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Modulating Role of Ipomoea batatas (L.) Lam. Leaves on Dissemination of Hepatocellular Carcinoma Induced in Rats: Protective and Therapeutic Study

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Abstract

The alternative and complementary application of natural compounds has generated optimism regarding the discovery of curative and therapeutic strategies for hepatocarcinogenesis. This study examined the protective and therapeutic efficacy of *Ipomoea batatas* (L.) Lam. leaves extract (IbLE) extract in countering hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DEN) at a dosage of 50 mg/kg and carbon tetrachloride (CCl_4) at 2 mg/kg in male rats, in comparison with the standard chemotherapeutic agent, Doxorubicin (DOXol) at 10 mg/kg. A total of forty eight rats were utilized and divided randomly into six groups of eight rats each. The extract was administered at a dosage based on its LD50. The results indicated that intraperitoneal (i.p.) injection of DEN/CCl_4 led to oxidative stress, as evidenced by an increase in malondialdehyde (MDA) levels, significant decrease in glutathione (GSH) content, detrimental effects on total antioxidant capacity (TAC) and induction of inflammation as evidenced by elevated levels of pro-inflammatory cytokines such as IL-6, BCl2, and TNF-α, along with increased serum levels of α-fetoprotein and α-fucosidase in hepatocellular carcinoma (HCC) induced rats .These alterations were accompanied by significant elevations in hepatic function biomarkers; transaminases (AST and ALT), alkaline phosphatase (ALP), and γ-Glutamyl transferase (γ-GT). Additionally, levels of blood urea nitrogen (BUN) and creatinine were found to be increased in HCC induced rats. Our findings demonstrated that both the prevention and treatment of HCC in rats using IbLE mitigated the rise in oxidative stress biomarkers, inflammation, and liver enzyme levels. Histopathological analysis documented the biochemical findings, revealing mild vacuolar degeneration of hepatocytes, mild portal fibrosis, and slight aggregation of neoplastic cells in the liver of HCC-induced rats treated with the extract. An immunohistochemical assessment of the neoplastic liver tissue from treated rats showed moderate positive expression of proliferating cell nuclear antigen (PCNA), whereas protective rats exhibited weak positive expression of PCNA. Quantification of the total phenolics and flavonoids content were determined. It could be concluded that *I. batatas* may serve as a promising candidate for the prevention and treatment of neoplastic liver status and its polyphenolic content may be accountable for its activity.

Keywords: *Ipomoea batatas*, Hepatocellular carcinoma, Diethylnitrosamine, Carbon tetrachloride, Oxidative stress, inflammatory cytokines.

1. Introduction

The predominant form of liver cancer globally is hepatocellular carcinoma (HCC), which ranks as the second leading cause of cancer-related fatalities in Egypt [1]. HCC is identified as the second most prevalent malignancy among males and the fifth among females [2]. Chronic liver diseases, particularly cirrhosis, are recognized as significant contributing factors to the onset of HCC [1]. Additionally, the presence of carcinogenic substances such as Aflatoxin and N-nitrosamines in contaminated food has been identified as a risk factor for HCC [3]. In the early stages of liver cancer, partial hepatectomy is the primary therapeutic approach. While this surgical intervention has demonstrated effectiveness and potential for cure, the rates of liver cancer recurrence post-surgery remain elevated. Consequently, enhancing survival outcomes will necessitate the development of more effective therapeutic agents to improve resection results [4, 5].

Among the most potent hepatocarcinogens in animal studies are N-nitrosamine compounds, particularly diethyl nitrosamine (DEN) [6]. Diethyl nitrosamine is frequently employed to initiate HCC, while carbon tetrachloride (CCl_4) is utilized to amplify the carcinogenic process [7]. Oxidative stress results from the generation of reactive oxygen species (ROS), which may contribute to the development of hepatocellular carcinoma induced by DEN [8]. The progression of HCC

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is associated with the formation of alkyl DNA-DEN adducts and the induction of various nuclear abnormalities in the liver of rats. Drug delivery embolization system known as drug-eluting beads has been incorporated into transarterial chemoembolization (TACE). This method, referred to as drug-eluting bead trans-arterial chemoembolization (DEB-TACE), utilizes microspheres as embolic agents that are infused with chemotherapeutic agents, typically doxorubicin, which are gradually released into the targeted tumor [9,10] .The application of drug-eluting beads containing Doxorubicin has demonstrated a reduction in systemic doxorubicin levels while enhancing tumor concentration compared to traditional lipiodol-based TACE approaches [11,12], thereby significantly minimizing systemic drug-related side effects [13] .

On the other side, several bioactive substances, such as flavonoids and particular phenolic compounds included in their structure, are responsible for the antioxidant qualities of *Ipomoea batatas* (sweet potato). Antioxidant properties have been favorably connected with the presence of total phenolics, hydroxycinnamic acid derivatives, and total anthocyanins, as well as a mixture of anthocyanins and phenolic acids, amino acids, and caffeoyl compounds. Numerous studies indicate that the detrimental consequences of free radical reactions are associated to degenerative diseases such cancer, diabetes, asthma, cardiovascular problems, inflammation, senile dementia, and ocular disorders [14]. Studies show that *Ipomoea batatas* (Family Convolvulaceae) leaves are more efficient at scavenging free radicals than the *Ipomoea batatas* (L.) Lam. extract's stalk, stem, skin, and flesh [15]. A notable positive correlation has been observed between the radical scavenging activity and the polyphenol content found in sweet potato (*Ipomoea batatas* (L.) Lam) leaves. These results indicate that sweet potato leaves represent a significant supplementary source of antioxidants. It is increasingly acknowledged that various dietary inhibitors counteract mutagens and carcinogens, thereby playing an essential role in reducing the risks associated with mutagenesis and carcinogenesis, while also offering hepatoprotective benefits, including those derived from sweet potato [16]. Numerous studies have indicated that anthocyanins extracted from purple sweet potatoes (PSP), possess hepatoprotective properties in healthy individuals with borderline hepatitis as well as in rats subjected to carbon tetrachloride exposure. Furthermore, purple sweet potato color (PSPC) has been demonstrated to protect mouse liver from damage induced by D-galactosamine by decreasing lipid peroxidation, boosting antioxidant enzyme activities, and inhibiting inflammatory responses [17] .The antioxidant capabilities of sweet potato *Ipomoea batatas* (L.) Lam. may be attributed to the presence of beta carotene, anthocyanins, caffeoyldaucic acid, and caffeoylquinic acid derivatives [18]. Anthocyanins have been recorded to exhibit several beneficial therapeutic effects, including radiation protection, vasotonic and vasoprotective properties, anti-inflammatory effects and chemoprotective actions against platinum toxicity in cancer therapy as well as hepatoprotective effects against carbon tetrachloride damage. Additionally, they may confer other advantageous effects through their interactions with various enzymes and metabolic processes [18].

Sweet potato leaves have been recognized as one of the richest and most abundant sources of anthocyanins among a variety of fruits and vegetables [19, 20]. Researchers have identified and characterized a total of fifteen distinct anthocyanin compounds within these leaves. The primary anthocyanins found are acylated cyanidin and peonidin, with cyanidin being present in significantly higher concentrations than peonidin. This suggests that the anthocyanin profile of sweet potato leaves is predominantly composed of cyanidin [19, 20]. Cyanidin-type anthocyanins are believed to exhibit superior antimutagenic properties in comparison to those of peonidin-type anthocyanins [21, 22] and demonstrate enhanced antioxidative effects. The anthocyanins extracted from sweet potatoes are acknowledged as widely occurring bioactive compounds, known for their potential antioxidant properties, which may provide cardioprotective, antidiabetic, and anticancer effects [16]. These advantageous effects may be influenced by the quantity of hydroxyl groups in their molecular structure. Specifically, cyanidin, which contains two hydroxyl groups, shows greater antimutagenic activity than peonidin, which has only one. Additional studies on enzyme activity have suggested that anthocyanins may protect against mutagenesis primarily through direct interactions with enzymatically activated carcinogens, such as heterocyclic amines, rather than through interactions with metabolic enzymes [23-28]. In human aortic endothelial cells, extracts from purple sweet potatoes (PSP) leaves significantly inhibited TNF-α-induced adhesion of monocytes to endothelial cells and reduced the expression of VCAM-1, IL-8, and CD40. The anti-inflammatory properties of PSP leaves extract are mediated through the modulation of NFκB and MAPK signaling pathways. Additionally, extracts from PSP leaves have been shown to down-regulate the expression of pro-inflammatory molecules such as TNF-α and IL-6 in adipocytes The anti-inflammatory activity of Ipomotaosides A-D, resin glycosides isolated from the dried aerial parts of IbLE, has been found to inhibit COX-1 and COX-2 [29] . The current study aims to assess the hepatoprotective and therapeutic effects of *I. batatas* (IbLE) in the context of liver carcinoma induced by DEN in rats through the determination of various biomarkers, including liver function enzymes, pro-inflammatory cytokines; IL-6, Bcl-2, and TNF-α, which were quantified using the ELISA method. The study was also focus on the measurement of tumor markers, including serum γ-Glutamyl transferase (γ-GT), alpha-fetoprotein (AFP) and α-L-fucosidase (AFU). Besides, non-enzymatic antioxidant; glutathione (GSH), lipid peroxide level (MDA), and total antioxidant capacity (TAC).

2 Methods

2.1 Plant material

In April 2022, leaves *Ipomoea batatas L. Lam.* (Family Convolvulaceae) are harvested from El Qanater El Khayreya in the El Qalyubiya Governorate, Egypt, while they are in the flowering stage. Mrs. Threase Labib, the Ministry of

Agriculture's plant taxonomy consultant, identified the plant materials. The NRC's Department of Chemistry of Natural Compounds housed the voucher specimens number IbL-22.

2.2 Apparatus

Automated UV-VIS spectrophotometer (Corporation Model V-730, JASCO, S.N. A112961798, Tokyo, Japan) for the determination of the polyphenols (total phenolics and total flavonoids content). Rotary vacuum evaporator (Laborota- 4011, Heidolph Co., Germany) was used for drying of the extract at 40 °C in reduced pressure. Continuous extraction apparatus was used for extraction of plant materials using aqueous alcohol.

