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PRECLINICAL EVALUATION OF ANTI-NEPHROTOXIC ACTIVITY OF *TEPHROSIA PURPURIA LINN* PLANT AQUEOUS EXTRACT BY CISPLATIN INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT

Background: *Tephrosia purpuria linn* plant was explored traditionally for its pharmacological activities for medicinal purpose. Review of literature suggested that regarding the nephroprotective activity of this plant few reported work is available, hence in the current investigation Anti-Nephrotoxic Activity of *Tephrosia purpuria linn* plant was carried out in albino rats. **Objectives:** The Anti-nephrotoxic activity of *Tephrosia purpuria linn* whole plant aqueous extract was conducted in albino rats by Cisplatin induced toxicity. **Methods:** Aqueous extract of *Tephrosia purpuria linn* whole plant was prepared by using maceration process. The aqueous extract was further used for acute toxicity studies in mice upto maximum dose level of 2000 mg/kg, p.o. Anti-nephrotoxic activity study was performed for aqueous extract of *Tephrosia purpuria linn* plant by Cisplatin induced nephrotoxicity. Bloog samples were excercised and centrifuged to collect serum. Samples were later analyzed for Alkaline phosphate, Blood urea nitrogen, Cholesterol, Creatinine,, Albumin and Uric acid in auto-analyzer. **Results and Discussion:** The acute toxicity studies revealed that no abnormal behavior and mortality rate in lab mice. *Tephrosia purpuria linn* plant Aqueous extract showed significant Anti-nephrotoxic activity. Parameters levels were also biochemically altered significantly after treatment with different doses of aqueous extract in nephrotoxic model. **Conclusion:** Plant Chemical constituent studies indicated availability of plant constituents like flavonoids, saponins, steroidal triterpines, tannins, carbohydrates and glycosides in plant aqueous extract. *Tephrosia purpuria linn* whole plant aqueous extract showed significant neproprotective action during studies.

KEYWORDS: Aqueous extract of *Tephrosia purpuria linn* plant, Chemical constituents, Anti-nephrotoxic activity, Cisplatin, Chemical parameters.

INTRODUCTION

The kidneys are essential for human life for their major function as they regulate the water content, mineral composition and acidity in the body and also involved in the excretion of waste metabolic products of various substances. The kidneys are affected by a variety of factors including infection, toxic substances, high blood pressure and diabetes etc further the body compensates for damage to the kidneys up to a certain extent and when it is extensive may leads to kidney failure. The symptoms include reduced urine output and pus in the urine. In patients with early kidney disease hypertension, endothelial dysfunction, dyslipidemia, inflammation and activation of rennin angiotensin system are the main causes for renal dysfunction and this could cause cardiovascular disease. It is believed that progressive alterations in kidney function or metabolism may cause progressive deleterious effects on cardiovascular status and nutrition.[1]

During the last decade, the deterioration in kidney function is strongly associated with increased cardiovascular morbidity, mortality and convincing evidence has been presented that moderate kidney disease as a strong independent risk factor for cardiovascular complications related death similar to that of diabetes mellitus.[2] Further the progressive loss of kidney function in patients with chronic kidney disease is associated with increase in number of complications including higher cases of cardiovascular mortality, anemia, hypoparathyroidism, inflammatory changes, metabolic acidosis and muscle weakness. Renal synthesis and excretion of ammonia is critical for efficient removal of acids from the body and intracellular ammonium levels are critical for protein turn over in kidney cells. Renal ammonia synthesis is increased in several clinical conditions associated with its hypertrophy. In tubular epithelial cells, the associated increase in ammonia

production rather than acidosis is responsible for tubular hypertrophy.[3] In metabolic acidosis the human kidney takes up higher amounts of glutamine and glycine from the blood and returns them to the systemic circulation, results in increased risk of kidney failure.[4]

Cisplatin (cis-diaminedichloroplatimum-II) is a heavy metal complex, one of the most effective and frequently used anti-neoplastic drugs for various malignancies including cancer of the testis, metastatic ovarian tumors, lung cancer, advanced bladder cancer and many other types of solid tumors. Although high doses are more effective in suppressing cancer however such doses also produce nephrotoxicity as a side effect.[3] Varied data indicate that cisplatin induce oxidative stress, lipid peroxidation, DNA damage and the underlying mechanisms of cisplatin-induced nephrotoxicity is still not fully understood. It has been suggested that binding of cisplatin to the renal base transport system and the following peroxidation of membrane lipids with the generation of free radicals may account for its nephrotoxicity. However, studies suggest lipid peroxidation as a pathway in the onset of cisplatin induced renal damage. In the earlier studies accumulation of lipid peroxides, depletion of glutathione and its related antioxidant enzymes in renal cortex supported the critical role of oxidative stress in cisplatin induced nephrotoxicity.[5]

