Evaluation of nephroprotective activity ethanolic extract of stem bark 

extract of Tephrosia purpurea

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ABSTRACT

Objective: To evaluate the nephroprotective activity of Ethanolic extract of Tephrosia purpurea (T.purpurea) (Fabaceae) in Gentamycin and Cisplatin-induced acute renal failure in rats.

Materials and Methods: Tephrosia purpurea (200 mg/kg and 400 mg/kg body weight), were administered orally to male Wistar albino rats in Gentamycin(GT) and Cisplatin(CP) was used to induce acute renal failure. The parameters studied included blood urea and serum creatinine and antioxidant parameters like malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) enzymes

Results: The results revealed that Tephrosia purpurea pretreatment significantly reduced blood urea and serum creatinine levels elevated by CP administration. Furthermore, Tephrosia purpurea significantly attenuated GT and CP-induced increase in MDA and decrease in reduced GSH, and CAT and SOD activities in renal cortical homogenates and it was showed that Tephrosia purpurea markedly ameliorated GT and CP-induced renal tubular necrosis.

Conclusion: The results indicate that the stem bark Tephrosia purpurea endowed with nephroprotective activity.

1. INTRODUCTION

Ayurveda is originated long back in India prevedic period. Ayurveda means ‘science of life’ as people are more concern about their future complications of people now refer ayurvedic treatment, medicines. Many natural drugs are shifts from edge to main stream to use herbal remedies for various disease with minimum side effects as compare to synthetic drugs. Recently attention has been paid to provide ecofreindly and biofreindly products to the people. In view of adverse effect of synthetic chemicals people are looking for safe and According to ayurvedic literature Tephrosia purpurea belongs to fabaceae have subfamily papillonaceae. There are approximately 400 species included in this genus. The plants in this genus are widely distributed in tropical, sub-tropical and arid regions of the world. It is an important constituent of some preparations such as Tephroli and Yakrifit used for liver disorders. Traditionally drug is used as liver tonic.

2. MATERIALS AND METHODS

Plant Material Collection: The plant material of Tephrosia purpurea (TP) was collected from Ranga Reddy dist, Telangana State in the month of August and was identified and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupathi, and Andhra Pradesh, India. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Extraction procedure3,4: Fresh plants material of Tephrosia purpurea (TP) were collected and dried under shade. The extracts used were prepared by using soxhelet apparatus by taking containing 500 ml of ethyl alcohol equivalent to two portions. Boiled up to 50-60°C for 4-5 hours, the filtrate was boiled until the concentrated residue is formed. Powdered Drug is extracted with ethyl alcohol yielding a crude extract. To
separate two portion of ethyl alcohol extraction of TP one portion used pharmacological screening second one used fractionated with chloroform and ethyl acetate extract

Preparation of ethanolic extract: The dried marc from the above process was extracted successively with Ethanol to get Ethanolic extract. The extract was collected and obtained dried extract used for further investigation.

Preliminary qualitative phytochemical analysis: The Ethanolic extract stem bark of *Tephrosia purperia* was subjected to qualitative examination for different phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Proteins, Lipids, and Reducing sugar, Phenol, Tannins, Saponins, Terpenoids and Steroids by using standard methods.

Acute Toxicity Study: The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD Guidelines 425). The animals were fasted overnight prior to the experiment. The first group was treated with oral dose of 1000 mg/kg body weight with the Ethanolic extract of the stem bark of *Tephrosia purperia*. The extracts were given in two different groups and the animals were observed continuously for 4-5 hours for general, behavioural, neurological, autonomic profiles and finally death after 24 hours. If there was no mortality and no sign of toxicity and the extract was found to be safe at that dose level, then a higher dose of 2000mg/kg body weight of the ethanolic extract was administered in another 2 groups. If no mortality was observed, the maximum tolerated dose level was taken as 2000 mg/kg body weight. The doses for pharmacological studies were taken as 400, 200, 100 mg/kg body weight i.e. 1/5th, 1/10th, 1/20th of the maximum tolerated dose (i.e. 2000mg/kg).