2.3 Chemicals and drugs

Diethyl nitrosamine (DEN) (CAS no. 55-18-5) and carbon tetrachloride (CCl_4) (CAS no. 56-23-5) were purchased from Sigma-Aldrich Chemicals Co (St Louis, MO, USA).Kits used for the quantitative determinations of different parameters were purchased from biodiagnostic Co. (Biogamma, Stanbio, West Germany).Reagents for ELIZA kits were obtained from Cloud – Clone Corp Co. (USA). Doxorubicin (DOXol), was supplied as vials (Oncotec Pharma Production GmbH Germany).The contents of vial were dissolved in saline. Quantitative colorimetric commercial kits (Biodiagnostic, ARE) were used as the biochemical markers such as serum ALT, AST, and ALP, BUN and creatinine. Aspectrum colorimetric kit for measuring blood γGT, total antioxidant capacity (TAC), lipid peroxide (MDA) and Glutathione (GSH). Alpha-fetoprotein (AFP) and α-L-fucosidase (AFU) as tumor markers, proinflammatory cytokines; TNF-α, BCl2, and IL6 were measured using ELISA Biocheck kits (USA) according to manufacturing instructions.

2.4. Preparation of *Ipomoea batatas* leaves extract (IbLE)

The total aqueous alcoholic extract of I. batatas leaves extract IbLE was prepared from 600 g of air-dried, finely powdered leaves by repeatedly extracting in a continuous extraction apparatus with ethanol (70%) until exhaustion [28]. The apparatus of rotatory evaporator was used to evaporate the mixture at a lower temperature and pressure. The extract was concentrating in vacuo at 50ºC The percentage yield was 7.44% of crude extract IbLE as a dark brown sticky residue of a stringent taste.

2.5. Determination of total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity.

The sample (0.1 mL), distilled water (2.8 mL), and Folin-Ciocalteau reagent (0.1 mL) were combined and vortexed. The mixture was then stirred with a 2 mL 7.5% (w/w) sodium carbonate solution, and it was then incubated for 60 minutes at 25°C in the dark. Finally, absorbance was measured at 750 nm using a spectrophotometer (Jenway 6305, England). Gallic acid (GA) standard curve was established, and TPC was expressed for samples as milligrams of GA equivalents per gram (mg GAE/g) [24].

2.6 Acute toxicity study

To assess the acute toxicity of I. batatas leaves extract, serial concentrations ranging from five hundred to three thousand mg/kg body weight were administered to four rats per group, totaling sixteen rats across all groups [30].

2.7 Animals

Forty-eight adults' male Wistar albino rats, weighing 120–150 grams, were obtained from the National Research Centre's animal facility in Dokki, Giza, Egypt, and housed in polypropylene cages in a clean air room with controlled environmental conditions. Throughout the experiment, the rats were given unrestricted access to a standard diet and water, which was provided by the National Research Centre and included 72.2% carbohydrates, 3.4% fats, 19.8% proteins, 3.65% cellulose, 0.5% vitamins, minerals, and 0.5% salts. The rats were given a two-week acclimatization period before the experimental protocol began, which lasted for six months. All animal treatments and experimental procedures were ethically approved by the National Research Center's Ethics Committee under approval number 04431223.

2.8 Induction of hepatocellular carcinoma

DEN was dissolved in corn oil and administered via intraperitoneal injection (i.p.) at a single dosage of 50 mg/kg of body weight [31]. Subsequently, two weeks later, the rats received a single i.p. of CCl4 at a dosage of 2 mL/kg to promote carcinogenesis induced by DEN [32].

2.9 Experimental design

Following a week of acclimatization, the rats were categorized into six distinct groups, each consisting of eight rats:

- Group 1: served as control rats. Group 2: normal healthy rats administered I. batatas extract orally at a dosage corresponding to the LD50 for one month (500 mg/kg body weight). Group 3: was designated for HCC induction; rats received a single i.p. of DEN (50 mg/kg) and, after a two-week period, were given a single oral dose of CCl4 (2 mL/kg) /kg) diluted 1:1 in corn oil [32] .They were monitored until the 24-week experiment, at which point all animals were euthanized if they exhibited inadequate physical condition or at the end of the study period. Group 4: protective group; received DEN and CCl4 injection as previously described, along with oral co-administration of I. batatas IbLE extract for six months (500 mg/kg body weight). Group 5: treated –HCC induced rats; where rats were given I. batatas IbLE extract orally (500 mg/kg body weight) for one month following the six-month period of DEN. Group 6: treated HCC-rats with Doxorubicin (DOXol) at a dosage of 10 mg/kg [33].

2.10 Biochemical Examination

Blood samples were taken from the retro-orbital plexus twenty-four hours after the experiment ended, and they were centrifuged for 15 minutes at 3000 rpm. Rats from different groups were subjected to cervical decapitation and livers were then removed.

Using quantitative colorimetric commercial kits (Biodiagnostic, ARE), the biochemical markers for early hepatic damage were identified as serum ALT, AST [34], and ALP [35]. Kidney function parameters BUN and creatinine were determined in accordance with Henry [36].Whereas the Egyptian Company for Biotechnology provided a spectrum kit for measuring blood γGT and liver cytosolic enzyme activity using Szasz's approach [37].ELISA was used to measure proinflammatory cytokines, including TNF-α, BCl2, and IL6. Reduced glutathione (GSH), a non-enzymatic antioxidant, was measured in liver tissue homogenate using quantitative colorimetric kits (Biodiagnostic, ARE) in accordance with Beutler et al. [38]. Ohkawa [39] method was used to measured lipid peroxidation (MDA) in liver tissue homogenate, and Rubio et al. [40] used their approach to measure total antioxidant capacity (TAC) in blood. Alpha-fetoprotein (AFP) and α-L-Fucosidase (AFU) tumor markers, were measured in serum using ELISA Biocheck kits (USA) [41, 42].

2.11 Liver tissue homogenate

Hepatic tissue was homogenized in a physiological saline solution (0.9% NaCl) at a ratio of 1:9 (w/v). The resulting homogenate was then centrifuged at 4 °C for 5 minutes at a speed of 3000 rpm. The supernatant obtained was utilized for the assessment of antioxidant parameters; MDA and GSH, while TAC was determined in serum.

2.12 Histopathological examination

Hepatic lobe specimens were preserved in a 10% formalin solution for the histological analysis of neoplastic nodules. Following preservation, the specimens were washed, dehydrated, cleared, and subsequently embedded in paraffin. They were then sectioned to a thickness of 5 microns and stained with Hematoxylin and Eosin for histopathological examination [43].

2.13 Histopathological lesion scoring

Histopathological alterations in the liver was evaluated and assigned scores. The grading system categorized changes as none (0), mild (1), moderate (2), and severe (3), based on the percentage of changes observed: changes less than 30% were classified as mild, changes between 30% and 50% as moderate, and changes exceeding 50% as severe [44].

2.14 Immunohistochemistry

The immunohistochemical study was carried out following the guidelines of Saleh et al. [45]. Sections of brain tissue were deparaffinized with xylene and then rehydrated with a succession of progressively stronger alcohol solutions. Thermo Scientific's Hydrogen Peroxide Block (USA) was used to suppress the body's natural peroxidase activity. The tissue sections were pretreated with 10 mM citrate in a microwave oven for 10 minutes in order to induce antigen retrieval. After that, the sections were incubated for two hours with either rabbit monoclonal proliferating cell nuclear antigen (PCNA) at a dilution of 1:4000 (13110) or rabbit polyclonal antibody targeting Ki-67 protein at a dilution of 1:100 (abx013129; Abbexa, Cambridge, UK). Following a PBS rinse, the sections were incubated for 10 minutes with goat anti-rat IgG H & L (HRP) (ab205718; Abcam, Cambridge, UK) before being rinsed with PBS once more. Incubation with 3, 3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) was the last step. After that, the slides were mounted and counterstained with hematoxylin. PBS was used in substitution of primary antibodies as negative controls.

2.15 Phytochemistry study

Qualitative phytochemical screening for major secondary metabolites

Qualitative phytochemical screening was carried out for the plant material of (*Ipomoea batatas*; sweet potato) IbLE for major secondary metabolites following standard procedures [46].

2.16.1 Total polyphenols content determination

The amount of the total polyphenols (TPC), expressed as gram sample per mg of gallic acid (GA). TPC of the sample was determined colorimetrically using the reagent of Folin-Ciocalteau according to the method described by Mythili [47]. The sample (0.1 g) was dissolved in methanol 98% (20 mL). The filter paper (Whatman No. 1, Grade 589/2) was used for the filtration of methanol extract. One ml of extract sample was mixed (at a rate of 1:10) with Folin-Ciocalteau reagent (1 mL) with distilled water for 3 min. Sodium carbonate (2%, 1 M, 3 mL) was added. For 15 min and at room temperature, the mixture was left. By an automated UV-VIS spectrophotometer (JASCO, Corporation Model V-730, S.N. A112961798, Tokyo, Japan), the polyphenols were determined at 765 nm. The results were calculated using a gallic acid calibration curve (0–100 mg/l). Using the same procedure (with 20µl of pure water), the blank was prepared in place of the extract. The result is expressed as equivalents to gallic acid (mg GAE/ 0.1 g sample). Using the standard linear equation (Y=1.0752X+0.0002; R2= 0.9999), the equivalent gallic acid content in the test sample was determined.

2.16.2 Determination of total flavonoids content (TFC)

According to the method described by Motevalli-Haghi et al. [48], TFC of the sample was determined. The sample (0.1 g) was dissolved methanol 98% (20 mL). The filter paper (Whatman No. 1, Grade 589/2) was used for the filtration of methanol extract. The sample (1 mL) was mixed with methanol (1.5 mL), 10% aluminium chloride (0.1mL), 1 M potassium acetate (0.1mL) and distilled water (2.8mL). It is left for 10min (at room temperature). On a UV/visible spectrophotometer

(JASCO, Corporation Model V-730, S.N. A112961798, Tokyo, Japan) and at 415nm, the absorbance of the mixture was measured. As a standard for the calibration curve, the quercetin (µg/mL) was used. Using the standard linear equation (A=0.022X+0.006; R2= 0.999), the equivalent quercetin content in the test sample was determined.

