



A REVIEW ARTICLE

Hepatoprotective Effects of β -Caryophyllene and Full-Spectrum Cannabis Extract Against Paracetamol-Induced Liver Injury in Mice



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Abstract

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Background: Paracetamol overdose is a primary cause of acute liver injury. While N-acetylcysteine is the standard care, its clinical utility is limited by adverse reactions. Cannabis-derived compounds, particularly β -caryophyllene (BCP), exhibit potent anti-inflammatory and antioxidant properties via cannabinoid receptor CB2 activation. **Objectives:** This study aimed to quantify cannabinoid constituents in commercially available Cannabis sativa samples and evaluate the comparative efficacy of BCP versus full-spectrum cannabis extract in mitigating paracetamol-induced hepatotoxicity in mice. **Methods:** Phytochemical profiles of four cannabis samples were determined using gas chromatography-mass spectrometry (GC-MS). Forty-two male albino mice were randomized into seven groups: negative and positive controls, paracetamol-induced toxicity, BCP or cannabis monotherapies, and combination regimens. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were quantified, and hepatic histopathology was evaluated using hematoxylin and eosin (H&E) staining. Data were analyzed via ANOVA with LSD post-hoc tests. **Results:** The concentrations of major cannabinoids exhibited considerable variability among the analyzed four samples, reflecting the heterogeneity of commercially available cannabis preparations. The biochemical and histopathological findings of the study demonstrate that both β -caryophyllene (BCP) and full-spectrum cannabis extract significantly attenuated paracetamol-induced liver injury, though the protective effect of BCP was markedly more pronounced.

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1. Introduction

Paracetamol (acetaminophen) is one of the most widely used analgesic and antipyretic agents globally and is generally regarded as safe when administered within therapeutic thresholds. However, it remains the leading cause of acute liver failure worldwide, primarily driven by accidental overdose or repeated supratherapeutic ingestion [1, 2]. Notably, standard therapeutic dosages can occasionally provoke idiosyncratic hepatotoxicity, with documented clinical cases of acute hepatic failure occurring in older patients receiving standard European oral regimens of 1 g four times daily [3]. The hepatotoxicity of paracetamol is largely attributed to its metabolic conversion via cytochrome P450 enzymes into the highly reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). At toxic thresholds, NAPQI depletes hepatic glutathione reserves, triggering mitochondrial dysfunction, severe oxidative stress, and extensive hepatocellular necrosis [1,4].

Currently, N-acetylcysteine (NAC) is the standard antidote for paracetamol poisoning, acting primarily by replenishing intracellular glutathione and enhancing non-toxic metabolic pathways [5]. Despite its established efficacy in reducing clinical morbidity, NAC therapy has several operational limitations. It is frequently associated with adverse reactions including nausea, vomiting, hypersensitivity, and potentially life-threatening anaphylactoid responses [6]. Furthermore, its therapeutic effectiveness is strictly time-dependent, rapidly declining if administration is delayed [5]. This narrow therapeutic window underscores the critical need for alternative or adjunctive hepatoprotective therapies with expanded windows of efficacy and improved safety profiles.

In recent years, increasing attention has focused on the regulatory role of the endocannabinoid system in liver pathophysiology. Both cannabinoid receptor type 1 (CB1) and type 2 (CB2) are differentially regulated during acute and chronic hepatic injury, directly modulating inflammatory cascades, oxidative stress, and fibrogenesis [7,8]. In this context, full-spectrum cannabis extracts have been proposed to exert enhanced therapeutic effects through synergistic chemical interactions among their multi-constituent compounds, a phenomenon known as the "entourage effect" [9]. Cannabis sativa produces hundreds of phytochemical constituents, most notably the non-psychoactive cannabinoid cannabidiol (CBD)—which has demonstrated clinical utility as an anticonvulsant—and Δ^9 -tetrahydrocannabinol (THC), the principal psychotropic component that interacts directly with central receptors. While global legislative shifts have expanded the medical use of cannabis-based medicines, concerns persist regarding their long-term safety, particularly the risk of cannabis use disorder [10]. Furthermore, clinical findings of elevated serum transaminases in recent trials have raised concerns regarding cannabinoid-induced hepatotoxicity [11]. Advanced microphysiological modeling, including human quad-culture liver chips and human-induced pluripotent stem cell-derived organoids, has been utilized to evaluate the specific toxicological fingerprints of major cannabinoids like CBD, cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG), highlighting variable margins of hepatic safety [12,13]. Concurrently, evidence indicates that the hepatic endocannabinoid system is heavily involved in the progression of chronic liver disorders, including metabolic dysfunction-associated steatotic liver disease (MASLD/NAFLD) and viral hepatitis, where targeted cannabinoid receptor modulation can alter metabolic and inflammatory pathways [10].

