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"PHARMACOLOGICAL EVALUATION OF THE NEPHROPROTECTIVE ACTIVITY OF LARREA TRIDENTATA LEAVES EXTRACT IN RATS"

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ABSTRACT
The present study investigates the Nephroprotective activity of *Larrea tridentata* leaves in rat models, aiming to explore its effectiveness and mechanism of action the term "nephrotoxicity" refers to the quickly declining kidney function brought on by the harmful effects of drugs and chemicals. Traditional medicine has long utilized various plant extracts for their Nephroprotective Activity, and *Larrea tridentata* is one such candidate with promising anecdotal evidence. To assess the diuretic active Nephrotoxicity, Wistar rats were administered varying doses of *Larrea tridentata* flower extract. The study employed a control group, Standard silymarin group, and two experimental groups receiving different concentrations of the extract. Urine output was measured over a 24-hour period. Additionally, parameters such as Estimation of Serum Creatinine and Estimation of Serum Blood urea nitrogen were monitored to assess the safety and mechanism of action of the extract. the rats treated with single dose of Gentamycin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomourular filtration rate, which is accompanied by elevated serum creatinine levels indicating induction of acute renal failure with *Larrea tridentata* at the dose level of 100 and 200 mg/kg body weight for 14 days significantly lowered the serum level of creatinine with a significant weight gain and increased urine output when compared with the nephrotoxic control group. Further studies are needed to isolate active compounds and understand their mechanisms, contributing to natural product pharmacology.

Keywords:
Nephroprotective activity, *Larrea tridentata*, Traditional medicine, Urine output, Electrolyte balance**.**

1. INTRODUCTION
The use of plants as medicines has increased as a result of the growing link between humans and plants. As our understanding of how to treat illnesses grew, so did the number of novel medications derived from plants **(Bernhoft *et al.*, 2010).** The growing global demand for natural plant treatments, particularly in Indian Traditional Medicine, has led to a surge in the popularity of herbal drugs due to their safety, efficacy, and cost-effectiveness **(Chauhan, and Johnson, 2009).** Early literatures have prescribed numerous herbal drugs for the treatment of renal disorders and also reported that concurrent administration along with different nephrotoxic agents reduced the toxic effects. **(Malathi, 2009).** An estimated 7800 medical medicine production facilities exist in India and these facilities are thought to use 2,000 tons of herbs a year on average **(Dubey *et al.*, 2004).** Nephrotoxicity is brought on by aminoglycosides and specifically targets the proximal tubule epithelial cells through the multi-ligand receptor megalin-mediated selective endocytosis and accumulation of aminoglycosides. Recently, a set of consensus phenotypic criteria for induced nephrotoxicity was presented. In observational studies, novel renal biomarkers have demonstrated potential in identifying proximal tubular injury sooner than established indicators. One such marker is kidney injury molecule-1. Further studies need to demonstrate a clear association with clinically relevant outcomes to inform translation into clinical practice **(Kuraishy *et al.*, 2019).**
The rounded, bushy shrub *Larrea tridentata* is an evergreen shrub growing *the Mojave, Sonoran, and Chihuahuan Deserts of western North America*. The whole plant exhibits a characteristic odor

of creosote, from which the common name derives. It has been found that leaves possess Antioxidant Activity, Antitumor Activity, and Neuroprotective Effects **(Winarno *et al.*, 2023).**

The goal of this study was to accurately evaluate the nephroprotective properties of *Larrea tridentata's* methanolic extract. By employing standardized experimental protocols, the research seeks to elucidate the efficacy and safety profile of the flower extract, providing a scientific basis for its use as a nephroprotective agent. The outcomes of this study could contribute to the development of novel, plant-based nephrotoxic drug that are both effective and have minimal side effects, thus offering a valuable alternative to synthetic diuretic drugs.