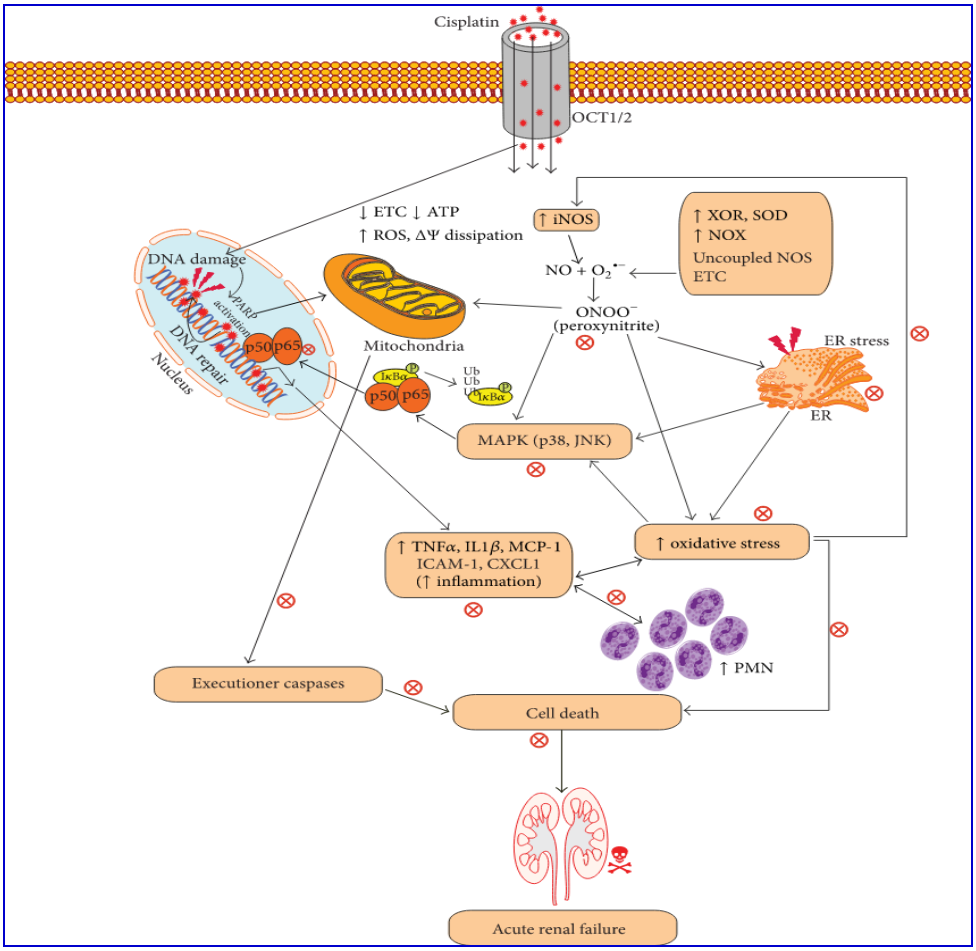

Fig No. 1: Cisplatin induced nephrotoxicity and acute renal failure.

Review of Literature

Studies on plant based products are going on throughout the world for the search of novel and highly effective/protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically with very little or no side effects exert during their function in the body. A number of herbs are traditionally used in different countries for treating chemical/drug or toxin induced hepatic and renal disorders.[6]

Currently, studies are being conducted worldwide to identify active molecules from the plant origin that can protect the kidney and other organs with few or no side effects. Therefore, this present study is planned to identify herbal medicines capable of protecting chemical/drug induced nephrotoxicity in experimental animals. To accomplish this, cisplatin is selected to induce oxidative stress on kidney cells, which was then planned to treat with various herbal extracts prepared as mentioned earlier to determine if any of these medicines exert a nephroprotective effect.[7]

