Protective efficacy of *Tephrosia purpurea* against Aflatoxin B1 induced oxidative damage in rats

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**ABSTRACT**

*Tephrosia purpurea* (TP), commonly known as wild indigo, has a unique quality of healing all kinds of wounds and it is also an important ingredient of many Ayurvedic formulations. Thus, the present investigation was designed to validate and evaluate protective efficacy of ethanolic extract of *Tephrosia purpurea* (TP) against Aflatoxin B1 (AFB1) induced renal dysfunction.

The ethanolic extract of *Tephrosia purpurea* was prepared by Soxhlet extraction method. For present study female rats (Wistar strain) were randomly divided into 6 groups with 6 animals in each group. The entire regime was of 28 days. AFB1 was administered at 200 µg/kg dose and TP was administered at three different doses (100, 200 and 300 mg/kg). 24 hours after last treatment the animals were euthanised various biochemical test were performed. AFB1 exposure significantly elevated uric acid, urea, creatinine in serum and lipid peroxidation in renal tissue indicating cellular damage. On the other hand, decline in activities of superoxide dismutase, catalase and reduced glutathione suggests exhaustion of endogenous defence system. Treatment with TP extract offers protection at all the three doses (100, 200 and 300 mg/kg). Although, maximum recovery was seen with 300 mg/kg dose of TP.

**Figures**: 05  **References**: 28  **Table**: 01

**KEY WORDS**: Aflatoxin B1, Antioxidants, Kidney, Oxidative damage, *Tephrosia purpurea*

**Introduction**

Aflatoxins (AF) are mycotoxins secreted by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are unavoidable contaminant of grains, nuts, milk and milk products. Out of 18 known Aflatoxins, Aflatoxin B1 (AFB1) is most toxic. Bioactivation of AFB1 generates AFB1 epoxide and free radicals which are responsible for triggering cascade of organ dysfunction. AFB1 epoxide binds with DNA and reactive oxygen species binds with cellular membrane thereby eliciting toxicological effects. Apart from hepatocellular carcinoma, renal dysfunction, immunosuppression, delayed implantation, lungs and gastrointestinal cancers are also induced by AFB1.

According to previous studies polyphenols and phytochemicals have shown ameliorating effects against AFB1 induced oxidative stress. Despite of ongoing research, there is no known antidote to limit and cure oxidative damage induced by AFB1. Plants of genus *Tephrosia* are rich in flavonoids, about 161 flavonoids have been identified and isolated so far. *Tephrosia purpurea* (TP), commonly known as “Sarapunkha” has a peculiar property of curing all types of wounds. Some researchers call *Tephrosia purpurea* a magical herb. TP possess antioxidant properties due to presence of various phytochemical such as alkaloids, amino acids, glycosides, sterols, rotenoids, isoflavones, flavanones, flavonoids, chalcones, flavanols, flavones, saponins, reducing sugars, terpenes and tannis. According to classical literature it is used in treatment of splenomegaly. TP significantly protects mitochondrial dysfunction, manages gout, maintain membrane integrity. Thus, the present investigation was carried out to explore and validate protective efficacy of TP against AFB1 induced oxidative damage. The oxidant/antioxidant status and serological markers were examined to determine the therapeutic potential of TP ethanolic extract.

**Material and Methods**

**Animals and Chemicals**

Female albino rats of *Wistar* strain were procured from animal house facility AIIMS, New Delhi. Animals were

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TABLE-1: Urea, Uric acid and creatinine in serum

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Uric Acid</th>
<th>Urea</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.51 ± 0.08</td>
<td>26.87 ± 1.48</td>
<td>0.28 ± 0.015</td>
</tr>
<tr>
<td><em>Perse</em></td>
<td>1.45 ± 0.08</td>
<td>26.70 ± 1.47</td>
<td>0.26 ± 0.014</td>
</tr>
<tr>
<td>A</td>
<td>6.84 ± 0.37#</td>
<td>47.04 ± 2.6#</td>
<td>1.32 ± 0.07#</td>
</tr>
<tr>
<td>A + TP 100 mg/kg</td>
<td>6.10 ± 0.33(13.88%)</td>
<td>42.35 ± 2.64(23.25%)</td>
<td>1.23 ± 0.06(8.65%)</td>
</tr>
<tr>
<td>A + TP 200 mg/kg</td>
<td>5.64 ± 0.31(22.51%)</td>
<td>39.27 ± 2.17(38.52%)</td>
<td>0.76 ± 0.04(53.84%)</td>
</tr>
<tr>
<td>A + TP 300 mg/kg</td>
<td>3.12 ± 0.17(69.79%)</td>
<td>30.64 ± 1.69(81.30%)</td>
<td>0.53 ± 0.02(71.96%)</td>
</tr>
<tr>
<td>F Value</td>
<td>86.45@</td>
<td>21.87@</td>
<td>113.18@</td>
</tr>
</tbody>
</table>

Values are Mean ± SE; N=6; @ = Significant at 5% for ANOVA # A vs Control; * A + Therapy vs A at P < 0.05

**Abbreviations:** TP = *Tephrosia purpurea*, A = Aflatoxin B1

Fig. 1: Lipidperoxidation Kidney
Aflatoxin B1 was procured from Hi-media Laboratories Ltd. Mumbai, India. All other chemicals were of analytical grade and purchased from Sigma-Aldrich Co., USA; Ranbaxy, New Delhi and Hi-media Laboratories Ltd. Mumbai, India.

