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NEPHROPROTECTIVE SCREENING OF *DELONIX REGIA* FLOWERS AND LEAVES AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN FEMALE WISTAR RATS

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ABSTRACT

Objective: To evaluate the nephroprotective effect of Ethanolic extract of Delonix regia leaves and flowers against cisplatin-induced nephrotoxicity in female Wistar rats. **Methods:** Nephrotoxicity was induced by Cisplatin at 7mg/kg body weight through the *intraperitoneal* route in rats. The nephroprotective activity of Ethanolic extract of Delonix regia leaves and flowers (Leaves 200mg/kg, Flowers 200mg/kg, Leaves + Flowers 200mg/kg at 1:1) included a nephrological source. Oxidative stress parameters like GSH and MDA and Biochemical parameters like BUN, SCr, serum uric acid, and total protein were determined and analyzed using one-way ANOVA followed by Tukey's test. **Results:** The flowers and leaves extract have anti-oxidant activity due to the presence of Gallic acid and flavonoids. The renal function markers like BUN, SCr, and Serum uric acid were found to be decreased significantly by Delonix regia flowers and a combination of leaves and flowers extract (at 1:1) treatment. **Conclusion:** In conclusion, the presence of Gallic acid, flavonoid, and vitamin E in Delonix regia flowers is likely responsible for the plant's nephroprotective properties. The flower extract of the plant and the combination of leaves and flower extract at 1:1 concentration have anti-oxidant and nephroprotective potential due to the presence of flavonoids and Gallic acid. The

administration of Ethanolic extract of Delonix regia flowers provides a preferable Lowering of plasma creatinine and blood urea nitrogen and serum uric acid levels as compared to the combination of leaves and flowers extract at 1:1 and leaves extract 200mg/kg.

KEYWORDS: Nephrotoxicity, *Delonix regia*, Cisplatin, Kidney.

INTRODUCTION

Cisplatin is indeed a widely used and effective chemotherapy medication, especially in the treatment of various types of cancer, such as pulmonary, stomach, and ovarian cancer.[1] However, nephrotoxicity is the primary adverse reaction of cisplatin, with a clinical risk of 20% to 35% and a risk of death in patients with acute renal injury (AKI).[2,3] Cisplatin is bad for the kidneys because it damages DNA, cause problems with cytoplasty and mitochondria, starts apoptotic pathways, causes oxidative stress, and inflammation. At present, no medicine can successfully manage or stop nephrotoxicity caused by cisplatin.[4] To address this issue, many high-efficacies, low-toxicity natural products have been designed to protect against Acute Kidney Injury. Examples of natural products that may be used as supplements include ginseng, Curcumin, pomegranate, and other antioxidants and anti-inflammatories (potentially acting as anti-inflammatory agents).[5]

The **Delonix regia**, commonly known as the Gulmohar tree, is a species of ornamental plant that belongs to the Fabaceae family, a subfamily of the Caesapiniaceae family. This tree is characterized by its deciduous leaves, which are fern-like in appearance. It is also referred to as the flame tree, Royal Poinciana, or the Peacock Flower. Various diseases are treated with the parts of this plant, such as its stem, bark, and leaves, as well as its flower and seed. The trees have wide, open, umbrella-shaped crowns and are almost always green, and it is known as the flame of the forest or flame tree. The therapeutics or medicinal value of plants is mainly due to the presence of many phytochemicals, like flavonoids, alkaloids, tannins, and phenolic compounds. They are essentially plant metabolites that are generated in every area of the plant body and have some apparent physiological function in animals as well as humans.[6] The genus name "Delonix" is derived from the Greek words "Delos," which means "visible," and "onyx," which means "claw," because the petals are so clearly clawed. The word "regia" is derived from the Latin word "regis."(royal, regal, magnificent).[7] The *Delonix regia* (Gulmohar) has various medicinal and traditional properties and various parts of this plant are used to treat different disorders. Traditionally, a leaf decoction has been used to alleviate stomach upset and joint discomfort associated with rheumatism. Diarrhoea and

gynecological issues were treated with flowers. Antibacterial, anti-inflammatory, and anti-diabetic properties have also been detected in the leaves.[8]

One of the most reported issues with the kidneys is nephrotoxicity which happens when a chemical or medication is ingested by the body. Nephrotoxicity refers to a drug's or another substance's detrimental impact on renal function. Numerous processes, such as renal tubular toxicity, glomerular injury, crystal nephropathy, and inflammation, contribute to nephrotoxicity. A significant variety of effective therapeutic medications, such as NSAIDs, chemotherapeutics, and antibiotics can harm the kidney and cause acute renal failure, chronic interstitial nephritis, and nephritis syndrome.[9]

MATERIALS AND METHODS

Chemicals and drugs

DPPH Reagent, Acetic Acid, Sodium Carbonate, Ellman Reagent, Sodium Hydroxide Pallets, NBT, Amlodipine, Formaldehyde Solution, Sodium Dodecyl Sulphate, TBA (Thiobarbituric Acid), Sodium Chloride, Sodium Dihydrogen Orthophosphate Formaldehyde, Xylene, Hematoxylin, Eosin, Paraffin wax, etc.

Identification Collection and Authentication of plant materials

Plant materials were collected from the ground of Meerut Institute of Engineering and Technology (MIET), Meerut. Plant materials were recognized and authenticated by the Department of Botany Ch. Charan Singh University, Meerut by Prof. Vijai Malik, Head Department of Botany on date-22/12/2022 Ref: Bot/PB.

Preparation of Ethanolic extract of Delonix regia leaves and flowers

5 kg of fresh Delonix regia leaves and flowers were collected, washed completely, and dried in the shade for 7 to 8 days. Dried leaves and flowers grinding into the mixture and powdered them. For a period of 36 hours, leaf powder (260g) was extracted with ethanol (500 ml) with the help of the Soxhlet apparatus. Flower powder (330g) was extracted for 36 hours in ethanol (500 ml) using a Soxhlet apparatus. The solvent was evaporated using a rotary evaporator and the extract left behind was stored. The extract yield in flowers was found to be 26.49%, while in leaves it was 12%.

