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Pharmacological Evaluation of Nephroprotective Activity of Adhatoda Vasica Leaf Extract in Wistar Rats

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Abstract: Acute renal failure is treated with a limited number of chemical agents. Studies show that synthetic nephroprotective agents have a negative effect in addition to reducing nephrotoxicity. The general public is becoming more interested in traditional medicine, particularly in the treatment of nephrotoxicity, owing to a lack of options in pharmacotherapy. Many plants have been used in traditional medical systems around the world to treat kidney failure. Plant preparation, along with dietary measures, was indeed the foundation of disease treatment until the introduction of allopathic medicine. Ethno medicinal plants can help prevent the need for dialysis by treating the causes and effects of renal failure, as well as reducing the numerous negative effects of dialysis. The methanolic extract of Adhatoda vasica, a plant known for its antioxidant properties, was found to be safe for oral acute toxicity studies up to 2000 mg/kg. The extract showed DPPH radical scavenging activity, with an IC 50 value of 57.10µg/ml. Cisplatin, a drug used in chemotherapy, can damage DNA and produce reactive oxygen species, potentially leading to nephrotoxicity. In rats treated with Adhatoda vasica, it reduced body weight, increased glomerular filtration rate, and increased serum creatinine levels, indicating acute renal failure.

Key Words:Nephrotoxicity, Adhatoda Vasica, Cisplatin, Lipoic acid, Biochemical labels.

1. INTRODUCTION:

Medicines are responsible for approximately 20% of acute renal failure cases that occur in hospitals and the general public.1-3 In elderly people, the prevalence of medication-induced nephrotoxicity may reach 66%.4 Cases are now older, more likely to have diabetes and cardiovascular disease, use more traditional medicines, and undergo more individual and rehabilitative procedures that may wreak havoc on order function than they were thirty years ago.5 Renal impairment can be costly and necessitate a variety of interventions, including hospitalization, even though it is always reversible if the offending drug is discontinued. [1-6]

1.1 Mortal medicine- convinced nephrotoxicity.

Aminoglycosides are ingested by proximal tubular epithelial cells after being filtered by the glomeruli, where they interact with phospholipids and alter phospholipid metabolism, resulting in their primary nephrotoxic effect.[7,8]

Aminoglycosides cause renal vasoconstriction in addition to their direct effect on cells. Dosing and treatment duration are two important factors in the development of acute kidney injury (AKI) secondary to aminoglycoside nephrotoxicity. As a saturable miracle, aminoglycoside uptake by the tubules is limited after a single cure. Thus, a single large cure is preferable to three lower bones throughout the day. Once the achromatic point is reached, it is possible that one cure per day leads to less accumulation in the tubular cells. [9-15]

1.2 Nephrotoxicity caused by amphotericin B

Amphotericin B has a broad antifungal spectrum due to its mode of action and increased membrane

permeability. It binds to cholesterol and ergo sterol in fungal cell walls, causing nephrotoxicity and electrolyte diseases like (dRTA) due to potassium and magnesium loss. Although lipid-based amphotericin B formulations are less harmful to feathers than traditional amphotericin B, they do not completely eradicate it. This might be due to the traditional medicine's direct nephrotoxic effects.[16-17]

1. 3. Nephropathy brought on by discrepancy

Differentiated nephropathy (CIN), a condition whose pathophysiology is still poorly understood, is most likely brought on by renal vasoconstriction and direct injury to renal tubular epithelial cells. In accordance with current theories, CIN toxin is brought on by a combination of direct cytotoxicity and post-ischemic reperfusion injury, which results in oxygen-free radicals and harms endothelial cells. [19-20]

1.4. Nephrotoxic goods of Calcineurin

Tacrolimus and cyclosporine cause efferent and sensory arteriolar vasoconstriction, which causes acute order damage (AKI). An ongoing injury may lead to interstitial fibrosis. Tacrolimus has been shown to cause thrombotic microangiopathy because it damages the endothelium. [21-22]

1.5. Nephrotoxicity brought on by cisplatin

This hydrolysed metabolite forms covalent bonds with these cellular components, leading to cell death. The primary mechanism of action of cisplatin is the formation of DNA adducts, which interfere with DNA replication and transcription, ultimately leading to cell cycle arrest and apoptosis.[23]

