



NEPHROPROTECTIVE ACTIVITY OF WHOLE PLANT OF *TEPHROSIA PURPUREA* BY CISPLATIN INDUCED METHOD

Dr. Dev Prakash^{*1}, Pavitra Solanki¹ and Dr. K. K. Sreenivasan²

¹Sanjay College of Pharmacy, Mathura, UP. India.

²Manipal College of Pharmaceutical Sciences, Manipal.

***Author for Correspondence: Dr. Dev Prakash**

Sanjay College of Pharmacy, Mathura, UP. India.

Article Received on 07/01/2016

Article Revised on 27/01/2016

Article Accepted on 17/02/2016

ABSTRACT

Tephrosia purpurea (Family *Fabaceae*) is commonly known as Sarphank. The plant has been claimed to cure diseases of kidney, liver, spleen, heart and blood. The aim of the present study was to determine the nephroprotective activity of ethanolic extracts of the whole plant of *Tephrosia purpurea*. The cisplatin induced nephrotic models were used for the study. Animals were divided into different group control, standard, and test group after IAEC approval. Final results of different groups were compared (results of the extract (250 mg/kg, 500 mg/kg) with that of standard drug. On the basis of experimental data obtained, it was observed that the whole plant extract at the dose of 250 mg/kg and 500 mg/kg showed significant nephroprotective activity and the activity was found to be maximum at 500 mg dose extract. It means that the effect was found to vary in a dose dependent way. The result suggests that the ethanolic extract from the whole plant of *Tephrosia purpurea* exhibited significant dose dependant nephroprotective activity.

KEYWORDS: *Tephrosia purpurea*, Nephroprotective, Cisplatin.

INTRODUCTION

Diseases of the kidneys are among the most important causes of death and disability in many countries throughout the world. For example, in 2004, more than 20 million adults in the United States were estimated to have chronic kidney disease. Severe kidney diseases can be divided into two main categories.

Acute renal failure, in which the kidneys abruptly stop working entirely or almost entirely but may eventually recover nearly normal functions and **Chronic renal failure**, in which there is progressive loss of functions of more and more nephrons that gradually, decreases overall kidney functions.

Nephrotic syndrome

It is characterized by loss of large quantities of plasma proteins into the urine. In some instances, this occur without evidence of the other major abnormalities of kidney function, but more often it is associated with some degree of renal failure. The cause of the protein loss in the urine is increased permeability of the glomerular membrane which can cause the nephrotic syndrome. Such diseases are as follows.

Drug induced renal diseases

Nephrotoxicity can be defined as a renal disease or dysfunction that arises as a direct or indirect results of

exposure to medicine and industrial or environmental chemicals. It has become increasingly apparent that a number of chemicals may adversely affect one or more of the anatomical demands of the kidney, such as glomerular, proximal, intermediate and distal tubule and medullary, endothelial and isothelial cells.^[1]

Methods to examine antioxidant activity

Methods to examine antioxidant activity of a sample can be divided in two major categories.

- i. Measuring its ability to donate an electron (or hydrogen atom) to a specific reactive oxygen species or to an electron acceptor.
- ii. Testing its ability to remove any source of oxidative initiation. e.g.- Inhibition of enzymes, chelation of transition metal ions and absorption of UV radicals.



Fig 1: Tephrosia purpurea.

MATERIALS AND METHODS

Collection and authentication of plants

The whole plants of *Tephrosia purpurea* was collected from Salam district, Tamilnadu. The plants was identified and authenticated by Dr. Raj Singh Saini, Head of the department of biotechnology, IIMT College of Medical Sciences, Meerut. Specimen's herbariums of the plants are kept for further reference.

Size Reduction of plant material

Whole plant material was collected dried under shade, powdered and extracted with ethanol.

Extraction of *T. purpurea* plant with ethanol

The powdered material of plant (2 kg in batches of 500g each) was extracted with the ethanol using Soxhlet's apparatus. Accurately weighed 500 g dried powder of whole plant material was packed in a thimble and 2 litre of ethanol was added in 5 litres round bottom flask. The Soxhlet assembly was set up to complete 20 cycles. The same procedure repeated thrice to complete the extraction of constituents. The crude extract was filtered and distilled under reduced pressure to concentrate. The dry extracts were kept in desiccator over calcium chloride for 3 days to dry completely. The yield obtained is *T. purpurea* (168g).

Fractionation

The ethanol extracts of plant were subjected to fractionation using different solvents in the increasing order of polarity.

- (a) Petroleum ether (60-80°C), (b) Chloroform
(c) Ethyl acetate, (d) Water Remnant

T. purpurea (80 g) were suspended in 100 ml of distilled water. The suspension was transferred to a separating funnel; petroleum ether 100 ml per batch was added and shaken thoroughly for 15 min in a separating funnel. The petroleum ether soluble fraction was separated and washed with 3 ml of water; these washings were transferred to the aqueous fraction. This aqueous soluble fraction was treated with 100 ml of chloroform per batch and shaken for 15 min thoroughly in a separating funnel. The chloroform soluble fraction was separated and washed with 3 ml of water; these washings were transferred to the aqueous fraction. Ethyl acetate (100 ml) was added per batch shaken for 15 min in a separating funnel. The ethyl acetate soluble fraction was separated and washed with 3 ml of water; these washings transferred to the aqueous fraction. The obtained fractions petroleum ether, chloroform, ethyl acetate and aqueous remnant were distilled under reduced pressure to a syrupy consistency and kept in a desiccator over anhydrous calcium chloride to obtain dry extract fractions. Yield of the different fraction of *T. purpurea* weight of the fractions were (a) Petroleum ether (12g), (b) Chloroform (18g), (c) Ethyl acetate (21g) (d) Aqueous fraction (26g).

ANTIOXIDANT ACTIVITY

Estimation of total phenolic content by Spectrophotometer

By Folin Denis Method the method is based on the oxidation of molecule containing a OH groups. The tannin and tannin like compound reduce Phosphotungstomolybdic acid in alkaline solution to produce a highly blue colored solution.^[2] 1ml of the aqueous and ethanolic extract that has adjusted to come under the linearity range i.e. (50 µg/ml) of both the extract was withdrawn in 10ml volumetric flask separately. To each flask 0.5ml of Folin-Denis reagent and 1ml of Sodium carbonate was added and volume is made up to 10ml with distilled water. The absorbance was measured at absorption maxima 700nm within 30 minute of reaction against the blank. The total phenolic content was determined by using calibration curve (5 to 30 µg/ml). Three readings were taken for each and every solution for checking the reproducibility and to get accurate result. The intensity of the solution is proportional to the amount of tannins and can be estimated against standard tannic acid, the total phenolic content, expressed as mg tannic acid equivalents per 100 g dry weight of sample.