Within this pharmacological landscape, β -Caryophyllene (BCP) a natural, bicyclic sesquiterpene abundant in medicinal plants such as clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum* spp.), and copaiba (*Copaifera* spp.) —presents a promising therapeutic profile. BCP acts as a selective, full agonist of the peripheral (CB2) receptor [9]. Because it does not bind centrally to (CB1), it lacks psychoactive properties, distinguishing it favorably from psychotropic cannabinoids [14]. Beyond its classical receptor-mediated actions, BCP is an FDA-approved food additive capable of modulating behavioral pathways, such as inhibiting methamphetamine-seeking behaviors in animal models [15].

Experimental studies demonstrate that BCP exerts potent anti-inflammatory, antioxidant, and cytoprotective properties across diverse pathologies [16,17]. These protective actions are primarily mediated through the simultaneous upregulation of the cytoprotective nuclear factor erythroid 2—related factor 2 (Nrf2) pathway and the suppression of the pro-inflammatory nuclear factor kappa B (NF- κ B) signaling cascade [10,14]. In various pre-clinical models of toxin-induced liver injury, BCP has consistently shown hepatoprotective potential by mitigating oxidative stress, downregulating pro-inflammatory cytokines, preserving tissue architecture, and reducing serum biochemical markers of hepatic necrosis [10,14,16,17]. Given that profound oxidative stress and acute inflammatory cascades represent the primary drivers of paracetamol-induced hepatotoxicity, the unique pharmacological profile of β -caryophyllene as a non-psychoactive with potent antioxidant capabilities makes it a strong therapeutic candidate. Therefore, it is hypothesized that β -Caryophyllene (BCP) administration will significantly mitigate paracetamol-induced liver injury.

The study aims to quantify the precise phytochemical concentrations of distinct cannabinoid and terpenoid constituents within four commercially available Cannabis sativa samples using gas chromatography–mass spectrometry (GC-MS) analysis. and evaluate and compare the therapeutic efficacy of β -Caryophyllene (BCP) versus a full-spectrum cannabis extract in attenuating paracetamol-induced hepatotoxicity in a murine model, utilizing serum biochemical transaminases (ALT and AST) and histopathological scoring as primary markers of hepatic injury.

2. Materials and Methods

2.1. Reagents and Plant Materials

Cannabis Material: Commercially available resinous *Cannabis sativa* samples (N = 4), commonly referred to colloquially as hashish) were obtained via local procurement from four distinct geographical sites within the Assiut Governorate, Egypt.

β -Caryophyllene (BCP): Powdered β -Caryophyllene was purchased from Sigma-Aldrich® (St. Louis, MO, USA) with a certified chemical purity Sigma– [Molecular formula; C₁₅H₂₄: molecular weight; 204, 36: Purity $\geq$ 98%.

Paracetamol (Acetaminophen): Reagent-grade paracetamol powder (1000 mg) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Diagnostic Assays: Serum biochemical parameters—including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin levels—were quantified using commercial colorimetric assay kits obtained from Sigma-Aldrich (St. Louis, MO, USA).

Vehicle Preparation and Administration: Prior to *in vivo* administration, all experimental compounds (powdered β -Caryophyllene, paracetamol, and the prepared cannabis extracts) were freshly dissolved or homogenously suspended in sterile normal saline (0.9 % NaCl) for standardized oral gavage delivery to the animals.

2.2. Experimental Design

The study was executed in two distinct experimental phases:

Phase I: Phytochemical profiling and characterization of *Cannabis sativa* samples using gas chromatography–mass spectrometry (GC-MS).

Phase II: An *in vivo* evaluation of the hepatoprotective efficacy of β -Caryophyllene (BCP) and full-spectrum cannabis extract against paracetamol-induced hepatotoxicity in mice.

2.2.1. GC-MS Instrumentation and Chromatographic Conditions

Phytochemical screening was performed in the Chromatography Laboratory, Faculty of Science, Assiut University. Extracts were analyzed using a gas chromatography–mass spectrometry (GC-MS) system (Thermo Fisher Scientific, Austin, TX, USA) integrated with a Direct Probe Controller (DPC; Model: DPC, Part No.: 1R119300-5000). The system operated at an AC input of 100–230 V, 50/60 Hz, with a maximum current of 2 A. Helium (high purity) was used as the carrier gas throughout the analysis.

2.2.2. Sample Preparation and Selection

Dried plant resin (0.25 g) from each of the four samples was weighed precisely and dissolved in 2 mL of analytical-grade chloroform within sealed volatile-safe vials. To ensure maximum extraction efficiency, samples underwent ultrasonic-assisted extraction for 30 min utilizing a Branson PC620 ultrasonic bath (Branson, USA) at ambient temperature.

Following extraction, the homogenate was centrifuged at 10,000 rpm for 15 min at 20 °C to precipitate insoluble particulate matter. The clear supernatant was harvested and passed through a 0.22 μ m polytetrafluoroethylene (PTFE) syringe filter. A 1 μ L aliquot of the clear filtrate was loaded into an autosampler vial and injected into the GC-MS system. Based on the resulting chromatograms, Sample 2 was selected for subsequent *in vivo* protocols due to its balanced, full-spectrum profile of major cannabinoids and essential terpenes.

