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Original Article

Tephrosia purpurea Ameliorates Oxidative and Histological Alterations Induced by Aflatoxin B1 in Rats

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ABSTRACT

Background: *Tephrosia purpurea* (TP), commonly known as wild indigo, is traditionally used in treatment of splenomegaly. It is an important ingredient of various Ayurvedic medicines used in treatment of liver diseases. **Objectives:** Thus, the study was undertaken to investigate the hepatoprotective efficacy of ethanolic extract of TP against Aflatoxin B1 (AFB1) induced liver injury. **Materials and Methods:** The ethanolic extract of TP was prepared by Soxhlet extraction method. Presence of polyphenols and antioxidant potential were assessed. The antiproliferative activity of extract was tested on HepG2 cells using MTT assay. For *in vivo* studies female *Wistar* rats were randomly divided into 6 groups with 6 animals in each. The entire regime was of 33 days. AFB1 was administered at 200 µg/kg dose and TP was administered at three different doses (100, 200 and 300 mg/kg). 24 hr after last treatment the animals were euthanised and liver and blood samples were collected. **Results:** 45.77± 2.53 µg/ml IC_{50} of extract was seen on HepG2 cells. A significant elevation in serum transaminases, triglycerides (TG) and LPO was seen after AFB1 intoxication. Whereas decline in activities of GSH, SOD, CAT, G6Pase and ATPase were observed. Treatment with different doses of TP restored its activities towards normal indices. Maximum recovery was seen with 300 mg/kg dose of TP. **Conclusion:** It may be concluded that TP possess hepatoprotective efficacy against AFB1 induced oxidative injury and it may prove to be of clinical use after further studies.

Keywords: Aflatoxin B1, Oxidative injury, Liver, *Tephrosia purpurea*, Antioxidant status.

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INTRODUCTION

Liver is a prime organ continuously involved in metabolism and drug detoxification. This unique quality of liver makes it vulnerable to chronic liver toxicity. About 800,000 deaths per year is caused by chronic liver dysfunctions. In African and Asian countries liver related disorders are prime cause of death.[1] Aflatoxins are mycotoxins produced by fungus of *Aspergillus* strain. Out of 20 known Aflatoxins, AFB1 is most common and toxic mycotoxin. It is predominantly found in staple foods like corn, peanuts and soybean due to improper storage and humid climatic conditions.[2] Maize samples from India showed concentration of AFB1 in range 48-58 µg/kg sample of grain.[3] Daily exposure of small amount of AFB1 weakens the antioxidant and immune capacity of body. According to IACR, it is listed as class I hepatocarcinogen. Its role in development and progression of hepatocellular carcinoma is extensively studied.[4-5] AFB1 itself is not toxic but when it undergoes oxidation via cytochrome 450 enzymes (CYP450) a reactive intermediate species named AFB1-8,9- epoxide (AFBO) is formed, which is responsible for toxicological manifestation. Metabolism of AFB1 generates reactive oxygen species (ROS), accumulation of these free radicals creates a condition of oxidative stress leading to loss of membrane integrity of hepatocytes.[6]

Despite of ongoing research there is no known antidote for managing AFB1 induced oxidative damage.[7] According to WHO a shift towards plant-based medicines is been observed in recent years due to its minimum side effects. Till date about 35,000-70,000 species of plants have been screened for their medicinal use.[8] Plants of genus *Tephrosia* are known to be rich in polyphenols, about 161 flavonoids have been identified and isolated. *Tephrosia purpurea* (TP), commonly known as wild indigo or "Sarapunkha" belongs to Fabaceae family. Extract of TP possess free radical scavenging activity and antioxidant properties due to presence of various phytochemicals like alkaloids, amino acids, glycosides, sterols, rotenoids, isoflavones, flavanones, flavonoids, chalcones, flavanols, flavones, saponins, reducing sugars, terpenes and tannis.[9-11] Due to presence of these phytochemicals, it has a peculiar property of curing all types of wounds. Hence, it is known as "*Sarwa wranvishapaka*". According to classical literature it is also used in treatment of splenomegaly.[12-13] Keeping in mind the antioxidant potential of TP, present study was hypothesized to explore and validate curative effect of TP against AFB1 induced injury. The oxidative stress, antioxidant markers, serological parameters and histological alterations were examined to determine the therapeutic potential of TP extract.

