https://doi.org/10.33451/florafauna.v29i2pp291-295
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2023 Vol. 29 No.2 PP 291-295 ISSN 0971 - 6920 (Print)

Therapeutic effect of *Bacopa monnieri* on Aflatoxin B1-induced oxidative damage in rats

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

UNESCO Trace Element Satellite Centre,
School of Studies in Zoology Jiwaji University,
GWALIOR-474011 (M.P.), INDIA
*Corresponding Author
E-mail: artirathore0207@gmail.com

Received **:** 25.08.2023; ***Accepted*** : 03.09.2023

ABSTRACT

Aflatoxin B1 is the one of most toxic and well-known mycotoxins, which contaminates staple food. *Bacopa monnieri* popularly known as Brahmi, is a well-documented nootropic drug in Ayurveda. Thus, the present study was designed to evaluate the protective efficacy of brahmi against liver damage induced within 4 weeks by oral doses 200 ìg/kg Aflatoxin B_1, to male rats. Resulting in increased lipid peroxidation, decline in reduced glutathione, and antioxidant enzymes in liver tissue, increased serum liver pathology markers in serum. Oral pretreatment with 20 – 40 mg/kg Brahmi for a week showed remarkable protection against the toxic effects of aflatoxin B_1.

Figure : 01 References : 23 Tables : 02
KEY WORDS : Aflatoxin B1, *Bacopa monnieri,* Liver, Oxidative injury

Introduction

Aflatoxins (AF) are secondary metabolites produced by the fungus *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are strong hepatocarginogens in model animals and a possible carcinogens in humans.[7] It contaminates food products like maize, nuts, rice, and wheat.[10] The four major naturally occurring Aflatoxins (AFs) are designated as B1, B2, G1, and G2.[13] Aflatoxin B1 (AFB1), which is present in maize, peanuts, and peanut oil, is the most hazardous Aflatoxin in terms of toxic potency and occurrence.[23] They have been linked to a variety of harmful effects in both animal and human health, including carcinogenicity, mutagenicity, teratogenicity, and immuno suppressive activities.[5] Maternal exposure to AFB1 and its metabolite AFM1 resulted in the toxins passing from the blood stream into milk and, as a result, changes in hippocampus neurogenesis with down-regulation of cholinergic signalling in their offspring. AFB1 metabolism is highly concerned in human liver. This extremely reactive metabolite forms adducts with DNA guanine, causing a harmful impact. AFB1 is very toxic and carcinogenic mycotoxin to the liver. The mechanism of AFB1-induced cellular damage is not entirely understood.

Brahmi (BM) is a well-known medicinal herb in India's traditional medical system. It is a member of the Scrophulariaceae family and is widely utilised in Ayurveda.[18] *Bacopa monnieri* has been used by Ayurvedic medicinal practitioners in India for about 3000

Fig. 1 : ***Bacopa monnieri***

ACKNOWLEDGEMENTS : The authors would like to thank the DRDO (LSRB scheme), New Delhi (No.0/0 DGTM/81/48222/LSRB-340/BTB/2019) for sponsoring the research and Jiwaji University Gwalior for providing lab space.

years and is classed as a *madhya rasayana*, a medicine used as a nervine tonic to strengthen intelligence and memory.[15] The existence of bioactive chemicals such as alkaloids, carbohydrates, glycosides, saponins, phenols, proteins, and amino acids was discovered through phytochemical screening.[22] *Bacopa monnieri*, is a well-known memory booster in the Indian medicine.[14] It grows natively in India and is used to cure a variety of problems, including those involving anxiety, intelligence, and bad memory. They also ameliorate neuronal dysfunctions caused by diabetic neuropathy. *Bacopa monnieri* leaf extract improves behavioural abnormalities, decreases oxidative stress, and inhibits apoptosis in Parkinson's disease brains.[11]

In recent years, there has been increasing interest in medicinal plants and phytochemical-based pharmaceuticals because they provide a holistic and harmonious balance of physical, biological, and mental processes *via* medical systems such as Ayurveda, Unani, and Siddha. In this work, an attempt was made to analyse the prophylactic profiling of BM against AFB1 produced hepatic abnormalities, emphasising the current era of phytomedicine.

