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Research Article

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Integrated in-Vitro and in-Vivo Evaluation of the Ethyl Acetate Fraction of *Basella Alba* Against Aflatoxicin B1- Induced Hepatocellular Carcinoma

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Abstract

Aflatoxin B1 (AFb1) is known to be a strong hepatocarcinogen, and hepatocellular carcinoma (HCC) continues to be one of the main causes of cancer-related death globally. The current work used integrated in-vitro and in-vivo methods to assess the ethyl acetate fraction of Basella alba (B alba) anticancer and hepatoprotective capabilities against AFb1-induced hepatocellular carcinoma. Standard cell viability, apoptosis, and oxidative stress tests were used to evaluate the ethyl acetate fraction's in vitro cytotoxicity and antiproliferative properties in human hepatocellular carcinoma cell lines. Strong anticancer efficacy was indicated by the fraction's dose-dependent suppression of cancer cell proliferation, induction of apoptosis, and notable decrease in reactive oxygen species. AFb1 was used to produce hepatocellular cancer in experimental rats for in vivo assessment. By examining biochemical liver function markers, antioxidant enzyme levels and histological alterations in hepatic tissue, the ethyl acetate fraction's therapeutic efficiency was evaluated. In comparison to the AFb1-induced control group, treatment with the ethyl acetate fraction considerably improved hepatic architecture, decreased lipid peroxidation, increased antioxidant defences, and restored abnormal liver enzymes. The results indicate that B. albaethyl acetate fraction has strong hepatoprotective and anticancer effects, which are probably caused by its pro-apoptotic, anti-inflammatory, and antioxidant qualities. The promise of B. alba as a viable natural treatment option for the treatment of hepatocellular carcinoma caused by AFb1 is supported by this study.

Keywords: B. Alba, AFb1, Hepatocellular Carcinoma, Ethyl Acetate Fraction, Anticancer activity, Hepatoprotection

1. Introduction

Ayurvedic medicine in India has used chemical extracts from *B. alba* leaves and stems for anticancer, including melanoma, according to preliminary phytochemical investigations.*B. alba* is used orally for the treatment of anal prolapse and hernia [1]. The current study used leaf extracts of Basella and Diclofenac sodium, a nonsteroidal anti-inflammatory drug (NSAID), as reference standard drugs. *B. alba* as a test medication on Enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and healing are all part of inflammation, which is a typical protective reaction to tissue damage [2]. Ayurveda and Siddha, the two primary traditional Indian medical systems, are mainly plant-based. The assessment of novel medications, particularly those derived from phytochemicals, has once again created a wide field for study and advancement [3]. Aflatoxins are widely known for being severe mutagens, carcinogenic, teratogenic, immunosuppressive, and inhibiting various metabolic processes, resulting in liver, kidney, and heart damage [4].

Several environmental factors influence the AFb1 production route, which is one of the known secondary fungal metabolic processes [5]. Among these toxins, AFb1 is regarded as the most common carcinogenic and immunosuppressive ability have been extensively reported in a wide range of animals, including chickens, trout, cattle, and rats [6]. Additionally, other aflatoxin-associated ailments have also been shown in humans and animals, including diseases connected to malnutrition, delayed physical and mental development, changes in reproduction, and disorders of the nervous system. Computational research can help comprehend the molecular process underlying the harmful consequences [7]. Scientists from all around the world have been examining the molecular pathways and detoxifying strategies connected to AFb1 toxicity because of its high-risk level and toxicity [8]. One of the fifth most common diseases worldwide is hepatocellular carcinoma (HCC), a primary tumor of the liver. After stomach, colorectal, and lung cancers, it is the third most common cause of cancer-related mortality. Malignant transformation of HCC is recognized to happen in an environment of inflammation and oxidative DNA damage, regardless of the etiological cause. Side effects, including but not limited to gastrointestinal toxicity, hepatotoxicity, cardiotoxicity, and neurotoxicity, are widespread among patients undergoing chemotherapies and radiotherapies against cancers, including HCC [9,10].

