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A STUDY ON EFFICACY OF ALPINIA GALANGA AGAINST CHEMICAL INDUCED KIDNEY DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

The present study throws light on the effect of the plant *Alpinia galanga* (L.) in reducing the nephrotoxic effect that has been induced by potassium dichromate as well as gentamicin. The results of phytochemical screening components of ethanolic and aqueous extracts of *Alpinia galanga* (L.) leaves might be accountable for the effective antioxidant capacity like Thiobarbituric acid reactive substance(TBARS), Super oxide dismutase(SOD), Catalase(CAT), Reduced glutathione(GSH), and to estimated the effect of *Alpinia galanga* on different oxidative stress parameters in Kidneys. Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced nephrotoxicity is analysed by the Creatinine (mg/dl), Urea (mg/dl), Blood Urea Nitrogen (mg/dl), Total protein (g/dl) levels.

KEYWORDS: Plant *Alpinia galanga* (L.), oxidative stress parameters in Kidneys, Antioxidants.

INTRODUCTION

Most scientists delineate acute kidney injury (AKI) as an unexpected decline in glomerular filtration rate (GFR) reflected by the doublings of serum creatinine and azotemia.[1] Nephrotoxicity is indeed an ancient discipline with a noble and distinguished legacy that spans at least three previous millennia. A bronze artefact closely resembling the human kidney and dating to 1300BC was excavated from the ruins of the temples of kition. Before the time of the Christ, Greek physician prescribed botanical material to promote diuresis and employed bloodletting and other means for removal of excess body fluids. Hippocrates (460-375BC) was skilled in microscopic detail of urine analysis. Artaeus of Cappadocia (30-90AD) and Galan (130-200AD) recognized kidney as the organ responsible for urine formation. By the middle of 1800, the structural complexity of mammalian kidney was revealed and unraveled through improved optics and microscopy. The few names had chosen among the pioneers, Marcello Malpigi and Lorenzo Bellin in Italy and Antoine Ferrein in France for the birth of renal anatomy, Sir William Bowman in England and Karl Ludwig in Germany for renal physiology and Richard Bright in London and Pierra Rayer in Paris for the Kidney disease.[2]

Drug nephrotoxicity is attributed with community based statistics to estimate the occurrence of AKI as being between 0% and 7% and in adults at about 20% the prevalence of all AKI in hospital attributed to drug nephrotoxicity. The NSAIDs (3–22%), antibiotics (3–11%), contrast media (2–12%) and angiotensin converting enzyme (ACE) inhibitors (0.5–7%) were regarded as most frequent crooks.[3]

From the survey by Ronco et al in 2001 it was confirmed that drug induced was caused by a higher frequencies of ACE inhibitors and NSAIDs. Hospital acquired AKI has been observing with one of three renal insults: sepsis, exposure to nephrotoxins, or a pre renal event. At least 25% of all cases of hospital acquired AKI is attributed to nephrotoxins, alone or in combination.

Besides liver, kidney is an indispensable excretory organ in the human body for the elimination of endogenous waste metabolites and foreign chemicals, detoxification but also to maintain the acid base balance, endocrine function like erythropoietin production. Therefore, the kidney can be considered as a major target organ for exogenous toxicants and hence like liver, the renal system also faces high risk of toxicity.[4,5,6]

Body unable to clear of surfeit urine and wastes from the body when kidney damage occurs and blood electrolytes such as potassium and magnesium all are grow to be raised abnormally.[7]

The tubular cells of the kidney are particularly susceptible to toxicant mediated injury due to their disproportionate exposure to circulating chemicals and transport processes that result in high intracellular concentrations. The parent chemical or a metabolite

initiates toxicity through its covalent or non covalent binding to cellular macromolecules or through their ability to produce reactive oxygen species. Furthermore cell injury was occurred by change in the activity of the macromolecule. For instance, mitochondrion, lysosome, plasma membrane of proteins and lipids, cytosol and nucleus all are the objects of toxicants. The toxicant cause oxidative stress in both lipid peroxidation and protein oxidation has been shown to contribute to cell injury.

Exposure to chemical reagents like ethylene glycol, carbon tetra chloride, potassium dichromate, sodium oxalate and heavy metals like cadmium, mercury, lead and arsenic also persuades nephrotoxicity leads to acute kidney injury (AKI).[8 to10]

MATERIALS AND METHODS

Collection, identification and authentification of plant material

An indigenous medicinal plant *Alpinia galanga L.* (Zingiberaceae) known by a local name called peddadumparashtram (Telugu). The fresh leaves of *Alpinia galanga (L.)* was collected in the month of June 2018 from village Pedur in Nellore district, Andhra pradesh, India. The plant material was taxonomically identified and authenticated by expert botanist Dr. CVS Bhaskar, Principal / Lecturer in-charge, department of botany, V.R College, Nellore. The voucher specimen of the plant was deposited at the college for further reference.