Anti-oxidant activity of plant materials

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay)

1. Prepare stock solutions of the sample at 1.0 mg/mL.
2. Dilute these solutions to obtain concentrations of 50, 40, 30, 20, and 10 µg/mL in ethanol.
3. Prepare a 0.3 mM DPPH solution in ethanol.
4. Mix 2.5 mL of each sample solution with 1 mL of the DPPH solution and let it react for 30 minutes in the dark palace.
5. Measure the absorbance at 517 nm to assess the extent of DPPH reduction.
6. Antioxidant activity (AA) was calculated as a percentage using the formula:[10]

$$AA\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Animals

Female Wistar rats weighing 150-200gm were given by Meerut Institute of Engineering and Technology's (MIET) animal house. The animals were kept in plastic cages in an air-conditioned environment with precise temperature, relative humidity, and a normal dark-light cycle. During the trial, the rats were fed a nutritious diet and given enough water. All surgical operations were carried out in a sterile environment. All animal procedures were approved by the Ethical Committee at the Institution proposal no **IAEC/MIET/ CPCSEA/Meeting No: 01/2023/Protocol No.102**

Experimental design

Induction of nephrotoxicity- cisplatin was used for inducing nephrotoxicity. Cisplatin was injected intraperitoneally in a single nephrotoxic dose of 7mg/kg.

Experimental design for nephroprotective activity

Animals were selected randomly in 6 groups, the control group contained 6 animals, and the rest of each group contained 8 animals for 14 days of study.

Group 1 (control group) - animals were treated with normal water 3ml/kg orally for 14 days.

Group 2 (Negative control group) - animals were treated with cisplatin 7mg/kg intraperitoneally.

Group 3 (test group 1) – animals were treated with leaves extract 200mg/kg orally and cisplatin 7mg/kg was injected intraperitoneally on day 7.

Group 4 (test group 2) – animals were treated with flower extract 200mg/kg orally and cisplatin 7mg/kg was injected intraperitoneally on day 7.

Group 5 (test group 3) – animals were treated with a combination of leaves and flower extract at 1:1 orally and cisplatin 7mg/kg was injected intraperitoneally on day 7.

Group 6 (standard group) – animals were treated with amlodipine 2mg/kg orally, and cisplatin 7mg/kg intraperitoneally on day 7.

Experimental protocols

Collection of sample

Blood was collected from the retro-orbital plexus, after the administration of anesthesia. These samples were then subjected to centrifugation at 3000 revolutions per minute to separate the serum from the whole blood, which was subsequently stored at a temperature of -20°C. All animals were humanely euthanized through cervical dislocation. The intact kidneys were carefully removed, cleansed with saline, and gently dried using filter paper. The kidneys were then halved for further processing:

1. One half was frozen at -20°C to enable the subsequent measurement of kidney biomarkers such as Malondialdehyde (MDA) and Glutathione (GSH). These markers are frequently used to evaluate oxidative stress and antioxidant levels within the kidney tissue.
2. The other half was preserved in 10% buffered formaldehyde for Histopathological analysis. This method preserves the tissue and makes it suitable for microscopic examination, allowing for the detection of any abnormalities or pathological conditions.[11]

Evaluation of serum creatinine and blood urea nitrogen concentrations

Alterations in the rate of excretion may serve as indicators of compromised kidney function. The process of measuring serum creatinine (Cr) and blood urea nitrogen (BUN) levels is a valuable approach for identifying renal diseases and estimating the degree of renal function impairment. The concentrations of serum creatinine and urea levels were determined using a commercial kit developed by ERBA, specifically the Urea (BUN) Kit (code no. 120214) and the Creatinine Kit. (Code no. 120246).[12]

Measurement of MDA level- MDA is an outcome of the peroxidation of polyunsaturated fatty acids within cells. An increase in free radicals can result in MDA overproduction. MDA

levels are frequently used to assess oxidative stress. The Okhawa Method is used to calculate MDA concentrations. The procedure involves the following steps:

1. Take a sample of kidney tissue and place it in test tubes.
2. Add 4.5 ml of phosphate buffer (pH 7.4) to the test tubes containing the tissue and then homogenize the mixture.
3. Centrifuge the homogenized mixture at 2000-3000 rpm for 10 minutes. This step separates the supernatant liquid from the solid components.
4. 100 µl of 8.1% Sodium Dodecyl Sulfate (SDS), 750 µl of 20% acetic acid, and 750 µl of 0.8% Thiobarbituric acid were added to the supernatant liquid.
5. Make up the total volume in the test tubes to 2 ml with distilled water.
6. Wrap the mouths of the test tubes with aluminum foil to prevent light exposure and heat the test tubes in a water bath at 95ºC for 60 minutes.
7. After heating, cool the test tubes under tap water. You'll notice a pinkish color developing in the sample.
8. Centrifuge the test tubes at 10,000 rpm for 10 minutes.
9. Take the supernatant liquid from the test tubes as the sample, and use distilled water as a blank.
10. Measure the absorbance of the sample at 532 nm using a UV spectrophotometer.
11. This process is used to quantify the levels of MDA in the kidney tissue, with the absorbance at 532 nm serving as an indicator of MDA concentration. It's a common method for assessing oxidative stress.[13]

Determination of GSH level in kidney homogenate

GSH serves as a crucial biomarker for antioxidant activity, acting as a scavenger for superoxide radicals. Its primary role lies in safeguarding thiol groups essential for preserving cell integrity against oxidative damage. The estimation of GSH levels follows the methodology introduced by Moron et al.[14]

The procedure involves the preparation of kidney tissue for analysis

1. First, ice-cold physiological saline is used to rinse the kidney. After that, the kidney is finely minced.
2. A homogenate of the kidney tissue is created using a phosphate buffer at pH 7.4.
3. This homogenate is subjected to centrifugation at 2000-3000 rpm for 10 minutes, and the resulting supernatant is collected for GSH determination.

To determine GSH levels, the following steps are carried out

4. A 500 µl portion of the supernatant is mixed with an identical amount of chilled sulfosalicyclic acid (5%).
5. The mixture is vortexed and placed on ice for 30 minutes to precipitate proteins.
6. After the incubation period, the mixture undergoes centrifugation at a speed of 10,000 revolutions per minute for a duration of 10 minutes.
7. In the GSH assay, a total of 50 µl of the supernatant is mixed with 450 µl of phosphate buffer at a pH of 7.4, followed by the addition of 1500 µl of Elman's reagent.
8. As for the blank, it consists of 500 µl of phosphate buffer (pH 7.4) and 1500 µl of Ellman's reagent.
9. Both the sample mixture and the blank are vortexed and then incubated at 37°C for 10 minutes.
10. Following incubation, the optical density is measured at 412 nm.

