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Therapeutic Efficacy of *Bacopa monnieri* against Aflatoxin B_1 Induced Toxicity in Rats

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Abstract

Aflatoxin B1 (AFB1), the most frequently discovered aflatoxin in tainted foods and feed, is considered the most important risk factor. Brahmi (*Bacopa monnieri*) is a well-known perennial, creeping herb in the Indian *Ayurvedic* system. Thus, the present study was designed to evaluate the protective efficacy of Brahmi against liver damage induced by Aflatoxin B1. The whole study was conducted in two experiments. The first experiment used phytochemical estimation of BM (*Bacopa monnieri*). In the second experiment, animals were randomly divided into six groups with six animals in each group. Group 1 served as the control. Group 2 served as *per se* and received the highest dose of therapy which was 40 mg/kg for 13 days, post orally. Group 3 received AFB1 (200 µg/kg/day) for 13 days, orally Groups 4 to 6 received different doses of Brahmi (20, 30, 40 mg/kg/day PO) for 3 consecutive days after 10 days of exposure to AFB1. All animals were sacrificed after 24 hr of the last treatment. DPPH free radical scavenging activity of the plant was reported in terms of IC_{50} (45.30 ± 2.52 µg/ml). The presence of flavonoids and protein in the plant was reported in 29.63 ± 1.63 µg Rutin /mg, and 59.72 ± 3.30 µg BSA / mg. Acute studies showed increased lipid peroxidation and a decline in antioxidant status. Alterations in the Liver Function Test (LFT) were also observed. Oral treatment with 20-40 mg/kg Brahmi showed remarkable protection against the toxic effects of Aflatoxin B1. Biochemical results of this study demonstrate that *Bacopa monnieri* extract possesses protective potential against AFB1-induced hepatotoxicity due to the presence of several bioactive phytochemicals. From this study, we can conclude that treating *Bacopa monnieri* can protect against AFB1 challenge rats.

Keywords: Aflatoxin B1, *Bacopa monnieri,* Liver, Oxidative Injury, Polyphenol

1. Introduction

Aflatoxins (AFs) are secondary metabolites synthesised by *Aspergillus* fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*. AFs are potent hepatocarcinogens in animal models and suspected carcinogens in humans[1]. It contaminates food products like maize, nuts, rice, and wheat. The four major naturally produced AFs are known as B1, B2, G1, and G2. The most important AF in terms of toxic potency and occurrence is AFB1, which is found in maize, peanuts, and peanut oil. AFs have been associated with toxic effects on animal and human

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health including carcinogenic, mutagenic, teratogenic, and immunosuppressive activities[2]. Maternal exposures to AFB1, as well as its metabolite AFM1, revealed the crossing of these toxins from the bloodstream into milk and subsequent modifications in hippocampal neurogenesis with down-regulation of cholinergic signals in their progenies[3]. AFB1 metabolism is of high concern in the human liver. This extremely reactive metabolite binds to DNA guanine to form adducts, causing toxic effects. AFB1 is a potent hepatotoxic and hepatocarcinogenic mycotoxin. The mechanism of cellular damage caused by AFB1 has not been fully elucidated. Thus the present study is designed to throw ought on made of AFB1 toxicity. Up to 4.5 billion humans are believed to be at risk of exposure to aflatoxins, which have deleterious effects on the health of both humans and animals. Multiple toxicities and mechanisms have been reported, especially carcinogenicity. The present study will also demonstrate the role of BM in the management of AFB1-induced toxicity.

Bacopa monnieri (BM) is a reputed medicinal herb documented in the traditional medicine system of India, belongs to the family Scrophulariaceae, and is prominently used in Ayurveda[4]. The herb was described in *Charaka Samhita, Susrutu Samhita, and Athar-Ved* to sharpen intellect and attenuate mental deficits[5]. Ayurveda medical practitioners in India have been using BM for almost 3000 years and classified it as a *madhyarasayana*, a drug used as a nervine tonic to sharpen intellect and memory. The phytochemical screening detected the presence of bioactive compounds like alkaloids, carbohydrates, glycosides, saponins, phenols, proteins, and amino acids[6]. BM, is a well-known memory booster in Indian medicine. It occurs naturally in India and is used in the treatment of several disorders, particularly those involving anxiety, intellect, and poor memory. They also ameliorate neuronal dysfunctions caused by diabetic neuropathy[7]. BM leaf extract improves behavioural abnormalities and reduces oxidative stress, and apoptosis in the brains of Parkinson's disease[8].

In recent years, there has been a renewed interest in medicinal plants and phytochemical-based drugs as they give a holistic and harmonious balance of physical, biological, and mental processes through systems of medicine like Ayurveda, Unani, and Siddha. Emphasizing the current era of phytomedicine, an attempt has been made in the present study to analyze the prophylactic profiling of BM against AFB1-induced hepatic abnormalities.