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Larrea tridentata* (300 gm) was collected. After cleaning, plant part (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant part (leaves) was stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected Medicinal plant *Larrea tridentata* was authenticated by a plant taxonomist (Dr. Jagrati Tripati, Govt. Botanist) in order to confirm its identity and purity. **(Authentication no.-AC/068/24).**

2.2 Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Larrea tridentata powder was added to a thimble of a soxhlet device. Petroleum ether was used as a non-polar solvent for soxhlation, which was carried out at 60°C. After being dried, the exhausted plant material (marc) was extracted again using an ethanol solvent. Each solvent's soxhlation was continued until no visible color change was seen in the siphon tube, and the extraction's completion was verified by the absence of any solvent residue after it evaporated. A Buchi-type rotating vacuum evaporator was used to evaporate the obtained extracts at 40°C. After the dried extract was weighed, each extract's % yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al.*, 2002).**

2.3 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were used **(Kokate *et al.*, 2000).**

2.4 Quantitative Phytochemical Estimation

2.4.1 TPC

The total phenolic content of *Larrea tridentata* extract was determined using the Folin-Ciocalteu Assay. The *Larrea tridentata* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. A 7 mL dilution of this mixture was made using distilled water. Following a 2-hour room temperature incubation period, the resultant solutions were subjected to spectrophotometric absorbance measurement at 760 nm. Standard solutions of Gallic Acid Equivalent (GAE) mg/gm were used to create calibration curves. Gallic aid concentrations of 20, 40, 60, 80, and 100 µg/mL were made. Polyphenols and other reducing agents can be detected by the Folin-Ciocalteu reagent. In response, they become blue in color. Using spectrophotometry, this blue color was measured (**Tangco *et al.*, 2015)**.

2.4.2 TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Larrea tridentata* extract solution was erratic with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride

(10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Rutin concentrations of 20, 40, 60, 80, and 100 µg/mL were generated, and calibration curves were constructed using standard solutions of Rutin Equivalent (RE) mg/gm.Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy S *et al.*, 2009).**

2.5 DPPH

The antioxidant activity of *Larrea tridentata* extract was determined by using the DPPH test for scavenging free radicals 1 mg/ml methanol solution of extracts/standard was prepared.

Different concentration of *Larrea tridentata* extracts /standard (20 – 100µg/ml) were prepared was vortexed, allowed to sit at room temperature in a somewhat dark environment for 30 minutes, and then its wavelength was measured at 517 nm using a UV spectrophotometer from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The resultant mixture (Shimadzu 1700). For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm **(Athavale *et al.*, 2012).**

Percentage antioxidant activity of sample/standard was calculated by using formula:

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

2.6 Acute Toxicity Study

The acute toxic class approach described in the guidelines is a step-by-step process that uses 3 animals of the same sex for each phase. It may take an average of 2-4 steps to determine the acute toxicity of the test drug, depending on the animals' mortality and/or moribund state. A set of experimental animals receives the drug orally at one of the specified dosages. A methodical process is employed to test the chemical, employing three animals of the same sex in each phase. The next stage will be determined by whether compound-related mortality of the animals dosed at one step is present or absent, meaning that if more testing is not required, three additional animals will be dosed at the same dose and three other animals will be dosed at the next higher or lower dose level. Every phase involves the use of three animals. One of four fixed dose levels—5, 50, 300, or 2000 mg/kg body weight—is chosen to constitute the initial dosage (**Guideline Document on 1996**).

2.7*In vivo* study

2.7.1 Experimental protocol

IAEC Approval All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). CPCSEA Approval No.- **PBRI/IAEC/21-03-24/025.**

The experimental protocol was designed for 14 days. Rats of either sex were split up into five groups, and each group was comprised of six rats. Rats in every group were given the oral preparations with the feeding tube **(Rad *et al.*, 2017).**

Group-I: Served as normal control which was given 1 mL/kg normal saline daily

Group-II: Served as Nephrotoxic control, received 40 mg/kg Gentamycin intraperitoneally (i.p.) at the same time for 14 days

Group-III: Received the standard Nephroprotective drug 200 mg/kg silymarin per oral

Group-IV: Gentamycin + *Larrea tridentata* extract (100mg/kg; p.o)

Group-V: Gentamycin + *Larrea tridentata* extract (200mg/kg; p.o)

3. RESULTS

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Larrea tridentata shown* in Table: 1

Table 1: Percentage Yield of crude extracts of *Larrea tridentata* extract

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield

1	*Larrea tridentata*	Pet ether	286	1.65	0.57%
2		Methanol	299	6.10	2.04%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

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

3.3 Quantitative Analysis

Preliminary phytochemical tests on crude extracts indicated the presence of phenolics and flavonoids in plant material. To assess their level, assays were done for total phenolic (TPC) and total flavonoid

content (TFC).

