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EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF MORNIDO CITROFOLIA IN ALBINO RATS

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Abstract:
Nephroprotective agents are material that has potential to minimize the effects of nephrotoxic agents. Medicinal plants have curative properties due to the presence of various complex chemical substances. Morinda citrifolia L (Noni) also known as Indian mulberry is a common plant known to grow in the tropical countries. The present study was focused on the investigation of Nephroprotective activity of the ethanolic extract of fruit Juice of Morinda citrifolia on cisplatin induced nephrotoxicity in Wistar albino rats. The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols and anthroquinone in EEMC. 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 EEMC showed significantly elevate the attenuated body weight, urine volume, creatinine clearance and significantly reduce in elevated serum creatinine level, which supports its Nephroprotective activity.

Key words: *Nephroprotective activity, Mornido citrofolia, Albino rats, phytochemical screenin*

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INTRODUCTION:

Nephrotoxicity occurs when kidney-specific detoxification and excretion do not function optimally due to the damage or destruction of kidney function by exogenous or endogenous toxicants [1]. Drug-induced nephrotoxicity remains a major problem as use of nephrotoxic drugs is unavoidable in clinical setting. Paracetamol (PCM) is a commonly used drug, well-known for its analgesic and antipyretic properties [2]. Indeed, overdose of PCM in human is relatively common due to self-administration and is often associated with hepatic [3–5] and renal damage [6–9]. Even if nephrotoxicity is less common than hepatotoxicity in PCM overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury [10–12] and can be fatal in humans and experimental animals [13–15].To date, numbers of studies have been published to prove nephroprotective effect of medicinal plants by using PCM-induced nephrotoxicity in rats. Various PCM dose and modes of administration were used in these studies. In majority of the studies a single dose of PCM in the range of 400 mg/kg to 2000 mg/kg was administrated by oral or intraperitoneal (IP) route to induce toxicity [16–19]. Nephrotoxicity can also be induced by administering lower but repeated dose as suggested by some published work [20, 21]. A repeated daily dose of 200 mg/kg of PCM for 14 days by IP route was selected to induce nephrotoxicity in the present study as the selected dose was proven to cause nephrotoxicity [21].

Nephroprotective agents are material that has potential to minimize the effects of nephrotoxic agents. Medicinal plants have curative properties due to the presence of various complex chemical substances [22]. Ethnomedicinal plants from the traditional system of medicine viz Ayurveda and Unani, which are acclaimed by the Ayurvedic and Unani physicians to have nephroprotective properties and commonly used to treat various renal disorders, have been extensively investigated for their significant nephroprotective effects [23–25].

Morinda citrifolia L (Noni) also known as Indian mulberry is a common plant known to grow in the tropical countries. The juice obtained from the Noni Fruit (NFJ) has been used in folk medicine since historic days for health promoting effects [26]. NFJ has been claimed to have antibacterial, antiviral, antifungal, antitumour, anthelmintic, analgesic, anti-inflammatory, antioxidant, hypotensive, immune enhancing and hepatoprotective effects [27]. The juice of the noni fruit has been consumed for its widely advertised medicinal qualities which include therapeutic benefits in arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, Acquired Immune Deficiency Syndrome, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, cancer and drug addiction [28]. The antioxidant, analgesic and anti-inflammatory properties [29], anti-fatigue and endurance promoting potentials [30], anti-tumour and immune enhancing activities of NFJ have been demonstrated in various animal experiments.

The present study was focused on the investigation of Nephroprotective activity of the ethanolic extract of fruit Juice of Morinda citrifolia on cisplatin induced nephrotoxicity in Wistar albino rats. The objectives of the present study were, Evaluation of Nephroprotective effect of EEMC.

MATERIALS AND METHODS:

Selection of the plant:

The medicinal plant Morinda citrifolia (Family: Rubiaceae) was selected for Nephroprotective activity based on the literature survey.

Collection and authentication of the plant:

Collection and Authentication of Noni Fruit Morinda citrifolia elite fruits were handpicked was collected in the months of May-June from plants maintain. These fruits authenticated from the expert Botanist

Processing of Plant Materials:

All fruits were cleaned and rinse with tap water, while rinsing the fruits found to float floats on water surface. Cleaned fruits chopped into thin slices of 2-5 mm in diameter (Figure 1) were air dried at room temperature (Figure 2). Dried fruits pulverized with help of commercial flouring mill and using electrical grinder. Thus, fruit powder obtain was sieved through different size mesh to obtain coarser and fine fruit powders. About 12.816 kg fruits in lots of 5 kg weighed at each time, individual fruit weighs also taken to determine moisture content in fruit. Taking difference in weight of individual fruit before and after drying, the moisture content was determined by subtracting the final weight of dried fruit from its initial weight.