Total tissue protein estimation

The task was carried out using Lowry's methodology. The fundamental approach utilized for protein analysis involves the process of acid hydrolysis, which is subsequently followed by the analysis of amino acids. This method involves the reaction of peptide bonds within proteins with copper ions in an alkaline environment, resulting in the formation of Cu+ ions. These Cu+ ions subsequently interact with the Folin reagent.[15]

Histopathological examination

The kidneys from each animal were removed and subsequently preserved in a 10% formalin-buffered solution. These specimens were then prepared for Histopathological analysis by undergoing a dehydration process using absolute ethanol and xylol. The paraffin sections, which were three micrometers thick, underwent staining with Hematoxylin and eosin using a conventional protocol.

Change in percentage of body weight

The body weight of each animal in the respective groups was measured. The body weight of the animals was measured every week, starting from the beginning of the study until its completion, and also before the sacrifice of the animals.

Measurement of food intake

Every day, food intake was recorded. To obtain an accurate food intake measurement, the spillage food was separated from the husk during the experimental animals' food consumption.[15]

Statistical analysis- The data was analyzed using a statistical method called one-way ANOVA. After that, Dunnett's multiple comparison test was used. The results were compared in two ways:

- [a]results were compared to the "Normal control group."
- [b]results were compared to the "Toxic control group."

RESULTS

Phytochemical study report

The preliminary qualitative phytochemical tests were carried out to analyze the various chemical groups that are present in the Ethanolic extract of the flowers and leaves of Delonix regia, including flavonoids, alkaloids, tannins, glycosides, phenolic compound, Gallic acid, vitamin E, etc.

Table 1: Preliminary phytochemical screening of Ethanolic extract of leaves and flowers of Delonix regia.

Compounds	Tests	Ethanolic extract of leaves	Ethanolic extract of flowers
Flavonoids	Shinoda test	+	+
	Alkaline reagent test	+	+
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
Glycosides	Molisch's test	+	—
	Borntrager's test	+	—
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Saponin	Foam test	+	+
Proteins	Biuret Test	—	+
	Ninhydrin test	—	+
Carbohydrates	Seliwanoff's test	—	—
Phenols	Ferric chloride test	—	+
	Liebermann's test	—	+

Anti-oxidant study report

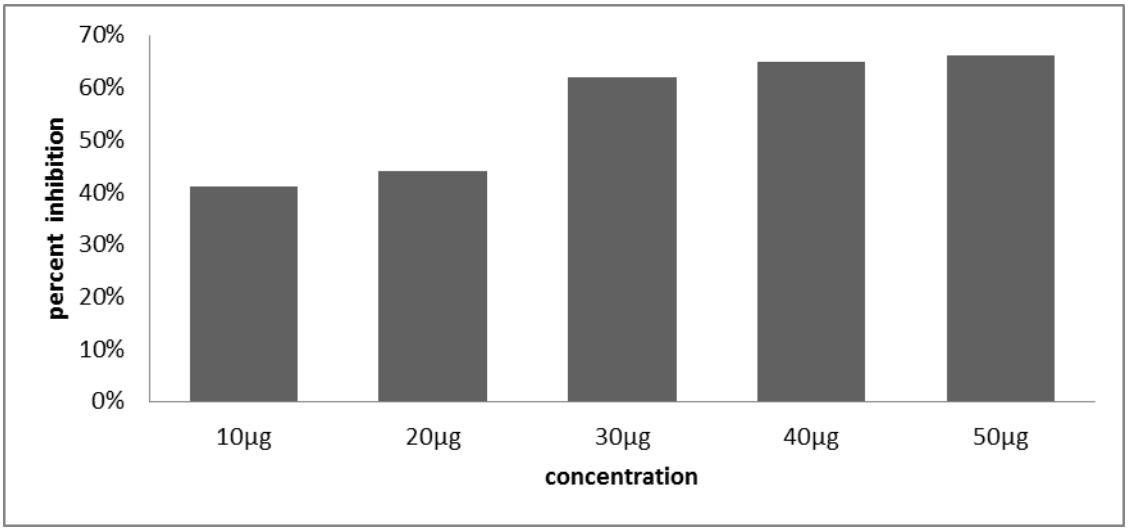
Tab. 2.

S.no.	Concentration	Absorbance at 517nm	Percent inhibition %
1	10µg	0.353	41%
2	20µg	0.332	44%
3	30µg	0.226	62%
4	40µg	0.207	65%
5	50µg	0.202	66%

$$\frac{\text{Absorbance of DPPH} - \text{Absorbance of DPPH with extract}}{\text{Absorbance of DPPH}} \times 100$$

Absorbance of DPPH (control) = 0.599

The absorbance of DPPH + leaf extract of different concentration



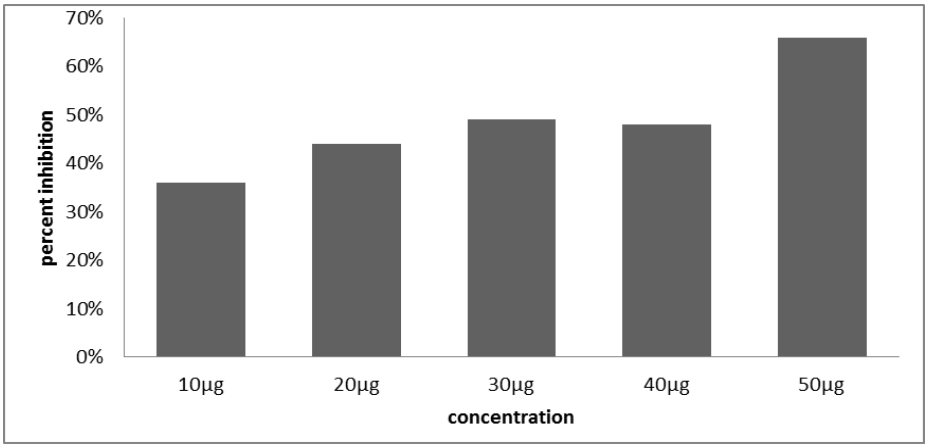
Graph 1: Percent inhibition% of leaves extract of *Delonix regia.*

Absorbance of DPPH (control) = 0.599

The absorbance of DPPH + flowers extract of different concentration

Tab. 3.