2. Materials and Methods

2.1 Animals and Chemicals

The animals were purchased from the central animal house facility, All India Institute of Medical Sciences, New Delhi, India. Male rats of the *Wistar* strain weighing between 150-200 g were used in this study. Animals were treated and cared for following the guidelines recommended by the Committee for Control and Supervision of Experiments on Animals (CPCSEA No-IAEC/JU/56). Aflatoxin B1 was procured from Sigma-AlFdrich (St. Louis, MO, USA). All other analytical chemicals were purchased from Sigma-Aldrich, New Delhi, and Hi-media Laboratories Ltd. Mumbai, India.

2.2 Collection and Preparation of Plant Extract

The whole plant of BM was collected in November (29°C -14°C temperature) on the campus of Jiwaji University Gwalior, Madhya Pradesh, India. The herbarium was submitted to SOS in Botany with accession number 5312/PP-49-50/03/12/2020. The plant was washed and dried in the shade. After drying whole plant was pulverized to a coarse powdered form by using an electric grinder and was stored in a desiccator. The aqueous extract was prepared; the extract was allowed to dry in the oven at 37°C for 15 days. The extraction was done by taking 10 g of powdered drug and extracting it using 100 mL of distilled water as a solvent in Soxhlet extractor on a water bath for 5 hr. Then extract was filtered and left to dry



Figure 1. An image of *Bacopa monnieri*.

under pressure. The aqueous extract thus obtained was kept in a closed bottle at 4°C until further use.

2.3 Experimental Design

2.3.1 Bioactive Compounds of BM Extract

DPPH free radical scavenging activity was also determined according to Singh[9]. solution of 0.1 mM DPPH dye in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract in water (10-100 µg/kg). Ascorbic acid is used as a standard. Then absorbance was recorded at λ 517 nm using a UV-Vis spectrophotometer.

The aqueous plant extract was subjected to various phytochemical analyses to quantify the amounts of polyphenols[10]. The aliquot of plant sample (100-1000 µg/kg) 0.3 ml of $NaNO_3$ and 0.3 ml $AlCl_3$ 5 minutes 2 ml NaOH was added and reading was recorded at 510 nm against blank and protein estimation[11].

2.3.2 Preparation of Doses

Aflatoxin B1 dose according to Vipin[12]: 200µl of AFB1 in 50ml double distilled water (stock) *Bacopa monnieri* dose according to Kamkaew[13]: 80mg of BM dissolved in 10ml double distilled water (stock)

$C_1V_1=C_2V_2$, according to this formula dilution was adjusted.

2.3.3 Animal Experimental Design

Animals were randomly divided into six groups with six animals in each group. Group 1 served as the control. Group 2 served as *per se* and received the highest dose of therapy which was 40 mg/kg for 13 days, post orally. Group 3 received AFB1 (200 µg/kg/day) for 13 days, orally Groups 4 to 6 received different doses of BM (20, 30, 40 mg/kg/day orally) for 3 consecutive days after 10 days of exposure to AFB1. All animals were sacrificed after 24 hr of last treatment.

2.3.4 Hematological Analysis

Blood was collected by puncturing the retro-orbital plexus of anaesthetized rats. 10% Ethylene Diamine Tetraacetic Acid (EDTA) was mixed in the blood to prevent clotting. Complete blood count was determined using apparatus ABX Micros 60.

2.3.5 Serological Analysis

The blood samples were incubated according to Riley, for 30 min and 37°C then centrifuged at 3000 rpm for 10 min to isolate serum. Serum samples were kept in the refrigerator under freezing conditions at -20°C. Activity of serum enzymes, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), activity of serum metabolites Triglycerides cholesterol, bilirubin, albumin, and Lactate Dehydrogenase (LDH), Gamma-glutamyl Transferase (γ- GT) and Alkaline Phosphatase (ALP) was estimated by the kit method using an auto-analyzer (Micro Lab 200) according to the directions given in the manual of the diagnostic kit (Erba).

2.3.6 Estimation of Oxidative Stress Markers

For Lipid Peroxidation (LPO) homogenates were made in 0.15M KCL solution. The extent of lipid peroxidation in liver samples was calculated spectrophotometrically by assessing the formation of Thiobarbituric Acid Reactive Species (TBARS) at λ 535 nm against blank[14].

2.3.7 Non-enzymatic Markers of Oxidative Stress

Reduced Glutathione (GSH) homogenates were prepared in 0.25M Sucrose solution. A yellow-coloured chromophore is formed when reduced glutathione reacts (GSH) with 5-5'-dithiobis 2-nitrobenzoic acid, which is measured at λ 412 nm[15].

2.3.8 Enzymatic Markers of Oxidative Stress

Superoxide Dismutase (SOD)[16] and Catalase (CAT)[17] homogenates were made in normal saline (0.9 NaCl) solution. SOD was estimated by assessing the rate of adrenochrome formation inhibition, it was recorded at λ 480 nm for 3 min. Catalase activity was determined by calculating the decomposition of H_2O_2 and was recorded at λ 240 nm for 3 min.