Preparation of Extracts:

The fruit powder (coarser and fine) was subjected to aque ous and hydroethanolic extraction. It was dissolved in dis tilled in pure water and in hydroethanolic solvent (50% v/v) respectively. The

extracts were prepared by maceration technique using material to solvent ratio 1:20 and 1:10 in water and ethanol respectively. Solvent mixtures macerate for 48 hr sat room temperature. To obtain hydroethanolic extract, 200 gm of each fruit powder (coarser and fine) was mixed in 2.0 L ethanolic solvent (1:10), meanwhile intermittently shaken mixture manually and with help of mechanical shaker. After complete maceration, macerates was strain through muslin cloth then filtered through What man No. 42 filter papers. The filtrates transfer to Petri plates (Figure 3) and air-dried at room temperature, then in hot air oven (43 0 C). The weights of extract determine taking difference in weight of Petri plate before and after drying extractive solvent (macerates). The final weight of extracts obtained by subtracting initial weight of Petri plates (Bank) from its final weight (with extract) at each trail and calculated extract yield (extractability). While handling the macerates and extracts precaution was taken for possibilities of contamination and loss of any extract during processing.

Petri plates and placed in airtight desiccators and kept in refrigerator. Similarly, the aqueous extracts were obtained using 50 gm powder material at each trial was macerate in 200 ml distilled water. Total 2.5 kg of fruit powder were used in ethanol extraction and 600 gm powder for aqueous extraction. The extraction yield, color, odor, taste, consistency, fluorescence, pH, optical density was determine for all extractsaccording to techniques.

Preliminary phytochemical screening:

The Ethanolic extract of the fruit pulp of Morinda citrifolia was subjected to a preliminary phytochemical screening to identify the active chemical constituents.

The Ethanolic extract of the fruit pulp of Morinda citrifolia was taken and dissolved in distilled water.

Pharmacological Evaluation:

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 reasons or 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. of ethanolic extracts of Morinda citrifolia .

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 albinos wistar rats of either sex, each group consisting of six animals.

Group-I : Served as normal control received 0.5 % DMSO (Dimethylsulphoxide) ; 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 Morinda citrifolia (200mg/kg; p.o) dissolved in DMSO for 15 days.
Group-V : Received ethanolic extract of Morinda citrifolia (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 Morinda citrifolia (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:
Preliminary phytochemical studies

Table no-5: Results of the Preliminary Phytochemical Constituents present inethanolic extract of Morinda centifolia.

Table-1: Results of preliminary phytochemical analysis of EEMC

Phyto-constituents	Morinda citrifolia
Tannins	Present
Flavonoids	Present
Anthroquinone	Absent
Saponins	Present
Reducing compounds	Present
Tri Terpenoids	Absent
Phytosterols	Absent

RESULT:

Ethanolic extract of the whole plants of Morinda centifolia (EEMC) was subjected to various phytochemical tests, which showed the presence of reducing sugars, tannins, flavonoids, Saponins, Anthroquinone.

Assessment of general biochemical parameters

Assessment of urine volume

The effects of the different doses of ethanolic extract of Morinda citrifolia on urine volume.

Table-2: Results of the effect of EEMC 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%) +EEMC (200mg/kg)	9.17±0.40*
V	Cisplatin (0.75%) +EEMC (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

Diagrammatic representation of EEMC on urine volume in cisplatin inducedNephrotoxic rats

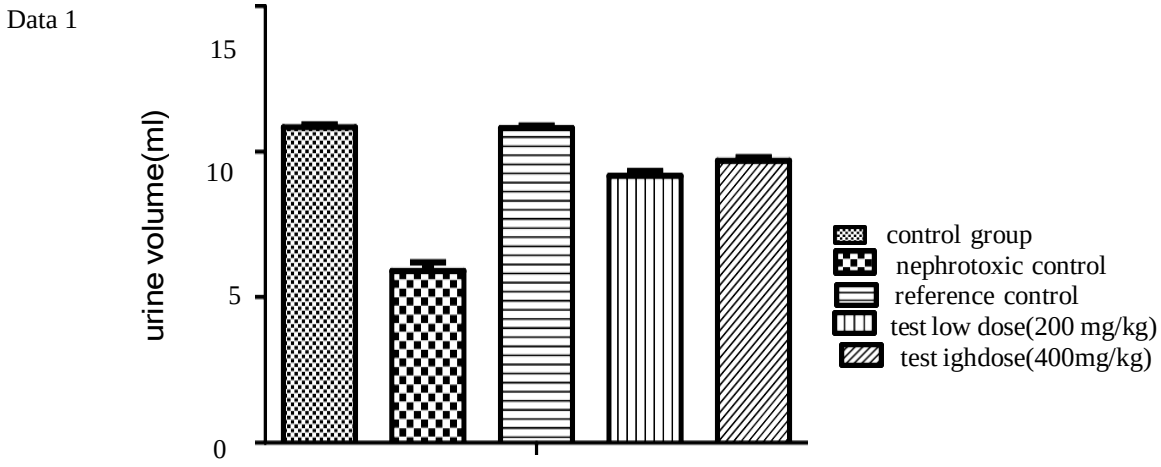


Fig. No: 1

Results:

The urine volume were measured were showed in Table no-7 and Fig. no-8.

The Nephrotoxic control (Group 2) showed significant decrease in urinevolume when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in urine volumewhen compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant increase inurine volume when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant increase inurine volume when compared to the Nephrotoxic control (Group 2).