Material and Methods

Animals and chemicals

The animals were obtained from the AIIMS animal home facility in New Delhi. Male *Wistar* rats weighing 150-200g were used in this study. The animals were kept in a university animal house facility under regular settings (25 ± 2f C temperature, 60-70% relative humidity, 14 hours of light and 10 hours of darkness). The rats were fed a conventional pellet diet and had free access to water. The animals were treated and cared for in compliance with the Committee for the Control and Supervision of Animal Experiments' (CPCSEA No-IAEC/JU/56) recommendations. Sigma Aldrich & Co. Aflatoxin B1 was made available in the United States. All of the other chemicals were of analytical quality and purchased from Sigma-Aldrich Co. in the United States, Ranbaxy in New Delhi, and HI media Laboratories Ltd. in Mumbai, India.

Collection and processing of plant extracts:

A taxonomist recognised the full BM plant, which was acquired from the Campus of Jiwaji University. SOS in Botany accepted the herbarium with accession 5312/PP-49-50/03/12/2020. In the shade, the plant was cleaned and dried. The plant was crushed to a coarse powder after drying. Aqueous extract was prepared; the extract was allowed to dry in oven at 37 f C. In a 250-ml Erlenmeyer flask, 10 g of powder was mixed with 100 ml of double distilled water and heated for 1-2 hours in a water bath at 60 °C. The mixture was filtered through muslin filter fabric after cooling to ambient temperature. The filtrate was then filtered again using 0.6 µm filters and stored at 4 °C in an airtight container for future research.

Experimental design:

Animal experimental design

The animals were placed into five groups, each with six animals. Group 1 was the control group. Group 2 served as *per se* and received highest dose of therapy which was 40 mg/kg for 28 days, post orally. Group 3 was given AFB1 (200 µg/kg/day) orally for 28 days. Groups 4–6 were given varied BM dosages (20, 30, 40 mg/kg/day orally) for 5 consecutive days before 23 days of exposure to AFB1. After 24 hours from the last treatment, all animals were sacrificed.

Serological analysis:

Blood samples were incubated for 30 minutes at 37 degrees Celsius before being centrifuged at 3000 revolutions per minute for 10 minutes to separate serum. Serum samples were kept at refrigerator under freezing conditions at 20ºC.

Serum enzyme activity, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured using a kit approach.

Serum metabolite activity Triglycerides were determined using the kit method on a Merck auto-analyzer (Micro Lab 200) in accordance with the instructions in the diagnostic kit's manual (E-Merck India Ltd.).

Tissue biochemical analysis:

Following necropsy, living tissues were extracted and washed in cold normal saline. Tissues were homogenised using the Remi Motor Homogenizer (RQ-122) had a glass tube and a Teflon pistle, were immediately processed to assess oxidative stress markers such as lipid peroxidation (LPO),[17] antioxidant enzyme markers such as reduced glutathione (GSH),[4] superoxide dismutase (SOD),[12], and catalase (CAT).[3]

Statistical Evaluation:

The data were presented as the mean standard deviation of six animals in each group. One-way analysis of variance (ANOVA) was used to compute the mean with significance set at P dŠ 0.05, and was then tested using the student t test.[27] The data are shown as the mean ± SE of six animals in each group.

Results and Discussion

Present study demonstrated protective effect of *Bacopa monnieri* against AFB1 induced hepatotoxicity. Pharmacological effects of polyphenols are associated with its antioxidant activity due to ability to scavenge free radicals and exerting synergistic effect.

