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RESEARCH ARTICLE
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"RENOPROTECTIVE ACTIVITY OF *AMORPHOPHALLUS PAEONIIFOLIUS* AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY IN RATS"

Shekshavali T[1*], Abdul Haseeb TS[2], T. Naga Aparna[3], Jaffer sadik Md[4], Konda Shravan kumar[5], V. Arun Reddy[6], E. Praveen[7], Shaik Rahaman[8]

[1]Assistant Professor, Department of Pharmacology, National College of pharmacy, Shivmogga, Karnataka, India
[2]Senior Pharmacist, Pharmacy service Department, Johns Hopkins Aramco Healthcare, Dharan, Saudi Arabia
[3,4]Associate Professor, Department of Pharmaceutics, Sri indu institute of Pharmacy, Hyderabad, Telangana, India
[5]Professor, Department of Pharmaceutics, Vision college of Pharmaceutical sciences and research, Hyderabad, Telangana, India
[6]Assistant Professor, Department of Pharmaceutical analysis, Bharat institute of Technology (Pharmacy), Mangalpaly, Hyderbad,India
[7]Assistant Professor, Department of Pharmaceutics, Sri indu institute of Pharmacy, Hyderabad, Telangana, India
[8]Department of Pharmacology, School of Pharmaceutical Education and research, Jamia Hamdard University, New Delhi, India

***Corresponding Author**: Dr.Shekshavali T
*Assistant Professor, Department of Pharmacognosy, National College of pharmacy, shivmogga, Karnataka. Mail id: Shahakeer@gmail.com

Abstract

To investigate the nephroprotective and antioxidant activities of methanol and aqueous extracts of *Amorphophallus paeoniifolius* on Gentamicin induced nephrotoxicity in male Wistar rats.In this model of nephrotoxicity, 24 adults male wistar rats (150-200gms) were evenly divided into 4 groups. Group-1 and 2 served as control and Gentamicin induced models respectively, while Group-3 and 4 are the treatment groups which were simultaneously treated with methanolic (250mg/kg) and aqueous (250 mg/kg) extracts of *Amorphophallus paeoniifolius* respectively, after each dose of Gentamicin (80 mg/kg, i.p.) for 10 days. On 11th day, blood samples for biochemical parameters, while the rat kidneys for histology were obtained under inhaled diethyl ether anesthesia.Co-administration of ME and AE extracts with Gentamicin decreased rise in serum urea and creatinine. *Amorphophallus paeoniifolius* extracts significantly show increased activities of renal Catalase and Glutathione, and significant decreased activity of lipid peroxidase. Apart from these, histopathological changes also showed the protective nature of *Amorphophallus paeoniifolius* extracts against Gentamicin induced necrotic damage of renal tissues.In comparison to aqueous extracts, histological and biochemical findings demonstrated that the methanolic extract of Amorphophallus paeoniifolius conferred greater nephro-protective and antioxidant effects against Gentamicin-induced nephrotoxicity in rats. In the future, Amorphophallus paeoniifolius might provide a clue for the development of a brand-new medication to treat drug-induced nephrotoxicity.

Key words: *Amorphophallus paeoniifolius*; nephro-protective; Gentamicin; antioxidant

INTRODUCTION

1. Introduction to herbal medicines

In the present situation, herbal medicine is gaining importance and it has become a topic of world. It is showing impact on both world health and international trade. In the developing countries large proportion of the population are using herbal medicine due to high cost of western pharmaceuticals in the health care[1].

Since times immemorial plants have been using as stores of potential biochemical factories. Man is able to extract many of the potential bio chemicals from the medicinal plants. These useful chemicals can be extracted from any part of the plant like bark, leaves, flowers, fruits, seeds, roots etc[2].

2. Introduction to Nephrotoxicity

Nephrotoxicity is important concern during drug development when selecting new drugs. Presently the world population is having lots of kidney disorders, which has several reasons to be explained for the causes of disorders. Kidneys are the major organs which are responsible for various functions in our body like regulate blood volume and composition, help to regulate blood pressure, synthesize glucose, release erythropoietin and excrete wastes in the urine. Liver and kidney are major organ systems physiologically involved in the metabolism and excretion of various xenobiotics, consequently they are exposed to oxidative stress and free radicals. This results in the tissue necrosis and damage of these organ systems. Therefore, several attempts are been made to protect these organs from the free radical challenges. Reactive oxygen species developed form the chemicals or drugs that are exposed to the renal cells will lead to the renal necrosis.

In our body free radicals are generated through several processes that might be exposure to ultraviolet radiation in sun light, exposure to x- rays and during normal metabolism of the cells. Chemical substances like carbon tetrachloride and various drugs which we are using for various diseases also produce free radicals when they are metabolized in the body[3]. There are several types of reactive oxygen species which include the hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. These reactive species will react with membrane lipids, proteins, nucleic acids, enzymes and other small molecules it may lead to the cellular damage. The cellular damage which is caused by these free radicals is the main reason for many degenerative diseases of ageing such as cardiovascular diseases, brain dysfunction, cancer, cataracts, and immune system decline etc. Free radicals are charged molecules which are having unpaired electrons, these charged molecules will try to get neutralized by capturing electrons from other substances. During neutralization process of these free radicals they will generate new free radicals, so it is a chain reaction which will generate thousands of free radicals within seconds. Antioxidants are able to stabilizing or deactivating those free radicals before they attack cells. So, antioxidants are essential for maintaining optimal cellular and systemic health. To protect from these free radical's human have evolved highly sophisticated antioxidant protection system. The protection system involves both exogenous and endogenous in origin.

These antioxidants include:

- **Nutrient derived antioxidants:** ascorbic acid, tocopherols, tocotrienols, carotenoids and low molecular weight compounds such as glutathione and lipoic acid.
- **Antioxidant enzymes:** these enzymes which catalyze free radical quenching reactions includes superoxide dismutase, glutathione peroxidise, and glutathione reductase.
- **Metal binding proteins:** these proteins will bind to the metal ions like iron and copper because metal ions will act as catalysts for oxidative reactions, such proteins are ferritin, lactoferrin, albumin, and ceruloplasmin.
- **Phytonutrients:** wide varieties of plant materials are having antioxidant compounds[3,4].

The inbuilt antioxidant systems like glutathione reductase, superoxide dismutase, catalase etc., will protect the cells in our body from free radicals. But, sometimes the generation of free radicals are so high such that they may over power the inbuilt antioxidant systems and damage the cells. So we need extra supplements of antioxidants to protect from these free radicals. By using vitamins and phytonutrients we can protect our body cells from these free radicals. As we know many plants contain phytochemicals constituents that possess medicinal properties, among them majority of plants shows antioxidant activity. Hence, the present study was conducted to evaluate the nephroprotective and antioxidant activity of *Amorphophallus paeoniifolius* Dennst. The main aim of the present study is to detect the nephroprotective activity of aqueous and methanolic extract of *Amorphophallus paeoniifolius* Dennst. The nephroprotective activity was screened against Gentamicin-induced nephrotoxiciy in rats. In addition, the antioxidant activity was found out by using *in-vitro* methods like serum enzymes estimation and *in-vivo* methods like tissue lipid peroxidation (LP), superoxide dismutase (SOD), catalase and glutathione (GSH).

Gentamicin is an aminoglycoside antibiotic widely used for treating gram negative infections. But it will cause nephrotoxicity which may occur 13 -30% treated patients. During Gentamicin treatment free radicals is going to be generated which are highly toxic to the tissues[5]. So the study was under taken to examine the preventive effect of extracts of *Amorphophallus paeoniifolius* on Gentamicin induced nephrotoxicity in albino rats Therefore, present study is to explore a possibility of using nephro-protective agents from natural sources along with nephrotoxic drugs so as to protect kidneys.

Anatomy and Physiology of Kidney:

The kidneys lie on the posterior abdominal wall, one on each side of the vertebral column, behind the peritoneum and below the diaphragm. They extend from the level of the 12th thoracic vertebra to the 3rd lumbar vertebra, receiving some protection from the lower rib cage. The right kidney is usually slightly lower than the left, probably because of the considerable space occupied by the liver (Fig no: 1)

Kidneys are bean-shaped organs, about 11 cm long, 6 cm wide, 3 cm thick and weigh 150g. They are embedded in, and held in position by a mass of fat. A sheath of fibro-elastic renal fascia encloses the kidney and the renal fat.

Kidney Toxicity Induced by Nephrotoxic Agents:

The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. In addition, there is failure of regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure (Herfindal *et al*)[,13].

Chronic renal failure (CRF) is an irreversible deterioration in the renal function which classically develops over a period of years, leading to loss of excretory metabolic and endocrine functions. Various causes of renal failure has been attributed like hypertension, diabetes mellitus, antineoplastic agents like cyclophosphamide, vincristin, Cisplatin etc.