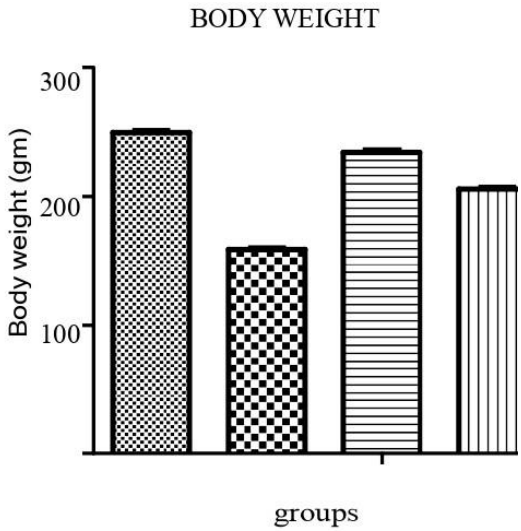
Assessment of Body weight:
The effects of the different doses of ethanolic extract of Morinda citrifolia on body weight.

Table-3: Results of the effect of EEMC 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%) + EEMC (200mg/kg)	205.83±3.43*
V	Cisplatin (0.75%) + EEMC (400mg/kg)	222±3.742***

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

Diagrammatic representation of EEMC on serum creatinine level on cisplatininduced Nephrotoxicity in rats



RESULTS:

The body weight were measured were showed in Table no-8 and Fig. no-9.

The Nephrotoxic control (Group 2) showed significant decrease in bodyweight when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in body weightwhen compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant increase inbody weight when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant increase inbody weight when compared to the Nephrotoxic control (Group 2).Assessment of serum biochemical parameters

Serum creatinine level

The effects of the different doses of ethanolic extract of Morinda citrifolia on serum creatinine level.

TABLE-4: Results of the effect of the EEMC on serum Creatinine on Cisplatin induced Nephrotoxicity 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%) + EEMC (200mg/kg)	1.47± 0.206*
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on serum creatinine level on cisplatininduced Nephrotoxicity in rats

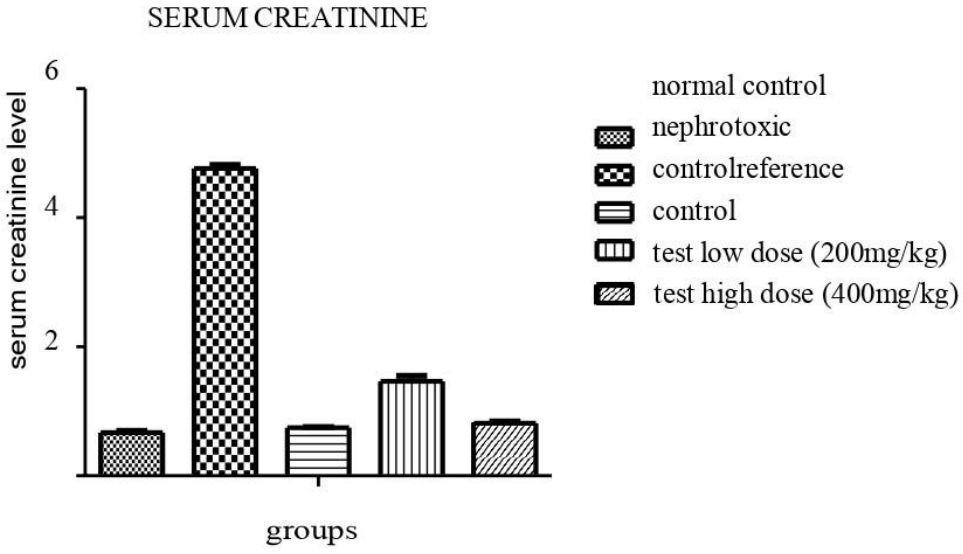


Fig. no-3

RESULTS:

The serum creatinine was measured were showed in Table no-9 and Fig. no- 10.

The Nephrotoxic control (Group 2) showed significant increase in serumcreatinine level when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in serumcreatinine level when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant decrease inserum creatinine level when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant decrease inserum creatinine level when compared to the Nephrotoxic control (Group 2).

7.3.2 Serum Blood urea nitrogen (BUN)

The effects of the different doses of ethanolic extract of Morinda citrifolia on serum Blood urea nitrogen (BUN) level.

Table-5: Results of the effect of the EEMC on serum Blood urea nitrogen on Cisplatin induced 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%) + EEMC (200mg/kg)	30.02±0.94*
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on serum Blood urea nitrogenparameters on cisplatin induced Nephrotoxicity in rats

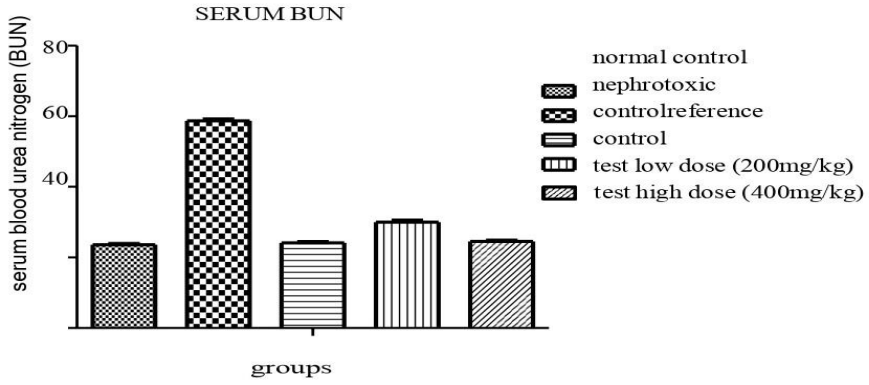


Fig. No: 4

RESULTS:

The serum Blood urea nitrogen (BUN) level were measured were showed in Table no-10 and Fig. no-11.

