RESEARCH PAPER

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# Nephroprotective and antioxidant activities of *Tephrosia purpurea* L. on paracetamol and gentamicin induced albino rats

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Nephroprotective and antioxidant activities of *Tephrosia purpurea* have been evaluated against paracetamol and gentamicin induced renal damage in male albino rats. Paracetamol (200mg/kg) and gentamicin (40mg/kg) induced renal damage was well manifested by significant increase in the levels of ALT, AST, ALP, urea, creatinine, sodium in serum. On the other hand, the of levels potassium, protein, albumin, enzymatic and non enzymatic antioxidants were lowered. The oral administration of varying doses of ethanolic extract of *Tephrosia purpurea* (5,10 and 15 mg/kg) for the period of 7 days reversed these altered parameters to normal levels indicating the antioxidative and nephroprotective efficacy of *Tephrosia purpurea L.* against paracetamol and gentamicin induced renal injury.

Key words : Acetaminophen, Carvedilol, Gentamicin and *Tephrosia purpurea*

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## INTRODUCTION

Antioxidants are substances that markedly delay or prevent the oxidation of the substrate. Antioxidants may help the body to protect itself against various types of oxidative damages caused by reactive oxygen species, which are linked to a variety of diseases including cancer, diabetes, shock, arthritis, nephrotic syndrome and acceleration of the ageing process. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Free radicals may also be involved in a number of diseases and tissue injuries (Shahidi, 1997). Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Antioxidants may act by decreasing singlet oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds, and chain breaking to prevent continued hydrogen abstraction from substrates. The hydroxyl radicals derived from superoxide radicals and hydrogen peroxide is the most potent reactive oxygen radical which causes DNA damage (Gutteridge, 1984).

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines, and industrial or environmental chemicals. It is well established that toxic nephropathies are not restricted to a single type of renal injury.The renal response to injury is dynamic, and the kidney adapts to maintain homeostasis during the cascade of repair and recovery that follows the primary insult (Bach *et al.,* 1989). Depending on the type and frequency of the damage, and the region of the kidney that is damaged, the organ can respond by a recovery, a reduced functional reserve, or by a progressive degenerative change.

Gentamicin, an aminoglycoside class of bactericidal antibiotic, is effective against Gram-negative bacterial infections (Martinez-Salgado *et al.,* 2007). In spite of inducing nephrotoxicity, gentamicin is used clinically due to its wide spectrum of activities against Gram-negative bacterial infections caused by *Pseudomonas, Proteus, and Serratia* (Del Valle *et al.,* 1969; Miglioli *et al.,* 1999; Hendriks *et al.,* 2004).The gentamicin – induced nephrotoxicity occurs by selective

accumulation of the drug in renal proximal convoluted tubules that leads to loss of its brush border integrity (Whiting and Brown, 1996). The gentamicin-nephrotoxicity involves renal free radical generation, reduction in antioxidant defense mechanisms, acute tubular necrosis and glomerular congestion (Martinez-Salgado *et al.,* 2007; Mingeot-Leclercq *et al.,* 1999; Elfarra *et al.,* 1994; Geleilete *et al.,* 2002; Abdel-Raheem *et al.,* 2009) resulting in diminished glomerular filtration rate and renal dysfunction.

Carvedilol is both a beta blocker ($\hat{a}_1$, $\hat{a}_2$) and alpha blocker ($\acute{a}_1$). Norepinephrine stimulates the nerves that control the muscles of the heart by binding to the $\hat{a}_1$- and $\hat{a}_2$-adrenergic receptors. Carvedilol blocks the binding to those receptors, (Stafylas and Sarafidis, 2008). Which both slows the heart rhythm and reduces the force of the heart's pumping. This lowers blood pressure and reduces heart failure.

