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PHYTOCHEMICAL INVESTIGATION AND NEPHROPROTECTIVE ACTIVITY OF PHYLLANTHUS EMBLICA FRUIT EXTRACT

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Abstract:

The present study was undertaken to scientifically evaluate thenephroprotective activity of the ethanolic extract of fruit pulps of Phyllanthus emblica. The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols and anthroquinone in EEPE.The administration of cisplatin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Therefore,it is an effective and an ideal model for nephrotoxicity research. The evaluation of renal parameters on nephrotoxic rats with EEPE showed significantly elevate the attenuated body weight, urine volume, creatinine clearance and significantly reduce in elevated serum creatinine level, which supports its Nephroprotective activity. The cisplatin induced rats showed elevated levels of serum blood urea nitrogen (BUN) and lipid peroxidation parameter like malondialdehyde (MDA) whichwas significantly decreased with treatment of EEPE, which proves it having Nephroprotective activity. The Nephrotoxic rats also showed the reduced levels of enzymatic antioxidant like sulphoxide dismutase (SOD), glutathione peroxidise (GPx) and Catalase (CAT), and non-enzymatic antioxidant like Reduced glutathione (GSH), which was significantly increased with treatment of EEPE, which showed its antioxidant activitydue to the Flavonoids which is present in the extract. Histopathological studies on isolated kidney revealed that the EEPE, reversed the kidney damage and also restored normal kidney architecture. The fruit pulp of Phyllanthus emblica in an ethanolic extract showed statistically significant nephroprotective activity.

Key words: *Phytochemical Screening, Nephroprotective Activity, Phyllanthus emblica , Fruit Extract, Cisplatin Induced Nephrotoxicity.*

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INTRODUCTION:

The kidneys play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance, and filtering and removing nitrogenous and other waste products [1,2]. Chronic kidney disease (CKD) is characterized by a progressive loss of functions while acute kidney injury (AKI) is an abrupt reduction in kidney function. Both CKD and AKI have increased worldwide and are considered one of the leading public health problems [2,3]. A projection of health concerns by 2040 ranked CKD as the fifth leading cause of death worldwide [3]. In addition, kidney diseases have been recognized as risk factors for severe forms of COVID-19 [4]. The increase of their prevalence is associated with the increase of diabetes Mellitus (DM) and hypertension as the main reported causes of kidney dysfunctions, although various factors can trigger this physiopathology. Factors are classified, based on the pathway they led to kidney damage, as pre-renal, intrinsic, and post-renal factors. According to clinical criteria, pre-renal diseases are related to a decrease in renal perfusion or alteration in the systemic circulation, which will first compromise the glomerular filtration rate (GFR) and secondly lead to more severe alterations in the kidney structure. These dysfunctions are reflected in clinical analyses by changes in biomarker levels; for example, an increase in serum creatinine; and fluctuations in urine flow [5,6]. Several diseases have been identified as pre-renal factors such as bleeding, trauma, shock, hypertension, cirrhosis, diabetes, systemic infections, hypotension, autoimmune diseases, rhabdomyolysis, disorders of the gut microbiota, liver damage, and intravascular volume depletion [7]. The factors that directly cause kidney damage are intrinsic, whether heavy metals, trauma, Wegener's granulomatosis, proteinuria, congenital abnormalities, drug toxicity, renal atheroembolic disease, arthralgias, lupus erythematosus, or kidney cancer. Histologically, the main diagnoses are ischemic acute tubular necrosis, nephrotoxic acute tubular necrosis, and glomerulonephritis [8]. Any other health disorders which can indirectly induce kidney failures that occur after the physiological action of the kidney are post-renal diseases. Among these post-renal factors, mainly related to urinary flow disorders, are the ureter and urethra obstruction due to blood clots, lithiasis, or tumor growth, which can lead to increased pressure inside tubules, and as a consequence, the GFR is compromised, and a urinary tract infection (UTI) [9] is caused.

Kidney diseases can be addressed at several levels according to the physiological pathway of the original cause. Each pre-renal and post-renal disease currently has pharmacological treatments; however, most of the drugs used cause adverse effects and sometimes lead to intrinsic kidney damage. Among them are non-steroidal anti-inflammatory drugs (NSAID), proton pump inhibitors, antibiotics, and chemotherapy [10,11,12,13]. Nephrotoxicity of drugs administrated as treatments for pre-renal and post-renal diseases, as well as for other diseases, is now considered as a risk factor of acute and chronic kidney conditions. To avoid the adverse effects caused by medication, different alternatives have been sought to treat these pathologies.