MATERIALS AND METHODS

Animals and Chemicals

Female rats of *Wistar* strain were purchased from animal house facility AIIMS, New Delhi. They were housed in university animal house facility under standard conditions (25 ± 2°C temp, 60–70% relative humidity and 12 hr photoperiod) and were treated in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals **(CPCSEA No-IAEC/JU/55)**.

Aflatoxin B1 was procured from Himedia Laboratories Ltd. Mumbai, India. All other chemicals were of analytical grade and purchased from Sigma-Aldrich Co., USA; Ranbaxy, New Delhi and Himedia Laboratories Ltd. Mumbai, India.

Collection and Preparation of Plant Extract

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

Phytochemical Studies

Ethanolic extract of plant was subjected to phytochemical analysis to quantify the amount of polyphenols[14] and free radical scavenging activity.[15]

Cell Culture and Condition

The HepG2 cell lines were obtained from National Centre for Cell Science (NCCS) Pune, and were maintained in DMEM medium supplemented with 10% foetal bovine serum, 100 µg/mL streptomycin and 100 U/mL penicillin. HepG2 cells were stored in T75 flask at 37°C and supplemented with 5% CO_2 in a humidified incubator. Cells were sub cultured once the 70% confluence was achieved.

Cell Viability Assay

Antiproliferative activity of TP was determined on HepG2 cells using MTT assay.[16] Approximately 5x10^4 cells were seeded in 96 well plate and incubated for at 37°C in CO2 incubator for one day. On following day treatment of TP extract was given in different concentration (25 to 50µg/ml) and the cells were maintained for 24 hr. Subsequently 20µl of MTT was added in each well and cells were incubated for 4 hr in dark. After incubation media was aspirated and formazan crystals were dissolved by adding 100 µl DMSO. After 5 min, absorbance was noted at 570nm.

Experimental Design

Animals were randomly divided into 6 groups with 6 animals in each group. Group III received AFB1 (200 µg/kg/day) for 33 days, post orally. Group IV to VI received AFB1 along with different doses of TP for 33 days (100, 200, 300mg/kg, respectively).

Group I: Control

Group II: Therapy *Perse* (post orally)

Group III: AFB1 200 µg/kg/day (post orally)

Group IV: AFB1 Same as group III+ TP 100 mg/kg (post orally)

Group V: AFB1 Same as group III+ TP 200 mg/kg (post orally)

Group VI: AFB1 Same as group III + TP 300 mg/kg (post orally)

Animals were euthanized after 24 hr of last treatment. Blood samples were collected from retro orbital plexus just before euthanisation by Riley[17] and liver samples were harvested and washed with normal saline.

Serological Analysis

Blood samples were incubated at 37°C for half an hour and centrifuged at 3000 rpm for 15 min, serum was collected and stored at -20°C. The harvested serum was used to analyse serum aminotransferases[18] and triglyceride were estimated using Erba diagnostics kits according to the given instructions.

Homogenate Preparation

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

Assessment of Oxidative Stress Markers

The extent of lipid peroxidation (LPO) in liver samples were calculated spectrophotometrically by assessing the formation of thiobarbiutric acid reactive species (TBARS) at λ 535 nm against blank.[19]

Estimation of Antioxidant Potential

A yellow-coloured chromophore is formed when reduced glutathione reacts (GSH) with 5-5'-dithiobis 2-nitrobenzoic acid, which is recorded at λ 412 nm.[20] Superoxide dismutase (SOD) was estimated by assessing rate of adrenochrome formation inhibition, it was recorded at λ 480 nm for 3 min.[21] Catalase (CAT) activity was determined by calculating decomposition of H_2O_2 and was recorded at λ 240 nm for 3 min.[22]

Estimation of Membrane Bound Enzymes

For estimation of adenosine triphosphatase (ATPase) activity, inorganic phosphate released from ATP is measured at λ 620 nm.[23] Glucose 6 phosphates (G6Pase) catalyses the hydrolysis of glucose 6 phosphate into glucose and inorganic phosphate, which reacts with ammonium molybdate to form blue coloured complex. Its intensity was recorded at λ 660 nm.[24]

Assessment of Protein

Protein was calculated according to Lowry[25] method.