Acute renal failure (ARF) refers to the sudden and usually reversible loss of renal function which develops over a period of days or weeks. There are many causes of acute renal failure which could be either pre-renal (55%), renal (40%), or post renal (5%). Among the renal causes of acute renal failure, acute tubular necrosis is more common accounting for 85% of incidence. Acute tubular necrosis occurs either due to ischemia or toxins. The toxin can be either exogenous or endogenous. The exogenous agents are radiocontrast agents, cyclosporin, antibiotics, chemotherapeutic agents, organic solvents, acetaminophen and illegal abortifacients[9,14].

Nephrotoxic Agents:

Drugs, diagnostic agents and chemicals are well known to be nephrotoxic. The following are the some of the important nephrotoxic agents[9,14].

A. Antineoplastic Agents: Alkylating Agents
Cisplatin, Cyclophosphamide, Nitrosoureas: Streptozotocin, Carmustine, Lomustine, Semustine.

Antimetabolites
High dose Methotrexate, Cytosine arabinose, High dose 6-Thioguanine, 5-Flurouracil.

Antitumor Antibiotics
Mitomycin, Mithramycin, Doxorubicin.

Biological Agents
Recombinant Leukocyte A Interferon

B. Antimicrobial Agents
Tetracycline, Acyclovir, Pentamidine, Sulphadiazine, Trimethoprim, Rifampin, Amphotericin B.

C. Aminoglycosides
Gentamicin, Amikacin, Kanamycin, Streptomycin, Toberamycin, Neomycin, Dibekacin.

D. Heavy Metals
Mercury, Arsenic, Lead, Bismuth.

E. Miscellaneous
Radio-contrast agents.

F. NSAIDS:
Paracetamol, Ibuprofen, Indomethacin, Aspirin etc.
Nephrotoxic agents can produce damage either by directly reacting with cellular macromolecules and membrane components or from metabolism within the tubular cells to toxic products. The agents which cause direct toxicity are heavy metals like Hg, Pb which interact with sulfhydryl groups, organic cations such as spermine, cationic amino acids, amino glycosides, which interacts with membrane phospholipids, polyene antibiotics like amphotericin B which interacts with membrane cholesterol. Fluoride and oxalates produced by hepatic metabolism of methoxyflurane, intermediates of Cisplatin, cystine conjugates, cephalodrine, acetaminophen induce damage by their metabolites. These toxic metabolites mainly include free radicals[15].
The nephrotoxins damage specific segment of the nephron to a greater extent than the other segments. The proximal tubule is the most commonly affected, because of the presence of inducible type of microsomal mixed function oxidases (cytochrome P. 450) which have been implicated in the toxic activation of various agents. This segment is also rich in glutathione and glutathione metabolizing enzymes. The other common sites which can be affected are renal medulla, distal tubule and loop of Henle. The renal medulla is affected mainly by polyene antibiotics and cyclosporine and that of distal tubule dysfunction is mainly due to non-steroidal anti-inflammatory agents, cyclosporine, pentamidine, trimethoprim, amphotericin, sulphamethaxozole, aminoglycoside antibiotics, lithium and demeclocycline.
The functional manifestations of nephrotoxicity can occur at several levels like tubular function abnormalities such as potassium, magnesium and sodium wasting, concentrating defects and reduction in glomerular filtration. However, there are no ideal clues to the occurrence or localization of tubular cell injury. The nephrotoxin induced changes in the tubule cells may be lethal or sublethal[15,16].

Mechanisms of drug induced renal damage:
a) Free radical production[17,18].
b) Disturbance of renal tubule cell energy metabolism[19].

c) Disrupted cell calcium homeostasis[20].
d) Alteration of membrane phospholipid metabolism[21,22].
e) Disruption of cellular monovalent cation volume and pH dependant degradation[23].
f) Disruption of cytoskeleton[23].
g) Abnormalities of cell proteases[24].
h) Abnormalities of protein and nucleic acid synthesis[25-27].
i) Distruption of lysosomal function[28].

a) Free Radical Production:

Free radicals may be defined as atoms or group of atoms having an unpaired number of electrons that may enter into covalent bond formation. These unpaired electrons are chemically unstable, highly reactive and may induce chemical chain reactions in susceptible compounds. Any aromatic compound can be involved in free radical formation. Biological important free radicals include metabolic products of chemicals and drugs, free radicals formed during metabolic reactions such as superoxide, hydroxyl radical and singlet oxygen (high energy state of oxygen). In kidney the metabolism of toxic agents intracellularly forms reactive metabolites, the free radicals causing toxic cell injury. The involvement of the free radical mechanisms in the co-oxidation process is strongly suggested by observations that free radical scavengers, such as Vit.E and butylated hydroxy toluene inhibit co-oxidation. Highest levels of prostaglandin endoperoxide synthetase in the kidney are found in medulla. A major deleterious effect of intracellular free radical generation is lipid peroxidation. Lipid peroxidation is the oxidative deterioration of poly unsaturated lipids.

Peroxidation of lipids usually involves reaction of free radicals and poly unsaturated lipids to form free radical intermediates and semistable hydroperoxide. The peroxidation of unsaturated lipids causes dramatic alteration in membrane structure and function due to loss of membrane lipids. Membranes of mitochondria endoplasmic reticulum contain large amount of unsaturated fatty acids making them important sites of lipid peroxidation damage. The free radical metabolite can also damage proteins and nucleic acids. Lipid hydroperoxidase are relatively stable but with decomposition in presence of metals like iron can leads to damage to the membrane. Lipid peroxidation of lysosomes enhances phospholipid degradation and production of lysophospholipids, suggesting an action of lipid peroxidation to enhance susceptibility of membrane phospholipids to phospholipases. Lipid peroxidation increases the mitochondrial membrane sensitivity to phospholipases. Toxic activation of free radical production ultimately leads to cell death. The two major processes implicated in the pathogenesis of cell injury is abnormal calcium metabolism and phospholipadase activation[13,29].

b) Disturbance of renal tubule cell energy metabolism:

Mitochondrion is an important intracellular site for cell energy metabolism. ATP is the energy currency of the cell. Renal tubule cells are abundantly supplied with mitochondria, which provides the controlled oxidative metabolism to the ATP required to drive transport and other energy consumption processes. Mitochondria comprise of about 30% of the protein and volume of renal proximal tubule cells. Kidney tubules normally respire at rates that are only about one-third of their maximal capacity for oxidative metabolism, suggesting the availability of redundant functional capacity.

The complex balance of transport functions at the inner mitochondrial membrane, requiring maintenance and precise regulation of its selective permeability properties, make it highly sensitive to perturbation by toxins. Following in vivo Exposure to a number of nephrotoxins like Cisplatin and Gentamicin, prior to the appearance of lethal cell injury, it has been observed that renal cortex ATP levels are reduced. Along with mitochondrial dysfunction due to decreased oxygen supply and changes in renal blood flow[32,33]. Agents with defined tubule toxicity that have distinct mitochondrial functional effects include arsenic, uranyl, cadmium, tin, copper, silver and gold. The importance of nephrotoxic mitochondrial effects in the pathogenesis of renal cell are:

- Interference with renal tubule cell oxidative metabolism can lead to functional changes, structural organization, and cell death.
- *In-vitro*, most nephrotoxins are capable of disrupting the integrated function of the inner mitochondrial membrane by means of mechanisms that appear to be relatively specific for the nephrotoxin.
- Available studies on isolated tubules and tissue slices suggest that the functional changes in isolated mitochondria are also expressed in situ.
- Nephrotoxins interact with renal tubule cell mitochondria in situ but, as is also the case with the classic metabolic inhibitors, the complex extracellular and intracellular milieus that must be crossed before the nephrotoxins reaches the mitochondria may markedly modulate the nature of this interaction. In remains difficult to assess whether changes in mitochondrial function seen after in vivo treatment, even when occurring early, are attributable to direct nephrotoxic activity at the mitochondrial membrane or are secondary to other actions.

c) Disrupted cell-cell calcium homeostasis:

The major cellular calcium pools are present in the following sites:

1. Calcium bound to the outer surface of the cell membrane.
2. Free cytosolic calcium.
3. Calcium bound to cytosolic surface of the membrane.
4. Calcium in the intracellular organelles, mainly mitochondria and endoplasmic reticulum.

During the development of the lethal cell injury, there is an increase in the cellular calcium concentration. Among the calcium pools active mitochondrial sequestration is the most important process accounting for major elevation of tissue calcium during injury[34].

The calcium dependent membrane bound phospholipases are present in the plasma membrane, membrane of mitochondria and endoplasmic reticulum. These calcium membranes bound lipases are proposed to have important role in mediating membrane injury.