Hot soxhlet extraction method: A weighed quantity (60 gm) of the powder extracted with 500 ml of ethanol and water in ration of 70:30 for 16 to 18 hours at 40 to 60^0C until it become colorless. The extracts subsequently concentrated by vacuum distillation until all the solvent has been removed to give an extract sample known as semisolid mass, kept in a petri dish and stored in refrigerator until use.

EXPERIMENTAL PROTOCOL

Wistar albino rats of either sex 12 weeks old weighing between 150 to 200 g was used for the study. The animals are pre treated with physiological saline (0.9% NaCl) to impose a uniform water and salt load at an oral dose of 0.15 ml / 10g body weight.

Effect of *Alpinia galanga* (L.) on gentamicin induced nephrotoxicity

The dose limits were chosen in rats on the basis of oral acute toxicity studies in accordance with the OECD guidelines. A total thirty six rats of the same age group were randomized and divided into six groups (1 to 6) of six animals (n=6) in each. The study carried out for nine days. Each animal received Gentamicin (GM) sulphate 80 mg/kg B.W/day of treatment intraperitoneally (I.P.) for 8 days except of normal control group. The protocol was designed as follows:

Group I (Normal control): Animals received normal food and water *ad libitum* for a period of 8 days.
Group II (Disease control): Animals received GM sulphate injection (80 mg/kg B.W/day I.P.) once daily for a period of 8 days.
Group III (Test control 1): GM sulphate injection (80 mg/kg B.W/day I.P.) was administered, prior to that animals received *Alpinia galanga* (L.) hydroalcoholic extract (200 mg/kg B.W/day PO) once daily for a period of 8 days.
Group IV (Test control 2): GM sulphate injection (80 mg/kg B.W/day I.P.) was administered, prior to that animals received *Alpinia galanga* (L.) hydroalcoholic extract (400 mg/kg BW/day PO) once daily for a period of 8 days.

Biochemical studies

At the end of experimental period, Blood samples were collected by retro orbital puncture under diethyl ether anesthesia using a fine capillary and centrifuged for 10 minutes using the table top centrifuge (REMI) at 3000 rpm to get serum for estimation of various biochemical parameters. The biochemical parameters including creatinine, urea, blood urea nitrogen and total protein were estimated. The biochemical estimations are done in a Biochemical semi auto analyzer by standard procedures using commercial kits called Span Diagnostic kits for assessment of renal toxicity.

Removal of kidneys

At the end of experimental period the rats were sacrificed by decapitation. the kidneys were excised carefully and washed with normal saline. one kidney from each group was sliced into two equal halves and one half was homogenized for estimating in vivo anti oxidant parameters and the other half was put in 10% formalin and used for the Histopathological studies.

Preparation of tissue homogenate

The removed kidney from the rats were weighed and tissue was homogenized with trichloroacetic acid (TCA) in 1:10 ratio and centrifuged at 3000 rpm 10 min after that the supernatant was collected and used for the estimation of anti oxidant parameters.

ESTIMATION OF ANTI OXIDANT PARAMETERS

Estimation of Thiobarbituric acid Reactive Substances (TBARS)

TBARS levels were determined by a modified version of the method described by Ohkawa et al, (1979).

Principle

Acetic acid was used to detach the lipid and protein of the tissue and the protein in the reaction mixture were dissolved by the addition of sodium lauryl sulphate. Thiobarbituric acid reacts with lipid peroxides, hydroperoxide and oxygen double bond to form a coloured adduct with absorption maximum at 532 nm, which was then measured.

Reagents
- Sodium lauryl sulphate - 8.1% (W/V)
- Acetic acid - 20% (pH – 3.5)
- Thiobarbituricacid - 0.8% (w/v)
- Butanol:Pyridine - (15:1) (v/v)
- Trichloracetic acid (TCA) - 10%

Procedure
Tissue was homogenized with10% Trichloroacetic acid (TCA) in 1:10 ratio (for 1 gm of tissue 10 ml of 10%TCA was added) and centrifuged at 3000 × g for 10 min. 0.2 ml of whole homogenate was taken to which 0.2 ml of 8.1% sodium lauryl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml 0.8% thiobarbituric acid were added. Volume was made up to 4 ml with double distilled water. It was heated at 95°C for 60 min. After cooling, 1 ml of double distilled H_2O and 5 ml of butanol–pyridine mixture was added. The solution was shaken vigorously in a vortex and centrifuged at 4000 rpm for 10 min in a cold centrifuge. The organic layer was separated and absorbance was observed at 532 nm in a spectrophotometer.