S.no.	Concentration	Absorbance at 517nm	Percent inhibition %
1	10µg	0.383	36%
2	20µg	0.334	44%
3	30µg	0.302	49%
4	40µg	0.307	48%
5	50µg	0.202	66%



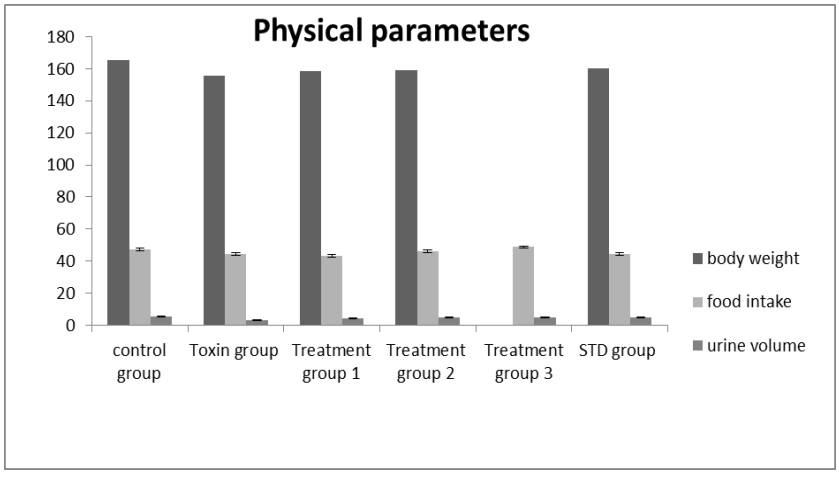
Graph 5.2: Percent inhibition% of flower extract of *Delonix regia.*

Evaluation of physical parameters

Table 5.4: Effect of Ethanolic extract of Delonix regia on physical parameters.

Groups	weight of body	food intake	urine volume
Normal Control	165.32 ± 8.32 [a]	47.33 ± 3.55 [a]	5.33 ± 0.32 [a]
Negative Control Cisplatin(7mg/kg)	145.53 ± 9.12 [b]	44.43 ± 4.55 [b]	3.22 ± 0.33 [b]
Treatment group 1 Leaves extract(200mg/kg)+Cisplatin	160.55 ± 10.13 [a]	43.31 ± 5.66 [a]	4.55 ± 0.87 [a]
Treatment group 2 Flowers extract(200mg/kg)+cisplatin	159.33 ± 10.99 [a]	46.31 ± 4.77 [a]	4.77 ± 0.98 [a]
Treatment group 3 Leaves + Flowers (200mg/kg)+ cisplatin	159.22 ± 9.33 [a]	48.76 ± 4.88 [a]	5.1 ± 0.43 [a]
STD group Amlodipine (2mg/kg)+cisplatin	160.33 ± 8.97 [a]	44.54 ± 5.88 [a]	4.89 ± 0.88 [a]

All values are shown as Mean ± SEM, n = numbers of animals



Graph 5.3- In comparison to the control group, no significant weight loss was observed after administration of CP 7mg/kg. There were no weight changes in the treatment and STD groups. Furthermore, there was no variation found in food consumption or urine production among the six groups.

Evaluation of Bio-chemicals parameters

BUN- (blood urea nitrogen)

The BUN level in blood is shown in Table 3.The BUN level significantly increased after administration of cisplatin. After administration of Amlodipine (2mg/kg) as STD drug and Ethanolic extract of Delonix regia flowers (200mg/kg) and a combination of leaves and flowers extract (200mg/kg) at 1:1 as test drug along with Toxin (cisplatin), the BUN level in serum significantly decreased.

Table 3: Effect of Ethanolic extract of Delonix regia leaves and flowers on Blood Urea Nitrogen (BUN).

Groups	BUN(mg/dl)
Normal Control	21.98± 1.31
Negative Control Cisplatin(7mg/kg)	40.92 ± 2.029[a]
Treatment group 1 Leaves extract(200mg/kg)+Cisplatin	32.75± 3.32[b]
Treatment group 2 Flowers extract(200mg/kg)+cisplatin	25.84 ± 2.71[b]
Treatment group 3 Leaves + Flowers (200mg/kg)+ cisplatin	28.79± 2.27[b]
STD group Amlodipine (2mg/kg)+cisplatin	27.33 ± 2.86[b]

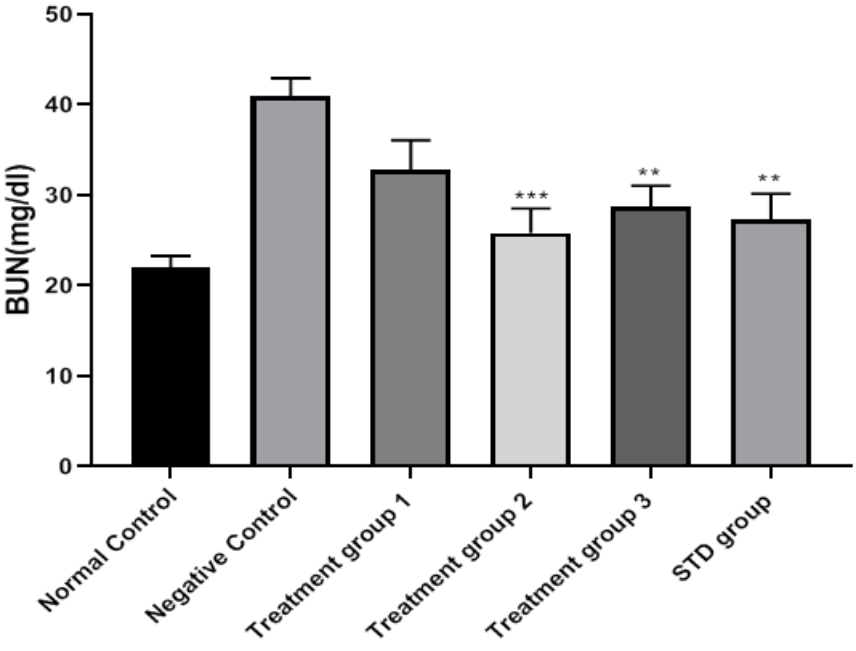


Fig. 2: Cisplatin administration significantly increased Bun level as compared to the control group. After administration of Amlodipine as an STD drug and Ethanolic extract of Delonix regia flowers in treatment group 2 and a combination of leaves and flowers extract at 1:1 in treatment group 3 along with toxin significantly reduced BUN level as compared to treatment group 1.