The Nephrotoxic control (Group 2) showed significant increase in serum Blood urea nitrogen (BUN) level when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).

Assessment of urine biochemical parameters:

Assessment of creatinine clearence

The effects of the different doses of ethanolic extract of Morinda citrifolia on creatinine clearance.

Table-6:Results of the effect of EEMC on creatinine clearence in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Creatinine clearance
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%) + EEMC (200mg/kg)	14.74± 0.746*
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on creatinine clearance in cisplatininduced Nephrotoxic rats

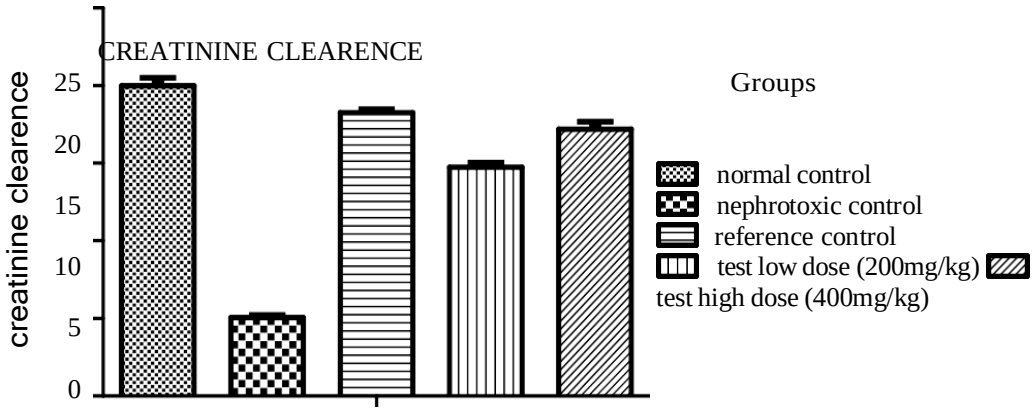


Fig. no- 5

RESULTS:

The creatinine clearence were measured were showed in Table no-11 and Fig. no-12.

The Nephrotoxic control (Group 2) showed significant decrease in creatinineclearence when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in creatinine clearence when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant increase in creatinine clearence when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant increase increatinine clearence when compared to the Nephrotoxic control (Group 2).

Assessment of oxidative stress parameter

Assessment of Malondialdehyde (MDA)

The effects of the different doses of ethanolic extract of Morinda citrifolia on malondialdehyde (MDA).

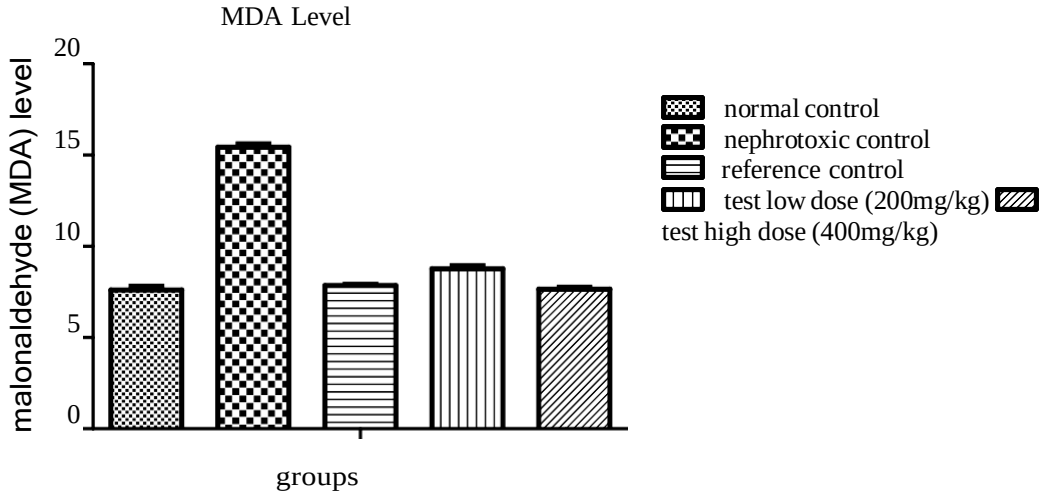
Table-7: Results of the effect of EEMC on Malondialdehyde (MDA) in cisplatin induced Nephrotoxic rats

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%) + EEMC (200mg/kg)	8.77±0.427**
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on Malondialdehyde (MDA) in cisplatininduced Nephrotoxic rats



RESULTS:

The malondialdehyde (MDA) were measured were showed in Table no-12 and Fig. no-13.