Total Flavonoid Content by Spectrophotometer Aluminum chloride colorimetric assay method

Total flavonoid contents were measured with the aluminum chloride colorimetric assay.^[3] Aqueous and ethanolic extracts that has been adjusted to come under the linearity range i.e. (400 µg/ml) and different dilution of standard solution of Quercetin (10-100 µg/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3ml of 5% NaNO₂ was added. After 5 minutes, 0.3ml of 10% AlCl₃ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample.

In-Vitro Antioxidant Study FRAP method^[4]

The ferric reducing property of the extract was determined by taking 1ml of different dilutions of standard solutions of Gallic acid (10 -100 µg/ml) or aqueous and ethanolic extract that has adjusted to come under the linearity range (500 µg/ml) was taken in 10 ml volumetric flasks and mixed with 2.5 ml of potassium buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. To the 2.5 ml of above solution 2.5 ml of distilled water is added and then 0.5 ml of 0.1% of FeCl₃ was added and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance obtained was converted to Gallic acid equivalent in mg per gm. of dry material (GAE/g) using Gallic acid standard curve.

Scavenging Activity Assays; Nitric oxide scavenging assay

Nitric oxide radical inhibition was estimated by the use of Griess Illosvory reaction.^[5] In this investigation, Griess Illosvory reagent was generally modified by using Naphthyl ethylene diamine dihydrochloride (0.1 %w/v) instead of the use of 1-naphthylamine (5%). The reaction mixture (3 ml) containing 2 ml of 10 mM sodium nitroprusside, 0.5 ml saline phosphate buffer and 0.5 ml of standard solution or aqueous and ethanolic extract of (50 -500 µg/ml) were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was mixed with 1 ml Sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for the completion of the reaction of diazotization. After that further 1ml of the Naphthyl ethylene diamine dihydrochloride was added, mixed and was allowed to stand for 30 min at 25°C. The concentration of nitrite was assayed at 546 nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but the same condition should be followed). Here the blank is taken as the buffer and make up solvents and the Ascorbic acid (10 -50 µg/ml) was taken as standard. The percentage inhibition was calculated using the formula:

$$\% \text{ Scavenging Activity} = \frac{\text{A control} - \text{A test or A Std}}{\text{A control}} * 100$$

Where, A control = absorbance of control A test or A Std = absorbance of test/stdnd.

Hydrogen Peroxide scavenging Assay^[6]

The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100 mM) was prepared instead of 40 mM in phosphate buffer saline of (PH 7.4), at various concentration of aqueous and ethanolic extract (50 -500 µg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. Results are provided in figures. The percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A₀ is the absorbance of the control and A₁ is the absorbance of extract/standard taken as Gallic acid (10 -100 µg/ml).

DPPH –RSA method

The free radical scavenging activity of aqueous and ethanolic extracts and the standard L-Ascorbic Acid (Vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH.^[8] Here, 0.1 mM solution of DPPH in alcohol was prepared and it must be protected from light influence by maintaining the dark condition and also fold by aluminum foil and 3 ml of this solution was added to

1 ml various conc.(100-2000 µg/ml) of extracts or standard solution of (10-100 µg/ml). Absorbance was taken after 30 min at 517 nm. The percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A₀ is the absorbance of the control and A₁ is the absorbance of extract/standard taken as Ascorbic acid.

NEPHROPROTECTIVE STUDY

Acute toxicity studies

Albino Wistar rats (150-200 gm body weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and no adverse effects/mortality were detected in both rats upto 3000 mg/kg p.o., during the 24-hr observation period. Based on the results obtained from this study, the doses for nephroprotective activity studies were narrowed down to 250 mg/kg and 500 mg/kg for dose-dependent study.^[8]

Procedure for Nephroprotective Study

Cisplatin (cis diamine dichloro platinum II) 6mg/kg body weight of the animal has given through intra peritoneal route as single dose to induce nephrotoxicity.

Nephroprotective study was carried out as per the proposal given below. Total 24 male albino wistar rats weighing 180-200 g were divided into groups of 6 animals each.

Group I: animals received distilled water, served as normal Control.

Group II: animal were treated with *Cisplatin*.

Group III a: animals were treated with ethanol extracts 250 mg/kg body weight for 12 days from the next day of *Cisplatin* administration. (*T. purpurea*)

Group III b: animals were treated with ethanol extracts 500 mg/kg body weight for 12 days from the next day of *Cisplatin* administration. (*T. purpurea*).

Route of Administration

Dose of 6 mg/kg body weight of *Cisplatin* was given through intraperitoneal route to all the groups. Extracts were given daily by oral route.

Body Weight Analysis

Body weights of the animals were recorded on the initial and final of the drug treatment Table 1 and Fig 1.

Urine collection

Animals were housed in the stainless steel metabolic cages with free access to food and water. Each urine sample was collected over 24 hrs on 5th & 16th day of the treatment, into 100 ml beakers mainted at 0-4°C avoiding fecal contamination. The volume of each sample was measured (and the values are recorded in Table 2 and fig 2) and centrifuged at 3000 rpm for 15 min. and the supernatant portion was removed and utilized for urea, creatinine and minerals (electrolytes) estimation.

Biological Estimation in Urine**Creatinine**

Creatinine level in urine sample was estimated by using Ecoline Diagnostic Kit.

Creatinine levels in urine sample were expressed in mg/dl and the values are recorded in the Table 3 and fig 3a.

Urea

Urea levels in urine were estimated using an Ecoline diagnostic kit. Urea catalysis the following reaction.



The reaction was estimated photo metrically at 340 nm (Sarre EM 1927). Urea levels in urine sample were expressed in mg/dl and the values are recorded in Table 3 and fig 3b.

Sodium ions

Urine sample were diluted to 1000 times and used to estimate sodium ion concentration by flame photometry. Sodium levels in urine sample were expressed in meq/day and the values are recorded in Table 4 and fig 4a.

Potassium Ions

Potassium levels in urine samples were expressed in meq/day and the values are recorded in Table 4 and fig 4b.

Calcium ions

Calcium levels in urine samples were expressed in meq/day and the values are recorded in Table 4 and fig 4c.

Anaesthesia

Urethane (1.25 gm/kg) was given through i.p. route to anaesthetize the animals (Tomohisa K 1994).

