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EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* IN GENTAMICIN AND CISPLATIN INDUCED NEPHROTOXICITY IN RATS

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Abstract:

Cisplatin induced nephrotoxicity is a major contributor to Acute Kidney Injury (AKI) resulting from free radicals induced oxidative stress. Tinospora cordifolia is an Indian medicinal plant, widely used because of its antioxidant activity. Due to limited scientific literature exploring its nephroprotective potential, the present study was designed to investigate the nephroprotective effect of aqueous extract of Tinospora cordifolia against Cisplatin induced nephrotoxicity in albino rats.

The study was commenced following approval from Institutional Animal Ethical Committee. Twenty-four rats were randomized into four groups of six animals each. Total duration of study was 21 days. Group I received normal saline p.o., group II received normal saline along with Cisplatin on last 5 days, group III and IV received Tinospora cordifolia in graded doses p.o. along with Cisplatin on last 5 days. Injection Cisplatin (10mg/kg) i.p. was given once daily for last 5 days to induce nephrotoxicity in rats of groups II, III and IV. The rats were sacrificed under anaesthesia, blood samples analyzed for blood urea nitrogen (BUN) and serum creatinine levels and histopathological changes were studied. Statistical analysis was done using ANOVA followed by post hoc test. Tinospora cordifolia pre-treated groups exhibited significant ($p<0.001$) limitation in rise in levels of BUN and serum creatinine in a dose dependent manner. Histopathological observations further corroborated the biochemical findings. The present study concluded that aqueous extract of Tinospora cordifolia possesses nephroprotective potential against gentamicin induced nephrotoxicity

Keywords: *Cisplatin, Tinospora cordifolia, Nephrotoxicity and Nephroprotective activity.*

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INTRODUCTION:

Kidney

Anatomy and physiology of kidney

Kidney is an important excretory organ in the human body. The function of kidney is not only to excrete the metabolic waste products, but also to maintain the acid base balance and endocrine functions like erythropoietin production (which stimulates the bone marrow to produce red blood cells), active form of vitamin D (calcitriol or 1,25 dihydroxy-vitamin D which regulates absorption of calcium and phosphorus from food, promoting formation of strong bone), renin (which regulates blood volume and blood pressure). The kidney receives blood supply from the renal artery, the branch of abdominal aorta and the venous drainage occurs through renal vein. The urine formed in the kidney gets drained through ureter into the urinary bladder. Kidneys are situated retroperitonially in abdominal cavity and has outer cortex and inner hypertonic medulla.The structural and functional unit of the kidney is nephron. Each human kidney has approximately about 1.3 million nephrons. Each nephron has glomerulus and renal tubules. The glomerulus is formed by invagination of tuft of capillaries into the dilated blind end of the nephron (Bowman's capsule); the capillaries are supplied by an afferent arteriole and drained by an efferent arteriole. The blind end of the nephron continues as the proximal convoluted tubule of 15 mm long and 55nm diameter. The convoluted portion of the proximal tubule drain into the straight portion which forms the first part of the loop of henle. The loop of henle continues with ascending loop of henle and further as distal convoluted tubule which opens into the collecting duct[1].

In the resting adult, the kidney receives 1.2 to 1.3 liters of blood per minute. Glomerular filtrate is formed by the blood in the glomerular capillaries by hydrostatic and osmotic pressure gradients. The glomerular membrane permits free passage of neutral substances with particle size up to 4nm in diameter and excludes such with diameter greater than 8nm like albumin. Approximately 120 ml of ultra filtrate is formed each minute, yet only 1 ml per minute of urine is produced. Therefore, greater than 99% of glomerular filtrate is reabsorbed. In the proximal convoluted tubule approximately 65% of filtrated solutes are reabsorbed and is highly permeable to water. In the loop of Henle there is reabsorption of Na+, Cl–, H2O and urea, about 25% of the filtrate is reabsorbed in this site. The distal convoluted tubule transports Na+ and Cl– and is impermeable to water. The collecting duct system of the kidney is an area of fine control of ultra filtrate composition and volume, where final adjustment in electrolyte composition is made by the action of mineralocorticoid (aldosterone) and antidiuretic hormone (ADH). The hyper tonicity of medullary interstitium plays an important role in concentrating the urine. Thus urine is formed by three processes that are glomerular filtration, tubular reabsorption and tubular secretion. Kidney not only excretes the metabolic substances, but also toxic agents from the body[1, 2]. Hence kidney becomes one of the important targets for the toxicity of agents more than other organs in the body. Kidney is particularly prone for the action of nephrotoxins because of following reasons.

