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EVALUATION OF *DELONIX REGIA* (LEAVES) WAS USED TO INVESTIGATE FOR THEIR PROTECTIVE ABILITY OF THE KIDNEY AGAINST GENTAMYCIN-INDUCED NEPHROTOXICITY

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ABSTRACT

The present study aims to examine the potential beneficial effects of Delonix regia (leaves) against gentamicin-induced renal injury in vitro and in vivo, as well as to investigate the main underlying pathogenic mechanisms. Gentamycin is a widely used aminoglycoside antibiotic that has proven efficiency against life-threatening Gram-negative bacterial infections. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and ground to powder. The air-dried and powdered defatted marc of the drugs was subjected to extraction with hydroalcoholic solvent (ethanol: water: 80: 20). Tested for the presence of alkaloids, glycosides, flavonoids, and carbohydrates etc. In conclusion Preliminary phtochemical investigation on 80:20 hydroalcoholic extract of Delonix regia leaves showed the presence of bioactive compounds such as Glycosides, flavonoids, diterpenes, phenolics, carbohydrates, saponins compounds and alkaloids, proteins and amino acids are absent. A group of natural polyphenols called “flavanoids” are of most popular interest because reserchers have found them to be of health contributing potenial. The total phenol content was 1.690 (mg GAE/100mg of leaves extract while the flavonoid content was 1.305 (mg QE/100mg) of hydroalacolic extract. Therefore, Delonix regia leaves represented a novel therapeutic tool in the prevention and treatment of gentamicin-induced nephrotoxicity in the clinical setting.

KEYWORDS: Gentamycin, nephrotoxicity, Delonix regia, kidney.

INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998).

The demand for medicinal plants is continuously increasing not only in developing countries, but also in developed countries as drug, food supplements (nutraceuticals) and cosmetics (Ramawat et al., 2004). Tyler defines herbal medicines as "crude drugs of vegetable origin utilised for the treatment of diseased state often of a chronic nature or to attain or maintain a condition of improved health". (Tyler; 1994). There are certain pockets in a country like India where the tribal people have no access to modern amenities like roads, telecommunications or electricity, and therefore, these communities rely only on their traditional knowledge of medicine for day-today requirements (Katewa et al., 2006). It is well established that industrialisation has many direct and indirect effects on the human population. Increased stress is the most evident, although this is offset by increased health awareness among the people and better medical facilities. Nevertheless, increases in the incidence of diseases (mostly in urban populations) such as coronary heart disease, diabetes, hyperlipidaemia, AIDS and cancer cannot be denied (Merillon et al., 2007).

The Indian Herbal Drugs Scenario in Global Perspectives 325 this plant was collected from forests, but it is now cultivated in several thousand hectares and consumed in tonnes, but nothing is known about its chemistry and pharmacology (Arora et al., 2004; Arora et al., 2006).

Anatomy and physiology of kidney

Paired kidneys are reddish bean shaped organs about 10-12 cm long, 5-7 cm wide and 3 cm thick and have a mass of 135-150 g (Tortora, 1988). The kidneys lie on the posterior abdominal wall, one on each side of vertebral column, behind the peritoneum and below diaphragm. Near the centre of concave boarder is a deep vertical fissure called the renal hilum, through which the ureter emerges from the kidney along with blood vessels, lymphatic vessels and nerves. The kidney consists of two distinct region, outer renal cortex and inner renal medulla. The urine collects to calyx and then to renal pelvis which empties into ureter.

The functional unit of kidney is nephron and there are about 1million nephron in each kidney. They extend from the level of 12th thoracic vertebrae to 3rd lumbar vertebrae. (Ross and Wilson, 1997).

Nephrotoxicity

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, NSAID's, chemotherapeutic agents have been added to the therapeutic arsenal in recent years. Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy (Nadkarni and Nadkarni, 1927). Nephroprotective agents are the substances which possess protective activity against Nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Early literatures have prescribed various herbs for the cure of renal disorders. Co- administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity. The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood5. In addition to this, there is a failure of regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure (Williams, 1995).