2.17 Statistical analysis

Using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA, software version 8), the results of the biochemical analysis were assessed using one-way analysis of variance (ANOVA) and post hoc comparisons using the Least Significant Difference (LSD) method, in conjunction with the Co-State computer program. Differences were deemed significant at $P \leq 0.05$, and quantitative values were presented as mean ± standard deviation (SD).

The percentage of change compared to control group is calculated as follows: (mean of test − mean of control/mean of control) × 100.

Percentage of improvement: (mean of diseased group − mean of treated group/mean of control) × 100

3. Results

3.1 Acute toxicity study

Acute toxicity was assessed using serial concentrations of IbLE up to 3000 mg/kg body weight, with no indications of mortality or toxicity observed over a 48-hour period, and no signs of behavioral or clinical issues were noted.

3.2 Qualitative phytochemical screening for major secondary metabolites

The plant materials of IbLE were subjected to qualitative phytochemical screening for major secondary metabolites, including flavonoids, coumarins, alkaloids, anthraquinones, triterpenes/steroids, saponins and tannins, following standard procedures [46] .Varied analytical responses such as formation of precipitate or intensity of a color were used. Positive results were obtained for all the major secondary metabolites except the triterpenes/steroids. Flavonoids were the major detected class of compounds and trace of coumarins was detected.

3.3 Determination of total phenols and flavonoids content

TPC of the sample (mg GAE /0.1 g sample) was 76.4508±6.2 and the TFC (µg QE/ 0.1g sample) was 4571.0358±27.5 (Table 1).

3.4 Liver function enzyme activities

The oral administration of IbLE to normal rats resulted in negligible alterations in the activities of AST, ALT, ALP, and GGT enzymes when compared to control group. In contrast, rats subjected to DEN/CCl_4 exhibited significant increase in the activities of these enzymes relative to the control group ($P \leq 0.05$), with percentage increases of 121.368%, 143.130%, 422%, and 269.714%, respectively (Table 2). The co-administration IbLE of alongside DEN in the protective group led to improvements in AST, ALT, ALP, and GGT activities, with amelioration percentages 69.74%, 39.13%, 283%, and 91.5%, respectively. In the therapeutic group, the percentages of improvement were even higher, reaching 78.95%, 63.04%, 297.92%, and 86.53% when compared to DOXol (69.74%, 67.4%, 339.5%, and 72.34%, respectively.

3.5 Kidney function

The oral administration of IbLE to normal rats resulted in insignificant alterations in BUN and creatinine levels when compared to control rats. While, rats injected with DEN/CCl_4 showed significant increase in BUN and creatinine levels compared to control group, with percentage increases of 320.35% and 875.57%, respectively (Table 3). The administration of IbLE in the protective group led to improvements in BUN and creatinine levels with percentages 134.80% and 431.03%, respectively. However, the improvement percentages of BUN and creatinine in the therapeutic HCC rats recorded 103.36% and 315.17% respectively, in comparison with the values of 110.62% and 379.31%, in the DOXol-treated group.

3.6 Hepatocellular carcinoma biomarkers

The oral administration of IbLE to normal rats resulted in insignificant change in AFP and AFU levels when compared to control group. While, HCC –induced rats declared a significant rise in AFP and AFU levels as compared to control (374.766% and 537.914%, respectively). In the protective group receiving I. batatas IbLE; there was a significant amelioration in AFP and AFU levels with percentages 195.33% and 194.64%, respectively. In the treated group, the percentages were even higher (228.82% and 294.35%, respectively), when compared with DOXol treated HCC rats (260.23% and 363.21%, respectively) (Table 4).

3.7 Pro-inflammatory cytokines in the different experimental groups

The oral administration of IbLE to normal rats resulted in undetectable significant changes in IL-6, Bcl-2 and TNF-α levels when compared with control rats. DEN/CCl4 intoxicated rats demonstrated a significant elevation in IL-6, Bcl-2 and TNF-α levels compared with control rats ($P \leq 0.05$), with percentage increases 292.383%, 319.837% and 230.575%, respectively. The administration of IbLE in both protective and therapeutic groups led to improvements in IL-6, Bcl-2 and TNF-α levels, with amelioration percentages reaching to 210.68%, 149.60% and 67.42% for the protective group, and 220.34%, 170.82% and127.04%, for the therapeutic group, respectively. In HCC rats treated with DOXol, the recorded improvement percentages were 232.74%, 176.92% and 131.06%, respectively (Table 5).

3.8 Oxidative stress biomarker

The oral administration of IbLE to normal rats resulted in no significant alteration in MDA levels when compared with control rats. HCC-induced rats showed significant increase in MDA levels relative to control rats (P≤0.05), with a percentage increase 1180.956%. The administration of IbLE in both protective and therapeutic groups recorded improvements in MDA levels, with percentages 724.08% and 609.78%, respectively, comparing with the standard drug DOXol(768.56%). Regarding to TAC and GSH levels, the oral administration of IbLE to normal rats (G2) resulted in a significant increase in both TAC and GSH levels when compared with control group (G1). While, DEN/CCl4 injected rats demonstrated a notable decrease in TAC and GSH levels with percentage reductions -26.871% and -1607.343%, respectively compared to control. Co- administration of IbLE in the protective group with DEN/CCl_4 led to improvements in TAC and GSH levels, with percentages 40.80% and 41.51%, respectively. While in the therapeutic HCC rats, the percentages reached to 51.32% and 56.23%, respectively, in comparison with DOXol(52.63% and 36.36%, respectively) (Table 6).

3.9 Histopathological examination

Histopathological examination (Fig.1) revealed an insignificant change in liver tissue of control and normal rats supplemented with the extract of IbLE (Fig.1,Photomicrographs 1,2) .While, livers of carcinogenic rats exhibited significant vacuolar degeneration of hepatocytes, karyomegaly, sinusoidal dilation, and the presence of newly formed bile ductules (Fig.1,Photomicrographs 3-6) . In contrast, livers of carcinogenic rats that protected with IbLE displayed only mild portal fibrosis accompanied by mononuclear infiltration, slight vacuolar degeneration of hepatocytes, and localized areas of neoplastic cells (Fig.1, Photomicrographs 7-9). Furthermore, livers of carcinogenic rats treated with IbLE showed clusters of neoplastic cells, vacuolar degeneration of hepatocytes, and portal fibrosis (Fig.1, Photomicrographs 10-12). Additionally, the livers of carcinogenic rats treated with a standard drug exhibited normal hepatocyte morphology along with mild portal fibrosis (Fig.1, Photomicrograph 13) (Table 7).

3.10 Immunohistochemical analysis

Immunohistochemical analysis (Fig.2) indicated that no marked alteration in immune response between control and normal rats administered IbLE (Fig.2, photographs 14, 15).While, carcinogenic rats exhibited a strong positive immune response for PCNA (Fig.2, photographs 16, 17). In contrast, carcinogenic rats that were protected with IbLE displayed a weak immune response for PCNA (Fig.2, photomicrograph 18). Carcinogenic rats treated with IbLE demonstrated a moderate immune response for PCNA (photomicrograph 19), whereas the standard drug resulted in a weak immune response (Fig.2, photomicrograph 20).

Table 1: Total phenols and flavonoids content of

***I. batatas* (IbLE)**	
Total phenols (mg GAE /0.1 g sample)	**Total flavonoids** (µg QE/ 0.1g sample)
76.4508±6.2	4571.0358±27.5

The mean± SD is considered average of 3 samples.

Table 2: Liver function enzymes in HCC- induced rats and different groups

Groups Parameters	G1	G2	G3	G4	G5
AST (U/l)	76.00±4.00a	79.00±5.00a	150.00±6.00b	97.00±4. 22c d	90.00±3. 10c
Percentage change	-	-27.947	-121.368	-63.24	-42.42 %
Percentage improvement	-	-	-	69.74%	78.95%
ALT (U/l)	46.00±6.00a	45.23±3,90a	87.00±6.32 b	69.00±5.00 c	58.00±5.00 d
Percentage change	-	-52.326	-143.130	-104.00%	-80.09%
Percentage improvement				39.13%	63.04%
ALP(U/l)	50.00±5.00a	47.00±3.00a	236.00±10.22b	94.50.00±5.90d	87.04±7.11d
Percentage change	-	-44	-422	-139.00%	-124.01%
%improvement	283%			283.00%	297.92%
GGT (IU/l)	35.25±2.80a	33.30±3.00a	107.50±7.33b	75.25±5.00d	77.00±5.00 c
Percentage change	-	-59.218	-269.714	-178.25%	-183.19%
Percentage improvement	-	-	-	91.50%	86.53%

The results of biochemical analysis was analyzed using computer program Statistical Package for the Social Sciences using one-way analysis of variance (ANOVA),SPSS computer program (SPSS, Chicago, IL, USA, software version 8) ,post hoc ;Least significant Difference (LSD),combined with co-state computer program ,where different letters at the same column are significant at p ≤0.05. Quantitative values were expressed by mean ± SD of 8 rats in each group. G1 (control), G2 (*I.*

batatas (IbLE)), G3 (DEN +CCl_4), G4 (DEN+CCl_4) + IbLE protective group, G5 (DEN+CCl_4) + IbLE treated group and G6 (DEN+CCl_4) + doxorubicin (DOXol).