SCr (serum creatinine) and SUA (Serum uric acid)

The SCr and SUA level in serum is shown in Table 4. Cisplatin administration significantly increased the SCr and SUA levels. After administration of Amlodipine (2mg/kg) as STD drug and Ethanolic extract of Delonix regia flowers and a combination of leaves and flowers extract at 1:1 as test drug along with Toxin (cisplatin), the SCr and SUA levels in serum significantly decreased.

Table 4: Effect of Ethanolic extract of Delonix regia leaves and flowers on Serum creatinine and Serum uric acid.

Groups	Serum creatinine(mg/dl)	Serum uric acid(µg/ml)
Normal Control	0.74 ± 0.151	19.24±2.64
Negative Control Cisplatin(7mg/kg)	2.995± 0.928 [a]	32.51±2.074 [a]
Treatment group 1 Leaves extract(200mg/kg)+Cisplatin	1.66 ± 0.53 [b]	27.61±3.12 [b]
Treatment group 2 Flowers extract(200mg/kg)+cisplatin	1.08 ± 0.50 [b]	22.49 ±1.47 [b]
Treatment group 3 Leaves + Flowers (200mg/kg)+ cisplatin	1.39± 0.598 [b]	25.84±2.27 [b]
STD group Amlodipine (2mg/kg)+cisplatin	1.11± 0.539 [b]	24.06 ±2.041b[b]

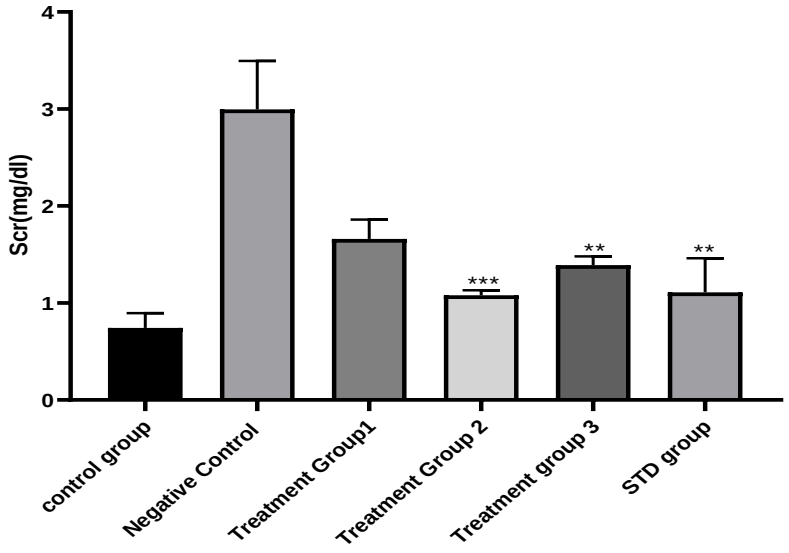


Fig. 3: Cisplatin administration significantly increased SCr level as compared to a control group. After administration of Amlodipine as an STD drug and Ethanolic extract of Delonix regia flowers in treatment group 2 and a combination of leaves and flowers extract at 1:1 in treatment group 3 along with toxin significantly reduced SCr level as compared to treatment group 1.

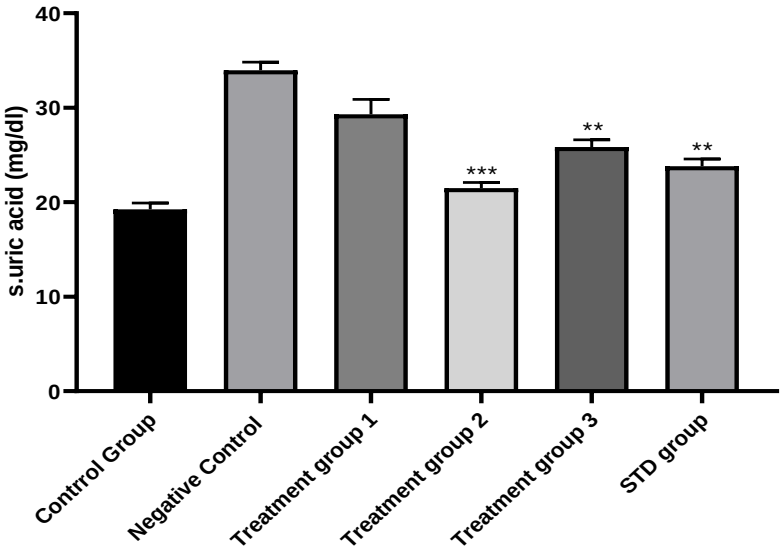


Fig. 4: Cisplatin administration significantly increased SUM level as compared to the control group. After administration of Amlodipine as an STD drug and Ethanolic extract of Delonix regia flowers in treatment group 2 and a combination of leaves and flowers extract at 1:1 in treatment group 3 along with toxin significantly reduced SUM level as compared to treatment group 1.

Total tissue protein

The Total protein level in serum is shown in Table 5. The Total tissue protein level significantly decreased by administration of cisplatin. After administration of Amlodipine as an STD drug Ethanolic extract of Delonix regia flowers and a combination of leaves and flowers extract at 1:1 as a test drug along with Toxin (cisplatin), the Total protein level in serum significantly increased.

Table 5.6: Effect of Ethanolic extract of Delonix regia leaves and flowers on Total protein.

Group	Total protein (mg/ml)
Normal Control	19.06±1.86
Negative Control Cisplatin(7mg/kg)	8.58±2.14 [a]
Treatment group 1 Leaves extract(200mg/kg)+Cisplatin	11.36±1.34 [b]
Treatment group 2 Flowers extract(200mg/kg)+cisplatin	17.15±1.23 [b]
Treatment group 3 Leaves + Flowers (200mg/kg)+ cisplatin	14.88±1.28 [b]
STD group Amlodipine (2mg/kg)+cisplatin	16.22±1.34 [b]

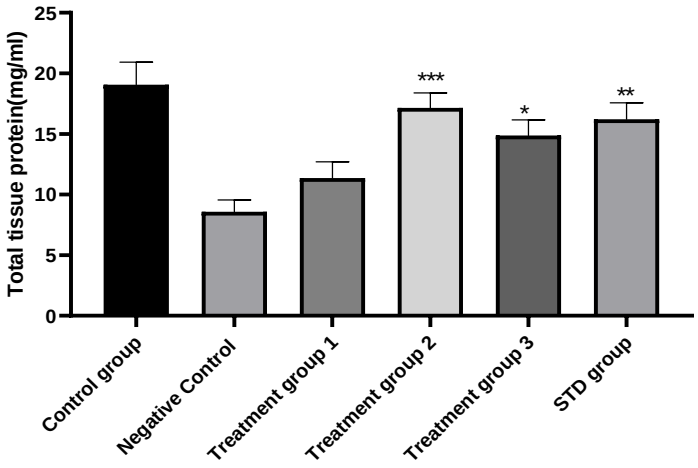


Fig. 5: Total protein level in the rats. Cisplatin administration significantly decreased Total protein level as compared to the control group. After administration of Amlodipine as an STD drug and Ethanolic extract of Delonix regia flowers in treatment group 2 and a combination of leaves and flowers extract at 1:1 in treatment group 3 along with toxin significantly reduced Total protein level as compared to treatment group 1.