The Nephrotoxic control (Group 2) showed significant increase in malondialdehyde (MDA) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in malondialdehyde (MDA) when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant decrease inmalondialdehyde (MDA) when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant decrease inmalondialdehyde (MDA) when compared to the Nephrotoxic control (Group 2).

Assessment of enzymatic antioxidant parameters

Assessment of superoxide dismutase (SOD)

The effects of the different doses of ethanolic extract of Morinda citrifolia on superoxide dismutase (SOD).

Table-8: Results of the effect of EEMC 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%) + EEMC (200mg/kg)	11.89±0.303*
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on superoxide dismutase (SOD) incisplatin induced Nephrotoxic rats

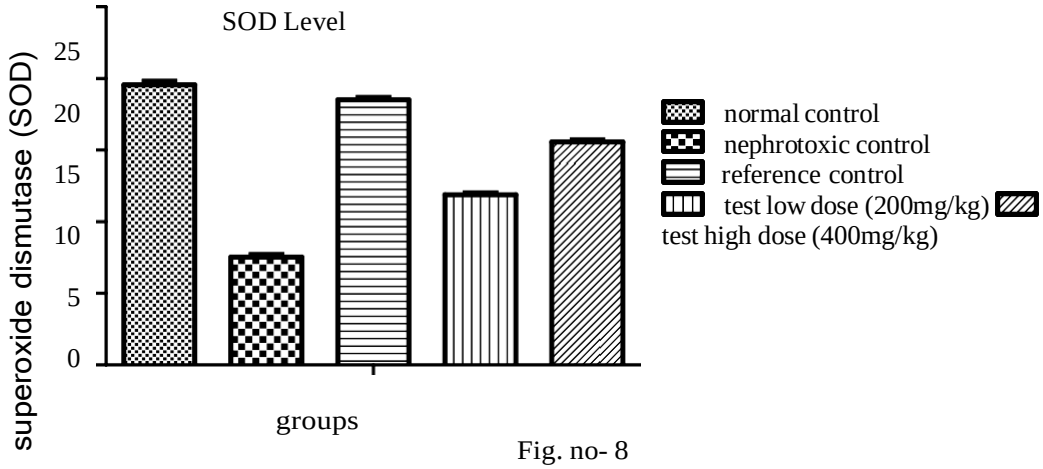


Fig. no- 8

RESULTS:

The superoxide dismutase (SOD) were measured were showed in Table no-13 and Fig. no-14.

The Nephrotoxic control (Group 2) showed significant decrease in superoxidedismutase (SOD) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in superoxidedismutase (SOD) when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant increase insuperoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant increase insuperoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).

Assessment of Catalase (CAT)

The effects of the different doses of ethanolic extract of Morinda citrifolia on Catalase (CAT).

Table-9: Results of the effect of EEMC on Catalase (CAT) in cisplatin inducedNephrotoxic rats

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

Values were given in Mean ±SEM;

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

Diagrammatic representation of EEMC on Catalase (CAT) in cisplatin inducedNephrotoxic rats

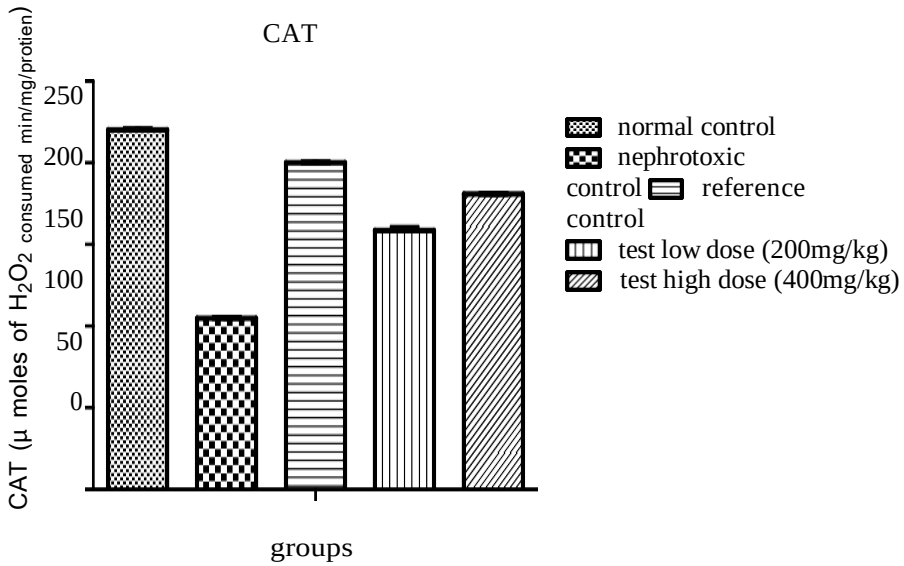


Fig. no- 9

RESULTS:

The Catalase (CAT) were measured were showed in Table no-14 and Fig. no-15.

The Nephrotoxic control (Group 2) showed significant decrease in Catalase(CAT) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Catalase (CAT)when compared to Nephrotoxic control (Group 2).