Table 3: kidney function biomarkers in HCC induced rats and different groups

Groups / Parameters	G1	G2	G3	G4	G5	G6
(BUN (mmol/l)	2.73±0.05a	3.05±0.03a	8.82±0.13	5.14±0.40d	6.00±0.44c	5.80±0.23c
Percentage change		-108.991	-320.346	-85.548%	-217.050%	209.724%
Percentage improvement	-	-	-	134.8%	103.36%	110.62%
Creatinine (mg/dl)	0.29±0.01a	0.30±0.01a	2.54±0.11b	1.29±0.01d	1.34±0.44ed	1.44±0.04ef
Percentage change	-	-103.158	-875.572	-444.537%	-461.778%	496.261%
Percentage improvement	-	-	-	431.03%	315.17%	379.31%

The results of biochemical analysis was analyzed using computer program Statistical Package for the Social Sciences using one-way analysis of variance (ANOVA),SPSS computer program (SPSS, Chicago, IL, USA, software version 8) ,post hoc ;Least significant Difference (LSD),combined with co-state computer program ,where different letters at the same column are significant at $P \leq 0.05$. Quantitative values were expressed by mean ± SD of 8 rats in each group. G1 (control), G2 (*I. batatas* (IbLE)), G3 (DEN +CCl_4), G4 (DEN+CCl_4) + IbLE protective group, G5 (DEN+CCl_4) + IbLE treated group and G6 (DEN+CCl_4) + doxorubicin (DOXol).

Table 4: Hepatocellular carcinoma biomarkers in HCC- induced rats and different groups

Groups / Parameters	G1	G2	G3	G4	G5	G6
AFP (IU/mL)	11.14 ± 0.800^{a}	11.22 ± 0.60^{a}	42.99 ± 2.00^{b}	21.23 ± 2.00^{c}	17.50 ± 1.00^{c}	14.00 ± 1.00^{d}
Percentage change	-	-89.578	-374.766	-179.434%	-145.95%	-114.53%
Percentage improvement	-	-	-	195.33%	228.82%	260.23%
AFU(U/l)	2.80 ± 0.11^{a}	2.65 ± 0.20^{a}	15.14 ± 1.22^{b}	9.69 ± 1.00^{d}	6.90 ± 0.10^{e}	4.97 ± 0.60^{f}
Percentage change	-	-91.842	-537.914	-343.271%	-243.63%	-174.07%
Percentage improvement	-	-	-	194.64%	294.35%	363.21%

The results of biochemical analysis was analyzed using computer program Statistical Package for the Social Sciences using one-way analysis of variance (ANOVA),SPSS computer program (SPSS, Chicago, IL, USA, software version 8) ,post hoc ;Least significant Difference (LSD),combined with co-state computer program ,where different letters at the same column are significant at $P \leq 0.05$. Quantitative values were expressed by mean ± SD of 8 rats in each group. G1 (control), G2 (*I. batatas* (IbLE)), G3 (DEN +CCl_4), G4 (DEN+CCl_4) + IbLE protective group, G5 (DEN+CCl_4) + IbLE treated group and G6 (DEN+CCl_4) + doxorubicin (DOXol).

Table 5: Pro- inflammatory cytokines levels in HCC- induced rats' ad different groups

Group Parameters	G1	G2	G3	G4	G5	G6
IL-6 (Pg/mL)	70.25 ± 4.00^{a}	56.75 ± 2.21^{b}	254.75 ± 8.43^{c}	106.75 ± 5.00^{e}	100.00 ± 10.00^{ef}	91.25 ± 6.3^{f}
Percentage change	-	-10.532	-292.383	-81.707%	-72.089%	-59.643%
Percentage improvement	-		-	210.68%	220.34%	232.74%
BCl-2(ng/mL)	3.77 ± 0.44^{a}	3.67 ± 0.21^{a}	12.20 ± 0.63^{b}	6.56 ± 0.45^{e}	5.76 ± 0.56^{d}	5.53 ± 0.45^{d}
Percentage change	-	-93.577	-319.837	-170.235%	-149.015%	-142.914%
Percentage improvement	-	-	-	149.60%	170.82%	176.92%
TNF-α(Pg/mL) units	328.75 ± 16.00^{a}	322.75 ± 15.88^{a}	1065.650 ± 21.0^{b}	844.00 ± 12.98^{cd}	648 ± 21.00^{f}	635.50 ± 13.9^{f}
Percentage change	-	230.575	4.597	72.019%	131.639%	135.442%
Percentage improvement	-	-	-	67.42%	127.04%	131.06%

The results of biochemical analysis was analyzed using computer program Statistical Package for the Social Sciences using one-way analysis of variance (ANOVA),SPSS computer program (SPSS, Chicago, IL, USA, software version 8) ,post hoc ;Least significant Difference (LSD),combined with co-state computer program ,where different letters at the same column are significant at P ≤0.05. Quantitative values were expressed by mean ± SD of 8 rats in each group. G1 (control), G2 (*I. batatas* (IbLE)), G3 (DEN +CCl_4), G4 (DEN+CCl_4) + IbLE protective group, G5 (DEN+CCl4) + IbLE treated group and G6 (DEN+CCl_4) + doxorubicin (DOXol).

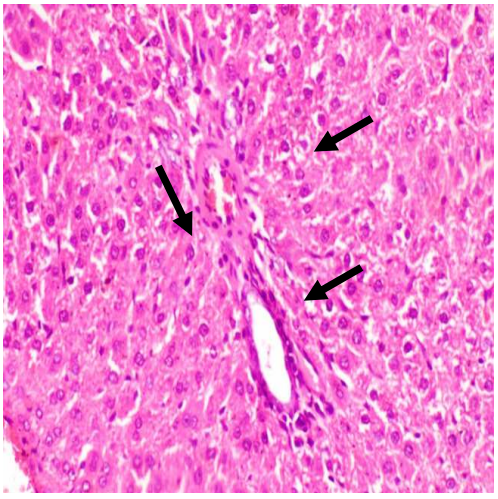
Table 6: Oxidative stress biomarkers in HCC-induced rats and different groups

Lesions	Control	+ ve Cancer	Standard drug	Protective IbLE	IbLE Treatment
Foci of neoplastic cells	0	2	0	1	1
Bridging fibrosis	0	3	0	0	1
Karyocytomegaly	0	3	1	1	2
Vacuolar degeneration	0	3	0	1	1
Portal fibrosis	0	3	1	1	1
Hyperplasia of bile ducts	0	2	1	1	1
Newly formed bile ductules	0	2	1	1	1

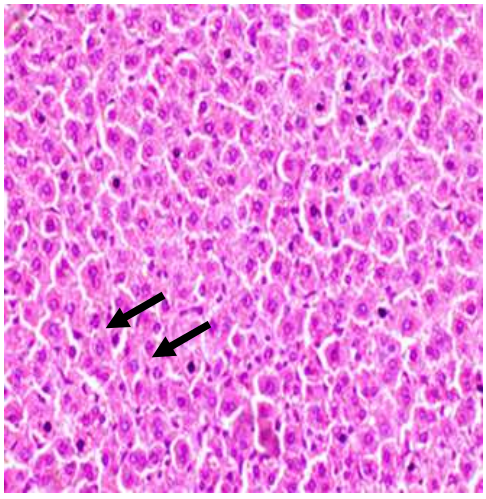
The results of biochemical analysis was analyzed using computer program Statistical Package for the Social Sciences using one-way analysis of variance (ANOVA),SPSS computer program (SPSS, Chicago, IL, USA, software version 8) ,post hoc ;Least significant Difference (LSD),combined with co-state computer program ,where different letters at the same column are significant at *P* ≤0.05. Quantitative values were expressed by mean ± SD of 8 rats in each group. G1 (control), G2 (*I. batatas* (IbLE)), G3 (DEN +CCl_4), G4 (DEN+CCl_4) + IbLE protective group, G5 (DEN+CCl_4) + IbLE treated group and G6 (DEN+CCl_4) + doxorubicin (DOXol).

Table 7: Scoring of histopathological alterations in the liver of all treated groups

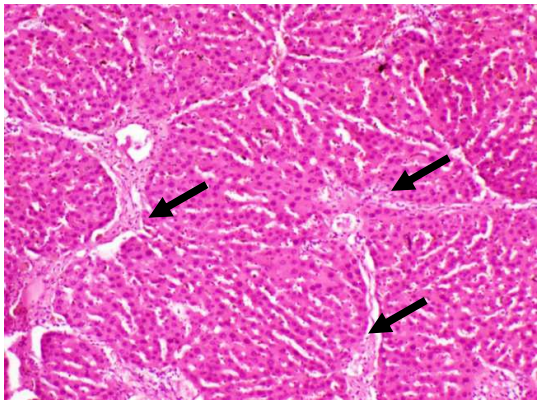
groups / Parameters	G1	G2	G3	G4	G5	G6
MDA (nMol/g tissue)	2.99±0.21^{a}	2.79±0.40^{a}	35.40±2.4	13.75±1.22^{d}	17.17±1.43^{c}	12.42±0.82^{d}
Percentage change	-	-90.321	-1180.956	-456.876%	-571.257%	-412.394%
Percentage improvement	-	-	-	724.08%	609.78%	768.56%
GSH (mMol/g tissue)	1646.25±19.88^{fa}	2125.00±26.54^{b}	640.50±12.00^{c}	1323.75±22.00^{d}	1566.2543.09^{f}	1239.75±17.09^{d}
Percentage change	-	1517.168	1607.343	1565.839%	1551.109%	1570.942%
Percentage improvement	-	-	-	41.51%	56.23%	36.36%
TAC (mM/l)	0.76±0.10^{a}	0.83±0.22^{a}	0.21±0.03^{b}	0.52±0.22^{c}	0.60±0.53^{d}	0.61±0.34^{d}
Percentage change	-	-108.450	-26.871	-67.661%	-78.187%	-79.503%
Percentage improvement	-	-	-	40.80%	51.32%	52.63%



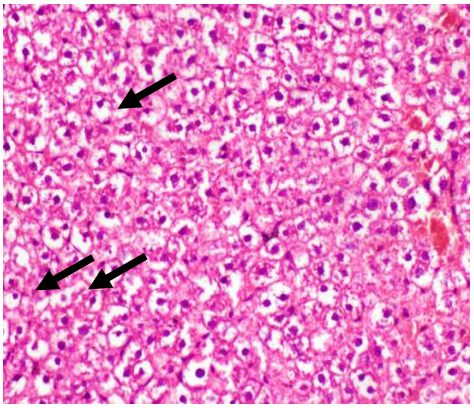
Photomicrograph 1: of control rat liver showing normal histological structure of hepatocytes (long arrow) and portal area (short arrow) (H&EX200)



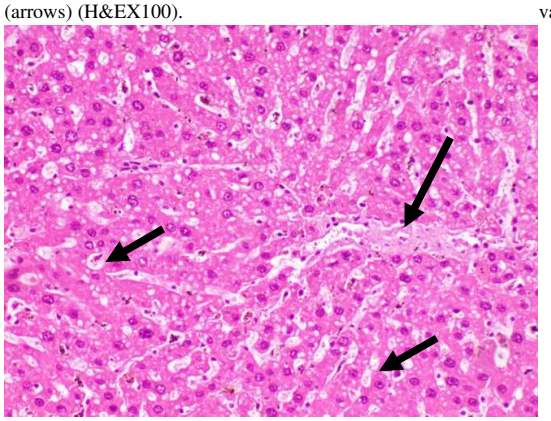
Photomicrograph 2: of normal rat liver treated with IbLE showing normal histological structure of hepatocytes (arrow) (H&EX200).



