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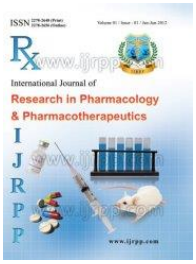

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Nephroprotective activity of *tamarindus indica* linn fruit extract on cisplatin induced nephrotoxicity in rats

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ABSTRACT

Nephrotoxicity is one of the important side effects of Cisplatin. The aim of the study was to determine the protective effect of *Tamarindus indica* Linn fruit pulp extract on Cisplatin induced nephrotoxicity in rats using biochemical approaches. The experiment was done in Cisplatin-induced acute renal failure in Albino wistar rats. Thirty rats were divided into five groups. Group 1 was given Dimethyl sulphoxide (DMSO) (5 ml/kg) per oral, group 2 with single dose of Cisplatin (7.5 mg/kg) by intra peritoneal injection into the peritoneal cavity, group 3 with Cisplatin and standard nephroprotective drug Lipoic acid (50 mg/kg) orally, group 4, with Cisplatin and ethanolic extract of *Tamarindus indica* Linn fruit pulp extract (EETI) 200 mg/kg and group 5, Cisplatin and EETI 400 mg/kg. Extract and standard drug Lipoic acid were given orally 120 min prior to Cisplatin injection. 24 h urine output, Animal body weight, serum creatinine, serum blood urea nitrogen and Creatinine clearence were measured for all the groups. Kidneys were examined for histopathological changes. The antioxidant activity of the extract was tested in vivo. Rats treated with EETI showed significant improvement in biochemical parameters and histopathological changes compared to Cisplatin treated group. The protective effect was highly significant at 400 mg/kg. The in vivo assays showed significant antioxidant activity. The EETI has nephroprotective effect in Cisplatin-induced acute renal failure.

Keywords: Nephrotoxicity, *Tamarindus indica* Linn, Fruit extract, Cisplatin

INTRODUCTION

The kidney is an excretory organ. It is located on the posterior abdominal wall, one on each side of the lumbar part of the vertebral column. The main function of the kidney is excretion of waste products like urea, uric acid, creatinine, etc, it regulates the blood contents of NaCl and other electrolytes, as well as the volume of extracellular fluid to maintain homeostasis and regulate blood pressure, and it also plays a crucial role in maintaining acid-base balance [1]. Nephrotoxicity is toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on renal function. There are varies forms, and some drugs may affect renal

function in more than one way. Kidney failure is the one of the most common diseases in India. The world health organisation recognizes four major groups of renal failure according to the predominant involvement of corresponding morphologic component. They are Glomouruler diseases, Tubular diseases, Interstitial diseases and Vascular diseases. Acute renal failure is a syndrome charecterised by rapid onset of renal dysfunction, chiefly oliguria or anuria, and sudden increase in metabolic waste product in the blood [2]. Cisplatin is a widely used and highly effective cancer chemotherapeutic agent. Which is used to treat various type of cancers. One of the limiting side effects of Cisplatin use is nephrotoxicity. Research over the past 10 years has uncovered many of the cellular mechanisms which underlie Cisplatin-induced renal cell death (Apoptosis and Necrosis). It has also become apparent that inflammation provoked by injury to renal epithelial cells serves to amplify kidney injury and dysfunction *in vivo* [3]. *Tamarindus indica* Linn (Fabaceae/ Caesalpinioideae) is a tree grown in villages and towns in Africa, America, Mexico, Asia and Arabian countries. Ethnobotanically, riped and un-riped fruits pulp mixed with milk, honey or lemon juice were used as laxative and constipation reliever. The leaves and bark were used as wound healer. Fresh fruits are used as antipyretic. Fruit pulp and leaves are used as anti-malarial agent. Flowers, leaves, bark and fruit pulps were used as aphrodisiac. Fresh bark or stem used as abdominal pain reliever. Fruit pulp with lemon or milk and leaf juice were used as anti-diarrheal and anti-dysentry agent. Bark and leaves are used as anti-asthmatic and anti-tussive. Leaves or fruits are used as anti-measles and against mumps. Leaf and bark decoctions were used as hepato-protective. Leaves are used as anti-diabetic. Fruits are used as antibacterial. Bark is used as anthelminitic. Fruits were used as preservative [4]. *Tamarindus indica* Linn possesses hepatoprotective activity, [5] invivo antioxidant activity, Antidiabetic and hepatoprotective activities, [6] Acute toxicity and antifungal studies, [7] Antimicrobial Activity, [8] Anti-inflammatory activity, [9] Analgesic activity, [10] anticancer activity, [11] anti-asthmatic and cough, [12] and wound healing capacity. [13] Literature showed that there are no works carried out to explore the protective effect of *Tamarindus indica* fruit pulp. We therefore investigated the Nephroprotective activity of the ethanolic extract of *Tamarindus indica* Linn against Cisplatin induced Nephrotoxicity in rats to ascertain its medical potentials. Preliminary phytochemicals study of *Tamarindus indica* have revealed presence of phenolic compounds, cardiac glycosides, mallic acid, tartaric acid,uronic acid, mucilage, pectin, arabinose, xylose, galactose and glucose [14]. Tamarind plant shows the presence of various essential elements like arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead and zinc. The ethanolic extract of *Tamarindus indica* showed the presence of fatty acid, out of which 21 are saturated fatty acids such as n-heptadeconate, hexadeconic acid, n-nonadecanoate, etc. along with 11 unsaturated fatty acids such as nenodecenoic acid, 10-octadecenoic acid, heptadeconoate, etc. Fruit pulp contains organic acids such as tartaric acid, acetic acid, citric acid, formic acid, mallic acid and succinic acid. And also the pulp shows the presence of high amounts of ascorbic acid, vitamin B_1, B_3, amino acids such as alanine, phenylalanine, proline, serine, leucine; volatile oils; pectin; proteins and fat. Pulp also contains alkaloids, glycosides, saponins, sesquiterpenes, flavonoids, tannins and phlobatannins.Whole plant: alkaloids e azimine, azacarpaine and carpaine. The objectives were to evaluate the nephroprotective activity of ethanolic extract of *Tamarindus indica* Linn fruit pulp in cisplatin-induced acute renal failure in albino wistar rats.