EEMC 200 mg/kg treated (Group 4) showed statistically significant increase inCatalase (CAT) when compared to the Nephrotoxic control (Group 2).

EEMC 400mg/kg treated (Group 5) showed statistically significant increase inCatalase (CAT) when compared to the Nephrotoxic control (Group 2).

Assessment of Glutathione peroxidise (GPx)
The effects of the different doses of ethanolic extract of Morinda citrifolia on Glutathione peroxidise (GPx).

Table-10: Results of the effect of EEMC 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%) + EEMC (200mg/kg)	16.33±0.399**
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on Glutathione peroxidise (GPx) incisplatin induced Nephrotoxic rats

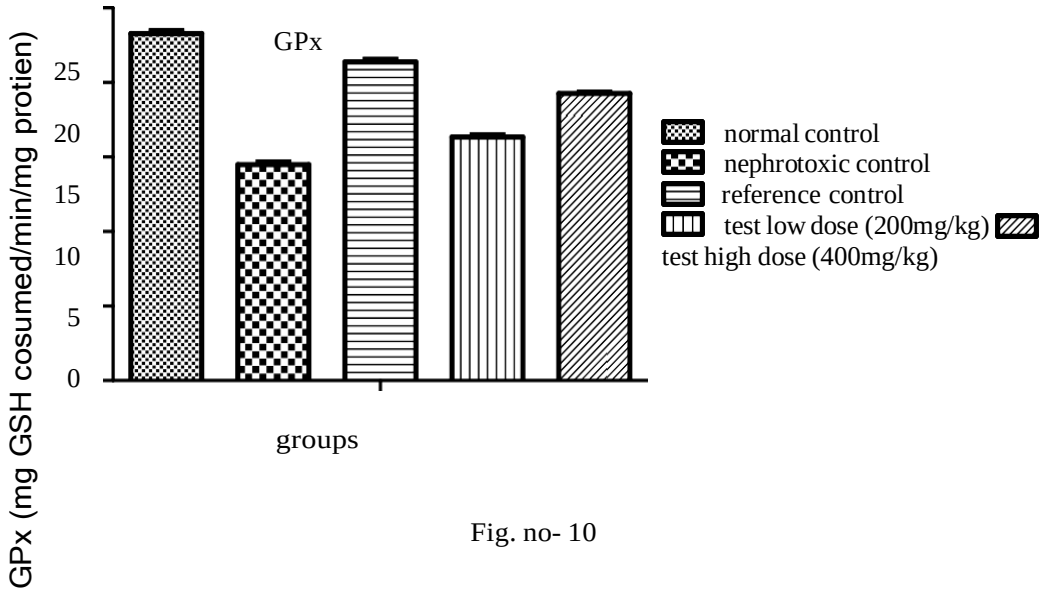


Fig. no- 10

RESULTS:
The Glutathione peroxidise (GPx) were measured were showed in Table no-15 and Fig. no-16.
The Nephrotoxic control (Group 2) showed significant decrease in Glutathioneperoxidise (GPx) when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in Glutathioneperoxidise (GPx) when compared to Nephrotoxic control (Group 2).
EEMC 200 mg/kg treated (Group 4) showed statistically significant increase inGlutathione peroxidise (GPx) when compared to the Nephrotoxic control (Group 2).
EEMC 400mg/kg treated (Group 5) showed statistically significant increase inGlutathione peroxidise (GPx) when compared to the Nephrotoxic control (Group 2).

Assessment of non-enzymatic antioxidant parameter
Assessment of Reduced glutathione (GSH)
The effects of the different doses of ethanolic extract of Morinda citrifolia on Reduced glutathione (GSH).

Table-11: Results of the effect of EEMC 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%) + EEMC (200mg/kg)	14.37±0.280**
V	Cisplatin (0.75%) + EEMC (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

Diagrammatic representation of EEMC on Reduced glutathione (GSH) incisplatin induced Nephrotoxic rats

