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Pharmacological evaluation and Nephroprotective activity of *Coccinia indica* leaves extract

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Abstract

Kidney diseases are a major problem of worldwide proportions, and renal damage is very common since kidney has the capacity to excrete toxic substances. This study aimed to evaluate the protective effect of the extract of *Coccinia indica* against gentamicin induced nephrotoxicity in rats. Nephroprotective activity was estimated by inducing gentamicin (40mg/kg) to all the groups of animals; acute kidney dysfunction is an evidenced by significant elevation of serum creatinine and decreased body weight with multiple histological damages. Treatment with the *Coccinia indica* has shown dose-dependent improvement in the body weight at the dose of 100 and 200 mg/kg and also shown significantimprovement by protecting the kidney from the oxidative stress. It is also identified that treatment with *Coccinia indica* significantly lowered the level of serum creatinine when compared with the toxic group. Nephroprotective activity of *Coccinia indica* treatment was found compared with the standard group (200 mg/kg silymarin) and control group againstthe toxic control group animals in parameters including serum creatinine, urine volume and body weights. The histopathological studies were also evinced the protective effect of *Coccinia indica*.

Keyword: Nephrotoxicity, Creatinine, Kidney, Gentamicin.

INRODUCTION

Nephrotoxicity is defined as the rapid decline of kidney function caused by the toxic effects of drugs and substances. There are several types, and some medications can alter renal function in more than one way. Nephrotoxins are chemicals that exhibit nephrotoxicity. Nephrotoxicity should not be confused with the fact that some drugs, such as heparin, are mostly excreted through the kidneys and require dose adjustments to account for impaired renal function. The nephrotoxic effect of most drugs is more profound in patients already

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suffering from kidney failure. About 20% of nephrotoxicity is induced and caused by drugs. Nephrotoxicity is more common in elderly people, and it is linked to lower glomerular filtration and (mostly cardiovascular) co-morbidities. Sex has a significant impact on medications, particularly weight and body composition. Men, on average, have a higher BMI and body surface area than women. Body size disparities lead to higher distribution volumes and faster overall clearance of most drugs in men compared to women. Greater body fat in women (till later in life) may enhance the distribution volumes of lipophilic medicines in female patients. Pre-treatment CKD is one of the most significant risk factors for nephrotoxicity. Diabetes mellitus raises the risk of nephrotoxicity, particularly the NSAID induced variety. Decreased hydration raises drug levels in the peritubular circulation as well as in urine discharged into the PT, resulting in increased nephrotoxicity via the mechanism of ATN or tubular obstruction by crystals or casts containing medicines and their metabolites. Sepsis is a factor that impairs kidney function for reasons other than hemodynamics, with endotoxins interacting synergistically with potentially harmful chemicals. Hypoalbuminemia raises the risk of nephrotoxicity linked with the use of cisplatin and aminoglycosides.

Nephrotoxicity is dose-dependent in medications that cause kidney injury via the mechanism of ATN or tubular blockage by crystals or casts containing drugs and their metabolites. Treatment duration is especially significant for aminoglycosides and amphotericin. When it comes to aminoglycosides, the frequency of administration is important.

MATERIAL AND METHOD

Plant collection

The medicinal plant Coccinia Indica (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. Medicinal plant Coccinia Indica was authenticated by a plant taxonomist in order to confirm its identity and purity.

Extraction

Plant material was extracted utilizing the continuous hot percolation method with Soxhlet equipment. Coccinia Indica powder was placed in the thimble of a soxhlet apparatus. Soxhlation was performed at 60°C with petroleum ether as the non-polar solvent. The exhausted plant material (marc) was dried and then re-extracted with methanol solvent. Soxhlation was maintained for each solvent until no visible color change was noticed in the siphon tube, and extraction was confirmed by the absence of any residual solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and each extract's % yield was calculated.

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Phytochemical investigation

An experiment was carried out to determine the presence or absence of several phytoconstituents using thorough qualitative phytochemical analysis.

Quantitative Phytochemical Estimation

TPC

The Folin–Ciocalteu Assay was used to assess the total phenolic content of Coccinia Indica extract. The Coccinia Indica extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. The solutions were then allowed to rest at room temperature for 2 hours before being measured spectrophotometrically at 760 nm. Calibration curves were created with standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic Aid was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically.