3.3.1 Total Phenolic content (TPC) and Total Flavonoid Content (TFC) estimation

Table 3 Standard table for Gallic acid and Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.143
2.	40	0.176
3.	60	0.191
4.	80	0.238
5.	100	0.273

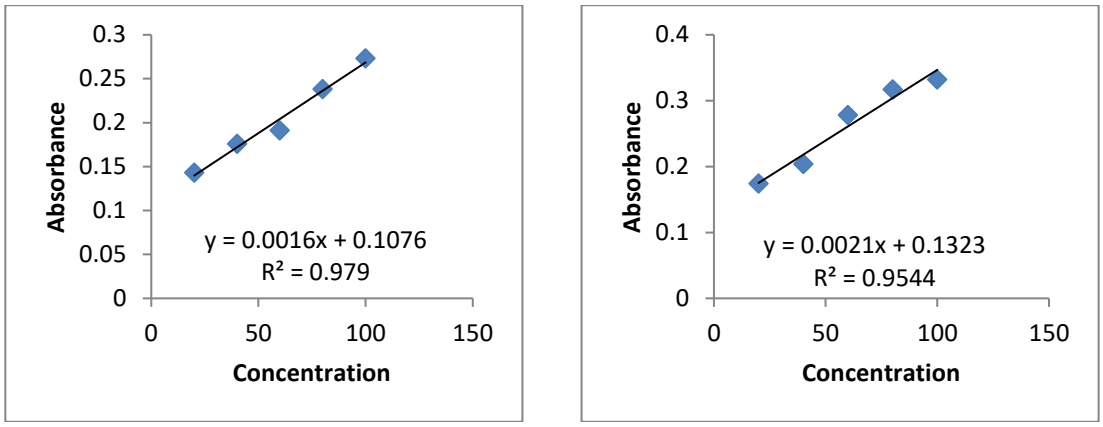


Figure 1: Represent standard curve of Gallic acid and Rutin

3.3.1.1 Total Phenolic Content in extract

Table 4: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.135	60.33 mg/gm
2	0.178	
3	0.189	

3.3.1.2 Total Flavonoid Content in extract

Table 5: Total Flavonoid Content

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.148	17.84 mg/gm
2	0.162	
3	0.193	

3.4 *In vitro* Antioxidant Assays

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

Table 6: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	51.111
40	0.435	56.060
60	0.344	65.252
80	0.285	71.212
100	0.145	85.353
Control	0.990	

IC50	22.24

Table 7: DPPH radical scavenging activity of methanol extract of *Larrea tridentata*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.519	43.831
40	0.465	49.675
60	0.454	50.865
80	0.413	55.303
100	0.367	60.281
Control	0.924	
IC50		49.84