*Tephrosia purpurea* Linn. (Leguminosae), commonly known in Sanskrit as Sharapunkha is a highly branched, suberect, herbaceous perennial herb (Chopra *et al.*, 1956). 30-60 cm in height with spreading branches; leaves imparipinnate, leaflets 11-21, narrow; flowers red or purple in extra axillary racemes; fruits slightly curved pods, 3-4.5 cm, long; seeds 5-10 per pod, grey, smooth. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity (Gokhale and Saraf, 2000). The phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols (Pelter *et al.,* 1981). The present study was undertaken to evaluate the nephroprotective and antioxidant activities of *Tephrosia purpurea* in paracetamol and gentamicin induced aibino rats.

## RESEARCH METHODOLOGY

Male wistar albino rats (100-140g) used were collected from Sri Venkateshwara Enterprises, Bangalore and maintained under standard conditions, fed with standard pellet diet and water *ad libitum*.

**Collection and extraction of plant material :**

*Tephrosia purpurea* plant materials were collected from the near by villages of Pattukkottai, Tamilnadu, India. The leaves were air dried for 72 hours, pulverized into fine powder and stored in a clean air tight container until use. The dried material (25g) was extracted with 50ml of 99.9 per cent of ethanol and filtered using Buckner funnel and Whatman NO.1 filter paper. The filtrate was evaporated at 55° C and used for further studies. The extract was administered in different dose (5, 10 and 15mg/kg b.w., orally) (Khan *et al.,* 2009).

**Experimental design :**

The rats were divided into six groups (n=4).

Group 1: Served as control.

Group 2:Treated with gentamicin (40mg/kg) and acetaminophen (200mg/kg) orally for 7 days.

Group 3: Renal toxicity induced with gentamicin, acetaminophen along with standard drug carvedilol 1mg/kg/day orally for 7 days as co-treatment for nephroprotection.

Group 4: Induced with gentamicin, acetaminophen co-treated with *Tephrosia purpurea* 5 mg/kg/day for 7 days.

Group 5: Induced with gentamicin, acetaminophen co-treated with *Tephrosia purpurea* 10 mg/kg/day for 7 days

Group 6: Induced with gentamicin, acetaminophen co-treated with *Tephrosia purpurea* 15mg/kg/day for 7 days.

**Study protocol:**

The standard and test formulations were administered for 7 days using oral gavages once in a day. At the end of experiment, rats were sacrificed by cervical decapitation. Blood was collected to separate the serum and plasma. The kidney tissue was dissected out, weighed and washed using ice cold saline solution. Tissues were homogenized with buffer solution, centrifuged and the resulting supernatant was used for various biochemical and antioxidant assay.

**Biochemical analysis**

Biochemical parameters such as ALT, AST, ALP, urea, creatinine, sodium, potassium, protein, albumin, TBARS, SOD, CAT, GSH, vit C and vit E were evaluated using standard procedures.

**Statistical analysis:**

The data obtained were subjected to statistical analysis. All results are expressed as Mean ± S.D. Statistical significance was ascertained by students 't' test using SPSS soft ware.

## RESULTS AND ANALYSIS

Nephroprotective and antioxidant activities of *Tephrosia purpurea* have been evaluated against paracetamol and gentamicin induced renal damage in male albino rats and the results are presented in Table 1 and 2. Gentamicin and acetaminophen significantly increased the levels of ALT, AST, ALP, urea, creatinine, sodium, TBARS and decreased the levels of potassium,protein, albumin, enzymatic and non enzymatic antioxidants when compared to normal group. Treatment with herbal drug *Tephrosia purpurea* at different dosage (5, 10,15 mg/kg b.w.,) brought back the altered parameters to near normal.