TABLE-1 : Serological investigation of BM therapeutic potential against AFB1-induced liver damage

Treatment	AST(IU/L))	ALT(IU/L)	Triglyceride(mg/dl)
Control	58±3.20	41.67±2.30	79.14±4.37
BM per se	58.43±3.23	42.05±2.32	80±4.42
AFB1	103.02±5.69#	89.94±4.97#	415±22.94#
AFB1+BM (20mg/kg) (% protection)	86.02±4.75* (37.76%)	75.32±4.16* (30.28%)	319±17.63* (28.58%)
AFB1+BM (30mg/kg) (% protection)	75.82±4.19* (60.41%)	67.47±3.72* (46.55%)	231±12.76* (54.78%)
AFB1 +BM (40mg/kg) (% protection)	68.34±3.77* (77.03%)	56.63±3.13* (69.007%)	171±9.45* (72.64%)
F value (at 5% level)	20.27@	34.41@	114.45@

Ayurveda is the oldest system of medicine in the world as its antiquity goes back to the Vedas. *Bacopa monnieri* (BM) is a well-documented nootropic herb in traditional Indian medical system. Moreover, the neuroprotective properties of *Bacopa monnieri* are widely recognized and have been discussed in studies[8] but very less focus has been given on hepatoprotective activity of *Bacopa monnieri* extract.

The activities of ALT, AST, and Triglyceride were significantly higher (p**dŠ**0.05) in the serum of untreated AFB1-intoxicated rats compared to the control (Table 1). Nonetheless, the activities of ALT, AST and Triglyceride were significantly decreased towards control values (p**dŠ**0.05) in rats injected with AFB1 pretreated of different dose BM compared to untreated AFB1-intoxicated rats.

Elevated levels of AST, ALT and triglyceride in the circulation are indicative of a hepatic injury after AFB1 intoxication. The leakage of AST and ALT from cytoplasm and Triglyceride from lipids into serum have been reported in many studies.[19] As in liver ailments such as hepatic lesions and parenchymal cell necrosis, these enzymes are released into the bloodstream. When the AFB1-treated group was compared to the control group, there was a significant rise in plasma AST, ALT, and Triglyceride activity. However, pretreatment with BM dramatically reduced liver damage, resulting in lower plasma levels of these markers. Thus, combining BM with AFB1 improves the physiological integrity of rat hepatocytes by normalising the levels of AST, ALT, and Triglyceride in blood serum. AFB1 treatment increased AST, ALT, and Triglyceride levels[1]. Typically, these findings demonstrated that it had hypofunction and degenerative alterations in liver tissue.

The quantity of TBARS in the liver of AFB1 intoxicated rats was considerably ((P**dŠ**0.05) increased, whereas the amount of GSH was significantly ((P**dŠ**0.05) decreased. When compared to untreated AFB1-intoxicated rats, pretreatment with BM resulted in a substantial (P**dŠ**0.05) drop in the TBARS level and a significant (P**dŠ**0.05) decrease in the GSH level of liver tissue. All three doses of BM therapy resulted in significant healing. According to ANOVA at 5% significant level, percent protection exhibited maximal recovery at 40 mg/kg doses (Table-2).

Lipid peroxidation (LPO) is one of the most common symptoms of mediated oxidative damage, and it has been linked to toxicity and carcinogenicity.[6] Several studies found that AFB1 exposure increased LPO in the liver. AFB1 treatment increased lipid peroxidation in the current investigation, as evidenced by a considerable increase in TBARS levels, which is a direct outcome of free radical-mediated toxicity.[18] To produce LPO, free radicals are known to target the cell membrane's highly unsaturated fatty acids. Moreover, pretreatment with BM prevents cellular integrity from damage caused by free radical caused by AFB1.

GSH is a crucial intracellular reductant in cells, protecting cells from free radicals and causing metabolic effects. Depletion of GSH can lead to liver tissue diseases and injuries. *Bacopa monnieri* exogenous

Table-2 : Therapeutic potential of BM on Oxidant and antioxidant potential against AFB1 induced toxicity

