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

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**ABSTRACT**

Aflatoxin B1 is one of the 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\(_1\).

**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 hepatocarcinogens 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.\(^9\) 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 years.

**Fig. 1**: *Bacopa monnieri*
years and is classed as *madhya rasayana*, a medicine used as a nerve tonic to strengthen intelligence and memory. The existence of bioactive chemicals such as alkaloids, carbohydrates, glycosides, saponins, phenols, proteins, and amino acids was discovered through phytochemical screening. *Bacopa monnieri* is a well-known memory booster in the Indian medicine. 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.

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 ± 2°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, Rahnax 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°C before being centrifuged at 3000 revolutions per minute for 10 minutes to separate serum. 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 20°C.

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

Serum metabolite activity Triglycerides and antioxidant activity such as reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were measured using a kit approach.

**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), antioxidant enzyme markers such as reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).

**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 < 0.05, and it was then tested using the Student' t test. 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.
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 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<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<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. 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. 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<0.05) increased, whereas the amount of GSH was significantly ((P<0.05) decreased. When compared to untreated AFB1-intoxicated rats, pretreatment with BM resulted in a substantial (P<0.05) drop in the TBARS level and a significant (P<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. 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. 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-1 : Serological investigation of BM therapeutic potential against AFB1-induced liver damage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
<th>Triglyceride(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58±3.20</td>
<td>41.67±2.30</td>
<td>79.14±4.37</td>
</tr>
<tr>
<td>BM per se</td>
<td>58.43±3.23</td>
<td>42.05±2.32</td>
<td>80±4.42</td>
</tr>
<tr>
<td>AFB1</td>
<td>103.02±5.69*</td>
<td>89.94±4.97*</td>
<td>415±22.94*</td>
</tr>
<tr>
<td>AFB1+BM (20mg/kg) (% protection)</td>
<td>86.02±4.75* (37.76%)</td>
<td>75.32±4.16* (30.28%)</td>
<td>319±17.63* (28.58%)</td>
</tr>
<tr>
<td>AFB1+BM (30mg/kg) (% protection)</td>
<td>75.82±4.19* (60.41%)</td>
<td>67.47±3.72* (46.55%)</td>
<td>231±12.76* (54.78%)</td>
</tr>
<tr>
<td>AFB1 +BM (40mg/kg) (% protection)</td>
<td>68.34±3.77* (77.03%)</td>
<td>56.63±3.13* (69.007%)</td>
<td>171±9.45* (72.64%)</td>
</tr>
<tr>
<td>F value (at 5% level)</td>
<td>20.27@</td>
<td>34.41@</td>
<td>114.45@</td>
</tr>
</tbody>
</table>
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. AFB1 was administered to rats, the SOD and CAT levels in the liver decreased dramatically (P < 0.05) as compared to the control group. When compared to untreated AFB1-intoxicated rats, pretreatment with BM resulted in a significant (P < 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 40 mg/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. SOD is in charge of catalysing the transformation of superoxide anions into hydrogen peroxide. CAT then decomposes it further into water and oxygen. 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.

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

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

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

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**