Notably, hepatitis viral infection is extremely difficult to eradicate; consequently, chemoprevention may be a better option for HCC, particularly in carriers of hepatitis B and C viruses [11]. While liver transplants and surgical resection have been utilized to treat early-stage HCC, these two therapeutic approaches are not appropriate for advanced HCC [12]. There are few effective treatment options for hepatocellular carcinoma (HCC), the well-known threat to the liver and the third most frequent cause of tumor mortality worldwide [13]. Medicinal plants' phytochemicals are the source of medications used to treat a variety of illnesses as well as possible sources for the creation of new drugs [14]. According to biochemical research, lead causes the liver's levels of lipids, cholesterol, and glycogen to decrease, but causes hepatic lipid peroxidation to rise [15]. The liver is a fascinating organ with intricate functions and a high capacity for regeneration. It serves as a biochemical barrier against harmful substances coming through the portal vein due to its advantageous placement in regard to the food supply and the distinct gene and protein expression patterns of hepatocytes, the primary functional cells of the liver [16].

Consuming phytochemical substances found in foods that can act as cellular antioxidants is one approach to accomplish this. Bioactive substances that inhibit the growth of cancer cells can alter the drug's detoxifying side, DNA repair, and cell proliferation while also interacting with cancer processes at the genetic level. One important way to stop cancer from developing is by dietary treatments that contain phytochemical substances that can act as cellular antioxidants [17]. Sterols, terpenoids, saponins, tannins, flavonoids, and phenolic chemicals can all be dissolved by the extraction solvent, ethyl acetate. Conversely, n-hexane was selected as a solvent due to its stability, volatility, selective dissolution of molecules, and ability to dissolve non-structural compounds such as terpenoids, lipids, and waxes [18]. Many malignancies are treated with cisplatin combination chemotherapy. Although many cancer patients will eventually relapse with cisplatin-resistant illness, platinum responsiveness is often high. Increased biotransformation and detoxification in the liver, altered cellular absorption and efflux of cisplatin, and an increase in DNA repair and anti-apoptotic processes are some of the suggested mechanisms of cisplatin resistance [19]. The ethyl acetate fraction may counteract AFb1-induced HCC through cytotoxic and antioxidant pathways, according to the integrated in-vitro cytotoxicity and in-vivo hepatoprotection [20].

2. Materials and Methods

2.1. Plant Material

2.1.1. Preparation of Plant Extract

Aerial part of *B. alba* plant was collected from the Botanical Garden of the National Botanical Research Institute in Lucknow, India (NBRI). Dr. Sayeeda Khatoon, a taxonomist, confirmed the plant material, and the voucher specimens (NAB2B04293, 200495, and 200492) were archived in the departmental herbarium and the Institutional Museum for future reference. *B. alba* fresh plant material was rinsed with distilled water to remove dirt and soil. *B. alba* powder (100g) was air-dried (30±20c) and extracted overnight with 10 ml ofethyl acetate. It was then centrifuged at 10000 rev/min on a Rota evaporator and dried in a lyophilizer (Labeonco, USA) under reduced pressure. The extract was then subjected to phytochemical and pharmacological testing.

2.1.2. In Vitro Study: HPTLC Analysis

Reflux 5 g of finely powdered medication with 25 ml of over a water bath for 25 minutes three times in a row, then filter and extract the solvent under low pressure. Dissolve 25 milligrammes of extractive in 20 millilitres of ethyl acetate. Using an automator applicator (CAMAG Linomat IV), apply 10 l of extract on Merck percoated silica gel 60 F254 plates with a 0.2mm thickness. The plates were then run using a fresh solvent system (ethyl acetate). Chloroform: Methanol (8.5:1.5) ratio for *B. alba* in a CAMAG twin through chamber up to a distance of approximately 9 cm, dry, and scan. UV 254 and visible light were used to view the plates. If necessary, spray the plate with anisaldehyde-sulphuric acid and heat at 110°C for 10 minutes. Record the Rf values and colour of the resolved bands, then use the Desaga video documentation unit to document the movie. The plates were densitometrically scanned with a CAMAG TLC scanner at the relevant wavelength [21].

2.1.3. Cell Viability Assay

HepG2 cells were grown at 37 °C in a humidified environment with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics. After being planted into 96-well plates, the cells were left to adhere overnight. To achieve varying quantities, the *B. alba* ethyl acetate extract was produced, dissolved in DMSO, and diluted with culture media, making sure the final DMSO content did not surpass 1% (v/v). Cells were exposed to the extract for 48 hours, whereas vehicle control cells were given 1% DMSO and control cells were

given simply culture media. Following incubation, the MTT test was used to measure cell viability by adding MTT reagent and incubated for four hours. A microplate reader was used to detect the absorbance at 570 nm after the formazan crystals were dissolved in DMSO. The percentage of cell viability in relation to the control group was computed [22].