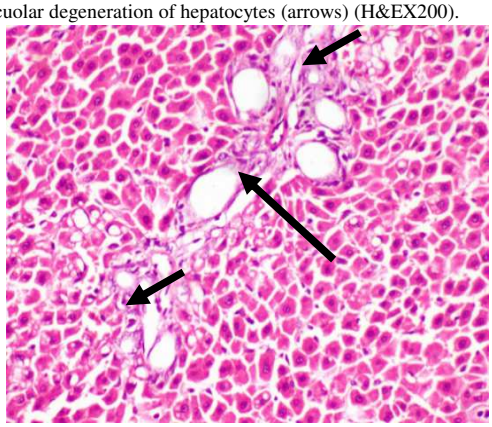
Photomicrograph 3: of liver rat showing bridging fibrosis (arrows) (H&EX100).



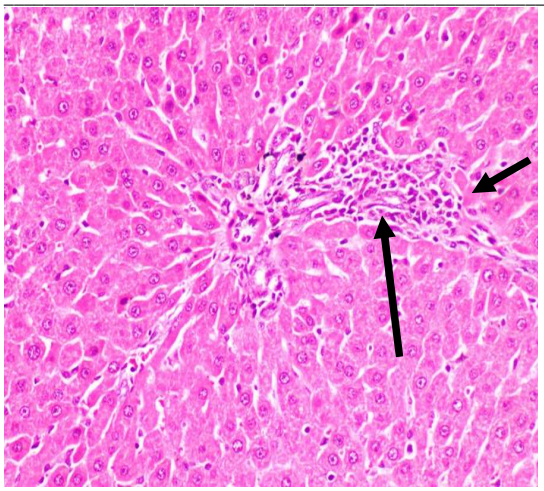
Photomicrograph 4: liver of carcinogenic rats showing severe vacuolar degeneration of hepatocytes (arrows) (H&EX200).



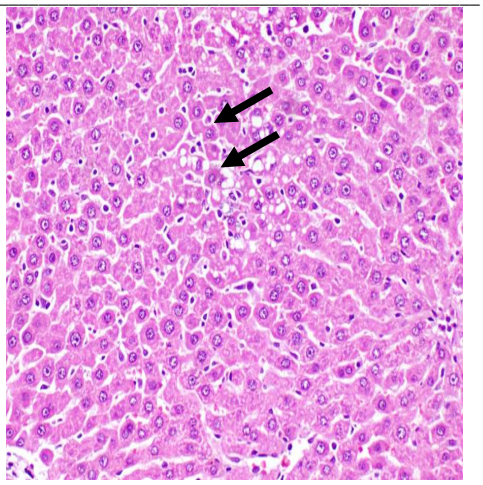
Photomicrograph 5: Carcinogenic liver rat showing vacuolar degeneration of hepatocytes (long arrow), karyocytomegaly (arrowhead) and sinusoidal dilatation (short arrow) (H&EX200).



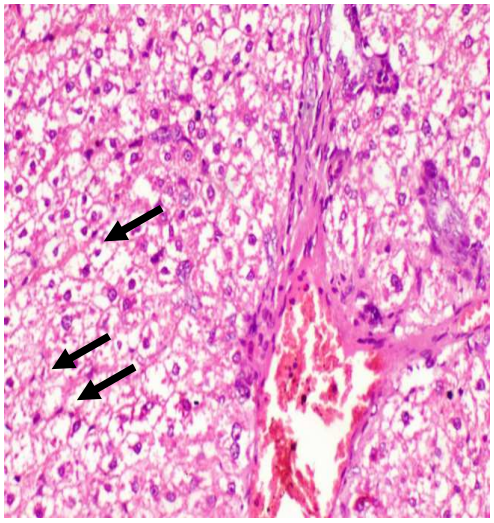
Photomicrograph 6: Carcinogenic liver rat showing newly formed bile ductules (arrows) (H&EX200).



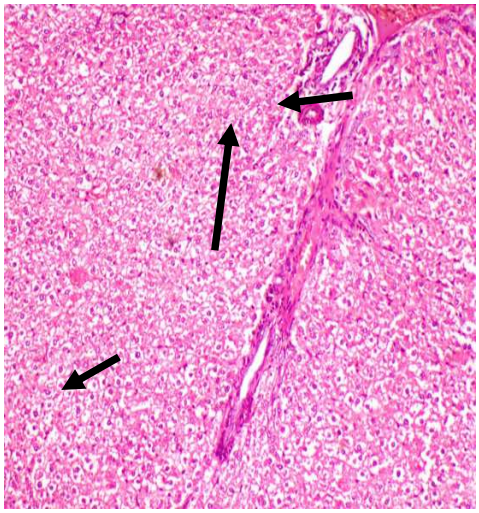
Photomicrograph 7: of carcinogenic liver rat protected with IbLE showing mild portal fibrosis with mononuclear infiltration (arrow) (H&EX200).



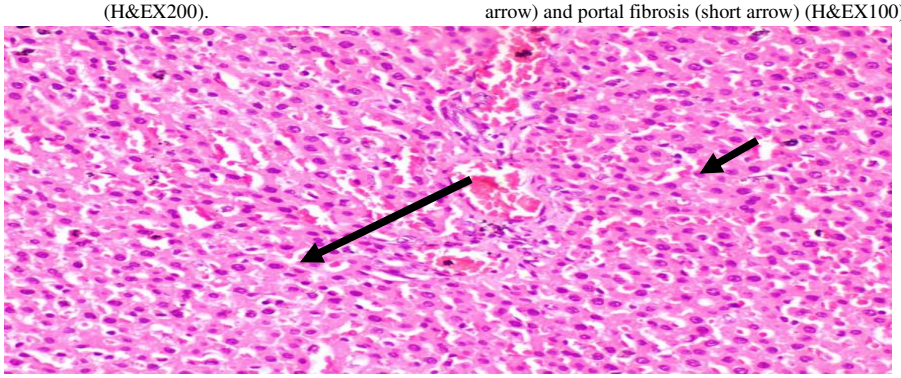
Photomicrograph 8: of carcinogenic liver rat protected with IbLE showing mild vacuolar degeneration of hepatocytes (arrow) (H&EX200).



Photomicrograph 9: of carcinogenic liver rat treated with IbLE, showing vacuolar degeneration of hepatocytes (arrow) (H&EX200).

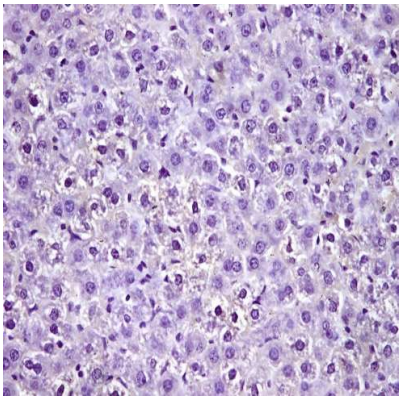


Photomicrograph 10: of carcinogenic liver rat treated with IbLE showing vacuolar degeneration of hepatocytes (long arrow) and portal fibrosis (short arrow) (H&EX100).

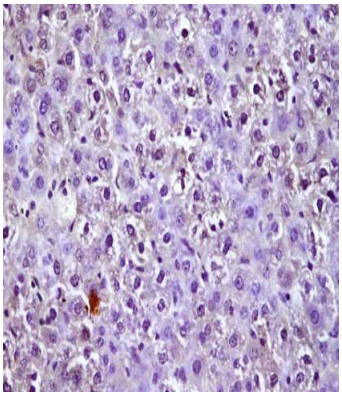


Photomicrograph 11: of carcinogenic liver rat treated with standard drug showing normal hepatocytes (long arrow) and mild portal fibrosis (short arrow) (H&EX200).

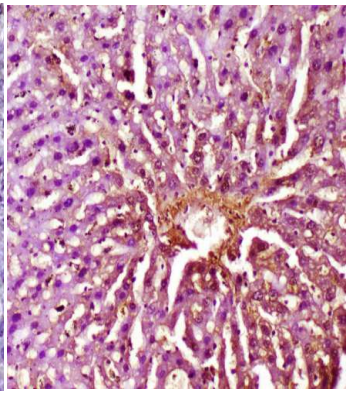
Figure 1: Histopathological alterations in HCC and IbLE protected and treated carcinogenic rats.



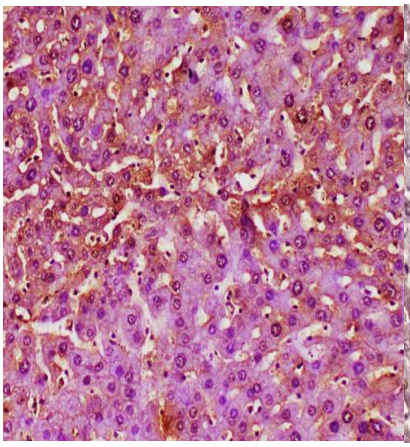
Photomicrograph 14: control of liver rat showing no immune- reactive cells in liver tissue (PCNA X200).