The polyphenolic compounds are found in fruits, vegetables, nuts and seeds as well as most types of tea and in red wine. Flavanoids have been reported to exhibit a wide range of biological effects and play a protective role against chronic diseases such as cancer and cardiovascular diseases. They have antioxidant and antiproliferative properties.[8]

The major components of most of the mushrooms extracts revealed that the component of the extracts contains polysaccharide, protein complex, terpenoids show a significant antioxidant activity. A polysaccharide protein complex isolated from *Pleurotus ostreatus* was reported to use as a nephroprotective agent.[9]

Literature has shown that *Harungana madagascariensis* contained high concentrations of glycosides, flavanoids, alkaloids, saponins and tannins. The protection offered by the aqueous extract could have been due to the presence of any of the active principles taking into account that flavonoids, particularly quercetin, in other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects. In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity. Any of these with their combination could be responsible for the nephroprotective effect. In view of the above, the possible mechanisms for nephroprotective actions of different extracts could be via their antioxidant and/or free radical scavenging activities.[10]

Several independent animal and human studies have reported and confirmed the high safety profile of the plant extracts in the treatment of various diseases. In addition, previous phytochemical studies of *Phyllanthus amarus* extract has reported with the isolation and structural determination of bioflavonoids (e.g. quercetin), lignans (e.g., phyllanthine and hypophyllanthine) and alkaloids. Taking into account that flavonoids particularly quercetin (isolated from other medicinal plants identified with nephroprotective) have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to its potent antioxidant free radicals scavenging effects.[11]

A diet rich in carotenoids is associated with a number of health benefits. Interestingly the use of tomatoes and tomato-based products has been increased as a consequence of many epidemiological studies showed the presence and protective action of phytoconstituents carotenoids, in particular lycopene on cancer and cardiovascular diseases. Lycopene is a major carotenoid, available primarily from tomatoes and its products. Of all carotenoids, lycopene has been shown to exhibit the highest physical quenching rate constant with ROS. The administration of carotenoids with fruits and vegetables instead of carotenoid supplements such as lycopene is rarely associated with nephroprotective effects.[12]

MATERIAL AND METHODS

Plant material: The whole plant of *Tephrosia purpuria* was collected from the fields in and around areas of Raichur, Karnataka in the month of August and September in 2011 and was authenticated by Dr. V.Hemanth Kumar, Professor and Head Department of Pharmacognosy and Phytochemistry, V.L. College of Pharmacy, Raichur, Karnataka.

Preparation of *Tephrosia purpuria* aqueous extract (*AQETP*): The whole plant was collected and dried under shade and subjected for size reduction to prepare coarse powder with mixer grinder. The bark powder was defatted with petroleum ether (1:4) to remove fatty material. Then about 100 g of dried powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of purified water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every 1 h in a closed vessel. Then the marc is removed by filtering the extract and then it is concentrated on a water bath maintained at < 50^0C to get aqueous extract *AQETP*. The *AQETP* was analysed for its colour and consistency and percentage yield was calculated with reference to the quantity used for extraction. The extract was stored in airtight container in a refrigerator at 4-8^0C.

Experimental Animal care: Male albino rats used for the acute and chronic experiments were handled in accordance with international principles guiding the use and handling of experimental animals. The albino rats were maintained on standard pellet feed and potable water *adlibitum* at ambient temperature 28 - 30 ºC and 55 ± 5% relative humidity and standard (natural) photoperiod of approximately 12 h of light (06:30 h – 18:30 h) alternating with approximately 12 h of darkness (18:30 h - 06:30 h)[1].

LD_{50} Studies: Acute toxicity study of methanol and aqueous extracts will be carried out by using male albino rats with body weight 120-150g, maintained under standard animal husbandry. The animals will be fasted for 3 h prior to the experiment and each extract has to be administering as single dose and observed mortality up to 48 h study period for acute toxicity and 14 days for chronic toxicity. Based on the acute toxicity profile the next dose was determined as per OECD guidelines (No.425).[13-16]

Cisplatin induced nephrotoxicity model for aqueous extract of *Tephrosia purpuria* (*AQETP*) for Nephroprotective activity: Wistar albino Rats were divided into 9 groups of 6 animals in each group (n=6).
Group I: Animals were treated with (vehicle) distilled water for 6 days and was kept as normal control.