Plants have been traditionally used as treatments for various diseases, among them several pathologies identified as pre-, intra-, and post-renal factors. The medicinal characteristics of plants have been attributed to their secondary metabolites, which can protect against pathogens or have important physiological benefits to prevent some diseases [14,15]. Plants provide a wide range of bioactive compounds which act as antioxidants, anti-inflammatory, diuretic, anticancer, and antimicrobial [16,17,18]. Further, nephroprotective agents from plants mitigate processes such as interstitial nephritis, altered intraglomerular hemodynamics, tubular necrosis, or glomerulonephritis [19].

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries. Since there are only few researches made on this field of nephroprotection, this present study of nephroprotective activity of Phyllanthus emblica

The present study was focused on the investigation of Nephroprotective activity of the ethanolic extract of fruit pulp of Phyllanthus emblica on cisplatin induced nephrotoxicity in Wistar albino rats.

MATERIALS AND METHODS:

SELECTION OF THE PLANT

The medicinal plant Phyllanthus emblica was selected for Nephroprotective activity based on the literature survey.

Collection and authentication of the plant

The fruit pulp of Phyllanthus emblica was collected from Chittor district, Andra pradesh. The plant Phyllanthus emblica was identified and

authenticated by Prof. Dr. Madhava Shetti

Shade drying and cutting of the fruit pulp

The fruit pulp of Phyllanthus emblica were collected and shade dried at the room temperature and then cut it in to small pieces, which was used for theextraction for further studies.

MACERATION
Fresh fruit pulps of Phyllanthus emblica were cut into small pieces, seeds were removed and air dried. The dried pieces of Phyllanthus emblica fruit pulp, weighing 100 g, were soaked in 500 ml of 95% ethanol in a round bottom flask for about 24 hours.

EXTRACTION
Extracting values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug

Ethanolic extract
Solvent - Ethanol

The process of extraction was done by reflux condensation method using soxhlet apparatus at 60-80 ºC for 9 hours. The extract was concentrated by distillation apparatus till a syrupy consistency was obtained. Finally, the extract was put in a china dish and evaporated at40-60 ºC temperature in a water bath, 22 gms of semisolid extract was obtained.

Table-1: Nature and colour of Ethanolic extract of Phyllanthus emblica

S. No	Name of the plant extract	Part used
1.	Phyllanthus emblica	Fruit pulp

Preliminary phytochemical screening
The Ethanolic extract of the fruit pulp of Phyllanthus emblica was subjected to a preliminary phytochemical screening to identify the active chemical constituents.

Pharmacological Evaluation
ACUTE TOXICITY STUDY

Whenever an investigator administers a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 (the dose has proved to be lethal (causing death) to 50% of the tested group of animals).

Determination of oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. This article reviews the methods of so far utilized for the determination of median lethaldose (LD50) and the new changes which would be made. This has to go through the entire process of validation with different categories of substances before its final acceptance by regulatory bodies.

Organisation for Economic co-operation and Development (OECD) regulates guidelines for oral acute toxicity study. It is an international organisation which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. To determine the acute oral toxicity OECD frames the following guideline methods.
OECD 401 – Acute Oral Toxicity

OECD 420 – Acute Oral Toxicity: Fixed Dose procedure OECD 423 –Acute Oral Toxicity: Acute Toxic Classic method OECD 425 – Acute Oral Toxicity: Up and own Procedure
In the present study the acute oral toxicity of Spermacoce ocymoides Burn f was carried out according to OECD 423 guideline (Acute Oral Toxicity: acute Toxic Classic Method).

ACUTE ORAL TOXICITY
Acute oral toxicity refers to those adverse effects that occur following oral administration of a single dose of a substance or multiple doses given within 24 hours.

LD_{50} (median lethal oral dose)

LD_{50} (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per

unit weight of test animal (mg/kg).

PRINCIPLE

It is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a step wise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lowerdose level.