2.3.9 Assessment of Membrane-Bound Enzymes

Adenosine triphosphates (ATPase)[18] and glucose 6 phosphate (G6Pase)[19] homogenates were prepared in a hypotonic solution. ATPase activity inorganic phosphate released from ATP is measured at λ 620 nm. G6Pase catalyzes the hydrolysis of G6Pase into glucose and inorganic phosphate, which reacts with ammonium molybdate to form a blue-coloured complex. Its intensity was recorded at λ 660 nm.

2.3.10 Assessment of Protein

Protein was calculated according to the Lowry method[11].

2.3.11 Statistical Analysis

Data were expressed as mean ± standard error of six animals in each group. The mean using one-way Analysis of Variance (ANOVA) was done considering significance at p≤0.05 followed by a student test[20]. The results are presented as mean ± SE of six animals in each group.

3. Results

3.1 Determination of Antioxidant Potential

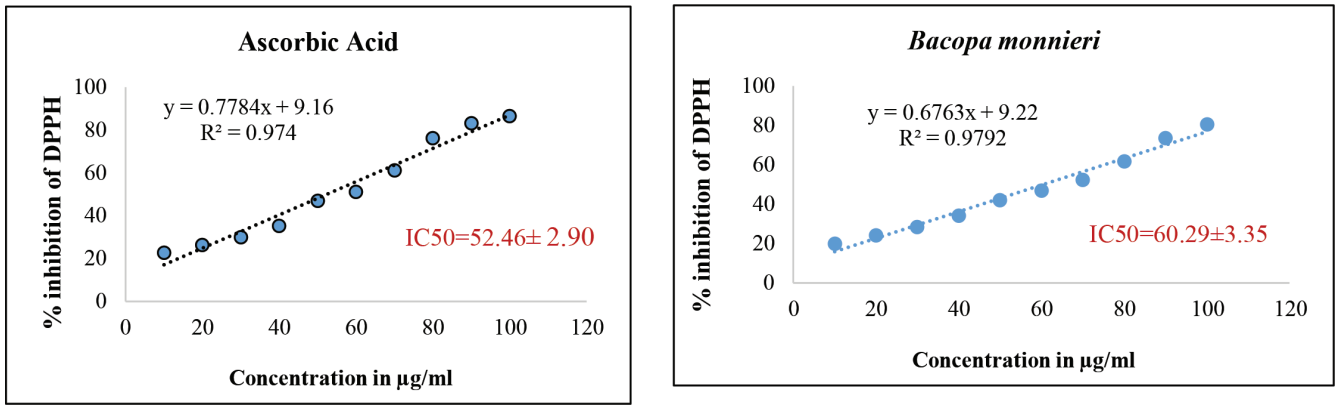
To investigate the antioxidant potential of plant extract in water at different concentrations (10-100 µl/ml) respectively, a DPPH free radical scavenging activity assay was performed, and the activity was reported in terms of % inhibition. The IC_{50} of extract was calculated using % inhibition, aqueous extract showed IC_{50} of 60.29± 3.35µg/ml as compared to 52.46 ± 2.90µg/ml of standard (Ascorbic acid) in Figure 2.

3.2 Determination of Total Flavonoid Content

The aqueous plant extract was analyzed for the presence of flavonoids and antioxidant potential was analyzed for the extract. To calculate total flavonoid content, the Rutin standard curve was plotted by taking concentration (100-1000 µl/ml) on the X axis and absorbance on the Y axis. The obtained equation line was Y= 0.0007X-0.0369, R2 = 0.9823 in Figure 3. The flavonoid content of the extract was 29.63 ± 1.63 µg Rutin /mg in Table 1.

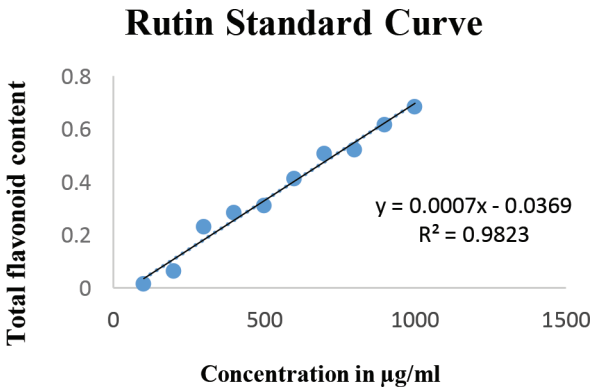
3.3 Protein Estimation

Aqueous BM extract was analyzed for the presence of proteins. To calculate protein estimation, the BSA



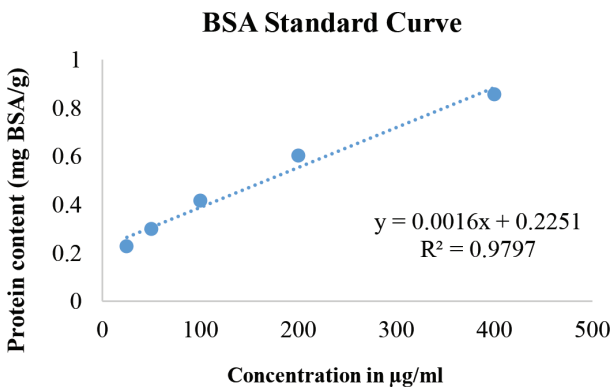
Values are mean ± SE for three experiments

Figure 2. DPPH Free radical scavenging activity.