Group II: Animals were treated with distilled water (vehicle) daily 1 h before cisplatin injection at a dose of (10 mg/kg i.p.) for 5 days as toxicant control.
Group III: Received with Rutin 20 mg/kg body weight daily 1 h before cisplatin injection for 5 days.
Group IV, V, VI: Animals were treated by oral route with different doses of aqueous extract (low, medium, high) respectively daily 1 h before cisplatin injection for 5 days.
Group VII, VIII, IX- Groups were treated daily orally with three different doses of (low, medium, high) aqueous extract for 7 days respectively on 8th day 1 h after extract treatment Cisplatin was administered via intra peritoneal route at a dose of 10 mg/kg body weight. Then the animals were fasted for 14 h and on 9th day animals anaesthetized with ether and the blood was collected from the retro-orbital puncture, centrifuged and the serum separated was subjected for the estimation of biochemical parameters like Alkaline phosphate, Blood urea nitrogen, Cholesterol, Creatinine, Albumin and Uric acid by using semi auto analyzer (Erba Diagnostics Mannheim GmbH, Germany).[13-16]

RESULTS

Table No. 1: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Alkaline Phosphate estimation (n=6).

a) Alkaline Phosphate						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	95.12	298.6	142.5	230.1	219.5	186.9
B	93.23	291.5	165.2	235	195.5	167.2
T	82.15	321	152.2	241.5	191.6	169.9
HB	89.69	312.5	173.5	221.5	211.5	190.9
BT	78.96	319.1	151.2	248.5	228.5	182.8
HT	87.21	313.6	143.5	220.5	225.1	172.3
Mean	**87.73**	**309.38*** **	**154.68*** **	**232.85** **	**211.95** **	**178.33*** **
SEM	6.28	11.77	12.30	11.07	15.42	9.83
P < 0.05*, 0.01** and 0.001***						

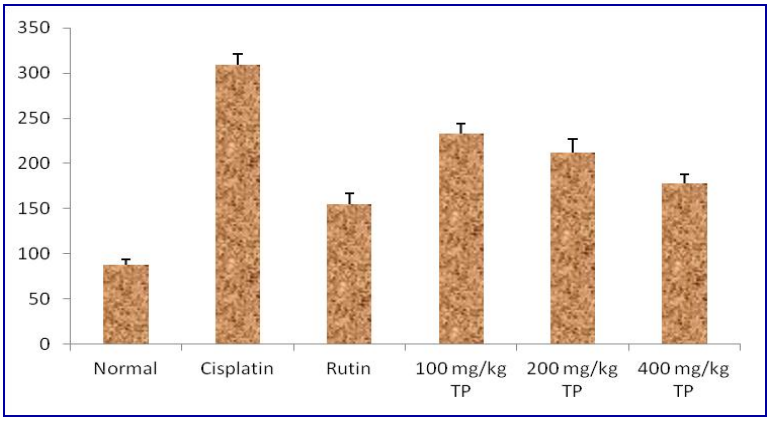

Fig No. 2: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Alkaline Phosphate estimation (n=6).

Table No. 2: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Blood Urea Nitrogen estimation (n=6).

b) Blood Urea Nitrogen (BUN)						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	7.82	48.6	19.8	32.5	26.8	21.3
B	6.75	38.9	21.3	28.9	22.9	19.2
T	7.45	37.8	25.6	24.2	20.1	23.5
HB	6.78	34.6	22.9	19.6	27.1	19.7
BT	7.41	41.9	18.9	29.1	24.6	18.2
HT	9.38	43.8	24.7	32.1	28.1	24.8
Mean	**7.60**	**40.93*** **	**22.2*** **	**27.73** **	**24.93** **	**21.12*** **
SEM	0.97	4.94	2.67	4.97	3.03	2.59

P < 0.05*, 0.01** and 0.001***

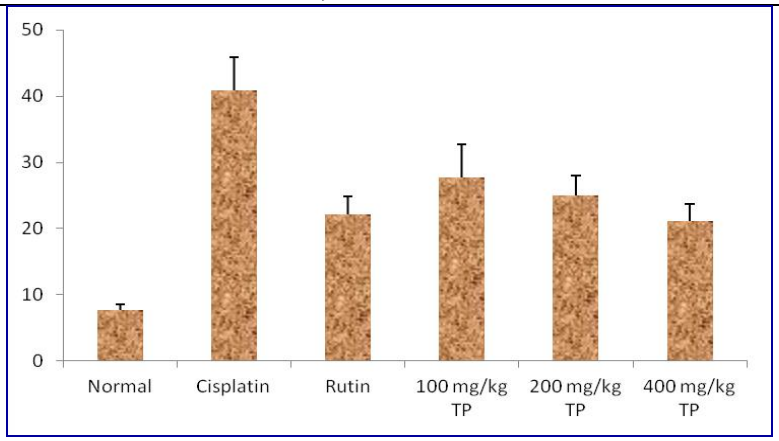

Fig No. 3: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Blood Urea Nitrogen estimation.