MATERIALS AND METHODS

The study was initiated after getting IAEC (Institutional Animal Ethics Committee) approval.

Preparation of *Tamarindus indica* fruit pulp extracts (EETI)

The fruit pulp of *Tamarindus indica* Linn was collected from Valanchery, Kerala. The plant *Tamarindus indica* Linn belonging to the family of *Fabaceae* was identified and authenticated by Prof. Dr. A. Balasubramanian, Ph.D., Director of A.B.S botanical conservation, Research and Training centre, kaaripatti, Salem district, Tamilnadu-636 106. The fruit pulps were shade dried for one month. The dried fruit pulps were further chopped into small pieces.

The 100 gm of small pieces of fruit pulps were macerated in 500 ml of 95% ethanol for 24 hours. The process of extraction was done by reflux condensation method using soxhlet apparatus at 60-80 ºC for 9 hours. The extract was concentrated by distillation apparatus till a syrupy consistency was obtained. Finally, the extract was put in a china dish and evaporated at 40-60 ºC temperature in a water bath until use for the proposed experiment.

Animals

Thirty healthy (both male and female) albino wistar rats were used for these experiments were obtained from King's institute, Guindy, Chennai, Tamilnadu, India. The animals were housed in standard cages and were maintained on a standard pelleted Feed and water ad libitum. Permission and approval for animal studies were obtained from Institutional animal ethical committee. (IAEC approval No: IAEC/XLVIII/03/CLBMCP/2016 dated on 04/05/2016).

Acute toxicity study

An acute oral toxicity study was carried out according to OECD-423 guidelines. 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. The ethanolic extract of fruit pulps of *Tamarindus indica* Linn 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.

No adverse effect was reported or mortality in albino wister rats up to 2000mg/kg p.o. of ethanolic extracts of *Tamarindus indica* Linn. Therefore, the maximum tolerated dose 200mg/kg & 400mg/kg was chosen for further studies.

In vivo evaluation

Induction of ARF: ARF was induced by intra peritonial injection of single dose of Cisplatin (7.5mg/kg) as per the standard methods prescribed.

Experimental design [15]

A total of 30 rats of both sexes were weighed and divided into five groups of 6 animals and treated as follows:

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

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

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

Group IV: Received ethanolic extract of Tamarindus indica Linn (200mg/kg; p.o) dissolved in DMSO for 15 days.

Group V: Received ethanolic extract of Tamarindus indica Linn (400mg/kg; p.o) dissolved in DMSO for 15 days.

On the 10^{th} day 2 hours after the administration of standard Nephroprotective drug (Lipoic acid) and *Tamarindus indica* fruit pulp extract (200 & 400 mg/kg) to the II-V groups received cisplatin (7.5mg/kg; i.p). At the end of the treatment period, the animals were weighed again and they 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 30 min at 37°C and then centrifuged to separate the serum to evaluate the biochemical markers. 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. A bit of tissue from each kidney was cut and fixed in bouin's fluid immediately after removal from the animal body for histological processes.

Estimation of General parameters

The general parameters such as urine output and animal body weight were estimated using the standard methods.

Estimation of Nephroprotective activity

The serum and urine samples were used for estimation of nephroprotective activity. The serum creatinine level [16] and serum blood urea nitrogen level [17] were determined by using serum samples and the creatinine clearance [18] was estimated in urine sample using standard methods prescribed.

Estimation of antioxidant activity

The homogenized kidney was used for estimation of antioxidants. The antioxidants, Malondialdehyde (MDA), [19] superoxide dismutase (SOD), [20] reduced glutathione (GSH), [21] glutathione peroxidase (GPx), [22] and catalase (CAT) [23] were determined using standard methods prescribed.

Histopathology

A bit of tissue from each kidney was cut and fixed in bouin's fluid immediately after removal from the animal body. The tissues were fixed in bouin's fluid for about 24 hours. The tissues were then taken and washed in glass distilled water for a day to remove excess of picric acid. The sections were stained with hematoxylin and eosin (H & E) stain, which were used to demonstrate different structures of the tissue.

Statistical analysis

Results were expressed as Mean ± SEM. The data obtained 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

General parameters

The body weight and urine output were analysed. The decreased levels of Body weight and urine volume in Cisplatin treated animals (Group 2) were seen compared with Dimethyl sulphoxide (DMSO) treated animals (Group 1) confirming renal failure. In Ethanolic extract of *Tamarindus indica* Linn (EETI) treated groups there was a significant increase ($p < 0.05$) in animal body weight (figure 1&2)