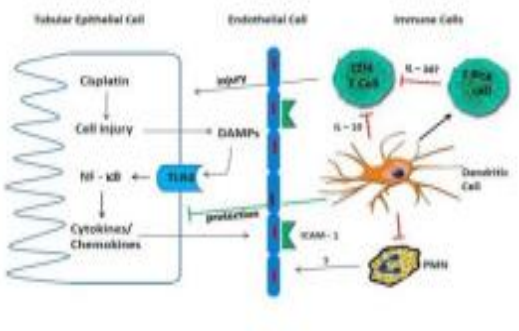

Figure 1: Cisplatin- convinced epithelial cell death pathways

Cisplatin enters renal epithelial cells via the OCT2 and, to a lesser extent, the Ctr1 transporters. Cisplatin, in addition to damaging mitochondrial and nuclear DNA, generates reactive oxygen species (ROS), which activate both mitochondrial and no mitochondrial pathways for apoptosis and necrosis. Cisplatin also disrupts mitochondrial energy products, which could be a factor in nephrotoxicity. The proximal tubule, which is where the order damage caused by cisplatin matures, receives the majority of its energy from adipose acids. Cisplatin reduces adipose acid oxidation in rat order and proximal tubule cells in culture by reducing PPAR-mediated expression of genes involved in cellular adipose acid use. Cisplatin causes DAMPs in renal epithelial cells, which activate TLR4.[24,25]

TNF- is one of many chemokines and cytokines produced when TLR4 is activated. These chemokines and cytokines stimulate adhesion molecule production and attract neutrophils and T cells, among other seditious cells, to the site of the damage.[29]

1.6. Nephrotoxic goods of Ifosfamide Cyclophosphamide

Ifosfamide, a well-known analog of cyclophosphamide, is poisonous to tubular cells due to its metabolite Chloroacetaldehyde, causing proximal tubule damage and performing in the Fanconi pattern.[24,26]

1.7. Nephrotoxicity caused by Foscarnet

The drug Foscarnet, which is used to treat resistant cytomegalovirus (CMV) infections, causes acute interstitial nephritis and intra-tubular demitasse conformation. Crystallization is not the only process that can have an impact.[27]

1.8. Nephrotoxicity brought on by demitasse-forming medicines

AKI, also known as acute kidney injury, is a condition that occurs due to tubular obstruction caused by certain substances present in the urine. These substances include sulfa drugs, acyclovir, methotrexate, ethylene glycol, and protease inhibitors such as indinavir. The blockage of the tubules leads to impaired kidney function and can result in severe damage if not promptly addressed.[28]

2. MATERIALS AND METHODS

2.1 Preparation of the extract

Coarsely plant parts of Adhatoda vasica (300 gm) were extracted using different organic solvents, defatted with petroleum ether (40-600C), and extracted with ethanol and distilled water for 36 hours using a soxhlet apparatus. To ensure complete extraction, each extract was evaporated to dryness using a rotary evaporator under reduced pressure, and the dried residue was stored in an airtight container for future use.[28,30]

Formula;

$$\%\ yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x}100$$

2.2.1 Test for Carbohydrates

- **Molisch'sTest:**

The Adhatoda vasica extract aqueous solution was mixed with a few drops of Molish reagent (Naphthol) and conc. H2SO4 (sulphuric acid) was added dropwise along the wall of the test tube. When two liquids combine, a purple color ring forms at the junction. It indicates that carbohydrates are present.

- **Fehling's Test:**

1ml of Fehling A and Fehling B solutions were mixed together, and 2ml of Adhatoda vasica extract aqueous solution was added. Cook for 5-10 minutes in a water bath. The presence of reducing sugar is indicated by the formation of a reddish brown precipitate as a result of cuprous oxide formation.

- **Benedict's test:**

In a test tube, an equal amount of Benedict's reagent and Adhatoda vasica extract were mixed and heated in a water bath for 5-10 minutes. The presence of reducing sugar appears green, yellow, or red depending on the amount of reducing sugar present in the test solution.[27,28]

- **Barfoed's Test:**

1 ml of Benedict solution was added to an aqueous solution of Adhatoda vasica extract and heated to boiling. Red color indication was observed in the presence of monosaccharides due to the formation of cupric oxide.