SELECTION OF ANIMAL SPECIES

The preferred rodent species was the rat. Normally females were used. Females were generally slightly more sensitive. Healthy young adult animals of commonly used laboratory strains were employed. Females were nulliparous and non-pregnant. Each animal, at the commencement of it's dosing, were between 8 to 12 weeks old.

ADMINISTRATION OF DOSES

The test substance was administered in a single dose by gavages using a oral feeding needle. Animals were fasted prior to dosing (e.g. with the rat,food but not water should be withheld over-night, with the mouse, food but not water was withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food was withheld for a further 3-4 hours in rats.

OBSERVATION

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasonsor are found dead. However, the duration of observation was not fixed rigidly. It was determined by the toxic reactions, time of onset and length of recovery period and extended when considered necessary. The times at which signs of toxicity appeared and disappeared were important, when toxic signs were to be delayed. All observations were systematically recorded with individual records being maintainedfor each animal.

Fixation of doses of the extract

An acute oral toxicity study was carried out according to OECD guidelines. No adverse effect was reported or mortality in albino wister rats up to 2000mg/kg p.o. ofethanolic extracts of Phyllanthus emblica .

Therefore, the maximum tolerated dose 200mg/kg & 400mg/kg was chosen for further studies.

Animals

Albino wistar rats of either sex (150-200 gm) were procured. Prior to the experiment the rats were housed in a clean polypropylene cage (6 rats/cages) for a period of 7days under temperature (25-30^{0}c), relative humidity (45-55%).

The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines.

Drugs and chemicals

All the drugs, chemicals and reagents were procured from S.D Fine chemicals, Mumbai, India. All chemicals and reagents used were of analytical reagent.

Experimental protocol

The Nephroprotective activity was tested on five groups of albino wistar rats of either sex, each group consisting of six animals.

Group-I : Served as normal control received 0.5 % DMSO (Dimethyl sulphoxide) ; for 15 days.

Group-II : Served as Nephrotoxic control, received vehicle (0.5% DMSO);for 15 days.

Group-III : Received the standard Nephroprotective drug, (Lipoic acid (50mg/kg; p.o)) dissolved in DMSO for 15 days.

Group-IV : Received ethanolic extract of Phyllanthus emblica (200mg/kg; p.o) dissolved in DMSO for 15 days.

Group-V : Received ethanolic extract of Phyllanthus emblica (400mg/kg; p.o) dissolved in DMSO for 15 days.

On the 10th day 2 hours after the administration of standard Nephroprotective drug (Lipoic acid) and Phyllanthus emblica (200 & 400 mg/kg) II-V groups received cisplatin (7.5mg/kg; i.p).

Blood collection techniques used in the present study

At the end of the experimental period, ie on the 15th day animals were sacrificed under mild ether anesthesia. The blood was collected by retro-orbital vein puncture using a fine capillary to an anticoagulant tube and allowed to stand for 30min at 37°C and then centrifuged to separate the serum to evaluate the biochemical markers.

Preparation of kidney homogenate

The kidney was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 minutes, supernatant was collected and used for various biochemical assays.

STATISTICAL ANALYSIS

Results were expressed as Mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. P value < 0.05 was considered as statistically significant. Data were processed with graph pad prism 5.0 software.

RESULTS:

Extracting values of crude drug were useful for their evaluation. Further, these values indicated the nature of the constituents present in the crude drug. The small pieces of the fruit pulps was subjected for continuous hot percolation using 95% ethanol as a solvent using a Soxhlet apparatus. The percentage of extract was calculated. The result was tabulated in the table-2.

Table-2: Results of the Percentage yield of EEPE

Drug	Phyllanthus emblica .
Solvent	Ethanol
Colour	Dark Brownish
Consistency	Semi solid
Percentage yield	22 % w/w

Preliminary phytochemical studies

Table no-3: Results of the Preliminary Phytochemical Constituents present inethanolic extract of Phyllanthus emblica .

Table-3: Results of preliminary phytochemical analysis of EEPE

Phyto-constituents	Phyllanthus emblica
Carbohydrate	Present
Reducing sugar	Present
Tannins	Present
Flavonoids	Present
Anthroquinone	Present
Saponins	Present
Alkaloids	Present

Glycosides	Present
Terpenes	Present
Phytosterols	Absent

RESULT:
Ethanolic extract of the whole plants of Phyllanthus emblica (EEPE) was subjected to various phytochemical tests, which showed the presence of carbohydrates, reducing sugars, glycosides, tannins, flavonoids, Anthroquinone, Saponins, Alkaloids, Glycosides and Terpenes.