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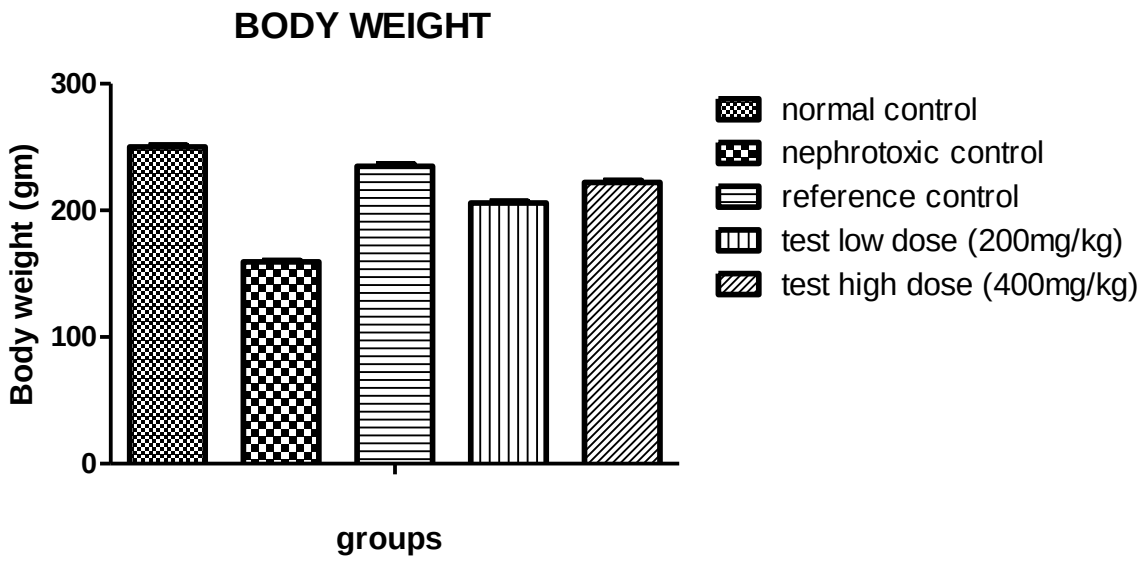


Fig.1 shows body weight of normal, nephrotoxic control and treated control

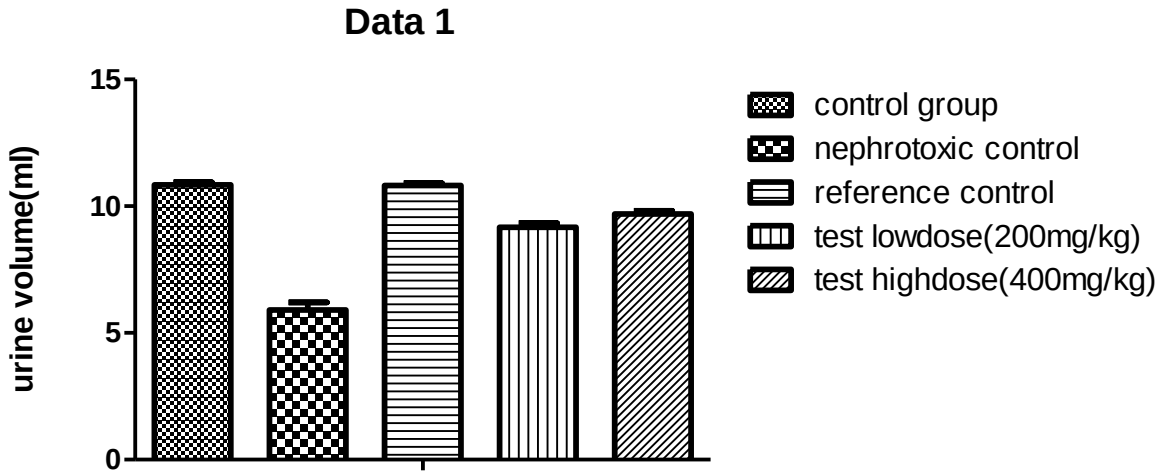


Fig.2 shows urine volume (ml) of normal, nephrotoxic control and treated control

Nephroprotective activity

Nephroprotective activity was assessed by the levels of serum creatinine, blood urea nitrogen, and Creatinine clearance as well as histopathological changes. Increased levels of serum creatinine and serum BUN, decreased level of creatinine clearance in Cisplatin treated animals were seen (group 2) compared with Dimethyl sulphoxide (DMSO) treated animals (group 1) confirming acute renal failure. In Ethanolic extract of *Tamarindus indica* Linn (EETI) treated groups there was a significant decrease ($p < 0.05$) in serum creatinine, blood urea nitrogen and increase in creatinine clearance at both the doses of 200 and 400 mg/kg. (Figure 3 & 4).

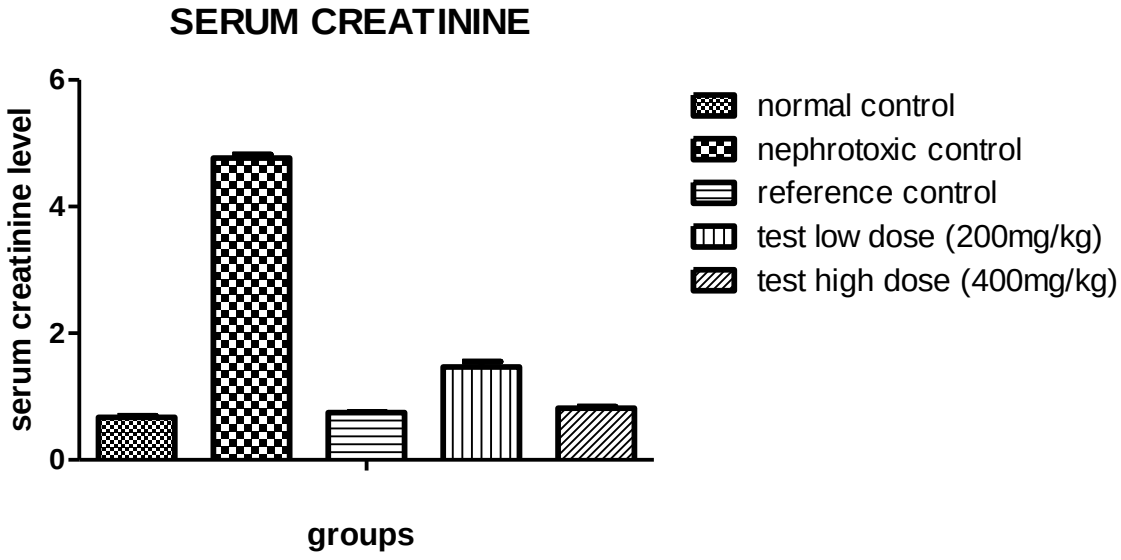


Fig.3 displays Serum creatinine of normal, nephrotoxic control and treated control