TFC

The flavonoid content was measured using the aluminum chloride technique. 0.5 mL of Coccinia Indica extract solution was combined 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. The mixture's absorbance at 510 nm was measured using a UV spectrophotometer. Calibration curves were created using standard solutions containing Rutin Equivalent (RE) mg/g. Rutin concentrations were prepared at 20, 40, 60, 80, and 100 µg/mL. The calibration curve was used to calculate the total flavonoid concentration, which was then expressed as mg Rutin equivalent per gram dry extract weight.

DPPH

The DPPH free radical scavenging assay was used to determine Coccinia Indica extract's antioxidant properties. 1mg/ml methanol solution of extracts/standard was prepared. To prepare different concentrations of Coccinia Indica extracts/standards (20-100µg/ml), a 1mg/mL stock solution was mixed with 2mL of 0.1mM DPPH solution. The mixture was vortexed, incubated for 30 minutes at room temperature in a dark place, and then read using a UV spectrophotometer (Shimadzu 1700) at 517 nm. For the control, add 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. The absorbance of the control was measured against methanol (as a blank) at 517 nm.

FT-IR

To establish the presence of the functional groups in Coccinia Indica methanolic extract, FT-IR spectroscopy

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was performed using Perkin Spectrum BX spectrophotometer. The sample was dried and ground with KBr pellets and analyzed. A disk of 100 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were collected in the area of 400–4,000 cm-1.

Experimental protocol

The experimental protocol was designed for 14 days. Rats of either sex were divided into five groups, and each group was comprised of six rats. Rats in every group were given the oral preparations with the feeding tube.

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 + Coccinia Indica extract (100mg/kg; p.o)

Group-V: Gentamycin + Coccinia Indica extract (200mg/kg; p.o)

Blood collection techniques used in the present study

At the end of the experimental period, ie on the 15th day animals were sacrificed under mild ether anesthesia. The blood was collected by retro-orbital vein puncture using a fine capillary to an anticoagulant tube and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to evaluate the biochemical markers.

Preparation of kidney homogenate

The kidney was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 minutes; supernatant was collected and used for various biochemical assays.

Analysis of general parameters

Estimation of urine volume

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume are taken after 24 hours. The food wastes and fecel matters are removed from the urine. And the volume of urine is measured by using measuring cylinder.

Estimation of Body weight

At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal are individually weighed and the weight were recorded.

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Analysis of serum biochemical parameters

Estimation of Serum Creatinine

Five test tubes were labelled as A, B, C, D and E. Where A&B is taken as standard, C &D taken as test and the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml serum and 1.5 ml of water, into A&B (standard), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added in all the five test tubes.

Estimation of Serum Blood urea nitrogen

The blood urea was calculated using the Berthelot method (Fawcett and Scott, 1960) with a commercially available kit (Kamineni Life Sciences Pvt. Ltd., Hyderabad, India). To create the test, standard, and blank, 1000 µl of working reagent-I with urease reagent, salicylate, hypochlorite, and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl), and 10 µl of filtered water. All test tubes were thoroughly mixed and incubated at 37°C for 5 minutes. All test tubes received 1000 µl of reagent-II-containing alkaline buffer and were incubated at 37°C for 5 minutes. Urease catalyzes the conversion of urea into ammonia and CO2. The released ammonia combines with a mixture of salicylate, hypochlorite, and nitroprusside to create indo phenol, a bluegreen colored chemical. The intensity of the color produced is related to the concentration of urea in the sample and evaluated spectrophotometrically at 578 nm. The blood urea was calculated using the following formula:

$$\text{Blood urea (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times 40$$

RESULTS

Percentage Yield

In phytochemical extraction was calculated the percentage yield with pet ether 0.57% and methanol 2.04%.