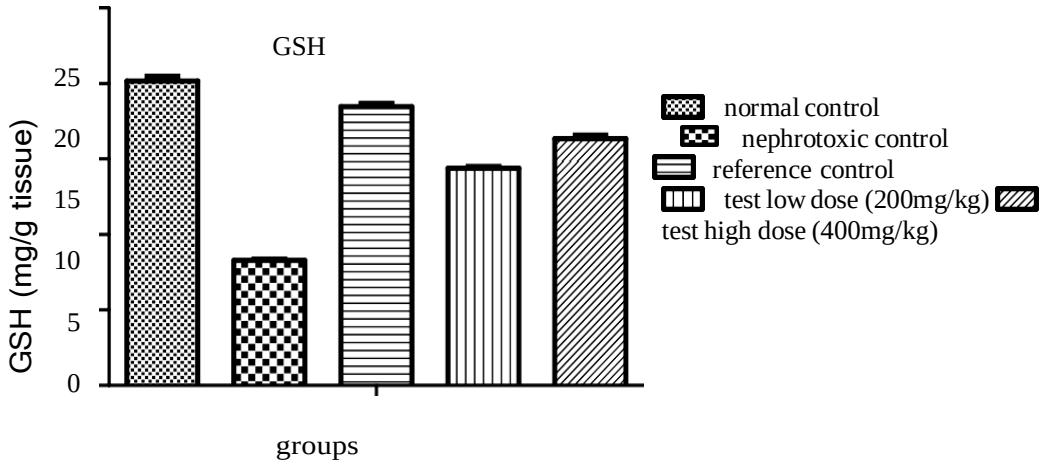


Fig. no- 11

RESULTS:
The Reduced glutathione (GSH) were measured were showed in Table no-16 and Fig. no-17.
The Nephrotoxic control (Group 2) showed significant decrease in Reducedglutathione (GSH) when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in Reduced glutathione (GSH) when compared to Nephrotoxic control (Group 2).
EEMC 200 mg/kg treated (Group 4) showed statistically significant increase in Reduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2).
EEMC 400mg/kg treated (Group 5) showed statistically significant increase in Reduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2).

HISTOPATHOLOGICAL STUDIES:

a) Normal group

Section of the kidney of normal control rat showed,

- Arrangement of nephrotic bundles appears normal, both cortex and medullaappear 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 EEMC showed

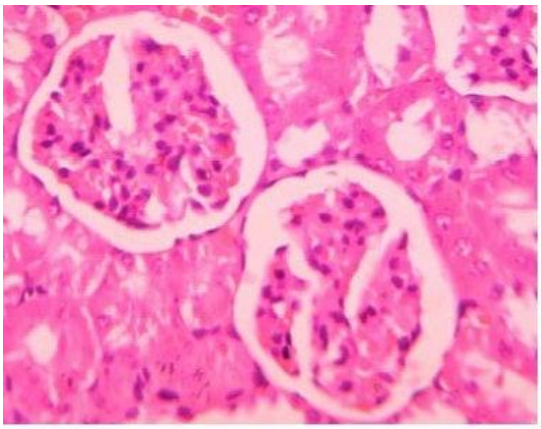
- Moderate tubular degeneration with mild edema and
- Necrotic changes with swollen tubular epithelium.

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

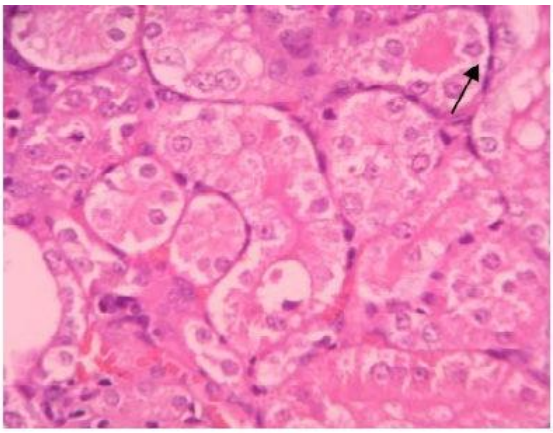
- Moderated signs of regeneration with occurrence of chromatolysis wasobserved in the tubular structure