Nephroprotective activity of *Tephrosia purperia*:

Gentamycin induced nephrotoxicity in rats: The Wistar rats (180-200g) of either sex was be randomly divided into 4 groups of 6 animals in each group. The different groups was be assigned as described below.

- **Group I**: Vehicle control
- **Group II**: Nephro toxic control (Gentamycin 100 mg/kg)
- **Group III**: *T. Purperia* stem bark ethanolic extract (200 mg/kg) + Gentamycin (100 mg/kg)
- **Group IV**: *T. Purperia* stem bark ethanolic extract (400 mg/kg) + Gentamycin (100 mg/kg)

Nephrotoxicity was be induced in all the animals except in Group-I by injecting 100 mg/kg of gentamycin by intraperitoneal route. All the treatments was be given orally 24 hours after intoxication and continued once daily for three weeks. Body weights were checked every till last day of study and on the last 16th day, urine was collected and estimated. After three weeks completion of the treatment, all the animals was be anaesthetized with anaesthetic ether and blood was be collected by retro-orbital puncture for serum estimations.

Collection and analysis of urine: Metabolic cages were cleaned to prevent contamination. The experimental animals were transferred to the separate metabolic cages after the last day administration. Twenty-four hours urine samples were collected. The collected urine samples were transferred to clean containers and mixed with suitable quantity of purified water.

A drop of concentrated HCL was added to the collected urine. This prevents the growth of microbes and also prevents metal hydrolysis. The collected urine was measured and transferred to a cleaned airtight container and used for the urine analysis. From the collected urine samples of rats, urine glucose, sodium, potassium, urinary creatinine and urinary urea were estimated using autoanalyzer and diagnostic kits (Erba and span diagnostic kits).

Collection of serum: The anesthesia was induced with the intraperitoneal administration of ketamine (60mg/kg) and xylazine (5mg/kg) after 24hours of the last dose administration. The retro orbital sinus puncture technique was followed for the collection of blood samples of experimental animals. After the blood collection, the blood was transferred to the clean container. The blood samples containers were kept at 37°C for 40min for blood coagulation. The clot was removed from the container and the remaining serum of the experimental animals was added to the centrifuge tube. The centrifuge tubes containing serum were subjected to centrifugation for ten min at 3000RPM. The resultant clear serum was transferred into the clean container and was kept in the refrigerator. From the serum, creatinine and urea were measured. Erba diagnostic kit and semi-auto analyzer are used for these estimations.

Cisplatin induced nephrotoxicity in rats: The Wistar rats (180-200g) of either sex was be randomly divided into 4 groups of six each. The different groups was be assigned as follows

- **Group I**: Vehicle control (10 ml distilled water)
- **Group II**: Nephro toxic control (cisplatin 5 mg/kg)
- **Group III**: *T. Purperia* stem bark ethanolic extract (200 mg/kg) + (cisplatin 5 mg/kg)
- **Group IV**: *T. Purperia* stem bark ethanolic extract (400 mg/kg) + (cisplatin 5 mg/kg)

Nephrotoxicity was be induced in all the animals except Group-I by injecting Cisplatin (5 mg/kg body weight) intraperitoneally. All the treatments was be given orally 24 hours after intoxication and continued once daily for three weeks. Body weight was be checked every day. On the 8th day, urine was be collected for urinary estimations. After three weeks, completion of the treatment, all the animals was be
anaesthetized with anaesthetic ether and blood was be collected by retro-orbital puncture for serum estimations.\textsuperscript{10}

Collection and analysis of urine: Metabolic cages were cleaned to prevent contamination. The experimental animals were transferred to the separate metabolic cages after the last day administration. Twenty-four hours urine samples were collected. The collected urine samples were transferred to clean containers and mixed with suitable quantity of purified water. A drop of concentrated HCl was added to the collected urine. This prevents the growth of microbes and also prevents metal hydrolysis. The collected urine was measured and transferred to a cleaned airtight container and used for the urine analysis.