Standard curve
Various concentrations of 1,1,3,3-Tetra methoxy propane (TMP) were used as external standard (1–10 nm) and were subjected to the steps mentioned in the procedure section. The readings of absorbance were plotted against the concentration of TMP to derive a linear standard graph. Data expressed as nmol/g wet wt. tissue.

CALCULATION
The concentration of TBARS was determined from the linear standard graph.

Super Oxide Dismutase (SOD)
SOD was estimated by the method of Misra and Fridovich (1972).

Principle
Rate of auto oxidation of epinephrine and the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8-10.2. O_2 generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2 introduced. The auto oxidation of epinephrine proceeds by at least two distinct pathways only one of which is free radical chain reaction involving O_2 and hence inhabitable by SOD.

Reagents
a. Carbonate buffer (0.05 M, pH 10.2): 16.8 gm of sodium bicarbonate and 22 gm of sodium carbonate was dissolved in 500 ml of distilled water and the final volume was made up to 1000 ml with distilled water.
b. Ethylene diamine tetra acetic acid (EDTA) (0.49 M): 1.82 gm of EDTA was dissolved in 1000 ml of distilled water.
c. Epinephrine (3 mM): 9.9 mg of epinephrine bitartarate was dissolved in 10 ml of 1 M HCl solution.
d. SOD standard: 1mg (1000 units/mg) of SOD from bovine liver was dissolved in 100 ml of carbonate buffer.

Procedure
0.5 ml of sample was diluted with 0.5 ml of distilled water. To this 0.25 ml ethanol, 0.5 ml of chloroform (all reagents chilled) were added. The mixture was shaken for 1 min and centrifuged at 2000 rpm for 20 min. The enzymatic activity in supernatant was determined. To it 0.05 ml of carbonate buffer (0.05 M, pH 10.2) and 0.5 ml of EDTA (0.49 M) was added. The reaction was initiated by the addition of 0.4 ml of epinephrine and the change in optical density/min was measured at 480 nm. SOD activity was expressed as units/mg protein change in optical density/min. 50% inhibition of epinephrine to adreno chrome transition by enzyme is taken the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

CATALASE (CAT)
Principle
Catalase was estimated by Hugo E. Aebi method; Hydrogen peroxide: hydrogen-peroxido reductase method.

In U.V range H_2O_2 can be followed directly by the decrease in absorbance (O.D 240) per unit time. It is the measure of catalase activity.

$$H_2O_2 \rightarrow H_2 + O_2$$

$$RDOH \rightarrow H_2O + ROH + A$$

Decomposition of H_2O_2 = Decrease in absorbance at 240 nm.

Reagents
1. Phosphate buffer (50 ml; pH 7.0):
a. Dissolve 6.81g KH_2PO_4 in water and make up to1000 ml.
b. Dissolve 8.9 g NaH_2PO_4. 2 H_2O in water and make up to1000 ml.
Mix the solutions (a) and (b) in proportion 1:1.5 (v/v).
2. Hydrogen peroxide (30 mm/l): Dilute 0.34 ml of 30% hydrogen peroxide with phosphate buffer up to 100ml.

Procedure
Dilute homogenate 20 times with phosphate buffer (pH 7.0).

Blank	**Test**
1. 4ml homogenate diluted	2ml homogenate diluted
2. 2ml Phosphate buffer pH 7.0	----------
3. ----------	1ml H_2O_2 (8.5 micro lit. in 2.5 ml phosphate buffer (50 mM/l; pH 7.0)).

H_2O_2 was added just before taking O.D at 240 nm. Take for 3 min with 15 sec interval.

Reduced Glutathione (GSH)

Reduced glutathione was determined by the method of Moran et al.

Reagents

1. Trichloroacetic acid 10% (TCA): Accurately weighed 10 gm trichloroacetic acid was dissolved in 100 ml of distilled water.
2. Phosphate buffer (0.2 M, pH 8).
3. DTNB reagent (0.6 M): 60mg of 5,5-dithobis (2-nitro benzoic acid) was dissolved in 100 ml of 0.2 M sodium phosphate (pH 8).
4. Standard glutathione: prepared by dissolving 10 mg of reduced glutathione in 100 ml of distilled water.