- Kidney receives 25% of the cardiac output hence high levels of toxins are delivered to the kidney. The large surface area of tubular epithelium provides sites for toxicin interaction and uptake.
- he availability of specific transport mechanisms that mediate cellular uptake.
- The normal concentrating mechanisms of the kidney can increase the concentration of the toxins.
- Due to the presence of the metabolic processes in the renal tubular cells, nephrotoxins an release toxic components and induce damage.

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) [2,3].

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 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.

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, Rifampicin, 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 sulphydryl 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, and acetaminophen induce damage by their metabolites.

These toxic metabolites mainly include free radicals[5].

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 P450) 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, sulphamethaxozole, amphotericin, aminoglycoside antibiotics, lithium, and demeclocycline.

The toxin can be either exogenous or endogenous. The exogenous agents are radio contrast agents, cyclosporine, antibiotics, chemotherapeutic agents, organic solvents, and acetaminophen and illegal abortifacients[2,4].

Nephrotoxic agents:

Drugs, diagnostic agents and chemicals are well known to be nephrotoxic. The important nephrotoxic agents are mentioned here under:

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 sub lethal[5,6].

Mechanisms of drugs induced renal damage:
a) Free radical production[7,8].
b) Disturbance of renal tubule cell energy metabolism[9].
c) Disrupted cell calcium homeostasis[10].
d) Alteration of membrane phospholipid metabolism[11,12].
e) Disruption of cellular monovalent cation volume and pH dependant degradation[13,14].

f) Disruption of cytoskeleton[13].
g) Abnormalities of cell proteases[14].
h) Abnormalities of protein and nucleic acid synthesis[15,16,17].
i) Distruption of lysosomal function.
Gentamicin induced renal injury

In 1982, aminoglycosides were described as the cornerstone of therapy against majority of aerobic Gram-negative organisms, responsible for serious sepsis. They still retain their position in the clinical armamentarium. Presently, amino glycosides are used in many types of infections, such as Gram-negative urosepsis and in febrile granulocytopenic patients, because of their established anti-pseudomonal activity.

Gentamicin is an extensively used aminoglycoside. One of the limiting side effects of amino glycoside is nephrotoxicity. The incidence of nephrotoxic reaction in aminoglycoside treated patients varies from 10-20% [19].

ce damaged tubular epithelium; (3) tubular obstruction by cellular debris or casts; and (4) reduction in capillary surface area available for glomerular filtration or reduction in glomerular capillary permeability.

The other mechanism attributed to gentamicin induced renal impairment is free radical generation. The kidney provides the principal excretory route for elimination of aminoglycoside antibiotics from the body. Pharmacokinetic studies in both experimental animals and humans have demonstrated that these are not metabolized and excreted primarily by glomerular filtration.

The majority of experimental evidence suggests that net absorption of aminoglycosides occurs in the proximal tubules by means of a high capacity transport system; there is little direct evidence for marked luminal concentration increase of aminoglycosides along with proximal tubule. Net drug secretion may occur in the early proximal tubule at high doses, while net secretion may occur in the late proximal tubule of juxtamedullary nephrons may occur at low doses. This nephronal heterogeneity in drug handling explains most features observed in the experimental setting. The combined processes of luminal reabsorption and basolateral uptake account for the high renal cortical tissue levels achieved in the experimental animals and humans. Once taken up by renal tubular cells, aminoglycosides reside in a poorly exchangeable pool, with tissue half lives exceeding those observed in serum by over a hundred fold[22].