Estimation of serum urea: As per the dosage schedule the treatments were made. The last day treatments followed by twenty four hours, the rats were subjected to the anesthesia with the intraperitoneal administration of ketamine (60mg/kg) and xylazine (5mg/kg). The retro orbital sinus puncture technique was followed for the collection of blood samples from the rats. The blood was transferred to clean container and the blood was kept for about 40min at 37oC for the blood coagulation. After the separation of clot the serum was transferred to another container and the serum was taken into a clean centrifuge tubes. The blood sample containing centrifuge tubes were subjected to centrifugation for ten minutes at 3000RPM. Then the clear supernatant serum was transferred to a clean container and was stored in cool place. The serum urea was estimated from this sample of serum using Erba diagnostic kit.

Estimation of rat urinary and serum creatinine: The collected urine was measured and transferred to a cleaned airtight container and used for the urine analysis. From the collected urine samples of rats, urine creatinine was measured using autoanalyzer and (Erba diagnostic kit). The rats were anesthetized with the intraperitoneal administration of ketamine (60mg/kg) and xylazine (5mg/kg). The retro orbital sinus puncture technique. The blood samples are collected into well cleaned containers and these containers were kept for 40min at 37°C for the development of coagulation of blood. Then the serum was separated and it is then was transferred in to well cleaned centrifuge tubes. The serum samples of the rats were subjected to centrifugation using cold centrifuge at 3000 RPM and for 40 minutes. Then the serum was transferred to another clean container after the filtration and was stored in the cool place. This is used for the measurement of serum urea using Erba diagnostic kit. The working reagent, standard and test solutions (rat urine/ serum) were prepared. The absorbance and concentrations (mg/dL) of the creatinine were measured directly from the autoanalyzer.

Estimation of lipid peroxidation (LPO): The reaction mixture was prepared by using 0.58ml phosphate buffer (0.1M,pH 7.4), 0.2ml PMS, 0.2ml ascorbic acid (1mM) and 0.02ml ferric chloride (100mM) in a total volume of 1 ml. The mixture was incubated at 37oC in a shaking water bath for 1hour Then 1ml 10% w/v trichloroacetic acid and 1ml of 0.67% of TBA was transferred to the tubes. For about 20 min all these tubes were kept in the water baths. The absorbance was measured at 535nm. The results were expressed as nM of MDA formed /100gm of tissue. The extinction coefficient of 1.56×105 M⁻¹cm⁻¹ was used to calculate the amount of MDA.\textsuperscript{11}

Calculation: The amount of serum MDA was calculated by using the formula as

\[
\text{Concentration of MDA} = \frac{\text{Absorbance of Test \times Molar Extinction coefficient}}{\text{Total volume} \times \text{Sample volume}}
\]

Estimation of Glutathion reductase: Reduced glutathione (GSH) in the kidneys was measured by precipitating PMS (10%) with 1ml sulphosalicylic acid (4%). The kidney PMS samples were stored at refrigerator temperature (4°C) for about1h. The sample was centrifuged c (1200RPM for 15min at 4°C). The clear sample of 0.1ml was added with 2.7ml of phosphate buffer (total 3ml). The absorbance of the developed yellow color was read at 412 nm using spectrophotometer. The GSH content in the kidney tissue was measured using molar extinction coefficient 1.36 x 104 in mcg/mg of protein.

Estimation of Superoxide dismutase: 100 μl of serum was taken in Beckman quartz cuvette of one cm path length. 1 ml of sodium carbonate and NBT (0.4 ml) was taken in the cuvette and the zero min reaction was performed at 560 nm. This reaction was preceded by the addition of 0.4 ml of hydroxylamine HCl to the test tube. The reaction mixture were then incubated at 37° C for 5 min, the reduction of nitro blue tetrazolium was read at 560 nm\textsuperscript{13}.