Blood Sampling and Serum Separation

Unhaemolysed sample of blood were collected from the clean tail tips of rats after urethane anesthesia on 5th, 16th day of the treatment. This was collected into Eppendrooff from the cleaned tail tips. This blood was allowed to clot at 37 °C and centrifuged at 2500 rpm for 7 min. to separate the serum and stored at -20°C until required.^[9]

Bio Chemical Estimation in Serum**Alkaline Phosphate (ALP)**

Alkaline Phosphate in serum was assayed using Ecoline Diagnostic Kit. Alkaline Phosphate levels in serum were expressed as Units/Litre and the values are recorded in Table 5 and fig 5a.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase level in serum was assayed using an Ecoline Diagnostic Kit. Lactate dehydrogenase levels in serum were expressed as units/liter and the values are recorded in Table 5 and fig 5b.

Gamma Glutamyl Transferase (GGT)

Gamma Glutamyl Transferase level in serum was assayed using Ecoline Diagnostic Kit the reaction was estimated photometrically at 405 nm. Gamma Glutamyl Transferees level in serum was expressed as Unit/Liter and the values are recorded in Table 6 and fig 6a.

Total Protein (TP)

Total protein level in serum was expressed as grams/liter and the values are recorded in Table 6 and fig 6b.

Creatinine

Creatinine levels in serum samle were estimated by using Ecoline Diagnostic Kit. Creatinine levels in serum sample were expressed in mg/dl and the values are recorded in Table 7 and fig 7a.

Urea

Urea levels in serum were estimated using an Ecoline diagnostic kit. Urea levels in serum sample were expressed as mg/dl and the values are recorded in Table 7 and fig 7b.

Blood Urea Nitrogen

Serum urea concentration was multiplied with 0.467 to get blood urea nitrogen level. Blood urea nitrogen levels were expressed as mg/dl^[10] and the values are recorded in Table 7 and fig 7c.

Euthanasia

Thiopental sodium overdose, through i.p. was given to induce euthanasia (painless killing).

Relative Kidney Weight

At the end of study, animals were euthanized. The kidney were immediately and the excised kidney were washed thoroughly with water, blotted free of water, weighed using Sartorius electric weighing balance and the values are recorded in Table 8 and fig 8.

Preparation of tissue homogenate

1 gram of kidney tissue was placed in a 7 ml glass homogenizing tube (Ten-broeck tissue grinder) and 10 ml KCl buffer (0.15M, pH 7.5) as added. The tissue was grind to a fine pulp by using Yarco high speed tissue homogenizer then centrifuged for 10 min at 2000 rpm. The supernatant was used for the Estimation of anti-oxidants, viz. catalase and SOD.

Enzymatic Anti-oxidants**Catalase**

Catalase was estimated by method.

Principles

The breakdown of hydrogen peroxide on addition of enzyme is followed by observing the decrease I light absorption of peroxidase solution in the ultraviolet (UV) region.

Reagent

- Phosphate Buffer: 0.3nm disodium hydrogen and 0.3 nm sodium dihydrogen phosphate were prepared and sufficient quantity of 0.3 nm sodium dihydrogen phosphate added till the required pH 7.5 is attained.
- Hydrogen peroxide (12.3 nm):- 0.14 ml of 30% H₂O₂ (commercially available) to 100 ml with distilled water.

Procedure

To 0.1 ml of 5% kidney homogenate in 0.15 M Kcl buffer was taken in a test tube, to this 1.9 ml of phosphate buffer was added, the reaction was initiated by addition of 1 ml of H₂O₂ the decreased of absorbance was measured at 240 nm at 30 second interval for 3 min. in Shimadzu UV-VIS 160^oA spectrometer. The activity was expressed as $\mu\text{mol H}_2\text{O}_2$ consumed/min/mg protein and the values are recorded in Table 9 and fig 9a.

Superoxide Dismutase (SOD)

Misra and Fridovich method was used to measure the activity of superoxide dismutase.

Principle

It is based on inhibition of epinephrine – adrenochrome transition by the enzyme.

Reagent

- Carbonate buffer: 0.3 M,(pH 10.2)
- EDTA: 0.6 mm
- Epinephrine: 3 nM
- Sodium carbonate: 0.25 M

Procedure

To 0.5 ml of tissue homogenate 0.5 ml of distilled water was added to dilute the sample. To this 0.25 ml of ice cold ethanol and 0.15 ml of chloroform were added. The mixture was shaken for a minute at 4°C and then centrifuged. The enzyme activity in the Supernatant was determined. Production of Adrenochrome in the reaction

mixture containing 0.2 ml of EDTA, 0.4 ml of sodium carbonate and 0.2 ml of epinephrine in a final volume of 2.5 ml was followed at 470 nm in Shimadzu UV-VIS 160A^o spectrophotometer. Transition of epinephrine to adrenochrome was inhibited by the addition of required quantity of enzyme. The amount of enzyme required to produce 50% inhibition of epinephrine to adrenochrome transition was taken as one enzyme unit. Activity of the enzyme was expressed as units/mg protein and the values are recorded in Table 9 and fig 9b.

ANTIOXIDANT ACTIVITY OF *T.PURPUREA***Total Phenolic content & Flavonoid content of *T. purpurea***

The quantitative determination of the total phenolic content of ethanolic extracts showed the content values of 15.40±0.31% w/w. Total flavonoid content of ethanolic extracts was 0.92±0.31% w/w. The above results showed that ethanolic extract having tannins and flavonoids.

FRAP method

By FRAP showed the results of ethanolic extracts is 51.21±0.26 mg GAE/g.

Nitric oxide scavenging assay

The extent of % inhibition of ethanolic (R²=0.9964) extract was 252.06 $\mu\text{g/ml}$ as compared to ascorbic acid 34.16 $\mu\text{g/ml}$ (R²=0.9999).

Hydrogen Peroxide scavenging

Scavenging of Hydrogen peroxide and its % inhibition of ethanolic extract showed I_{C50} value 244.21 $\mu\text{g/ml}$ (R²=0.9991). Gallic acid 60.00 $\mu\text{g/ml}$ (R²=0.9997).

Hydrogen Peroxide scavenging assay**DPPH –RSA**

DPPH-RSA method showed % inhibition of ethanolic extracts (I_{C50} values) 241.11 $\mu\text{g/ml}$ (R²=0.9993). Ascorbic acid showed 51.12 $\mu\text{g/ml}$. (R²=0.9998).