2.2.2 Tests for Alkaloids

- **Dragendorff's Test:**

1 mL of Adhatoda vasica extract was used. Alcohol was mixed and thoroughly shaken with a few drops of acetic acid and Dragendroff's reagent. The presence of alkaloids is indicated by the presence of an orange-red precipitate.

- **Wagner's Test:**

1 mL of Adhatoda vasica extract was dissolved in acetic acid. A few drops of Wagner's reagent were added. The presence of alkaloids indicated the presence of a reddish-brown precipitate.

- **Mayer's Test:**

1 mL of Adhatoda vasica extract was dissolved in acetic acid, along with a few drops of Mayer's reagent. The formation of a dull white precipitate indicated the presence of alkaloids.

- **Hager's Test:**

Acetic acid was used to dissolve 1-2 ml of Adhatoda vasica extract. 3 mL of Hager's reagent was added to it, and the presence of alkaloids resulted in the formation of yellow precipitate.

2.2.3 Test for Saponins

- **Froth Test:**

1ml of Adhatoda vasica extract was mixed into 1ml of distilled water and thoroughly shaken. The presence of saponin was indicated by the formation of a stable froth.

2.2.4 Test for Triterpenoids and Steroids

- **Libermann-Burchard Test:**

The Adhatoda vasica extract was dissolved in chloroform. It was heated on a water bath and then cooled after 1 mL of acetic acid and 1 mL of acetic anhydride were added. Then, along the sides of the test tube, add a few drops of concentrated sulphuric acid. The presence of steroids is indicated by the appearance of a bluish green color.

- **Salkowski Test:**

The Adhatoda vasica extract was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added. The presence of steroids was indicated by the formation of bluish red to cherry red coloration in the chloroform layer and green fluorescence in the acid layer.

2.2.5 Test for Tannin and Phenolic Compounds

- **Ferric Chloride Test:**

A small amount of Adhatoda vasica extract was dissolved in distilled water. Add a few drops of diluted ferric chloride solution to it. The presence of tannins was indicated by the formation of a dark blue color.

- **Gelatin Test:**

The extract in Adhatoda vasica was dissolved in distilled water 2ml of 1% gelatin solution containing 10% sodium chloride was added. The presence of phenolic compounds is indicated by the formation of a white precipitate.

- **Lead Acetate Test:**

A test tube was filled with distilled water, a small amount of Adhatoda vasica extract, and a few drops of lead acetate solution. The presence of phenolic compounds is indicated by the formation of a white precipitate.

2.2.6 Test for Flavonoids

- **Shinoda's Test:**

Adhatoda vasica extract in alcohol was diluted to 1 ml with a few magnesium turnings and a few drops of concentrated hydrochloric acid. A water bath was used to heat it.Indicating the presence of flavonoids was the formation of a red to pink color.[16,17]

2.2.7 Test for Glycosides

- **Borntragers Test:**

To 3 ml of the test solution, diluted sulfuric acid was added. The filtrate was then obtained after it had been boiled for 5 minutes. Equal parts of benzene or chloroform were added to the cold filtrate and thoroughly mixed. After the organic solvent layer had been separated, ammonia had been added to it. The formation of a pink-to-red color in the ammonia layer was indicated by the presence of anthraquinone glycosides.[18]

- **Keller Killiani Test:**

A test tube was filled with 2 ml of the test solution, 3 ml of glacial acetic acid, and 1 drop of 5% ferric chloride. 0.5 ml of concentrated sulfuric acid should be added slowly. The development of a blue hue in the acetic acid layer served as a sign of the presence of cardiac glycosides.

2.2.8 Test for fats and oils

- **Solubility test:**

- Chloroform was added in small amounts to a 2-3 ml alcoholic solution of Adhatoda vasica extract, and solubility was noted.
- To 2–3 ml of an alcoholic extract of Adhatoda vasica. A few ml. of 90% ethanol were added, and solubility was seen. extract. a few ml. of 90% ethanol are added before extracting. Solubility was observed after adding a few ml. of 90% ethanol.[18,19]

2.3 Acute Toxicity Study

The acute toxic class method is a stepwise procedure that involves administering a substance to a group of experimental animals at a defined dose. The process involves using three animals of a single sex per step, with 2-4 steps required depending on the mortality and moribund status of the animals. If no compound-related mortality occurs, the next step involves dosing three additional animals with the same dose or at a higher or lower dose level. The starting dose is selected from four fixed levels: 5, 50, 300, and 2000 mg/kg body weight.[19]

2.4 DPPH

The DPPH free radical scavenging assay was used to determine the antioxidant activity of Adhatoda vasica extract. Extract/standard methanol solution of 1 mg/ml was prepared.