MATERIAL AND METHODS

Leaves of Delonix regia are quite evident from the literatures surveyed that these plant number of antioxidant activity. The aim of our study is to provide scientific evidence concerned to the antioxidant values of this herb. Physicochemical investigations were performed on the hydroalcoholic extract of these plants using systemic as follows:

Plant material collection and authentication

The plant Delonix regia (leaves) was collected from local area of Bhopal (M.P.) in the month of January, 2019. The plant has been selected on the basis of its availability and Folk use of the plant. Drying of fresh plant parts was carried out in sun but under the shade. Dried

Delonix regia (leaves) was preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction Procedure

Following procedure was adopted for the preparation of methanol extracts from the shade dried and powdered herbs. Delonix regia (leaves) was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Dried powdered of Delonix regia (leaves) has been extracted with hydroalcoholic solvent (ethanol: water: 80: 20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 400C (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{Weight\ of\ Extract}{Weight\ of\ powder\ drug\ Taken} \times 100$$

Preparation of reagents Mayers Reagents

Dissolve 1.358g of HgCl2 in 60ml of water and pour into a solution of 5g of KI in 10ml of H2O Add sufficient water to make 100 ml.

Dragendorff's Reagents

Mix 850 mg of bismuth subnitrate with 40 mL of water and 10ml of glacial acetic acid (solution A). Dissolve 8g of potassium iodide with 20 mL of water (solution B). Mix equal portion of solution A and solution B to obtain a stock solution which can be stored for several months in a dark bottle. Mix 10 mL of stock solution with 20 mL of glacial acetic acid and dilute with water to make 100 mL.

Hagers regents

Dissolve 1g of picric acid in 100 ml of water.

Wagner' reagents

Dissolve 25g of citric acid and 1g of salicylic acid in water and dilute to 1 liter. Use 50 ml of the reagent.

Molish Reagents

Dissolve 15g of α-naphthol in 100ml of alcohol or chloroform. α-Nitroso-β-naphthol. Make a saturated solution in 50 % acetic acid (1 part of glacial acetic acid with 1 part of water).

Fehling's test

a. Copper sulfate solution: Dissolve 34.66g of CuSO4 5H2O in water and dilute to 500 ml.
b. Alkaline tartrate solution:Dissolve 173g of potassium sodium tartrate (Rochelle salts, KNaC4H4O6.4 H2O) and 50g of NaOH in water and dilute when cold to 500 ml. For use, mix equal volumes of the two solutions at the time of using.

Benedict's test

With the aid of heat, dissolve 173g of sodium citrate and 100g of Na2CO3 in 800ml of water. Filter, if necessary, and dilute to 850 ml. Dissolve 17.3 g of CuSO4.5H2O in 100ml of water. Pour the latter solution, with constant stirring, into the carbonate-citrate solution, and make up to 1 liter.

Million's reagent

Dissolve 1 part of mercury in 1 part of cold fuming nitric acid. Dilute with twice the volume of water and decant the clear solution after several hours.

Phytochemical Screening

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods.

- Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
  - Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
  - Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

- Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

➢ Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
- Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
- Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

➢ Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

- Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

➢ Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

➢ Detection of saponins

- Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
- Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

- Detection of phytosterols
  - Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
  - Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

- Detection of phenols
  - Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

- Detection of tannins
  - Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

- Detection of flavonoids
  - Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
  - Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
  - Detection of proteins and aminoacids
  - Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
  - Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

- Detection of diterpenes
  - Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Roopashreeet al., 2008; Obasi et al., 2010; Audu et al., 2007).

- Total Phenolic content estimation
  - The total phenolic content of the extract was determined by the modified Folin- Ciocalteu method.
  - Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.
  - Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1 mg/ml) of this solution was used for the estimation of phenol.
  - Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5 g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Olufunmiso and Anthony, 2011).

- Total flavonoids content estimation
  - Determination of total flavonoids content was based on aluminium chloride method
  - Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.
  - Preparation of extract: 10 mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1 mg/ml) of this solution was used for the estimation of flavonoid. Procedure:1 ml of 2% AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Qualitative chromatographic analysis

Thin layer chromatography

Thin layer chromatography: TLC is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase

Steps involved in TLC

- Preparation of plates
- Activation of plates
- Preparation and saturation of chamber
- Sample application and development
- Detection and calculation of Rf Value TLC of extract was performed and reported.

Preparation of Plates

- Silica gel, the most frequently used stationary phases, was employed as such for adsorption TLC
- To reduce the band broadening the stationary phase should consist of small particles of uniform size so as to provide a large surface area for interaction and a small void volume.
- Silica Gel was mixed with water and made into slurry.
- Coated the slurry by spreading the slurry on the plate uniformly.
- Firstly air dried the plate for some time and then kept for activation.