Lots of evidences have claimed to support the role of disordered cell calcium homeostasis in toxic cell injury in the kidney[36].

d) Alteration of membrane phospholipids metabolism:

The membrane phospholipids have been shown to play an important role in regulating membrane permeability and the activity of the membrane bound enzymes++37 such as Na K ATPase . The cell membrane has intrinsic membrane phosphol passes which is responsible for membrane phospholipids degradation. The degraded products like lysophospholipids and free fatty acids have toxic detergent properties, which contributes to the membrane injury. The cell viability is determined mainly on the integrity of cell membrane. Any disruption of phospholipids metabolism by means of abnormal increase in phospholipases, decreases normal phospholipids synthetic activity or combination of these two events those are critically important in the pathogenesis of cell injury. The impairment of the regulation of the activity of the phospholipase or failure of normal compensatory synthetic mechanism from salvaging phospholipids degradation products and reconstituting membrane of phospholipids leads to cell damage. Amino glycosides not only affects plasma membrane bound phospholipases but also cytosolic and lysosomal phospholipases enzyme which further contributes to cell damage[37,38].

e) Disruption of cell monovalent cation volume and pH degradation:

Nephrotoxin induced of cellular monovalent cation homeostasis can impair the normal resorptive function of renal tubule resulting in cation wasting and volume dysregulation, there by promoting cell injury. Mercury and organic mercurial are known to increase membrane monovalent cation permeability. Heavy metals directly affect numerous enzymes including Na -K ATPase. Cisplatin and Gentamicin are found to inhibit Na -K ATPase. Alterations of cell pH during toxic injury have not been well documented.

However, studies with both renal tubules and other all types demonstrated important modulating effects on cellular susceptibility to injury.

Causes of Elevated BUN and Creatinine Levels:

There are many causes of elevated BUN and Creatinine levels in blood, those are as follows.

1. There may be a blockage of blood flow to or from the kidney. This may be caused by kidney stones or a tumor. Low blood pressure or irregular heart rhythms may be preventing blood flow to the kidneys and may produce signs of kidney problems.
2. In the case of dehydration.
3. In the case of urinary infections or nephritis (inflammation of the kidneys).
4. Drug toxicity.

- Chemotherapy drugs such as: Cisplatin, Carboplatin, Carmustine, Mitomycin, high-dose Methotrexate.
- Biologic therapy such as Interleukin-2, or Interferon Alfa.
- Antibiotics (such as Amphotericin B, Gentamicin and Vancomycin.
- Angiotensin-Converting Enzyme (ACE) Inhibitors - used in heart failure or after a heart attack.
- Non-steroidal Anti-inflammatory Drugs (NSAID's).
- Some diuretics - such as Furosemide - may cause kidney failure.
- Intravenous radio-contrast dye - certain "dyes" may be injected into our bloodstream during a radiology procedure to improve the "picture" that is seen on CT scan, MRI or x-ray. These dyes, if the patient is at risk for kidney failure, or when given in combination with certain other medications, may cause further kidney problems.

5. An enlarged prostate gland in men.
6. In case of heart attack or in congestive heart failure.
7. In the case of bleed in the gastrointestinal tract or in the stomach.
8. When the diet is having lots of protein.
9. Long-standing low blood pressure levels.
10. Diabetes mellitus (diabetic nephropathy)[5].

The Nephro-protective and antioxidant plants are:

- *Bauhinia variegate* (Linn.) whole plant extract[62].
- *Cassia auriculata* (Linn.) root extract[63].
- *Aerva lanata* extrac[64]
- Green tea extract[65].
- *Rubia cordifolia* extract[66]
- Garlic oil[67].

Introduction to *Amorphophallus paeoniifolius*[68]:

Figure No. 7: Whole plant of *Amorphophallus paeoniifolius*

Figure No. 8: Tubers of *Amorphophallus paeoniifolius*

Name of the plant: *Amorphophallus paeoniifolius*
Family: Araceae
Vernacular names[68,69]**:**
English - Elephant foot yam
Sanskrit - Surunah
Hindi - Jamikand, Suran
Kannada - Choorana, Choorana gedde, Kandagedde Telugu - Duladumpa, Kandagadda, Manchikanda Marathi - Sooran
Mizoram - Hlochangvawn, Khatual Oriya - Suruni kanda Malayalam - Cena
Tamil - Karanaikkilanku

Habitat and distribution[70]**:**
Elephant foot yam is a crop of South East Asian origin. The plant prefers growing in secondary forests, shrub forests and grasslands of arid valley areas at an elevation of 700 meters. This foot yam grows in wild form in Indonesia, Malaysia, Philippines, and several other parts of Southeast Asian countries. In several parts of India like West Bengal, Kerala, Maharashtra, and Orissa, Amorphophallus Paeoniifolius is widely found. In India it is popularly known as Suran.

Description[69]**:**
Shrubs, annual, high ± 1 m, cylindrical, forming a tuber, green. Stem is single, menjari, flat edge, pointed tip, base grooved, length + 50 cm, width of ± 30 cm, stem hugging the stem, cylindrical, ± 30 cm long, green white spots, green. Leaves are compound, androgynous, head shape, length + 7.5 cm, would be tightly coiled fruit, the stigma two to the league, anthers circular, crown red, red. Fruit is buni, oval, red. Seed is round and red. Roots are fiber, dirty white. A perennial, stemless herb. corm is globose, up to 30cm in diameter. The leaf stalk develops from the corm, usually about 1 meter high. Leaves are solitary, blades upto 1meter in diameter, trisected with dichotomous segments. Spathe is sessile campanulate, and purplish up to 30cm in diameter. The spadix (a spike of flowers contained in the spathe) sulcate and depressed, up to 15cms long, are malodorous when flowering.

Chemical composition[69]**:**
Amorphophallus paeoniifolius tubers contain saponins and flavonoid, stems and leaves contain saponins and polyphenols. Corm is 74% moisture; 0.73% ash; 5.1% protein; 18% carbohydrate providing about 1,000 calories per kilo; comparable in food value to kalabasa, superior to singkamas.

Medicinal uses[71]**:**
The tubers of wild plants are highly acrid and cause irritation in throat and mouth due to excessive amount of calcium oxalate present in the tubers. The tubers are anodyne, anti- inflammatory, anti-haemorrhoidal, haemostatic, expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic, aphrodisiac, emmenagogue, rejuvenating and tonic. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, haemorrhoids, haemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis, hepatopathy, spleenopathy, amenorrhea, dysmenorrhoea, seminal weakness, fatigue, anaemia and general debility. The tuber is reported to have anti-protease activity, analgesic activity, and cytotoxic activity.

OBJECTIVES

The literature survey of *Amorphophallus paeoniifolius* reveals that tuber part has been used to treat variety of diseases like anti-inflammatory, anti-hemorrhoidal, hemostatic, expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic, aphrodisiac, emmenagogue, rejuvenating and tonic. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis hepatopathy, spleenopathy, amenorrhea, dysmenorrhea, seminal weakness, fatigue, anemia and general debility. The tuber is reported to have anti-protease activity, analgesic activity, and cytotoxic activity[71]. However, the tuber part of *Amorphophallus paeoniifolius* has been not evaluated and not reported for nephro-protective property, in view of this the present work undertaken to evaluate nephro-protective property of *Amorphophallus paeoniifolius*. The work was carried out in the following phases:

Phase I:

- Collection and authentication of plant material.
- Preparation of *Amorphophallus paeoniifolius* methanol, petroleum ether and chloroform extracts using soxhlet apparatus.
- To investigate preliminary phytochemical constituents, present in the extract.
- Determination of LD_{50} to fix therapeutic dose for the study of nephro-protective activity. On the basis of LD_{50} the two doses were taken for the study.

Phase II:

To evaluate the nephro-protective activity of extracts in experimental animal model which include:

- Gentamicin induced nephrotoxicity in rats.

Parameters to be studied:

- Serum analysis- Urea, Creatinine.

***In-vitro* studies:**

- DPPH method

***In-vivo* Antioxidant studies:**

- Lipid peroxidation (LPO)
- Glutathione (GSH)
- Catalase (CAT)

MATERIALS AND METHODS

1.1 List of Materials and Equipments used in Experiment:

Table No. 3: List of Chemicals Used

Sl. No	Name of the Materials and Equipments
1	Dilute hydrochloride acid(Bangalore fine chemicals)
2	Albino rats (National college laboratory animal facilities)
3	Anesthetic ether (Kabra drugs Ltd)
4	Ascorbic acid (Sd fine chemicals Ltd)
5	Chemicals for creatinine in serum (Coral clinical systems)
6	Chemicals for urea in serum (Span diagnostics Ltd)
7	Cisplatin (Biochem Pharmaceutical Industries)
8	Deoxyribose (Bangalore fine chemicals)
9	Disodium hydrogen phosphate (Sd fine chemicals Ltd)
10	DPPH (Aldrich)
11	EDTA (Spectrum reagents and chemicals Pvt. Ltd)