**Collection and preparation of plant extract**

The whole plant of TP was collected from Jiwaji University campus and identified by taxonomist. The herbarium was submitted to SOS in Botany, Jiwaji University, Gwalior (M.P.) with accession number (5313/PP- 49-50/03/12/2020). The plant was washed, shade dried, grinded and sieved to obtain fine powder. Subsequently the powder was extracted with 70% ethanol. The extract was dried at 37°C and stored at 4°C till its use.

**Experimental Design**

Animals were randomly divided into 6 groups with 6 animals in each group. Group III received AFB1 (200 ìg/kg/day) for 28 days, post orally. Group IV to VI received AFB1 along with different doses of TP for 28 days (100, 200, 300mg/kg, respectively).

- Group I: Control
- Group II: Therapy Perse (post orally)
- Group III: AFB1 200 ìg/kg/day (post orally)
- Group IV: AFB1 + TP 100mg/kg/day
- Group V: AFB1 + TP 200mg/kg/day
- Group VI: AFB1 + TP 300mg/kg/day

**Fig. 2 : Reduced Glutathione Kidney**

Values are Mean ± SE; N=6; *=Significant at 5% for ANOVA

# A vs Control; * A vs Therapy vs A at P ≤ 0.05

**Abbreviations:**

TP = *Tephrosia purpurea*, A = Aflatoxin B1, N = Normal
Blood samples were collected from retro orbital plexus just before euthanization, kidney samples were harvested, washed with normal saline and were kept in sterile conditions for further investigation.

**Serological analysis**

Blood samples were incubated at 37°C for half an hour and centrifuged at 3000 rpm for 15 minutes, serum was collected and stored at -20°C. The harvested serum was used to analyse urea, uric acid and creatinine, which were estimated using Erba diagnostics kits according to the given instructions.

**Homogenate preparation**

After necropsy kidney samples were excised and cleaned with cold normal saline. The kidney samples were homogenized using Remi motor homogenizer. The homogenates were made according to the need of particular protocol.

**Renal oxidant and antioxidant status**

Oxidant and antioxidant status of renal tissues were estimated by lipid peroxidation, reduced glutathione and superoxide dismutase.
Statistical analysis

Data are expressed as mean ± standard error of six animals in each group. Analysis of variance (ANOVA) was done considering significance at p ≤ 0.05 followed by student t test. Statistical analysis was done with help of Graph Pad Instat software.

Results and Discussion

In a tropical country like India, AFB1 is prevalent contaminant of grains. The unavoidable exposure of AFB1 to humans led to organ dysfunction. AFB1 is metabolised by cytochrome P450 enzymes to from AFB1 epoxide and reactive oxygen species. AFB1 epoxide is highly lipophilic and binds with lipids of cellular membrane. Similarly reactive oxygen species also binds with lipids of membrane thereby initiating lipid peroxidation. An increased lipid peroxidation is clearly evident in cohorts exposed with AFB1 in present study (Fig. 1). Leakage of urea, uric acid and creatinine in serum are markers of kidney dysfunction and are also an indication of compromised membrane integrity due to free radicals (Table 1). Treatment with TP at different doses offered protection against free radicals at all the doses. However maximum protection was seen at 300 mg/kg dose of TP. The endogenous deposits of antioxidants like glutathione, superoxide dismutase, catalase, adenosine triphosphatase and assessment of protein.

Abbreviations: TP = Tephrosia purpurea, A = Aflatoxin B1, N = Normal

Fig. 4: Catalase Kidney
Fig. 5: ATPase Kidney

ANOVA Kidney

F values 3.56

Values are Mean ± SE; N=6. * = Significant at 5% for ANOVA # A vs Control; + A + Therapy vs A at P ≤ 0.05

Abbreviations: TP = Tephrosia purpurea, A = Atlatoxin B1, N = Normal

reduced glutathione, superoxide dismutase and catalase counters generation of free radicals during AFB1 metabolism. The exhaustion of reduced glutathione, superoxide dismutase and catalase can be associated with consumption of antioxidants during quenching of free radicals and converting it into harmless metabolites. In present study AFB1 challenged cohorts showed altered activities of reduced glutathione, superoxide dismutase and catalase. Treatment with TP extract significantly resorted their activities towards normal. Maximum restoration in terms of percent protection was seen with 300 mg/kg dose of TP (Figs. 2, 3 and 4). The extend of mitochondrial dysfunction in renal tissues can be estimated by adenosine triphosphatase (ATPase). ATPase, is a mitochondrial membrane bound enzyme, which plays an important role in maintaining ionic balance in cell. In current study rats exposed to AFB1 showed a decline in activity of ATPase. TP extract was able to maintain the activity of ATPase towards control at all the three doses but 300 mg/kg dose was most effective in terms of percent protection (Fig. 5). In present study the TP extract was able to limit the effect of AFB1 epoxide and reactive oxygen species this could be due to presence of flavonoids and other phytochemicals. Flavonoids and polyphenols act as quenchers of free radicals formed during AFB1 metabolisms, thereby preventing accumulation and binding of these free radicals to macromolecules. The present investigation is supported
by earlier investigator who explained role of gallic acid in management of oxidative damage caused in hepatic tissues during AFB1 metabolism.

**Conclusion**

From this investigation it may be concluded that phytochemicals present in ethanolic extract of TP offers protection against AFB1 induced renal toxicity by modulating endogenous deposits of antioxidants and by quenching free radicals generated during AFB1 metabolism.

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injury in rats via inhibiting cytochrome P450 enzyme, activating Nrf2/GST pathway and regulating mitochondrial