Estimation of Catalase: 100 μl of serum was added to 1.9 ml of phosphate buffer and the absorbance was estimated at 240 nm. This mixture is added with 1 ml of H₂O₂was added and the absorbance noted after standing for 1 min at 240 nm using phosphate buffer as blank. The molar extinction coefficient of hydrogen peroxide was found to be 43.6 M cm⁻¹ and this was utilized for the determination of the catalase activity and was expressed as μmol/mg protein. The amount of serum catalase was calculated by using the formula\textsuperscript{14}.

3. RESULTS AND DISCUSSION

Preliminary Qualitative Phytochemical Screening of extracts and Formulation: The preliminary qualitative phytochemical analysis of ethanolic extract of T. Purperia, was carried out. The ethanolic extract of T. Purperia showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids,
steroids, fats and oils, and saponins, triterpenoids and glycosides.

**Acute toxicity study:** The Ethanolic extracts of Stem bark extract of *Tephrosia purpurea* was found to be safe at the maximum dose of 2000 mg/kg body weight by oral route. After 24 hours animals were found well tolerated. There was no mortality and no signs of toxicity. General behavior, neurological, autonomic profiles were found to be normal and the both the extracts were found to be safe.

### Table 1. The effect of *Tephrosia purpurea* on urine volume, urine urea, urine creatinine, serum urea and serum creatinine in gentamycin induced nephrotoxicity rats (Nephroprotective activity of *Tephrosia purpurea*)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urine volume (ml/day)</th>
<th>Urine urea (mg/dl)</th>
<th>Urine creatinine (mg/dl)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.95±0.18</td>
<td>35.05±0.05</td>
<td>93.50±3.21</td>
<td>42.50±2.59</td>
<td>0.69±0.01</td>
<td>16.69±2.48</td>
</tr>
<tr>
<td>Group II</td>
<td>2.63±0.12*</td>
<td>57.59±2.37*</td>
<td>244.17±4.40*</td>
<td>94.17±3.71*</td>
<td>1.02±0.08*</td>
<td>37.23±1.36**</td>
</tr>
<tr>
<td>Group III</td>
<td>2.91±0.07</td>
<td>41.01±1.59**</td>
<td>201.0±4.65**</td>
<td>63.83±5.04**</td>
<td>0.79±0.07**</td>
<td>24.54±2.85**</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.65±0.20</td>
<td>41.11±1.86**</td>
<td>162.1±3.31**</td>
<td>57.17±2.93**</td>
<td>0.83±0.04**</td>
<td>20.21±3.38**</td>
</tr>
</tbody>
</table>

The values are shown in mean ±SEM. Data was analyzed by one way ANOVA followed by Tukey’s test. Values of * = p<0.05, **= p<0.01, ***=p<0.001 were considered as significant. Mean percentage ±SEM; n = 6 animals in each group

A significant decrease in the urine volume in the gentamicin induced nephrotoxic rats (2.63±0.12 ml/day) was observed when compared to the normal rats (2.95±0.18 ml/day). The gentamicin, *Tephrosia purpurea* administered rats have shown an insignificant increase in the urine volume (2.91±0.07, 2.65±0.20 ml/day) when compared gentamicin induced nephrotoxic rats (2.63±0.12 ml/day).

There is a significant increase in the urine urea in the gentamicin induced nephrotoxic rats (57.59±2.37 mg/dL) was observed when compared to the normal rats (35.05±0.05 mg/dL). The gentamicin *Tephrosia purpurea* administered rats have shown significant decrease in the urine urea (41.01±1.59, 41.11±1.86 mg/dL) when compared gentamicin induced nephrotoxic rats (57.59±2.37 mg/dL).

A significant increase in the urine creatinine in the gentamicin induced nephrotoxic rats (244.17±4.40 mg/dL) was observed when compared to the normal rats (93.50±3.21 mg/dL). The gentamicin *Tephrosia purpurea* administered rats have shown significant decrease in the urine creatinine (201.0±4.65, 162.17±4.31 mg/dL) when compared gentamicin induced nephrotoxic rats (244.17±4.40 mg/dL). A significant increase in the urine and serum creatinine in the gentamicin induced nephrotoxic rats was observed when compared to the normal rats.