Activation of Plates

1. By heating the plates in an oven at 100 to 110oc for 30 minutes

- Activation is necessary for linear movement of solutes over stationary phase.

Preparation and Saturation of Chamber

1. Prepared the solvent system.
2. Poured it into the chamber and saturated the chamber by lining the chamber with a piece of filter paper that has been wet with the mobile phase

Sample application and development

- After plates were activated, the sample, which may be range from a few µg to mg. was applied on the plates with the help of capillary tube.
- Plates were placed in the chamber that contains developing solvent to a depth of about 0.5 cm.
- Plates were then removed from the chamber, the mobile phase front is marked by scratching the surface, and the solvent was evaporated in an oven.

Detection and Calculation of Rf. Value

1. Once the chromatogram was developed the Rf Value of the spot was calculated using the formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

In Vivo Study

Animals

Albino wistar rats weighing 200 g (±20 g) gm were used for all experiments in present study. Animals were collected at random from Animal house of PBRI, Bhopal. All animal

experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal. Animals were housed in a group of four in separate cages under controlled conditions of temperature (22± 2°C). All animals were given standard diet (golden feed, New Delhi) and water ad libitum. Animals were kept at 12:12, light: dark cycle. Animals were further divided in 6 groups with four animals in eachgroup.

Experimental protocol

Animals were divided randomly into four groups of 6 animals each and placed in separate cages. The extract of DRL and gentamicin were administered for a total duration of 8 days. The test extract was started 3 days prior to the commencement of the study (Jabbari et al., 2011).

Toxological Study

The dose of delonix regia was selected on the basis of the research article which has reported the dose, 100 mg/kg. The LD50 Dose was 2500 mg/kg. So the dose selected for extract as per OECD guidelines No. 420; fixed dose method was mentioned below. 100 mg/kg (1/25th of 2500 mg/kg). The dose of delonix regia was selected on the basis of the research article which has reported the dose, 200 mg/kg. The LD50 Dose was 4000 mg/kg. So the dose selected for extract as per OECD guidelines No. 420; fixed dose method was mentioned below. 100 mg/kg (1/5th of 4000 mg/kg). The dose of Gentamycin (80 mg/kg/day) was selected on the basis of research article which has reported the dose.

Table No. 1: Grouping of animals were as follows.

Group I received control was given normal saline in the dose of 30ml/Kg, twice a day
Group II received daily intraperitoneal injections of gentamicin (80 mg/ kg).
Group III received test extract DRL orally at 100 mg/kg b.w.
Group IV received test extract DRL orally at 200 mg/kg b.w.
Animals of group three and four were administered 80 mg/kg of gentamicin i.p. along with extract for 8 days.

Biochemical assays

After 24hrs of the last day, blood samples were collected by retro orbital puncture.

The serum was rapidly separated and processed for determination of serum creatinine, serum urea, serum uric acid and blood urea nitrogen (BUN) as an indicator of kidney damage, using commercially available kits from Span Diagnostics Private Ltd. The animals were sacrificed and kidney was isolated for histopathological examination.

Table No. 2: Groups and Treatment.

S. No.	Groups	Treatment
1.	Control	Normal saline in the dose of 30 mg/Kg i.p
2.	Gentamycin Group	(80 mg /kg, i.p.)
3.	Sample DRL 100	Gentamycin + Sample 1 (100 mg/kg, b.w)
4.	Sample DRL 200	Gentamycin + Sample 1 (200 mg/kg, b.w)

RESULTS AND DISCUSSION

Determination of Percentage Yield

Yield of Extraction: The crude extracts so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant. The yield of extract obtained from leaves of Delonix regia using hydroalcoholic as solvent is depicted in the table below:

Table No. 3: % Yield of Delonix regia(leaves).

S. No.	Solvent	% Yield (W/W)
1	Hydroalcoholic (80:20)	3.4%

% Yield of Hydroalcoholic extract of Delonix regia was found 3.4 W/W%.

Phytochemical screening of extract

A small portion of the dried extracts were subjected to the phytochemical test using (Kokate, 1994) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extract of all samples. Small amount of extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table below.

Table No. 4: Result of Phytochemical Screening of hydroalcoholic extract of Delonix regia (leaves).