Antioxidants play an important role in protecting health. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus, inhibit the oxidative mechanisms that lead to degenerative diseases (Halliwell and Gutteridge, 1984). These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals (Pourmorad *et al.,* 2006). Gentamicin is known as one of the most common causes of acute renal failure, which occurs in about 10–30 per cent of patients receiving the drug (Mathew, 1992). Gentamicin is known to generate ROS associated with an increase in lipid peroxidation and decrease in antioxidant enzymes in the intestine and kidney (Banday *et al.,* 2008). Another mechanism by which gentamicin induces nephrotoxicity is by causing renal phospholipidosis through inhibition of lysosomal hydrolases, such as sphingomyelinase and phospholipases in addition to causing oxidative stress (Lindquist,1986; Cojocel *et al.,* 1997).

Acetaminophen is an effective, well-tolerated, household, analgesic and antipyretic alternative to aspirin. Its ingestion in large doses or chronic use is commonly associated with hepatotoxicity and nephrotoxicity in humans and animals (Schnellman, 2001). In the present study, acetaminophen and gentamicin were found to cause

**Table 1 : Levels of biochemical parameters in control and experimental groups**

| arameters | Groups | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
| ALT (IU/L) | 81.5 ± 1.91 | 149.5 ±14.71$^{*}$ | 97.5 ± 3.42$^{**}$ | 115 ± 12.99$^{ns}$ | 106.25 ± 5.91$^{ns}$ | 97 ± 10.52$^{ns}$ |
| AST (IU/L) | 74.5 ± 2.65 | 185.5 ± 8.66$^{*}$ | 87.5 ± 6.81$^{**}$ | 93 ± 2.58$^{ns}$ | 85 ± 2.6$^{ns}$ | 79.8 ± 1.71$^{ns}$ |
| ALP (IU/L) | 127 ± 2.6 | 252.3 ± 19.8$^{*}$ | 135.25 ± 7.65$^{**}$ | 145.25 ± 2.87$^{ns}$ | 128.3 ± 2.98$^{ns}$ | 128.5 ± 3.87$^{ns}$ |
| Urea (mg/dl) | 25.5 ± 1.3 | 80.75 ± 3.1$^{*}$ | 47.25 ± 1.7$^{**}$ | 55.5 ± 3.42$^{ns}$ | 44.5 ± 2.65$^{**}$ | 39 ± 3.92$^{ns}$ |
| Creatinine (mg/dl) | 0.7 ± 0.26 | 2.5 ± 0.3$^{*}$ | 1.8 ± 0.22$^{ns}$ | 1.5 ± 0.2$^{ns}$ | 1.3 ± 0.22$^{ns}$ | 1.2 ± 0.17$^{ns}$ |
| Sodium (meq/l) | 127 ± 2.16 | 178 ± 14.58$^{*}$ | 143 ± 8.77$^{ns}$ | 149 ± 2.89$^{ns}$ | 137.5 ± 2.65$^{ns}$ | 132.5 ± 3.42$^{ns}$ |
| Potassium (meq/l) | 5.53 ± 0.17 | 3.15 ± 0.13$^{*}$ | 4.5 ± 0.21$^{**}$ | 4 ± 0.18$^{ns}$ | 4.42 ± 0.17$^{ns}$ | 4.7 ± 0.18$^{ns}$ |
| Protein (g/dl) | 6.3 ± 0.18 | 2.4 ± 0.32$^{*}$ | 4.75 ± 0.13$^{**}$ | 4.52 ± 0.3$^{ns}$ | 5 ± 0.18$^{ns}$ | 5.6 ± 0.17$^{ns}$ |
| Albumin (g/dl) | 4 ± 0.27 | 1.77 ± 0.17$^{*}$ | 3.63 ± 0.2$^{**}$ | 3.85 ± 0.13$^{ns}$ | 3.25 ± 0.129$^{ns}$ | 3.43 ± 0.125$^{ns}$ |

