group-II) may be because of higher ROMs production, especially O_2 which itself affects directly the CAT activity [25]. The decreased activity of Gpx is observed in the present study (Group II) may be due to the low availability of the substrate GSH. Treatment with the *Alpinia purpurata* extracts afforded maximum protection to the antioxidant enzymes such as SOD, CAT and GPX by influencing the GSH [26]. The activities of antioxidant enzymes in the kidney are represented in table. 3.

The *Alpinia purpurata* extracts acts as an antioxidant, prevented the decrease in GSH and GST levels and the increase in MDA levels and CAT activity and inhibits lipid peroxidation and prevents cell injury and have decreases tubular necrosis, irreversible cell damage. The group IV and group V showed no significant difference level of enzymes and LPO when compared with the control (group I).

Conclusion

The present study reveal the protective effect of ethanolic extracts of *Alpinia purpurata* against the kidney damage induced by the gentamicin respectively. The result from the present study clearly revealed that the *Alpinia purpurata* extracts at a concentration of 200, 400 and 600mg when administered to the rats (group III, IV and group V) could protect the damage induced by gentamicin.

Conflicts of interest

All authors have none to declare.

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