It was observed that there is a significant increase in the serum urea in the gentamicin induced nephrotoxic rats (94.17±3.71 mg/dL) was observed when compared to the normal rats (42.50±2.59 mg/dL). The gentamicin *Tephrosia purpurea* administered rats have shown a significant decrease in the serum urea (63.83±5.04, 57.17±2.93 mg/dL) when compared gentamicin induced nephrotoxic rats (94.17±3.71 mg/dL).

A significant increase in the serum creatinine in the gentamicin induced nephrotoxic rats (1.02±0.08 mg/dL) was observed when compared to the normal rats (0.69±0.01 mg/dL). The gentamicin, *Tephrosia purpurea* administered rats have shown significant decrease in the serum creatinine (0.79±0.07, 0.83±0.04, mg/dL) when compared gentamicin induced nephrotoxic rats (1.02±0.086 mg/dL).

A significant increase in the serum blood urea nitrogen (BUN) in the gentamicin induced nephrotoxic rats (37.23±1.36 mg/dL) was observed when compared to the normal rats (16.69±2.48 mg/dL). The gentamicin, *Tephrosia purpurea* administered rats have shown significant decrease in the serum creatinine (24.54±2.58 and 20.21±3.39 mg/dL) when compared gentamicin induced nephrotoxic rats (16.69±2.48 mg/dL). (Table 1)

### Table 2. The effects *Tephrosia purpurea* on lipid peroxidation, superoxide dismutase, catalase and glutathione on gentamicin induced nephrotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxide mM/100gm of tissue</th>
<th>SOD (U/mg of protein)</th>
<th>Catalase (U/mg of protein)</th>
<th>GSH nM/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>27.70±0.79</td>
<td>3.49±0.33</td>
<td>19.77±0.56</td>
<td>21.38±1.01</td>
</tr>
<tr>
<td>Group II</td>
<td>41.59±0.91*</td>
<td>1.98±0.11*</td>
<td>10.30±0.88*</td>
<td>10.10±0.32*</td>
</tr>
<tr>
<td>Group III</td>
<td>34.71±0.80*</td>
<td>4.03±0.39**</td>
<td>14.61±0.60**</td>
<td>18.56±0.67**</td>
</tr>
<tr>
<td>Group IV</td>
<td>28.69±1.79**</td>
<td>4.75±0.66**</td>
<td>13.58±0.31**</td>
<td>17.85±0.74**</td>
</tr>
</tbody>
</table>

The values are shown in mean ±SEM. Data was analyzed by one way ANOVA followed by Tukey’s test. Values of * = p<0.05, **= p<0.01, ***=p<0.001 were considered as significant. Mean percentage ±SEM; n = 6 animals in each group.
A significant increase in the lipid peroxide in the gentamicin induced nephrotoxic rats (41.59±0.91 mM/100g tissue) was observed when compared to the normal rats (27.70±0.79 mM/100g tissue). The gentamicin/Tephrosia purpurea administered rats have shown a significant decrease (34.71±0.80 and 28.69±1.79 mM/100g tissue) in the lipid peroxide when compared gentamicin induced nephrotoxic rats (41.59±0.91 mM/100g tissue).

A significant increase in the lipid peroxide in the gentamicin induced nephrotoxic rats was observed when compared to the normal rats.