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation

Table 1: Standard table for Gallic acid

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.143
2.	40	0.173
3.	60	0.192

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4.	80	0.234
5.	100	0.275

Total Phenolic Content in extract

Table 2: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.137	61.66 mg/gm
2	0.178	
3	0.185	

Table 3: Total Phenolic Content of extract Coccinia Indica

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	61.66

Total Flavonoids content (TFC) estimation

Table 4: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.174
2.	40	0.200
3.	60	0.278
4.	80	0.317
5.	100	0.330

Total Flavonoid Content in extract

Table 5: Total Flavonoid Content

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	**0.148**	**18.33 mg/gm**
2	**0.162**	
3	**0.193**	

Table 6: Total Flavonoid Content of extract Coccinia Indica

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Extracts	Total Flavonoid content (mg/gm equivalentof rutin)
Methanol	18.33

In vitro Antioxidant Assays

In the present investigation, the in vitro anti-oxidant activity of extracts of Coccinia Indica was evaluated by DPPH radical scavenging activity. The results are summarized in Tables.

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

Table 7: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	51.356
40	0.432	56.582
60	0.346	65.226
80	0.285	71.356
100	0.148	85.125
Control	0.995	
IC50		21.33

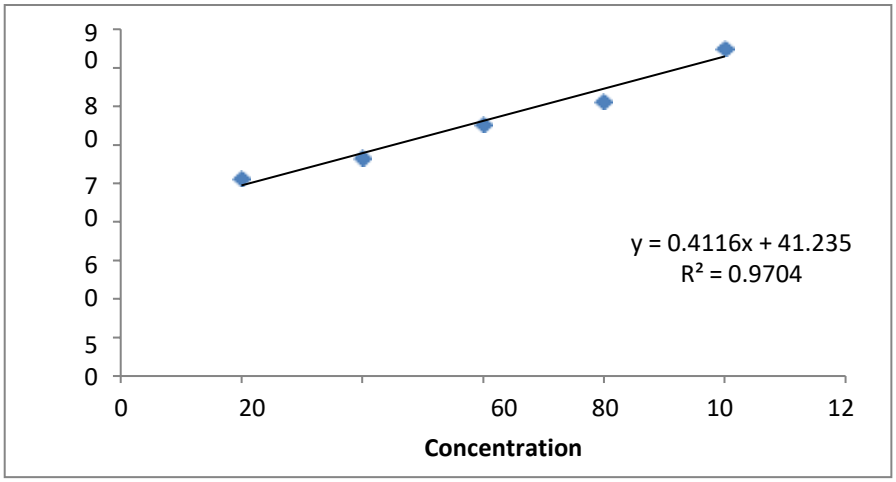

Figure 1: DPPH radical scavenging activity of Std. Ascorbic acid

Table 8: DPPH radical scavenging activity of methanol extract of *Coccinia Indica*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.518	43.878
40	0.467	49.404
60	0.456	50.595
80	0.414	55.146
100	0.368	60.130