12	Ethanol (Changshu yangyuan chemicals)
13	Ferric chloride (Spectrum reagents and chemicals Pvt. Ltd)
14	Formalin (Sd fine-chemicals Ltd)
15	Gentamicin (Randox Chemicals Ltd)
16	Griess reagent (Bangalore fine chemicals)
17	Hydrochloric acid (Sd. Fine chemicals Ltd)
18	Hydrogen peroxide (Merck specialties Pvt Ltd)
19	Methanol (Sd fine-chemicals Ltd)
20	NADH (Bangalore fine chemicals)
21	NBT (Bangalore fine chemicals)
22	Paracetamol (A TO Z pharmaceuticals Pvt Ltd)
23	PMS (Bangalore fine chemicals)
24	Potassium ferricyanide (Sd fine chemicals Ltd)
25	Sodium nitroprusside (Sd fine chemicals Ltd)
26	Thiobarbituric acid (Bangalore fine chemicals)
27	Trichloroacetic acid (Sd. Fine chemicals Ltd)
28	Bovine Serum Albumin (Hi-media Labs Pvt. Ltd, Mumbai)
29	Di-sodium Hydrogen ortho phosphate (Nice chemicals Pvt Ltd, Cochin)
30	Orthophosphoric acid (Nice chemicals Pvt Ltd, Cochin)
31	Reduced Glutathione (GSH) (Loba chemicals Pvt. Ltd, Mumbai)
32	2-thiobarbituric acid (TBA) (Hi media labs Pvt. Ltd, Mumbai)
33	Trichloro acetic acid (TCA) (Hi media labs Pvt. Ltd, Mumbai)
34	5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB) (Hi media labs Pvt. Ltd, Mumbai)

1.2 Collection and Authentication of plant material:

The tubers of the plant *Amorphophallus paeoniifolius* used for the present study were collected from Shimoga district of Karnataka. The whole plant was identified, confirmed and authenticated by Rudrappa, Department of Botany, DVS College, Shimoga . The whole plant material were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

1.2.1 Preparation of tuber extracts:

The powder was extracted directly with methanol, petroleum ether, chloroform and aqueous extracts which were used for biological investigations and *in-vitro* antioxidant studies, after subjecting it to preliminary qualitative phytochemical studies[90]. The extract was concentrated under reduced pressure and stored in vacuum desiccators.

The percentage yield of methanol, petroleum ether, chloroform and aqueous extracts were reported.

1.3 Preliminary phytochemical screening of the extracts[91]:

Phytochemical analysis was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, phytosterols/terpenes, proteins Tannins and saponins were qualitatively analyzed.

- **Alkaloids:**

The extracts were dissolved in dil. H_2SO_4 and filtered. The filtrate was treated with Dragendroff's, Hager's, Mayer's and Wagner's reagents separately. Appearance of orange brown, yellow, cream, pink and reddish brown precipitates in response to the above reagents respectively indicate the presence of alkaloids.

- **Carbohydrates:**

The extracts were treated with Benedict's, Fehling's, Molisch's and Barfoed's reagents under suitable conditions. Appearance of red, brick red, purple colour in response to the above reagents respectively indicates the presence of carbohydrates.

- **Flavonoids:**

The extracts along with few ml of alcohol were heated with magnesium ribbon and concentrated Hcl under cooling. Appearance of pink colour indicates the presence of flavonoids. A few ml of extract is treated with $FeCl_3$ an appearance of intense green colour was observed. The extract treated with few ml of aqueous NaOH, appearance of yellow colour and changes to colourless with Hcl indicate the presence of flavonoids. Extracts were treated with the lead acetate and formation of yellow precipitate indicates the presence of flavonoids.

- **Glycosides:**

Small quantities of extracts were subjected to Balijet, Borntrager's, Keller- Killiani, Legal and Modified Borntrager's tests under suitable conditions. Appearance of red, pink-violet brown, pink-red and rose pink-cherry red colour in response to the above tests respectively indicates the presence of glycosides.

- **Phytosterols/Terpenes:**

The extracts were treated with Libermann-Burchard and Salkowski's tests under suitable condition. Appearance of blue–emerald green and yellowish colour with green fluorescence in response to the above tests respectively indicates the presence of phytosterols/terpenes.

- **Proteins:**

The extracts were subjected to Biuret, Million's, Xanthoproteic and Ninhydrin tests. Appearance of pink-purple, violet, red and blue-purple colour in the above reactions respectively indicates the presence of proteins.

- **Tannins:**

Small quantity of extracts were dissolved in water and to that $FeCl_3$ (5%) or gelatin solution (1%) or lead acetate solution (10%) was added. Appearance of blue colour with $FeCl_3$ or precipitation with other reagents indicates the presence of tannins and phenols.

- **Saponins:**

Small quantity of extracts were mixed with water in a test tube and shaken well for 15 minutes. Foam was observed, it indicates presence of saponins.

1.4 *In-vitro* Antioxidant Studies:

The *In-vitro* methods for antioxidant activity are based on inhibition of free radicals. Samples are added to a free radical-generating system and inhibition of the free radical action is measured. This inhibition is related to antioxidant activity of the sample. Methods vary greatly as to the generated radical, the reproducibility of the generation process and the end point that is used for the determination.

4.4.1. DPPH Assay[92]:

I% = (Ablank – Asample / Ablank) X 100

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

a) Reagents

2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 M): 22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 M DPPH solution.

b) Preparation of test solutions:
21 mg each of the MEAP and AEAP extracts were dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.
Even though, *in-vitro* methods provide a useful indication of antioxidant activities, data obtained from *in-vitro* methods are difficult to apply to biological systems and do not necessarily predict a similar *in-vivo* antioxidant activity. All the methods developed have strengths and limitations and hence a single measurement of antioxidant capacity usually is not sufficient. A number of different methods may be necessary to adequately assess *in-vitro* antioxidant of a specific compound or antioxidant capacity of a biological fluid.
In the present study, MEAP and AEAP extracts used for their *in-vitro* antioxidant activity using several standard methods. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the formula.

c) Preparation of standard solutions:
10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 0.95 ml of Dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

d) Procedure:
The assay was carried out in a 96 well microtitre plate. To 200 of DPPH solution, 10 of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 g/ml. The plates were incubated at 37° C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

1.5 Experimental Animals:
Albino Wistar rats weighing 150-250g was procured from Central animal house, National College of Pharmacy, Shimoga. Animals were maintained under controlled condition of temperature at 27° ± 2° C and 12-h light-dark cycles. They were housed in polypropylene cages and had a free access to standard pellets and water *ad libitum*.
All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC), National College of Pharmacy, Shimoga (NCP/IAEC/CL/21/05/2011-12) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Determination of Acute Toxicity (LD_{50})[93]:
Animals:
Female Albino rats weighing 160-220g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

Preparation of Dose:
MEAP and AEAP extracts were dissolved in distilled water to prepare a dose of 2500 mg/kg. The doses were selected according to the OECD guideline no. 425.

Procedure:
The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2500 mg/kg was selected for the test, as the test item was a source from herb. After

administration of extract, food was withheld for 3- 4 hrs. If the animal dies, conduct the main test to determine the LD_{50}. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD_{50} is greater than 2500 mg/kg, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors. ***In-vivo*** **Antioxidant and Nephroprotective Activities:**

1.5.1 Evaluation of Nephroprotective Activity in Gentamicin Induced Nephrotoxicity[17]**.**

In the dose response experiment, albino rats were randomly assigned into 4 groups of 6 animals each.

Group I: Normal saline was administered daily once throughout the experiment.
Group II: This group of animals received daily i.p. injection of Gentamicin (80mg/kg b.w.) for eight days. This dose has already been shown to produce nephrotoxicity.
Group III: The animals of this group received 80 mg/kg b.w. of Gentamicin i.p. nearly for eight days in addition to this they also received 250 mg/kg i.p. MEAP respectively.
Group IV: Animals of this group were given 80 mg/kg b.w. of Gentamicin i.p. for eight days in addition to this they also received 250 mg/kg i.p. of AEAP which was given simultaneously for ten days with Gentamicin.
At the last day the animals were sacrificed under mild ether anesthesia and the kidney tissues, serum and blood samples were collected and assessed.

1.6 Biochemical parameters in serum:

1.6.1 Estimation of Creatinine[94, 95]**:**

Principle:

Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline picrate $\longrightarrow$ Orange coloured complex

Procedure:

Picric acid and buffer reagents are mixed with 0.5ml of blank, test and standard samples respectively. 0.1ml of test and standard solutions are taken. Mix well and read initial absorbance A_1 for the standard and test after exactly 30 seconds. Read another absorbance A_2 of the standard and test exactly 60 seconds later. Calculate change in the absorbance ΔA for both the standard and test.
For Standard $\Delta AS=A_2S-A_1S$ For Test $\Delta AT=A_2T-A_1T$ Urine creatinine (mg/dl) = $\Delta AT/\Delta AS\times 2$.