NEPHROPROTECTIVE ACTIVITY**NEPHROPROTECTIVE ACTIVITY OF *T. PURPUREA* RESULTS ARE TABLED AS FOLLOWS****Table 1: Effect of ethanolic extracts of *T.purpurea* on body weight changes.**

Groups	Treatment	Body Weight (Grams)	
		6 th day	12 th day
Gr. 1	Normal	+1.69 ±0.12	+2.64±0.16
Gr. 2	Cisplatin	-9.72±0.49	-27.96±0.18
Gr. 3a	Cisplatin +T.P250 mg/kg	-8.98±0.55	-12.88±0.54
Gr. 3b	Cisplatin +T.P500 mg/kg	-7.42±0.38	-9.46±0.36
F-Value		452.61	5733.8
P-Value		< 0.001	< 0.001

Table 2: Effect of ethanolic extracts of *T. purpurea* on water and feed intake and urine excretion.

Groups	Treatment	Water intake (ml/day)			Food intake (ml/day)			Urine Excretion (ml/day)		
		0 th	6 th	12 th	0 th	6 th	12 th	0 th	6 th	12 th
Gr. 1	Normal	32.4	31.7	35.1	16.5	15.3	17.8	11.56 ±0.94	11.86 ±0.14	11.34 ±0.46
Gr. 2	Cisplatin	34.8	26.2	21.6	17.3	09.6	08.2	10.92 ±0.84	06.85 ±0.34	03.40 ±0.26
Gr. 3a	Cisplatin+T.P 250 mg/kg	34.2	35.4	40.2	17.8	09.8	13.1	11.90 ±0.76	14.40 ±0.68	12.90 ±0.64

Gr. 3b	Cisplatin+T.P 500 mg/kg	34.5	36.1	46.4	16.6	10.8	14.8	11.28 ±0.62	14.60 ±0.52	10.30 ±0.58
F-Value								6.536	1069.	1133.8
P-Value								0.0029	< 0.001	< 0.001

Table 3: Effect of ethanolic extracts of *T. purpurea* on urine analysis.

Groups	Treatment	Creatinine Excretion (mg/dl)			Urea Excretion (mg/dl)		
		0 th	6 th	12 th	0 th	6 th	12 th
Group 1	Normal	18.34 ±0.98	16.40 ±0.84	17.80 ±0.96	36.40 ±0.82	42.60 ±0.92	35.10 ±0.98
Group 2	Cisplatin	17.40 ±0.78	08.70 ±0.88	4.30 ±0.92	37.30 ±1.04	12.23 ±0.94	7.68 ±0.82
Group 3a	Cisplatin+T.P 250 mg/kg	18.50 ±1.10	14.30 ±0.92	12.40 ±0.82	35.60 ±1.42	18.20 ±1.10	21.80 ±0.98
Group 3b	Cisplatin+T.P 500 mg/kg	18.20 ±0.86	14.80 ±0.64	16.20 ±0.72	34.40 ±1.34	22.20 ±0.98	26.80 ±0.74
F-Value		1.631	98.74	294.46	6.536	1069.1	1133.8
P-Value		0.2139	< 0.001	< 0.001	0.0029	< 0.001	< 0.001

Table 4: Effect of ethanolic extracts of *T. purpurea* on mineral excretion.

Groups	Treatment	Na ⁺ Excretion (meq/day)			K ⁺ Excretion (meq/day)			Ca ⁺ Excretion (meq/day)		
		0 th	6 th	12 th	0 th	6 th	12 th	0 th	6 th	12 th
Gr. 1	Normal	0.86 ±0.6	0.84 ±0.4	0.88 ±0.6	0.69±0 .68	1.05±0. 12	0.98±0. 08	0.12±0.6 4	0.14±0. 001	0.13±0. 002
Gr.2	Cisplatin	0.78 ±0.46	0.38±0 .2	0.30 ±0.22	0.97±0. 82	1.70±0. 21	1.78±0. 07	0.14±0.0 04	0.07±0. 002	0.04±0. 002
Gr.3a	Cisplatin+T.P 250 mg/kg	0.82 ±0.16	0.56±0 .07	0.68 ±0.08	0.98±0. 36	0.82±0. 28	0.88±0. 12	0.11±0.0 07	0.09±0. 002	0.08±0. 002
Gr.3b	Cisplatin+T.P 500 mg/kg	0.84 ±0.38	0.58 ±0.08	0.72 ±0.12	0.96±0. 68	0.88±0. 06	0.86±0. 04	0.09±0.0 08	0.10±0. 001	0.11±0. 002
F-Value		0.0377	4.074	3.376	2.437	27.825	170.05	0.02528	2080.0	2080.0
P-Value		0.9899	0.0207	0.0386	0.0945	< 0.001	< 0.001	0.9944	< 0.001	< 0.001

Table 5: Effect of ethanolic extracts of *T. purpurea* on serum biochemical parameters.

Groups	Treatment	ALP(U/L)			LDH(U/L)		
		0 th	6 th	12 th	0 th	6 th	12 th
Gr.1	Normal	205.11 ±11.12	198.12 ±10.12	236.15 ±10.16	178.12±1 2.32	182.33 ±14.12	186.41 ±10.02
Gr. 2	Cisplatin	202.12 ±10.20	406.14 ±11.20	478.11 ±8.10	184.21±1 3.24	368.34 ±14.34	464.31 ±12.32
Gr.3a	Cisplatin+T.P25 0 mg/kg	197.00 ±14.10	248.13 ±12.24	282.21 ±14.12	172.34±1 5.12	250.36 ±14.26	224.24 ±12.58
Gr. 3b	Cisplatin+T.P50 0 mg/kg	202.12 ±11.86	260.16 ±12.22	242.17 ±11.32	186.24±1 2.24	274.42 ±11.82	298.65 ±10.24
F-Value		0.4799	364.49	630.00	1.346	189.87	705.89
P-Value		0.6999	< 0.001	< 0.001	0.2879	< 0.001	< 0.001

Table 6: Effect of ethanolic extracts of *T. purpurea* on serum biochemical parameters.

Groups	Treatment	GGT(U/L)			TP(gms/L)		
		0 th	6 th	12 th	0 th	6 th	12 th
Gr.1	Normal	5.10 ±0.46	5.00 ±0.34	5.48 ±0.32	7.52± 0.007	7.32± 0.004	7.48± 0.002
Gr. 2	Cisplatin	4.96 ±0.42	12.10 ±0.30	22.12 ±0.28	6.88± 0.008	4.86± 0.004	3.86± 0.002
Gr.3a	Cisplatin+T.P250 mg/kg	5.31 ±0.68	11.10 ±0.62	09.70 ±0.46	7.82± 0.006	5.60± 0.004	6.1± 0.002
Gr.3b	Cisplatin+T.P500 mg/kg	5.02 ±0.72	10.32 ±0.66	08.82 ±0.36	7.54± 0.006	6.54± 0.002	6.89± 0.003
F-Value		0.456	530.76	2443	205.1	5335.	28742
P-Value		0.7154	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 7: Effect of ethanolic extracts of *T.purpurea* on serum creatine, urea and Bun.