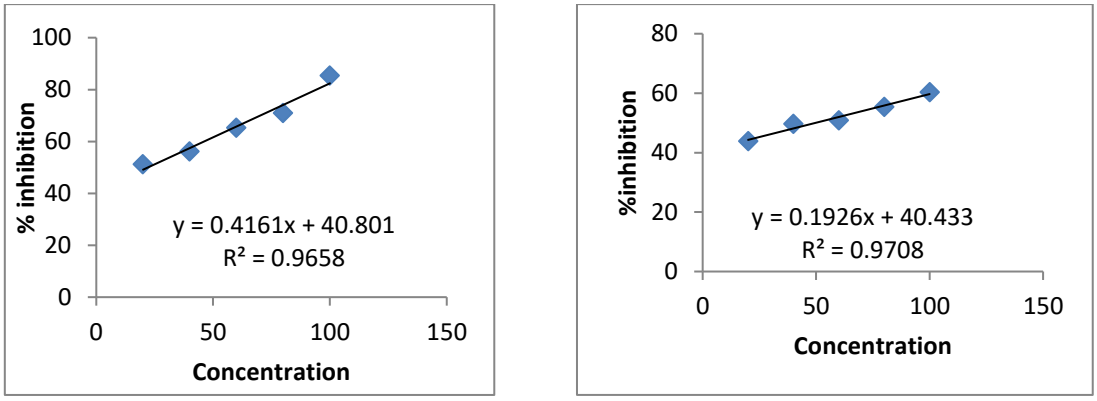


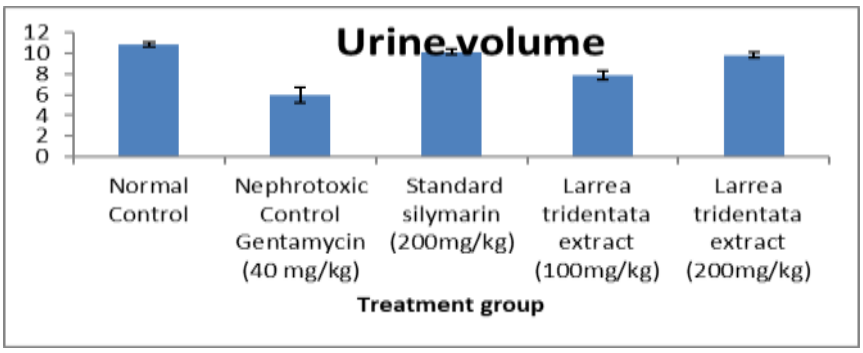
Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid and extract of *Larrea tridentata*

3.5 Analysis of general parameters

3.5.1 Estimation of urine volume

Table 8: Urine volume

Groups	Urine volume
Normal Control	10.88±0.23
Nephrotoxic Control Gentamycin (40 mg/kg)	5.98±0.75
Standard silymarin (200mg/kg)	10.14±0.25**
Larrea tridentata extract (100mg/kg)	7.87±0.41**
Larrea tridentata extract (200mg/kg)	9.80±0.28**



Graph 1: Urine volume

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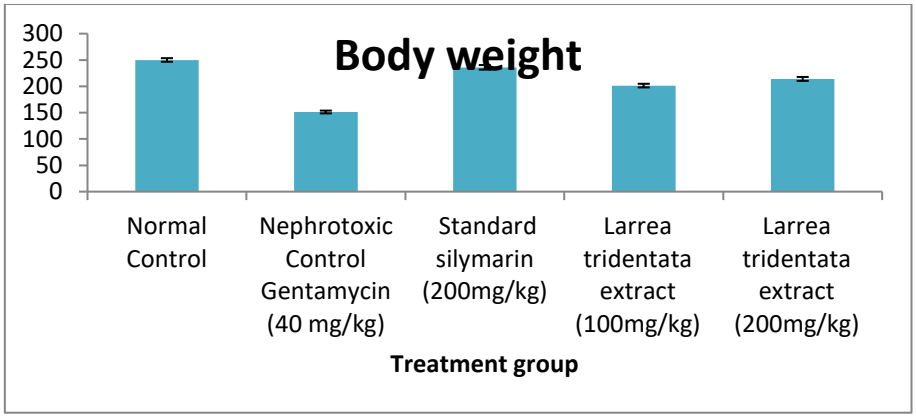
The urine volume of the group III and V is more significant than the group IV, *P<0.05, **P<0.01, ***P<0.001 when compared to control group.

3.5.2 Estimation of Body weight

Table 9: Body weight

Groups	Body weight
Normal Control	250±3.403
Nephrotoxic Control Gentamycin (40 mg/kg)	151.22±2.656
Standard silymarin (200mg/kg)	236±4.353**
Larrea tridentata extract (100mg/kg)	201.32±3.43**
Larrea tridentata extract (200mg/kg)	214±3.740**