2.1.4. In Vivo Study: Animals

Studies are carried out on rats weighing between 140 and 160 g. They were obtained from the toxicity control animal house at the Central Drug Research Institute in Lochnow, as well as cattle raised in the departmental animal facility. The rats were housed in polyacrylic cages with no more than 6 animals per cage and kept under typical laboratory conditions (temperature 25 ± 20C, 12h dark/light cycle). They have free access to a standard dry pellet meal (Amrut, India) and unrestricted tap water. The institutional committee for the ethical use of animals reviewed and approved all of the disclosed procedures. All experiments conducted were in accordance with the institutional ethical committee and the Institutional Animal Care Committee, CPCSEA, India (Reg. No.: 221/2000/CPCSEA).

2.1.5. Dose Administration

The rats were randomly assigned to the experimental and control groups (n=6). Group 1 rats were given 0.9% normal saline. Group II rats with chemically induced HCC (AFb1) were given a single oral administration of AFb1 mg/kg b.w. Group III rats received 100 mg/kg b.w.i.p. of *B. alba* in ethyl acetate). Groups IV and V were given i.p injections of 200 and 400 mg/kg/b. w, respectively, whereas Group VI rats received 6 mg/kg b.w of cisplatin.

2.1.6. Laboratory Investigations

Body weights were measured on the day of receipt, before randomisation, on the day of dosing, and weekly thereafter for the treatment and recovery groups, while food and drink consumption were documented daily and reported weekly. All animals had their blood drawn for haematology and clinical biochemistry. Animals were placed in metabolic cages and fasted overnight before blood sample, but they had unlimited access to water. Blood was drawn from the retroorbital plexus using a micro-hematocrit heparinised glass capillary tube. Potassium EDTA was employed as an anticoagulant during haematology tests. Blood samples were stored in serum tubes at room temperature for around 30 minutes before being aliquoted. After clotting, the blood tubes were centrifuged at 3000 rpm for 15 minutes. The supernatants were decanted and stored at 700°C for subsequent analysis.

2.1.7. Biochemical Marker Estimation

The biochemical markers like SGOT(U/l), SGPT(U/l), SALP(U/l), Bilirubin level (U/l) and Gamma glutamyl transpeptidase, GGT (U/l) were determined for both control and treated groups by using standard biochemical method [23].

2.1.8. Estimation of Free Radical Generation

The liver homogenate (5%) in ice-cold phosphate buffer was centrifuged at 800X g for 10 minutes, followed by centrifugation of the supernatant at 12,000X g for 15 minutes to obtain the mitochondrial fractions, which were used to measure lipid peroxidation (LPO), superoxide dismutase activity (SOD), catalase activity (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPX) [24].

2.1.9. Hematological Estimation

Red blood cell counts, White blood cell counts and hemoglobin were estimated with the help of hematology analyzer (Medonic CA620, Boule, Sweden) [25].

2.1.10. Estimation of Ethyl Acetate Extract of *B. alba* on Level of Nucleic Acids (DNA, RNA, and Protein)

After the experiment, the liver tissues from the ethyl acetate extract of *B. alba*-treated and control groups were removed, cleaned with ice-cold saline, blotted, and weighed. To get the supernatant, the tissues were homogenized in cold buffer and centrifuged. The diphenylamine and orcinol techniques were used to estimate the amounts of DNA and RNA, respectively, and the results were expressed as mg/g wet tissue. Using bovine serum albumin as the standard, the Lowry technique was used to calculate the protein content, which was then represented as g/dL. A UV-visible spectrophotometer was used to quantify absorbance, and each analysis was carried out in triplicate [26].

2.1.11. Histopathological Analysis

The livers were removed immediately upon autopsy for histological examination, and the tissues were stored in 10% formalin for at least 24 hours. The paraffin sections were then treated (Automatic Tissue Processor, Lipshaw) and sliced into 5 um-thick sections using a rotary microtome. The sections were then stained with haematoxilin-eosin dye (Merck) and mounted on Canada balsam. The histology slides were examined and photographed under a picture zoom microscope (3.2X10 and 10X10) [27].

2.1.12. Statistical Analysis

Data are expressed as mean +SEM (standard error of mean). The difference among means has been analyzed by an unpaired Student's t-test.

