

Ameliorative Effect of *Tephrosia Purpurea* in Arsenic-induced Nephrotoxicity in Rats

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ABSTRACT

Objectives: The present investigation was conducted to evaluate the nephroprotective activity of *Tephrosia purpurea* (TPE) against arsenic-induced toxicity. **Materials and Methods:** Twenty four number of wistar rats were equally divided into three groups. Sodium arsenite (10 mg/kg) was orally given to group I for 28 days, additionally group II was orally treated with TPE (500 mg/kg), while the control group was kept untreated with neither arsenic nor TPE. Serum biomarker levels, oxidative stress indices and arsenic concentration in kidney were estimated. Histopathology of kidney was also conducted. **Results:** Group II animals show significantly reduced blood urea nitrogen and plasma creatinine, and increased serum albumin level compared to group I. The higher lipid peroxidation with exhausted superoxide dismutase activity and reduced glutathione level were noticed in group I compared to group II. There was no significant difference in arsenic accumulation in kidneys between the two arsenic treated groups, but the histopathology of kidney of group II rats revealed reduced necrosis and intact tubular architecture as compared to group I. **Conclusions:** *Tephrosia Purpurea* extract has a significant role in protecting the animals from arsenic-induced nephrotoxicity.

Key words: Arsenic, nephrotoxicity, rats, sub-acute toxicity, *Tephrosia purpurea*

INTRODUCTION

Arsenic, a ubiquitous element belonging to group V of the periodic chart, persists in organic, inorganic or elemental form in nature. Among different forms the trivalent inorganic species is the most toxic.^[1] Humans and animals are generally exposed to arsenic by consumption of contaminated ground water or through food chain.^[2]

Arsenic has an affinity toward the SH group of proteins^[3] that leads to inhibition of cellular respiration, impaired glycolysis and oxidative process^[4] and finally death of cells. Though almost all the systems are being affected, liver and kidneys are most susceptible to arsenic toxicity.^[5] During a metabolic process arsenic gets methylated in liver by arsenic methyltransferase to form organoarsenics that are excreted by kidney through urine.^[6] Continuous exposure to arsenic damages the kidney through generation of excessive free radicals inside the nephrons.^[7] Medicinal plants of India are having proven efficacy against different ailments both in human and animals.^[8] *Tephrosia purpurea* is such a plant of fabaceae family being used for many years in traditional Indian medicine due to its rich flavonoid and polyphenol content.^[9,10] It is distributed widely in India, China and Sri Lanka.^[11] This plant has been extensively used in the treatment of jaundice, gastritis, dyspepsia,

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diarrhoea, tumors, bronchitis, asthma, rheumatism, urinary and kidney disorders.^[12,13] To the best of our knowledge there is no scientific reference regarding the effect of *T. purpurea* extract (TPE) against arsenic-induced kidney toxicity in rats. So, the present investigation was carried out for evaluation of nephroprotective activity of *T. purpurea* against induced arsenic toxicity in wistar albino rats.

MATERIALS AND METHODS

Chemicals

Sodium arsenite was procured from Himedia (Mumbai, India) and used for arsenic intoxication to the experimental animals. ERBA Diagnostics kits (Mannheim, Germany) were procured for estimation of the serum bio-marker levels.

Experimental animals

Healthy male and female wistar albino rats (140-150 g) were purchased from Laboratory Animal Research Station, I.V.R.I, Izatnagar, India, and housed in propylene cages under standard laboratory conditions and allowed for acclimatization for a period of 15 days before starting the experiment. Rats were offered standard diet and water was given ad-libitum during the period of acclimatization and experiment. The experimental protocol was approved by Institutional Animal Ethical Committee with approval no. 528/RVC/IAEC/119.

Arsenic solution

Sodium arsenite was dissolved in distilled water @ 10 mg/kg and orally administered with oral gavage to the rats. The dose was calculated according to the 1/4th of oral LD50 of sodium arsenite in rats.^[14]

Plant extract

The aerial parts of *T. purpurea* were collected, washed with distilled water, shade dried, pulverized and freshly prepared powder (25 g) was immersed in a hydro-alcoholic solution (40% distilled water + 60% ethanol) in a flask stoppered and was kept at room temperature for 48 hours at 150 rpm in orbital shaker. The contents were filtered through the muslin cloth and then through whatman No. 1 filter paper. The extract was then dried in a petridish at room temperature and used along with gum acacia @ 500 mg/kg body weight to individual animal with oral gavage needle.^[15]

Experimental design

A total 24 number of rats were equally divided into three groups. Sodium arsenite @ 10 mg/kg was orally given to group I for 28 days. Group II was orally treated with

TPE @ 500 mg/kg and the same dose of sodium arsenite as in group I for the entire study period of 28 days. The control group was kept untreated with either arsenic or TPE and was given normal diet and water throughout the study period.

