https://doi.org/10.33451/florafauna.v28i1pp137-144
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2022 Vol. 28 No. 1 PP 137-144 ISSN 0971 - 6920 (Print)

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

***Shamli S. Gupte, Arti Rathour, Sadhana Shrivastava and Sangeeta Shukla**

UNESCO Trace Element Satellite Centre,
School of Studies in Zoology,
Jiwaji University GWALIOR (M.P.), INDIA
*Corresponding Author:
E-mail : sg2761@gmail.com

Received **:** 11.01.2022; ***Accepted*** : 08.03.2022

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[3]. AFB1 epoxide binds with DNA and reactive oxygen species binds with cellular membrane thereby eliciting toxicological effects[10]. Apart from hepatocellular carcinoma, renal dysfunction, immunosuppression, delayed implantation, lungs and gastrointestinal cancers are also induced by AFB1[7].

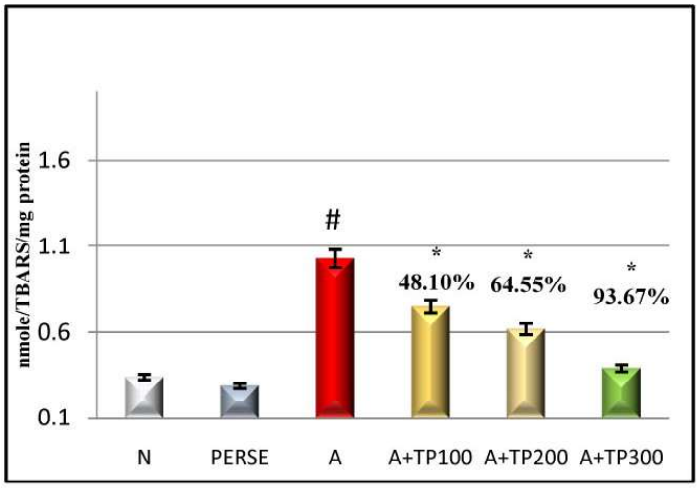
According to previous studies polyphenols and phytochemicals have shown ameliorating effects against AFB1 induced oxidative stress[24]. Despite of ongoing research, there is no known antidote to limit and cure oxidative damage induced by AFB1. Plants of genus *Tephrosia* are rich in flavonoids, about 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[18]. According to classical literature it is used in treatment of splenomegaly[6]. TP significantly protects mitochondrial dysfunction[8], manages gout[13], maintain membrane integrity[15]. 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

ACKNOWLEDGEMENT : Author is thankful to DRDO (LSRB scheme), New Delhi (No.0/0 DGTM/81/48222/ LSRB-340/BTB/2019) for funding the research work and Jiwaji University Gwalior for providing lab facility.



ANOVA	Kidney
F values	81.91 @

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, N= Normal

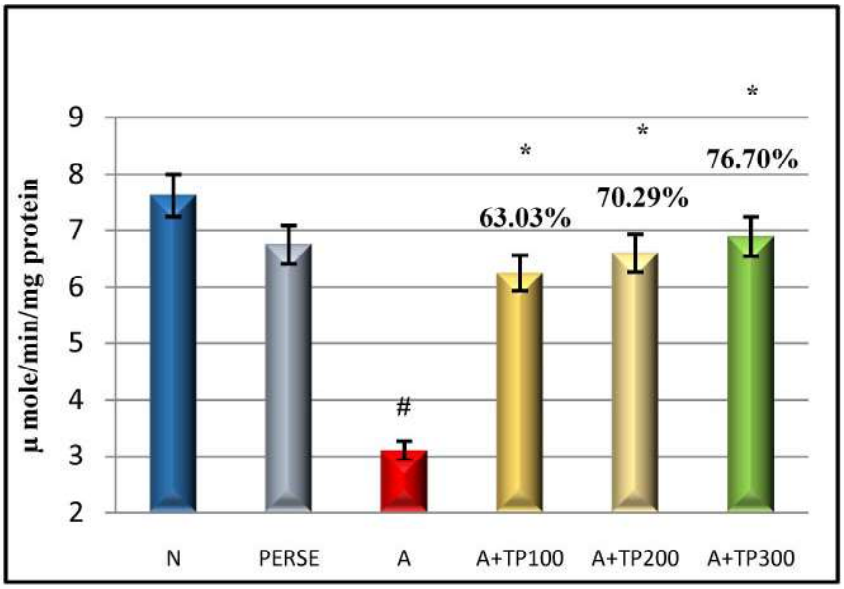
Fig. 1 : Lipidperoxidation Kidney

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

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

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

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



52.35

ANOVA	Kidney
F values	30.09 @

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, N=Normal

Fig. 2 : Reduced Glutathione Kidney

housed, treated and cared 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 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 power. Subsequently the powder was extracted with 70% ethanol. The extract was dried at 37f C and stored at 4^{f} C till its use.