1.6.2 Estimation of Urea[96, 97]**:**

Principle:

Urea is hydrolysed in presence of water and urease to produce ammonia and carbon dioxide.
Under alkaline conditions, Ammonia so formed reacts with hypochlorite and phenolic chromogen to form coloured indophenol, which is measured at 578nm. Sodium nitroprusside act as catalyst. The intensity of colour is proportional to the concentration of urea in the sample.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

$$Ammonia + Phenolic\ chromogen + Hypochlorite \xrightarrow{Sodium\ nitroprusside} Indophenol$$

Procedure:

Reagent 3 and serum are prepared by using 10 µl of standard and test solutions respectively. Solution 1 is prepared by mixing 1000 µl of the samples. Mix well and incubate at 37^0C for 3 minutes, then

solution 2 is prepared. Mix well and incubate at 37^0 C for 5 minutes. Read the absorbance at 570-600 nm.

$$\text{Urea concentration} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 50$$

1.7 Homogenization

10% kidney homogenate was prepared with ice-cold phosphate buffered saline using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4^0 C for 15 min (Remi Motors Ltd., Mumbai) and the pellet was discarded. The supernatant obtained was used for biochemical estimations.

1.7.1 Estimation of Lipid peroxidation (LPO)[98]:

The assay is based on the reaction of Thiobarbituric acid (TBA) with malondialdehyde (MDA), a breakdown product derived from many oxidized molecules. The resulting chromogen formed is measured at its maximum absorbance 532 nm. Briefly, to a test tube containing 0.1 ml homogenate, 1 ml of TBA reagent containing equal proportions of 0.375% TBA, 15% TCA and 0.25N HCl was added and placed in a boiling water bath for 30 min. Then the mixture w as placed in crushed ice for 10 min followed by centrifugation at 6000 rpm for 5 min. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using = 1.56 x 105 M-1 cm-1 and the results were expressed as μ moles of MDA/mg protein.

1.7.2 Estimation of Glutathione (GSH)[99]:

The assay is based on the principle of Ellman's reaction. The sulphydryl group of glutathione reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1ml of 25% TCA to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extinction coefficient 13.6 x 103 M-1 cm-1. The values are expressed as n moles/mg protein.

4.8.4 Estimation of Catalase (CAT)[100]:

Catalase measurement was done based on its ability to decompose hydrogen peroxide (H_2O_2). Briefly, to 0.95 ml of 10 mM H_2O_2 in 60 mM phosphate buffer (pH =7.0) 50 l of the tissue supernatant was added and the rate of degradation of H_2O_2 was followed at 240 nm per min. Catalase content in terms of U/ml of protein was estimated from the rate of decomposition of H_2O_2 using the formula k = 2.303/ t x log (A1/A2) s-1 (A unit of catalase is defined as the quantity which decomposes 1.0 mole of H_2O_2 per min at pH=7.0 at 25C).

1.8 Histopathological Study:

Processing of Isolated Kidneys:

Animals were sacrificed and the kidneys of each animal were isolated. The isolated kidneys were preserved and fixed in 10% formalin for two days. Following this was the washing step where by the kidney pieces were washed in running water for about 12 hours. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then the final dehydration is done using absolute alcohol with about three changes for 12 hours each.

The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After clearing, the kidney pieces were subjected to paraffin infiltration in automatic tissue processing unit. The kidney pieces were washed with running water to remove formalin completely. To remove the water, alcohol of increasing strengths was used since it is a dehydrating agent. Further alcohol was

removed by using chloroform and chloroform removed by paraffin infiltration.

Embedding in Paraffin Vacuum:
Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The kidney pieces were then dropped into the molten paraffin quickly and allow cooling.

Sectioning:
The blocks were sectioned by using microtome to get sections of thickness of 5µ. The sections were taken on a micro slide on which an egg albumin (sticking substance) was applied. The sections were allowed to remain in an oven at 60^{o}C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixes tissues to slide.

Staining:
Eosin is an acid stain. Hence it stains all the cell constituents pink which are basic in nature, eg: Cytoplasm. Haematoxylin basic stain which stains all the acidic cell components blue eg: DNA in the nucleus.

Procedure:
1. De-paraffinized the sections by washing with chloroform for about 15 minutes.
2. Hydrate the sections by washing in isopropyl alcohol of decreasing strength (100%, 90%, 80%, 70%).
3. Finally washed with water.
4. Stained with haematoxylin for 15 minutes.
5. Rinsed in tap water.
6. Differentiated in 1% acid alcohol by 10 quick dips. Checked the differentiation with a microscope. Nuclei were distinct and the back ground was very light (or colourless).
7. Washed in tap water.
8. Dipped in (Lithium carbonate) until sections become bright blue (3-5 dips).
9. Wash in running tap water for 10 to 20 minutes, if washing is inadequate eosin will not stain evenly.
10. Stained with eosin for 15 seconds – 2 minutes depending on the age of the eosin and the depth of the counter stain desired. For even staining results, dip slides several times before allowing them to set in the eosin for the desired time.
11. Dehydrated in 95% isopropyl and absolute isopropyl alcohol until excess eosin is removed, 2 changes of 2 minutes each (check under microscope).
12. An absolute isopropyl alcohol 2 changes of 3 minutes each.
13. Chloroform 2 changes of 2 minutes each.
14. Mounted in DPX (Desterene dibutyl phthalate xylene).

All the sections of the tissues were examined under microscope for analyzing the altered architecture of the kidney tissue due to Gentamicin treatment and improved kidney architecture due to pretreatment with test extracts.

Statistical Analysis:
The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.001$.

RESULTS
In the present study, phytochemical investigation was carried out for different extracts of the tuber *Amorphophallus paeoniifolius* and biochemical and histopathological studies were carried out for nephro-protective activity.

1.9 Collection of the plant:

The tuber of the plant, *Amorphophallus paeoniifolius* used for the present study was collected from Shimoga district of Karnataka. The whole plant was identified, confirmed and authenticated by Rudrappa, Department of Botany, DVS College, Shimoga. The whole plant material were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

1.10Extraction of plant material and phytochemical investigation of extracts of *Amorphophallus paeoniifolius*:

Table No. 4: Percentage yield of extracts of tubers of *Amorphophallus paeoniifolius*

Sl. No.	Extract	Color & consistency	% yield
1.	Chloroform extract	Pale yellow and viscous	2.5%
2.	Petroleum ether extract	Yellowish brown and viscous	1.3%
3.	Methanol extract	Reddish, viscous and pasty mass	4.76%
5.	Aqueous extract	Reddish brown and highly viscous	5.39%

Table No. 5: Phytochemical investigation of extracts of *Amorphophallus paeoniifolius*

Chemical Constituent	Tests	Chloroform extract	Petroleum Ether extract	Methanol extract	Aqueous extract
Alkaloids	1. Mayer's test	+ve	+ve	+ve	-ve
	2. Dragendorff's test	+ve	+ve	+ve	-ve
	3. Wagner's test	+ve	+ve	+ve	-ve
	4. Hager's test	-ve	+ve	+ve	-ve
Carbohydrate	1. Molisch's test	-ve	-ve	-ve	+ve
	2. Benedict's test	-ve	-ve	-ve	+ve
	3. Fehling's test	-ve	-ve	-ve	+ve
Glycosides	1.Modified Borntrager's	-ve	-ve	-ve	-ve
	2. Legal test	-ve	-ve	-ve	-ve
	4.Baljet test	-ve	-ve	-ve	-ve
Saponins	1. Foam test	+ve	-ve	-ve	+ve
	1. Salkowski test	+ve	-ve	-ve	+ve
Phytosterols	2.Libermann Burchard	-ve	+ve	+ve	-ve
Triterpenoids	1.Salkowski test	-ve	+ve	+ve	-ve
	2.Tschugajew's test	-ve	+ve	+ve	-ve
Phenols	Ferric Chloride test	+ve	-ve	+ve	+ve
Tannins	1. Gelatin test	-ve	-ve	-ve	+ve
Flavanoids	1. Lead acetate test	-ve	-ve	+ve	+ve
	2. Shinoda test	-ve	-ve	+ve	+ve
Proteins	1. Xanthoproteic test	-ve	-ve	-ve	+ve
	2. Ninhydrin test	-ve	-ve	-ve	+ve
Fixed oils	1.Stain test	-ve	+ve	-ve	-ve
	2.Soap test	-ve	+ve	-ve	-ve

1.11Evaluation of Nephroprotective activity:

1.11.1 Biochemical parameters:

In Gentamicin treated group of animals the concentration of serum urea and creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating (group 3 & 4) with methanol extract of IA showed significant decrease ($p<0.001$) in concentration of serum urea and creatinine compared to Gentamicin treated group 2. Nevertheless the concentration of uric acid not so much considerably increased in the Gentamicin treated groups (group 2) than control group (group1). Treatment with methanol extract of IA significantly ($p<0.05$) decreases the uric acid levels in group 3 & 4 ($p<0.01$) compared to Gentamicin treated group (group 2).