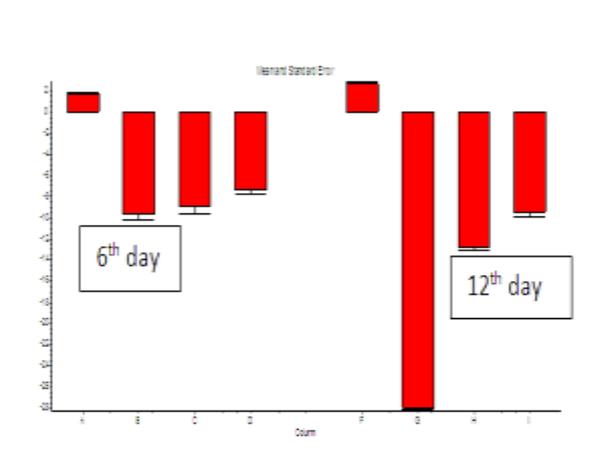
Groups	Treatment	Creatinine (mg/dl)			Urea(mg/dl)			Bun(mg/dl)		
		0 th	6 th	12 th	0 th	6 th	12 th	0 th	6 th	12 th
Gr.1	Normal	5.89 ±0.04	5.74 ±0.21	6.10 ±0.31	47.61±1 .20	45.14±1. 12	46.48± 1.03	20.78± 1.08	22.68± 1.64	21.13 ±1.24
Gr.2	Cisplatin	5.70 ±0.11	12.34 ±0.22	17.23 ±0.43	50.52±1 .43	242.0±2. 40	288.6± 2.84	22.24± 1.88	124.2± 2.26	134.1 ±2.10
Gr.3a	Cisplatin+T P250 mg/kg	6.12 ±0.26	5.02 ±0.40	3.78 ±0.46	49.77±1 .48	32.87±1. 12	26.22± 1.08	19.98± 0.78	15.62± 0.46	12.22 ±0.26
Gr.3b	Cisplatin+T P500 mg/kg	5.88 ±0.23	2.82 ±0.25	1.88 ±0.34	42.74±2 .20	29.34±1. 20	26.32± 1.16	21.16± 0.64	14.22± 0.38	12.88 ±0.28
F-value		5.298	1280.4	2710.5	28.028	25919	33876	3.701	8423.8	13945
P-value		0.0075	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0288	< 0.001	< 0.001

Table 8: Effect of ethanolic extracts of *T. purpurea* on relative kidney weight.

Groups	Treatment	Relative Kidney Weight (g)	Enzymtic Antioxidation		Lipid Peroxidation
			Catalase(μ mol H ₂ O ₂ consumed /min/mg protein)	SOD (U/mg protein)	TAB-RS μ mol/gm of wet tissue
			12 th	12 th	12 th
Gr.1	Normal	0.58±0.001	19.24±0.98	0.98±0.001	0.14±0.006
Gr. 2	Cisplatin	0.69±0.02	10.88±0.88	0.04±0.004	0.31±0.008
Gr. 3a	Cisplatin+T.P 250 mg/kg	0.62±0.03	14.22±0.78	0.72±0.024	0.28±0.07
Gr.3b	Cisplatin+T.P50 0 mg/kg	0.61±0.07	16.12±0.72	0.84±0.004	0.21±0.06
F-Value		8.386	159.86	6855.8	16.093
P-Value		0.008	< 0.001	< 0.001	< 0.001

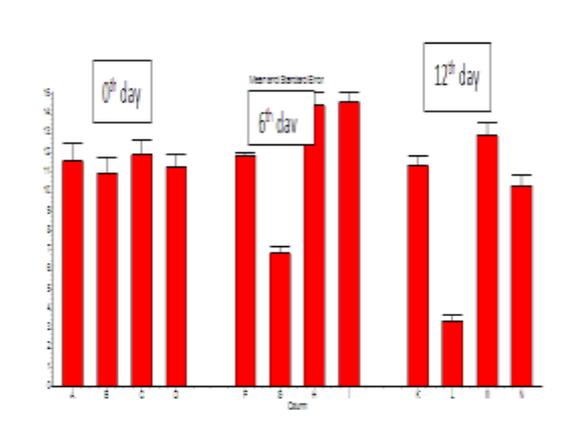
Table 9: Effect of ethanolic extracts of *T. purpurea* on creatinine and urea clearance.

Groups	Treatment	Creatinine Clearance (ml/min)			Urea Clearance (ml/min)		
		0 th	6 th	12 th	0 th	6 th	12 th
Gr.1	Normal	2.31±0.14	2.56±0.20	2.12±0.13	0.82±0.08	0.72±0.06	0.93±0.04
Gr. 2	Cisplatin	1.9±0.12	0.42±0.01	0.17±0.01	0.79±0.06	0.01±0.05	0.007±0.02
Gr.3a	Cisplatin+T.P 250 mg/kg	2.16±0.02	1.40±0.04	2.86±0.24	0.89±0.09	0.38±0.04	0.52±0.02
Gr. 3b	Cisplatin+T.P 500 mg/kg	2.48±0.21	1.82±0.16	2.24±0.72	0.92±0.12	0.56±0.09	0.41±0.08
F-Value		18.494	284.39	54.873	2.683	141.41	392.75
P-Value		< 0.001	< 0.001	< 0.001	0.0743	< 0.001	< 0.001



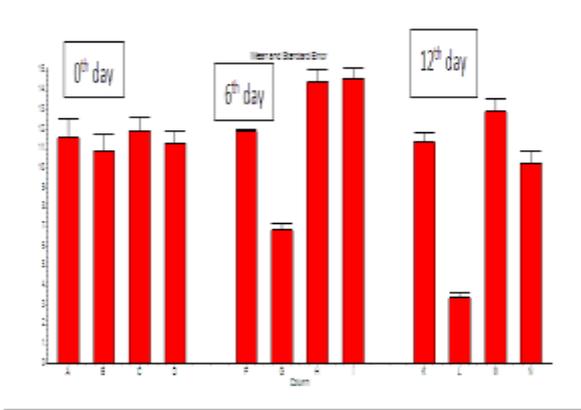
Gr 1: A,F Gr 2: B,G Gr 3a: C,H Gr 3b: D,I

Fig 1: Effect of ethanolic extracts of *T. purpurea* on body weight.