Histopathology

After necropsy liver slices were fixed immediately in Bouin's fixative and were regularly washed with 70% alcohol, after clearing of tissues, paraffin blocks were formed. These blocks were cut into sections of 5 µm thickness. These sections were stained with Hematoxylin-eosin and were observed under light microscope (Leica).

Statistical Analysis

Data is expressed as mean ± standard error of six animals in each group. Analysis of variance (ANOVA) was done considering significance at $p \leq 0.05$ followed by student t test. Statistical analysis is done with help of Graph Pad Instat software. Percent (%) protection was calculated by the following formula:

$$\%\,\text{protection} = \left\{1 - \frac{X - C}{Y - C}\right\} \times 100$$

Where, X = AFB1 + TP, Y = AFB1, C = Control

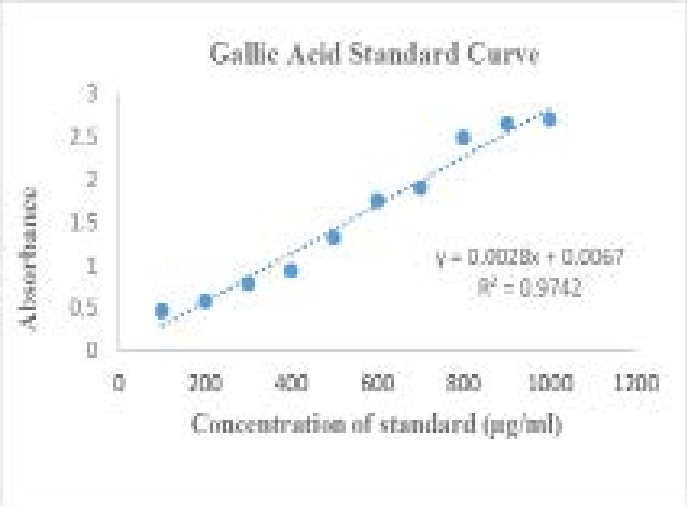
RESULTS

Phytochemical Studies: 3.1.1. Determination of total phenolic content:

The ethanolic extract was analysed for presence of polyphenols. To calculate total phenolic content, gallic acid standard curve was plotted by taking concentration on X axis and absorbance on Y axis. The obtained equation line was Y = 0.0028x + 0.0067R^2 = 0.9742 (Graph 1). The phenolic content of extract was 195.771 ± 10.82 µg Gallic acid /mg (Table 1).

Determination of antioxidant potential

To investigate antioxidant potential of plant sample, DPPH free radical scavenging activity assay was performed and the activity was reported



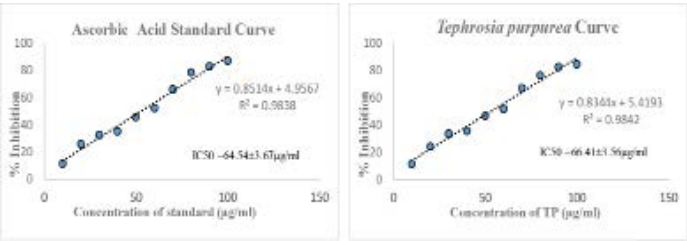
Graph 1: Gallic acid curve.

Table 1: **Phenolic Content.**

Total Phenolic Content		
Sl. No.	Extract	Total Phenolic (µg Gallic acid /mg)
1	Tephrosia purpurea	195.77 ± 10.82

Values are the mean ± SE for three observations

DPPH Assay



Graph 2: Free radical scavenging activity.
Values are the mean ± SE for three observations.

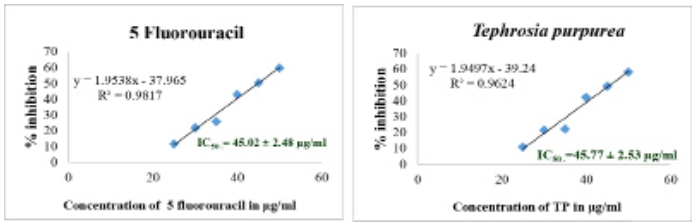
in terms of % inhibition. The inhibitory concentration 50 (IC_{50}) of each extract was calculated using % inhibition. Ethanolic extract showed IC_{50} of 66.41 ± 3.56 µg/ml whereas standard (Ascorbic acid) showed IC_{50} 64.54 ± 3.67 µg/ml (graph 2).