Table No. 6: Effect of tuber extracts of Amorphophallus paeoniifolius on serum urea and creatinine in Gentamicin treated rats

Sl. No.	Groups	Drug treatment	Serum urea (mg/dl)	Serum creatinine (mg/dl)
1.	Control	5ml/kg, i.p, NS	42.20±07146	1.212±0.01302
2.	Gentamicin treated	80 mg/kg, i.p	71.37±0.8204*** a	1.710±0.01612***a
3.	Gentamicin + MEAP	80mg/kg, i.p +250mg/kg	55.18±1.021*** b	1.348±0.02892***b
4.	Gentamicin + AEAP	80mg/kg, i.p + 250mg/kg	62.47±0.7455*** b	1.512±0.03516***b

Data was analyzed by One way ANOVA test followed by Dunnets test n=6 animals in a group; Values are expressed as Mean ± SEM; [a] comparison with control group and [b] comparison with toxic group *p<0.05, **p<0.01, ***p<0.001 significant; ns indicates no significant.
*

Figure No. 9: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum urea in various groups of rats

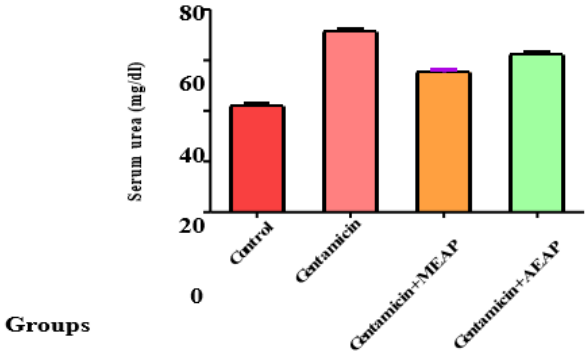


Figure No. 10: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum creatinine in various groups of rats

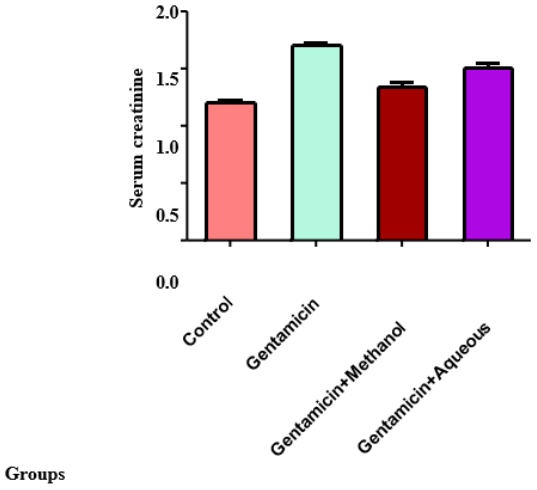


Table No. 7: Effect of tuber extracts of AP on serum enzymes in Gentamicin treated rats
Data was analyzed by One way ANOVA test followed by Dunnets test

Sl. No	Groups	Drug treatment	Amylase	SGOT	SGPT	ALP
1.	Control	5ml/kg, i.p, NS	102.7±0.5350 a	902.3±2.108	991.8±6.509	254.5±1.432
2.	Gentamicin treated	80 mg/kg, i.p	65.83±1.078*** b	266.0±2.251*** a	199.3±4.462*** a	157.0±1.461*** a
3.	Gentamicin +	80 mg/kg,	96.47±1.961*** b	577.5±2.849*** b	543.3±2.246*	205.2±1.167*** b

	MEAP	i.p + 250mg/k g			** [b]	
4.	Gentamicin + AEAP	80 mg/kg, i.p + 250mg/k g	96.50±1.232*** [b]	376.3±5.333*** [b]	455.8±6.645** [b]	237.5±2.172*** [b]

n=6 animals in a group; Values are expressed as Mean ± SEM; [a] comparison with control group and [b] comparison with toxic group

*p<0.05, **p<0.01, ***p<0.001 significant; ns indicates no significant.

Figure No. 11: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum Amylase in various groups of rats

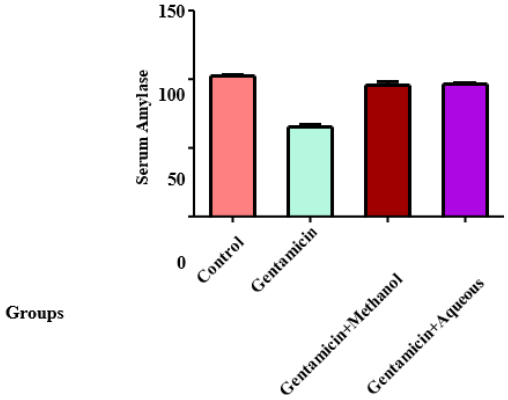


Figure No. 12: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum SGPT in various groups of rats

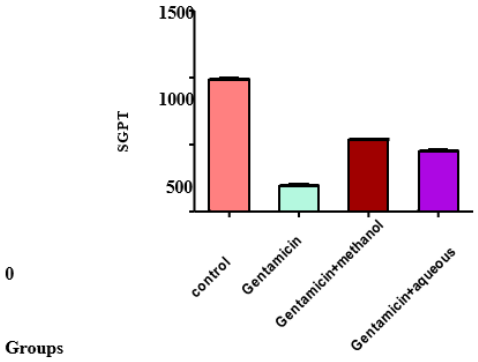


Figure No. 13: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum SGOT in various groups of rats

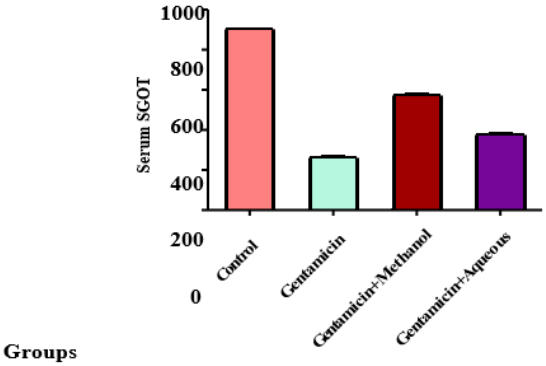
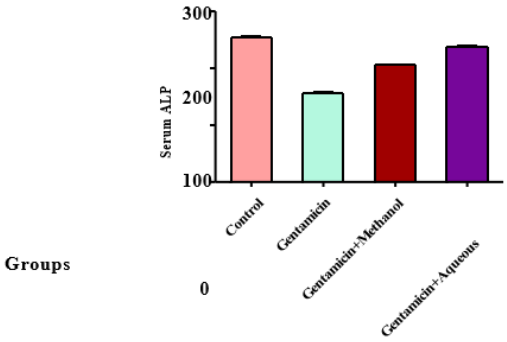


Figure No. 14: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on serum ALP in various groups of rats



1.11.2 Antioxidant parameters:

1.11.2.1 In-vitro anti-oxidant parameters:

DPPH radical scavenging activity:

Table No. 8: DPPH radical scavenging activity of MEAP and AEAP extracts

Sl. No.	Concentration	Methanol	Aqueous
1.	1000	74.6	23.8
2.	500	70.7	7.4
3.	250	52.2	6.2
4.	125	30.0	0.0
5.	61.5	18.2	0.0
6.	31.2	9.7	0.0
7.	15.6	7.4	0.0
8.	**Control**	0.0	0.0

Table No. 9: IC_{50} values of DPPH in comparison with rutin

Samples	IC_{50} values □g/ml by methods	
	Methanolic	Aqueous
DPPH	237.5	>1000.00
Standard	Rutin	
	4.2	

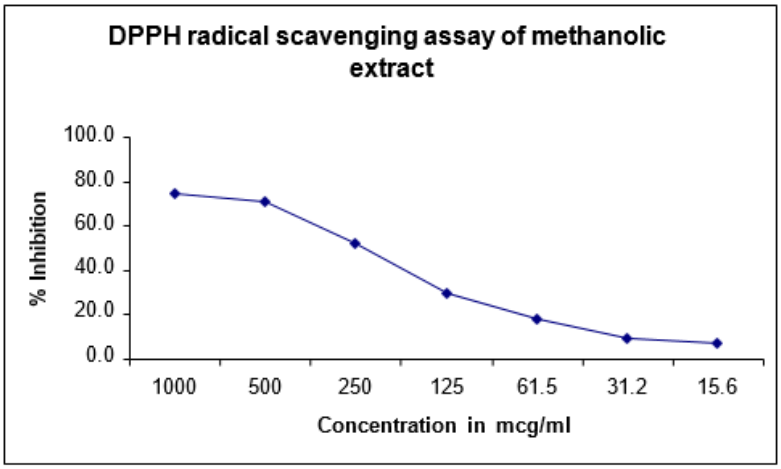


Figure No.15: Histogram showing DPPH radical scavenging assay of methanolic extract