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Control	0.923	
IC50		49.84

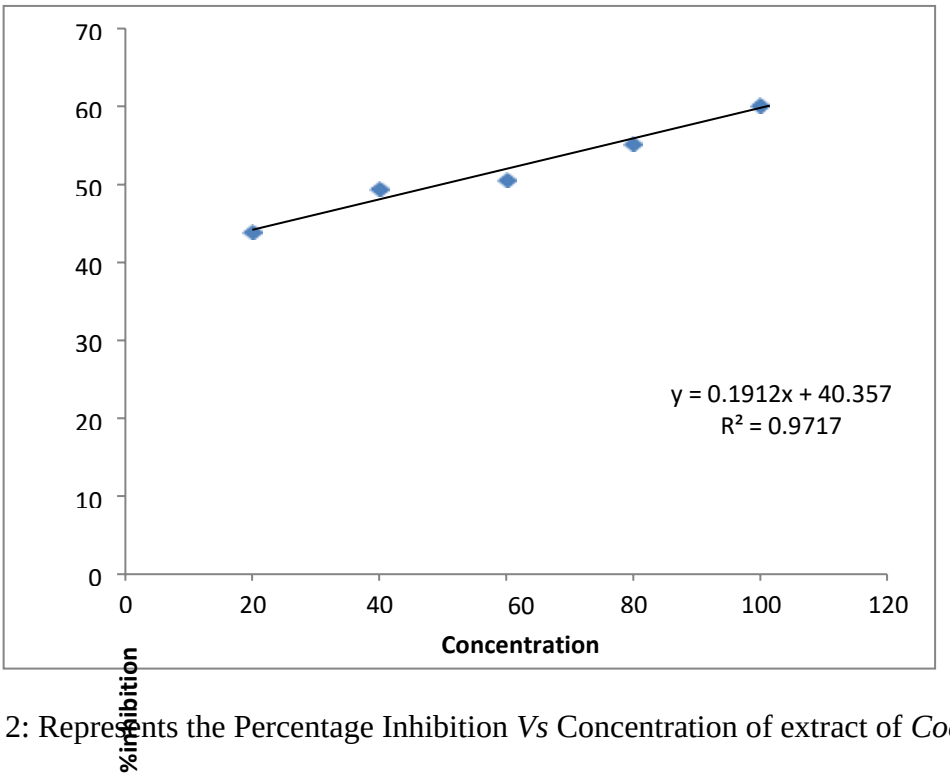

Figure 2: Represents the Percentage Inhibition *Vs* Concentration of extract of *CocciniaIndica*

Functional group identified by FTIR Study

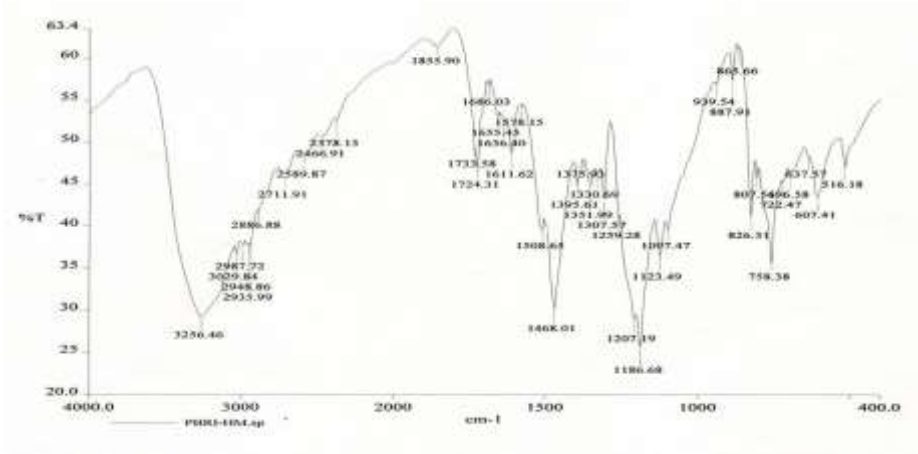

Figure 3: FTIR of *Coccinia Indica*

Analysis of general parameters

Estimation of urine volume

Table 9: Urine volume

Groups	**Urine volume**
Normal Control	10.88±0.24
Nephrotoxic Control Gentamycin (40 mg/kg)	5.96±0.75
Standard silymarin (200mg/kg)	10.12±0.23