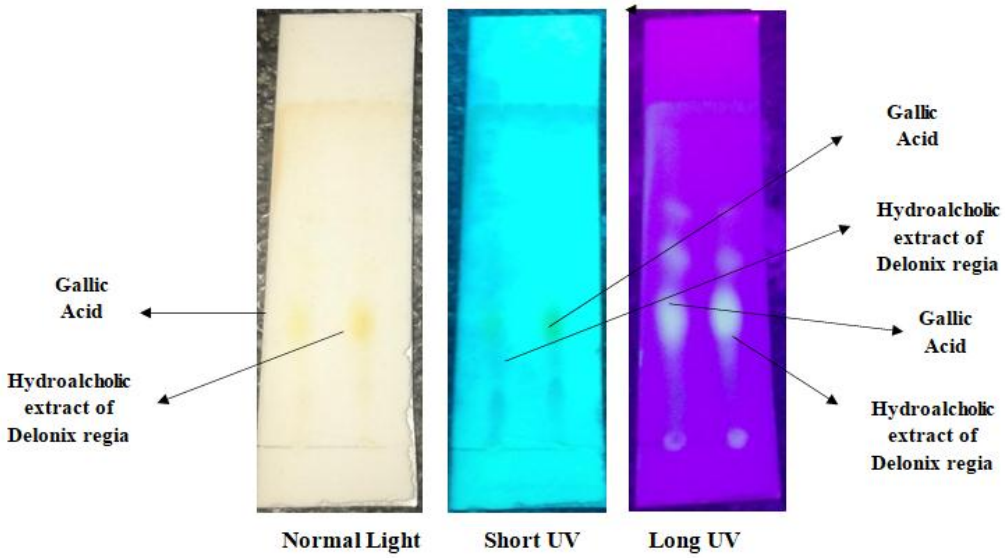
S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	-
2.	Glycosides	+
3.	Flavonoids	+
4.	Diterpenes	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	+
8.	Proteins	-
9.	Saponins	+

Phytochemical analysis is done for analyzing secondary metabolites which are responsible for curing elements: - An initial screening of effectiveness of different solvent extracts was done by Phytochemical analysis. The results of phytochemical analysis of Hydroalcoholic extract of Delonix regia shows the presence of bioactive compounds such as flavonoids, diterpenes, phenolics, carbohydrate, saponins, compounds and alkaloids, proteins and amino acids are absent.

Results of Thin Layer Chromatography of hydroalcoholic extract of Delonix regia (leaves)

Table No. 5: Calculation of Rf. Value.

S. No.	Compound	Extract Rf Value
1.	Gallic acid	Toluene: Ethyl acetate: Formic acid (7:5:1)0.68
2.	Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)0.78

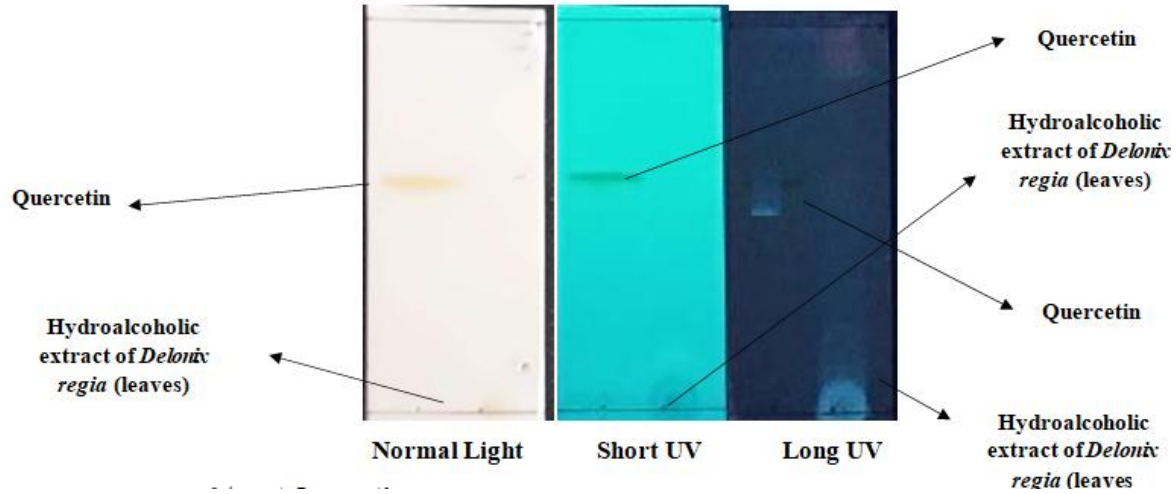


1st spot Gallic acid

2nd spot hydroalcoholic extract of Delonix regia (leaves)

Figure 1: Photograph of TLC (Gallic acid).