Aminoglycosides produce tubular cell necrosis, which is largely confined to the proximal convoluted tubule and pars recta. The earliest lesion, seen by electron microscopy, is an increase in the number and size of secondary lysosomes, also called cytosegrosomes or phagosomes. These lysosomal alterations probably represent autophagic vacuoles arising from sequestration of fragments of membranes and organelles, which were damaged in developing toxicity and are undergoing lysosomal processing[23].

Aminoglycosides nephrotoxicity is characterized by a variety of renal functional alterations. Renal excretory failure with near cessation of effective granular filtration rate is the final manifestation of this clinical disorder.

Prior to this event more subtle nephronal deragements, predominated by proximal tubular abnormalities, occur both in humans and animals. The initial renal manifestation is enzymeuria. Deterioration in the proximal tubular transport processes develops during toxicity and glycosurea, aminoacidurea, tubular proteinurea with B2-microglobulinurea and transport defects consistent with a Fanconi-like syndrome.

Several approaches have been tried to minimize the aminoglycoside nephrotoxicity in animal models. The main are reducing the intrinsic toxicity of the drug, decreasing the renal accumulation of the drug by saturating the uptake mechanism and co-administration of nephroprotectants that neutralize the toxic effects of the drug in renal cells. The nephroprotective agents like poly aspartic acid has been reported to completely protect rats from all signs of aminoglycoside nephrotoxicity. However clinical results are awaited. Probucol, a known hypolipidemic agent and an antioxidant has been recently shown to prevent both functional and histological renal changes induced by gentamicin in rats. Cyclodextrin sulfates interact electro statically with gentamicin and interfere with the binding of gentamicin in an in vitro study. Thereby they would likely to affect the interaction of gentamicin with negatively charged phospholipid. Laurent et al. proposed that cyclodextrin sulfates enhanced the renal tissue repair their by prevent the gentamicin induced tubular necrosis leading to functional

impairment. However the pharmacokinetic and toxicological data is incomplete regarding cyclodextrin sulfates.

Paracetamol induced nephrotoxicity: Paracetamol also known as acetaminophen is widely used as analgesic and antipyretic drug. An acute paracetamol over dosage can lead to potentially liver and kidney failure in humans and experimental animals and in severe cases to death due to renal failure[31,32]. Paracetamol is a phenacetin metabolite[26]. Phenacetin was considered one of the most nephrotoxic analgesics and has now been withdrawn from the market in many countries[27].

MATERIALS AND METHODS:

5.1 MATERIALS –

CHEMICALS REQUIRED:

- NaOH (Merck, Sura labs, Dilsukhnagar, Hyd)
- Formalin 10% (Merck, Sura labs, Dilsukhnagar, Hyd)
- Tween 80 2% (Merck, Sura labs, Dilsukhnagar, Hyd)
- Distilled water

EQUIPMENTS:

- Microscope
- Centrifuge
- Animal weighing balance
- Mechanical stirrer

APPARATUS REQUIRED:

- Burette
- Pipette
- Conical flask
- Petridish
- Surgical equipment's

5.2 Collection of plant material:

Tinospora cordifolia used for the present studies was collected Local market. The bark was 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.

5.3 Preparation of ethanolic Extract:

Preparation of plant extract the aqueous extract of *Tinospora cordifolia* (AETC) stem was prepared by taking 100 grams of shade dried and powdered plant material and mixing with 500mL of distilled water in a Soxhlet apparatus for 20-24hours. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4ºC until required.