Table No. 3: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Creatinine estimation (n=6).

c) Creatinine						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	0.91	1.82	1.06	1.58	1.32	1.21
B	0.82	1.54	1.21	1.54	1.28	1.19
T	0.95	1.63	1.12	1.48	1.35	1.28
HB	0.96	1.79	1.28	1.52	1.31	1.29
BT	0.79	1.65	1.35	1.49	1.25	1.12
HT	0.82	1.59	1.22	1.59	1.39	1.14
Mean	**0.88**	**1.67*****	**1.21****	**1.53**	**1.32***	**1.21****
SEM	0.07	0.11	0.11	0.05	0.05	0.07
P < 0.05*, 0.01** and 0.001***						

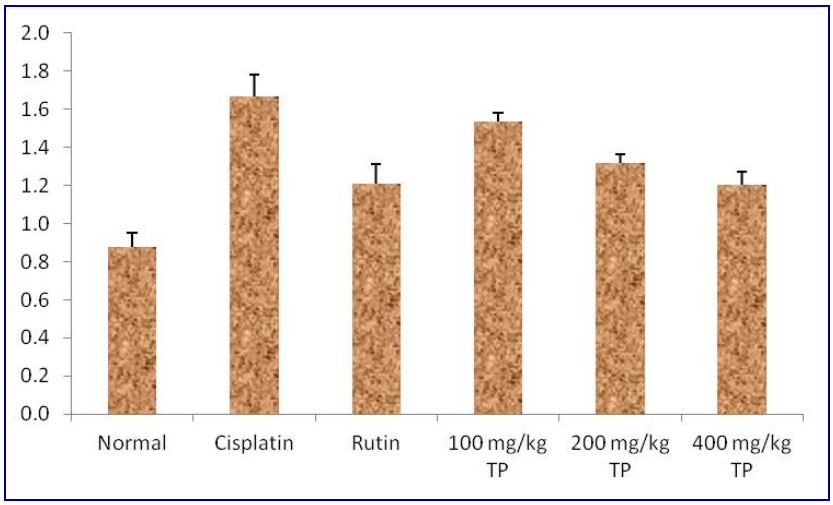

Fig No. 4: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Creatinine estimation.

Table No. 4: Nephroprotective activity of AQETP by Cisplatin induced toxicity Cholesterol estimation (n=6).

d) Cholesterol						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	192.5	287.2	184.1	256.5	227.9	194.8
B	172.1	295.3	181.9	235.8	221.3	187.4
T	175.2	301.4	192.5	252.6	232.8	192.1
HB	180.1	319.8	172.4	248.5	215.4	183.2

BT	158.9	279.5	169.9	239.2	229.8	187.9
HT	165.8	298.6	183.2	241.1	228.1	178.6
Mean	**174.1**	**296.97****	**180.67****	**245.62***	**225.88***	**187.33****
SEM	11.66	13.76	8.29	8.17	6.37	5.87
P < 0.05*, 0.01** and 0.001***						