Values are mean ± SE for three experiments

Figure 3. Estimation of total flavonoid content in *Bacopa monnieri* extract.



Values are mean ± SE for three experiments

Figure 4. Estimation of total protein content in *Bacopa monnieri* extract.

Table 1. Total flavonoid content

Sr. No.	Extract	Total Flavonoids (µg Rutin / mg)
1	*Bacopa monnieri*	29.63 ± 1.63
Values are mean ± SE for three experiments		

Table 2. Protein content

Sr. No.	Extract	Protein content (µg BSA / mg)
1	*Bacopa monnieri*	59.72 ± 3.30
Values are mean ± SE for three experiments		

Table 3. The therapeutic effect of BM against AFB1 on complete blood count

Treatment	Blood Biochemistry				
	WBCs (10000/3/ul)	RBCs (10000/3/ul)	HGB (%)	HCT (%)	Platelet (10^{10}/µl)
Control 10.2 ± 0.56 6.8±0.37			16.1±0.89	33.2 ±1.83	7.59±41
BM *per se*	11.01 ± 0.60	5.88± 0.32	15.7± 0.86	32.1 ± 1.34	7.12 ± 0.39
AFB1	21.3 ± 1.17$^{\#}$	5±0.27$^{\#}$	13.8±0.76$^{\#}$	24.4 ±1.77$^{\#}$	3.98 ±0.22$^{\#}$
AFB1+BM (20mg/kg) (% protection)	14.2±0.78* (63.96%)	5.21±0.28* (11.66%)	14.6±0.80* (34.78%)	28.2 ±1.55* (83.85%)	6.45 ±0.35* (68.42%)
AFB1+BM (30mg/kg) (% protection)	13.6±0.75* (69.36%)	5.43±0.30* (23.88%)	14.87±0.82* (46.52%)	30.2 ±1.66* (90.31%)	6.88 ±0.38* (80.33%)
AFB1+BM (40mg/kg) (% protection)	12±0.66* (83.78%)	5.79±0.32* (43.88%)	15.2±0.84* (60.86%)	31.1 ±1.71* (93.21%)	7.02±0.38* (84.21%)
F value (at 5% level)	31.31$^{@}$	4.93 $^{@}$	1.15$^{@}$	4.39$^{@}$	5.25 $^{@}$

Data are Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1at p≤0.05
Abbreviations: BM = *Bacopa monnieri*, AFB1 =Aflatoxin B1

standard curve was plotted by taking concentration (25-400 µl/ml) on the X-axis and absorbance on the Y-axis. The obtained equation line was Y = 0.0016x + 0.2251, R^2 = 0.9797 in Figure 4. The protein content of the extract was 59.72 ± 3.30 µg BSA /mg in Table 2.

3.4 Hematological Analysis

The results of the hematological analysis are shown in Table 3. White Blood Cells (WBCs) of rats in group 3 were above the reference range, while Red Blood Corpuscles (RBCs), Haemoglobin (HGBs), Hematocrit (HCTs) and platelet values were found to be decreased. Animals from Groups 4 to 6 showed recovery in the above hematological profiling ($p < 0.05$).

3.5 Serological Analysis

Tables 4, 5 demonstrated that the activities of Triglycerides (TG), cholesterol, bilirubin, albumin, Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were significantly increased (p≤0.05) in the serum of AFB1-intoxicated rats compared with control and per se. However, activities of the above liver function test markers were significantly decreased towards control values (p≤0.05) in AFB1-intoxicated rats treated with BM in a dose-dependent manner, a higher dose of therapy shows maximum improvement.

Table 4. The therapeutic effect of BM against AFB1 on liver function test biomarkers

Treatment	AST (IU/L))	ALT (IU/L)
Control	52±2.87	39.26±2.17
BM per se	53.34±2.94	40.35±2.23
AFB1	101.03±5.58$^{\#}$	97.69±5.40$^{\#}$
AFB1+BM (20mg/kg) (% protection)	76.03±4.20* (52.90%)	89.36±4.93* (14.26%)
AFB1+BM (30mg/kg) (% protection)	64.53±3.56* (75.44%)	74.47±4.11* (39.73%)
AFB1 +BM (40mg/kg) (% protection)	59.46±3.28* (85.37%)	64.53±3.56* (56.75%)
F value (at 5% level)	27.57$^{@}$	46.19$^{@}$

Data are Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1at p≤0.05
Abbreviations: BM = *Bacopa monnieri*, AFB1 =Aflatoxin B1