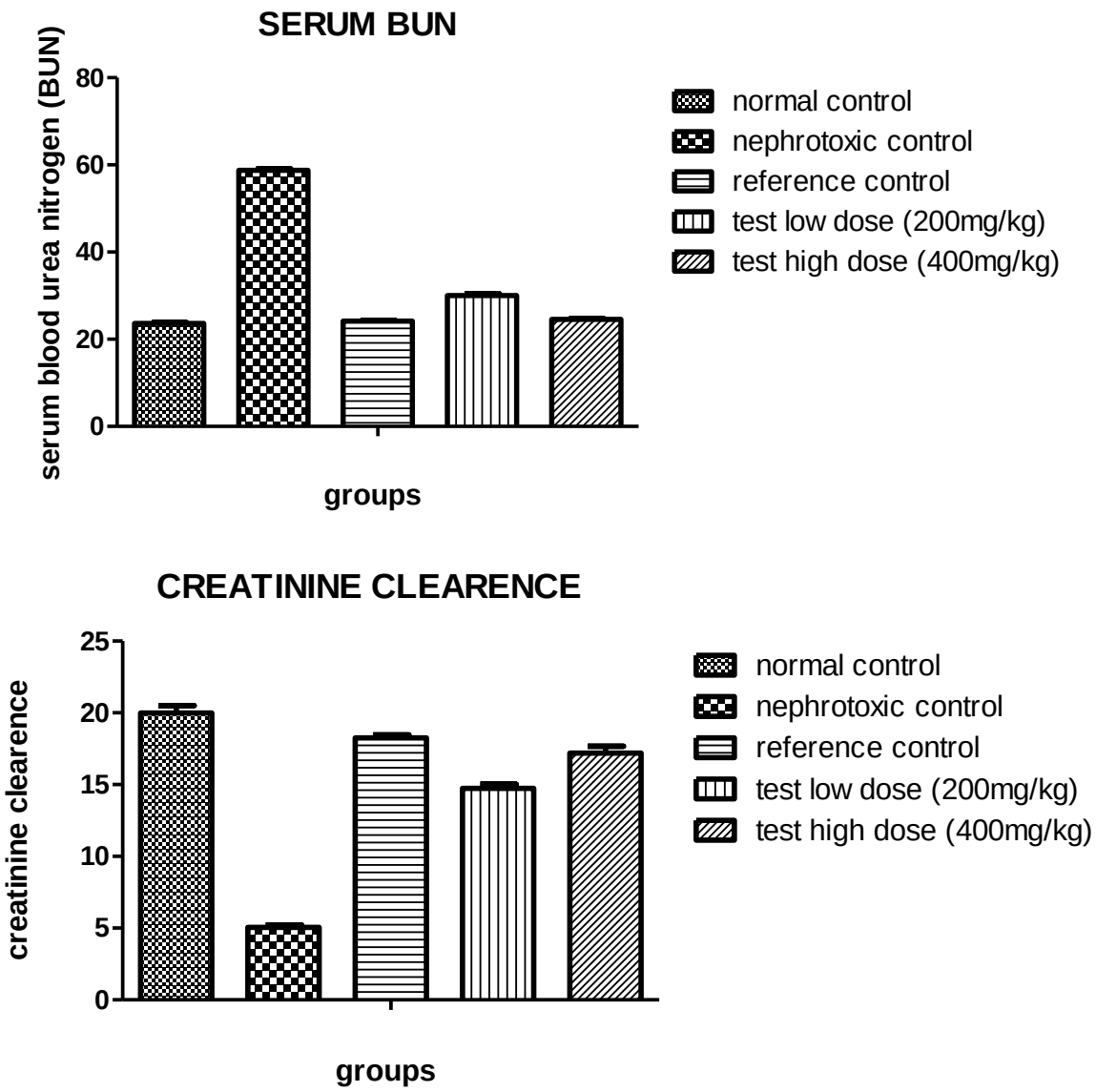


Fig.4 shows creatinine clearance of normal, nephrotoxic control and treated control

Enzymatic and non-enzymatic antioxidants

MDA levels were significantly ($p < 0.05$) decreased in group 4 and 5 compared with group 2 and SOD, GSH, GPx and CAT levels were significantly ($p < 0.05$) increased in group 4 and 5 compared with group 2. Ethanolic extract of *Tamarindus indica* Linn at 400 mg/kg had shown higher antioxidant levels than 200 mg/kg (Figures 5-9).