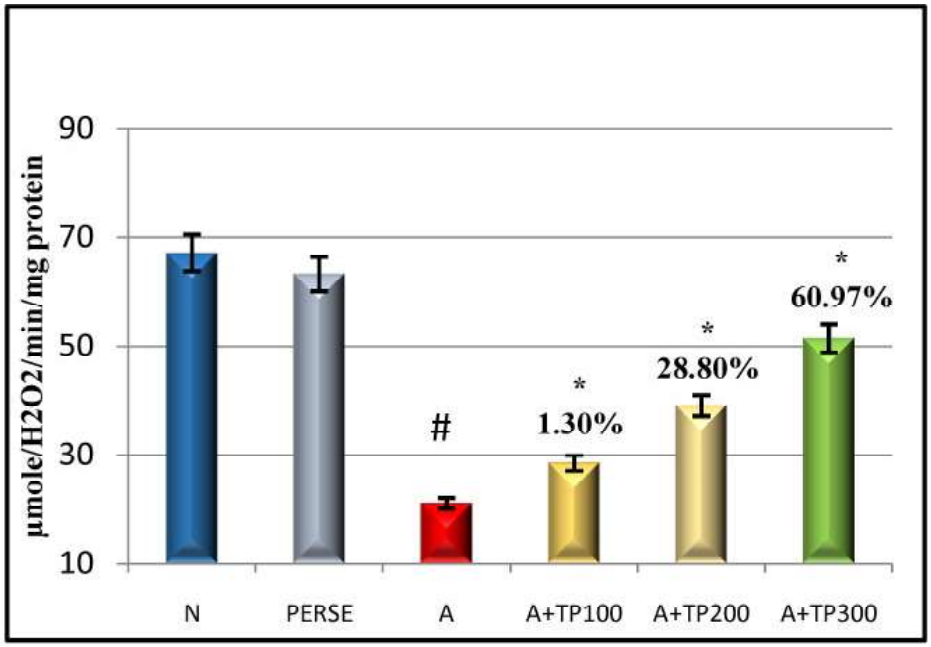
Experimental Design

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)



ANOVA	Kidney
F values	59.02@

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, N= Normal

Fig. 3 : Superoxide Dismutase Kidney

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)

Blood samples were collected from retro orbital plexus just before euthanization[16], kidney samples were harvested, washed with normal saline and were kept in sterile conditions for further investigation.

Serological analysis

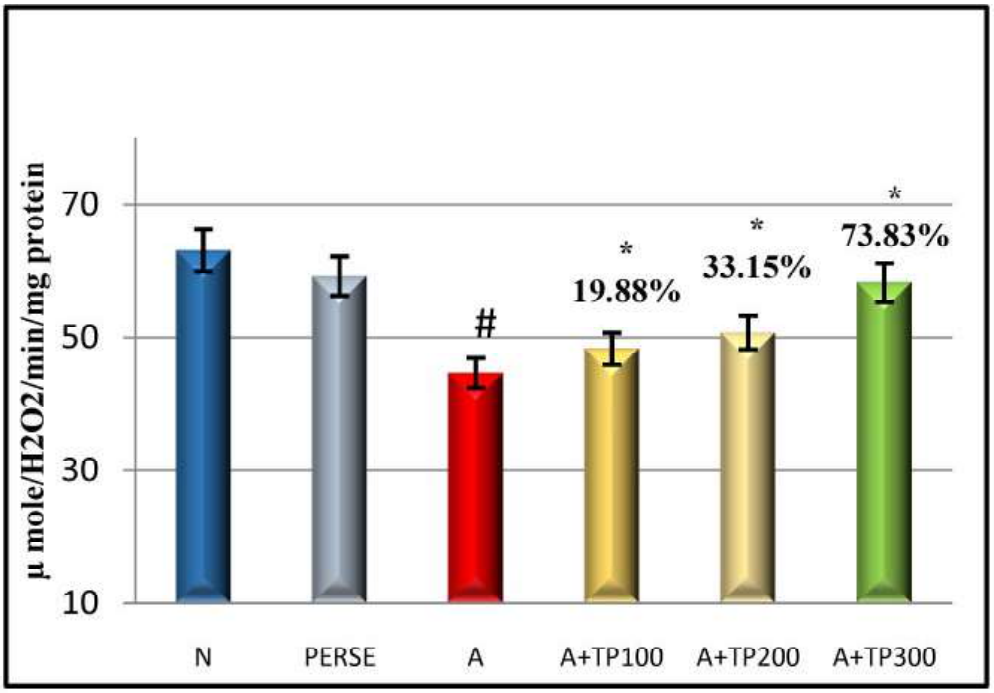
Blood samples were incubated at 37f C for half an hour and centrifuged at 3000 rpm for 15 minutes, serum was collected and stored at -20 ºC. The harvested serum was used to analyse urea, uric acid and creatinine, which were estimated using Erba diagnostics kits according to the given instructions.