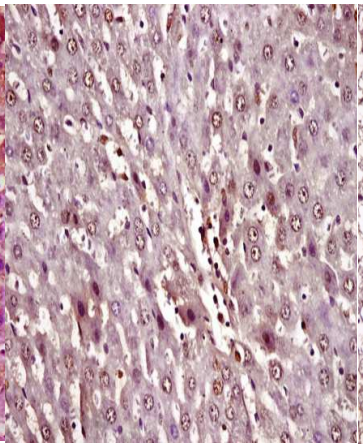
Photomicrograph 15: normal rat supplemented with IbLE showing no immune- reactive cells in liver tissue (PCNA X200).



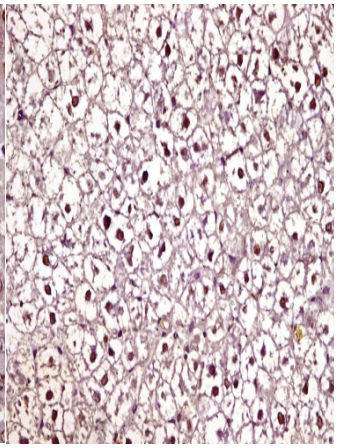
Photomicrograph 16: of carcinogenic rats showing strong positive immune expression (PCNA X200).



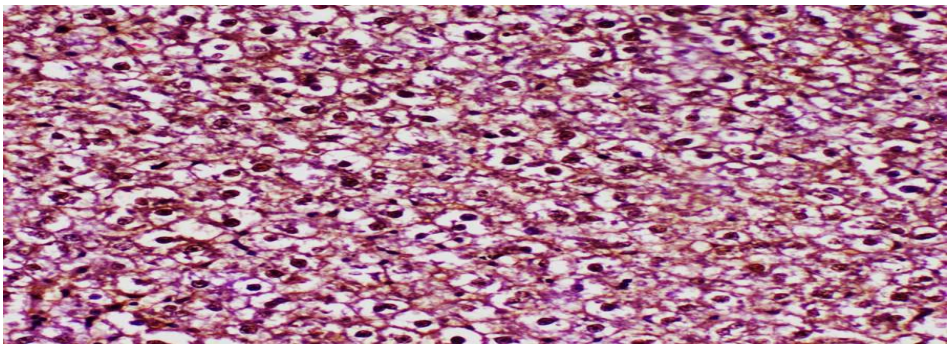
Photomicrograph 17: of carcinogenic liver showing strong positive immune expression (PCNA X200).



Photomicrograph 18: of carcinogenic liver protected by IbLE showing weak positive immune expression (PCNA X200)



Photomicrograph 19: Carcinogenic rat treated with IbLE showing moderate positive immune expression (PCNA X200).



Photomicrograph 20: of carcinogenic liver treated with standard drug showing weak positive immune expression (PCNA X200).

Figure 2: Imunohistochemical examination of PCNA in HCC and IbLE protected and treated carcinogenic rats.

The score system was designed as: score 0 = absence of the lesion in all rats of the group (n = 5), score 1 = (<30%), score 2 = (<30% - 50%), score 3= (>50%).

4. Discussions

Acute toxicity was assessed using serial concentrations of IbLE up to 3000 mg/kg body weight, with no indications of mortality or toxicity observed over a 48-hour, and no signs of behavioral and clinical issues were noted. The dosage employed for this experiment was 500 mg/kg body weight [30]. Our results confirmed the safety of the extract at doses reaching 3000 mg/kg body weight, consistent with earlier studies. In particular, Suhendy et al. [17] and Majid et al. [49] found that the plant extracts did not cause any notable changes in behavior, toxicity, or mortality during the acute toxicity evaluation phase. The administered doses, varying from 100 to 2000 mg/kg in rats, were considered safe since no deaths occurred in the experimental groups, and no noticeable signs of toxicity were detected [49]. Moreover, research conducted with doses between 300 and 4000 mg/kg in rats indicated that specific extracts of IbLE, such as IPT-EA, IPT-M, IPR-EA, and IPR-M, were safe even at the highest dose of 4000 mg/kg weight [50]. The LD_{50} for the purple sweet potato (PSP) extract was determined to be over 5,000 mg/kg body [51]. Additionally, the aqueous extracts from leaves and stems showed no mortality at doses up to 12 g/kg body weight, with an acute oral LD_{50} value measured at 12.0 ± 1.2 g/kg [52].

The current results revealed that, rats subjected to DEN/CCl_4 showed significant increase in the activities of liver enzymes relative to control group (Table 2). While, the co-administration of IbLE alongside DEN in the protective or in therapeutic groups showed ameliorations in the enzymes level compared to standard drug .Our results are in agreement with Zhang et al. [53] ,who illustrated the i.p injection of DEN to normal rats involve cellular mechanism of toxicity resulting in considerable elevation in liver enzymes; AST, ALP , ALT and GGT , confirmed the hepatotoxic effect of DEN that leads to the release of the liver enzymes into the circulation accompanied with their decrease in the hepatic tissue. Moreover, Ahmedy et al. [54] illustrated that during the carcinogenic process; AST, ALT, ALP and GGT elevation indicated DEN induced acute liver injury and necrosis and these enzymes can be used as markers for HCC response to treatment. Carbon tetrachloride is recognized for its toxic effects on the liver, as its metabolite, the trichloromethyl free radical (CCl3•), can cause acute hepatitis and lead the liver to produce increased amounts of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT).The improvement in liver function biomarkers observed in both the protective and therapeutic groups aligns with findings from Suda et al. [55], in their study involving rats demonstrated that administration of carbon tetrachloride resulted in acute hepatitis, accompanied by increased serum levels of GOT and GPT. Notably, the elevation of these enzymes in mice that were concurrently given a purple sweet potato (PSP) beverage was significantly mitigated. Furthermore, the PSP beverage was shown to lower the serum levels of hepatitis-related enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and particularly γ-glutamyl transferase (γ-GT), attributed to the antioxidant properties of purple sweet potato anthocyanins, as noted by Suda et al. [56]. Additional research has indicated that PSP exhibits a considerable protective effect against liver damage induced by cholesterol and D-galactose, as reported by Suhendy et al. [17] and Han et al. [57]. Additionally, the supplementation of anthocyanin-rich purple sweet potato extract (APSPE) was shown to boost superoxide dismutase (SOD) activity, raise GSH and reduce MDA levels in the livers of mice, suggesting a potential therapeutic benefit against CCl_4-induced liver injury, as highlighted by Islam et al. [23]. The observed anti-cytolytic effects may be attributed to the presence of anthocyanins, as noted by Seniuk et al. [58]. The inhibition in liver biomarkers levels observed in carcinogenic rats with sweet potatoes supplementation may be attributed to the presence of various

phytochemical substances, including phenolic substances, flavonoids, anthocyanins, carotenoids, and tannins found in their roots, tubers, leaves, and stems [26,59] . Also, sweet potatoes are loaded with important nutrients like vitamin C, protein, fiber, carbohydrates, β-carotene, amylose, amylopectin, fats, and minerals [18, 60, 61].

Suhendy and colleagues [17] suggested that the leaves of IbLE have healing potential for lowering lipids and preventing artery hardening in traditional medicine. After a four-week treatment using IbLE leaves extract at doses of 500 and 600 mg/kg body weight, notable decreases were seen in factors like low-density lipoprotein cholesterol (LDL-c) to high-density lipoprotein- cholesterol (HDL-c) ratios, along with total cholesterol (TC) to HDL-c ratios ($p < 0.01$). Flavonoid substances, including luteolin and luteoloside, have shown positive effects on blood lipid levels and hepatic steatosis buildup. The mechanisms underlying these advantages may be associated with their antioxidant properties and their capacity to modulate enzymes involved in the metabolism of fatty acids, cholesterol, and triglycerides in the liver [62].The same author pointed out that the ethanolic extract of *I. batatas* leaves extract (IbLE) showed properties like lower blood sugar level in streptozotocin (STZ)-induced rats, which is linked to the effect of flavonoids as antioxidants, showing a direct relation between the amount of flavonoid antioxidant agents and their blood sugar-lowering effects. The compounds of anthocyanin found in IbLE help to regulate blood sugar levels by inhibiting excess sugar absorption and protecting pancreatic beta cells [63]. Consistent with our results, Suhendy et al. [17] illustrated that the oral supplementation of sweet potato leaves extract (SPLE) led to a significant decrease ($P<0.05$) in serum SGPT and SGOT. In the current study, TFC (µg QE/ 0.1g sample) was 4571.0358±27.5 (Table 1). Flavonoids including quercetin are thought to play an important role in many aspects of plant life, including interactions with the environment, where they protect plants from various stresses. As an antioxidant, the quercetin found in SPLE, efficiency scavenging free radicals to shield the liver

from oxidative damage. This mechanism demonstrates decrement in SGOT and SGPT enzymes activity, indicating its liver-protective properties [64]. Our present study showed that the TPC of the sample (mg GAE /0.1 g sample) was 76.4508±6.2 and the TFC (µg QE/ 0.1g sample) was 4571.0358±27.5. Based on these obtained results, the activity shown by lowering the serum levels of hepatitis-related enzymes, including AST, ALT, and particularly GGT, attributed to the antioxidant properties of purple sweet potato polyphenols(PSPP) including phenolic substances, flavonoids, anthocyanins, carotenoids, and tannins [17] .Polyphenols content (as flavonoids and anthocyanins), was attributed to the anti-cytolytic effect of IbLE, as well as the reduction in liver biomarkers observed in carcinogenic rats following the supplementation of leaves extract [17,58].

In a related study, Suhendy et al. [17] found that administration of high-dose of purple sweet potato color (PSPC) to rats in a high-fat diet (HFD) significantly (P<0.01) lowered obesity when compared to HFD-treated rats, with no significant difference between the PSPC and control groups. The anti-obesity effects of PSPC may be due to flavonoids such as anthocyanins, which help to reduce oxidative stress and attenuate signaling of leptin/AMPK in the hypothalamus [65]. Combining sweet potato leaves into HFD may yield anti-obesity benefits. The group consuming a 5% freeze-dried powder of sweet potato leaves (SPLP) exhibited a significant reduction in weight gain and adipose tissue weight without a decrease in feed intake. The polyphenols present in SPLP may affect the composition of blood lipid and dietary fiber [66]. PSP anti-obesity properties may be attributed to their ability to accelerate browning of adipose tissue and produce antioxidant effects, thereby protecting mice from the detrimental impacts of HFD [67].