2.2.3. Animal Husbandry and Environmental Conditions

Forty-two adult male Swiss albino mice (20–25 g) were procured from a certified institutional animal facility. The animals were acclimated for one week under controlled laboratory environmental conditions: (12 h light/dark cycle, temperature 22 $\pm$ 2°C, and relative humidity at 50–60%). Mice had *ad libitum* access to a standard pellet diet and purified water. All protocols were conducted in strict accordance with institutional ethical guidelines for the care and use of laboratory animals.

2.2.4. Experimental Grouping and Dosing Regimen

The animals were randomized using a weight-stratified approach into seven experimental groups (n = 6 per group). The study followed a subacute, repeated-dose exposure model, wherein treatments were administered via oral gavage once daily for three consecutive days as outlined below:

Group	Nomenclature	Daily Protocol (Orally for 3 Consecutive Days)	Rationale & Reference
1	Negative Control	Untreated baseline control	To establish reference physiology.
2	Positive Control	Normal saline vehicle (10 mL/kg)	To control for vehicle administration stress.
3	Paracetamol Toxicity	Paracetamol (250mg/kg/day)	Induces reproducible acute hepatic injury [1].
4	BCP Monotherapy	β -Caryophyllene (100 mg/kg/day)	Evaluation of standalone safety and baseline profile.
5	Cannabis Monotherapy	Full-spectrum extract (300 mg/kg/day)	Evaluation of baseline systemic and hepatic impacts.
6	BCP Intervention	Paracetamol (250 mg /kg/day) + BCP (100 mg/kg/day)	Tests CB2 mediated hepatoprotection [14].
7	Cannabis Intervention	Paracetamol (250 mg/kg/day) + Extract (300 mg/kg/day)	Tests synergistic phytocannabinoid "entourage" protection.

Note on Dosing: Selected dosages were determined from established empirical models. Paracetamol at 250 mg/kg consistently challenges hepatic glutathione reserves without inducing mass mortality [4], while BCP at 100 mg/kg activates peripheral CB2 receptors effectively [9].

2.2.5. Sample Harvesting

Twenty-four hours following the final treatment delivery, mice were deeply anesthetized. Whole blood samples were collected via cardiac puncture into separator tubes and centrifuged to isolate serum for immediate liver function testing. Concurrently, whole livers were rapidly excised, rinsed in ice-cold normal saline, and fixed in an alcohol-based or buffered fixative solution for subsequent histopathological processing.

2.2.6. Laboratory and Analytical Procedures (*Serum Biochemical Assays (ALT and AST Quantification)*)

Quantitative activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured colorimetrically using the standardized Reitman-Frankel endpoint method. Briefly, serum was incubated with specific substrates (L-alanine for ALT; L-aspartate for AST) in the presence of 2-oxoglutarate. The enzymatic transfer of amino groups yields pyruvate or oxaloacetate, respectively. These intermediate keto-acids were reacted with 2,4-dinitrophenylhydrazine to synthesize colored hydrazone complexes. Absorbance intensities were measured spectrophotometrically at 546 nm and were directly proportional to systemic transaminase activity (U/L).

2.2.7. Histopathological Processing

Fixed liver samples were dehydrated through a graded series of alcohols, cleared in xylene, and embedded in paraffin wax blocks. Transverse tissue blocks were sectioned at a thickness of 3 μ m using a rotary microtome and mounted on glass slides. Following deparaffinization and rehydration, the specimens were stained with Hematoxylin and Eosin (H&E). Structural alterations, including inflammatory cell infiltration, sinusoidal congestion, and centrilobular necrosis, were evaluated and imaged under a light microscope (Olympus, Hamburg, Germany).

2.3. Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Statistical differences among the seven experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons via SPSS software. Variations were considered statistically significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Tables and Figures

Phase I: Phytochemical GC–MS Analysis Cannabis sativa samples : As illustrated in **Figure 1**, the concentrations of major cannabinoids exhibited considerable variability among the analyzed

four samples, reflecting the heterogeneity of commercially available cannabis preparations. Specifically, Δ^9 -tetrahydrocannabinol (THC) concentrations ranged from 0.00% to 37.43%, while cannabidiol (CBD) levels varied between 19.18% and 26.95%. Cannabinol (CBN) concentrations ranged from 6.86% to 31.0%, whereas β -caryophyllene (BCP) was detected at comparatively lower concentrations ranging from 0.00% to 1.33%.