Cell Viability Assay

TP extract was analysed for its antiproliferative activity against HepG2 cell line at 6 different concentrations (25 µg/ml to 50 µg/ml). The antiproliferative activity was determined by MTT assay. TP extract showed inhibition at IC_{50} of 45.77± 2.53 µg/ml whereas 5 FU showed inhibition at IC_{50} of 45.02 ± 2.48 µg/ml (Graph 3).

Serological Analysis

Exposure of AFB1 significantly ($p \leq 0.05$) altered the levels of liver specific markers (AST, ALT, and TG) in circulation when compared to control. All the 3 doses of TP (100, 200, 300 mg/kg) restored the deviated level towards normal (Table 2).



Graph 3: Effect of 5 FU and TP on HepG2 cells
Values are the mean ± SE for three observations.

Table 2: **Effect of TP on serological markers.**

Treatments	AST	ALT	TG
Control	60 ± 3.31	41± 2.26	79.14 ± 4.37
Perse	64 ± 3.53	43± 2.37	81.02 ± 4.47
AFB1	108 ± 5.95#	96.3± 5.32#	336.75 ± 18.61#
AFB1+TP 100 mg/kg	104 ± 5.72* (60.64%)	75.9 ± 4.19* (36.96%)	208.54 ± 11.52* (49.76%)
AFB1+ TP 200 mg/kg	78.4 ± 4.33* (61.35%)	69.1 ± 3.81* (49.25%)	203.41 ± 11.24* (51.76%)
AFB1 + TP 300 mg/kg	69 ± 3.81* (81.12%)	53.6 ± 2.96* (77.21%)	152.13 ± 8.40* (71.66%)
F Value	24.15@	41.06@	95.56@

Values are the mean ± SE; N=6; @ = Significant at 5% for ANOVA; # AFB1 vs Control;

* AFB1 + Therapy vs AFB1 at $p \leq 0.05$

Abbreviations: TP= Tephrosia purpurea, AFB1: Aflatoxin B1

Serological analysis
Oxidant and antioxidant potential

Table 3: **Effect of TP on oxidant and antioxidant potential.**

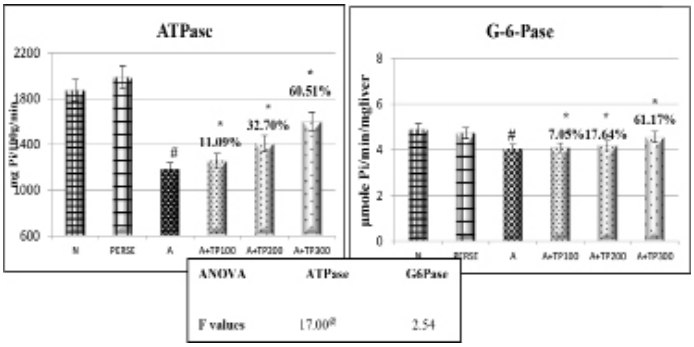
Treatments	LPO	GSH	SOD	CAT
Control	0.35 ± 0.019	7.62 ± 0.42	66.4 ± 3.67	62.9 ± 3.47
Perse	0.32 ± 0.017	8.57 ± 0.47	66.3 ± 3.66	62.05 ± 3.43
AFB1	1.16 ± 0.064#	3.10 ± 0.17#	28 ± 1.55#	30.3 ± 1.67#
AFB1+TP 100 mg/kg	0.58 ± 0.032* (70.85%)	6.25± 0.34* (63.03%)	39.1 ± 2.16* (28.95%)	39.3 ± 2.17* (27.50%)
AFB1+ TP 200 mg/kg	0.43 ± 0.023* (89.63%)	6.6 ± 0.36* (70.51%)	43.7 ± 2.41* (40.71%)	45.97± 2.54* (48.03%)
AFB1 + TP 300 mg/kg	0.38 ± 0.021* (95.12%)	6.89 ± 0.38* (76.70%)	52.1 ± 2.88* (62.79%)	57.4 ± 3.17* (83.11%)
F Value	107.54@	30.09@	35.19@	26.57@

Values are Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1 at $p \leq 0.05$

Abbreviations: TP = *Tephrosia purpurea*, AFB1 =Aflatoxin B1

Oxidative stress marker

LPO in liver samples were significantly elevated after AFB1 exposure ($p \leq 0.05$, F = 107.54). All the three doses decreased LPO when compared to control. However maximum recovery was seen with 300 mg/kg dose (Table 3).