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[20], reduced



ANOVA	Kidney
F values	6.85 @

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, N= Normal

Fig. 4 : Catalase Kidney

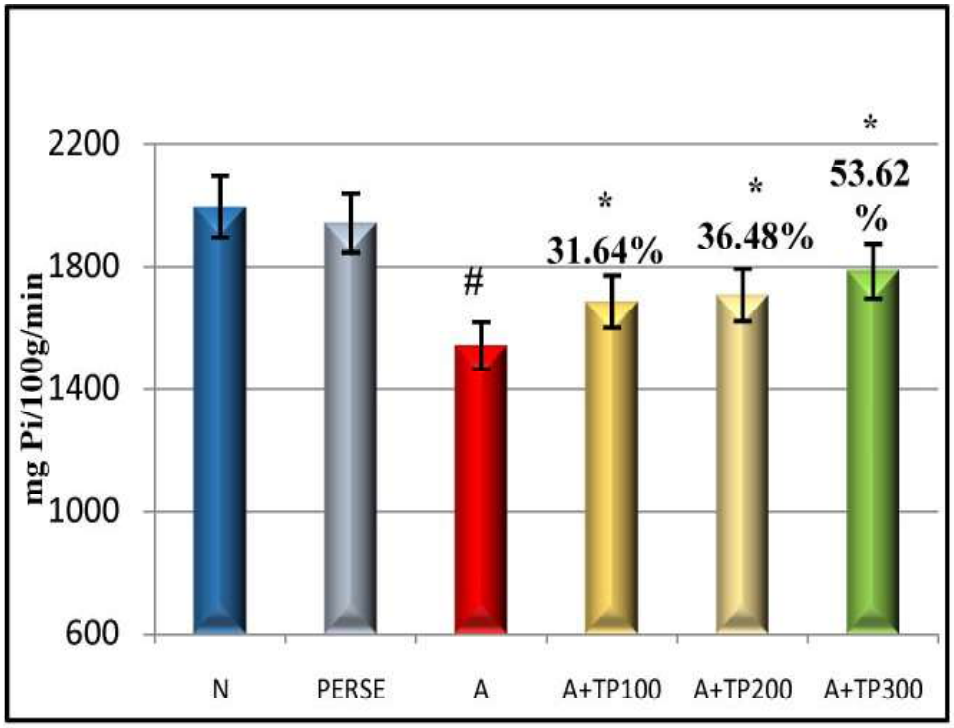
glutathione[5], superoxide dismutase[12], catalase[1], adenosine triphosphatase[19] and assessment of protein[11].

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 d" 0.05 followed by student t test[21]. 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[22]. 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[23]. 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[17] (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



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 =Aflatoxin B1, N= Normal

Fig. 5 : ATPase Kidney

reduced glutathione, superoxide dismutase and catalase counters generation of free radicals during AFB1 metabolism[1]. 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[7]. 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[4]. 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[9]. The present investigation is supported

by earlier investigator[14] 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.