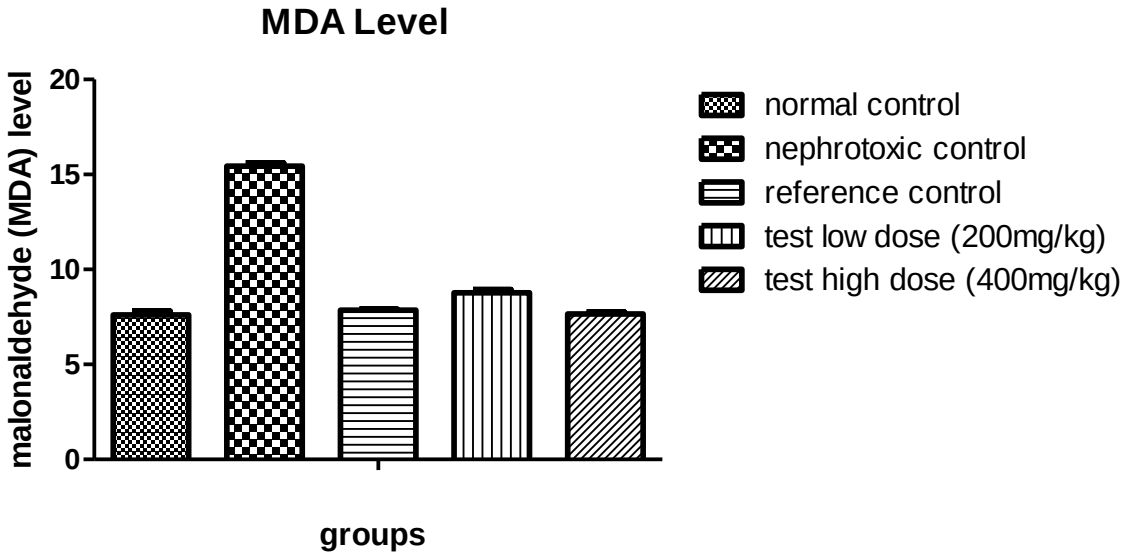


Fig.5 shows MDA level of normal, nephrotoxic control and treated control

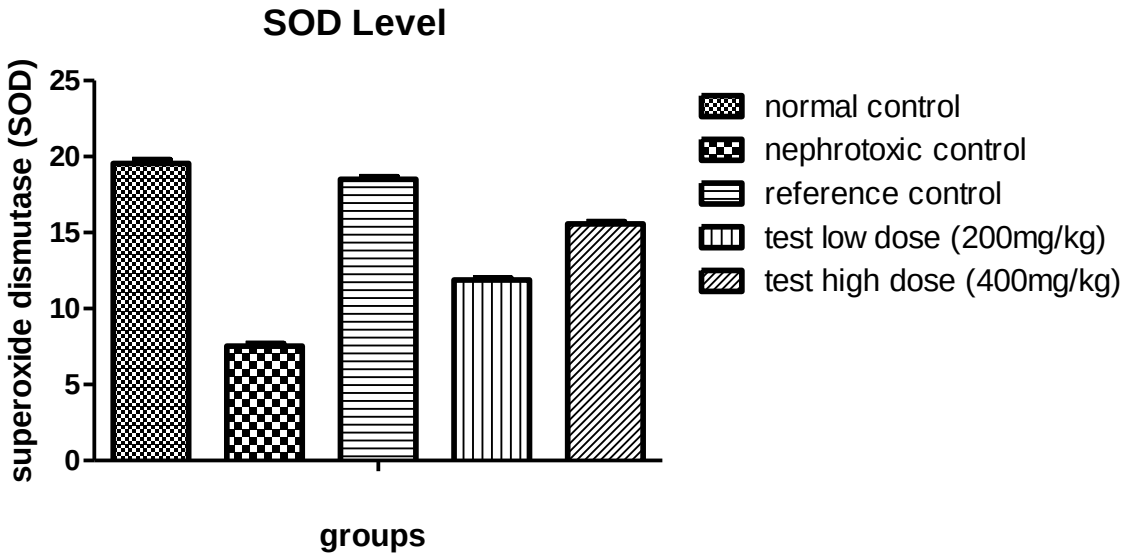


Fig.6 shows SOD level of normal, nephrotoxic control and treated control

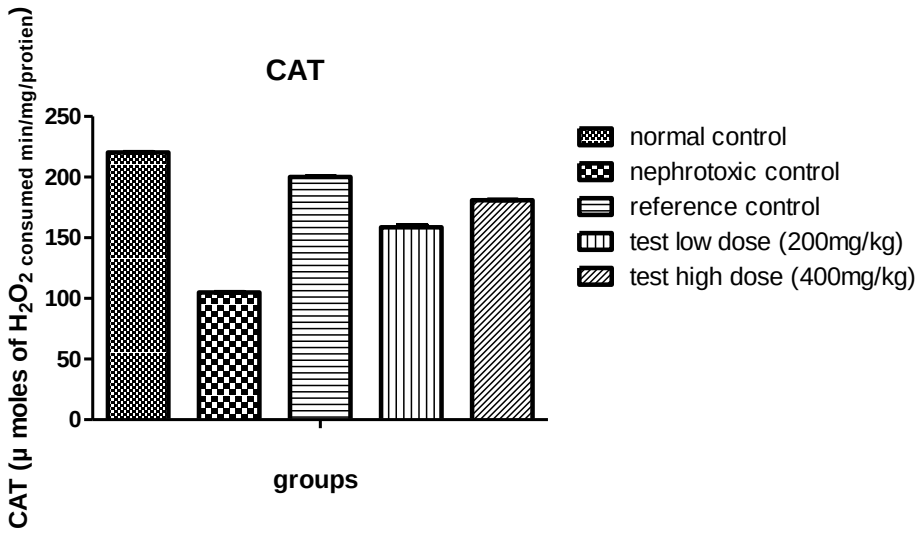