Gallic acid was used as standard for phenol. Rf value 0.68 was identified as Gallic acid. The solvent system used was (Toluene: Ethyl acetate: Formic acid (7:5:1).



1st spot Quercetin

2nd spot hydroalcoholic extract of Delonix regia (leaves)

Figure 5.2: Photograph of TLC (Quercetin).

Quercetin was used as standard for flavonoid. Rf value 0.78 was identified for flavonoid. The solvent system used was (Toluene: Ethyl acetate: Formic acid (5:4:1).

Results of Estimation of Total Phenolic and Flavonoids Contents

Total Phenolic content estimation (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.011X+0.011, R2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

S. No.	Concentration	Absorbance
0	0	0
1	10	0.135
2	20	0.247
3	30	0.364
4	40	0.474
5	50	0.581

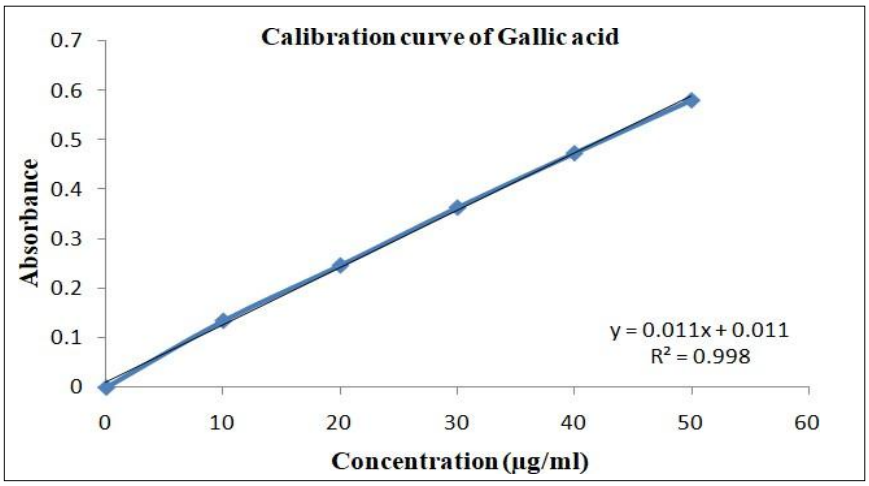


Figure 2: Graph of calibration curve of Gallic acid at λmax 765 nm.

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.040X + 0.009, R2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 6: Preparation of calibration curve of Quercetin at λmax 420 nm.

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

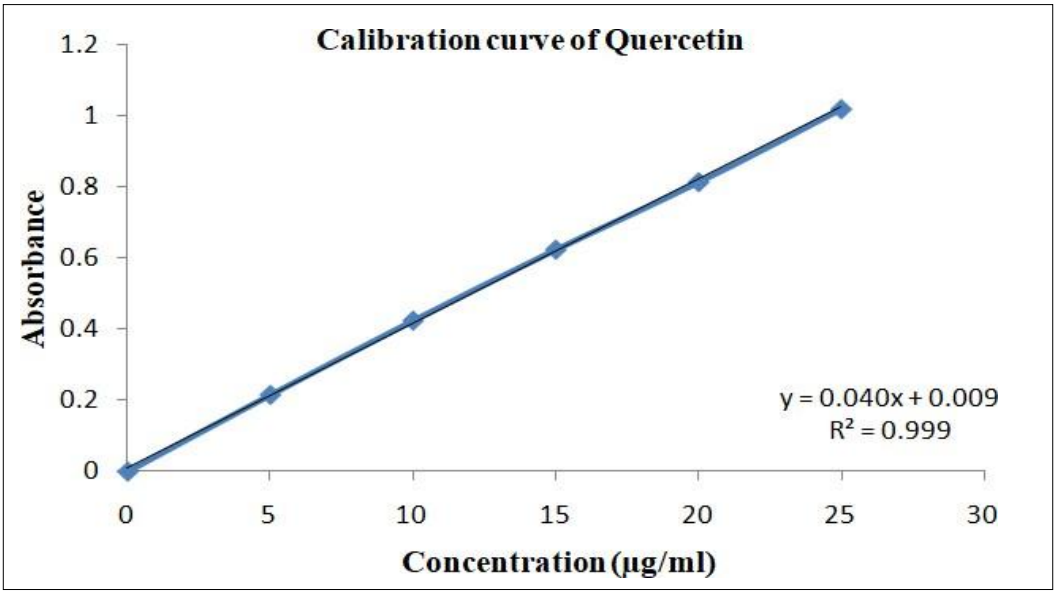


Figure 3: Graph of calibration curve of Gallic acid at λmax 765 nm.