5.4 Qualitative phytochemical screening:

The following tests were carried out on the standardized herbal extract to detect various phytoconstituents present in them.

i.Molisch's test
ii.Fehling's test
iii.Barfoed's test

1. Detection of carbohydrates

Small quantity of the extract was dissolved in distilled water and filtered. The filterate was subjected to

i. Molisch's test

To the filtrate few drops of alcoholic α-napthol was added and 2ml of conc sulphuric acid was added slowly through the slides of the test tube. No purple-colored ring was formed at junction of the two layers, which indicates absences of carbohydrates.

ii. Fehling's test

Small portion of the extract was treated with fehling's solution I and II and then heated on water bath. No brick red colored precipitate was formed, which indicates absence of carbohydrates.

iii. Barfoeds test

Small portion of the extract was treated with barfoed's reagent. No red precipitate formed, which indicates absence of carbohydrates.

2. Test of starch

A small amount of powdered drug was treated with diluted iodine solution. No blue color was observed, which indicates absence of starch.

3. Detection of proteins and amino acids

Small quantity of extract was dissolved in few ml of water and was subjected to million's, biuret and ninhydrin test.

a) Million's test:

The extract was treated with million's reagent. No white precipitate was produced, shows the absence of proteins and free amino acids.

b) **Biuret test:**

To the extract equal volume of 5%w/v NaOH and four drops of 1%w/v $CuSO_4$ solution were added. No pink or purple color was formed indicating the absence of proteins.

c) Ninhydrin test:

The extract was treated with ninhydrin reagent. No purple color was produced, indicating the absence of proteins.

4. Detection of phenolic compounds and tannins

The decoction was diluted with distilled water and filtered. The filtrates were treated with following reagent.

a) Ferric chloride test:

The filtrate was treated with 5% of ferric chloride solution. No black precipitate was found in the decoction of the plant, indicating the absence of tannins and phenolic compounds.

b) Test with Lead acetate Solution:

Few ml of filtrate was treated with lead acetate solution. No white precipitate was produced in the decoction of plant.

c) Gelatin test:

To the filtrate of decoction, add 1ml of 1% solution of gelatin. No white precipitate was seen, which indicates absence of tannin in plant.

a) Salkowski test

b) Libermann – Burchards test

5. Test for phytosterols

Small quantity of decoction was dissolved in 5ml of chloroform separately. Then these chloroform layer subjected to,

a) Salkowski test:

To 1ml of the above prepared chloroform solutions, few drops of conc H_2SO_4 was added. Red color produced in the lower layer, shows the presence of phytosterols.

The above chloroform solution was treated with few drops of conc H_2SO_4 followed by 1ml of acetic anhydride solution. Green color was produced, shows the presence of phytosterols.

b)Libermann – Burchards test:

6. Test for fixed oils and fats

a) Spot test:

A small quantity of extract was pressed between two filter papers. Oil stain was observed, show presence of fixed oils.

b) Saponification:

Few drops of 0.5N alcoholic potassium hydroxide was added to extract along with a few drops of phenolphthalein. The mixture was heated on a water bath for about 1 – 2 hours. Formation of soap or a partial neutralization of alkali indicated the presence of fixed oils and fats.

7. Test for alkaloids

Small amount of extract was stirred with a few ml of dil HCl and filtered. The filtrate was teste with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

a) Mayer's test:

To the small amount of filtrate add few drops of Mayer's reagent. A white color precipitate was formed, indicating the presence of alkaloids.

b) Dragendroff's test: (potassium bismuth iodide)

To the small amount of filtrate add few drops of Dragendroff's reagent. An orange red color precipitate was formed, indicating the presence of alkaloids.

c) Wagner's test:

To the small amount of filtrate add few drops of Wagner's reagent. A brown color precipitate was formed, indicating the presence of alkaloids.

d) Hager's test: (picric acid)

To the small amount of filtrate add few drops of Hager's reagent. An yellow crystalline precipitate was formed, indicating the presence of alkaloids.