Procedure

To 1ml of sample, 1ml of 10% TCA was added. The precipitated fraction was centrifuged and to it 0.5 ml supernatant, 2 ml of DTNB reagent was added. The final volume was made up to 3 ml with phosphate buffer. The colour developed was read at 412 nm. The amount of glutathione was expressed as µg of GSH/mg protein. Reduced glutathione was used as standard (100 µg/ml).

Statistical analysis

All the results were expressed as Mean ± SEM for six animals in each group. Statistical significance was carried out using one way ANOVA followed by Dunnet's test using computer based fitting program (Graph pad prism version 5.0) and significance was set accordingly.

RESULTS

Percentage yield

The percentage yield of hydroalcoholic extracts of leaves of *Alpinia galanga* (L.) is 18.764 w/w.

Table No.1: Percentage yield of leaf extracts of *Rumex vesicarius* (L.).

S. No	Solvent	Weight of extracts (gm)		Percentage yield (% w/w)
		Practical yield	Theoretical yield	
1.	Hydroalcoholic	20.64	110	18.764

Pharmacological evaluation of nephroprotective activity by gentamicin induced nephrotoxicity

Biochemical Parameters

Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on serum creatinine in gentamicin induced nephrotoxicity

The serum creatinine is significantly increased in gentamicin treated group (2.852±0.125; p<0.001) when compared to normal group (0.940±0.144; p<0.001). *Alpinia galanga* (L.) in high dose (400 mg/kg B.W) and low dose (200 mg/kg B.W) of hydroalcoholic extract has shown greater significance (1.182±0.138 and 1.563 ±0.115; p<0.001), in their creatinine levels when compared to disease control.

Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on serum urea in gentamicin induced nephrotoxicity

The serum urea is significantly increased in gentamicin treated group (51.87±1.862; p<0.001) when compared to normal group i.e. 33.69±2.841 (p<0.001). It is exemplified from Table No.6.32. *Alpinia galanga* (L.) in high dose (400 mg/kg B.W) and low dose (200 mg/kg B.W) of hydroalcoholic extract significantly decreased the serum urea (36.04±1.633 and 39.91±1.425; p<0.001) respectively when compared to gentamicin treated group.

Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on blood urea nitrogen in gentamicin induced nephrotoxicity

Compared to normal group (16.83±0.974; p<0.001), the blood urea nitrogen is significantly increased in gentamicin treated group (30.06±0.907; p<0.001). *Alpinia galanga* (L.) in high dose (400 mg/kg B.W) and low dose (200 mg/kg B.W) of hydroalcoholic extract has revealed higher significance (18.25±0.603 and 20.92±0.788; p<0.001), in their blood urea nitrogen levels when compared to disease control. It is explained from Table No.6.33.

Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on total protein in gentamicin induced nephrotoxicity

The total protein is significantly decreased in gentamicin treated group (4.517±0.224; p<0.001) when compared to normal group (8.314±0.105; p<0.001). *Alpinia galanga* (L.) in high dose (400 mg/kg B.W) of has shown better significance (7.512±0.170 p<0.001), in low doses extract (6.392±0.229; p<0.01) in their protein levels when compared to disease control.

Table No.2: Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced nephrotoxicity.

Parameters	Normal control	Gentamicin treated	AG(200 mg/kg) hydroalcoholi Ext.	AG(400 mg/kg) hydroalcoholic Ext.
Creatinine (mg/dl)	0.940±0.144	2.852±0.125^{**a}	1.563±0.115^{**b}	1.182±0.1 38^{**b}
Urea (mg/dl)	33.69±2.841	51.87±1.862^{**a}	39.91±1.425^{**b}	36.04±1.633^{**b}
BUN (mg/dl)	16.83±0.974	30.06±0.907^{**a}	20.92±0.788^{**b}	18.25±0.603^{**b}
Total protein (g/dl)	8.314±0.105	4.517±0.224^{**a}	6.392±0.229^{**b}	7.512±0.170^{**b}

Values are expressed as mean ± SEM; n=6 animals in a group; One Way ANOVA followed by Dunnet's t-test.
*= p<0.01, ** = p<0.001
a = comparison to normal control group.
b = comparison to gentamicin treated group.

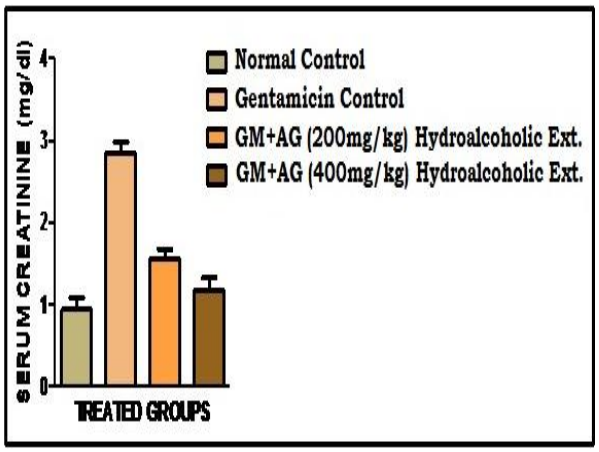

Figure No. 1: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced elevated in serum creatinine levels.