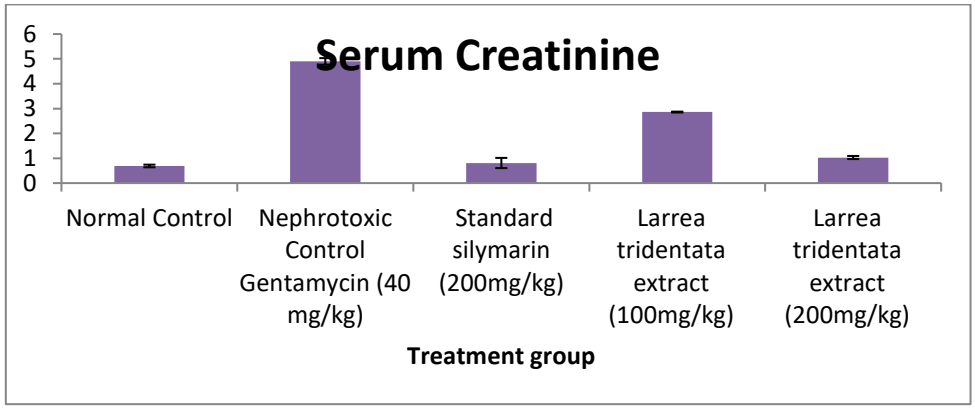
Graph 2: Body weight

3.6 Analysis of serum biochemical parameters

3.6.1 Estimation of Serum Creatinine

Table 10: Serum Creatinine

Groups	Serum Creatinine
Normal Control	0.69±0.053
Nephrotoxic Control Gentamycin (40 mg/kg)	4.90±0.131
Standard silymarin (200mg/kg)	0.81±0.205**
Larrea tridentata extract (100mg/kg)	2.86±0.018**
Larrea tridentata extract (200mg/kg)	1.03±0.062**



Graph 3: Serum Creatinine

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, **P< 0.001, compared to the normal control P< 1.00

3.6.2 Estimation of Serum Blood urea nitrogen (BUN)

Table 11: Serum Blood urea nitrogen

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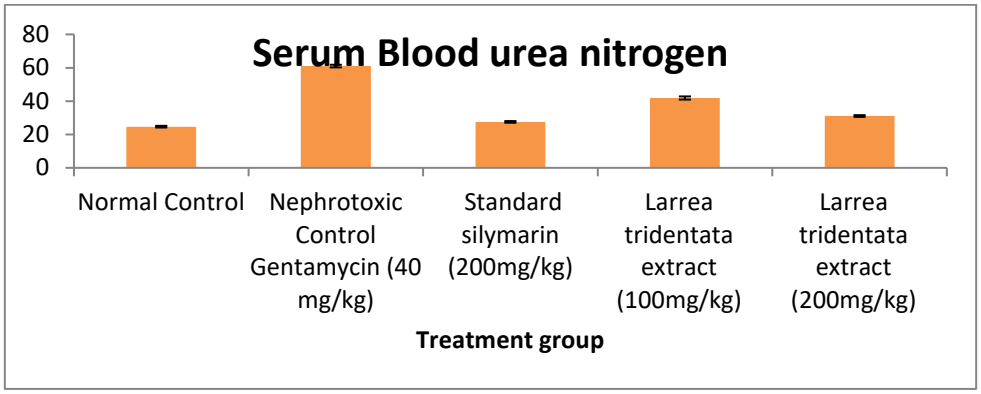
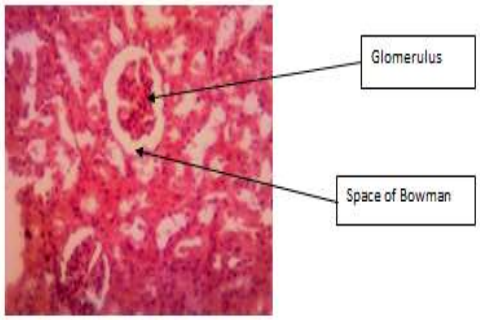
Groups	Serum Blood urea nitrogen
Normal Control	24.68±0.503
Nephrotoxic Control Gentamycin (40 mg/kg)	61±0.792
Standard silymarin (200mg/kg)	27.54±0.50**
Larrea tridentata extract (100mg/kg)	41.87±0.94**
Larrea tridentata extract (200mg/kg)	31.06±0.53**



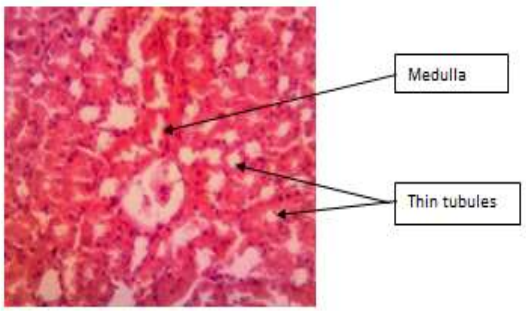
Graph 4: Serum Blood urea nitrogen

All the results are expressed as Mean ± SD (n=6), for each experimental group. The statistical analysis was carried out using one way ANOVA method. Significant after analysis of variance (ANOVA) followed by Bonferroni's test. *P<0.05, **P<0.01, ***P<0.001 when compared to control group.