Fig.7 Exhibits the CAT of normal, nephrotoxic control and treated control

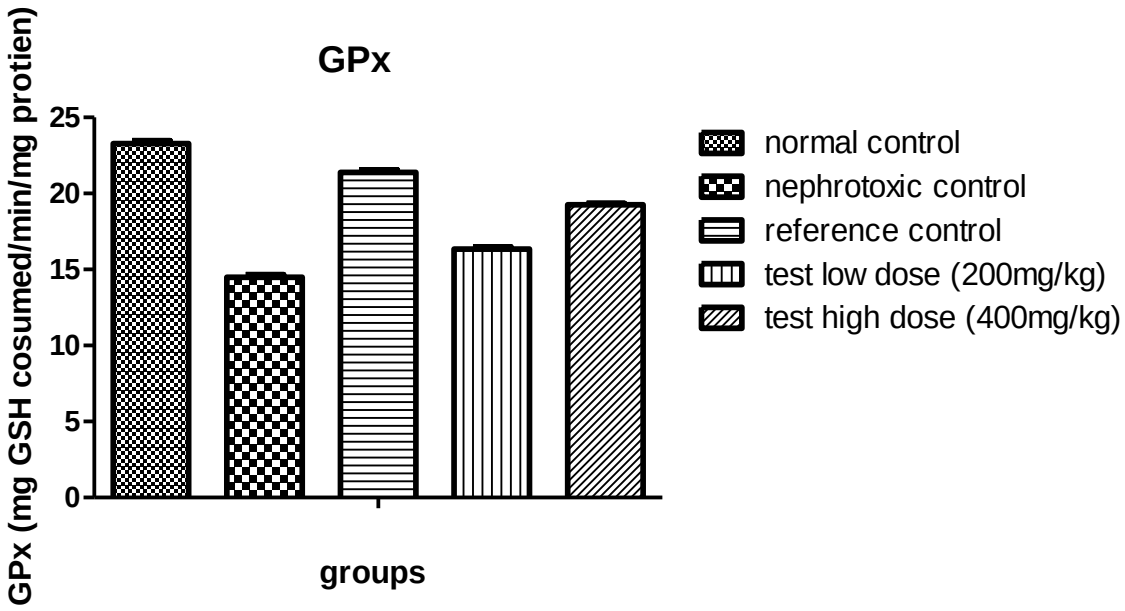


Fig.8 shows GPx of normal, nephrotoxic control and treated control

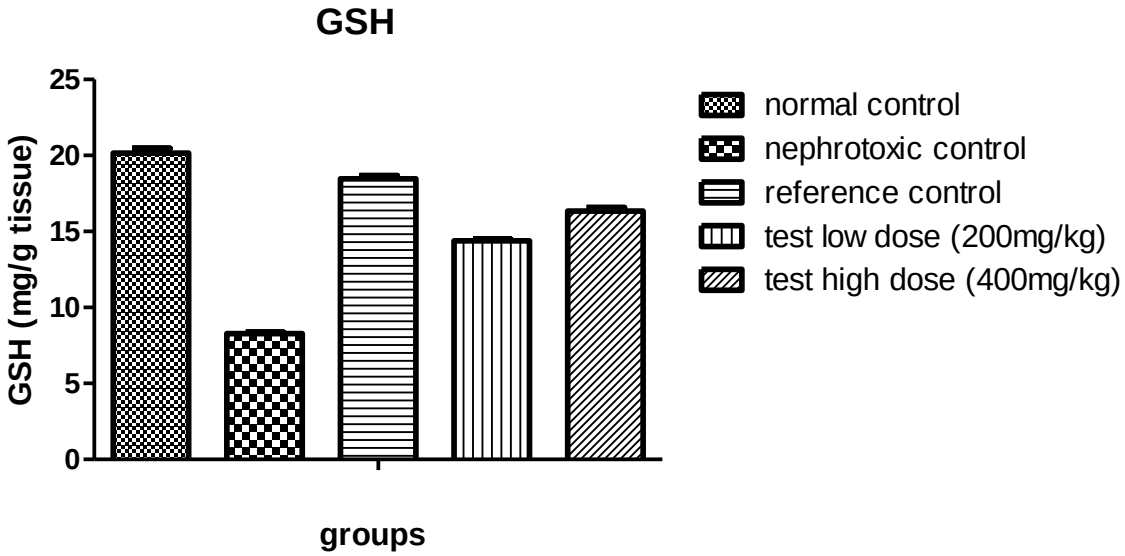
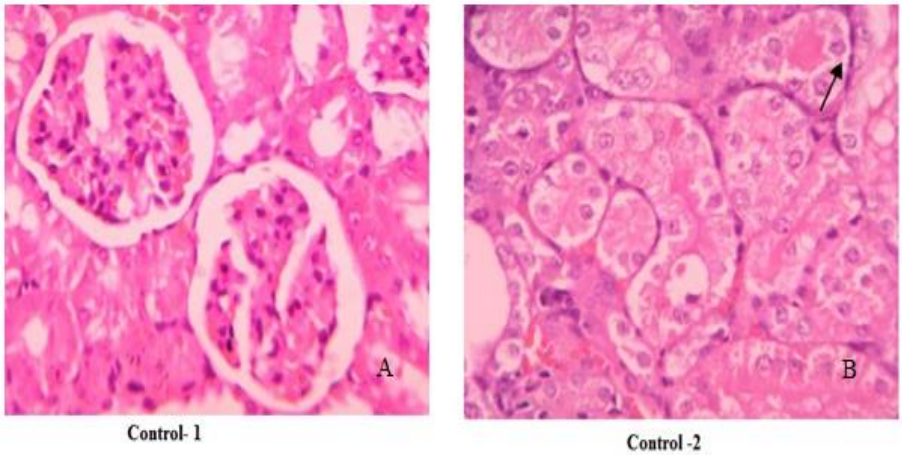