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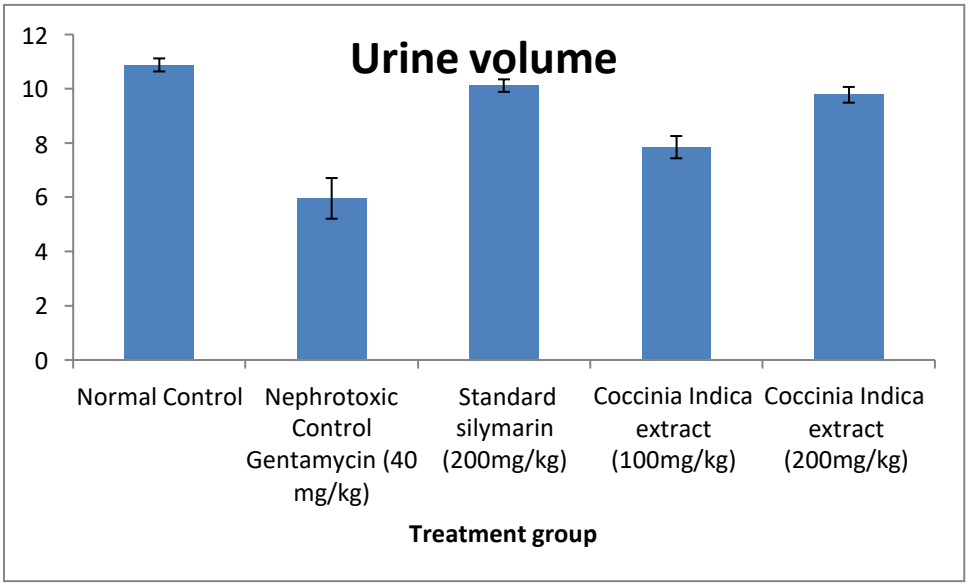
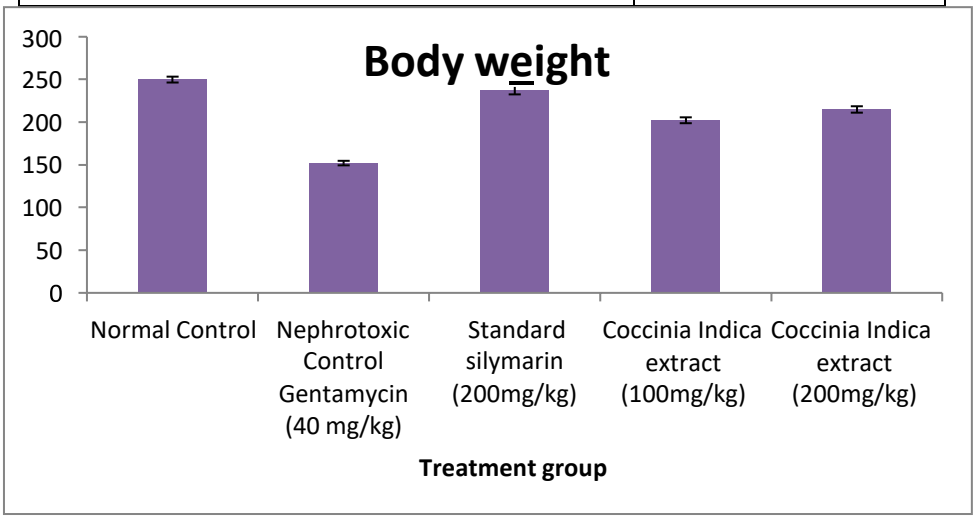
Coccinia Indica extract (100mg/kg)	7.85±0.41
Coccinia Indica extract (200mg/kg)	9.78±0.29



Estimation of Body weight

Table 10: Body weight

Groups	**Body weight**
Normal Control	250±3.406
Nephrotoxic Control Gentamycin (40 mg/kg)	152.22±2.657
Standard silymarin (200mg/kg)	237±4.353
Coccinia Indica extract (100mg/kg)	202.32±3.44
Coccinia Indica extract (200mg/kg)	215±3.742



Analysis of serum biochemical parameters

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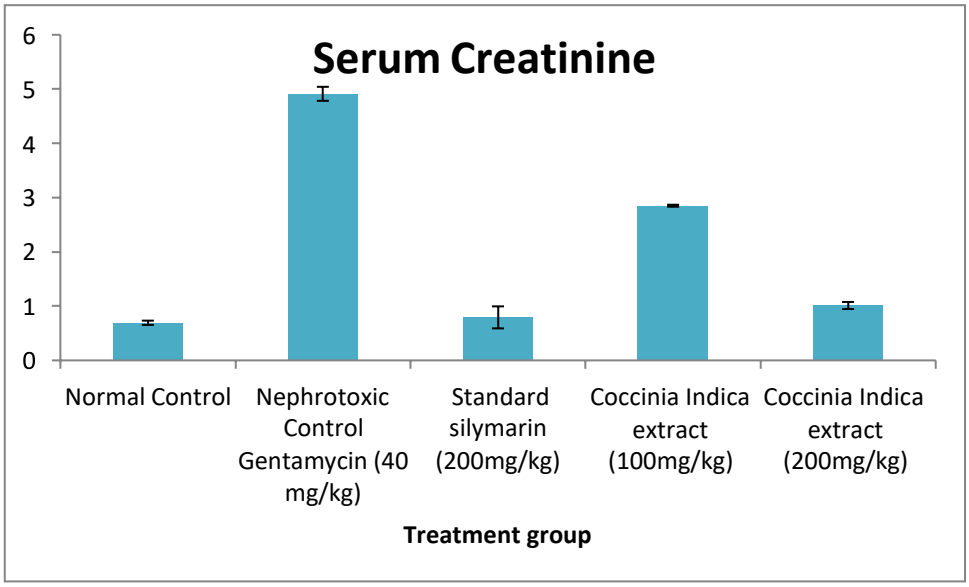
Estimation of Serum Creatinine