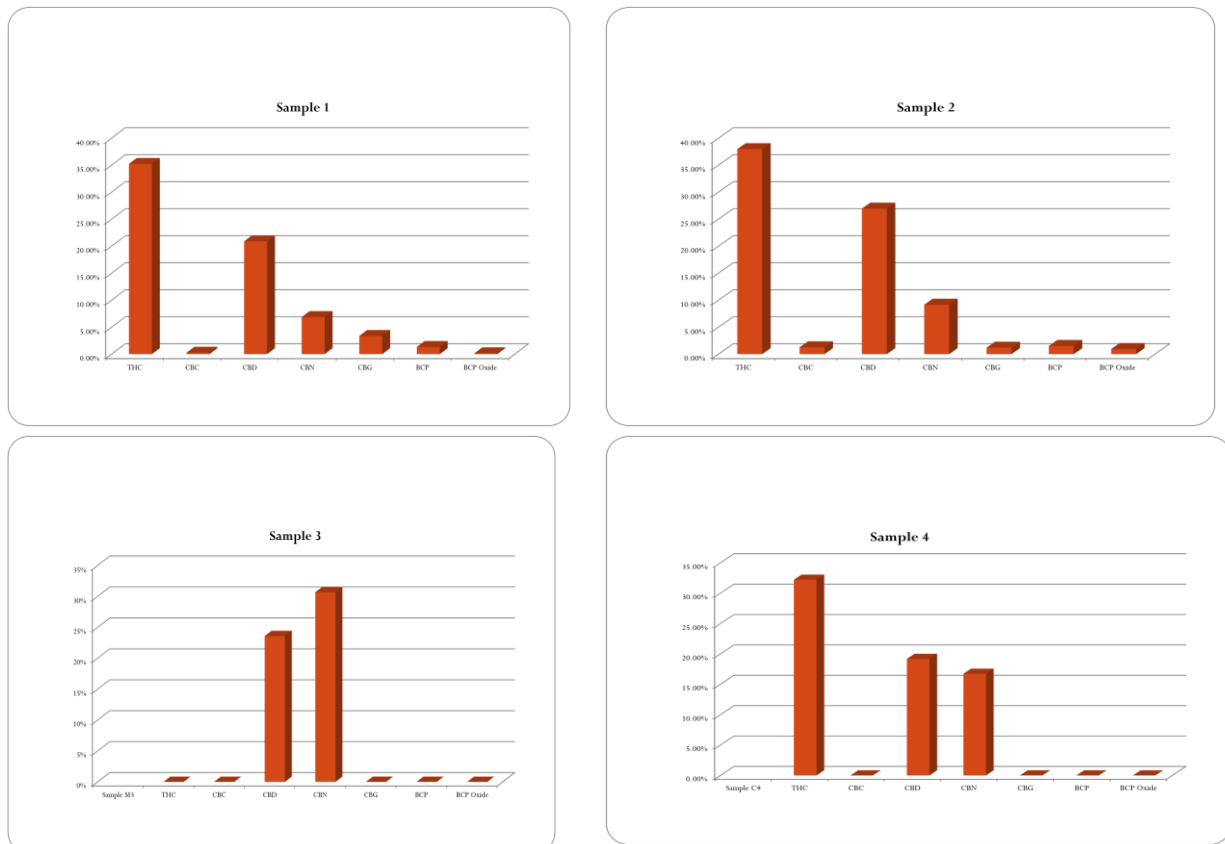


Figure 1. The concentrations of major cannabinoids in four cannabis commercial samples

Phase II: Evaluation of Therapeutic Effects of β -caryophyllene (BCP) and Full-Spectrum *Cannabis sativa* Extract

The effects of the experimental treatments on serum biomarkers of hepatic injury are presented in Figure 2.

Induction of Hepatotoxicity: Oral administration of paracetamol resulted in profound hepatic injury, characterized by a significant elevation in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities compared to both the negative and vehicle-positive control groups ($p \leq 0.05$).

- This marked increase confirms acute hepatocellular damage and the leakage of these transaminases from the necrotic parenchyma into systemic circulation.
- Attenuation of Injury by Interventions: In contrast, concomitant treatment with either full-spectrum *Cannabis sativa* extract or β -caryophyllene (BCP) significantly attenuated paracetamol-induced hepatotoxicity. Mice in these intervention groups exhibited significantly reduced serum levels of both ALT and AST ($p \leq 0.05$) relative to the untreated paracetamol group, demonstrating the potent hepatoprotective properties of both substances.
- Comparative Superiority of BCP: Notably, BCP monotherapy demonstrated superior efficacy compared to the full-spectrum extract. The BCP-treated cohort exhibited significantly lower transaminase levels, moving substantially closer to baseline control values and indicating a more pronounced capacity to preserve hepatocyte membrane integrity against toxic metabolic insults.