Fig.9 Shows GSH of normal, nephrotoxic control and treated control

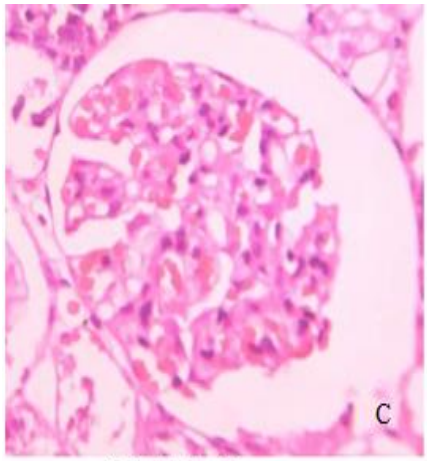
Histopathology

Section of the kidney of normal control rat (Group 1) showed, normal arrangement of nephrotic bundles, normal appearance of both cortex and medulla, normal glomerular structure with regularly arranged podocytes, there is no signs of degeneration and edema and no signs of inflammation like glomerulonephritis, normal and intact appearance of Proximal and Distal convoluted tubule, and no signs of karyolysis.

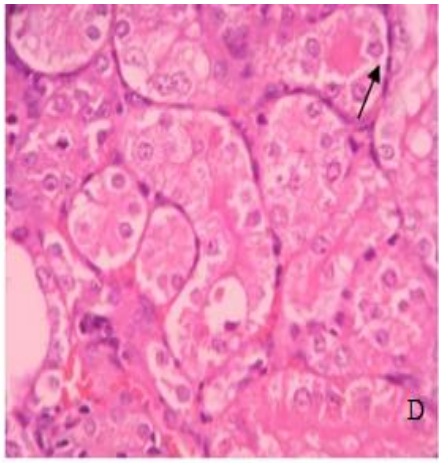
Section of the kidney of Nephroprotective control rat (Group 2) showed, coagulative and diffused necrosis, Severe Glomerulonephritis- Glomerular condensation and appearance of inflammatory cells and marked signs of hemorrhage, edema and narrowed renal arterioles. Section of the kidney of lipoic acid treated group (Group 3) rat showed normal histology of kidney and absence of necrosis. Section of the kidney treated with low dose (200mg/kg) of *EETI* (Group 4) 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 *EETI* (Group 5) showed, Moderated signs of regeneration with occurrence of chromatolysis in the tubular structure and Stripping of tubular epithelium with inter tubular edema shown in figure10. (Fig. 4- histopathology)



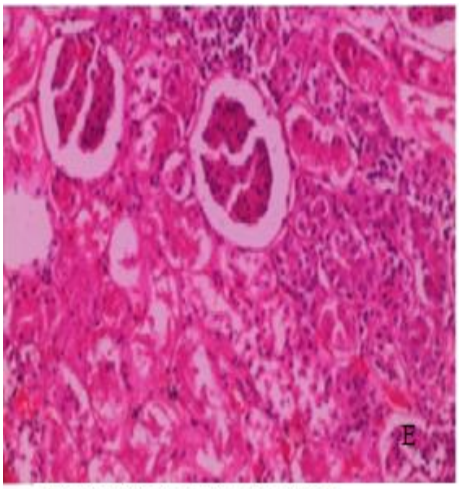
Control- 1 Control -2



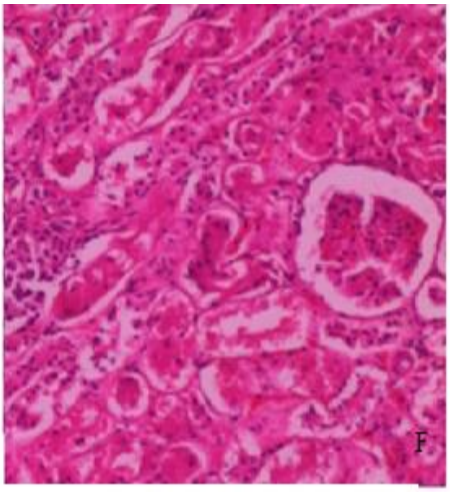
Cisplatin induced 1



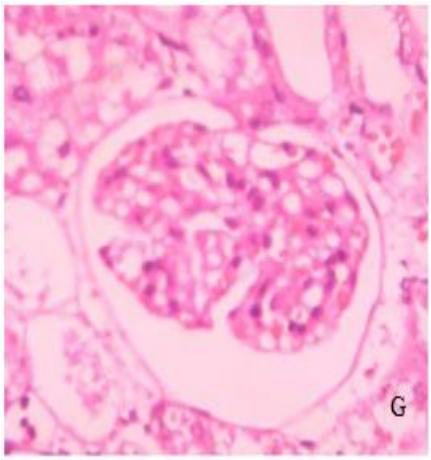
Cisplatin induced 2



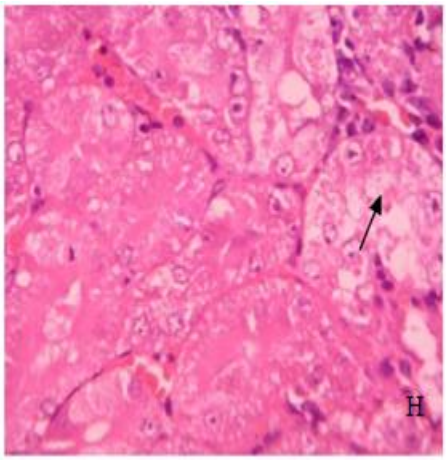
Standard (lipoic acid) + Cisplatin 1



Standard (lipoic acid) + Cisplatin 2



Plant extract 200mg + Cisplatin - 1(Kidney)