Table 5. Therapeutic potential of BM on serological analysis against AFB1-induced hepatic toxicity

Treatment	LFT						
	Triglyceride (mg/dl)	Cholestrol (mg/dl)	Bilirubin (mg/dl)	Albumin (mg/dl)	LDH (IU/L))	γ- GT (IU/L)	SALP (mg pi/100 ml/h)
Control	84.12±4.65	2.90±0.16	0.35±0.01	2.90±0.16	45.2±2.49	36.5±2.01	190.3±10.51
BM *per se*	96.80 ± 5.35	2.62 ± 0.14	0.34± 0.04	2.62 ± 0.14	43.5±2.40	37.1±2.05	195.1±10.78
AFB1	398±22.01#	1.6±0.08#	0.22±0.01#	1.6±0.08#	132.1±7.30#	83.7±4.62#	560.1±30.96#
AFB1+BM (20mg/kg) (% protection)	291±16.08* (34.08%)	1.87±0.10* (20.76%)	0.27±0.01* (38.46%)	1.87±0.10* (20.76%)	80.3±4.43* (59.69%)	63.5±3.51* (42.79%)	240.3±13.28* (86.47%)
AFB1+BM (30mg/kg) (% protection)	231±12.49* (54.79%)	2.07±0.11* (36.15%)	0.31±0.01* (69.23%)	2.07±0.11* (36.15%)	62.5±3.45* (80.09%)	41.2±2.27* (90.04%)	198.4±10.01* (97.80%)
AFB1+BM (40mg/kg) (% protection)	171±9.23* (73.59%)	2.11±0.11* (39.23%)	0.33±0.01* (84.61%)	2.11±0.11* (39.23%)	48.7±2.69* (95.97%)	39.2±2.16* (94.27%)	196.7±10.87* (98.26%)
F value (at 5% level)	105.19@	18.18@	10.29@	18.18@	79.84@	51.62@	96.48@

Data are Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1at p≤0.05
Abbreviations: BM = *Bacopa monnieri*, AFB1 =Aflatoxin B1

3.6 Calculation of Percent Protection

The protective percentage of test drugs was calculated by the following equation:

$$\text{Formula per cent protection} = \left\{1 - \frac{Tx - C}{Th - C}\right\} \times 100$$

where, Tx = AFB1 + TP, Th = AFB1, C = Control

3.7 Markers of Oxidative Stress and Non-enzymatic Antioxidant

The level of TBARS was significantly (p≤0.05) increased and the GSH level was significantly (p≤0.05) decreased in the liver of AFB1 intoxicated rats as compared to the control. Graph pretreated with different doses of BM showed a significant decline in TBARS levels and recoupment in the

Table 6. 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 H_2O_2/ min/mg protein)
Control	0.37±0.020	8.64±0.47	73.36±4.055	73.51±4.06
BM *per se*	0.38±0.021	8.15±0.45	68.6±3.79	73.16±4.04
AFB1	1.03±0.051#	2.3±0.12#	15.71±0.86#	21.01±1.16#
AFB1+BM (20mg/kg) (% protection)	0.86±0.047 * (70.48%)	7.1±0.39 * (71.16%)	38.88±2.14 * (40.19%)	47.5±2.62 * (50.45%)
AFB1+BM (30mg/kg) (% protection)	0.74±0.040* (77.71%)	7.4±0.40* (76.77%)	47.46±2.62* (55.07%)	51.9±2.86* (58.85%)
AFB1+BM (40mg/kg) (% protection)	0.58±0.032* (87.34%)	7.8±0.43* (84.26%)	58.21±2.44* (73.72%)	68.13±3.76* (89.75%)
F value (at 5% level)	55.95@	40.50@	61.01@	46.72@

Data are Mean ± SE; N=6; @=Significant at 5% for ANOVA # AFB1 vs Control; * AFB1 + Therapy vs AFB1at p≤0.05
Abbreviations: BM = *Bacopa monnieri*, AFB1 =Aflatoxin B1

Table 7. Therapeutic potential of BM on membrane-bound enzymes (ATPase and G6Pase) against AFB1-induced toxicity

Treatments	ATPase (mg pi/100 g/min)	G6Pase (µmole pi/min/g liver)
Control	1749.9±96.73	4.96±0.27
BM *per se*	1728±95.52	4.86±0.26
AFB1	1012±55.94#	1.50±0.08#
AFB1+BM (20mg/kg) (% protection)	1264±69.87* (34.15%)	4.34±0.23 * (82.08%)
AFB1+BM (30mg/kg) (% protection)	1371±75.79* (48.65%)	4.43±0.24* (84.68%)
AFB1+BM (40mg/kg) (% protection)	1407±77.78* (53.53%)	4.63±0.25* (90.46%)
F value (at 5% level)	14.93@	36.32@

Data are Mean ± SE; N=6; @=Significant at 5% for ANOVA
AFB1 vs Control; * AFB1 + Therapy vs AFB1at p≤0.05
Abbreviations: BM = *Bacopa monnieri*, AFB1 =Aflatoxin B1

activity of GSH. However max recovery was shown by 40 mg/kg dose. The perse group showed no adverse effect. Significant recovery was seen after BM therapy at all three doses. Percent protection showed maximum recovery at higher most 40 mg/kg doses, as shown by ANOVA at a 5% significance level in Table 6.