Graph 4: Effect of TP on membrane bound enzymes.

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.

Antioxidant Potential

A significant reduction ($p \leq 0.05$) in activities of GSH, SOD and CAT were observed after AFB1 exposure. Treatment with all the three doses of TP recouped its level towards normal indices, however maximum recovery was seen at 300 mg/kg dose. ($p \leq 0.05$, $F_{GSH} = 30.09$, $F_{SOD} = 35.19$, $F_{CAT} = 26.57$). No adverse effect on activity of antioxidants were observed in *perse* group (Table 3).

Membrane Bound Enzymes

The activity of ATPase and G6Pase was significantly reduced after AFB1 intoxication (p≤ 0.05). Treatment with TP restored activity of ATPase (F= 17.00) and G6Pase (F= 2.54) at all the doses (Graph 4).

Histological Observations

The photomicrograph of control (Figure 1 A) and *perse* group (Figure 1 B), showed normal structure of hepatocytes and well-arranged hepatic chords with clear central vein. Figure 1 (C), showed significant deformities in hepatic architecture of AFB1 administered group. Derbies in central vein was clearly visible, with significant kupffer cells infiltration. Treatment with 100 mg/kg of TP showed mild recovery with lesser kupffer cells infiltration, but still congestion in central vein was persistent (Figure 1 D). Treatment with 200 mg/kg dose of TP showed improved hepatocytes with cordially arranged hepatic chords, congestion in central vein is visible (Figure 1 E). TP (300 mg/kg) showed well maintained hepatic chords, well-formed hepatocytes with prominent and clear central vein (Figure 1 F).

Histology of Liver

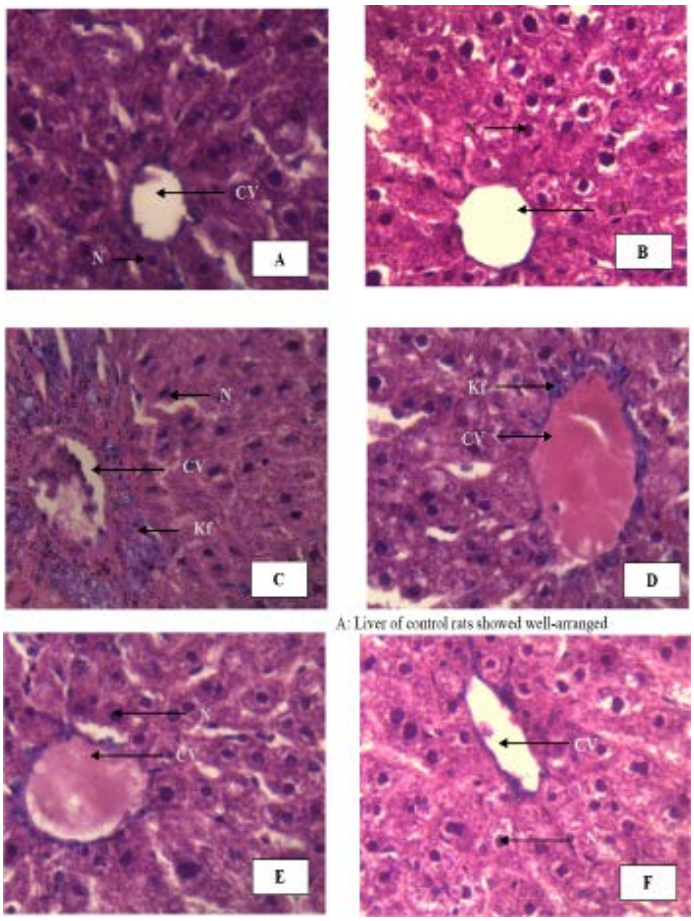


Figure 1: A: Liver of control rats showed well-arranged hepatocytes, clear central vein (CV) and prominent nucleus (N) (400X). B: Therapy perse group showed well maintained hepatocytes, prominent clear central vein (CV) and nucleus (N). C: Liver of Aflatoxin B1 challenged rats showed congestion in central vein (CV) and kupffer cell infiltration (Kf). D: Therapy of TP at 100 mg/kg showed mild infiltration of kupffer cells (Kf) and congestion in central vein (CV). E: Treatment with 200 mg/kg TP showed well-formed hepatocytes, fairly less kupffer cell infiltration (Kf) and congestion in central vein (CV). F: Liver of 300 mg/kg TP treated group showed decent protection with cordially arranged hepatocytes and clear central vein (CV).