From a 1mg/mL stock solution and 2mL of 0.1mM DPPH solution, different concentrations of Adhatoda vasica extracts/standard (20-100g/ml) were prepared. The obtained mixture was vortexed, incubated for 30 minutes at room temperature in a relatively dark location, and then measured at 517 nm with a UV spectrophotometer (Shimadzu 1700). Take 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. At 517 nm, the absorbance of the control was measured against methanol (as a blank).[32]

The percentage antioxidant activity of the sample/standard was calculated using the following formula:[20, 21]

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.5*In vivo* study

2.5.1 Animals

Animals will be chosen at random from the Pinnacle Biomedical Research Institute (PBRI) animal house in Bhopal, India, and divided into various treatment groups before being housed in a propylene cage with sterile husk as bedding. In the animal house, relative humidity of 30.7% at 22°C and a 12:12 light and dark cycle will be maintained, and standard pellets (Golden Feeds, New Delhi, India) and water will be available ad libitum. Before the experiments, rats will be acclimatized to laboratory conditions for 7 days. Each set of experiments will use a separate group of rats (n=6). The Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal will approve the animal experiments.[22-26]

Animals used

- Strain - *Albino Wistar* rats
- Age - 5-6 weeks
- Sex - either sex
- Body weight - 250±50 g

2.5.2 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 (Cisplatin)

Group-III: Received the standard Nephroprotective drug, (Lipoic acid (50mg/kg; p.o))

Group-IV: Received methanolic extract of *Adhatoda vasica*(200mg/kg; p.o)

Group-V: Received methanolic extract of *Adhatoda vasica*(400mg/kg; p.o).[26,27]

2.5.3 Blood collection techniques used in the present study

At the end of the experiment, on the 15th day, animals were sacrificed under mild ether anaesthesia. The blood was collected via retro-orbital vein puncture with a fine capillary into an anticoagulant tube, allowed to stand for 30 minutes at 37°C, and then centrifuged to separate the serum to evaluate the biochemical markers.[28]

2.5.4 Preparation of kidney homogenate

The kidney was quickly removed and immediately perfused with ice-cold saline (0.9% NaCl). Using a homogenizer, a portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4). After centrifuging the homogenate for 10 minutes at 5000 rpm, the supernatant was collected and used for various biochemical assays.[29]

2.6 Analysis of general parameters

2.6.1 Estimation of urine volume

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume is measured after 24 hours. Food waste and feces are removed from the urine. And the volume of urine is measured with a measuring cylinder.[30]

2.6.2 Estimation of Body weight

At the end of the experiment, each group of animals was kept in their own cage. After removing the food and water, each animal was individually weighed and its weight was recorded.[31]

2.7 Analysis of serum biochemical parameters

2.7.1 Estimation of Serum Creatinine

Five test tubes were labelled A, B, C, D, and E. Where A&B are taken as standard, C&D are taken as test, and E is taken as blank. Pipette out 2 ml of distilled water into E (blank), 0.5 ml serum and 1.5 ml of water into C&D (test), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl) into A&B (standard), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl). In each of the five test tubes, 6 mL of picric acid and 0.4 mL of sodium hydroxide (NaOH) (2.5M) were added.[32, 33]