3. Results

3.1. HPTLC Analysis of Ethyl Acetate Extract of *B. alba*

A distinctive fingerprint with thirteen well-resolved peaks was revealed by HPTLC analysis of the ethyl acetate extract of *B. alba*, suggesting the presence of several phytoconstituents. The compounds' Rf values ranged from 0.17 to 1.15, with a notable peak at Rf 0.73–0.86 displaying the largest area percentage (35.79%), indicating that it is the main component as shown in (Table 1). Differences in the relative abundance of the separated compounds are reflected in the variance in peak height and area percentages shown in figure 1. This HPTLC profile verifies the extract's chemical complexity and could be used as a guide for quality assurance and additional phytochemical research.

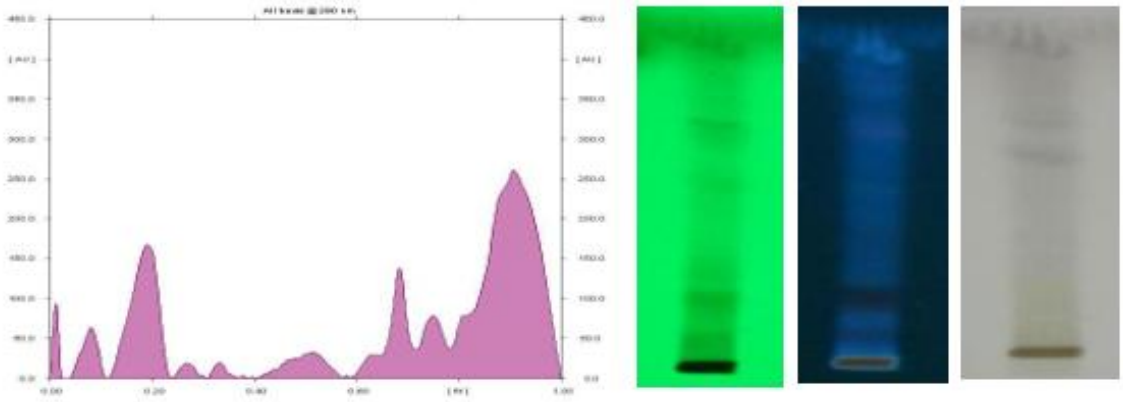


Figure 1: HPTLC Fingerprint of Ethyl Extract of Aerial Parts B. alba. Solvent: Chloroform: Methanol (8.5:1.5)

Peak	Start Rf	Height %	End Rf	Area %
1	0.17	17.08	0.19	12.98
2	0.20	23.104	0.21	14.53
3	0.21	13.46	0.25	11.52
4	0.28	11.72	0.35	11.57
5	0.38	11.31	0.41	10.67
6	0.41	11.44	0.45	11.10
7	0.55	12.103	0.62	13.35
8	0.62	17.89	0.73	13.49
9	0.73	24.24	0.86	35.79
10	0.87	18.10	0.92	15.54
11	0.92	18.26	0.100	19.09
12	0.101	18.78	0.108	19.45
13	0.102	32.31	1.15	31.58

Table 1: HPTLC Values

3.2. Cell Viability in Control and Ethyl Acetate Extract of *B. alba*

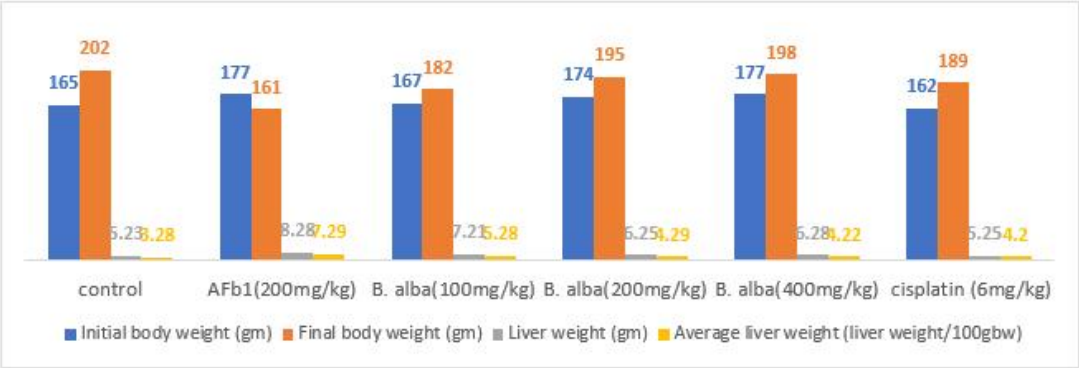
Cell viability was significantly and dose-dependently inhibited by the *B. alba* ethyl acetate fraction. There was no cytotoxicity in the control or DMSO (1% v/v) groups. *B. alba* treatment produced percentage inhibitions of 24.22 ± 1.29% at 20 µg/ml, 34.34 ± 1.34% at 40 µg/ml, and 69.41 ± 1.46% at 60 µg/ml, 84.52 ± 1.55% at 80 µg/ml, and 101.56 ± 1.42% at 100 µg/ml. In comparison to the control, all treated groups demonstrated statistically significant suppression (aP < 0.001), suggesting that *B. alba* has a potent cytotoxic and antiproliferative effect against cancer cells in a concentration-dependent manner, as shown in (Table 2).