8. Test for glycosides

A small amount of the extract was hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to

a) Legal's test
b) Balget's test
c) Borntrager's test
d) Modified borntrager's test.

a) Legal's test:

To the hydrolysate 1ml pyridine few drops of sodium nitroprusside solution was added and then made alkaline with NaOH solution. Pink color was obtained showing the presence of glycosides.

b) Balget's test:

To a solution of extract sodium picrate solution was added. Yellowish orange color was obtained showing, the presence of glycosides.

c) Borntrager's test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink color
was observed in ammoniacal layer, confirms the presence of glycosides.

d) Modified borntrager's test:

The extracts were boiled with few ml of dil HCl and 5ml of ferric chloride solution. The contents are cooled and shaken with organic solvent. Organic layer was separated and to this equal volume of ammoniacal solution was added. The ammoniacal layer showed pink color. In this test, addition of ferric chloride was added to break the C – C linking of glycosides which is a stronger than C = O linkage.

9. Test for flavonoids

The extract was dissolved in ethanol and then subjected to the following tests.

a) Ferric chloride test:

To a small quantity of Methanolic solution of extract few drops of neutral ferric chloride was added. Blackish red color was observed, showing the presence of flavonoids.

b) Shinoida's test:

To the alcoholic solution a small piece of magnesium ribbon was added along with conc HCl. Magenta color was formed, showing the presence of flavonoids.

c) Fluorescence test:

Alcoholic solution was seen under ultra violet light. Green color fluorescence was observed, indicating the presence of flavonoids.

d) Reaction with alkali and acid:

With sodium hydroxide solution the extracts gave yellow color. Extract gave orange color with conc H_2SO_4 indicating the presence of flavonoids.

e) Zinc, HCl reduction test:

To a small quantity of extract, a pinch of zinc dust was added. Then add few drops of conc HCl. Magenta color was produced, the presence of flavonoids.

f) Lead acetate solution:

To a small quantity of extract a few drops of 10% lead acetate solution was added. Yellow precipitate was produced, shows presence of flavonoids.

10. Detection of saponins

The extracts were diluted, with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. A one-centimeter layer of foam was formed, indicating the presence of saponins.

11. Detection of coumarins

To a small quantity of extract were dissolved in alcohol and exposed to UV
light, shows green fluorescence.

To small quantity of extract were dissolved in alcohol and add ferric chloride solution, shows green color, indicating the presence of coumarins.

5.5 EXPERIMENTAL ANIMALS:

Experimental animals Twenty-four healthy Wistar albino rats of either sex, weighing 150-200gm were grouped and housed in polypropylene cages in CPCSEA approved Central Animal House. The rats were maintained under standard laboratory conditions of alternating periods of light and darkness of 12 hours each, temperature (25±2ºC) and relative humidity (45 to 55%). The rats had free access to standard rat pellet diet and tap water ad libitum. After one week of acclimatization, the animals were considered suitable for study. Pregnant female rats were not included in the study.

5.6 METHODS:

Acute Toxicity Studies:
Animals will be fasted prior to dosing, food but not water should be withheld overnight. Following the period of fasting, the animals will be weighed and the test substance will be administered. After the substance is administered, food may be withheld for a further 3-4 hrs. As a dose is administered in fractions over a period, it may be necessary to provide the animals with food and water depending on the length of the period.
Three animals will be used for each step. The dose level used as the starting dose will be selected from one of the four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dose animals. The animals will be observed for behavioral changes for 4 hrs and 48 hrs for mortality.

5.7 *In vivo* model:

Nephroprotective studies

Effect of *Tinospora cordifolia* on Gentamicin-induced Nephrotoxicity

The study was commenced after getting approval from Institutional Animal Ethical Committee, registered under CPCSEA India. The study was conducted in the Department of Pharmacology.
The total duration of study was 21 days, wherein 24 rats were randomly divided into four groups of six animals each. Drugs were administered per orally to the rats after a fasting period of 4 hours to ensure proper absorption.

Group-I: Control group was given 0.9 % NaCl solution in a single oral dose of 2ml/kg/day for 21 days.

Group-II: In addition to 0.9% NaCl solution, the animals of this group received injection Cisplatin (10 mg/kg) intraperitoneally (i.p.) once daily for last 5 days.