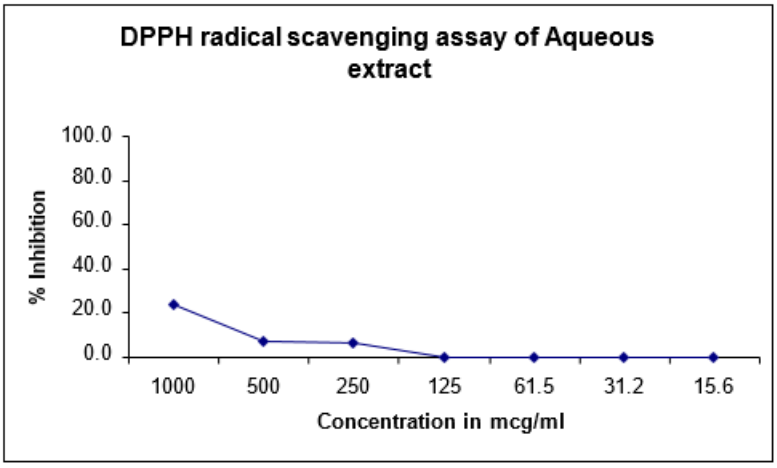


Figure No. 16: Histogram showing DPPH radical scavenging assay of aqueous extract

1.11.2.2 Antioxidant enzyme estimation:

Table No. 10: Effect of tuber extracts of AP on antioxidant enzymes in Gentamicin treated rats

Sl. No.	Groups	Drug treatment	CAT	GSH	LPO
1.	Control	5ml/kg, i.p, NS	407.7±6.469	101.6±3.482	12±0.5774
2.	Gentamicin treated	80 mg/kg, i.p	186.3±0.7106***a	27.96±0.4182***a	52.01±0.2659***a
3.	Gentamicin + MEAP	80 mg/kg, i.p,+ 250mg/kg	292.0±1.713***b	86.30±0.3477***b	21.67±0.7149***b
4.	Gentamicin + AEAP	80 mg/kg, i.p,+ 250mg/kg	254.5±1.258***b	82.67±0.3241***b	28.13±0.1458***b

Data was analyzed by One way ANOVA test followed by Dunnets test n=6 animals in a group; Values are expressed as Mean ± SEM; [a] comparison with control group and [b] comparison with toxic group * p<0.05, **p<0.01, ***p<0.001 significant; ns indicates no significant.

Figure No. 17: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on Catalase (CAT) in various groups of rats

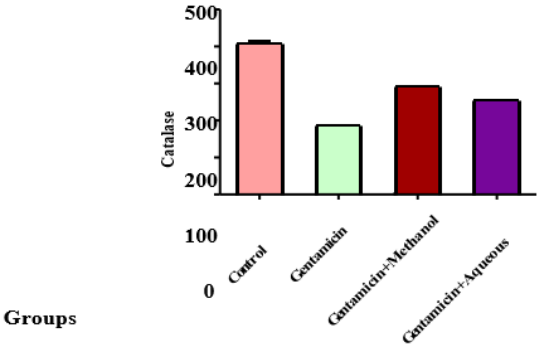


Figure No. 18: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on Glutathione (GSH) in various groups of rats

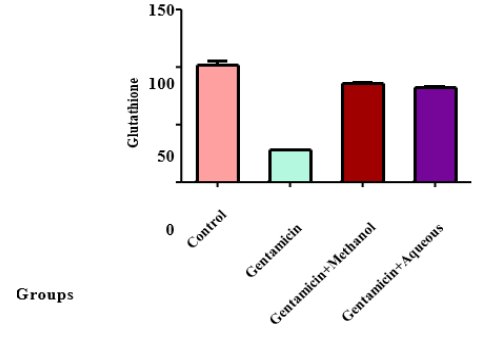


Figure No. 19: Histogram showing effect of tuber extracts of *Amorphophallus paeoniifolius* on Lipid peroxidase (LPO) in various groups of rats

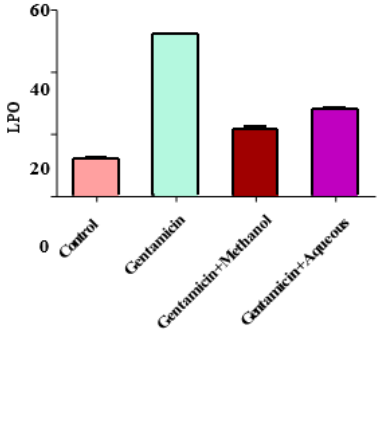


Figure No. 20: Histopathological studies of rat kidneys of various groups:

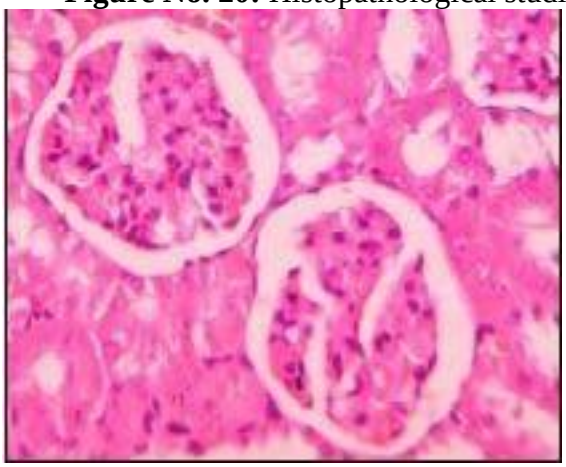


Figure 20(a) Glomerulus of control rat kidney (10x)

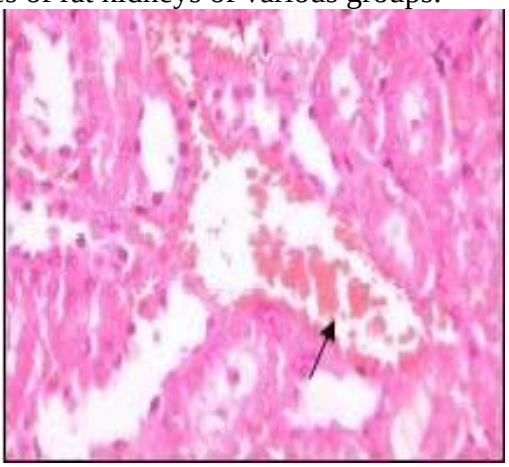


Figure 20(b) Glomerulus of control rat kidney (40x)

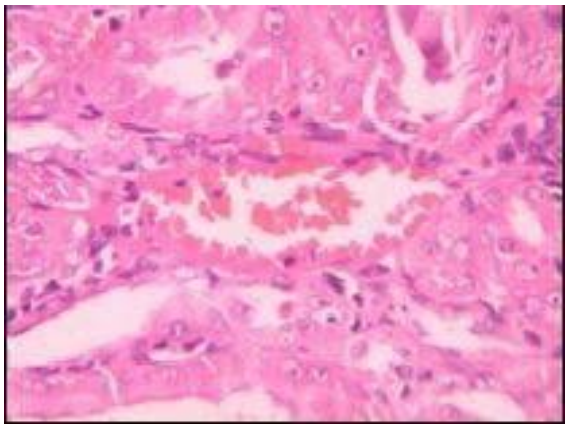


Figure 20(c) Glomerulus of Gentamicin treated kidney (10x)

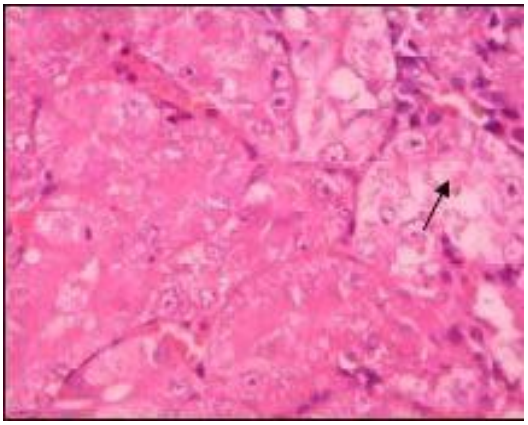


Figure 20(d) Glomerulus of Gentamicin treated rat kidney (40x)

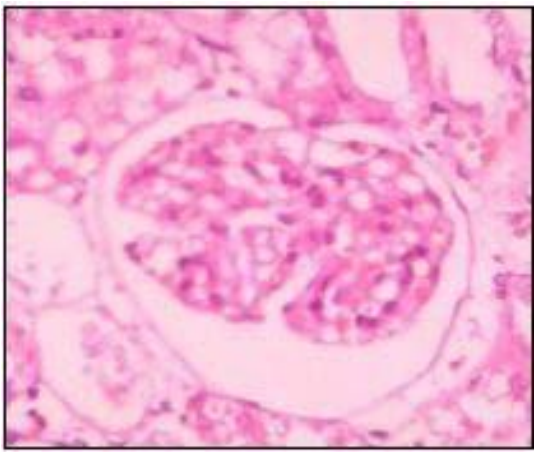


Figure 20(e) Glomerulus of G+MEAP rat kidney (10x)