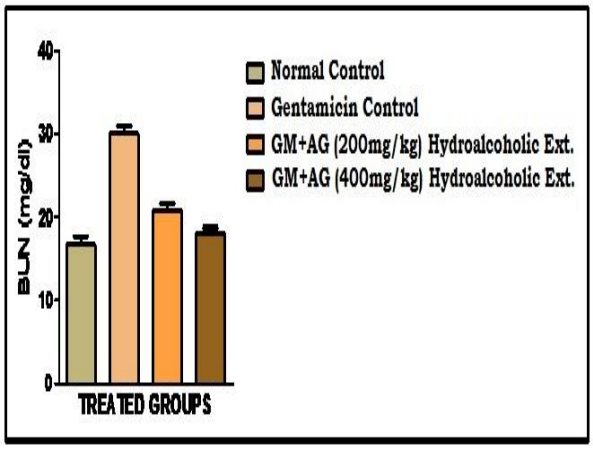

Figure No. 3: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced elevated in blood urea nitrogen levels.

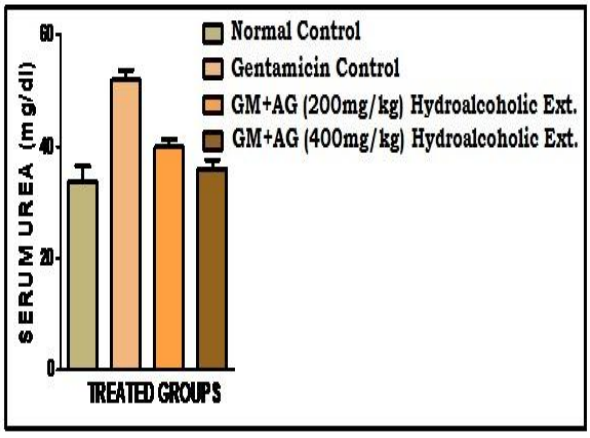

Figure No. 2: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced elevated in serum urea levels.

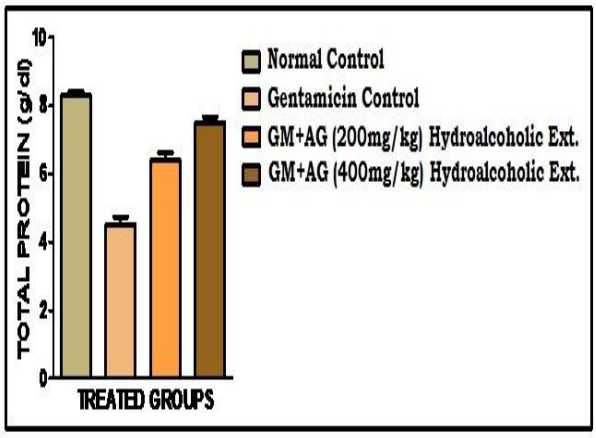

Figure No. 4: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on gentamicin induced reduction in total protein levels.

Table No.3: Effect of hydroalcoholic extract of *Alpinia galanga* (L.) leaves on on tissue parameters

S. No	Treatment	TBARS (mmol/g)	GSH (ug/g)	SOD (IU/dl)	Catalase (IU/dl)
1	Group I	29.8±0.7	67.1±5.2	409±35	54.2±0.71
2	Group II	108±8.0*	23.3±6.5**	160±6.3***	19.2±8.06***
3	Group III	33.2±3.6**	56.8±3.3*	171±11***	48.4±3.6***
4	Group IV	24±1.9***	55.3±3.1*	331±22***	48.2±1.9***

All values are expressed as mean ±SEM, one way Analysis of variance, followed by Dunnets,*p<0.05, **p<0.001, ***p<0.0001, ns-non significant when compared Group II with normal control and remaining groups with disease control.