Group-III: This group was treated with aqueous extract of *Tinospora cordifolia* (AEHI) in the dose of 250 mg/kg body weight after making a suspension in normal saline per orally for 21 days. Injection Cisplatin (10 mg/kg) was administered intraperitoneally (i.p.) once daily for last 5 days.

Group-IV: This group was treated with aqueous extract of *Tinospora cordifolia* (AEHI) in the dose of 500 mg/kg body weight after making a suspension in normal saline per orally for 21 days. Injection Cisplatin (10 mg/kg) was administered intraperitoneally (i.p.) once daily for last 5 days.
After fasting for 24 hrs after the last dose of Cisplatin, with free access to water ad libitum, the rats were sacrificed under anaesthesia produced by intraperitoneal injection of Ketamine (75 mg/kg) and Xylazine (10 mg/kg). Blood samples for performing biochemical tests were collected from abdominal aorta and the kidneys were dissected out for histopathological study. The collected blood samples were centrifuged at about 2500 rpm for 10 minutes.
The serum so separated was used for estimation of BUN and serum creatinine levels using assay kits spectrophotometrically.
Rats from each group were sacrificed and the kidneys were excised for histopathological examination. The kidneys were washed with normal saline. A section of kidney was dissected, fixed in neutral buffered formalin (10%) and then dehydrated with grades of ethanol (70%, 80%, 90%, 95% and 100%). Dehydration was followed by clearing the samples in xylene. Tissue samples were then embedded in paraffin wax from which blocks were prepared. Sections of 5µm thickness were prepared from the blocks using a microtome. Tissue samples were stained with Haematoxylin and Eosin stain and subjected to histopathological examination.
Statistical analysis
The study data were expressed as mean ± standard error of mean (SE). The statistical analysis was carried out using one way analysis of variation (ANOVA) followed by Tukey's post hoc test. p-value

RESULTS
The results of qualitative phytochemical analysis of the crude powder and the aqueous extract of *Tinospora cordifolia* are shown in Table.

Table ---: Preliminary qualitative phytochemical analysis of *Tinospora cordifolia*

Phytochemical	Test	Methanolic extract
Alkaloids	Dragandroffs test	+
	Mayers test	+
	Wagners test	-
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Cardiac glycosides	Keller-kilianni test	+
Phlobotannins	HCl test	+
Saponins	Frothing test	+
Steroids	Libbermann-Burchard test	-
Tannins	$FeCl_3$ test	-
Triterpenes	H_2SO_4 test	+

(-): absent, (+): present.

In methanol extract maximum amount of tannins, alkaloids, Flavonoids, phlobotannins, saponins and triterpenes were present. Cardiac glycosides and steroids were absent.

In Cisplatin treated group of animals the concentration of serum urea and Creatinine were considerably increased than the normal animals which indicates severe Nephrotoxicity. Treating (group 4 & 5) with aqueous extract of showed significant decrease ($p<0.001$) in concentration of serum Creatinine compared to Cisplatin treated group 2. Nevertheless, the concentration of uric acid not so much considerably increased in the Cisplatin treated groups than control group (group1). Treatment with aqueous extract of significantly ($p<0.05$) decreases the Creatinine levels in group 4 & 5 ($p<0.01$) compared to Cisplatin treated group.

Effect of *Tinospora cordifolia* on blood urea nitrogen (BUN) and serum creatinine levels

BUN level, as depicted in Table 1, in normal saline treated group was 22.63±0.01 mg/dl. It was found to be significantly increased ($p<0.001$) after administration of Cisplatin to 62.13±1.15 mg/dl. *Tinospora cordifolia* exhibited a dose dependent limitation of BUN rise after Cisplatin administration. At the dose of 250mg/kg for 21days *Tinospora cordifolia* displayed a highly significant ($p<0.001$) prevention in BUN rise (43.36±1.02 mg/dl) when compared to Cisplatin treated group. However, in the dose of 500 mg/kg for 21 days the *Tinospora cordifolia* extract showed much more efficacy, in limiting the BUN rise after Cisplatin administration, to 30.10±0.11mg/dl, and it was also found to be highly significant ($p<0.001$).