DISCUSSION

Indian folk medicinal system or *Ayurveda* is *reservoir* of well documented plants and herbs *Tephrosia purpurea* (TP) been one of them.[26] TP is rich in presence of polyphenolic compounds, these compounds are responsible for antioxidant potential and broad spectrum of biological activities.[13] When ethanolic extract was subjected to total phenolic and DPPH assay, it revealed the presence of polyphenols and antioxidant potential in TP extract. Our findings are similar to Sreenivasan and Subburaju.[27]

Humans are exposed to AFB1 unknowingly through consumption of contaminated food. Continuous detoxification of AFB1 creates a stressful situation for liver.[28] Under normal circumstances AFB1 is metabolised via CYP 1A, 3A, 2B and 2C to form AFQ1 and AFM1. These metabolites under goes phase II transformation and forms glucuronyl adduct which is then excreted out of body. But under overwhelming conditions AFB1 is metabolised by CYP 2C11, CYP 3A2 and CYP 1A2 to form AFB1-8,9- epoxide (AFB1 epoxide). Formation of epoxide- GSH adduct is catalysed GST, and this adduct is excreted out of body. Under continuous AFB1 stress, the level of GSH decreases and AFB1 epoxide binds with DNA, RNA, protein and other macromolecules.[29-30] CYP 450 mediated metabolism of AFB1 generates free radicals, these free radicals bind with lipids and initiates lipid peroxidation and cellular damage.[31] The present study was designed to evaluate therapeutic potential of *Tephrosia purpurea* in management of AFB1 induced oxidative damage.

Ethanolic extract of TP showed anti-proliferative effect in dose dependent manner on HepG2 cells. A significant anti-proliferative activity was seen at 50μg/ml dose. Similar results were documented by Padmapriya.[32] Gulecha and Sivakuma,[33] reported antiproliferative activity of TP on MCF-7 cells. According to them flavonoids may act as chemoprotective drugs as they modulate proliferation and angiogenesis, in cancer cells.

Aminotransferases (AST and ALT), are key enzymes involved in citric acid cycle and are metabolised in liver.[8] Level of TG in blood is regulated by hepatic lipase. A sharp increase in level of AST, ALT and triglyceride was seen in AFB1 administered cohort, which could be due

to compromised membrane integrity by free radicals.[34-35] Treatment with ethanolic extract of TP showed recovery at all the doses, maximum recovery was seen in 300mg/kg treated group. This recovery is possible due to maintenance of membrane integrity by flavonoids.[13] Our results are in accordance with Abdel-Wahhab[36] and Rastogi.[31]

According to Ajiboye,[7] disruption of membrane integrity is caused by free radicals generated by AFB1 during its course of metabolism. After treatment with TP extract a reduction in LPO was seen this could be possible because of free radical scavenging activity of flavonoids present in TP. Our results are similar to Gora[37] and Khatri.[38] GSH is cysteine containing non enzymatic antioxidant. –SH group of cysteine binds with free radical to from GSH adduct and helps in its elimination.[2] Our investigation showed exhaustion of GSH content in AFB1 administered cohort. Treatment with different doses of TP showed recovery but significant recovery was seen with 300mg/kg dose of TP. This could be due to presence of phenols. Our findings are similar to that of Pan.[39]

SOD and CAT are the first line defence antioxidant enzymes.[40] SOD has ability to convert superoxide radical into H_2O_2 which is further converted into H_2O by activity of CAT.[7] A significant decline in SOD and CAT activity is associated with their consumption during quenching of free radicals and converting it into harmless metabolites. Various reports (El Nekeety;[4] Kanchana)[6] have mentioned depletion in activities of SOD and CAT after AFB1 administration. The cohort treated with different doses of TP showed restoration of SOD and CAT activities in tissues. The restoration in level of antioxidant enzymes may be due to presence of flavonoids in TP extract.[39] Our findings are similar to Uthra[41] and El-Nekeety.[42]