Evaluation of oxidative parameters

GSH and MDA

GSH levels were significantly reduced by cisplatin administration in comparison to the control group. Groups receiving Amlodipine as STD and Ethanolic extract of Delonix regia flowers and a combination of leaves and flowers extract along with toxin showed a significant improvement observed in the glutathione (GSH) levels within the renal tissue. MDA levels in kidney tissue increased after the administration of cisplatin as a toxin. After receiving Amlodipine as an STD drug Ethanolic extract of Delonix regia flowers and a combination of leaves and flowers extract as a test drug along with toxin showed significant changes in MDA level in kidney tissue.

Table 5.7: Effect of Ethanolic extract of Delonix regia leaves and flowers on GSH and MDA.

Group	GSH(µmol/mg protein)	MDA(µmol/mg protein)
Normal Control	50.43 ± 3.79	98.93 ± 5.19
Negative Control Cisplatin(7mg/kg)	22.035 ± 2.23 [a]	149.49± 12.58 [a]
Treatment group 1 Leaves extract(200mg/kg)+Cisplatin	32.94± 3.99 [b]	136.79 ± 5.41 [b]
Treatment group 2 Flowers	43.18 ± 2.90 [b]	115.51± 5.66 [b]

extract(200mg/kg)+cisplatin		
Treatment group 3 Leaves + Flowers (200mg/kg)+ cisplatin	37.56± 3.12 [b]	127.75 ± 5.63 [b]
STD group Amlodipine (2mg/kg)+cisplatin	41.25 ± 3.70 [b]	107.05± 5.51 [b]

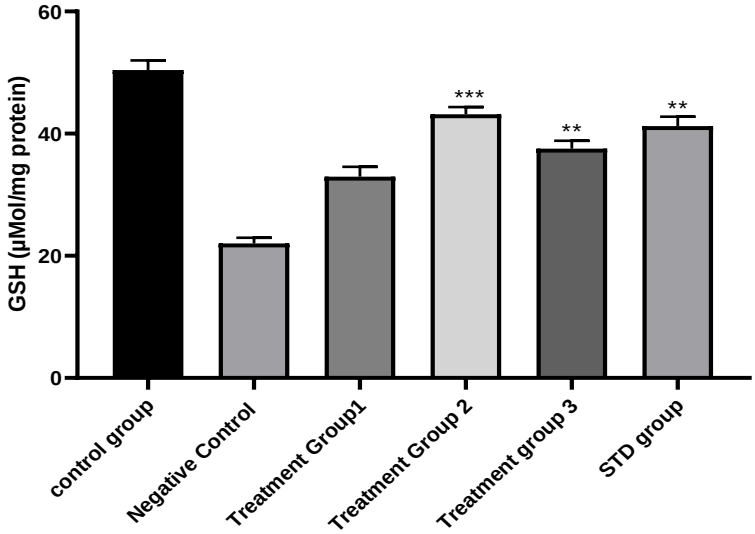


Fig. 6: The administration of Cisplatin led to a notable reduction in GSH levels when compared to the control group. However, groups receiving Amlodipine as an STD drug and the Ethanolic extract of Delonix regia flowers, and a combination of leaf and flower extracts in conjunction with the toxin demonstrated a significant restoration of GSH levels in the kidney tissue.

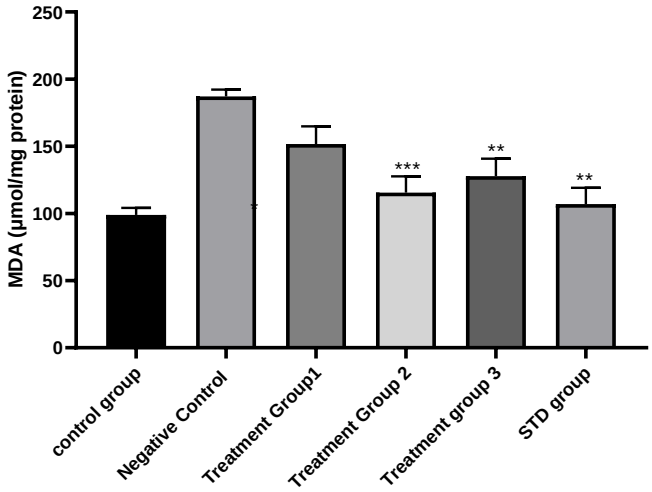


Fig. 6: Cisplatin administration resulted in a significant elevation in MDA levels when compared to the control group. However, groups that received Amlodipine as an STD drug the Ethanolic extract of Delonix regia flowers, and a combination of leaf and

flower extracts alongside the toxin exhibited a substantial reduction in MDA levels in the kidney tissue.