Table: Effect of *Tinospora cordifolia* on Cisplatin induced changes in BUN levels (Mean±SE) (n=6).

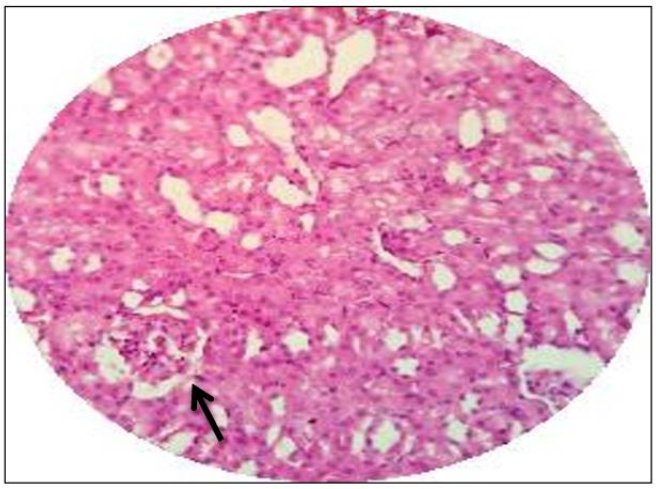
Group	Treatment	BUN (mg/dl), Mean±SE
I	Normal Saline (2ml/kg, p.o.)	22.63±0.01
II	Normal Saline (2ml/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	62.13±1.15*
III	*Tinospora cordifolia* (250mg/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	45.19±1.10#
IV	*Tinospora cordifolia* (500mg/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	33.36±0.36#

*$p<0.001$ as compared to Normal saline treated group, #$p<0.001$ as compared to Cisplatin treated group

Table 2: Effect of *Tinospora cordifolia* on Cisplatin induced changes in serum creatinine levels (Mean±SE) (n=6).

Group	Treatment	BUN (mg/dl), Mean±SE
I	Normal Saline (2ml/kg, p.o.)	0.65±0.013
II	Normal Saline (2ml/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	1.16±0.013*
III	*Tinospora cordifolia* (250mg/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	0.83±0.030#
IV	*Tinospora cordifolia* (500mg/kg, p.o.) + Cisplatin (10mg/kg, i.p.)	0.63±0.060#

*$p<0.001$ as compared to Normal saline treated group, #$p<0.001$ as compared to Cisplatin treated group



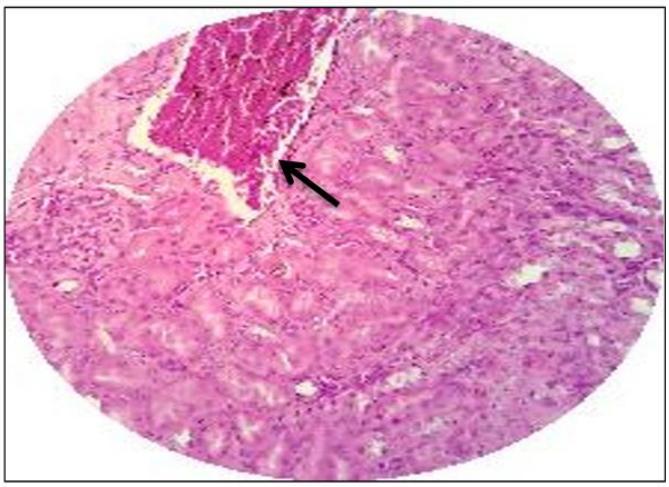
Normal histological architecture of glomerulus (arrow) and renal tubules in rats of control group (Oral saline 2ml/100g/day).

Figure 1: Light micrograph of section from kidney stained with Haematoxylin-Eosin (40X).