2.7.2 Estimation of Serum Blood urea nitrogen (BUN)

The blood urea level was determined using the Berthelot method (Fawcett and Scott, 1960) and a commercially available kit (Kamineni Life Sciences Pvt. Ltd., Hyderabad, India). To prepare the test, standard, and blank, 1000 l of working reagent-I containing urease reagent and a mixture of salicylate, hypochlorite, and nitroprusside was added to 10 l of serum, 10 l of standard urea (40 mg/dl), and 10 l of purified water. All of the test

tubes were thoroughly mixed and incubated for 5 minutes at 37 °C. The test tubes were then filled with 1000 l of reagent-II containing alkaline buffer and incubated at 37 °C for 5 minutes. Urease is a protein that catalyzes the conversion of urea to ammonia and carbon dioxide. The ammonia produced reacts with a mixture of salicylate, hypochlorite, and nitroprusside to produce indo phenol, a bluegreen compound. The intensity of the color produced is proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm. The blood urea was calculated using the following formula:[32,35]

$$\text{Blood urea (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Std}} \times 40$$

3 RESULTS

3.1 Percentage Yield

In phytochemical extraction, the percentage yield is critical in determining the standard extraction efficiency for a specific plant, different sections of the same plant, or different solvents used. Table shows the yield of extracts obtained from Adhatoda vasica.

Table 1:Percentage Yield of crude extracts of *Adhatoda vasica*extract

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Adhatoda vasica*	Pet ether	300	1.21	0.40 %
2		Methanol	283.02	5.99	2.11 %

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Present
2.2	Legal's test	Present	Present
2.3	Killer-Killiani test	Present	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Present	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present
8.2	Libbermann-Burchard's test	Present	Present

3.3 *In vitro* Antioxidant Assays

The *In vitro* anti-oxidant activity of Adhatoda vasica extracts was assessed using DPPH radical scavenging activity in the current study. Tables summarize the findings.

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3.3.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 3: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.454	52.757
40	0.414	56.919
60	0.330	65.660
80	0.276	71.279
100	0.131	86.368
Control	0.961	
IC50		**19.36**

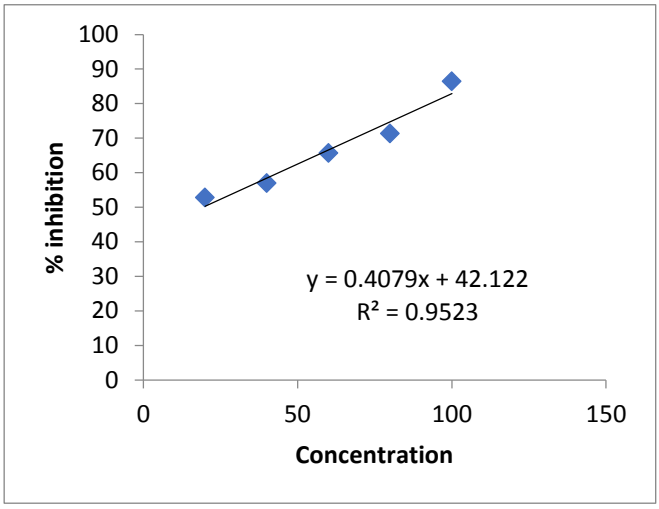


Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid

Table 4: DPPH radical scavenging activity of methanol extract of *Adhatoda vasica*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.520	42.919
40	0.484	46.871
60	0.457	49.835
80	0.426	53.238
100	0.363	60.153
Control	0.911	
IC50		**57.10**