Conversely, the administration of doxorubicin to rats intoxicated with DEN/CCl_4 resulted in enhanced liver function, evidenced by a notable reduction in the serum levels of liver enzymes. Doxorubicin, an anticancer agent, effectively eliminates tumor cells and mitigates the oxidative stress associated with such conditions. As a result, improvements in hepatocyte regeneration and integrity were observed [68].

The oral administration of IbLE to normal rats resulted in insignificant alterations in BUN and creatinine levels when compared to control group. DEN/CCl_4 induced rats exhibited a significant increase in BUN and creatinine levels relative to the control group, (Table 3). The administration of IbLE in the protective group and treated one led to improvements in BUN and creatinine levels in comparison to the DOXol-treated group. In a prior investigation by Gray and Cooper [69], it was established that elevated levels of urea and creatinine in hyperglycemic rats serve as indicators of compromised renal function. Diabetic individuals frequently exhibit increased concentrations of nitrogenous compounds, such as urea and creatinine, in both plasma and urine, which arise from diminished protein synthesis and heightened muscle proteolysis. However, the current findings revealed that the levels of creatinine and BUN were normalized in carcinogenic rats treated with IbLE, suggesting an enhancement in renal activity. Esatbeyoglu et al. [70] and Herawati et al. [71], presented that the intake of anthocyanin from PSP led to a reduction in body weight and a decrease in the urine albumin-to-creatinine ratio in mice subjected to a high-fat diet. Notably, there was a significant decline in urea and creatinine levels following the administration of anthocyanin derived from purple sweet potato (APSP). The anthocyanins present in PSP are primarily 3, 5-diglucoside derivatives of cyanidin or peonidin, along with caffeoyl, feruloyl, and p-hydroxybenzoyl [72]. Anthocyanins have garnered significant interest due to their potential biological and pharmacological advantages. These compounds exhibit a range of beneficial activities, including antioxidant properties [73, 74], anti-cancer effects [75], antidiabetic actions [76-78], anti-inflammatory responses [79], and hepatoprotective effects [80-82]. These bioactive substances may positively influence the reduction of kidney failure and extracellular dehydration [83, 84]. Anthocyanins derived from PSP may mitigate diabetic renal injury by inhibiting reactive oxygen species (ROS) and safeguarding mitochondrial function [85]. Moreover, the high-soluble dietary fiber (HSDF) found in *I. batatas* has been shown to reduce oxidative stress and prevent lead-induced renal damage [86]. In earlier research, Pérez-Beltrán et al. [87] demonstrated that a puree rich in anthocyanins from fruits lowered plasma glucose, urea, and creatinine levels in hyperglycemic rats. Recently, Suhendy et al. [17] investigated the aqueous extract of *I. batatas* root (AEIB) and found that a dose of 400 mg/kg (p.o) significantly (P<0.01) elevated diuresis, as measured by the total urine volume in mL/100 g/h. This diuretic effect may be attributed to the presence of carbohydrates, flavonoids, and tannins [88]. The precise mechanism of action for the diuretic effects of AEIB remains unclear. The total content of polyphenols has been attributed to various health benefits such as anti-inflammatory, antioxidant, and protective effects against oxidative stress [17, 89]. Multiple research teams reported that some flavonoids, such as quercetin and kaempferol may have diuretic activity by interacting with the adenosine A1 receptor, which is connected to diuretic effects [17, 90].

Furthermore, intoxicated rats with DEN showed a significant rise in AFP and AFU levels compared to control group (Table 4). The rise in AFP serves as an indicator not only of hepatic injury but also of the potential onset of HCC due to DEN/CCl_4 exposure. These results align with earlier investigations conducted by Zhang et al. [53], Soresi et al. [91], Kadasa et al. [92], documented elevated AFP levels in rats subjected to DEN intoxication in contrast to normal rats. In the protective and therapeutic groups receiving IbLE, there was a marked ameliorations in AFP and AFU levels when compared with the DOXol standard group .The observed improvement in AFP and AFU levels in both the protective and therapeutic groups can be elucidated by the findings of Suhendy et al. [17]. As they reported that compounds such as daucosterol linolenate (DLA) ($C_{53}H_{88}O_7$), daucosterol linoleate (DL) ($C_{53}H_{90}O_7$), and daucosterol palmitate (DP) ($C_{51}H_{90}O_7$), which are present in the tubers

of IbLE, exhibit inhibitory effects on the proliferation of breast cancer cells. *In vitro* investigations revealed that all three compounds demonstrated MCF-7 breast cancer cell inhibitory effects, with DL showing the most pronounced inhibition. Furthermore, in vivo studies indicated that DLA, DL, and DP can impede the progression of MCF-7 breast cancer in nude mice xenograft models [93]. As a member of the phytosterol family, daucosterol was found to induce cell growth inhibition in a dose-dependent manner, primarily through mechanisms involving cell cycle arrest and apoptosis. Additionally, it led to the depolarization of mitochondrial membrane potential and an increased Bax to Bcl-2 protein ratio [94]. Additionally, the anthocyanins found in the leaves and tubers of IbLE demonstrated a comparatively enhanced capacity to promote apoptosis in MCF-7 breast cancer cell lines. Nonetheless, the anthocyanins from the leaves exhibited greater apoptotic effect on the HCT-116 and HeLa cell lines [59]. Diets comprising 10% purple sweet potato flesh, 10% purple sweet potato skin, or 0.12% anthocyanin-rich extract over a period of 18 weeks resulted in a decrease in adenoma count in APCMIN mice. This decrement was related to the suppression of cell proliferation of tumor [95]. Anthocyanins, which are flavonoid compounds, display anticancer properties [96], across different types of cancer. In the context of breast cancer, anthocyanins, as part of the flavonoid class, can enhance the expression of miR-27a. This increase in miR-27a expression mitigates carcinogenesis, suggesting a promising therapeutic advantage in cancer treatment and prevention [97].

The present results indicated the significant increase in IL-6, Bcl-2 and TNF-α levels in the carcinogenic rats (G3) compared to control group (G1). The administration of *I. batatas* extract in both protective and therapeutic groups showed noticeable improvements in all cytokines levels comparing with standard group(G6) (Table 5). It has been proposed that elevated levels of circulating CRP, rather than IL-6, TNF-α, or soluble TNFR2, are significantly linked to an increased risk of ovarian cancer. These findings suggested that inflammatory processes may play a role in the pathogenesis of ovarian cancer [98]. TNF-α is primarily secreted by macrophages and lymphocytes in response to cellular damage resulting from infection or malignant transformation [99]. However, it can also be produced by various other cell types and tissues, including adipocytes [100]. TNF-α is similarly IL-6 proinflammatory cytokine with a wide range of functions, including cytotoxic and cytostatic effects against cancer cells. The reduction in proinflammatory cytokines observed in our findings may be linked to the properties of purple sweet potato anthocyanins (PSPAs), which are known for their anti-inflammatory, anti-cancer, hypoglycemic effects, and their ability to enhance intestinal microecology. The research by Wang et al. [79] demonstrated that PSPAs markedly decreased the levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) in the brain cells of rats subjected to cumulative lipopolysaccharide exposure, thereby mitigating acute brain inflammation. In a separate study, Li et al. [29] extracted concentrated anthocyanins from purple sweet potato and treated BIU87 cells with varying concentrations (100, 200, 400, 800 μg/mL) for 48 hours. Their results indicated that increasing anthocyanin concentrations led to a reduction in the number of BIU87 cells, a decrease in cell volume, an increase in cell spacing, impaired cell adhesion, and altered cell morphology. The anti-inflammatory properties of purple sweet potato leaves extract are thought to be mediated through the modulation of NFκB and MAPK signaling pathways [101]. Additionally, extract from PSP leaves have been shown to down-regulate pro-inflammatory markers such as TNF-α and IL-6 in adipocytes [29]. Zengin et al. [102] declared that sweet potato leaves extract, at concentrations ranging from 100 to 1000 μg/mL, acts as anti-inflammatory agents by dose-dependent, lowering nitrite and iNOS gene expression in liver samples from male C57BL6 mice treated with LPS. The anti-inflammatory effects are possibly attributed to the presence of phenolic acids, anthocyanins, and flavonoids in the leaves extract [71, 98,102]. In agreement with previously reported literature, the activity may be attributed to the high quantities of phenolic compounds [102]. In a good agreement with our results, Suhendy et al. [17], found that the ethanol extract from purple sweet potato tubers, rich in flavonoids and anthocyanins, was effective in reducing TNF-α and IL-6 levels in the liver. This finding indicates that the extract may play a role in mitigating inflammation, thereby supporting its anti-inflammatory properties [103]. Moreover, compounds from sweet potato leaves, particularly caffeic acid at a concentration of 0.2 mg/mL, notably reduced the production of nitric oxide (NO) and affected the expression of various inflammation-related factors, such as nitric oxide synthase (iNOS), TNF-α and IL-6, and nuclear factor kappa B (NF-kB). Additionally, this extract showed a beneficial effect on the integrity of Caco-2 monolayers, underscoring its potential as an anti-inflammatory agent [104].

The HCC bearing rats showed a significant increase in MDA level, while marked reduction in GSH and TAC as compared to control rats (Table 6). The supplementation of IbLE in both protective and therapeutic groups led to improvements in oxidative stress markers. Our study indicated that the administration of DEN followed by CCl_4 in rats resulted in an increase in oxidative stress within the liver (G3), which was associated with a compromised antioxidative defense system. This was evidenced by a significant reduction in TAC and GSH levels when compared to control group (G1). The injection of DEN resulted in the generation of reactive oxygen (ROS) and nitrogen species (RNS), contributing to oxidative stress that is pivotal in the development of HCC [53]. ROS were implicated in tumor promotion by DEN through their interaction with key macromolecules, including DNA, lipids, and enzymes involved in DNA repair [53]. The same authors observed a significant decline in the activity of antioxidant enzymes, specifically catalase (CAT) and SOD, following DEN administration in the rats.