A significant decrease in the superoxide dismutase in the gentamicin induced nephrotoxic rats (1.98 ± 0.11 units/mg of protein) was observed when compared to the normal rats (3.49 ± 0.33 units/mg of protein). The gentamicin, Tephrosia purpurea administered rats have shown a significant increase in the SOD levels (4.03 ± 0.39 and 4.75 ±0.66 units/mg of protein) when compared to the normal rats. The gentamicin, Tephrosia purpurea administered rats have shown a significant increase in the glutathione levels (10.30±0.84 U/mg of protein units/mg of protein) when compared gentamicin induced nephrotoxic rats (10.10±0.32 nM/mg protein units/mg) (Table.2)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urine volume (ml/day)</th>
<th>Urine urea (mg/dl)</th>
<th>Urine creatinine (mg/dl)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.34±0.25</td>
<td>45.05±0.56</td>
<td>83.30±3.31</td>
<td>40.24±0.02</td>
<td>0.85±0.09</td>
</tr>
<tr>
<td>Group II</td>
<td>2.09±0.15*</td>
<td>63.54±0.23*</td>
<td>211.3±1.20*</td>
<td>106.10±0.28*</td>
<td>1.78±0.98*</td>
</tr>
<tr>
<td>Group III</td>
<td>2.67±0.22</td>
<td>45.01±1.12**</td>
<td>187.00±4.65**</td>
<td>80.49±0.38**</td>
<td>1.23±0.73**</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.56±0.23</td>
<td>39.11±1.86**</td>
<td>123.17±4.31*</td>
<td>74.18±0.18**</td>
<td>1.05±0.43***</td>
</tr>
</tbody>
</table>

The values are shown in mean ±SEM. Data was analyzed by one way ANOVA followed by Tukey’s test. Values of *= p<0.05 , **= p<0.01 , ***=p<0.001 were considered as a significant.Mean percentage ±SEM; n =6 animals in each group

A significant decrease in the urine volume in the Cisplatin induced nephrotoxic rats (2.09±0.15 ml/day) was observed when compared to the normal rats (3.34± 0.25 ml/day). The Cisplatin and Tephrosia purpurea administered rats have shown an insignificant decrease in the urine volume (2.67± 0.22, 2.56± 0.23) when compared with cisplatin induced nephrotoxic rats (2.09± 0.15 ml/day).

There is a significant increase in the urine urea in the Cisplatin induced nephrotoxic rats (63.54±2.32 mg/dL) was observed when compared to the normal rats (45.04±0.56 mg/dL). The Cisplatin and Tephrosia purpurea administered rats have shown significant decrease in the urine urea (45.01± 1.12, 39.11+ 1.86 mg/dL) when compared Cisplatin induced nephrotoxic rats (45.04±0.56 mg/dL).

A significant increase in the urine creatinine in the Cisplatin induced nephrotoxic rats (211.34±1.20 mg/dL) was observed when compared to the normal rats (83.03±3.31mg/dL). The Cisplatin Tephrosia purpurea, administered rats have shown significant decrease in the urine creatinine (187.00+ 4.65, 123.17+ 4.31 mg/dL) when compared Cisplatin induced nephrotoxic rats (211.34±1.20 mg/dL). A significant increase in the urine and serum creatinine in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats.

It was observed that there is a significant increase in the serum urea in the Cisplatin induced nephrotoxic rats (106.10±0.28 mg/dL) was observed when compared to the normal rats (40.24±0.02 mg/dL). The Cisplatin and Tephrosia purpurea administered rats have shown a significant decrease in the serum urea (80.49 ±0.38 74.80±0.18 mg/dL) when compared Cisplatin induced nephrotoxic rats (106. 10±0.02 mg/dL).

A significant increase in the serum creatinine in the Cisplatin induced nephrotoxic rats (1.78±0.98 mg/dL) was observed when compared to the normal rats (0.85±0.09 mg/dL). The Cisplatin and Tephrosia purpurea administered rats have shown a significant decrease in the serum creatinine (1.23±0.73,1.05±0.43 mg/dL) when compared Cisplatin induced nephrotoxic rats (1.78±0.98 mg/dL).(Table.3)
There is a significant decrease in the catalase in the Cisplatin induced nephrotoxic rats (10.12± 0.26 U/mg of protein) was observed when compared to the normal rats (18.32± 0.32 U/mg of protein). The Cisplatin and *Tephrosia purpurea* administered rats have shown significant increase in the catalase levels (16.17± 0.93 and 17.38± 0.62U/mg of protein) when compared Cisplatin induced nephrotoxic rats (10.12± 0.26 U/mg of protein units/mg of protein). The Cisplatin and *Tephrosia purpurea* administered rats have shown a significant increase in the glutathione levels (14.61±0.53, 16.20±0.68 nM/mg protein) when compared Cisplatin induced nephrotoxic rats (10.40±0.28 nM/mg protein units/mg).