3.8 Enzymatic Antioxidant Markers

The SOD and CAT activity drastically declined (p≤0.05) in the liver after AFB1administation when compared to the control group. Treatment of BM caused a significant (p≤0.05) elevated toward the normal value of SOD and CAT in liver tissue when compared to AFB1-intoxicated rats. Therapy of BM showed restored values at all doses. The maximum restoration was seen in a 40 mg/kg dose. Results were analyzed by ANOVA at a 5% significance level in Table 6.

3.9 Membrane-Bound Enzymes

The activity of Adenosine Triphosphatase (ATPase) and glucose 6 phosphatase (G6Pase) was significantly (p≤0.05) reduced after AFB1 administration. Treatment with BM restored the activity of adenosine triphosphatase and glucose 6 phosphatase at all doses. The maximum restoration was seen in a 40 mg/kg dose. In terms of present protection in the 40 mg/kg dose in Table 7.

4. Discussion

The present study demonstrated a protective effect of BM against AFB1-induced hepatotoxicity. The pharmacological effects of polyphenols are associated with their antioxidant activity due to their ability to scavenge free radicals and exert a synergistic effect[21].

Ayurveda is the oldest system of medicine in the world as its antiquity goes back to the Vedas. BM, is a well-documented nootropic herb in the traditional Indian medical system. Moreover, the neuroprotective properties of BM are widely recognized and have been discussed in various studies[22,23], but very little focus has been given on the hepatoprotective activity of BM extract[24].

BM is a potent natural scavenger of free radicals[25]. DPPH antioxidant assay is commonly used to determine free radical scavenging properties of pure and natural compounds. The strength of antioxidants present in an extract is determined by IC_{50}. In this study, BM exhibited a concentration-dependent increase in the per cent scavenging of DPPH free radicals which is following the previous study.

Blood acts as a primary pathological indicator of the status of exposed animals to xenobiotics. The parameters usually measured during blood toxicity are red blood cells, white blood cells or leucocytes, haemoglobin and hematocrit values[24]. Hematological disorders indicating alteration because of AFB1 is an indication of blood toxicity of AFB1 and its metabolites, while treatment with BM prevents this alteration showing the therapeutic value of the extract.

Our results showed a significant upsurge in liver function test marker enzymes (ALT, AST, ALP and LDH) in the AFB1 group. The upsurge of these liver enzymes (ALT, AST and LDH) indicates damage to hepatocytes and impairment of liver functions due to hepatotoxicity caused by any xenobiotics[26]. The mechanism of ALP's high level may result from faulty liver discharges or increased ALP generation of hepatic parenchymal cells[27]. Our results showed improvement after treatment with BM. Recovery towards normalization of these liver function test marker enzymes following BM treatment after AFB1 exposure suggested that BM has a role in maintaining the structural integrity of the hepatocellular membrane and preventing these enzymes from leaking into circulation. This recovery is the possible presence of saponins for the maintenance of membrane integrity[28]. Our results are with support the hypothesis that transaminase levels return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes[29].

Lipid Peroxidation (LPO) is one of the main manifestations of AFB1-mediated oxidative damage and it has been found to play an important role in toxicity and carcinogenicity[30]. Several reports indicated that exposure to AFB1 increased LPO in the liver. In the current study, AFB1 administration enhanced lipid peroxidation as indicated by the significant increase in TBARS level which is a direct result of free radical-mediated toxicity. The free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce LPO. Moreover, treatment with BM prevents cellular integrity from damage caused by free radicals caused by AFB1[31].

The endogenous deposits of enzymatic antioxidants like Superoxide Dismutase (SOD) and Catalase (CAT) counters free radicals generated during AFB1 metabolism. SOD is responsible for catalyzing the conversion of superoxide anions into hydrogen peroxide. Which, is further decomposed to water and oxygen by CAT. The activities of SOD and CAT significantly decline in AFB1-treated rats. BM treatment showed a protective effect by improving SOD and CAT levels confirming its efficient protective mechanism in response to ROS. These findings also indicate that BM may be associated with decreased oxidative stress and free radical-mediated tissue damage[32].