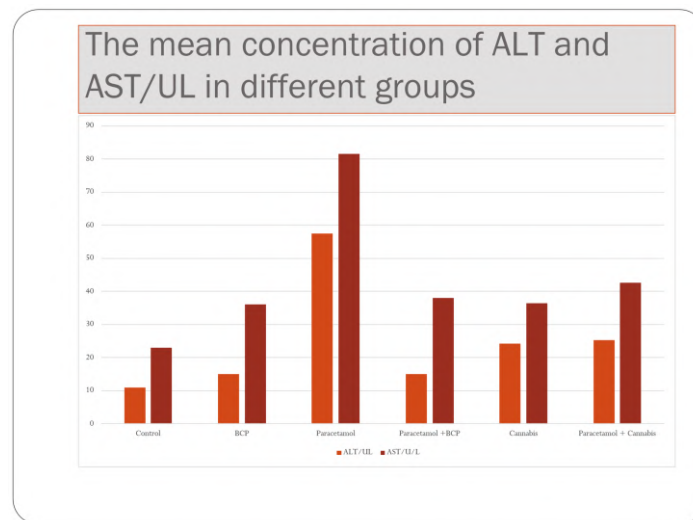


Figure 2. Serum levels of ALT and AST in different groups

Table 1. Descriptive Analysis of ALT and AST values

Group	ALT (U/L) Mean ± SD	AST (U/L) Mean ± SD
Positive Control	10.50 ± 0.71	23.00 ± 5.66
BCP	15.00 ± 4.24	36.00 ± 8.49
Cannabis	24.00 ± 11.58	36.50 ± 5.80
Paracetamol	57.50 ± 26.16	81.50 ± 43.13
Paracetamol + Cannabis	25.33 ± 12.22	42.67 ± 8.08
BCP + Paracetamol	15.00 ± 4.36	38.00 ± 6.24

Data are expressed as mean ± standard deviation (SD)

Serum ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) levels varied among the experimental groups, with the Paracetamol group exhibiting the highest mean enzyme values (ALT: 57.5 U/L; AST: 81.5 U/L), indicating significant hepatocellular injury. In contrast, the Positive Control, BCP, and BCP + Paracetamol groups showed relatively low enzyme levels that remained near baseline values. Treatment with cannabis extract, either alone or in combination with paracetamol, resulted in moderate reductions in liver enzyme levels, suggesting partial hepatoprotective activity. Overall, both ALT and AST followed a similar pattern, characterized by marked elevation in the Paracetamol group and subsequent reduction in the treated groups.

Table 2. AST/ALT ratio and interpretation in different groups

Group	AST/ALT Ratio	Interpretation
Positive Control	2.19	Within expected physiological range
BCP	2.40	Mild elevation
Cannabis	1.52	Moderate elevation
Paracetamol	2.53	Marked enzyme elevation
Paracetamol + Cannabis	1.68	Partial normalization
BCP + Paracetamol	1.42	Near normalization

Table 3. One-way ANOVA for AST Levels

Source of Variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	2674.98	5	534.996	4.05	0.0286
Within Groups	1322.50	10	132.25		
Total	3997.48	15			

Effect Size (for manuscript text)

ALT: $\eta^2 = 0.669$ (large effect) ; AST: $\eta^2 = 0.642$ (large effect)

Effect of treatments on serum ALT and AST levels:

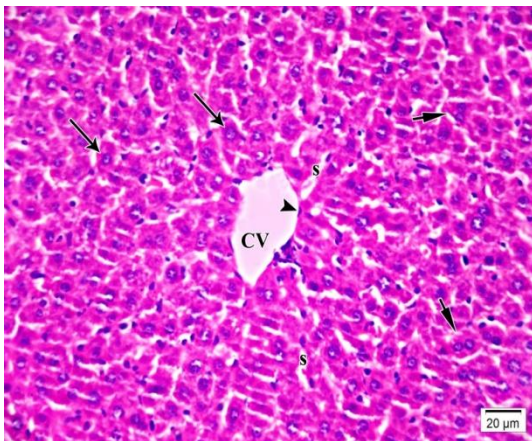
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the experimental groups. Data are presented as mean ± standard deviation (SD). Statistical

analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Groups that do not share the same letter (a, b, c) differ significantly at $p < 0.05$.

Suggested lettering (based on your data trend):

- Positive Control → a
- BCP → a
- BCP + Paracetamol → a
- Cannabis → ab
- Paracetamol + Cannabis → b
- Paracetamol → c

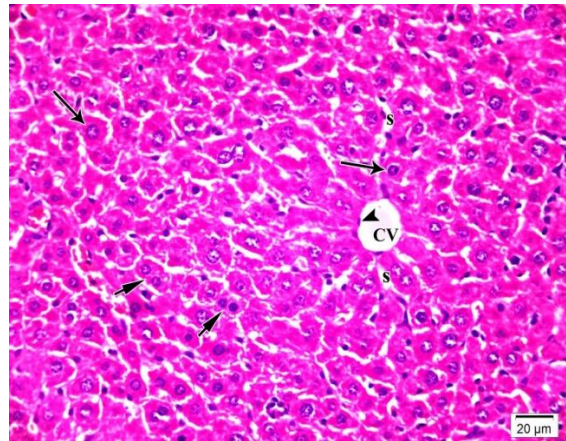
(This reflects: lowest → intermediate → highest enzyme levels)