Observation Table of Total phenolic and Flavonoids Content

Table No. 7: Estimation of Total phenolics and Total flavonoids content.

S.No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/100mg of driedextract)
1	*Delonix regia* (leaves)	7.81	1.94

A group of natural polyphenols called "flavonoids" are of most popular interest because researchers have found them to be of health-contributing potential. The total phenol content was 1.690 (mg GAE /100mg) of leaves extract while the flavonoid content was 1.305 (mg QE /100mg) of hydroalcoholic extract.

Observation table of In vivo Nephroprotective activity of Delonix regia (leaves)

Table No. 8: In Vivo Study results.

S.No.	Treatment/Group	BUN Mean ± SEM	Creatinine Mean ± SEM	Urea Mean ± SEM	Uric acid Mean ± SEM
1	Control	35.91±2.156	0.568±0.142	60.61±0.48	1.075±0.256
2	Gentamycin	47.765 ± 9.150	0.880± 0.292	105.51 ± 8.911	3.406 ± 0.994
3	Sample DRL 100 + G	44.432 ± 9.382**	0.786 ± 0.136*	92 .845 ±8.512*	2.441 ± 0.917*
4	Sample DRL 200 + G	39.248 ± 2.573***	0.670 ± 0.164**	65.530±6.120***	1.936±0.315**

Values are expressed as mean ± S.E.M (n = 4).Values are statistically significant at***P< 0.001; **P < 0.01; *P < 0.05 vs. control group (One-way ANOVA followed by Tukey's post hoc test).

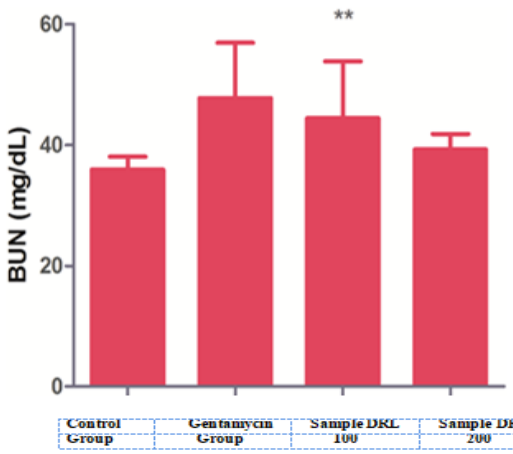


Figure 5.5: Blood Urea Nitrogen (mg/dL)

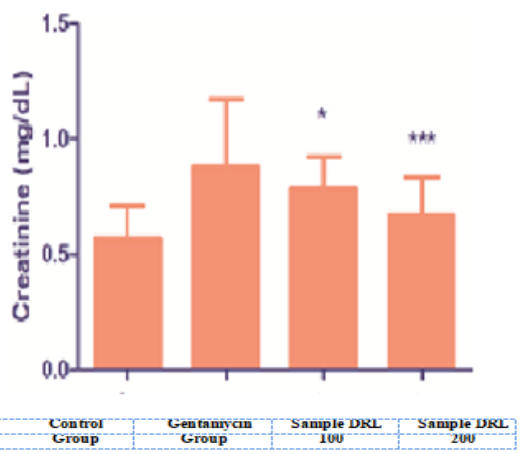


Figure 5.6: Creatinine (mg/dL)