Table 11: Serum Creatinine

Groups	Serum Creatinine
Normal Control	0.69±0.054
Nephrotoxic Control Gentamycin (40 mg/kg)	4.91±0.130
Standard silymarin (200mg/kg)	0.79±0.203
Coccinia Indica extract (100mg/kg)	2.85±0.018
Coccinia Indica extract (200mg/kg)	1.01±0.065



Estimation of Serum Blood urea nitrogen (BUN)

Table 12: Serum Blood urea nitrogen

Groups	Serum Blood urea nitrogen
Normal Control	24.68±0.504
Nephrotoxic Control Gentamycin (40 mg/kg)	61±0.792
Standard silymarin (200mg/kg)	25.54±0.50
Coccinia Indica extract (100mg/kg)	39.87±0.94
Coccinia Indica extract (200mg/kg)	29.06±0.53

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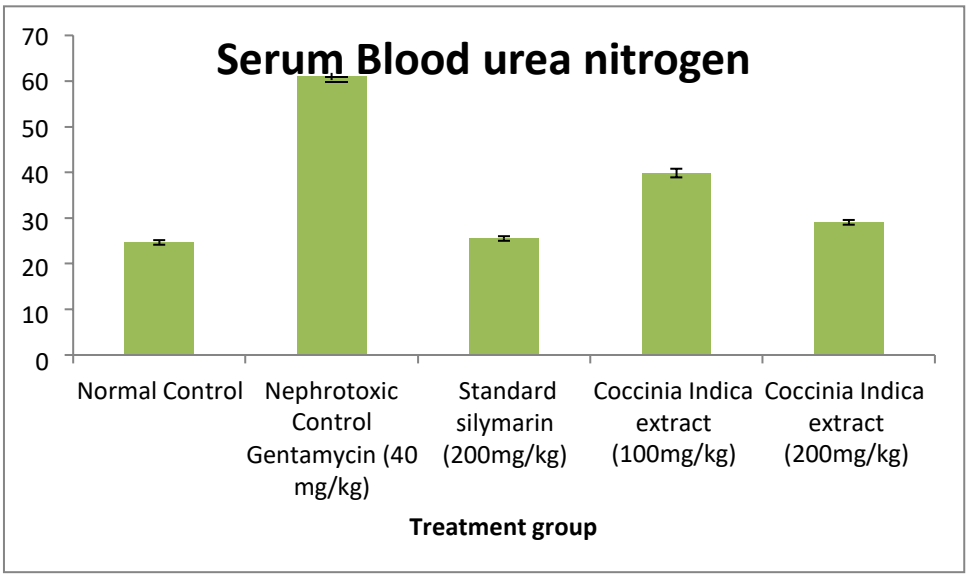

DISCUSSION

The phytochemical study of *Coccinia Indica* methanolic extract revealed the presence of alkaloids, phenolics, flavonoids, saponins, glycosides, 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. *Coccinia Indica* extract inhibited DPPH radicals by 60.28%, with an IC 50 value of 49.84µg/mL. Ascorbic acid was utilized as a reference molecule, exhibiting 85.46% inhibition and an IC 50 value of 21.98µg/ml. In the acute toxicity investigation, no symptoms of toxicity were seen up to a dose of 2000 mg/kg body weight. 100 mg/kg and 200 mg/kg have been fixed for study. In the current study, the rats treated with a single dose of Gentamycin showed a marked reduction in body weight as compared with the normal group, which is accompanied by a risein serum creatinine level, indicating induction of acute renal failure with Coccinia Indica at the dose level of 100 and 200 mg/kg body weight for 14 days significantly lowered the serumlevel of creatinine with a significant weight gain and increase. Gentamycin given to control rats caused a typical pattern of nephrotoxicity, as evidenced by a significant increase in serumblood urea nitrogen (BUN). *Coccinia Indica* supplementation to Gentamycin treated rats recorded decrement in levels of blood urea nitrogen (BUN) in plasma. The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties.

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