All the animals were slaughtered on day 29 to evaluate the serum bio-marker levels, kidney oxidative stress parameters and arsenic concentration in kidney. The histopathological study of kidney was also conducted.

Serum enzyme estimation

Serum blood urea nitrogen (BUN), creatinine, and serum albumin were estimated using the Erba-semi autoanalyzer (Mannheim, Germany).

Oxidative stress analysis

The pieces of kidney thus collected after the sacrifice of the experimental animals were washed in ice cold saline and 200 mg of kidney tissue sample was weighed and kept in 2 ml of ice-cold saline. For estimation of GSH, 200 mg of kidney tissue sample was kept in 0.02M EDTA. The homogenate was prepared in the Remi-Homogeniser and was centrifuged at 3000 rpm for 10 min. The supernatant was used for estimation of following oxidative stress indices. Superoxide dismutase (SOD) was estimated as per the method of Madesh *et al.*^[16] The extent of lipid peroxidation was evaluated in terms of MDA production and determined by the thiobarbituric acid method.^[17] The reduced glutathione (GSH) level was assessed by using the DTNB method^[18] in kidney homogenate.

Analysis of arsenic

Total arsenic in kidney was quantified by digestion, using a tri-acid mixture of nitric acid, perchloric acid and sulphuric acid (10:4:1) following the method of Datta *et al.*^[19] The digested samples were diluted with deionized Millipore water, passed through Whatman filter paper No. 4 (Rankem, India) and made the volume to 10 ml. Concentrated hydrochloric acid (5 ml) was added to it and shaken well. Then after 1 ml of potassium iodide (5% w/v) and ascorbic acid (5% w/v) mixture was added and the aliquot was incubated for 45 min for transformation of arsenate to arsenite.^[20] The final volume was made up to 50 ml with Millipore water and arsenic concentration read on the Varian AA240 model AAS Atomic Absorption Spectrometer (AAS) equipped with vapour generation accessories. The operating parameters were: lamp, arsenic hollow cathode lamp; wavelength, 193.7 nm; slit width, 0.5 nm; lamp current, 10.0 mA; vapor type, air/acetylene; air flow, 10.00 L/min; inert gas for hydride generation, Argon. Reducing agent (Aqueous solution of 0.6% sodium borohydride was prepared in 0.5% w/v sodium hydroxide) and 40% HCl were freshly

prepared before use. The working standards were 5, 10, 20 and 40 µg/L and prepared by the same procedure as the test sample.

Histopathology

Histopathology of buffered formalin-fixed kidney samples were routinely processed, cut at 5 µm and stained with H and E stain.^[21]

Statistical analysis

Statistical analysis was done by ANOVA using SPSS software version 17.0. A value $P < 0.05$ (*) and $P < 0.01$ (**) were considered significant at 5% and 1% level, respectively.

RESULTS

Serum biochemical parameters

A significant increase ($P < 0.01$) in serum levels of BUN and creatinine were observed in group I compared to the control group [Table 1]. Again, group II showed a significantly lower BUN ($P < 0.01$) and creatinine ($P < 0.05$) than group I [Table 1].

A significant decrease ($P \leq 0.01$) in serum albumin level was observed in group I as compared to the control group [Table 1]. Serum albumin level was significantly higher ($P \leq 0.05$) in group II compared to group I [Table 1].

Oxidative stress indices

GSH and SOD levels in kidney were significantly ($P \leq 0.01$) decreased in group I compared to control. Both the parameters were improved in group II (Tephrosia treated) in comparison to group I [Table 1].

Malonaldehyde (MDA) production in kidney of group I was significantly ($P \leq 0.01$) higher in comparison to both control and Tephrosia-treated (group II) group [Table 1].