Treatments	Lipid Peroxidation (n mole TBARS/mg protein)	Reduced Glutathione (µ mole/ g)	Superoxide Dismutase (U/ min/ mg protein)	Catalase (µ mole H2O2/ min/ mg protein)
Control	0.35±0.019	7.64±0.42	63.36±3.50	63.51±3.51
BM per se	0.37±0.020	7.15±0.39	58.6±3.23	63.16±3.49
AFB1	1.25±0.069#	3.3±0.18#	25.71±1.42#	31.01±1.71#
AFB1+BM (20mg/kg) (% protection)	0.85±0.046 * (45.55%)	6.1±0.33 * (64.51%)	28.88±1.59 * (8.41%)	37.5±2.07 * (19.96%)
AFB1+BM (30mg/kg) (% protection)	0.63±0.034* (68.88%)	6.4±0.35* (71.42%)	34.46±1.90* (23.24%)	51.9±2.86* (64.27%)
AFB1+BM (40mg/kg) (% protection)	0.39±0.021* (95.55%)	6.8±0.37* (80.64%)	44.21±2.44* (49.13%)	62.13±3.43* (95.75%)
F value (at 5% level)	96.75@	22.69@	47.93@	28.06@

Legends to tables

Data are presented as Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1 At p d" 0.05

Abbreviations: AFB1 = Aflatoxin B1, BM = *Bacopa monnieri*

aqueous extract may help recover reduced GSH levels and prevent these issues. A study using AFB1-induced rats demonstrated the extract effectiveness in reducing GSH levels.[9]

AFB1 was administered to rats, the SOD and CAT levels in the liver decreased dramatically (P**dŠ**0.05) as compared to the control group. When compared to untreated AFB1-intoxicated rats, pretreatment with BM resulted in a significant (P**dŠ**0.05) increase in SOD and CAT levels in liver tissue. At all doses, BM therapy recovered values. The maximum restoration was seen at a dosage of 40mg/kg. ANOVA was used to analyse the results at a 5% significance level (Table 2).

Endogenous antioxidants such as superoxide dismutase (SOD) and catalase (CAT) counteract free radicals produced during AFB1 metabolism.[2] SOD is in charge of catalysing the transformation of superoxide anions into hydrogen peroxide. CAT then decomposes it further into water and oxygen.[16] SOD and CAT activities were shown to be considerably reduced in AFB1-treated rats. The BM therapy improved SOD and CAT levels, suggesting its effective protective mechanism in response to ROS. These data suggest that BM may be linked to lower oxidative stress and free radical-mediated tissue damage.

The findings indicate that a BM dose of 40 mg/kg is effective in controlling all serological and biochemical changes. BM helps to achieve a physiological state of reduced oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and activating antioxidant enzymes. The antioxidant and free radical scavenging properties of BM provides protection against AFB1 induced hepatic dysfunction.

Conclusion

From this study we can conclude that pretreatment of BM is able to protect AFB1 challenges. Biochemical results of this study demonstrated that BM extract possess protective potential against AFB1

induced hepato toxicity due to presence of several bioactive phytochemicals. However, further studies may be performed to isolate lead compounds from this extract as protective agent against free radical mediated injuries.