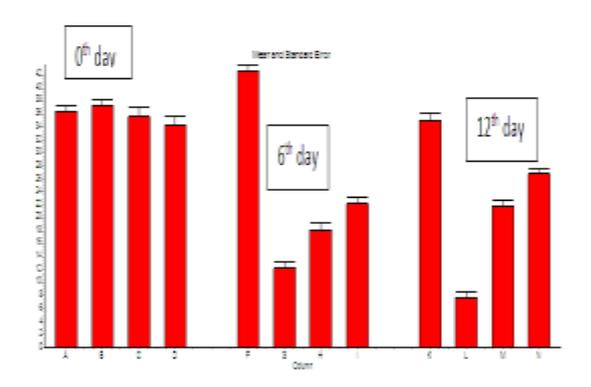


Gr 1: A, F, K Gr 2: B, G, L Gr 3a: C, H, M Gr 3b: D, I, N

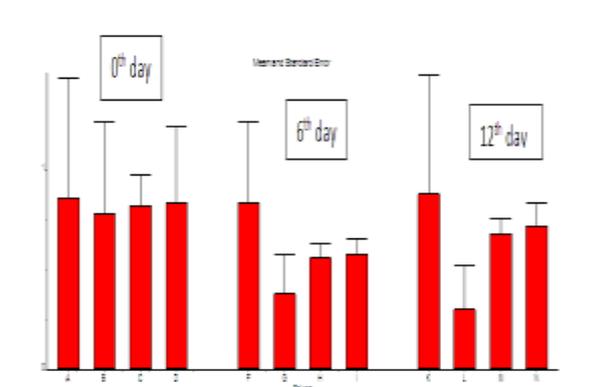
Fig 2: Effect of ethanol extract of *T.purpurea* on urine excretion.



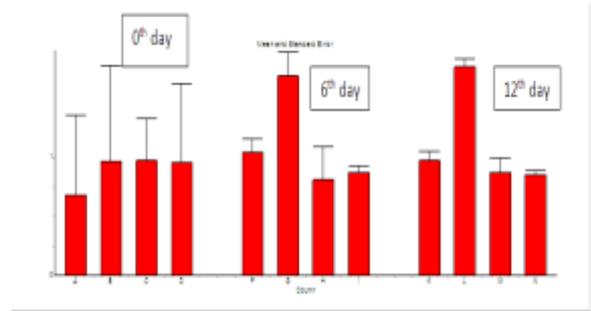
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 3a: Effect of ethanol extract of *T.purpurea* on creatinine excretion.



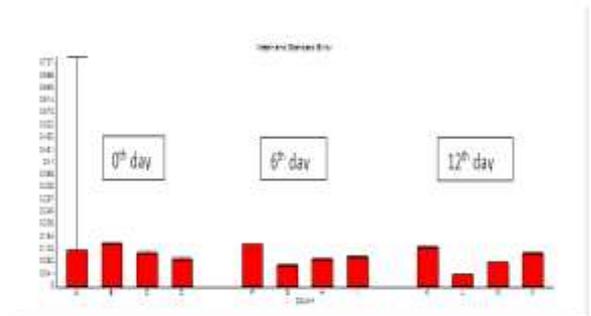
Gr 1: A, F, K .Gr 2: B, G, L Gr 3a: C, H, M Gr 3b: D, I, N
Fig 3b: Effect of ethanolic extract of *T.purpurea* on urea excretion.



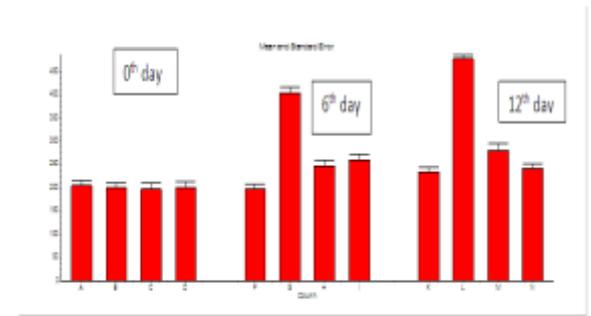
Gr 1: A,F,K Gr 2: B,G,L Gr 3a:C,H,M Gr 3b: D,I,N
Fig 4a: Effect of ethanol extract of *T.purpurea* on sodium excretion.



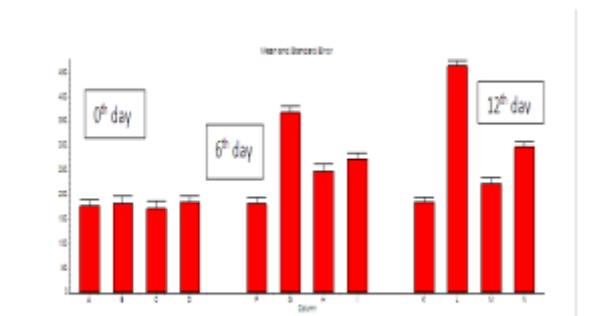
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 4b: Effect of ethanol extract of *T.purpurea* on potassium excretion.



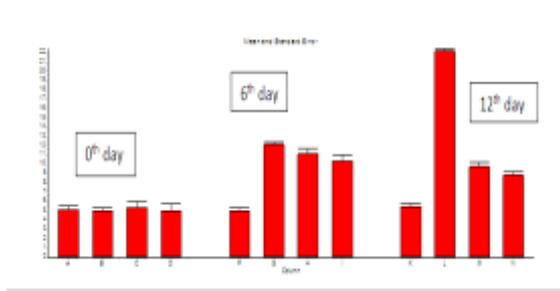
Gr 1: A,F,K Gr 2: B,G,L Gr 3a:C,H,M Gr 3b: D,I,N
Fig 4c: Effect of ethanolic extract of *T.purpurea* on calcium excretion.



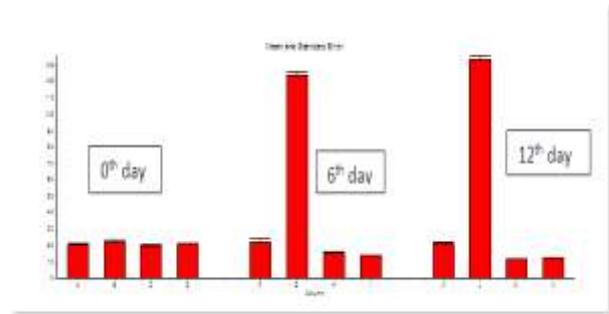
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 5a: Effect of ethanolic extract of *T.purpurea* on serum biochemistry parameter ALP.