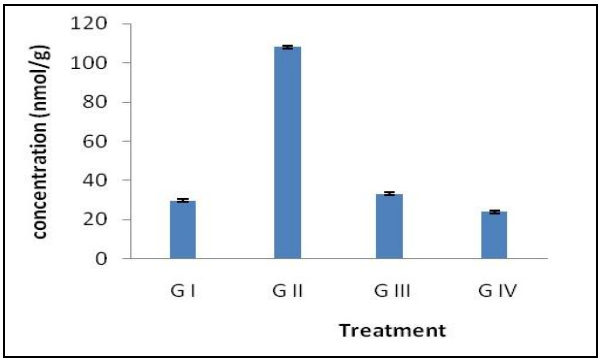

Figure No. 5: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on TBARS

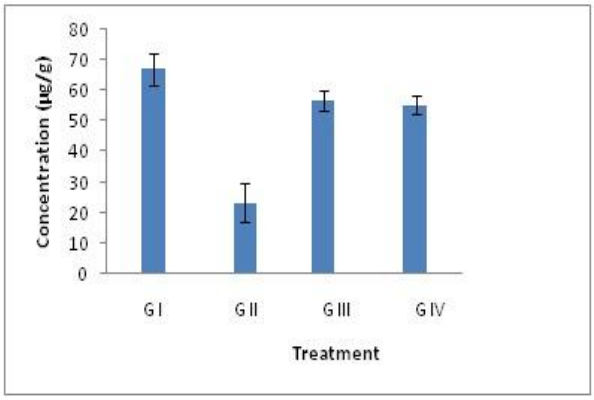

Figure No. 6: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on GSH

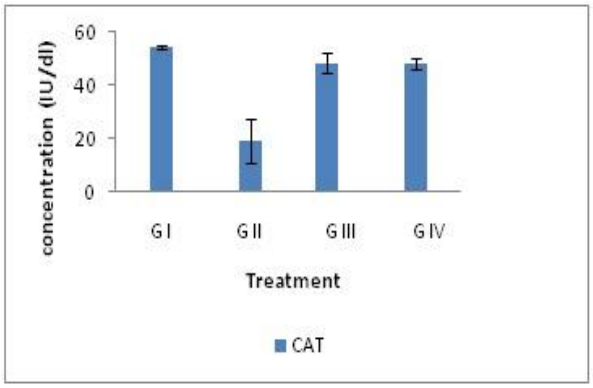

Figure No. 7: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on CAT

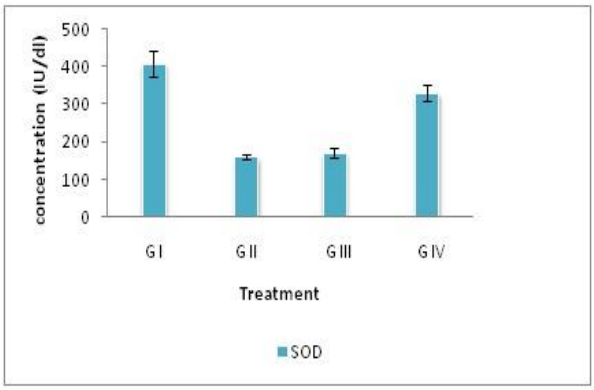

Figure No. 8: Effect of Hydroalcoholic extract of *Alpinia galanga* (L.) leaves on SOD

Effect of *Alpinia galanga* on different oxidative stress parameters in Kidneys

Gentamicin elevated the levels of TBARS and reduced the levels of GSH, SOD and CAT significantly compared to control group. Treatment with extract of *Alpinia galanga* (L.) at a dose of 200 mg/kg significantly reduced the levels of TBARS and non significant rise in levels of GSH, SOD and CAT levels. But the extract of doses 200 and 400 mg/kg decreased the levels of TBARS and increased the levels of SOD, GSH and CAT levels.

Thiobarbituric acid reactive substances

TBARS levels in gentamicin induced group (108±8.0 nmol/gm wet.wt) were significantly high ($p<0.01$) compared to control (29.8±0.7nmol/gm wet wt). TBARS levels in low dose extract treated (33.2±3.6 nmol/gm wet wt) and high dose extract treated groups (24±1.9 nmol/gm wet wt) were significantly high ($p<0.001$) compared to disease control.

Reduced glutathione

GSH levels in gentamicin treated group (23.3±6.5 µg/gm wet wt.) were significantly high ($p<0.001$) compared to normal control (67.1±5.2 µg/gm wet wt.). GSH levels in treated group ($p<0.05$), low dose extract treated group (56.8±3.3 µg/gm wet wt.) ($p<0.001$) showed significant increase compared to disease control.

Superoxide dismutase

SOD levels in gentamicin induced group (160±6.3 IU/dl) had shown significant increase compared to control group (409±35 IU/dl) ($p<0.001$). High dose extract treated (331±22 IU/dl) ($p<0.0001$) showed significant increase in SOD levels compared to gentamicin induced group i.e. disease control.