The increase in antioxidant enzyme levels following the administration of *I. batatas* extract in the current study(Table 6) may be attributed to the extract's antioxidant properties resulted from the presence of phenolic compounds [17, 105]. These phenolic compounds contain hydroxyl groups attached to an aromatic ring, enabling them to function as hydrogen donors in the neutralization of free radicals. Additionally, phenolic compounds serve as electron donors, facilitating the reduction of metal ions. Consequently, it is posited that the phenolic content is fundamental to the antioxidant properties of *I. batatas* extract [106]. Flavonoids also demonstrate antioxidant capabilities through various mechanisms, such as inhibiting the production ROS via inhibition of enzyme, free radicals scavenging, and modulating antioxidant defenses [89]. The primary antioxidant mechanism of phenolic compounds is believed to involve radical scavenging through hydrogen atom donation [107].*I batatas* tubers and roots demonstrated antioxidant properties across various in vitro assays, showing differing levels of antioxidant capacity when compared to the ethanolic extract. The antioxidant potential of the plant extracts was largely attributed to the biologically active polyphenols [49]. A strong direct relation was observed between the concentration of phenolic compounds and the inhibitory activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), suggesting that increased levels of phenolic compounds enhance antioxidant activity [102]. Our present study has demonstrated the high content of phenolic compounds. TPC of the sample (mg GAE /0.1 g sample) was 76.4508±6.2 and the TFC (µg QE/ 0.1g sample) was 4571.0358±27.5 (Table 1). An *in silico* analysis revealed the antioxidant effects of the aqueous extract from *I. batatas* tubers, which was effective in normalizing marker enzymes level, lipid peroxidation, ROS, mitochondrial tricarboxylic acid (TCA) cycle components, antioxidant enzymes and mitigating histopathological alterations induced by bisphenol A (BPA) [108].The antioxidant activity of *I. batatas* is linked to the presence of phenolic compounds such as flavonoids and anthocyanins [109]. Anthocyanin-rich APSP has been identified as a potential therapeutic agent for mitigating reproductive toxicity caused by lead exposure, functioning through modulation of the JNK signaling pathway and exhibiting antioxidant effects [110]. Furthermore, Suhendy et al. [17], reported that the ethyl acetate extract from purple and orange *I. batatas* tubers (PO2) displayed the highest antioxidant activity, characterized by the lowest IC_{50} value for DPPH (10.54 µg/mL) and the lowest EC_{50} value for FRAP (11.14 µg/mL). Hwang et al. [111], demonstrated that in both HepG2 cell models and rat studies, PSPAs are capable of significantly mitigating oxidative damage caused by tert-butyl hydroperoxide, as well as notably decreasing the occurrence of liver lesions. Additionally, Zhang et al. [53], indicated that PSPAs efficiently suppress ROS generation in mice and significantly counteract obesity and associated liver fat accumulation induced by a high-fat diet (HFD). This result comes from the triggering of the adenosine-monophosphate activated protein kinase signaling route and the control of fat cell formation, which in turn reduces the response to oxidative stress and insulin resistance in the liver.

On the other side, histopathological examination documented our biochemical finding showed carcinogenic rats with vacuolar degeneration of hepatocytes, karyomegaly, sinusoidal dilation, and the presence of newly formed bile ductules (Fig.1,Photomicrographs 3-6). While, carcinogenic rats protected with IbLE displayed only mild portal fibrosis accompanied by mononuclear infiltration, slight vacuolar degeneration of hepatocytes, and localized areas of neoplastic cells (Fig.1, Photomicrographs 7-9). Also, carcinogenic rats treated with *I. batatas* showed clusters of neoplastic cells, vacuolar degeneration of hepatocytes, and portal fibrosis (Fig.1,Photomicrographs 10-12), compared to HCC rats treated with standard drug which revealed normal hepatocyte morphology along with mild portal fibrosis (Fig.1,Photomicrograph 13) (Table 7). Wang et al. [81,82], provided support for our research by demonstrating that the rat liver index was significantly elevated and subsequently reduced by PSPA at both moderate (100 mg/kg body weight) and high doses (200 mg/kg body weight). At the cellular level, PSPA exhibited protective effects against liver injury across all three administered doses. The low dose of PSPA (50 mg/kg body weight) notably mitigated macrovesicular steatosis, which was prevalent in the CCl4-treated group; however, signs of inflammatory cell infiltration, hepatic fibrosis, and necrosis persisted, correlating with elevated serum enzyme and total bilirubin levels. The moderate dose of PSPA resulted in a marked reduction in macrovesicular steatosis and hepatic necrosis. In contrast, the high dose of PSPA nearly eliminated hepatic necrosis, with only minimal macrovesicular steatosis and hepatic fibrosis remaining. The effects of silymarin appeared to parallel those observed with the moderate and high doses of PSPA. Further histopathological analysis is warranted to elucidate these findings, along with additional references to support the discussion.

Immunohistochemical analysis (Fig.2) indicated that carcinogenic rats exhibited a strong positive immune response for PCNA, while protected and treated rats with *I. batatas* displayed a weak to moderate PCNA expression. It was noted that sweet potato roots fiber (SFE) improved the production of IgM in HB4C5 cells post treatment of heat and dialysis, thereby positively affecting immunostimulatory response *in vitro* [112]. The homogeneous 18.3 kDa polysaccharide component obtained from purple sweet potato tuber (PSPP-1) was found to boost the immune features of RAW264.7 cells, particularly regarding phagocytic activity, production of nitric oxide, ROS, and cytokine production. Furthermore, PSPP-1 was demonstrated to trigger toll-like receptor 2 and toll-like receptor 4-mediated pathways, resulting in significant increase in the proteins expression incorporated in the signaling of MyD88-dependent, mitogen-activated protein kinase (MAPK), NF-κB, AP-1 signaling, and TRIF-dependent pathways [113] . Sweet potato glycoprotein (SPG-1) was shown to enhance immune activity in a dose-dependent manner, as indicated by the assessment of serum lysozyme activity and T cell immune response [114]. Aligning with our findings, Wang et al. [115], demonstrated that PCNA is crucial in the regulation of DNA synthesis

and cell proliferation. According to Takasaki et al. [116], PCNA expression reaches its peak during the G1/S transition of interphase, subsequently declining to undetectable levels during mitosis when assessed through immunohistochemical techniques. Consequently, PCNA is recognized as an intrinsic histological marker for the G1/S phases of the cell cycle. Recent investigations have established a notable correlation between PCNA expression and both mitotic activity and tumor grading in solid tumors, as noted by Robbins et al. [117]. The proliferation activity, as indicated by PCNA immunohistochemistry, serves as a valuable marker in various malignant tumors, including gastric carcinomas [118], gastrointestinal lymphomas [119], and colorectal cancers [120].In comparison to mitotic counts, PCNA immunohistochemical staining has demonstrated greater efficacy and reliability. Overall, cell proliferation plays a significant role in determining the degree of malignancy, with less differentiated cells exhibiting a heightened capacity for proliferation, thereby increasing tumor aggressiveness. Furthermore, PCNA expression has been significantly associated with the differentiation level of hepatocellular carcinoma (HCC), with numerous PCNA-positive nuclei notably present in tumor thrombi and regions of extracapsular tumor growth. A significant relationship was also identified between the PCNA labeling index and factors such as tumor size, histological grade, tumor metastasis, and mitotic count, corroborating findings previously reported by Taniai et al. [121].

Ng et al. [122], conducted a study involving 72 patients who underwent surgical resection for hepatocellular carcinoma (HCC). Their findings indicated that patients with a proliferating cell nuclear antigen (PCNA) score of 200 or lower experienced significantly improved disease-free survival rates compared to those with scores exceeding 200. This underscores the critical significance of PCNA immunohistochemical staining in forecasting the prognosis of HCC. Consequently, PCNA staining serves as a dependable indicator of tumor proliferation in HCC. This methodology not only aids in assessing tumor invasion and metastasis but also plays a crucial role in predicting the prognosis for patients with HCC [123]. Ma et al. [124], declared a study on the expression and importance of PCNA in hepatocellular carcinoma (HCC), subsequently examining its impact on clinical outcomes. The findings revealed that the expression level of PCNA is significantly elevated in HCC tissues compared to paracarcinoma tissues. Furthermore, this expression was found to correlate with factors such as alpha-fetoprotein (AFP), albumin levels, number of tumors, clinical grade, vascular invasion, and the tumor-node-metastasis (TNM) staging.

From our biochemical, histopathological and immunochemical investigations, the protected HCC rats with IbLE showed better results in improving kidney function BUN and creatinine levels , histopathological architectures of liver tissue and weak response of PCNA .However , all other examined biochemical markers indicated more effective results in therapeutic HCC rats; liver enzymes ,oxidative stress ,inflammatory cytokines and tumor markers.

5. Conclusion

This study offers significant insights into the potential protective and therapeutic effects of IbLE, especially concerning the prevention and management of conditions associated with hepatic cancer, oxidative stress, and inflammation. The extract has been shown to normalize liver function, levels of BUN and creatinine, suppresses inflammatory cytokines levels; IL6 and BCl2 along with returned tumor markers ; TNF-α , AFP and AFU levels to more or less normal values. Additionally, the extract enhances antioxidant levels and improves histopathological structures, as well as PCNA immune-expression in rat model of liver carcinoma. Therefore, IbLE may serve as a promising nutraceutical for the prevention and treatment of hepatocarcinogensis. Further research is necessary to explore the underlying mechanisms and to validate these findings through in vivo studies.

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Author contributions

HFA and HIA: sampling, performing the experiments, data analysis, preparing the first draft of the manuscript and supervision. HHM and ASG, supervision and revision of the manuscript. FAF: sampling, performing the experiments, revision of the manuscript. All authors have read and approved the final manuscript.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval consent to participate

All animal treatments and experimental procedures were ethically approved by the National Research Center's Ethics Committee under approval number 04431223.

Consent for publication

None

Competing interests

The authors declare that they have no competing interests.

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