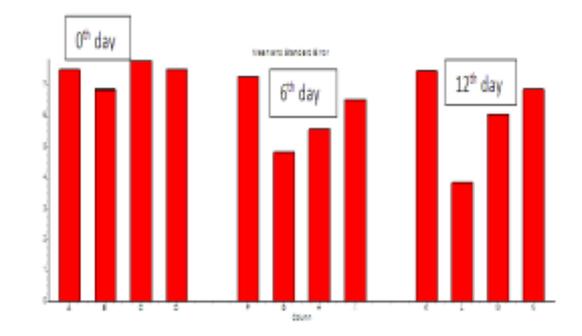
Gr 1: A,F,K Gr 2: B,G,L Gr 3a:C,H,M Gr 4b: D,I,N
Fig 5b: Effect of ethanolic extract of *T.purpurea* on serum biochemistry parameter LDH.



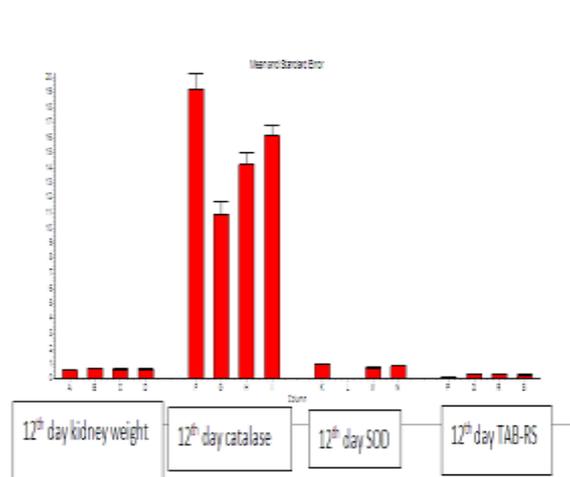
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 6a: Effect of ethanolic extract of *T.purpurea* on serum biochemical parameter GGT.



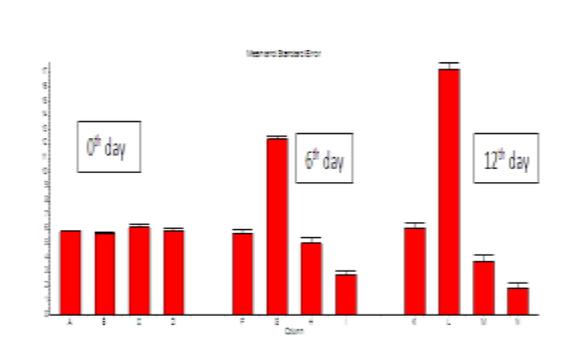
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 7c: 58 Effect of ethanolic extract of *T.purpurea* on serum BUN.



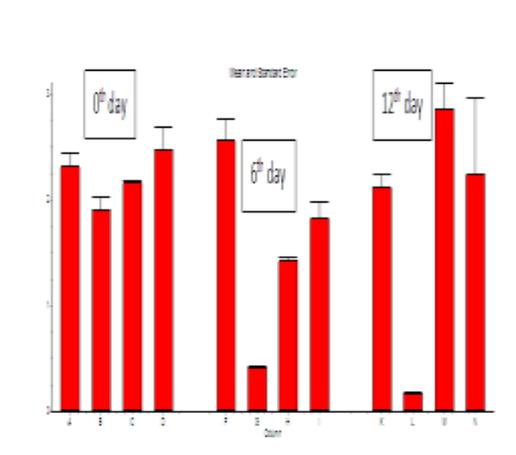
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 6b: Effect of ethanolic extract of *T.purpurea* on serum biochemical parameter Total protein.



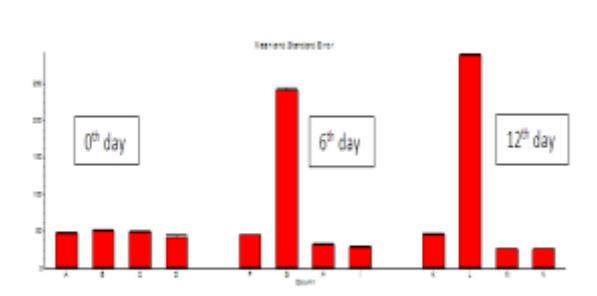
Gr 1: A,F,K,P Gr 2: B,G,L,Q Gr 3a: C,H,M,R Gr 3b: D,I,N,S
Fig 8: Effect of ethanolic extract of *T.purpurea* on relative kidney weight.



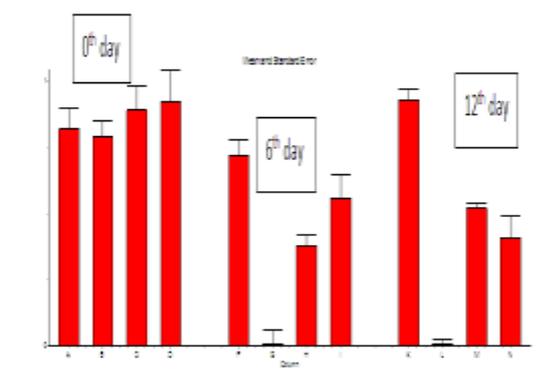
Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 7a: Effect of ethanolic extract of *T.purpurea* on serum creatinine.



Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 9a: Effect of ethanolic extract of *T.purpurea* on creatinine clearance.



Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
Fig 7b: Effect of ethanolic extract of *T.purpurea* on serum urea.



Gr 1: A,F,K Gr 2: B,G,L Gr 3a: C,H,M Gr 3b: D,I,N
 Fig 9b: Effect of ethanolic extract of *T.purpurea* on urea clearance.

HISTOPATHOLOGY OF KIDNEY

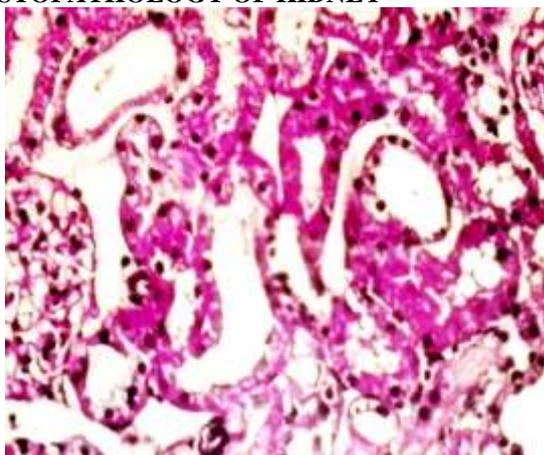


Fig 10a: Sections of the Kidney from Normal Rats Group 1(Normal) showed normal architecture of the kidney cells H & E 500.