Catalase

Catalase levels in STZ induced group (19.2±8.06) has shown significant compared to normal control (54.2±0.71). High dose extract treated (48.2±1.9) group has shown significant increase compared to the disease control group.

Histopathology of Kidney of The Experimented Animals

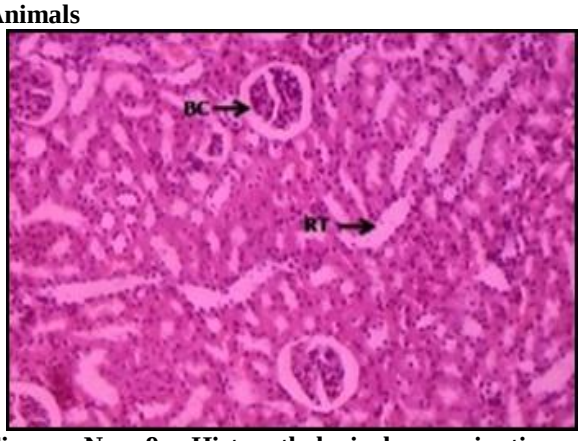

Figure No. 9: Histopathological examination of Group I.

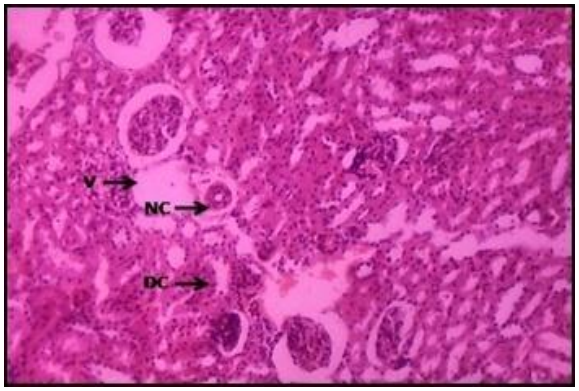

Figure No. 10: Histopathological examination of Group II.

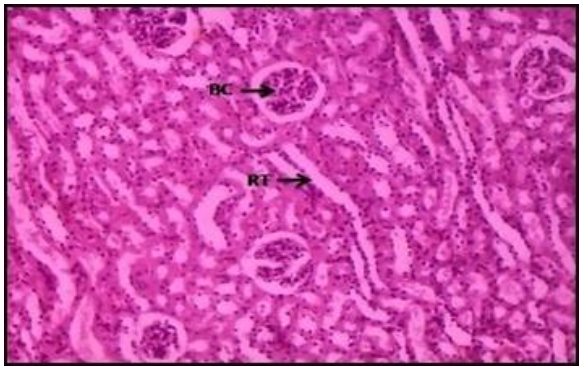

Figure No. 11: Histopathological examination of Group III.

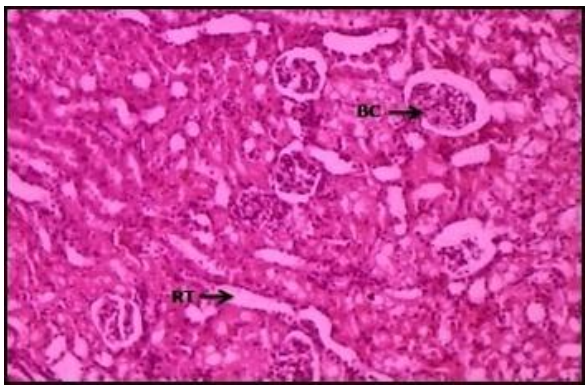

Figure No. 12: Histopathological examination of Group IV.

Group 1 (Normal Control): The normal architecture of the kidney tissue

Group II (Gentamicin treated): The degenerative changes in kidney tissue take place and show vacuolization. Necrosis and degenerative Bowman's capsule.

Group III {AG (200 mg/kg) Hydroalcoholic Ext.}: Mild regeneration of tissue shows normal cyto architecture of the kidney tissue.

Group Group IV {AG (400 mg/kg) Hydroalcoholic Ext.}: Regenerative changes in kidney tissue take place and shows normal cyto architecture

DISCUSSION

Phytochemical screening

The phenolic compounds like flavonoids and tannins are a major group of compounds which are known for primary antioxidants or free radical scavengers. Since these compounds were found in the leaves of *Alpinia galangal* (L.). Hence it might be answerable for the effective nephroprotective activity.