Treatment	Concentration	Percentage of inhibition
Control	-	0
DMSO	1%(v/v)	0
Basellaalba	20(µg/ml)	24.22 ± 1.29a
Basellaalba	40(µg/ml)	34.34 ± 1.34a
Basellaalba	60(µg/ml)	69.41 ± 1.46a
Basellaalba	80(µg/ml)	84.52 ± 1.55a
Basellaalba	100(µg/ml)	101.56 ± 1.42a
Values are mean ± SEM; n=6 [a]P<0.001 compared with control group		

Table 2: Estimation of Cell Viability in Control and Ethyl Acetate Extract of B. alba Treated Hep G2cells After 48 H of Exposure

3.3. Effect of Ethyl Acetate Extract of B. albaon Body Weight, Liver Weight and Average Liver Weight in (AFb1) Induced HCC Rat

50% ethanolic extracts of *B. Alba*, at a dose of 100, 200 mg and 400 mg once daily for 28 days and standard Cisplatin at a dose of 6 mg/kg were subjected for studying the body weight, liver weight and average liver weight in HCC rats. The study showed that the liver weights were significantly increased from 19.3,15.33 to 22.8,15.78 in AFb1 group to 21.1,15.36 to 19.8, 10.66 in *B. alba* treated animal. whereas, standard drug Cisplatin 6 mg/kg showed a significant reduction in liver weight compared to the AFb1 group Figure 2.



Values are mean ±of 6 rats in each group

Figure 2: Effect of Ethyl Acetate Extract of *B. alba* on Body Weight, Liver Weight and Average Liver Weight in (AFb1) Induced HCC Rat

3.4. Effect of Ethyl Acetate Extract of *B. albaon* SGOT, SGPT, SALP, BL and GGT Against AFb1-Induced HCC

In the AFb1group, the level of SGOT (212.20-374.21, P<0.001), SGPT (103.21-391.53, P<0.001), SALP (254.14-459.31, P<0.01), BL (0.72-1.46, P<0.001) and GGT (52.4-164.2, P<0.001). in contrast, the groups treated with *B. alba* extract at dose of (100-400 mg/kg) once daily for 28 days prevented the incidence of cancer of in a dose related manner. The ranges of protection in the serum marker were found to be SGOT (276.34-222.81, P<0.05 to p<0.001), SGPT (266.38-129.06, P<0.05 to p<0.001), SALP (374.24-269.96, P<0.01 to p<0.001), BL (0.111-0.102, p<0.001), and GGT (168.8-98.1, P<0.001) respectively. The protection of cisplatin ranged for SGOT (374.21-318.32, p<0.001), SGPT (391.53-112.34, p<0.001), SALP (459.31-262.26, p<0.001), BL (1.46-0.98, p<0.01) and GGT (164.2-72.4, p<0.001), respectively, as shown in Table 3.