Creatinine level, as depicted in Table 2, in normal saline treated group was 0.65±0.013 mg/dl. It was found to be significantly increased ($p<0.001$) after administration of Cisplatin to 1.16±0.013mg/dl. *Tinospora cordifolia* demonstrated a dose dependent limitation of creatinine rise after gentamicin administration. At the dose of 250mg/kg for 21 days *Tinospora cordifolia* showed a highly significant limitation ($p<0.001$) of creatinine rise (0.83±0.030 mg/dl) when compared to Cisplatin treated group. However, in the dose of 500 mg/kg for 21 days the *Tinospora cordifolia* extract had much more efficacy, in limiting the creatinine rise after Cisplatin administration, to 0.63±0.060 mg/dl. This observation was also highly significant ($p<0.001$).

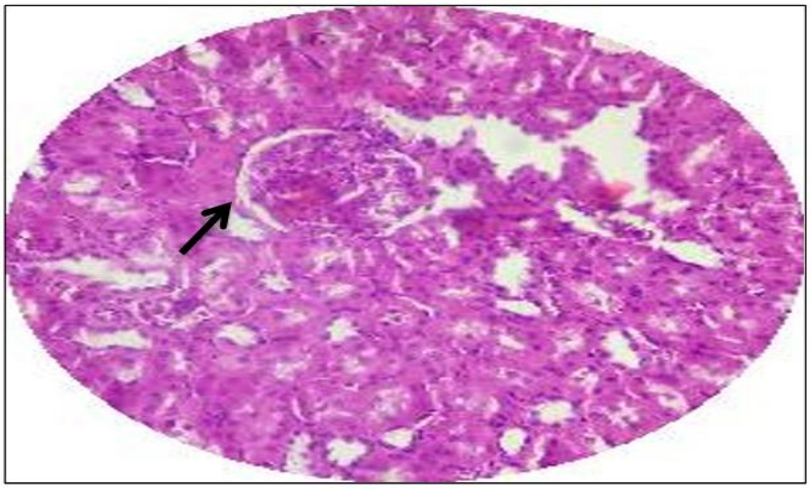
Histological studies

The histopathological sections of kidneys of normal control rats presented with normal morphological appearance of cortex and medulla with normal glomerular and tubular structures after 21 days (Figure 1). Whereas, in gentamicin treated group the kidney tissue sections of rats revealed hemorrhagic foci, renal tubular degeneration and leucocytic infiltration (Figure 2). The histological sections of kidneys of *Tinospora cordifolia* treated groups exhibited almost normal architecture of kidney with minimal leucocytic infiltration after 21days of pretreatment (Figure 3), with absence of haemorrhage, tubular casts, glomerular congestion or tubular necrosis. Thus, the nephroprotective effect was confirmed by the histopathological examination of the kidney, wherein the normal cellular architecture was found to be restored.



Haemorrhagic foci (black arrow) and renal tubular degeneration (blue arrow) in cortex of rats in Cisplatin group (Cisplatin 10mg/kg/day i.p. for last 5 days).

Figure 2: Light micrograph of section from kidney stained with Haematoxylin-Eosin (40X).



Nearly preserved histological architecture of kidney showing normal glomerulus (arrow) in rats treated with *Tinospora cordifolia* (500mg/kg/day p.o. for 21 days + Cisplatin 10mg/kg/day i.p. for last 5 days).

CONCLUSION:

It was concluded from the present study that pre-treatment with *Tinospora cordifolia* significantly reduced Cisplatin induced renal damage. However, further studies are needed to identify and characterize the phytoconstituents from *Tinospora cordifolia*; and also, to explore the exact mechanism by which it acts as nephroprotective agent, before it can be introduced in a clinical setting. The study also carries further scope for assessment of *Tinospora cordifolia* with its other extracts, dose levels, extended test durations and other biochemical parameters, in order to conclusively establish *Tinospora cordifolia* as a nephroprotective drug.

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