Assessment of general biochemical parameters
Assessment of urine volume

The effects of the different doses of ethanolic extract of Phyllanthus emblica on urine volume.

Table-4: Results of the effect of EEPE on urine volume in cisplatin induced Nephrotoxicrats

Groups	Drug Treatment	Urine Volume
I	Normal Control(0.5% DMSO)	10.85±0.223
II	Nephrotoxic ControlCisplatin (0.75%)	5.90±0.762
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	10.81±0.24***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	9.17±0.40*
V	Cisplatin (0.75%) + EEPE (400mg/kg)	9.70±0.28***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of Body weight
The effects of the different doses of ethanolic extract of Phyllanthus emblica on body weight.

Table-5: Results of the effect of EEPE on Body weight in cisplatin induced Nephrotoxicrats

Groups	Drug Treatment	Body weight
I	Normal Control(0.5% DMSO)	250±3.406
II	Nephrotoxic ControlCisplatin (0.75%)	159.33±2.658
III	Reference Control Cisplatin (0.75%) +Lipoic acid (50mg/kg)	234.83±4.355***
IV	Cisplatin (0.75%) +EEPE (200mg/kg)	205.83±3.43*
V	Cisplatin (0.75%) +EEPE (400mg/kg)	222±3.742***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of serum biochemical parameters
Serum creatinine level

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on serum creatinine level.

TABLE-6: Results of the effect of the EEPE on serum Creatinine on Cisplatin inducedNephrotoxicity in rats

Groups	Drug Treatment	Serum creatinine
I	Normal Control(0.5% DMSO)	0.67±0.055
II	Nephrotoxic ControlCisplatin (0.75%)	4.77±0.131
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	0.75±0.020***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	1.47±0.206*
V	Cisplatin (0.75%) + EEPE (400mg/kg)	0.82±0.062***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Serum Blood urea nitrogen (BUN)

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on serum Blood urea nitrogen (BUN) level.

Table-7: Results of the effect of the EEPE on serum Blood urea nitrogen on Cisplatininduced Nephrotoxicity in rats

Groups	Drug Treatment	Serum blood ureanitrogen (BUN)
I	Normal Control(0.5% DMSO)	23.66±0.505
II	Nephrotoxic ControlCisplatin (0.75%)	58.77±0.792
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	24.15±0.50***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	30.02±0.94*
V	Cisplatin (0.75%) + EEPE (400mg/kg)	24.55±0.55***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of urine biochemical parameters
Assessment of creatinine clearence
The effects of the different doses of ethanolic extract of Phyllanthus emblica Linn on creatinine clearance.

Table-8: Results of the effect of EEPE on creatinine clearence in cisplatin inducedNephrotoxic rats

Groups	Drug Treatment	Creatinineclearance
I	Normal Control(0.5% DMSO)	19.80±1.302
II	Nephrotoxic ControlCisplatin (0.75%)	5.05±0.445
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.265±0.512***
IV	Cisplatin (0.75%) +EEPE (200mg/kg)	14.74±0.746*
V	Cisplatin (0.75%) +EEPE (400mg/kg)	17.20±1.146***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of oxidative stress parameter
Assessment of Malondialdehyde (MDA)

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on malondialdehyde (MDA).

Table-9: Results of the effect of EEPE on Malondialdehyde (MDA) in cisplatin inducedNephrotoxic rats

Groups	Drug Treatment	Malondialdehyde(MDA)
I	Normal Control(0.5% DMSO)	7.61±0.470
II	Nephrotoxic ControlCisplatin (0.75%)	15.44±0.409
III	Reference Control Cisplatin (0.75%) + Lipoic acid (mg/kg)	7.86±0.118***
IV	Cisplatin (0.75%) +EEPE (200mg/kg)	8.77±0.427**
V	Cisplatin (0.75%) +EEPE (400mg/kg)	7.66±0.238***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control Group 2).

Assessment of enzymatic antioxidant parameters
Assessment of superoxide dismutase (SOD)

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on superoxide dismutase (SOD).