Pharmacological evaluation

Gentamicin is a known nephrotoxic agent. There is positive correlation between oxidative stress and nephrotoxicity. Gentamicin induces a significant degree of nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydroxylase, such as sphingomylinase and phospholipidase in addition to causing oxidative stress through induction of reactive oxygen species such as free radical, superoxide, hydroxyl radical anion and hydrogen peroxide may produce cellular injury and necrosis by means of several mechanisms including membrane lipids peroxidation, protein denaturation and DNA damage.

In this study, nephrotoxicity was developed by single daily I.P. injection of gentamicin at a dose of 80 mg/kg for 8 days. This toxicity was characterised by ($p<0.001$) decrease in body weight, serum albumin, total protein and increase in kidney weight; elevation in the circulating level of serum creatinine, serum urea, and blood urea nitrogen in group II, when compared to untreated group I rats. These changes were attenuated by the co treatment of hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) in high and low dose significantly ($p<0.001$) lowered acute elevation of serum markers of kidney function as compared to, when compared with group II. Apart from the direct nephrotoxic effect of gentamicin in group II, the acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state in rats due to the prolonged anorexia associated with gentamicin.

Gentamicin induced nephrotoxicity significantly ($p<0.001$) increase the serum creatinine level when compared to group I normal rats. Serum creatinine is a prominent indicator of renal failure. When kidney functions abnormally, creatinine levels in the blood rises as it is not adequately excreted by the kidney. Extract in high and low dose significantly lowered the elevated serum concentration of creatinine compared, maintaining their values within the normal range when compared to toxic control rats group II.

In case of serum urea, there was a significant ($p<0.001$) increase in the circulating level of serum urea in gentamicin treated group when compared to normal group I which may be due to damage in proximal tubular epithelial cells which affect the tubular secretion of urea, elevates the serum urea level. Nephroprotective activity of the extract ($p<0.001$) was found to be superior as

compared to control by decreasing the levels of urea within the normal range.

Even during blood urea nitrogen estimation, there was a significant ($p<0.001$) increase in BUN level in gentamicin treated group when compared to normal group I. It rises with renal failure, increased protein breakdown and fluid volume depletion. If kidney is unable to excrete urea from the blood normally, the BUN rises. Increase in BUN may be due to increased catabolic state of the rats due to the prolonged anorexia. Nephroprotective activity of the extract ($p<0.001$) was found to be superior), maintaining their values within the normal range compared to group II.

Discussing total protein content, there was a significant ($p<0.001$) decrease in the total protein level in gentamicin induced nephrotoxic rats compared to normal group I. It may be due to the breakdown of proteins which will decrease the total protein level. There is an elevation of total protein level in co administration of hydroalcoholic extract in high dose treated group shows better significant ($p<0.001$) activity, maintaining the values within the normal range when compared to group II nephrotoxic rats.

Hence nephroprotective activity of the hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) in high (400 mg/kg B.W) and low dose (200 mg/kg B.W) has shown better significance ($p<0.001$) was may be due to the presence of bio active principles such as flavonoids, alkaloids, phenols, tannins, saponins etc against potassium dichromate and gentamicin induced nephrotoxicity.

Histopathological evaluation

Extracts were able to prevent cell injury such as cellular infiltration, degenerative Bowman's capsule and blood vessel congestion. From the histopathological result it can be stated that hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) have protective effect against degenerative damage caused by potassium dichromate.

In rats treated with gentamicin, the nephrotoxicity was evidenced by expanded mesangium in glomeruli along with which distorted tubular shape, presence of inflammatory cells, vacuolization and necrotic epithelial cell in tubular lumen (Figure 6.24). Treatment with the hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) leaves (200 mg/kg, PO) reduced such pathological changes in kidney histology induced by gentamicin (Figure 6.25) which was more profound in higher dose (400 mg/kg, PO).

Oral administration of extracts could prevent cell injury such as expanded mesangium, vacoulization, tubular damage and necrotic epithelial cell. Histopathological result revealed that hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) in both doses have protective effect against degenerative damage caused by gentamicin.

CONCLUSION

According to the *in-vivo* results it can be stated that hydro alcoholic extract of *Alpinia galanga* (L.) had offered protective effect against the deleterious renal side effect of Gentamicin was exemplified by the alterations in physical and biochemical parameters as compared. On the other hand, the ability of the constituents of the extract to scavenge free radicals may possible be involved in the protection.

Hence; the hydro alcoholic extract of *Alpinia galanga* (L.) leaves can be advocated as nephro protective agent. Further studies required which compound is accountable for the nephro protective activity.

The efficacy of *Alpinia galanga* (L.) for curing or alleviating chronic renal failure (CRF) may be a light for developing a potential herbal medicine for the future.

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