Groups	Treatment	Dose	SGOT	SGPT	SALP	BL	GGT
I	Control	--	212.20±1.64	103.21±1.60	254.14±10.34	0.72±0.02	52.4±5.4
II	AFb1	200mg/kg AFb1	374.21±36.31z	391.53±42.72z	459.31±28.31z	1.46±0.08z	164.2±12.8z
III	*Basellaalba*	100mg/kg	276.34±22.52a	266.38±34.29a	374.24±24.34a	0.111±0.07b	168.8±10.8
IV	*Basellaalba*	200mg/kg	238.51±20.39b	201.14±36.29b	319.28±21.54b	0.107±0.06b	141.4±9.6
V	*Basellaalba*	400mg/kg	222.81±15.71b	129.06±21.34c	269.96±18.28c	0.102±0.04c	98.1±7.7c
VI	Cisplatin	6mg/kg	318.32±10.78b	112.34±23.48c	262.26±18.72c	0.98±0.03c	72.4±8.1c
Values are mean ± S.E.M. of 6 rats in each group P-values: z<0.001compared with respective control group P-values: a<0.05, b<0.01, c<0.001compared with group II (AFb1)							

Table 3: Effect of the Ethyl Acetate Extract of *B. albaon* SGOT(U/l), SGPT(U/l), SALP(U/l), Bilirubin Level (U/l) and Gamma Glutamyl Transpeptidase, GGT (U/l) in Serum of Rat

3.5. Effect of Ethyl Acetate Extract of *B. Alba* on LPO, SOD, CAT, GPX, GST and GSH Against AFb1-Induced HCC

Administration of AFb1 led to increase in the levels of LPO (0.66-4.84, p<0.001), and decrease in SOD (124.4-68.20, p<0.001), CAT (48.8-20.24, p<0.001), GPX (3.74-1.62, p<0.001), GST (1.26-0.67, p<0.001) and GSH (0.56-0.24, P<0.001) levels in the 5% w/v liver homogenate. Treatment of rats with 50% ethanolic extract of *B. albaat* dose of (100-400 mg/kg b.w) markedly prevented the AFb1 induced alterations of various parameters LPO (3.82-1.22, p<0.05 to p<0.01), SOD (98.12-118.52, p<0.05 to p<0.001), CAT (32.19-41.39, p<0.05 to p<0.001), GPX (1.70-3.44, p<0.001), GST (0.80-0.114, p<0.001) and GSH (0.30-0.77, p<0.001) respectively.

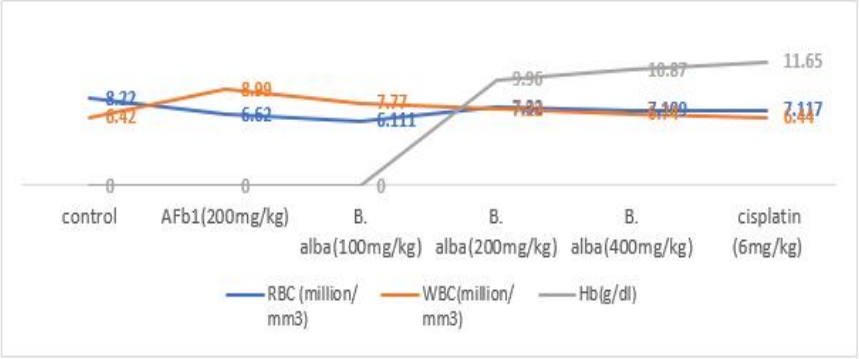
The protection of Cisplatin ranged for LPO (4.84-0.108, p<0.05 to p<0.01), SOD (68.20-122.31, p<0.05 to p<0.001), CAT (20.24-44.09, p<0.05 to p<0.001), GPX (1.62-3.62, p<0.001), GST (0.67-0.117 p<0.001) and GSH (0.24-0.52, p<0.001) respectively as shown in Table 4.

Groups	Treatment	Dose	SOD	CAT	LPO	GPX	GST	GSH
I	Control	---	124.4±9.1	48.8±1.2	0.66±0.04	3.74±0.04	1.26±0.12	0.56±0.02
II	AFb1	200 mg/kg AFb1	68.20±9.14z	20.24±2.2z	4.84±1.21z	1.62±0.02z	0.67±0.02z	0.24±0.01z
III	*Basellaalba*	100mg/kg	98.12±5.31a	32.19±1.2a	3.82±1.14	1.70±0.02a	0.80±0.04a	0.30±0.02a
IV	*Basellaalba*	200mg/kg	111.21±6.26b	26.19±1.30b	2.36±0.98	2.114±0.03c	0.102±0.05c	0.38±0.03b
V	*Basellaalba*	400mg/kg	118.52±4.21c	41.39±0.93c	1.22±0.36a	3.44±0.02c	0.114±0.08c	0.77±0.04c
VI	Cisplatin	6mg/kg	122.31±5.24c	44.09±2.12c	0.108±0.22b	3.62±0.02c	0.117±0.09c	0.52±0.05c
Values are mean ±S.E.M. of 6 rats in each group P values: z<0.001compared with respective control group P values: a<0.05, b<0.01, c<0.001 compared with group II (AFb1)								