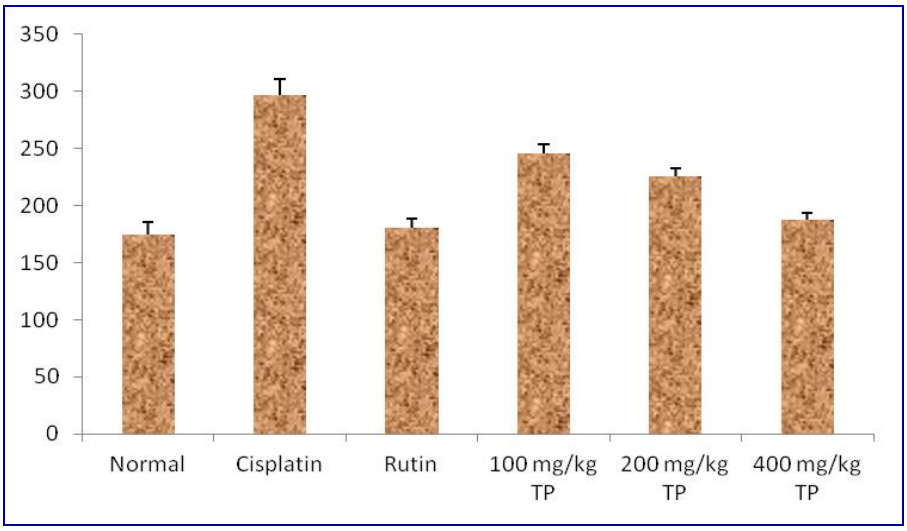

Fig. No. 5: Nephroprotective activity of AQETP by Cisplatin induced toxicity Cholesterol estimation.

Table No. 5: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Albumin estimation (n=6).

e) Albumin						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	3.51	2.32	4.73	3.14	3.35	3.92
B	3.53	2.28	4.9	3.28	3.43	3.86
T	3.97	2.33	4.82	3.19	3.38	3.7
HB	5.02	2.21	3.98	3.25	3.45	3.92
BT	3.87	2.39	4.85	3.3	3.37	3.71
HT	3.98	2.24	4.97	3.24	3.48	3.97
Mean	**3.98**	**2.30****	**4.71*****	**3.23***	**3.41****	**3.85****
SEM	0.55	0.07	0.37	0.06	0.05	0.12
P < 0.05*, 0.01** and 0.001***						

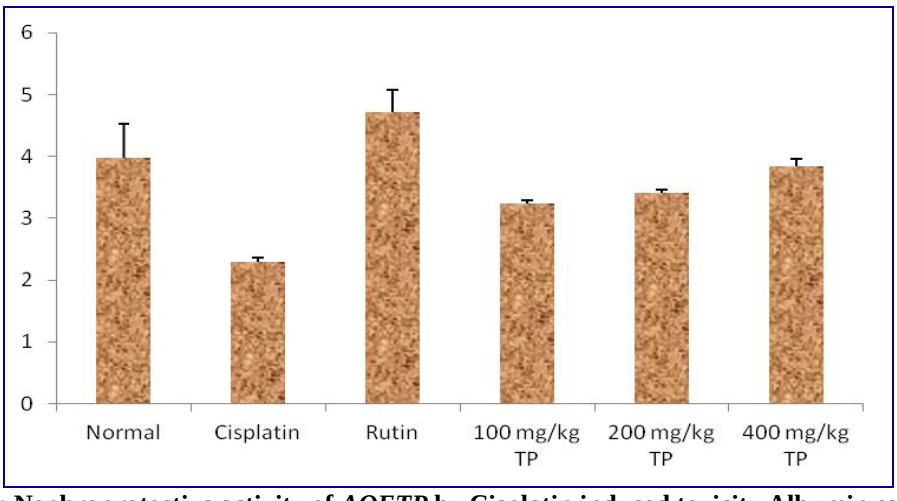

Fig No. 6: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Albumin estimation.

Table No. 6: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Uric acid estimation (n=6).

f) Uric acid						
Animals	**Normal**	**Toxicant (mg/kg)**	**Standard (mg/kg)**	***AQETP* dose (mg/kg)**		
		Cisplatin	**Rutin**	**Low**	**Medium**	**High**
		10	**20**	**100**	**200**	**400**
H	5.69	13.68	9.47	10.87	10.65	10.13
B	6.38	12.96	9.89	11.69	10.52	10.18
T	6.28	13.59	9.64	11.86	9.51	9.51
HB	5.92	13.92	9.43	10.69	9.79	9.68
BT	6.13	11.78	9.53	11.39	9.92	9.82
HT	5.89	14.2	9.25	10.93	10.19	9.49
Mean	**6.05**	**13.36*****	**9.54****	**11.24**	**10.10***	**9.80****
SEM	0.26	0.88	0.22	0.48	0.44	0.30
P < 0.05*, 0.01** and 0.001***						