A significant increase in the lipid peroxide in the Cisplatin induced nephrotoxic rats (36.58 ± 0.36 mM/100g tissue) was observed when compared to the normal rats (26.33±0.31mM/100gm tissue). The Cisplatin and *Tephrosia purpurea* administered rats have shown a significant decrease (30.86 ± 0.78 and 28.55 ± 0.56 mM/100g tissue) in the lipid peroxide when compared Cisplatin induced nephrotoxic rats (36.58 ± 0.36 mM/100g tissue).

A significant decrease in the superoxide dismutase in the Cisplatin induced nephrotoxic rats (1.49± 0.14 units/mg of protein) was observed when compared to the normal rats (3.42±0.22 units/mg of protein). The Cisplatin and *Tephrosia purpurea* administered rats have shown a significant increase in the SOD levels (2.80± 0.21 and 3.03±0.21 units/mg of protein) when compared Cisplatin induced nephrotoxic rats (1.49± 0.14 units/mg of protein). The significant decrease in the superoxide dismutase in the Cisplatin induced nephrotoxic rats was observed when compared to the normal rats. (Table 4)

**Discussion:**

**Gentamycin Induced Nephrotoxicity:** The aim of the present study was to investigate the possible defending effects of *Tephrosia purpurea*on gentamicin-induced nephrotoxicity in rats owed to their structural similarities. Aminoglycosides are totally eliminate by glomerular filtration and reabsorbed by the proximal tubules. Even therapeutic doses of these antibiotics commonly lead to proximal tubular cell necrosis in humans. This pathology was known due to the increase of gentamicin in renal cortex.15,16 parallel results were obtained in this study and recognition of nephrotoxicity criterias were found to be similar. The administration of gentamicin in rats induced a reduction in glomerular filtration rate as exposed by a reduced creatinine clearance and increased serum creatinine. This renal injury in turn manifests the movement of monocytes and macrophages to the site of injury by stimulating intercellular bond molecule and monocyte chemoattractant protein17,18 while a number of other studies reported the role of reactive oxygen species in implicate the pathogenesis of gentamicin induce nephrotoxicity.19 Gentamicin intoxicated nephrotoxicity is functionally evident by the elevated serum levels of urea, BUN, uric acid, and creatinine; structurally characterize by tubular necrosis, glomerular atrophy, mononuclear cell infiltration, intertubular hemorrhage, and hyaline casts. Similar sort of alterations were observed with the gentamicin treatment in GM and treatment groups. As an investigative of decrease in glomerular filtration rate, there was an increase in the serum creatinine levels in the GM group,21 but the serum urea and BUN were found to be increased as an indicative of parenchyma tissue injury after tubular necrosis.22

The serum uric acid levels were found to be increased because of accumulation by the decrease in glomerular filtration rate in gentamicin. Intoxicated rats, but with the supplementation of *Tephrosia purpurea* dose dependently ameliorated the gentamicin, Induced elevated serum levels of urea, BUN, uric acid, and creatinine in *Tephrosia purpurea*200 and *Tephrosia purpurea*400 groups. However, in *Tephrosia purpurea*400 group, the nephroprotective property was found to be very prominent with high dose when compared with *Tephrosia purpurea*200 group. These results notify the improved renal function by the successful clearance of urea, BUN, creatinine, and uric acid.