Table 4: Effect of Ethyl Acetate Extract of *B. alba* on LPO, SOD, CAT, GPX, GST and GSH against AFb1 Induced HCC

3.6. Effect of Ethyl Acetate Extract of *B. alba* on Haematological Parameters (RBC, WBC and Hb) of Control and AFb1 Induced HCC

The Figure 3 shows the level of Hb, RBC counts, all of which were significantly decreased (8.22-6.62, p<0.001) and (11.107-8.72, p<0.05) and with a simultaneous increase in WBC (6.42-8.99, p<0.01) with respect to control. In contrast, the groups treated with *B. alba* extract at a dose of (100-400 mg/kg b.w) once daily for 28 days prevented the cancer in a dose related manner. The range of protection in the Hb, RBC and WBC show (8.111-10.87, p<0.05), (6.111-7.109) and (7.77-6.74, p<0.01). The protection of Cisplatin ranged for Hb (8.72-11.65, p<0.01), RBC (6.62-7.117, p<0.05) and WBC (8.99-6.44, P<0.01) respectively.



Values are mean ± of 6 rats in each group

Figure 3: Effect of Ethyl Acetate Extract of *B. alba* on Haematological Parameters (RBC, WBC and Hb) of Control and AFb1 Induced HCC

3.7. Effect of Ethyl Acetate Extract of B. Alba on the Level of Deoxyribonucleic Acid, DNA (mg/g Wet Tissue) and Ribonucleic Acid, RNA (mg/g Wet Tissue) and Protein (g/dl):

When AFb_1 was administered, DNA and RNA levels significantly increased and protein content significantly decreased in comparison to the control group ($P < 0.001$), suggesting increased nucleic acid production and protein depletion. These changes were significantly and dose-dependently altered by treatment with Basella alba ethyl acetate extract at dosages of 100, 200, and 400 mg/kg. When compared to the AFb_1-treated group, the extract decreased the high amounts of DNA and RNA and brought the protein content back to normal. This effect was most noticeable at 400 mg/kg ($P < 0.001$) as shown in Table 5. B. alba preventive effect was similar to that of the common medication cisplatin, indicating that it may play a part in controlling protein synthesis and nucleic acid metabolism during carcinogenic stress.

Groups	Treatment	Dose	DNA	RNA	Protein
I	Control	---	5.61± 0.44	7.72± 0.24	8.24± 1.03
II	AFb1	200mg/kg (AFb1)	7.78± 0.24z	9.102± 0.46z	6.47± 0.72x
III	*Basella alba*	100mg/kg	6.107± 0.28	8.34± 0.38a	6.93± 0.95
IV	*Basella alba*	200mg/kg	5.88± 0.32c	7.108± 0.42b	7.65± 1.02
V	*Basella alba*	400mg/kg	5.72± 0.26c	7.82± 0.30b	7.107± 1.23
VI	Cisplatin	6mg/kg	5.68± 0.22c	7.78± 0.28b	7.75± 1.44
Values are mean ± S.E.M. of 6 rats in each group P values: x < 0.05, z < 0.001 compared with respective control group P values: a < 0.05, b < 0.01, c < 0.001 compared with group II (AFb1)					

Table 5: Effect of Ethyl Acetate Extract of Basella Alba on the Level of Deoxyribonucleic acid, DNA (mg/g Wet Tissue) and Ribonucleic Acid, RNA (mg/g Wet Tissue) and Protein (g/dl)

3.8. Histopathological Analysis

The control group's liver tissues showed normal hepatic architecture with intact hepatocytes and neatly arranged hepatic cords, according to histopathological analysis. The AFb1-treated group, on the other hand, displayed substantial liver damage marked by dysplastic alterations suggestive of hepatocellular carcinoma, disturbed architecture, cellular degeneration, necrosis, and inflammatory infiltration. The ethyl acetate fraction of *B. alba*treatment significantly improved liver histology, demonstrating hepatic structural restoration, decreased inflammation and necrosis, and a notable reduction in neoplastic changes. These results validate *B. alba* hepato protective and anticancer properties against AFb1-induced liver carcinogenesis.