**Table 2 : Levels of enzymatic and non enzymatic antioxidants in control and experimental groups**

| Parameters | Groups | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
| TBARS (mM/g) | 0.6 ± 0.183 | 1.88 ± 0.17$^{*}$ | 1.33 ± 0.38$^{ns}$ | 1.3 ± 0.42$^{ns}$ | 0.9 ± 0.22$^{ns}$ | 0.8 ± 0.22$^{ns}$ |
| SOD (U/mg) | 84.8 ± 4.11 | 30.25 ± 3.3$^{*}$ | 62.5 ± 6.5$^{**}$ | 50 ± 1.83$^{ns}$ | 59.8 ± 2.75$^{ns}$ | 73.8 ± 5.44$^{ns}$ |
| CAT (µM) | 74.75 ± 2.22 | 33.5 ± 5.45$^{*}$ | 55.75 ± 5.56$^{**}$ | 44.5 ± 8.5$^{ns}$ | 50.25 ± 3.30$^{ns}$ | 68 ± 2.58$^{ns}$ |
| GSH (µM/mg) | 83.75 ± 6.23 | 34.75 ± 5.73$^{*}$ | 73 ± 3.65$^{**}$ | 66.25 ±3.77$^{ns}$ | 71.25 ± 2.75$^{ns}$ | 78 ± 2.9$^{ns}$ |
| Vit C (mg/dl) | 78.5 ± 3.42 | 37.5 ± 4.12$^{*}$ | 63 ± 3.16$^{**}$ | 54.5 ±4.51$^{ns}$ | 57.25 ±1.71$^{ns}$ | 68 ±2.58$^{ns}$ |
| Vit E (mg/dl) | 177.5 ± 3.51 | 65.25 ± 5.61$^{*}$ | 100.3 ± 7.9$^{**}$ | 75.5 ±3.11$^{**}$ | 88 ± 2.58$^{ns}$ | 114.75 ± 4.86$^{ns}$ |

Values are expressed in Mean ± S.D; * - Significant different from Group I Vs Group II ($p<0.001$), **- Significant different from Group II Vs Group III, IV,V, VI ($p<0.001$) ns – not significant, Group 1: Control, Group 2 : Gentamicin and acetaminophen, Group 3 : Carvedilol, Group 4 : , *Tephrosia purpurea* 5 mg/kg b.w., Group 5 : *Tephrosia purpurea* 10 mg/kg b.w., Group 6 : *Tephrosia purpurea* 15 mg/kg b.w.,

significant elevations in the levels of serum AST, ALT and ALP. Mild alteration in the cells may be responsible for this elevation. These drug-induced nephrotoxicities were often associated with marked elevations in serum urea, creatinine. (Verpooten *et al*., 1998). It also augmented plasma sodium levels, while potassium level was decreased which might be due to glomerular dysfunction. There was significant decrease in the level of protein and albumin in nephrotoxic group of animals which might be due to loss of structural intergrity and diminished function of tubules augmenting leakage of protein via urine (Shah, 2007). The changes of membrane lipid composition may be induced by free radical-initiated lipid peroxidation. This view is supported by increased TBARS level. The balance between oxidants and antioxidants is crucial for the maintenance of the biological integrity of the tissues (Naziroglu *et al.,* 2004). The depletion of SOD, CAT,GSH, vit C and vit E appears to be an early and necessary event occurring in acetaminophen and gentamicin induced lipid peroxidation and subsequent toxicity. Oral administration of *Tephrosia purpurea* at graded doses brought back the altered parameters to near normal. This ameliorative potential was also comparable with the standard drug carvedilol (Gupta *et al.,* 1980). In conclusion, it has been shown that ROS participate in acetaminophen and gentamicin induced kidney injury and continual cell injury induces DNA lesions and interaction of protein cross-linkages. If intracellular free oxygen radicals increase, irreversible cellular injury process begins but treatment with *Tephrosia purpurea* reduces lipid peroxidation and increases antioxidant status. The free radical-scavenging property of *Tephrosia purpurea* is the basis of decreased tubular necrosis, tubular vacuolization and reduced parietal cell hyperplasia in nephrotoxin induced rats. Therefore, it was concluded that *Tephrosia purpurea* decelerates the development of acetaminophen and gentamicin induced nephrotoxicity in rats.

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