Plant extract 200mg + Cisplatin - 2 (Kidney)

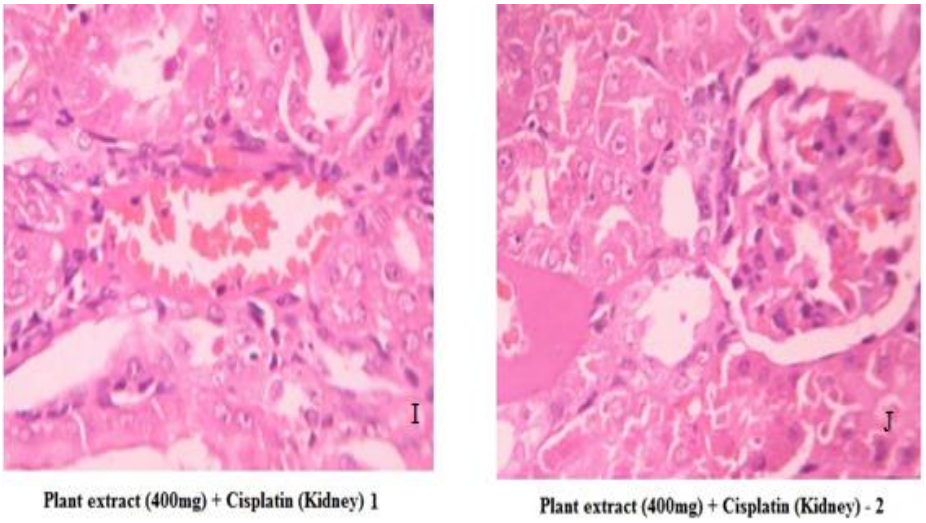


Fig. 10 (A-J) shows histopathology of normal, nephrotoxic control and treated kidneys

DISCUSSION

Nephrotoxicity is a common clinical syndrome defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted. There are few chemical agents to treat acute renal failure. Studies reveal back synthetic nephron protective agents have adverse effect besides reduce nephrotoxicity.

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of treatment of disease until the introduction of allopathic medicine. Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverse effect of dialysis.

The phytochemicals found to be present in the fruit pulp extract are the flavanoids, terpenoids, alkaloids, tannins, saponins and anthraquinones. Among them tannins, triterpenoids, flavanoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity. [14] Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of *Tamarindus indica* Linn. There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an LD_{50} dose of 2000 mg/kg and above was considered as unclassified so the ethanolic extract of *Tamarindus indica* Linn was found to be safe.

Cisplatin causes damage to nuclear and mitochondrial DNA and production of reactive oxygen species (ROS) which lead to activation of both mitochondrial and non-mitochondrial pathways of apoptosis and necrosis. Mitochondrial energetic are also disrupted by cisplatin and may contribute to nephrotoxicity. [24]

In present study, the rats treated with single dose of Cisplatin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomourular filtration rate, which is accompanied by increase in serum creatinine level and declain in creatinine clearence indicating induction of acute renal failure [25] with *Tamarindus indica* Linn at the dose level of 200 and 400 mg/kg body weight for 15 days significantly lowered the serum level of creatinine with a significant weight gain, increased urine output and creatinine clearence when compared with the nephrotoxic control group. Cisplatin administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum blood urea nitrogen (BUN). [26] *Tamarindus indica* Linn supplementation to Cisplatin treated rats recorded

decrement in levels of blood urea nitrogen (BUN) in plasma.

The elevated level of malondialdehyde (MDA), a marker of lipid preroxidation, indicates increased free radical generation in the Cisplatin induced nephrotoxicity. Cisplatin induced increment in malondialdehyde (MDA) content of plasma was significantly prevented by *Tamarindus indica* Linn treatment in the present study. Therefore, the significantly lower levels of malondialdehyde (MDA) in the kidney tissues of treated groups as compared with the Cisplatin group indicate attenuation of lipid peroxidation. This was probably due to less damage by oxygen free radicals with *Tamarindus indica* Linn. The involvement of oxygen free radicals in tissue injury is well established [27]. Decrement in activity levels of renal Superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH) following Cisplatin treatment are in accordance with previous report on Cisplatin induced suppression of endogenous enzymatic antioxidant machinery. [27] *Tamarindus indica* Linn treatment efficiently prevented Cisplatin induced decrease in activity levels of superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH). [28] A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators such as GPX system. Glutathione peroxidise (GPx) is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria, It catalyses the reaction of hydro-peroxides with reduced Glutathione to form Glutathione disulphide (GSSG) and the reduction product of the hydro-peroxide.[29]Effect of *Tamarindus indica* Linn on Glutathione peroxidise (GPx) in experimental rats study were significantly reduced in cisplatin treated rats than in the experimental control rats. Decrement in the activity of renal GPx following cisplatin treatment are due to suppression of endogenous enzymatic antioxidant machinery. Supplementation with *Tamarindus indica* Linn to Cisplatin treated rats resulted in near normal activity of glutathione peroxidise (GPx). Based on the above results, it was concluded that *Tamarindus indica* Linn exerted statistically significant Nephroprotective activity against cisplatin induced Nephrotoxic rats.

CONCLUSION

The *EETI* is found to have nephroprotective effect in Cisplatin-induced acute renal failure in albino wistar rats. *EETI* is found to have significant action at 400 mg/kg compared to 200 mg/kg.

Conflicts of interest

None declared.

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