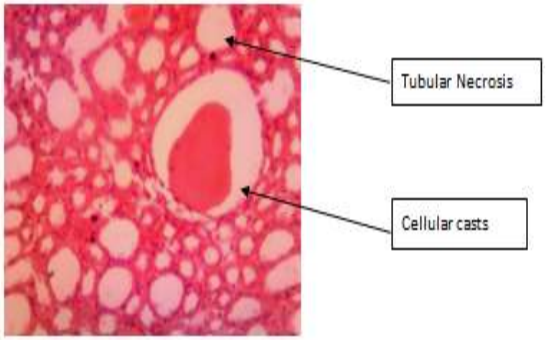
3.7 Histopathology



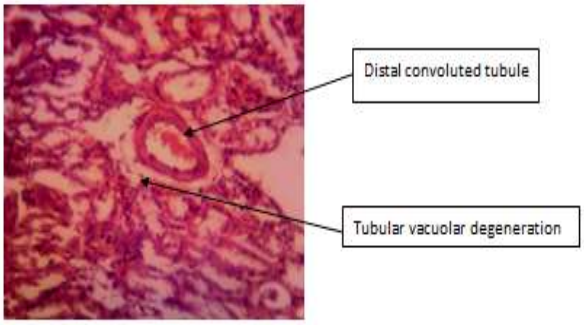
Group I: Normal



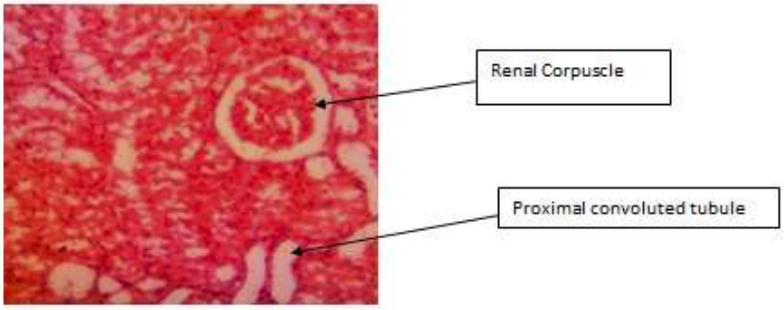
Group II: Standard



Group III: Induced



Group IV: 100mg of Extract



Group V: 200mg of extract

4. DISCUSSION

Phytochemical analysis of methanolic extract of *Larrea tridentata* showed the presence of alkaloids, phenolics, flavonoid, saponins, glycoside, saponin, tannin and phenolic. TPC and TFC were calculated as part of a quantitative phytochemical experiment. The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. Results shown in Table 7 & table10. DPPH action that scavenges radicals of *Larrea tridentata* extract exhibited percent inhibition 60.28% and its IC 50value was found to be 49.84µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 85.35% and showed IC 50 value of 22.24µg/ml. In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study. the rats treated with single dose of Gentamycin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomourular filtration rate, which is accompanied by elevated serum creatinine levels indicating induction of acute renal failure with *Larrea tridentata* at the dose level of 100 and 200 mg/kg body weight for 14 days significantly lowered the serum level of creatinine with a significant weight gain and increased urine output when compared with the nephrotoxic control group. When gentamycin was administered to control rats, a characteristic pattern of nephrotoxicity was observed, characterized by a significant rise in serum blood urea nitrogen (BUN). *Larrea tridentata* supplementation to Gentamycin treated rats recorded decrement in levels of blood urea nitrogen (BUN) in plasma. All the results are expressed as Mean ± SD (n=6), for each experimental group. The statistical analysis was carried out using one way ANOVA method. Significant after analysis of variance (ANOVA) followed by Bonferroni's test. *P<0.05, **P<0.01, ***P<0.001 when compared to control group.

5. CONCLUSION

The goal of this study was to accurately evaluate the nephroprotective properties of *Larrea tridentata's* methanolic extract. Histopathological studies on isolated kidney revealed that the *Larrea tridentata* reversed the kidney damage and also restored normal kidney architecture as a diuretic. The *Larrea tridentata* methanolic extract showed statistically significant nephroprotective activity. The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties.

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