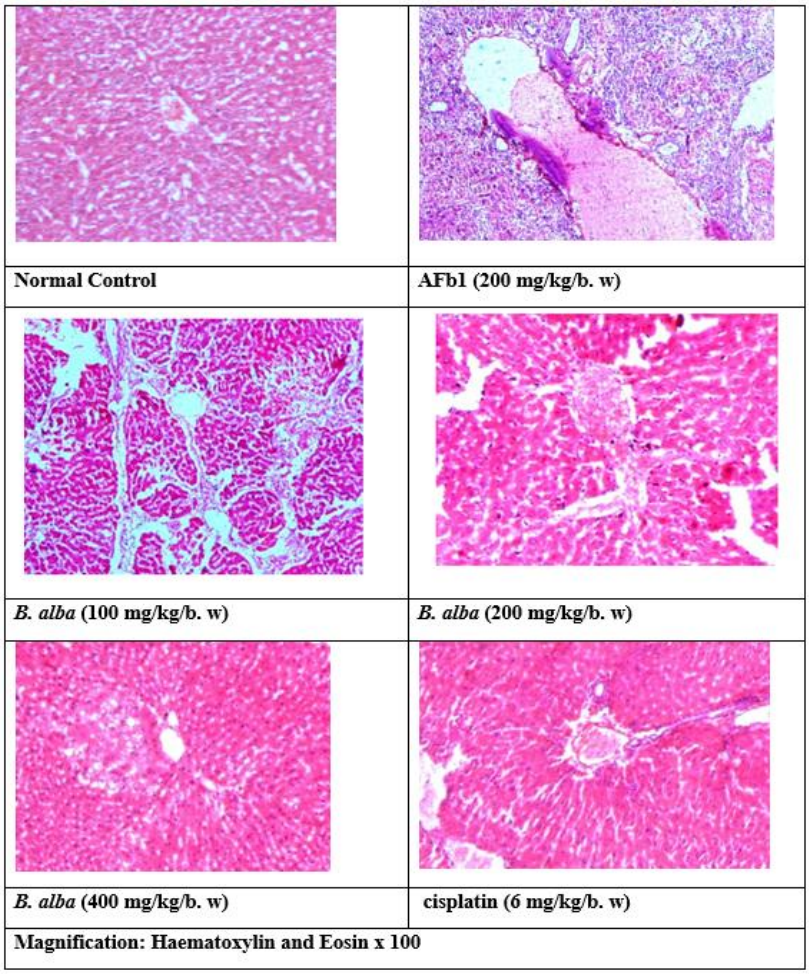


Figure 4: Effects of Ethyl Acetate Fraction of *B. alba* on AFb1 Induced Hepatocellular Carcinoma

4. Discussion

In an additional study, B. albaanti-inflammatory properties in vitro using a human blood cell membrane stabilization technique. They saw found, when compared to the common medication diclofenac, both the aqueous and methanol extracts of B. albaleaves had notable membrane stabilizing properties [28]. The antioxidant properties of polyphenols, which are mediated by hydroxyl groups that scavenge free radicals and/or chelate metal ions, account for the majority of the possible health benefits of polyphenols reported by HPLC analysis. A naturally occurring phenolic molecule, chlorogenic acid can treat or prevent a number of pathological disorders, such as inflammation and oxidative stress [29]. AFb1 is a strong hepatocarcinogen that causes DNA adducts and mutations to initiate HCC [30]. B. albahas been shown to have hepatoprotective properties in animal models of chemically induced liver injury [31]. In experimental rats, aqueous B. alba leaf extracts have shown demonstrated protective properties against liver damage [32].

5. Conclusion

The combined in-vitro and in-vivo results show that the ethyl acetate fraction of B. albahas strong anticancer and hepatoprotective properties against hepatocellular carcinoma caused by AFb_1. In a dose-dependent manner, the fraction efficiently decreased the viability of liver cancer cells, prevented their growth, and triggered apoptosis, most likely by modifying oxidative stress and apoptotic pathways. By stabilizing liver enzymes, lowering oxidative stress, boosting antioxidant defenses, and improving hepatic histoarchitecture, it significantly reduced AFb_1-induced hepatic damage in vivo. These findings point to B. albaas a promising plant-based option for managing and preventing aflatoxin-associated liver cancer, which calls for additional mechanistic and clinical research.

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