Table 1: Effect of of NaAsO₂ alone and with TPE on various parameters in rats (mean±S.E) (n=8)

Parameters	Control	Group I	Group II
BUN (mg/dl)	16.54±1.79	38.87±2.69 ^a	28.58±1.95**
Creatinine (mg/dl)	0.62±0.07	1.40±0.15 ^a	0.90±0.16*
Albumin (g/dl)	3.06±0.18	2.33±0.17 ^a	2.89±0.13*
GSH (µmol/gm of protein)	7.97±1.01	3.47±0.57 ^a	5.86±0.55*
LPO (nM/MDA/g)	0.93±0.23	6.43±0.51 ^a	4.34±0.55**
SOD (U/mg of protein)	5.38±0.63	3.01±0.49 ^a	4.67±0.50*
As (ppm)	0.14±0.09	7.94±2.53 ^a	6.96±0.77 ^{NS}

**($P < 0.01$); *($P < 0.05$); NS: non-significant, when compared with group I.

^a($P < 0.01$); when compared with the control group, TPE=*T. purpurea* extract, BUN=Serum blood urea nitrogen, GSH= Reduced glutathione, SOD=Superoxide dismutase, LPO=Lipid peroxidation

Arsenic concentration

The mean concentration (ppm) of arsenic in kidney of rats in control group, groups I and II was 0.14 ± 0.09 , 7.94 ± 2.53 and 6.96 ± 0.77 ppm, respectively. Though a significant ($P \leq 0.01$) amount of arsenic was deposited in kidney of both the group I and group II compared to the control group, but the accumulation was not varied significantly in between the group I and group II.

Histopathology

The microscopic section of kidney of rats fed with sodium arsenite @ 10 mg/kg BW exhibited varying degree of degenerative changes in tubular epithelium. There was also the presence of vacuolar changes along with necrosis, desquamation and distorted cellular architecture [Figures 1 and 2]. On the other hand, microscopic examination of kidney of rats treated with sodium arsenite @ 10 mg/kg and TPE @ 500 mg/kg showed lesser desquamation and necrotic changes [Figures 3 and 4] than group I.

DISCUSSION

Arsenic is metabolized in the liver and its methylated species are excreted through urine by kidney. So, it is considered as primary organ of target in arsenic toxicity.^[22] In this study, we tried to evaluate the nephroprotective activity of TPE against induced arsenic toxicity. Exposure of arsenic for 28 days has significantly increased the serum level of BUN and creatinine, and decreased serum albumin in rats [Table 1]. Earlier reports also documented a significant rise in the levels of BUN and creatinine following exposure of heavy metals.^[23] Ammonia, a product of protein metabolism, is converted into urea in liver, which is then transported to kidneys and finally excreted in the urine. Creatinine is the end product of protein metabolism that also excreted through urine from the body. The increase of creatinine concentration might be due to the loss of kidney function and considered as functional evidence of arsenic-induced nephrotoxicity.^[24-26] There was a decrease in serum albumin in rats of group I compared to the control group which might be due to the severe nephrotoxic effect of arsenic resulting a drainage of protein through urine. There was a significant decrease in BUN and creatinine levels in the Tephrosia-treated (group II) animals [Table 1] with respect to group I. Previously it has been suggested that *T. purpurea* is useful in kidney disorders^[27] and could reduce the elevated BUN, creatinine levels in N-diethylnitrosamine and potassium bromate-induced renal toxicity.^[28] Moreover, a comparatively higher albumin level in group II rats than group I is an indicative of glomerular membrane stabilizing activity of Tephrosia.

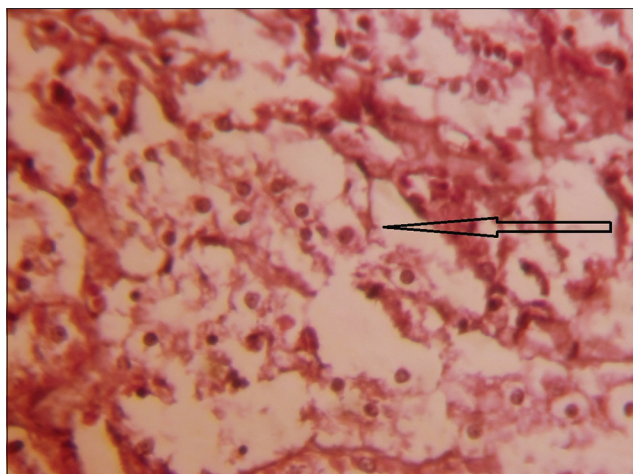


Figure 1: Microphotograph showing desquamation and necrosis in kidney of group I rat (x400)