To determine the severity of hepatocellular damage activity of G6Pase and ATPase was estimated. G6Pase is an endoplasmic reticulum (ER) membrane bound enzyme that plays key role in glucose metabolism. It converts glucose-6-phosphate into glucose and phosphate.[43] ATPase, is a mitochondrial membrane bound enzyme, which plays an important role in maintaining ionic balance in cell. Brand and Nicholls,[44] reported that free radicals cause oxidative damage to membrane bound cell organelles, endoplasmic reticulum and mitochondria which in turn decreases activity of G6Pase and ATPase. After treatment with TP a steady recovery was seen in dose dependent manner. In terms of percent protection maximum recovery was seen in 300 mg/kg dose. Our results are similar to Pamu,[45] who explained role of flavonoids and isoflavonoids present in TP, in improving the integrity of membrane and activity of membrane bound enzymes.

The biochemicals results are further strengthen and sustained by histological studies of liver. In the present study, AFB1 exposed rats showed an increase in serum concentration of AST, ALT and TG. These increase level could be due to AFB1 induced hepatocytes damage and subsequent release of AST, ALT and TG in blood stream. In the current investigation, it is also revealed that AFB1 challenged cohorts showed an increase in level of LPO and a significant decline in activities of antioxidants. The free radicals generated during AFB1 metabolism binds with cellular poly unsaturated fatty acids and produces some toxic intermediates which are responsible for inhibition of endogenous antioxidants. Histological evidence shows that AFB1 challenged group have damaged hepatocytes and hepatic chords with infiltration of kupffer cells and congested central vein. Treatment with different doses of TP reverted alterations or damage towards normal indices. Therapy of TP (100 and 200 mg/kg) mildly restored hepatic architecture. Treatment of TP (300 mg/kg) fairly maintained hepatic chords and clear central vein was recorded without any kupffer cells infiltration. Similar findings were reported by El-Nekeety,[4] Bard and Naeem.[46]

The results suggest that 300 mg/kg dose of TP to be effective in managing all the serological, biochemical and histological alterations towards control. This could be due to presence of phenolic compounds and flavonoids in TP extract. According to El-Nekeety[4] and Kulanthaivel[30] flavonoids and polyphenols acts as quenchers of reactive oxygen species and reactive intermediate formed during AFB1 metabolisms, thereby preventing binding of free radicals to macromolecules. The antioxidant and free radical scavenging properties of *Tephrosia purpurea* may offer protection against Aflatoxin B1 induced hepatic damage. Our results are further sustained by El-Nekeety,[42] who explained role of quercetin in management of oxidative damage caused in hepatic tissues during AFB1 metabolism.

CONCLUSION

To conclude, present study suggests that *Tephrosia purpurea* extract at 300mg/kg was effective in managing Aflatoxin B1 induced liver injury. The findings of current study strengthen the medicinal values *Tephrosia purpurea* and may be used as a remarkable alternative medicine in management of hepatic damage. Although the intensive preclinical and clinical trials are required to support the above said claim.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethical Statement

All the animals used in the experiment were cared and maintained according to CPCSEA guidelines (CPCSEA No- IAEC/JU/55). No human samples were used in the above experiments.

ABBREVIATIONS

AFB1: Aflatoxin B1; **TP:** *Tephrosia purpurea*; **IC_{50}:** Half maximal inhibitory concentration; **IACR:** International Agency for Research on Cancer; **CYP450:** Cytochrome P 450; **AFBO:** AFB1 Epoxide; **ROS:** Reactive Oxygen species; **WHO:** World Health Organisation; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **NCCS:** National Centre for Cell Sciences; **DMEM:** Dulbecco's Modified Eagle Medium; **TBARS:** Thiobrabiutric Acid Reactive Species; **GSH:** Reduced Glutathione; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **H_2O_2:** Hydrogen peroxide; **ATPase:** Adenosine Triphosphatase; **G6Pase:** Glucose 6 Phosphatase; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **5FU:** Fluorouracil; **AST:** Aspartate aminotransferase; **ALT:** Alanine transaminase; **GST:** Glutathione S Transferase; **TG:** Triglyceride; **AFB1:** Aflatoxin B1.

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