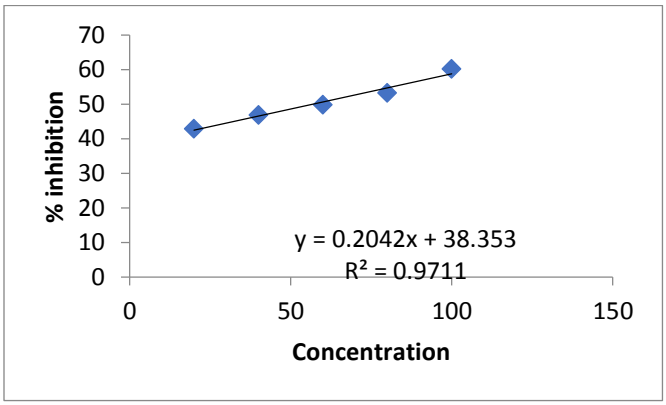


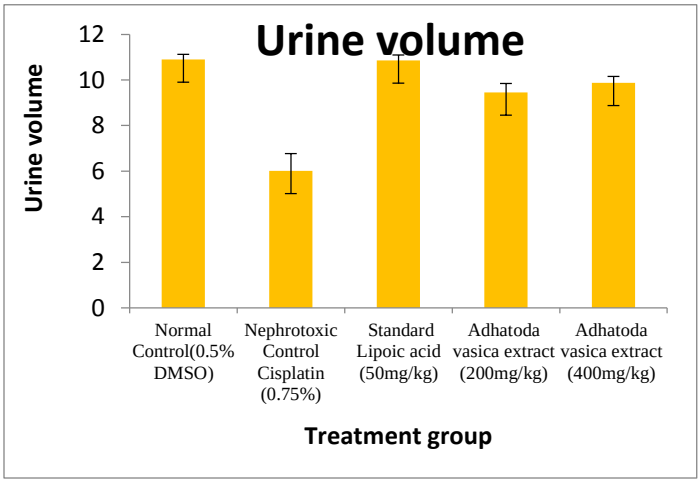
Figure 3: Represents the Percentage Inhibition Vs Concentration of extract of*Adhatoda vasica*

3.4 Analysis of general parameters

3.4.1 Estimation of urine volume

Table 5: Urine volume

Groups	Urine volume
Normal Control(0.5% DMSO)	10.90±0.223
Nephrotoxic Control Cisplatin (0.75%)	6.01±0.762
Standard Lipoic acid (50mg/kg)	10.86±0.24
*Adhatoda vasica*extract (200mg/kg)	9.45±0.40
*Adhatoda vasica*extract (400mg/kg)	9.87±0.28



Graph 1: Urine volume

3.4.2 Estimation of Body weight

Table 6: Body weight

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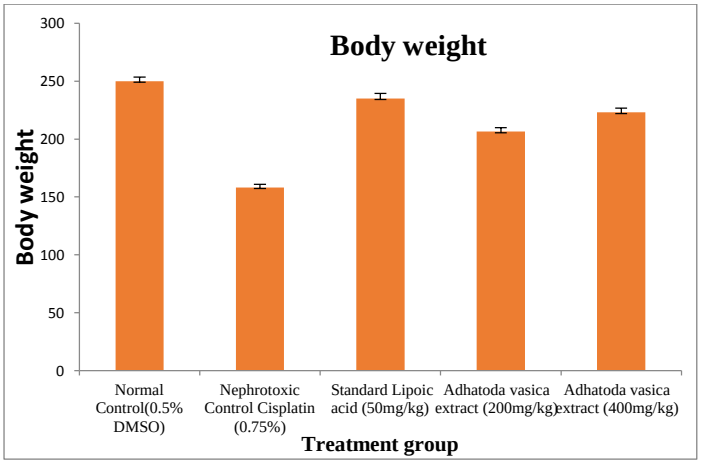
Groups	Body weight
Normal Control(0.5% DMSO)	250±3.406
Nephrotoxic Control Cisplatin (0.75%)	158.22±2.658
Standard Lipoic acid (50mg/kg)	235.12±4.355
*Adhatoda vasica*extract (200mg/kg)	206.54±3.43
*Adhatoda vasica*extract (400mg/kg)	223±3.742