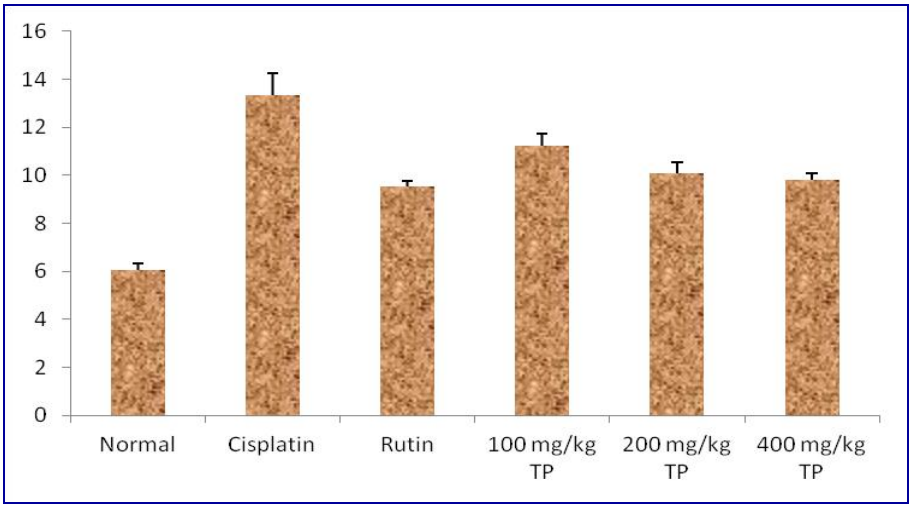

Fig No. 7: Nephroprotective activity of *AQETP* by Cisplatin induced toxicity Uric acid estimation.

DISCUSSION

A significantly increased level of serum alkaline phosphatase was observed in blood serum samples of the cispaltin treated group in comparison to the normal group (Table 1 and figure 2). However, supplementation with aqueous extract of *Tephrosia purpuria* at 100, 200 and 400 mg/kg doses significantly diminished ALP compared to cisplatin group. Highest effect was observed with 400 mg/kg of extract.

In cisplatin treated group of animals the concentration of BUN was considerably increased than the normal animals which indicated severe nephrotoxicity (Table 2 and figure 3). Treating aqueous extract of *Tephrosia purpuria* at 100, 200 and 400 mg/kg doses significantly decreased the concentration of serum BUN compared to cisplatin treated group.

Cisplatin administered rats had encountered nephrotoxicity as evidenced by elevation in the levels of creatinine compared to normal animals (Table 3 and figure 4). Treatment with aqueous extract of *Tephrosia purpuria* at 100, 200 and 400 mg/kg significantly lowered the levels of creatinine when compared with the toxic control group.

Single intraperitoneal administration of cisplatin induced significant rise in serum Cholesterol as seen in the cisplatin treated rats. However, elevation in the level of Cholesterol was significantly attenuated by treatment with the aqueous extract of *Tephrosia purpuria* at 100, 200 and 400 mg/kg in dose related fashion (Table 4 and figure 5). The effect of 400 mg/kg extract was comparable to that of standard rutin.

Quantification of serum biomarkers revealed a sharp rise in albumin in the cisplatin alone-treated group (Group-II) on comparing with the normal group indicating the induction of nephrotoxicity. However, in a dose-dependent fashion, the albumin level significantly decreased presenting the potency of aqueous extract of *Tephrosia purpuria* towards nephrotoxicity (Table 5 and figure 6).

A considerable enhancement in the levels of uric acid was observed for the cisplatin alone-treated group on comparing with the normal group (Table 6 and figure 7). Contrarily, a significant decline ($p<0.01$) was noted for uric acid with an administration of the aqueous extract of *Tephrosia purpuria* at 100, 200 and 400 mg/kg, in the experimental model after inducing nephrotoxicity with

cisplatin, indicating the efficiency of the aqueous extract of *Tephrosia purpuria* with respect to the nephroprotective activity.

CONCLUSION

In the present investigation, the Anti-nephrotoxic activity of aqueous extract of *Tephrosia purpuria* on cisplatin induced nephrotoxicity was evaluated by studying various biochemical parameters alterations in albino rats. The acute toxicity studies indicated no abnormal behavior and mortality rate. Aqueous extract of *Tephrosia purpuria linn* plant exhibited significant Anti-nephrotoxic activity. Several biochemical parameters levels were also altered significantly after treatment with aqueous extract. Chemical constituent studies indicated availability of Nephroprotective chemicals like flavonoids, saponins, steroidal triterpines, tannins, carbohydrates and glycosides in plant aqueous extract. *Tephrosia purpuria linn* whole plant aqueous extract showed significant Anti-nephrotoxic activity.

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