Histopathological examination

The kidneys of Normal control group animals treated with normal saline (1ml/100 g) showed normal histology Fig 5.1 whereas toxic control group animals treated with **cisplatin (7mg/kg) showed narrowing of Bowman's capsule spaces, damaged glomeruli and tubular necrosis** as shown in fig.5.2. In STD group animals were treated with cisplatin (7mg/kg) followed by amlodipine 2mg/kg, reduces necrosis and inflammation fig 5.3. In treatment group 1 animals were treated with cisplatin (7mg/kg) followed by leaves extract (200 mg/kg) and **showed mild provocative cells and swelling in bowman capsule** fig.5.4. In treatment group 2 animals were treated with cisplatin (7mg/kg) followed by flowers extract (200mg/kg) **showing almost normal glomeruli cells and inflammation** fig.5.5. In the treatment group 3 animals were treated with cisplatin (7mg/kg) followed by a combination of leaves and flowers extract at 1:1 (200mg/kg) show **normal histology with mild swelling in Bowman's capsule spaces** fig.5.6

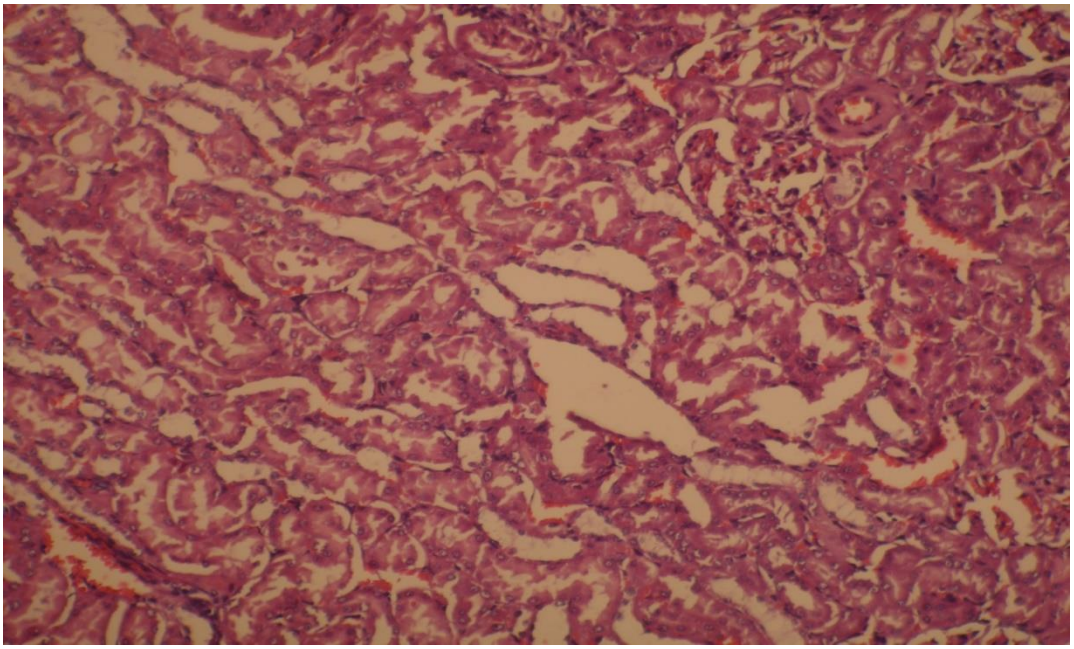


Figure 5.1: Histological examination of the renal cells of the rat of the Normal control group.

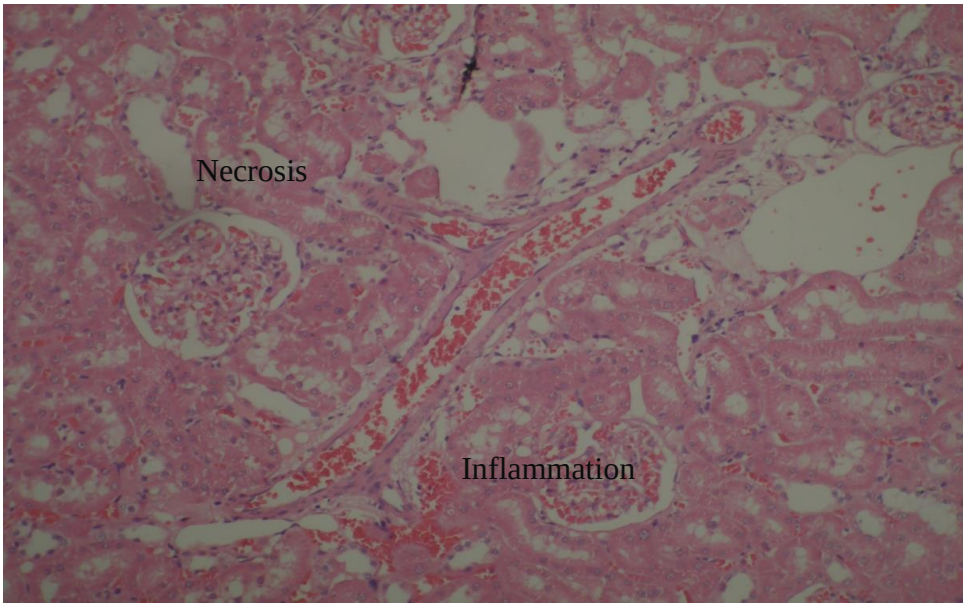


Fig. 5.2: Histopathology of renal showing necrosis and inflammation in the Negative control group, rats received cisplatin 7mg/kg/day here; the orange indicates the inflammation and necrosis represented by the black arrow.

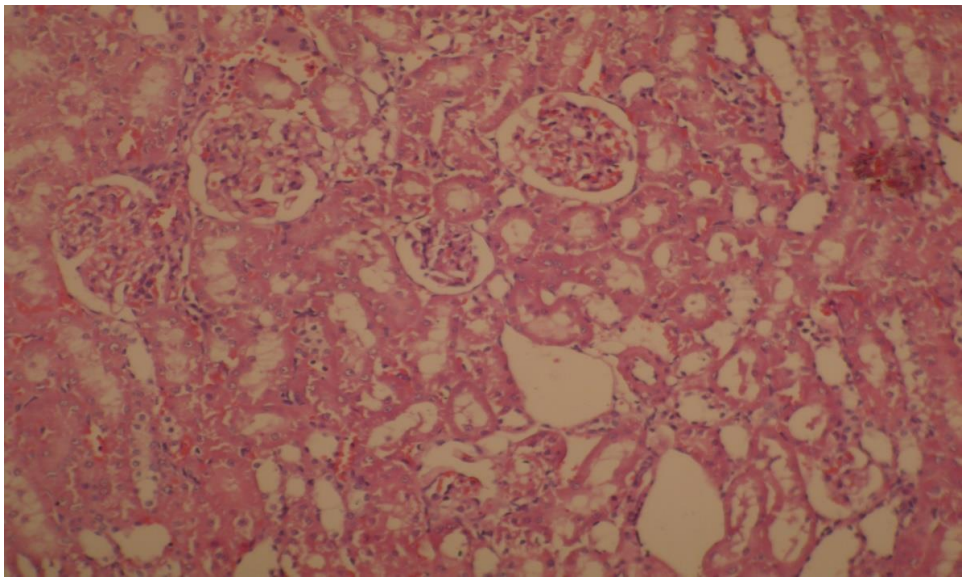


Fig.5.3 Analysis of the Kidney Histology in Rats of Treatment group 1 showing the alleviation of necrosis and inflammation, rats treated orally with leaves extract of plants 200mg/kg/day+ cisplatin 7mg/kg *i.p.*