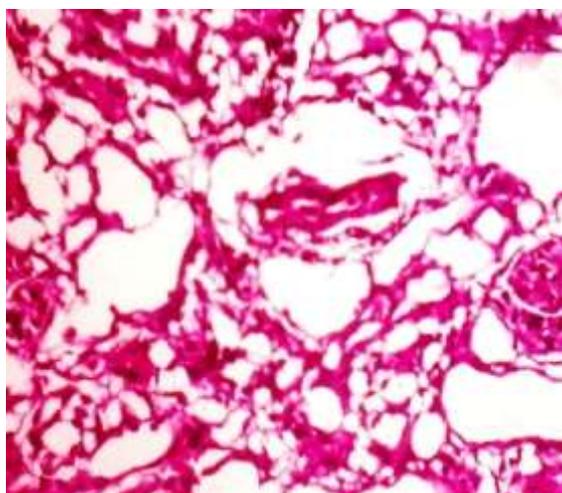


Fig 10b: Sections from Kidney Group 2(Cisplatin 6mg /kg) showed diffuse marked vacuolar degenerative changes in the tubular epithelial cells. Congestion of the interstitial vessels and vacuolar changes in the glomerular tuft.

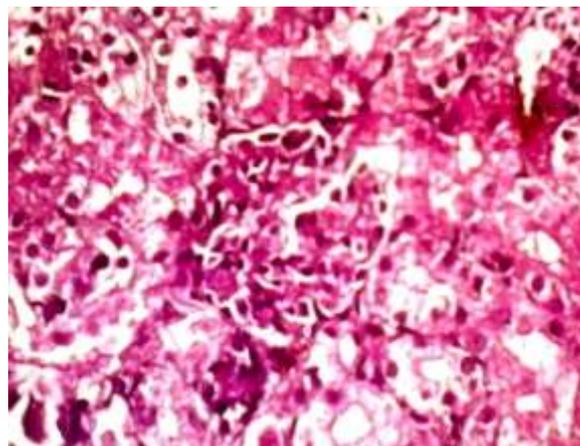


Fig 10c: Sections from Kidney of Group 3a (Ethanolic extract of *T. purpurea* 250mg/kg treated rats) showed diffuse congestion with mild degeneration in the tubular epithelial cells.

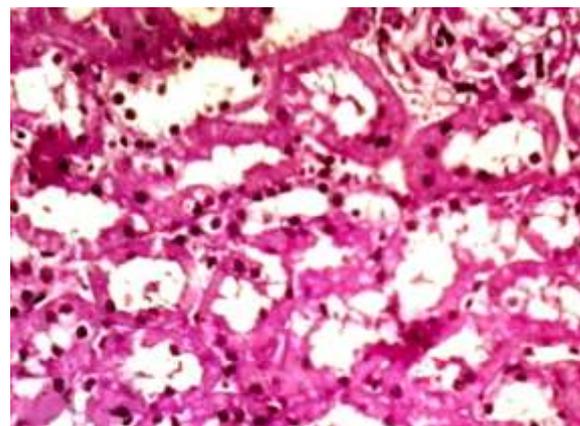


Fig 10d: Sections from Kidney of Group 3b (Ethanolic extract of *T. purpurea* 500mg/kg treated rats) showed very mild degenerative changes in the tubular epithelial cells and vacuolar degenerative changes still evidenced in glomerular tuft.

CONCLUSION

T. purpurea possesses antioxidant substances which may help in treatment of various ailments. It is evident plant aqueous extract is more potent as an antioxidant in comparison to the ethanolic extract. The total flavonoids content in aqueous extract is more than the ethanolic extract. Flavonoids are well known for their antioxidant properties. The flavonoid rutin was isolated and characterized. The plant showed significant diuretic potential and nephroprotective activity (500mg/kg) which is time and Dose dependent. These findings in recovery of renal dysfunction by the administration of the plants extracts in the cisplatin induced nephrotoxicity male albino wistar rats are very well comparable with the histopathological findings.

REFERENCES

1. Abayasekara CL, Rangama BNLD, Panagoda GJ, Senanayake MRDM, Antimicrobial activity of *Tephrosia purpurea* and *Mimusops elengi* against

- some clinical bacterial isolates. *J. Natn. Sci.*, 2009; 37(2): 139-45.
2. Ahmad S. Natural occurrence of Tephrosia flavones. *Phytochemistry*, 1986; 25(4): 955-958.
 3. Ahmad VU, Ali Z, Hussaini SR, Iqbal F, Zahid M, Abbas M et al. Flavonoids of *T. purpurea*. *Fitoterapia*, 1999; 70: 443-45.
 4. Akthar R, Ahmad S, Shaikh T, Mujawar T, Antihyperlipidemic effect of ethanolic extract of *T. purpurea* Linn in dexamethasone induced rats. *IJRAP*, 2011; 2(1): 264-266.
 5. Al-Shamaony, L, Al-Khazraji, S.M., Twaij, H.A., 1994. Hypoglycaemic effect of *Artemisia herba alba*. 11. Effect of a valuable extract on some blood parameters in diabetic animals. *Journal of Ethnopharmacology*, 43: 167-171.
 6. Allen S. 1989. Chemical analysis of ecological materials (2). London: Butter & Tanner. Anonymous, Useful Indian Medicinal Plant, PID (CSIR), New Delhi 1994; 53. Anon. 1976. The wealth of India 10: 451-457. CSIR, New Delhi, India.
 7. Asuntha G, Prasannaraju Y, Sujatha D, Prasad KVSRG. Assessment of ethanolic extract of *T. purpurea*, activity on lithium-pilocarpine induced Status Epilepticus and oxidative stress in Wistar rats. *Braz J. Pharmacog.* 2009; 22: 56-61.
 8. Baerheim SA, Scheffer JJ. Natural products in therapy. Prospects, goal and means in modern research. *Pharm Wkly [Sci]*, 1982; 4: 93-103.
 9. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant Chemicals: sources of industrial and medicinal materials. *Science*, 1985; 228: 1154-60.
 10. Bergmeyer HO, *Clin*, 1972; 18: 1305.