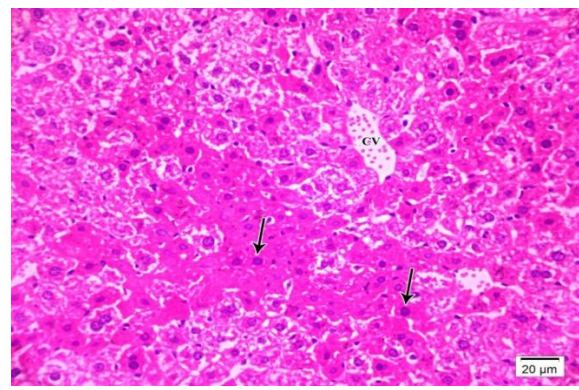
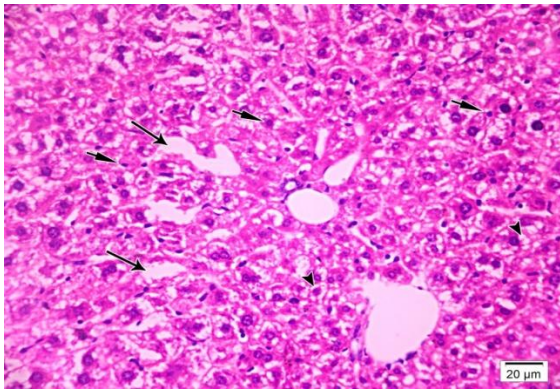
(A) Control groups: Photomicrograph of liver tissue stained with hematoxylin and eosin (H&E) (×400) showing normal hepatic architecture, with well-arranged hepatocytes, intact central vein (CV), and portal structures.



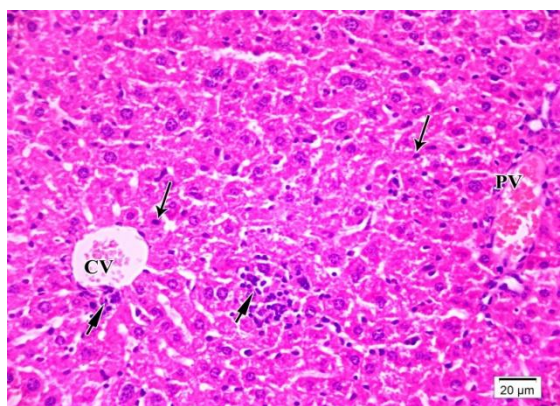
(B) (BCP) group: Photomicrograph (H&E, ×400) showing a nearly normal histological architecture of liver tissue, closely comparable to the control group, with no apparent pathological alterations.



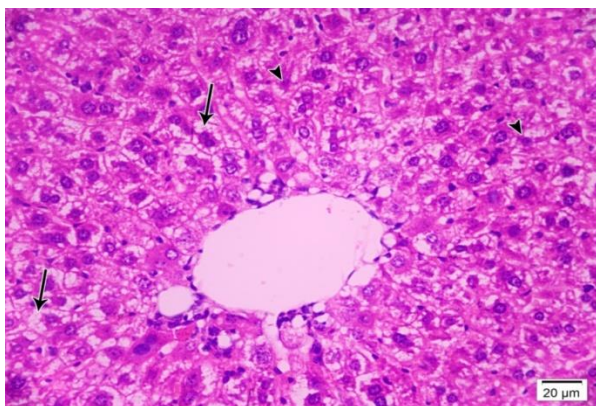
(C) Cannabis extract group: Photomicrograph (H&E, ×400) showing generally preserved and organized hepatic parenchyma. Mild inflammatory cell infiltration is observed surrounding the central vein (short arrows), along with a few scattered apoptotic cells exhibiting deeply stained pyknotic nuclei (arrowheads).



(D) Paracetamol group: Photomicrograph (H&E, ×400) showing marked disorganization of hepatic architecture, with diffuse hepatocellular destruction, particularly in the centrilobular region surrounding the central vein (CV). Hepatocytes appear widely spaced (arrows) with vacuolated cytoplasm (arrowheads), along with numerous apoptotic cells exhibiting shrunken, deeply stained nuclei (short arrows). Occasional relatively normal hepatocytes are interspersed between damaged cells.



E) Paracetamol + (BCP): Photomicrograph (H&E, ×400) showing predominantly preserved hepatic architecture, with many hepatocytes appearing normal. A few degenerated cells with shrunken, deeply stained nuclei are present (arrows), along with mild inflammatory cell infiltration between hepatic cords (short arrows). Mild hemorrhage is observed in the central vein (CV) and portal vein (PV).



(F) Paracetamol + cannabis extract: Photomicrograph (H&E, ×400) showing disorganized hepatocytes, particularly in the centrilobular region, with cytoplasmic vacuolation (arrows). Some cells exhibit deeply stained, degenerated nuclei (arrowheads), indicating partial but limited hepatoprotection.

4. Discussion

4.1. Characterization of the Paracetamol-Induced Hepatotoxicity Model

Paracetamol-induced hepatotoxicity remains one of the most extensively validated experimental models of acute liver injury [18].

In the present study, repeated oral administration of a toxic dose of paracetamol for three consecutive days produced significant hepatic injury. This pathology was evidenced by marked elevations in serum ALT and AST levels, alongside severe histopathological alterations characterized by hepatocellular degeneration and centrilobular disorganization.

These findings align with the established metabolic cascade of paracetamol toxicity, where cytochrome P450-mediated metabolism converts the parent drug into the highly reactive electrophile *N*-acetyl-*p*-benzoquinone imine (NAPQI). At supratherapeutic concentrations, NAPQI rapidly depletes hepatic glutathione (GSH) reserves, initiating widespread mitochondrial dysfunction, oxidative stress, and eventual centrilobular hepatocyte necrosis [1,2].