Activities of membrane enzyme ATPase were decreased considerably in the liver after AFB1 exposure. Free radicals are produced inside mitochondria and are frequently released into the cytosol. The AFB1-induced decrease in the activities of glucose-6-phosphatase and ATPase also supported mitochondrial dysfunction. G-6-Pase hydrolyzes G-6-P to glucose and inorganic phosphate, and the enzyme is considered to be important in the release of glucose from certain cells. A greatly increased G-6-Pase activity has been reported in connection with an increased glycogen breakdown in the liver cells. It is noteworthy that increased membrane-localized ATPase activity is seen at a time in hypovolemia when there is a dynamic alteration of fluid flux across membranes and that the increase in ATPase activity is most intense in the metabolically active centrilobular zones of the liver. The increased G-6-Pase activity occurs at a time when there is rapid glycogenolysis, increased glucose uptake by muscle, and decreased glucose transport by the gut. While the apparent increase in the activity of these enzymes relates logically to known alterations of carbohydrate metabolism and fluid dynamics in shock, biochemical verification of these findings is required before an actual increase in hepatic enzyme activity can be postulated[33].

5. Conclusion

From this study, we can conclude that the treatment of BM can protect against AFB1 challenges. Biochemical results of this study demonstrated that BM extract possesses protective potential against AFB1-induced hepatotoxicity due to the presence of free radical scavenging bioactive phytochemicals. However, further studies may be performed to isolate lead compounds from this extract as a protective agent against free radical-mediated injuries.

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8. References

1. Caglayan C, Kandemir FM, Darendelioğlu E, Yıldırım S, Kucukler S, Dortbudak MB. Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. J of Trace Eleme in Medi and Biolo. 2019; 56:60-8. https://doi.org/10.1016/j.jtemb.2019.07.011 PMid: 31442956.
2. Chen J, Ye Y, Huang D, Pan D, Qiu X, Lei L, *et al*. The mediating role of coagulation function on the association of prenatal exposure to aflatoxin B1 and postpartum hemorrhage in Guangxi, China. Enviro Sci and Pollu Res. 2022;29(25):37543-55. https://doi.org/10.1007/s11356-021-18186-8 PMid: 35066843.
3. Chen X, Li X, Xu X, Li L, Liang N, Zhang L, *et al*. Ferroptosis and cardiovascular disease: role of free radical-induced lipid peroxidation. Free Radical Res. 2021; 55(4):405-15. https://doi.org/10.1080/10715762.2021.1876856 PMid: 33455488.
4. Singh B, Pandey S, Rumman M, Kumar S, Kushwaha PP, Verma R, *et al*. 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. Fronti in Pharma. 2021; 12:616413. https://doi.org/10.3389/fphar.2021.616413 PMid: 33796021 PMCid: PMC8007855.
5. Kongkeaw C, Dilokthornsakul P, Thanarangsarit P, Limpeanchob N, Scholfield CN. Meta-analysis of randomized controlled trials on cognitive effects of *Bacopa*