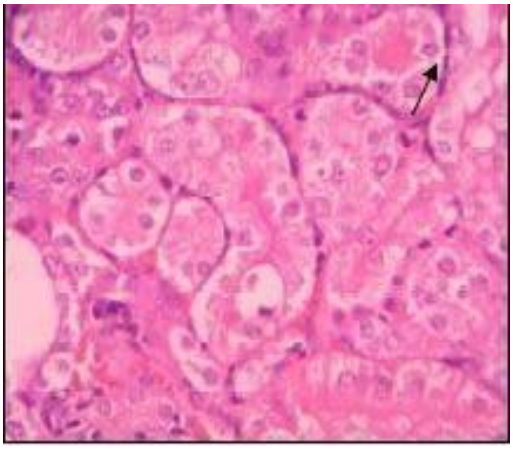


Figure 20(f) Glomerulus of G+MEAP rat kidney (40x)

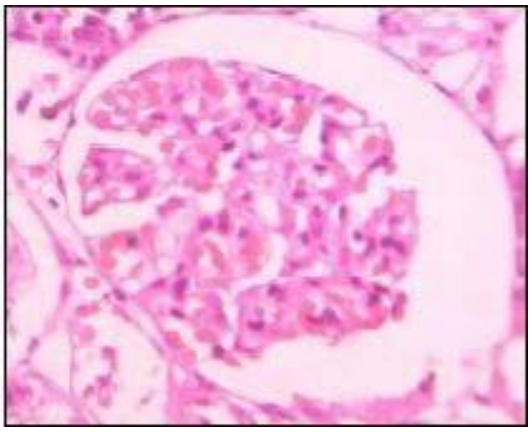


Figure 20(e) Glomerulus of G+AEAP rat kidney (10x)

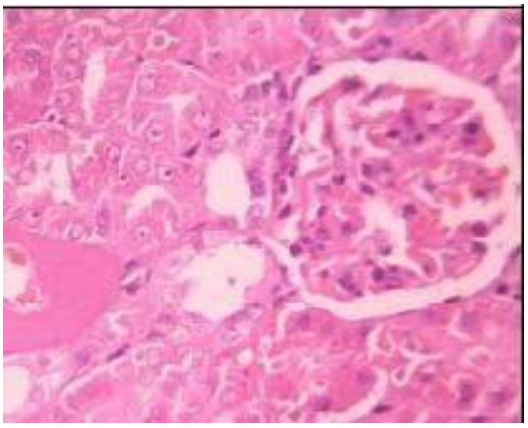


Figure 20(f) Glomerulus of G+AEAP rat kidney (40x)

Treatment and its microscopy:

Groups:

I) Normal saline - 5ml/kg i.p:

Section studied shows renal parenchyma with intact normal architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels are dilated and congested (Figure.20 (b) arrow) within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium.

Important Highlights: Some blood vessels show congestion.

II) Gentamicin 80mg/kg i.p for 10days daily:

Section studied shows renal parenchyma with intact architecture. There are seen diffuse glomerular congestion (Figure.20(c)) occasional tubular casts (Figure.20 (d) arrow), focal hydropic degeneration of the tubular epithelial cells and peritubular congestion. Some of the tubules show partial desquamation of the epithelial cells. Also seen are blood vessel congestion and scattered mononuclear inflammatory cell infiltrations within the interstitium.

Important Highlights: Diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion.

III) Gentamicin 80mg/kg i.p for 10 days with simultaneous administration of MEAP extract - 250mg/kg daily for 10 days:

Section studied shows renal parenchyma with intact architecture. There are seen focal glomerular

congestion (Figure.20(e)), few the tubular epithelial cells show hydropic degeneration (Figure.20(f) arrow) and peritubular congestion. Also seen are few scattered mononuclear inflammatory cell infiltrations within the interstitium.

Important Highlights: Focal glomerular congestion, Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells.

IV) Gentamicin 80mg/kg i.p for 10 days with simultaneous administration of AEAP extract - 250 mg/kg daily for 10 days:

Section studied shows renal parenchyma with intact architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels are dilated and congested (Figure. 20(h) arrow) within the interstitium. Few scattered mononuclear inflammatory infiltration were seen within the interstitium.

Important Highlights: Some blood vessels show congestion.

2. DISCUSSION

The use of Gentamicin, an aminoglycoside antibiotic with a wide spectrum of activities against Gram-positive and Gram-negative bacterial infections but with high preference for latter[101] is equally associated with nephrotoxicity as its side effect[102-103]. Thus, Gentamicin induced nephrotoxicity is well established experimental model of drug induced renal injury[104-105]. Many animal experiments have demonstrated overwhelmingly, the positive correlation between oxidative stress and nephrotoxicity[106]. Gentamicin induced nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomylinase and phospholipases in addition to causing oxidative stress[105, 107].

Drug induced nephrotoxicity are often associated with marked elevation in serum urea, serum creatinine and acute tubular necrosis[108]. So, these biochemical parameters have been used to investigate drug induced nephrotoxicity in animal and man[109]. In the present study drug induced nephrotoxicity were established by single daily intraperitoneal injection of the Gentamicin, for 10 days. This toxicity characterized by marked elevation in the circulating levels of serum urea, creatinine and histological features of tubulonephritis in the Gentamicin induced model (group 2) rats when compared to control (group 1) rats. However, these changes were attributed by pre-treatment with single daily graded doses of *Amorphophallus paeoniifolius* extract for 10 days. Administration of plant extracts significantly decreases the urea and creatinine level in both treatment groups when compared to toxicant group. Apart from the direct nephrotoxic effect of Gentamicin in group 2 rats, the acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolong anorexia associated with Gentamicin nephrotoxicity.

In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance[110]. Elevation of urea and creatinine levels in serum was taken as the index of nephrotoxicity[111-113]. Creatinine derived from endogenous sources by tissue creatinine breakdown[112]. Thus, serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of uric acid is non-significantly increased in the toxicant group when compared to control. Administration of plant extract significantly decreases the uric acid level in both treatment groups when compared to toxicant group.

It was established that Gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR[114].

Gentamicin is known to decrease the activities of catalase, glutathione peroxidase and the level of reduced glutathione[115]. Therefore it is no doubt to assume that the nephroprotection showed by *Amorphophallus paeoniifolius* extract in Gentamicin induced nephrotoxicity is mediated through its potent antioxidant effect. A relation between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. Administration of superoxide dismutase and

vitamin E significantly reduced the nephrotoxic symptoms produced by adriamycin[116-117]. In Gentamicin treated rats there was a significant increase in lipid peroxidation products (MDA) suggesting the involvement of oxidative stress. A role of lipid peroxidation in Gentamicin induced acute renal failure has been described by evaluating effect of diphenyl-phenylenediamine and vitamin E[118]. In these studies both the agents prevented Gentamicin induced lipid peroxidation. In addition alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity[119]. The presence of alkaloids could be the reason of protection offered by the extract might be due to its ability to activate anti-oxidant enzymes[120].

In histopathological study of saline treated group showing some blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. Gentamicin treated group showing diffuse glomerular congestion, tubular casts, peritubular congestion, epithelial desquamation, blood vessel congestion. While treatment group (250 mg/kg, Group III) shows focal glomerular congestion, peritubular congestion, focal hydrophic degeneration of tubular epithelial cells and treatment groups (250 mg/kg, Group IV) shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. From histopathological results, we can conclude that both *Amorphophallus paeoniifolius* extracts MEAP and AEAP at dose of 250 mg/kg, have significant protective effect on Gentamicin induced nephrotoxicity.

The findings suggest the potential use of methanol extract of *Amorphophallus paeoniifolius* a therapeutically useful nephro-protective agent. Therefore, further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

3. CONCLUSION

The nephro-protective effect of tuber extracts of *Amorphophallus paeoniifolius* was confirmed by the following measures:

In case of Gentamicin treated group there was rise in serum markers such as serum urea and creatinine and decrease in the level of protein. The same was observed in kidney diseases in clinical practice and hence are having diagnostic importance in the assessment of kidney function.

In the present study, the tuber extracts of *Amorphophallus paeoniifolius* significantly reduced the toxicant elevated levels of above-mentioned serum markers and increase in the levels of protein. Hence, at this point it has been concluded that the extract of *Amorphophallus paeoniifolius* offers nephro-protection.

In Gentamicin treated animals there was glomerular, peritubular and blood vessel congestion and result in presence of inflammatory cells in kidney sections. The same was observed in case of humans who were suffering from major kidney disorders.

In the present study, the extracts of *Amorphophallus paeoniifolius* treated group animals were found to reduce such changes in kidney histology induced by Gentamicin, indicating nephro-protection.

Further documented reports reveal that, plant material containing phenols, flavonoids, alkaloids and saponins offers organ protection by virtue of their free radical scavenging activity. The extract under study upon phytochemical analysis showed the presence of before mentioned phytoconstituents. Hence, the role of these phytoconstituents as free radical scavengers and consequent nephro-protection cannot be ruled out.

Based on improvement in serum marker levels, histopathological studies, level of antioxidant enzymes and presence of phytoconstituents, it has been concluded that the methanol extract of *Amorphophallus paeoniifolius* possesses significant nephro-protective activity when compared to aqueous extract and thus supports the traditional application of the same under the light of modern science.

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