4.2. Localized Socio-Legal Context and Cannabinoid Phytochemical Heterogeneity

While recent years have witnessed a widespread relaxation of legal restrictions on medicinal cannabis and industrial hemp across numerous global jurisdictions [19], strict regulatory prohibitions in Egypt have historically limited the advancement of localized cannabinoid research. Consequently, regional scientific interest has focused primarily on the harms associated with cannabis abuse, overshadowing a purported therapeutic history that dates back centuries [20]. This restrictive statutory environment has resulted in a notable deficit of baseline data regarding the phytochemistry, chemotypic classification, and therapeutic parameters of regionally accessible *Cannabis sativa* strains. The current study bridges this knowledge gap, establishes essential data regarding the commercial and medicinal profiles of these preparations.

Our initial gas chromatography–mass spectrometry (GC-MS) analysis revealed substantial heterogeneity in cannabinoid profiles and constituent concentrations across the sampled batches. This chemical variability is consistent with established literature demonstrating that the secondary metabolic output of *Cannabis sativa* is highly dependent on distinct genetic strains, chemotypic designations, environmental microclimates, anatomical plant organs, and specific cultivation conditions [10,17].

Because such profound phytochemical fluctuations directly dictate therapeutic efficacy and alter predictable safety margins, these findings underscore the absolute necessity of implementing rigorous standardization protocols and quality control metrics for future cannabis-derived medical applications.

4.3. Comparative Efficacy of Treatments and Histopathological Correlation

The biochemical findings of this study demonstrate that both β -caryophyllene (BCP) and full-spectrum cannabis extract significantly attenuated paracetamol-induced liver injury, though the protective effect of BCP was markedly more pronounced. Animals treated with paracetamol in combination with either BCP or cannabis extract showed significantly reduced ALT and AST levels compared with the paracetamol-only group, indicating partial preservation of hepatic membrane integrity. The large effect size observed in statistical analysis supports the biological significance of these changes, confirming that the choice of treatment modality was a major determinant of hepatic enzyme variation [21].

Histopathological examination of the present study directly corroborated these serum biochemical observations. Liver sections from the untreated paracetamol group showed marked hepatic lobular disarrangement, hepatocellular degeneration, and widespread necrotic changes. Conversely, the BCP-treated intervention cohort demonstrated substantial preservation of hepatic architecture and minimal cellular damage. In contrast, the paracetamol + cannabis extract group exhibited only partial histopathological improvement, with persistent cytoplasmic vacuolation and degenerative nuclear alterations remaining prominent in the centrilobular regions. Taken together, these findings reveal that the pure sesquiterpene BCP provides more effective hepatoprotection against acute paracetamol toxicity than the crude, multi-constituent cannabis extract. These results agreed with many preclinical studies indicate that β -caryophyllene (BCP) exhibits potent hepatoprotective effects. It works by reducing oxidative stress, preventing liver inflammation, and modulating lipid metabolism [22, 23].

4.4. Molecular Targets and Mechanisms of β -caryophyllene Hepatoprotection

The superior hepatoprotective effect of BCP observed in this study can be attributed to its unique multi-target pharmacological profile. Previous literature demonstrates that BCP acts as a selective, full agonist at peripheral cannabinoid type 2 receptors, a G-protein coupled receptor representing an important therapeutic target in inflammatory diseases [9]. Crucially, the activation of receptors is entirely devoid of the psychotropic adverse effects associated with receptor stimulation [14]. Beyond classical cannabinoid signaling, BCP acts as a functional polypharmacological agent. It activates peroxisome proliferator-activated receptor (PPAR) isoforms, specifically PPAR- and PPAR-, and suppresses downstream inflammatory signaling pathways triggered by the toll-like receptor complex [21]. Through these coordinated pathways, BCP actively suppresses the activation of nuclear factor kappa B (NF- κ B), resulting in the downregulated production of pro-inflammatory cytokines, alongside a substantial reduction in oxidative stress markers [6, 24]. BCP also acts as an antagonist at homomeric nicotinic acetylcholine receptors, while remaining devoid of disruptive effects at serotonergic or GABAergic pathways [24].

Our findings are highly consistent with previous experimental studies documenting the tissue-protective properties of this sesquiterpene. For instance, BCP has been shown to attenuate thioacetamide-induced liver fibrosis in rats by reducing inflammatory cytokine expression and collagen deposition [25]. Similarly, it exerts antioxidant, antifibrotic, and anti-inflammatory effects through -mediated signaling pathways, suppressing hepatic stellate cell activation and restoring functional baseline architecture [22, 26, 27, 28]. Furthermore, BCP has been shown to ameliorate aflatoxin -induced hepatic injury by reinforcing endogenous antioxidant defenses and eliminating free radicals [29].