Table-10: Results of the effect of EEPE on superoxide dismutase (SOD) in cisplatininduced Nephrotoxic rats

Groups	Drug Treatment	Superoxide dismutase (SOD)
I	Normal Control(0.5% DMSO)	19.56±0.591
II	Nephrotoxic ControlCisplatin (0.75%)	7.53±0.423
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.50±0.44***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	11.89±0.303*
V	Cisplatin (0.75%) + EEPE (400mg/kg)	15.57±0.375***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of Catalase (CAT)

The effects of the different doses of ethanolic extract of Phyllanthus emblica Linn on Catalase (CAT).

Table-11

Results of the effect of EEPE on Catalase (CAT) in cisplatin inducedNephrotoxic rats

Groups	Drug Treatment	Catalase (CAT)
I	Normal Control(0.5% DMSO)	220.31±0.52
II	Nephrotoxic ControlCisplatin (0.75%)	104.94±0.37
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	200.03±0.612***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	158.39±4.091**
V	Cisplatin (0.75%) + EEPE (400mg/kg)	181±0.265***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of Glutathione peroxidise (GPx)

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on Glutathione peroxidise (GPx).

Table-12: Results of the effect of EEPE on Glutathione peroxidise (GPx) in cisplatininduced Nephrotoxic rats

Groups	Drug Treatment	Glutathione peroxidise (GPx)
I	Normal Control(0.5% DMSO)	23.29±0.45
II	Nephrotoxic ControlCisplatin (0.75%)	14.48±0.448
III	Reference Control Cisplatin (0.75%) +Lipoic acid (50mg/kg)	21.39±0.37***
IV	Cisplatin (0.75%) +EEPE (200mg/kg)	16.33±0.399**
V	Cisplatin (0.75%) +EEPE (400mg/kg)	19.26±0.228***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Assessment of non-enzymatic antioxidant parameter

Assessment of Reduced glutathione (GSH)

The effects of the different doses of ethanolic extract of Phyllanthus emblica

Linn on Reduced glutathione (GSH).

Table-13: Results of the effect of EEPE on Reduced glutathione (GSH) in cisplatininduced Nephrotoxic rats

Groups	Drug Treatment	Reduced glutathione (GSH)
I	Normal Control(0.5% DMSO)	20.15±0.776
II	Nephrotoxic ControlCisplatin (0.75%)	8.28±0.201
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.47±0.488***
IV	Cisplatin (0.75%) + EEPE (200mg/kg)	14.37±0.280**
V	Cisplatin (0.75%) + EEPE (400mg/kg)	16.33±0.566***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

HISTOPATHOLOGICAL STUDIES

a) Normal group

Section of the kidney of normal control rat showed,

- Arrangement of nephrotic bundles appears normal, both cortex and medullaappears normal.
- Normal glomerular structure with regularly arranged podocytes was observed.
- No signs of degeneration and edema and no signs of inflammation likeglomerulonephritis.
- Proximal and Distal convoluted tubule appears normal and intact.
- No signs of karyolysis.

b) Nephrotoxic group

Section of the kidney of Nephroprotective control rat showed the following,

- Appearance of coagulative and diffused necrosis
- Severe Glomerulonephritis- Glomerular condensation and appearance ofinflammatory cells
- Marked signs of hemorrhage, edema and narrowed renal arterioles.

c) Standard group

Section of the kidney of lipoic acid treated group rat showed normal histologyof kidney and a20bsence of necrosis.

d) Extract treated groups

Section of the kidney treated with low dose (200mg/kg) of EEPE showed

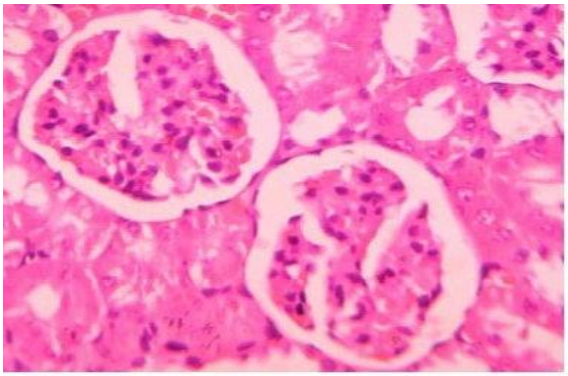
- Moderate tubular degeneration with mild edema and
- Necrotic changes with swollen tubular epithelium.