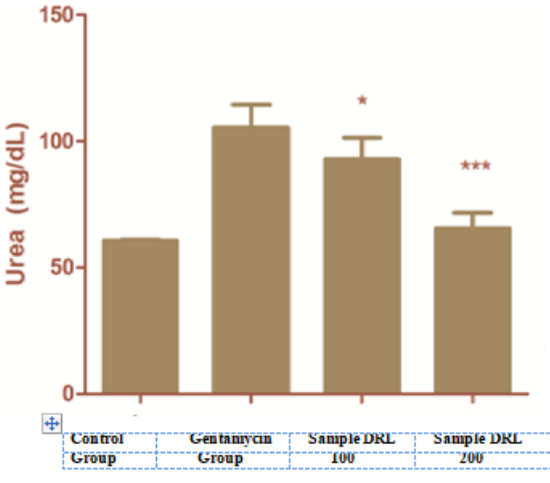


Figure 5.7: Urea (mg/dL)

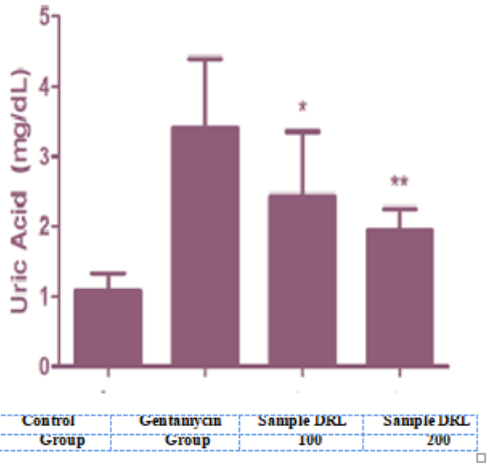


Figure 5.8: Uric Acid (mg/dL)

Gentamycin administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum BUN and in levels of blood urea nitrogen in plasma. Gentamicin induced renal injury was evidenced by the elevated biochemical markers [blood urea, serum creatinine] and by the histopathological features of acute tubular necrosis. Treatment with Delonix regia leaves extract was found to almost normalize the elevated blood urea, serum creatinine and bring about a marked recovery in kidneys as evidenced microscopically. However, morphological changes in kidneys were because of Gentamycin injection, but these changes tended to be considerably mild in Gentamycin plus sample treatment. In the conclusion, these finding powerfully supports that sample extract acts in the kidney to prevent the toxic effects of Gentamycin both in the biochemical and histopathological parameters and thus validate its ethnomedicinal use. The findings suggest that the potential use of Delonix regia leaves extract therapeutically used as a nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

Statistical Analysis

The result data are expressed as mean ± SEM for BUN, Creatinine, urea and uric acid was analyzed by one way analysis of variance followed by Tukey's test. The statistical significance was performed by using Graph Pad software and accepted at $P< 0.001$.

CONCLUSION

Preliminary phtochemical investigation on 80:20 hydroalcoholic extract of Delonix regia leaves showed the presence of bioactive compounds such as Glycosides, flavonoids, diterpenes, phenolics, carbohydrates, saponins compounds and alkaloids, proteins and amino acids are absent. A group of natural polyphenols called “flavanoids” are of most popular interest because reserchers have found them to be of health contributing potenial. The total phenol content was 1.690 (mg GAE/100mg of leaves extract while the flavonoid content was 1.305 (mg QE/100mg) of hydroalacolic extract. In histopathological study, these sections were examined under a photomicroscope for the presence of glomerulus, proximal convoluted tubule (PCT), and distal convulated tubule (DCT), tubular degeneration, mononuclear/polymorponuclear cell and narrowing of Bowman’s capsule. Histological study of the kidney tissues indicated that cytoarchitecture of the glomerulus, PCT, DCT, and tubular degeneration was maintained in group-I (normal) while cellular necrosis and glomerular hypercellularity were observed in group-II (gentamycin treated group). Rats which were administered with Delonix regia extract showed nearly normal glomerulus, PCT, DCT structures and renal tubules. The study measured the renal function markers lik BUN, creatinine, urea and uric acid level, which was found to be significantly ($P< 0.001$) higher in the Gentamycin treated animals when compared to that of normal group. This increased level was found to be decreased significiantly ($p<0.01$) by hydroalcoholic extract treatment. Gentamycin induced nephrotoxicity is characterized by elevated levels of urea and creatinine in plasma as well as urine, severe proximal tubular necrosis, renal failure were found to be significantly increased in rats treated with only Gentamycin. Similar pattern of changes were also observed in our study following Gentamycin treatment. Sample to Gentamycin treated rats recorded decrement in levels of urea and creatinine in plasma as well as urine. These observations indicate at an improved renal function in form of effective clearance of urea and creatinine.

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Conflict of interest

The Authors declare no conflict of interest.

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