monnieri extract. J of Ethnopharma. 2014; 151(1):528-35. https://doi.org/10.1016/j.jep.2013.11.008 PMid: 24252493.
6. 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. 2021; 11(1):64. https://doi.org/10.3390/plants11010064 PMid: 35009068.
7. Akorede GJ, Ambali SF, Olatunji AO, Aremu A, Basiru A, Ahmed AO, *et al*. Mitigative potentials of methanol leaf extract of *Moringa oleifera* on chronic carbamazepine-induced haemo-biochemical and thyrotoxicity in male Wistar rats. Pharmaco Rese-Modern Chine Medici. 2022; 2:100055. https://doi.org/10.1016/j.prmcm.2022.100055.
8. Malik J, Choudhary S. Indian traditional herbs and alzheimer's disease: Integrating ethnobotany and phytotherapy. Eviden Based Valida of Tradition Medi: A compreh Approach. 2021:1129-51. https://doi.org/10.1007/978-981-15-8127-4_50 .
9. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S. Anti-free radical activities of kaempferol isolated from *Acacia nilotica* (L.) Willd Ex Del Toxico *In Vitro*. 2008; 22(8):1965-70. https://doi.org/10.1016/j.tiv.2008.08.007_PMid: 18805478.
10. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Ameri J of Enology and Viticulture. 1977; 28(1):49-55. Corpus ID: 102040202.
11. Lowry OH. Protein measurement with the Folin phenol reagent. J biol Chem. 1951; 193(1):265-75 PMid: 14907713.
12. Vipin AV, Rao R, Kurrey NK, KA AA, Venkateswaran G. Protective effects of phenolics rich extract of ginger against Aflatoxin B1-induced oxidative stress and hepatotoxicity. Biomedi and Pharmacothe. 2017; 91:415-24. https://doi.org/10.1016/j.biopha.2017.04.107_PMid: 28475920.
13. Kamkaew N, Scholfield CN, Ingkaninan K, Maneesai P, Parkington HC, Tare M, *et al*. *Bacopa monnieri* and its constituents is hypotensive in anaesthetized rats and vasodilator in various artery types. J of Ethnopharma. 2011; 137(1):790-5. https://doi.org/10.1016/j.jep.2011.06.045 PMid: 21762768.
14. Sharma SK, Murti CK. Production of lipid peroxides by brain. J of Neurochemi. 1968; 15(2):147-9. https://doi.org/10.1111/j.1471-4159.1968.tb06187.x PMid: 5637724.
15. Brehe JE, Burch HB. Enzymatic assay for glutathione. Analyti Biochem. 1976; 74(1):189-97. https://doi.org/10.1016/0003-2697(76)90323-7 PMid: 962073.
16. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J of Biolo Chemis. 1972;247(10)3170-5. https://doi.org/10.1016/S0021-9258(19)45228-9 PMid: 4623845.
17. Aebi H. Catalase *in vitro*. Meth in Enzym 1984; 105:121-126. https://doi.org/10.1016/S0076-6879(84)05016-3 PMid: 6727660.
18. Seth PK, Tangri KK. Biochemical effects of some newer salicylic acid congeners. J of Pharmacy and Pharmaco. 1966; 18(12):831-3. https://doi.org/10.1111/j.2042-7158.1966.tb07822.x PMid: 4381672.
19. Baginski ES, Foa PP, Zak B. Glucose-6-phosphatase. InMethods of enzymatic analysis 1974; 876-880. https://doi.org/10.1016/B978-0-12-091302-2.50072-4.
20. Snedecor GW, Cochran WG. Statistical Methods. 8th ed. IOWA State University Press Ames, Iowa, USA; 1994.
21. Ghosh T, Maity TK, Das M, Bose A, Dash DK. *In vitro* antioxidant and hepatoprotective activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts.
22. Aguiar S, Borowski T. Neuropharmacological review of the nootropic herb *Bacopa monnieri*. Rejuvenation Res. 2013; 16(4):313-26. https://doi.org/10.1089/rej.2013.1431 PMid: 23772955 PMCid: PMC3746283.
23. Hosamani R, Krishna G, Muralidhara. Standardized *Bacopa monnieri* extract ameliorates acute paraquat-induced oxidative stress, and neurotoxicity in prepubertal mice brain. Nutritional Neurosci. 2016;19(10):434-46. https://doi.org/10.1179/1476830514Y.0000000149 PMid: 25153704.
24. Gupte SS, Rathour A, Gupta D, Soni R, Shrivastava S, Bhaduria M, *et al*. *Tephrosia purpurea* Ameliorates Oxidative and Histological Alterations Induced by Aflatoxin B1 in Rats. Interna J of Pharmaceuti Invest. 2022; 12(3). https://doi.org/10.5530/ijpi.2022.3.57.
25. Anand T, Naika M, Swamy MS, Khanum F. Antioxidant and DNA damage preventive properties of *Bacopa monniera* (L) Wettst. Free Radical and Antio. 2011; 1(1):84-90. https://doi.org/10.5530/ax.2011.1.13
26. Kucukler S, Darendelioğlu E, Caglayan C, Ayna A, Yıldırım S, Kandemir FM. Zingerone attenuates vancomycin-induced hepatotoxicity in rats through regulation of oxidative stress, inflammation and apoptosis. Life Scie. 2020; 259:118382. https://doi.org/10.1016/j.lfs.2020.118382 PMid: 32898532.
27. Cao W, Yu P, Yang K, Cao D. Aflatoxin B1: metabolism, toxicology, and its involvement in oxidative stress and cancer development. Toxico Mechani and Methods. 2022;32(6):395-419. https://doi.org/10.1016/0003-2697(76)90323-7 PMid: 34930097.
28. Shahid M, Subhan F, Ullah I, Ali G, Alam J, Shah R. Beneficial effects of *Bacopa monnieri* extract on opioid induced toxicity. Heliyon. 2016;2(2). https://doi.org/10.1016/j.heliyon.2016.e00068 PMid: 27441247 PMCid: PMC4945900.
29. Azarmehr N, Afshar P, Moradi M, Sadeghi H, Sadeghi H, Alipoor B, *et al*. Hepatoprotective and antioxidant activity of watercress extract on acetaminophen-induced hepatotoxicity in rats. Heliyon. 2019 ;5(7). https://doi.org/10.1016/j.heliyon.2019.e02072 PMid: 31334381 PMCid: PMC6624241.

30. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-9. https://doi.org/10.1016/S0899-9007(02)00916-4 PMid: 12361782.
31. Sekhar VC, Viswanathan G, Baby S. Insights into the molecular aspects of neuroprotective bacoside A and bacopaside I. Current Neurophar. 2019; 17(5):438-46. https://doi.org/10.2174/1570159X16666180419123022 PMid: 29676230 PMCid: PMC6520587.
32. 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. J of Plant Interac. 2021; 16(1):201-12. https://doi.org/10.1080/17429145.2021.1922771.
33. Mishra AK, Mishra A, Verma A, Chattopadhyay P. Effects of calendula essential oil-based cream on biochemical parameters of skin of albino rats against ultraviolet B radiation. Scien pharmace. 2012; 80(3):669-84. https://doi.org/10.3797/scipharm.1112-18 PMid: 23008814.