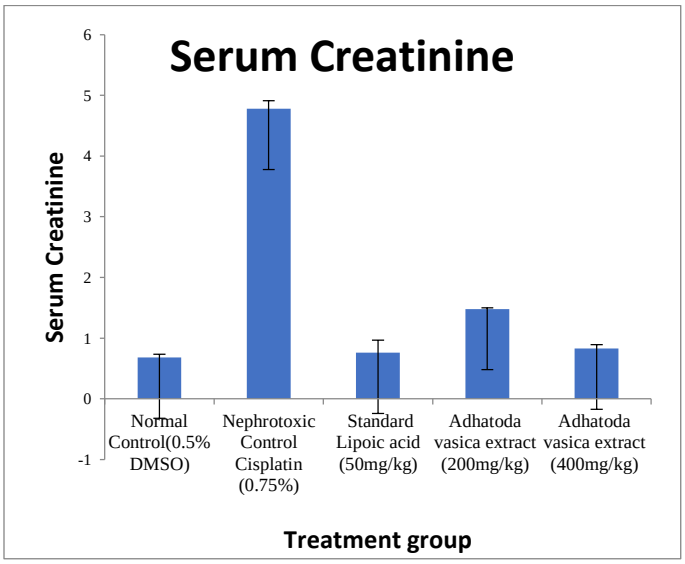
Graph 2: Body weight

3.5 Analysis of serum biochemical parameters

3.5.1 Estimation of Serum Creatinine

Table 7: Serum Creatinine

Groups	Serum Creatinine
Normal Control(0.5% DMSO)	0.68±0.055
Nephrotoxic Control Cisplatin (0.75%)	4.78±0.131
Standard Lipoic acid (50mg/kg)	0.76±0.206
*Adhatoda vasica*extract (200mg/kg)	1.48±0.020
*Adhatoda vasica*extract (400mg/kg)	0.83±0.062



Graph 3: Serum Creatinine

3.5.2 Estimation of Serum Blood urea nitrogen (BUN)

Table 8: Serum Blood urea nitrogen

Groups	Serum Blood urea nitrogen
Normal Control(0.5% DMSO)	24.66±0.505
Nephrotoxic Control Cisplatin (0.75%)	59.77±0.792
Standard Lipoic acid (50mg/kg)	25.15±0.50
*Adhatoda vasica*extract (200mg/kg)	31.02±0.94
*Adhatoda vasica*extract (400mg/kg)	25.55±0.55

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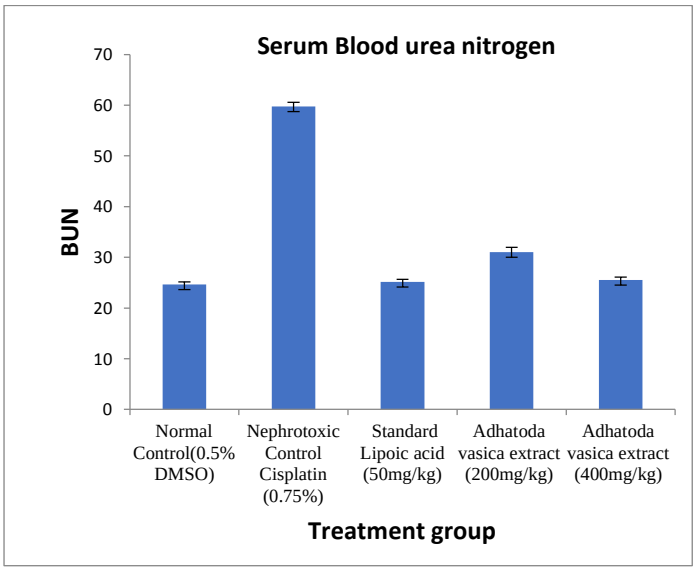
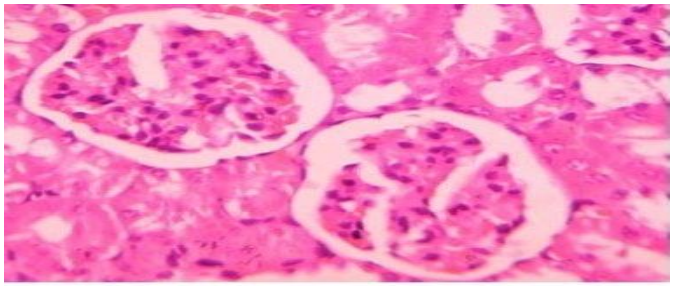


Graph 4: Serum Blood urea nitrogen

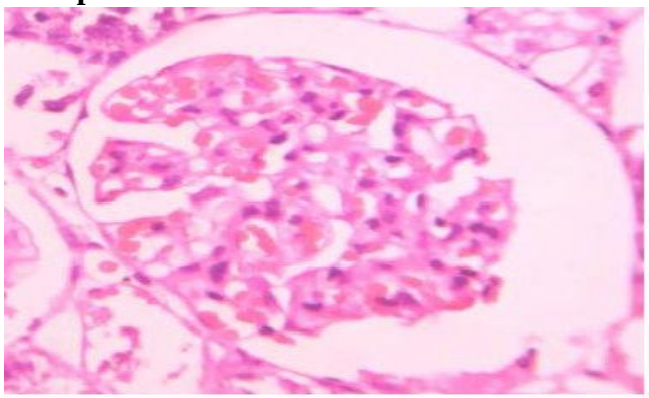
3.6 Histology

Group 1:



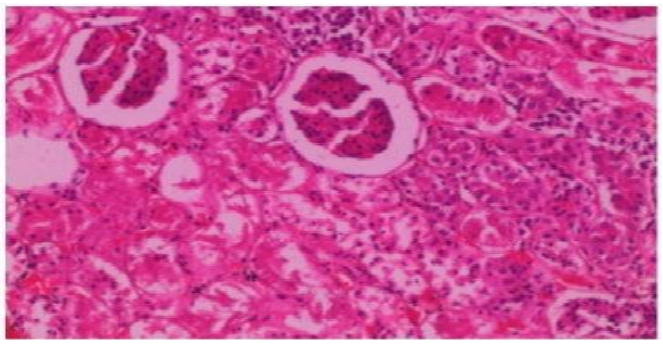
Normal Control

Group 2:



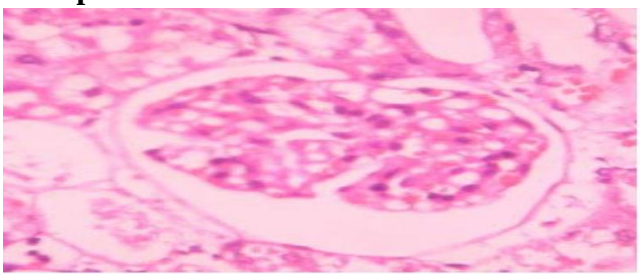
Nephrotoxic Control Cisplatin

Group 3:



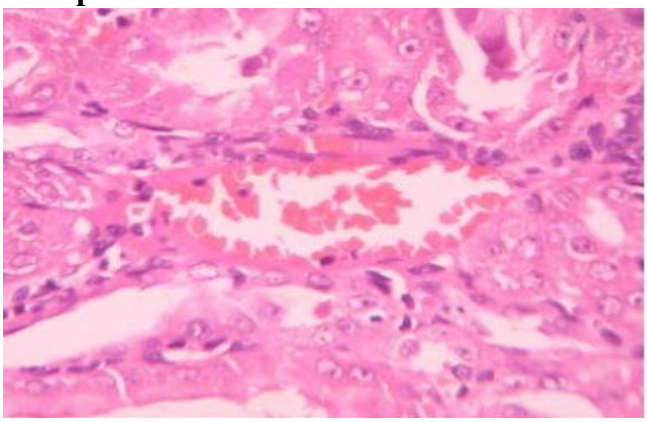
Standard Lipoic acid

Group 4:



***Adhatoda vasica*extract (200mg/kg)**

Group 5:



***Adhatoda vasica*extract (400mg/kg)**

4. DISCUSSION & CONCLUSION

Nephrotoxicity is a common clinical syndrome causing rapid decline in renal function, leading to abnormal retention of creatinine and blood urea. Chemical agents are limited in treating acute renal failure, and synthetic nephroprotective agents have adverse effects. Traditional medicine, including plants, has gained interest due to limited pharmacotherapy options. Ethno medicinal plants can help prevent dialysis.

The methanolic extract of Adhatoda vasica, containing carbohydrate, alkaloids, phenolic,

tannin, flavonoids, and glycoside, was found safe up to 2000 mg/kg in oral acute toxicity studies.

Adhatoda vasica extract inhibited DPPH radical scavenging activity by 60.15%, while ascorbic inhibited it by 86.36%. Cisplatin causes DNA damage, reactive oxygen species, and the activation of apoptosis and necrosis pathways, which can lead to nephrotoxicity.

In the current study, rats given a single dose of Cisplatin had a significant reduction in body weight compared to the control group, which was accompanied by an increase in serum creatinine levels, indicating the induction of acute renal failure.When compared to the nephrotoxic control group, 241 with Adhatoda vasica at dose levels of 200 and 400 mg/kg body weight for 15 days significantly reduced serum creatinine levels with a significant weight gain and increased urine output.

Cisplatin administration to control rats resulted in a typical pattern of nephrotoxicity, as evidenced by a significant increase in serum blood urea nitrogen (BUN).65 Adhatoda vasica supplementation to Cisplatin-treated rats resulted in a decrease in blood urea nitrogen (BUN) levels in plasma.

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