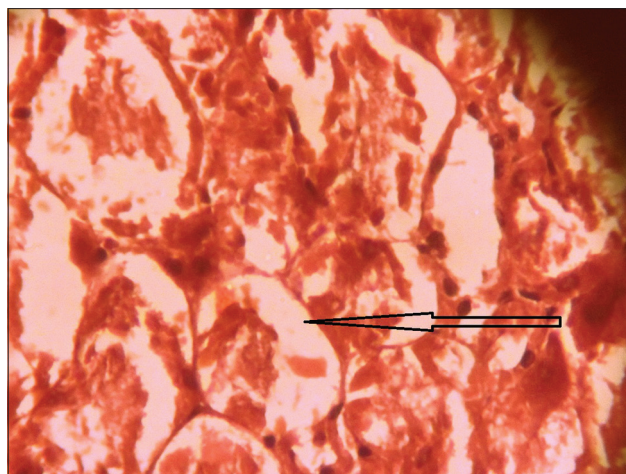


Figure 2: Microphotograph showing desquamation in kidney of group I rat (x400)

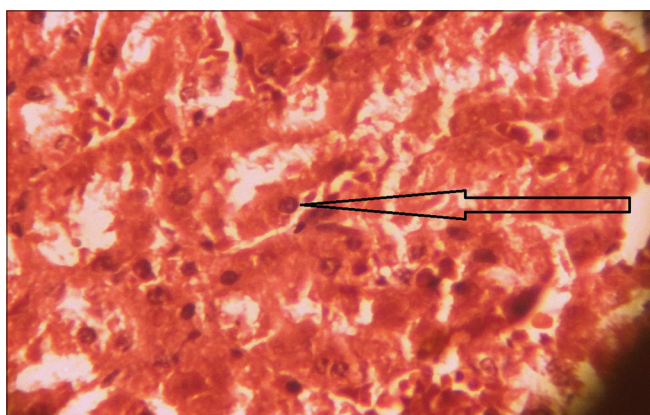


Figure 3: Microphotograph showing mild desquamation and necrosis in kidney of group II rat (x400)

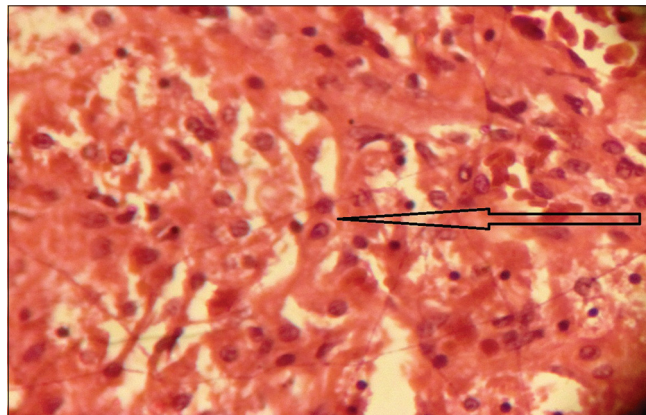


Figure 4: Microphotograph showing mild desquamation and reduced necrosis in kidney of group II rat (x400)

In the present study, arsenic significantly increases MDA production and decreases GSH and SOD in the kidney of both the arsenic treated groups; however, the damage is significantly lower in the Tephrosia-treated group (group II) compared to only arsenic-treated one (group I) [Table 1]. Arsenic attaches to the glomerular membrane due to its lipophilicity and increases lipid peroxidation.^[29] Again, GSH is having a sulphhydryl (-SH) group in it and acts as a scavenger of generated free radicals and also decreases lipid peroxidation.^[30] As already stated, arsenic has the affinity to react with the sulphhydryl group-containing compounds.^[31] So, it inhibits the GSH reductase and produces excessive ROS in the kidney which will lead to damage of the respective organ.^[32] SOD is an important antioxidant enzyme responsible for the elimination of superoxide radical and plays an important role in maintaining cellular ROS balance. A decrease in its level in group I compared to control is probably due to the over production of free radicals in the kidney.^[33,34] *T. purpurea* is having a potent antioxidant property and contains two biologically active flavonoidal compounds

quercetin and rutin.^[35] Flavonoids act against free radicals^[36] and decrease lipid peroxidation and reduces oxidative stress in the body. Earlier it has been reported that TPE reduces MDA levels and increases GSH levels significantly in gentamicin-induced acute renal injury in albino rats.^[37] Relatively reduced level of MDA along with improved SOD and GSH in group II than group I as evidenced from our study [Table 1] may be due to the presence of phenolic compounds and flavonoids like quercetin and rutin in *T. purpurea*.^[38,39] The reduced degree of necrosis and desquamation [Figures 3 and 4] in group II rats in the present experiment again established the nephroprotective effect of *T. purpurea*.

CONCLUSION

Therefore, it may be concluded from the present study that supplementation of *Tephrosia purpurea* extract significantly protects kidney from arsenic-induced nephrotoxicity by reducing the oxidative stress and restoration of serum bio-marker levels.

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