References

1. Abdulmajeed NA. Therapeutic ability of some plant extracts on aflatoxin B1 induced renal and cardiac damage. *Arabian Journal of Chemistry.* 2011; **4**(1): 1-10.
2. Aebi M, Kirchner G, Chen JY, Vijayraghavan U, Jacobson A, Martin NC, Abelson J. Isolation of a temperature-sensitive mutant with an altered tRNA nucleotidyltransferase and cloning of the gene encoding tRNA nucleotidyltransferase in the yeast Saccharomyces cerevisiae. *Journal of Biological Chemistry.* 1990; **265**(27): 16216-16220.
3. Alm-Eldeen AA, Basyony MA, Elfiky NK, Ghalwash MM. Effect of the Egyptian propolis on the hepatic antioxidant defense and pro-apoptotic p53 and anti-apoptotic bcl2 expressions in aflatoxin B1 treated male mice. *Biomedicine Pharmacotherapy.* 2017; **87**: 247-55.
4. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. *Biochemical Journal.* 2011; **435**(2): 297-312.
5. Brehe JE, Burch HB. Enzymatic assay for glutathione. *Analytical biochemistry.* 1976;**74**(1):189-197.
6. Chen Y, Yan T, Gao C, Cao W, Huang R. Natural products from the *genus Tephrosia. Molecules.* 2014; **19**(2) : 1432-58.
7. El-Nekeety AA, Salman AS, Hathout AS, Sabry BA, Abdel-Aziem SH, Hassan NS, Abdel-Wahhab MA. Evaluation of the bioactive extract of actinomyces isolated from the Egyptian environment against aflatoxin B1-induce cytotoxicity, genotoxicity and oxidative stress in the liver of rats. *Food and Chemical Toxicology.* 2017 ; **105**: 241-55.
8. Kesh S, Kannan RR. Neuroprotective Effect of Tephrosia purpurea Against Mitochondrial Dysfunction by Regulation of the Caspase3/9 and Pink1/Parkin Complexes. *International Journal of Nutrition, Pharmacology, Neurological Diseases.* 2021; **11**(2):137.
9. Kulanthaivel L, Srinivasan P, Shanmugam V, Periyasamy BM. Therapeutic efficacy of kaempferol against AFB1 induced experimental hepatocarcinogenesis with reference to lipid peroxidation, antioxidants and biotransformation enzymes. *Biomedicine & Preventive Nutrition.* 2012; **2**(4): 252-259.
10. Lokesh KN, Chaudhuri T, Lakshmaiah KC, Babu KG, Dasappa L, Jacob LA, Babu MS, Rudresha AH, Rajeev LK. Advanced hepatocellular carcinoma: A regional cancer center experience of 48 cases. *Indian Journal of Cancer.* 2017; **54**(3): 526.
11. Lowry O, Rosebrough N, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry.* 1951; **193**(1) : 265-75.
12. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry.* 1972; **247**(10) : 3170-3175.
13. Nipate SS, Yelmar PS. Therapeutic potential of ethyl acetate fraction of *Tephrosia purpurea* Linn. leaves in a rat model of gout. *Journal of Integrative Medicine.* 2019; **17**(6) : 455-460.
14. Owumi SE, Adedara IA, Akomolafe AP, Farombi EO, Oyelere AK. Gallic acid enhances reproductive function by modulating oxido-inflammatory and apoptosis mediators in rats exposed to aflatoxin-B1. *Experimental Biology and Medicine.* 2020; **245**(12) : 1016-1028.
15. Pamu P, Shanmugam M, Subramanian S. Beneficial effects of *Tephrosia purpurea* ethanolic seed extract on lipids and membrane bound enzymes in experimental diabetic rats. *Journal of young pharmacists.* 2017; **9**(4) : 550.

16. Rotimi OA, Rotimi SO, Goodrich JM, Adelani IB, Agbonihale E, Talabi G. Time-course effects of acute aflatoxin B1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. *Frontiers in pharmacology.* 2019; **10** : 467.

17. Samuel VJ, Mahesh AR, Murugan V. Phytochemical and pharmacological aspects of Tephrosia genus: A brief review. *Journal of Applied Pharmaceutical Science.* 2019; **9** : 117-25.

18. Riley V. Adaptation of orbital bleeding technic to rapid serial blood studies. *Proceedings of the Society for Experimental Biology and Medicine.* 1960; **104**(4) : 751-4.

19. Seth PK, Tangri KK. Biochemical effects of some newer salicylic acid congeners. *Journal of pharmacy and pharmacology.* 1966; **18**(12) : 831-3.

20. Sharma SK, Murti CK. Production of lipid peroxides by brain. *Journal of Neurochemistry.* 1968; **15** (2) : 147-149.

21. Snedecor GW, Cochran WG. Statistical Methods. 8th Edn IOWA State University Press. Ames, Iowa, USA. 1994.

22. Sreenivasan KK, Subburaju T. Comparative antioxidant studies of ethanol extract and fresh aqueous extract of *Vetiveria zizanioides*. *International journal of pharmacy and pharmaceutical sciences.* 2011; **3** (SUPPL. 5) : 325-331.

23. Ugbaja RN, Okedairo OM, Oloyede AR, Ugwor EI, Akinloye DI, Ojo OP, Ademuyiwa O. Probiotics consortium synergistically ameliorates aflatoxin B1-induced disruptions in lipid metabolism of female albino rats. *Toxicon.* 30; **186** : 109-119.

24. Wang X, He Y, Tian J, Muhammad I, Liu M, Wu C, Xu C, Zhang X. Ferulic acid prevents aflatoxin B1-induced liver injury in rats via inhibiting cytochrome P450 enzyme, activating Nrf2/GST pathway and regulating mitochondrial pathway. *Ecotoxicology and Environmental Safety.* 2021; **224** : 112624.