4.5. Limitations of Full-Spectrum Extracts and Phytocannabinoid Risks

The results of the present study revealed moderate and comparatively limited hepatoprotection offered by the full-spectrum cannabis extract likely stems from its complex, non-standardized chemical composition. While full-spectrum matrices contain multiple bioactive constituents (cannabinoids, terpenes, and flavonoids) capable of synergistic interactions, individual compounds can also exert opposing or toxic biological effects. Although clinical data supporting the therapeutic benefit of major phytocannabinoids specifically, Δ^9 -tetrahydrocannabinol and CBD, many literatures focusing on their limited clinical utility, especially when attempting to avoid the psychoactive liabilities of [20].

This has driven the next wave of cannabinoid therapeutics to explore isolated, non-psychoactive agents like BCP [30]. Furthermore, while cannabis is frequently assumed to possess a low risk of acute organ toxicity in healthy cohorts, evidence reveals that crude cannabinoids can exhibit direct hepatotoxic effects under certain dosing conditions or pre-existing disease states [30]. For example, CBD-associated liver injury has been clearly documented in clinical trials, where high doses (or) frequently induce dose-dependent elevations in serum transaminases and clinical signs of drug-induced liver injury requiring rigorous metabolic monitoring [11, 32, 33]. These experimental insights are supported by advanced physiologically based pharmacokinetic (PBPK) modeling and human-derived organoid systems assessing human liver efflux transporter inhibition and baseline cellular toxicity [12, 34, 35]. Similarly, cannabinoids can exert highly variable pharmacological effects based on dose, delivery formulation, and duration of exposure, which may undermine the net protective capacity of raw extracts [36, 37]. Emerging network toxicology models and experimental verifications indicate that exposures can cause abnormal expression across a network of ten crucial hepatic genes—including *ERBB2*, *GPX1*, *MAPK14*, *NR1H4*, *SOD1*,

CXCR2, *PPARG*, *EGFR*, *TYMS*, and *KDR*—disrupting vital antioxidant and metabolic pathways and elevating hepatotoxic risk [38]. Preliminary in vivo models have also confirmed unexpected interactions between hepatotoxic chemotherapeutics and, generating marked elevations in genotoxicity markers and transaminases [39].

4.6 Study Limitations and Future Research Directions

Despite these promising findings of the present study, several experimental limitations must be noted. The relatively small sample size per group may have limited statistical power during certain conservative post-hoc comparisons, contributing to wider confidence intervals. Additionally, this exploratory protocol relied on conventional serum liver enzymes and fixed histopathological grading, without directly quantifying specific tissue oxidative stress biomarkers (such as malondialdehyde or glutathione levels), pro-inflammatory cytokines, or downstream protein signaling expressions.

Future studies incorporating larger experimental cohorts, detailed mechanistic profiling, and extensive gene-expression analysis will provide deeper insights into the precise pathways driving BCP-mediated hepatoprotection.

5. Conclusions

Overall, the present study demonstrates that both β -caryophyllene and full-spectrum cannabis extract exert protective effects against paracetamol-induced hepatotoxicity, with BCP demonstrating superior biochemical and histopathological efficacy. These findings support the potential therapeutic value of BCP as a safe, non-psychoactive cannabinoid receptor modulator capable of dampening the destructive inflammatory and oxidative cascades that drive acute hepatic injury.

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Conflict of interests

Due to the difficulty of obtaining commercial cannabis samples through legal channels, the samples used in this study were sourced from the black market. This limitation may have impacted the consistency and traceability of the materials.

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التأثير الوقائي لمركب البيتا-كاريوفيلين والمستخلص الكامل لنبات القنب (الكانابيس) في الحد من تلف الكبد الناتج عن الباراسيتامول لدى الفئران

الملخص

تُعد السمية الكبدية الناتجة عن الجرعات المفرطة من عقار الباراسيتامول (Paracetamol) أحد الأسباب الرئيسية المؤدية لإصابة الكبد الحادة عالميًا. وعلى الرغم من الاعتماد السريري لمركب "إن-أسيتيل سيستين (NAC)" كعلاج قياسي ومضاد لهذه السمية، إلا أن استخدامه يواجه بعض القيود العلاجية والآثار الجانبية المصاحبة. في المقابل، تُشير الدراسات الحديثة إلى أن المركبات الطبيعية المشتقة من نبات القنب (*Cannabis sativa*)، ولا سيما "البيتا-كاريوفيلين" Beta-Caryophyllene، تمتلك خواصاً مضادة للأكسدة والالتهاب عالية الفعالية، ويُعزى ذلك بشكل رئيسي إلى قدرتها على التنشيط الانتقائي لمستقبلات القنب من النوع الثاني (CB2) علاوة على ذلك، يبرز في هذا المجال مفهوم "تأثير التآزر (Entourage effect)"، والذي يفترض أن التفاعل الديناميكي بين المكونات الكيميائية المتعددة للنبات يعزز من الفعالية العلاجية مقارنة بالمركبات الأحادية.

الكلمات المفتاحية: الباراسيتامول، نبات القنب، البيتا-كاريوفيلين، السمية الكبدية