HISTOPATHOLOGICAL STUDIES

Group I

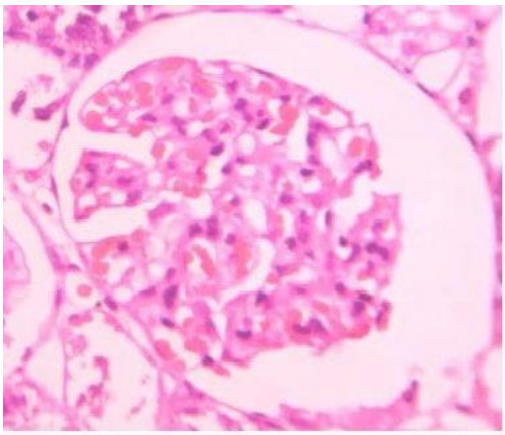


Control- 1

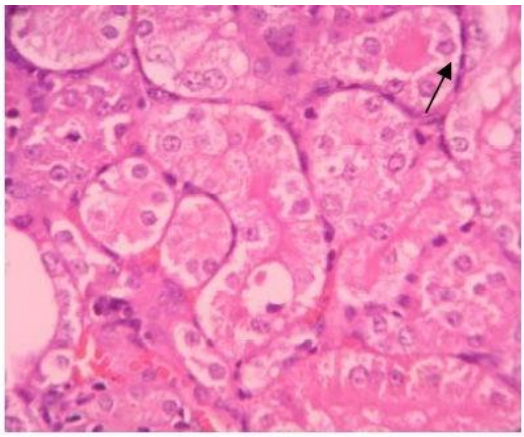


Control -2

Group II

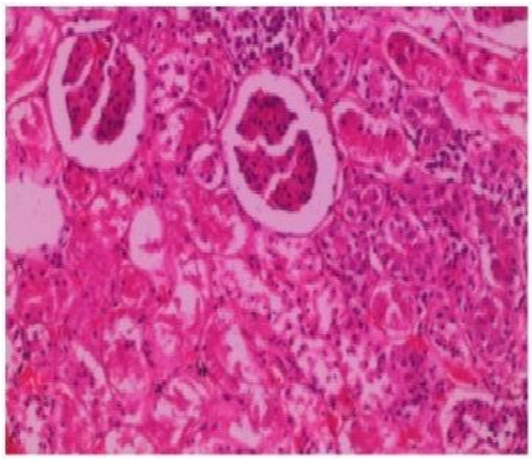


Cisplatin induced 1

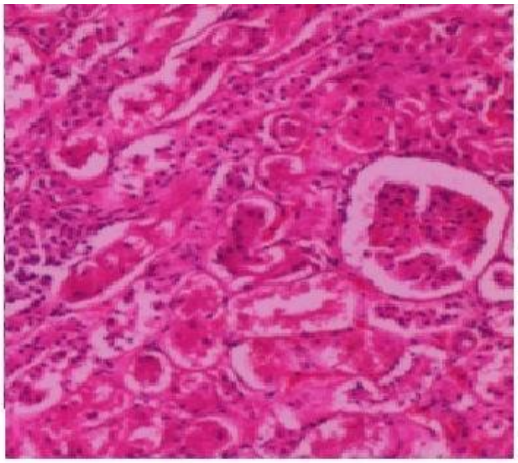


Cisplatin induced 2

Group III

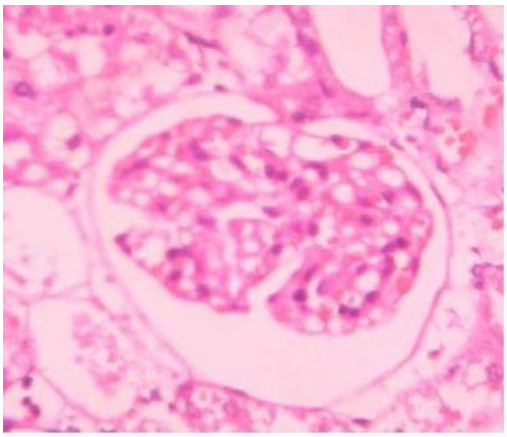


Standard (lipoic acid) + Cisplatin 1

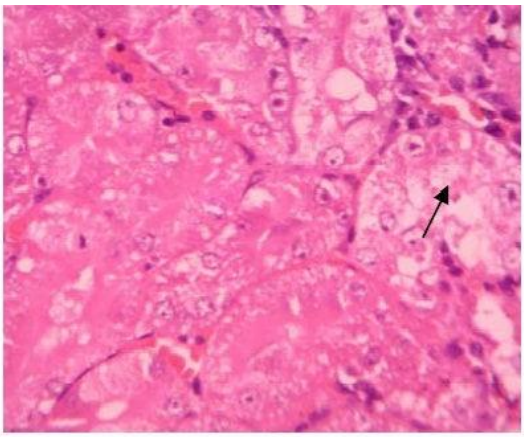


Standard (lipoic acid) + Cisplatin 2

Group IV

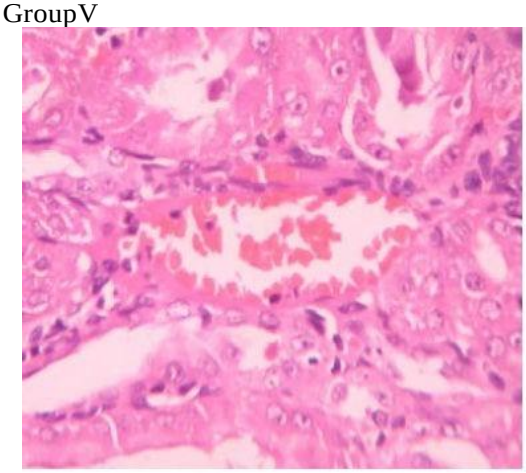


Plant extract 200mg + Cisplatin - 1 (Kidney)

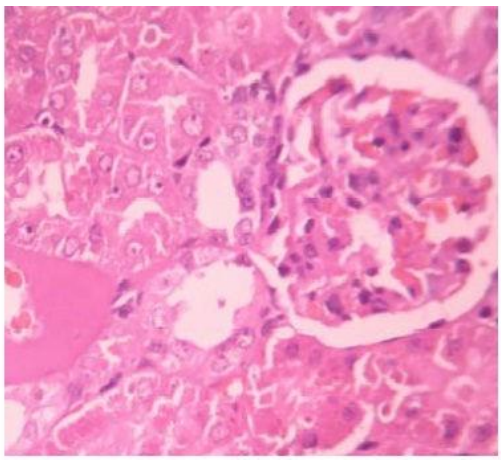


Plant extract 200mg + Cisplatin - 2 (Kidney)

GroupV



Plant extract (400mg) + Cisplatin (Kidney) 1



Plant extract (400mg) + Cisplatin (Kidney) - 2

Fig. no- 12: Photomicrographs of kidney tissue section Group I- Normal control, Group II- Nephrotoxic Control, Group III- Reference Control,Group IV- EEMC (200mg/kg), Group V- EEMC (400mg/kg)

SUMMARY AND CONCLUSION:

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

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

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 EEMC 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) which was significantly decreased with treatment of EEMC, 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 EEMC, which showed its antioxidant activity due to the Flavonoids which is present in the extract.

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

In summary, the fruit pulp of Morinda citrifolia 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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