Section of the kidney treated with high dose (400mg/kg) of EEPE showed

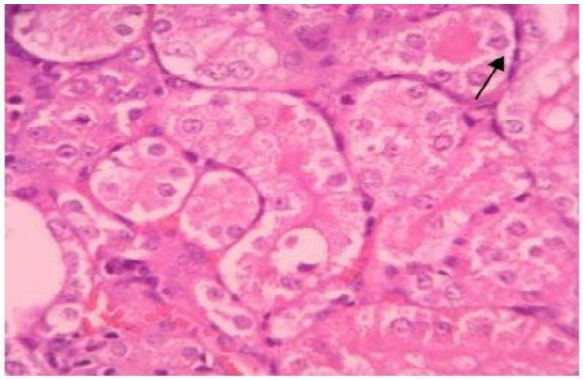
- Moderated signs of regeneration with occurrence of chromatolysis wasobserved in the tubular structure.

HISTOPATHOLOGICAL STUDIES

Group I

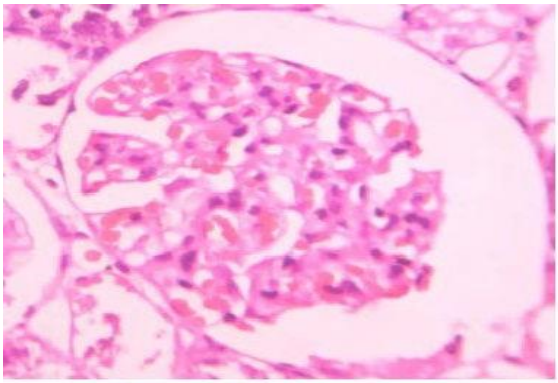


Control- 1

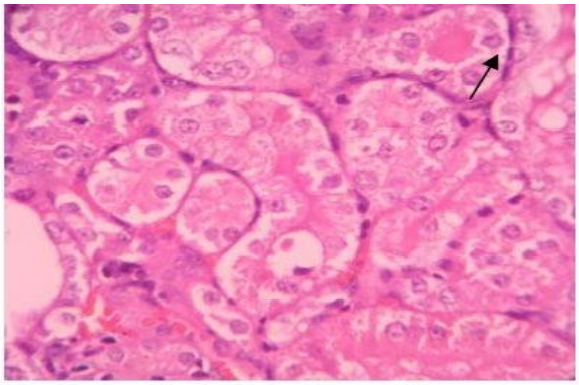


Control -2

Group II

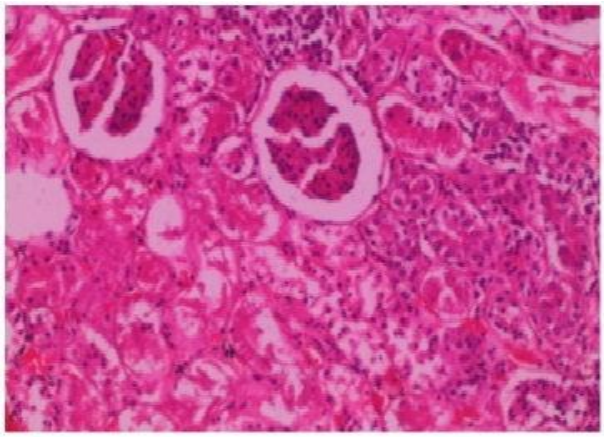


Cisplatin induced 1

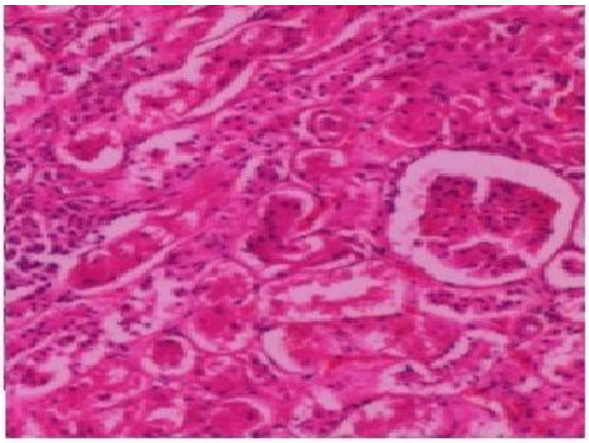


Cisplatin induced 2

Group III

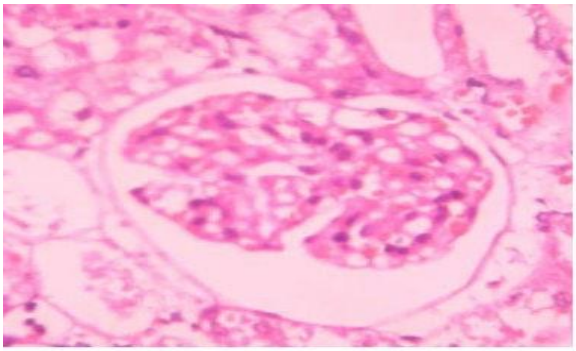


Standard (lipoic acid) + Cisplatin 1

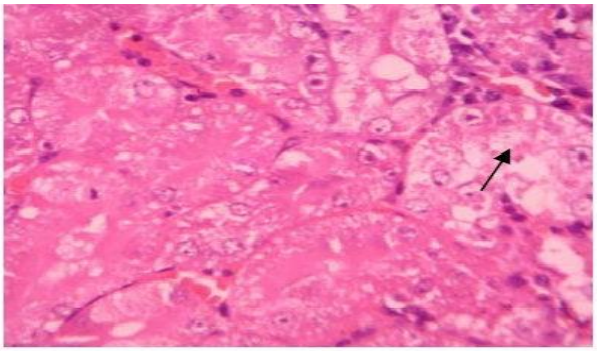


Standard (lipoic acid) + Cisplatin 2

Group IV



Plant extract 200mg + Cisplatin - 1 (Kidney)



Plant extract 200mg + Cisplatin - 2 (Kidney)

GroupV

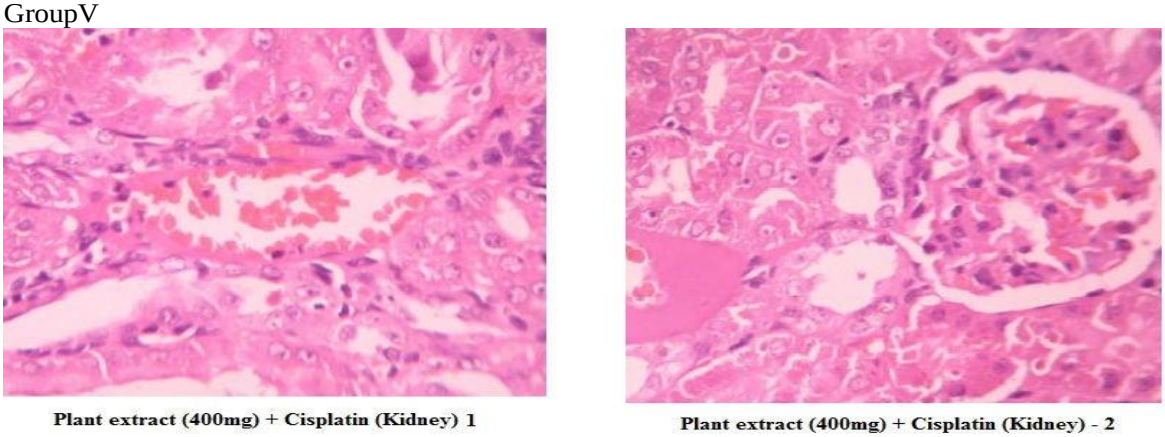


Fig. no- 11: Photomicrographs of kidney tissue section Group I- Normal control, Group II- Nephrotoxic Control, Group III- Reference Control,Group IV- EEPE (200mg/kg), Group V- EEPE (400mg/kg)

DISCUSSION:

Nephrotoxicity is a common clinical syndrome defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted.

There are few chemical agents to treat acute renal failure. Studies reveal back synthetic nephroprotective agents have adverse effect besides reduce nephrotoxicity.

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of treatment of disease until the introduction of allopathic medicine.

Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverseeffect of dialysis.

The phytochemicals found to be present in the fruit pulp extract are the flavanoids, terpenoids, alkaloids, tannins, saponins and anthraquinones. Among them tannins, triterpenoids, flavanoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity.[216]

Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of Phyllanthus emblica . There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an LD_{50} dose of 2000 mg/kg and above was considered as unclassified so the ethanolic extract of Phyllanthus emblica was found to be safe.