References

1. Abdel-Wahhab MA, Ahmed HH. Protective effect of Korean *Panax ginseng* against chromium *e!* toxicity and free radicals generation in rats. *Journal of Ginseng Research*. 2004; **28**(1): 11-17.
2. Abdulmajeed AM, Alnusairi GS, Alharbi MH, Almushhin A, Hasan MM, Soliman MH. Alleviation of copper phytotoxicity by acetylsalicylic acid and nitric oxide application in mung bean involves the up-regulation of antioxidants, osmolytes and glyoxalase system. *Journal of Plant Interactions*. 2021; **16**(1): 201-212.
3. Aebi H. Catalase *in vitro*. *Methods Enzymology*. 1984; **105**: 121-126.
4. Brehe JE, Bruch HB. Enzymatic assay for glutathione. *Analytical Biochemistry*. 1976; **74**: 189 –197.
5. Cao W, Yu P, Yang K, Cao D. Aflatoxin B1: metabolism, toxicology, and its involvement in oxidative stress and cancer development. *Toxicology Mechanisms and Methods*. 2022; 1-25.
6. El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. *Toxicon*. 2007; **50**(2): 256-269.
7. Gupte SS, Rathour A, Gupta D. *Tephrosia purpurea* Ameliorates Oxidative and Histological Alterations Induced by Aflatoxin B1 in Rats. *International Journal of Pharmaceutical Investig*ation. 2022; **12**(3): 340-345.
8. Hosamani R, Krishna G, Muralidhara. Standardized *Bacopa monnieri* extract ameliorates acute paraquat-induced oxidative stress, and neurotoxicity in prepubertal mice brain. *Nutritional neuroscience*. 2016; **19**(10): 434-446.
9. Jollow DJ. Glutathione thresholds in reactive metabolite toxicity. Archives of toxicology. Supplement. = Archiv fur Toxikologie. *Supplement.* 1980; **3**: pp.95-110.
10. Liu M, Zhao L, Gong G, Zhang L, Shi L, Dai J, Han Y, Wu Y, Khalil MM, Sun L. Remediation strategies for mycotoxin control in feed. *Journal of Animal Science and Biotechnology*. 2022; **13**(1): 1-16.
11. Malik J, Choudhary S. Indian Traditional Herbs and Alzheimer's Disease: Integrating Ethnobotany and Phytotherapy. In *Evidence Based Validation of Traditional Medicines*. 2021; 1129-1151.
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. Nazareth TM, Torrijos R, Bocate KP, Manes J, Luciano FB, Meca G, Vila-Donat, P. Development of an Antifungal Device Based on Oriental Mustard Flour to Prevent Fungal Growth and Aflatoxin B1 Production in Almonds. *Toxins*. 2022; **14**(1): 5.
14. Panda D, Barik JR, Barik J, Behera PK, Dash D. Suitability of Brahmi (*Bacopa monnieri* L.) cultivation on fly ash-amended soil for better growth and oil content. *International journal of phytoremediation*. 2021; **23**(1): 72-79.
15. Sasikumar P, Aswathy M, Prem PT, Radhakrishnan KV, Chakrapani PSB. Plant derived bioactive compounds and their potential to enhance adult neurogenesis. *Phytomedicine Plus*. 2022; **2**(1): 100191.
16. Shaeer A, Aslam M, Rashid NA. highly stable manganese catalase from *Geobacillus thermopakistaniensis:* molecular cloning and characterization. *Extremophiles*. 2019; **23**(6): 707-718.
17. Sharma SK, Murti CK. Production of lipid peroxides by brain. *J Neurochem*1968; **15**(2): 147-149.
18. Shukla P, Sahu NK, Kumar R, Dhalla DK, Rakshit S, Bhadauria M, Nirala SK. Quercetin ameliorates acute acrylamide induced spleen injury. *Biotechnic & Histochemistry*. 2023; **98**(4): 221-229.
19. Singh S, Kumar D, Nirala SK, Bhadauria M. Hepatoprotective potential of propolis. *IP Int J Compr Adv Pharmacology.* 2023; **8**(2): 91-97.
20. Singh B, Pandey S, Rumman M, Kumar S, Kushwaha PP, Verma R, Mahdi AA. Neuroprotective and neurorescue mode of action of *Bacopa monnieri* (L.) Wettst in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease: an *in silico* and *in vivo* study. *Frontiers in Pharmacology*. 2021; **12**: 169.
21. Snedecor GW, Cochran WG. Statistical Methods. 8th Edn IOWA State University Press. 1994; *Ames, Iowa, USA*.
22. Todorova V, Ivanov K, Ivanova S. Comparison between the Biological Active Compounds in Plants with Adaptogenic Properties *(Rhaponticum carthamoides, Lepidium meyenii, Eleutherococcus senticosus and Panax ginseng). Plants*. 2022; **11**(1): 64.
23. Yang B, Li L, Geng H, Wang G, Zhang C, Yang S, Zhao Y, Xing F, Liu Y. Detoxification of aflatoxin B1 by H2SO3 during maize wet processing, and toxicity assessment of the transformation product of aflatoxin B1. *Food Control*. 2022; **131**: 108444.