The renal antioxidant status, such as SOD, CAT, activities, and reduced GSH concentration are significantly decreased in the GM treated group of animals compared to the control group. The declined antioxidant status partially with previous findings. The pre-treatment of *Tephrosia purpurea* provides a significant protection against GM -induced...
nephrotoxicity, with lowering the level of plasma creatinine and blood urea in GM treated animals. Decreased concentration of GSH increases the sensitivity of organs to oxidative and chemical injury. The role of GSH, non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites, most often formed by cytochrome P-450-linked monooxygenase, is well-known. Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion. Reduced renal GSH can markedly increase the toxicity of GM. The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the GM-treated group.

Decreased SOD activity could cause the beginning and propagation of lipid peroxidation in the GM treated group. This may be either due to loss of copper and zinc, necessary for the activity of enzyme, or due to ROS induced inactivation of enzyme proteins. The decrease in activities of CAT explains the mechanism of nephrotoxicity induced by GM. The renal accumulation of platinum and covalent binding of renal protein may also play a role in the nephrotoxicity.

Several investigators have shown that GM nephrotoxicity is associated with LPO in renal tissue. LPO is ascribed to free radical-mediated chain reaction that damages cell membranes, and inhibition of this process by *Tephrosia purpurea* is mainly credited to the ability of scavenger free radicals. In the present investigation, pre-treatment with *Tephrosia purpurea* inhibited the increase in LPO induced by GM in renal tissue, indicating antioxidant activity of *T. Purpurea*.

**Cisplatin Induced Nephrotoxicity:** In this present study, rats treated with CP shown a decrease in body weight and it was not reported. This body weight loss was observed, but not completely prevented by pre-treatment of *T. Purpurea*. Suggesting that CP-induced weight-loss may be due to gastrointestinal toxicity and reduced ingestion of food. The destruction of kidney function by CP is recognized as the main side-effect and the most important dose restrictive factor associated with its clinical use, a number of investigators reported that the alterations induced by CP in the kidney functions were characterized by signs of injury, such as increase of products of lipid peroxidation (LPO) and changes in GSH levels in kidney tissue, creatinine and urea levels in plasma.

However, in *Tephrosia purpurea* and silymarin pretreated groups the body weight of animals in comparison with CP treated group representing prevention of gastrointestinal toxicity or maintenance of normal diet. The renal antioxidant status, such as SOD, CAT, activities, and reduced GSH concentration are significantly decreased in the CP treated group of animals compared to the control group. The declined antioxidant status partially with previous findings. The pre-treatment of *Tephrosia purpurea* provides a significant protection against CP-induced nephrotoxicity, with lowering the level of plasma creatinine, blood urea in CP treated animals. The decreased concentration of GSH increases the sensitivity of organs to oxidative stress. The role of GSH, non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites, most frequently formed by cytochrome P-450-coupled monooxygenase, is well-known. Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion. Reduced renal GSH can distinctly increase the toxicity of CP. The depletion of GSH also seems to be a prime factor that permit the lipid peroxidation in the CP-treated group.

Decreased SOD activity could cause the initiation and propagation of lipid peroxidation in the CP treated group. This may be either due to loss of copper and zinc, necessary for the activity of enzyme, or due to ROS induced inactivation of enzyme proteins. The decrease in activities of CAT explains the mechanism of nephrotoxicity induced by CP. The renal increase of platinum and covalent binding of renal protein may also play a role in the nephrotoxicity.

In the present study, increased serum creatinine and urea were observed in CP treated rats may be due to reduction in glomerular filtration rate.

Several investigators have shown that CP nephrotoxicity is associated with LPO in renal tissue. LPO is ascribed to a free radical-mediated chain reaction that damages cell membranes, and inhibition of this process by *Tephrosia purpurea* is mainly attributed to the ability of scavenger free radicals. In the present investigation, pre-treatment with *Tephrosia purpurea* inhibited the increase in LPO induced by CP in renal tissue, indicating antioxidant activity of *T. Purpurea*.

**REFERENCES**