Cisplatin causes damage to nuclear and mitochondrial DNA and production of reactive oxygen species (ROS) which lead to activation of both mitochondrial and non- mitochondrial pathways of apoptosis and necrosis. Mitochondrial energetic are also disrupted by cisplatin and may contribute to nephrotoxicity.[47]

In present study, the rats treated with single dose of Cisplatin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomourular filtration rate, which is accompanied by increase in serum creatinine level and declain in creatinine clearence indicating induction of acute renal failure.[241] with Phyllanthus emblica at the dose level of 200 and 400 mg/kg body weight for 15 days significantly lowered the serum level of creatinine with a significant weight gain, increased urine output and creatinine clearence when compared with the nephrotoxic control group.

Cisplatin administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum blood urea nitrogen (BUN).[65] Phyllanthus emblica supplementation to Cisplatin treated rats recorded decrement in levels of blood urea nitrogen (BUN) in plasma.

The elevated level of malondialdehyde (MDA), a marker of lipid preroxidation, indicates increased free radical generation in the Cisplatin induced nephrotoxicity. Cisplatin induced increment in malondialdehyde (MDA) content of plasma was significantly prevented by Phyllanthus emblica treatment in the present study. Therefore, the significantly lower levels of malondialdehyde (MDA) in the kidney tissues of treated groups as compared with the Cisplatin group indicate attenuationof lipid peroxidation. This was probably due to less damage by oxygen free radicals with Phyllanthus emblica . The involvement of oxygen free radicals in tissue injuryis well established.[66]

Decrement in activity levels of renal Superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH) following Cisplatin treatment are in accordance with previous report on Cisplatin induced suppression of endogenous enzymatic antioxidant machinery. Phyllanthus emblica treatment efficiently prevented Cisplatin induced decrease in activity levels of superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH). A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators such as GPX system. Glutathione peroxidise (GPx) is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria, It catalyses the reaction of hydro-peroxides with reduced Glutathione to form Glutathione disulphide (GSSG) and the reduction product of the hydro-peroxide.[243]Effect of Phyllanthus emblica on Glutathione peroxidise (GPx)in experimental rats study were significantly reduced in cisplatin treated rats than in the experimental control rats. Decrement in the activity of renal GPx following cisplatin treatment are due to suppression of endogenous enzymatic antioxidant machinery. Supplementation with Phyllanthus emblica to Cisplatin treated rats resulted in near normal activity of glutathione peroxidise (GPx).

Based on the above results, it was concluded that Phyllanthus emblica exerted statistically significant Nephroprotective activity against cisplatin induced Nephrotoxic rats.

SUMMARY AND CONCLUSION:

The present study was undertaken to scientifically evaluate the nephroprotective activity of the ethanolic extract of fruit pulps of Phyllanthus emblica .

The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols and anthroquinone in EEPE.

The administration of cisplatin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Thereforeit is an effective and an ideal model for nephrotoxicity research.

The evaluation of renal parameters on nephrotoxic rats with EEPE showed significantly elevate the attenuated body weight, urine volume, creatinine clearance and significant reduce in elevated serum creatinine level, which supports its Nephroprotective activity.

The cisplatin induced rats showed elevated levels of serum blood urea nitrogen (BUN) and lipid peroxidation parameter like malondialdehyde (MDA) which was significantly decreased with treatment of EEPE, which proves it having Nephroprotective activity.

The Nephrotoxic rats also showed the reduced levels of enzymatic antioxidant like sulphoxide dismutase (SOD), glutathione peroxidise (GPx) and Catalase (CAT), and non-enzymatic antioxidant like Reduced glutathione (GSH), which was significantly increased with treatment of EEPE, which showed its antioxidant activitydue to the Flavonoids which is present in the extract.

Histopathological studies on isolated kidney revealed that the EEPE, reversedthe kidney damage and also restored normal kidney architecture.

In summary, the fruit pulp of Phyllanthus emblica in an ethanolic extract showed statistically significant nephroprotective activity.

The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties.

There is a scope for further investigation on the histopathology of liver and spleen and clinical studies that are required to elucidate the active phytoconstituents with potent nephroprotective activity.

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