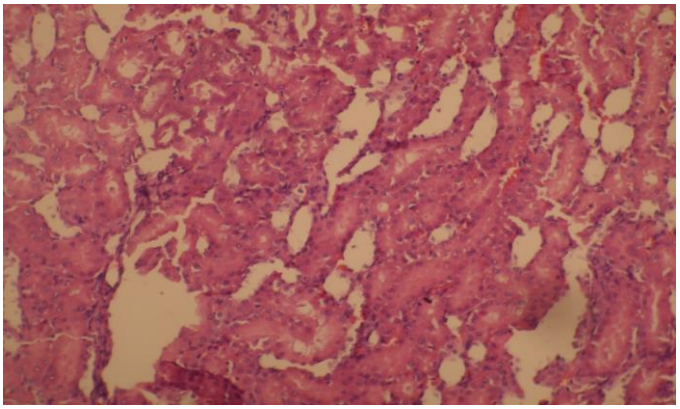


Fig. 5.4: Analysis of the Kidney Histology in Rats of Treatment Group 2 Showing the alleviation of necrosis and inflammation, rats treated orally with flower extract of plants 200mg/kg/day+ cisplatin 7mg/kg *i.p.*

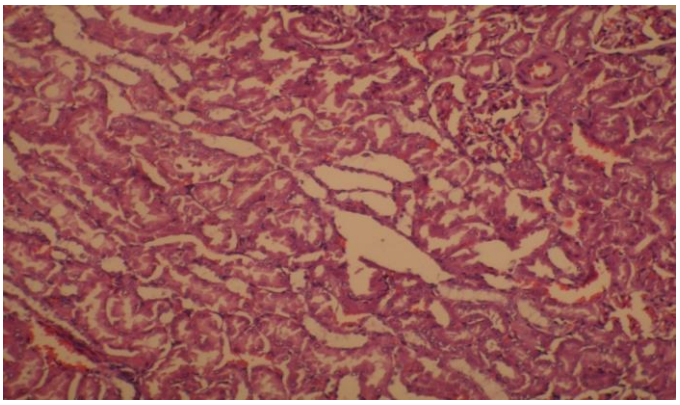


Fig. 5.5: Histopathology of the renal of treatment group 3 Showing the alleviation of necrosis and inflammation, rats treated orally with leaves extract of plants 100mg/kg/day+ flowers extract of plants 100mg/kg/day+ cisplatin 7mg/kg *i.p.*

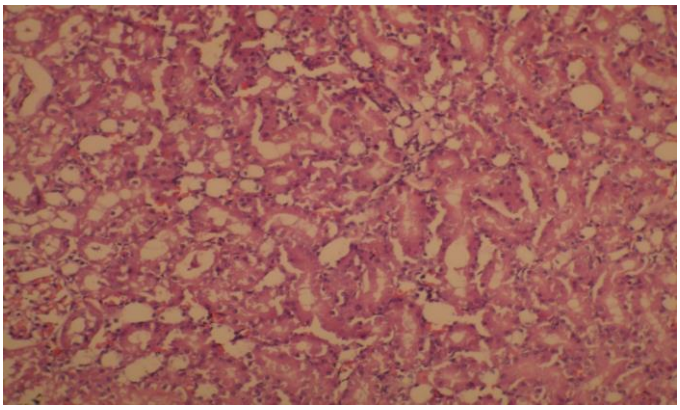


Fig. 5.6: Amlodipine was administered orally at a dose of 2 mg/kg per day, while cisplatin was administered intraperitoneally at a dose of 7 mg/kg. Histopathology analysis of the rat kidney from the STD group showed that the medication reduced necrosis and inflammation in renal tissue.

DISCUSSION

The impairment of kidney function by cisplatin is recognized as the main side effect and the most important dose-limiting factor associated with its clinical use. Several investigators reported that the alterations induced by cisplatin in the kidney functions were characterized by signs of injury, such as changes in GSH and MDA levels in kidney tissue, serum creatinine blood urea nitrogen serum, and uric acid levels in plasma.[16] Cisplatin has a well-established role in the treatment of solid cancer in many patients; however, its use is limited because of its toxicity to various organs including renal toxicity, gastrointestinal toxicity, and ototoxicity.[17] Although the standard approach for preventing cisplatin-induced nephrotoxicity is administering lower doses of cisplatin in combination with intensive intravenous isotonic saline with the resultant diuresis.[18] Studies with several models showed that the metabolism of chemotherapeutics and xenobiotics often produced GSH depletion. Reduced renal GSH can markedly increase the toxicity of cisplatin. The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the cisplatin-treated group. This may be due to the loss of copper and zinc, which are essential for the activity of enzymes, or the ROS-induced inactivation of enzyme proteins.[19]

CONCLUSION

In conclusion, the presence of Gallic acid, flavonoid, and vitamin E in Delonix regia flowers is likely responsible for the plant's nephroprotective properties. The flower extract of the plant and the combination of leaves and flower extract at 1:1 concentration have anti-oxidant and nephroprotective potential due to the presence of flavonoids and Gallic acid.

The administration of Ethanolic extract of Delonix regia flowers provides preferable prevention of cisplatin-induced nephrotoxicity by reducing plasma creatinine and blood urea nitrogen levels, serum uric acid as compared to a combination of leaves and flowers extract at 1:1 and leaves extract 200mg/kg.

The administration of Ethanolic extract of Delonix regia flowers provides preferable Prevention of cisplatin-induced nephrotoxicity through the reduction of malondialdehyde (MDA) levels, and increasing level of GSH as compared to a combination of leaves and flowers extract at 1:1 and leaves extract 200mg/kg.

Histopathology of treatment groups' kidney preparations showed reduced cisplatin-induced tubular necrosis and inflammation. Cisplatin causes renal vascular resistance and proximal

tubular cell histopathology. However, renal tissue GSH levels increased, and MDA levels decreased after treatment with Ethanolic extract of Delonix regia flowers.

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Conflict of interest

The Authors declare no conflict of interest.

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