

Original Research Article

Prevention of dimethyl hydrazine-induced liver damage by β -caryophyllene in rats

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Abstract

Purpose: To investigate the hepato-protection of beta-caryophyllene (BCP) in rats exposed to 1,2-dimethylhydrazine (DMH).

Methods: Sixty male Sprague-Dawley rats were distributed into six cohorts and administered 0.5 mL distilled water, 20 mg/kg DMH, 5 mg/kg BCP, 10 mg/kg BCP, 20 mg/kg DMH + 5 mg/kg BCP, and 20 mg/kg DMH + 10 mg/kg BCP. Liver damage was induced by weekly intraperitoneal DMH administration for 20 weeks, with once daily oral BCP treatment. After treatment, the rats were sacrificed, after which liver function markers, oxidative stress markers, and trace metals were assessed in blood and liver. The liver DNA fragmentation and histopathology were also determined.

Results: The DMH-treated group exhibited significant elevations of alanine transaminase, aspartate transaminase, malondialdehyde, and glutathione-S-transferase ($p < 0.05$), coupled with significant decreases in hepatic superoxide dismutase, catalase, and glutathione peroxidase ($p < 0.05$). The DMH perturbed hepatic trace metal homeostasis by significantly reducing Ca, Cu, and Zn levels ($p < 0.05$), while Fe was markedly raised ($p < 0.05$). A higher frequency of DNA fragments, portal inflammation, and hepatocellular necrosis was found in DMH-treated group. However, co-treatment with BCP led to a remarkable restoration of the markers to near-control values and prevented DMH-induced lesions.

Conclusion: These findings demonstrate that the preventive action of beta-caryophyllene is related to modulation of antioxidant defenses, metal homeostasis, and apoptosis.

Keywords: Liver, 1,2-dimethyl hydrazine, Beta-caryophyllene, Trace metals, Oxidative stress, DNA fragmentation, Histopathology

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INTRODUCTION

Cancer is a leading global cause of death, responsible for 10 million deaths in 2022, with liver cancer accounting for 758,725 deaths and ranking third among cancer-related fatalities [1]. Despite efforts to control it, liver cancer incidence

continues to rise, particularly among nations with low and moderate incomes, where it accounts for 4.3 % of new cancer cases [1]. New cases are projected to reach one million annually.

Liver cancer often results from chronic liver damage due to viruses, drugs, metabolic

diseases, alcohol, and environmental carcinogens. Hepatocellular carcinoma, the most diagnosed hepatic cancer, continues to be the leading cause of liver cancer-related deaths [2]. While liver transplantation is the gold standard for treatment, it is limited by cost, donor availability, and rejection. Other options, including surgery, radiotherapy, and chemotherapy, have significant side effects, driving research into plant-derived chemopreventive agents.

The 1,2-dimethylhydrazine (DMH) is an environmental contaminant that causes cancer in multiple organs, including the liver. The DMH is metabolized in the liver via CYP2E1, generating reactive metabolites such as azoxymethane, methyl-azoxymethane acetate, methyl diazonium, and methyl ions, which form DNA adducts, leading to severe hepatic damage and oxidative stress, which are key factors in hepatocellular injury and carcinogenesis [3]. Consequently, DMH is widely used as a model for studying liver-related pathologies and oxidative stress interventions [4].

Beta-caryophyllene (BCP), a sesquiterpene cannabinoid (CB) found in black pepper, cloves, and cinnamon, has gained attention for its antioxidant, anti-inflammatory, and anticancer properties [5]. Studies show that BCP enhances liver antioxidant defences, reduces oxidative stress, and protects against inflammation by acting as a selective CB2 receptor agonist, which has been shown to prevent liver damage by inhibiting Kupffer cell activation. Given these attributes, this study investigates the chemopreventive potential of BCP in mitigating DMH-induced liver toxicity.

EXPERIMENTAL

Chemicals

Sigma-Aldrich (St. Louis, USA) was the source of DMH, BCP, 2-mercaptobarbituric acid, 2,4-dinitrochlorobenzene, and reduced glutathione (GSH). Kits for liver function test were acquired from Cobas Laboratories (MA, Germany), and other reagents utilized were of analytical quality.

Animal handling

Sixty healthy male Sprague Dawley rats weighing around 170 grammes at 12 weeks of age were acquired from the Animal Holding Facilities at the University of Ibadan's Faculty of Basic Medical Sciences in Ibadan, Nigeria, and acclimatized for one week at the vivarium of Bells University of Technology located in Ota.

Maintained under conventional conditions ($25 \pm 5^\circ\text{C}$, $55 \pm 5\%$ humidity, 12-h light/dark cycle), they had unrestricted access to water and commercially available feed (Hybrid Feeds, Nigeria). Animal care followed National Research Council guidelines [6], and ethical approval was obtained from the Federal Medical Center, Abeokuta (approval no. FMCA/470/HREC/01/2023/12).

Treatment schedule

Rats were randomized into six groups ($n = 10$), receiving: 0.5 mL distilled water, 5 mg/kg BCP, 10 mg/kg BCP, 20 mg/kg DMH, 5 mg/kg BCP + DMH, or 10 mg/kg BCP + DMH. The BCP was orally administered once daily for 20 weeks, while DMH was administered intraperitoneally as per Agner *et al* [7]. After the final dose, rats were weighed before sedation with intraperitoneal injection of ketamine and xylazine at 45 mg/kg and 5 mg/kg, respectively. Thereafter, blood was obtained using ocular puncture for liver function analysis. Cervical dislocation was the method of euthanasia for the rats and the liver was excised, rinsed, blotted, weighed, and divided for oxidative stress, histopathology, and metal analysis.

Evaluation of liver parameters

Liver weight

Liver weight was recorded as the absolute weight (W) in grams, and Final body weight (F) was recorded, while the relative liver weight (Rw) was determined using Eq 1.

$$Rw (\%) = (W/F)100 \dots\dots\dots (1)$$

Determination of liver function markers

Alanine transferase (ALT), aspartate aminotransferase (AST), total protein, and albumin were determined using a Roche/Hitachi C311 analyzer (Mannheim, Germany).

Preparation of post-mitochondrial fraction and determination of oxidative stress

Liver pieces were subjected to homogenization in 0.1 M TRIS buffer at a ratio of five times their weight and spun for fifteen minutes at 10,000g and 4°C . Supernatants were utilized for oxidative stress tests. Malondialdehyde (MDA) levels were measured following the method of Ester Bauer and Cheeseman [8]. Catalase, superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione peroxidase (GPX) were also assessed using Beers and

Sizer [9], Sun and Zigma [10], Beutler *et al* [11], and Rotruck *et al* [12], respectively.

Determination of trace metals

Hepatic samples were assessed for the presence of trace metals using a previously described method with slight modification [13]. Aqua regia, which is a 4:1 mixture of concentrated nitric acid and perchloric acid (HClO₄) in a ratio of 4:1, respectively, was used to digest the liver. Briefly, the liver of each rat was soaked with 10x aqua regia (w/v) in a boiling tube overnight. Digestion was completed by heating the tube for thirty minutes in a water bath set at 100 °C. A clear, digested solution obtained was filtered, and the levels of calcium (Ca), copper (Cu), zinc (Zn), selenium (Se), iron (Fe), manganese (Mn), and magnesium (Mg) were assessed using Optima 8000 Perkin Elmer ICP-OES (MA, USA).

DNA fragmentation

The DNA fragmentation was assessed using a modified protocol based on Afsar *et al* [14]. For this, 100 mg of liver tissue was homogenized in TTE lysing buffer containing 10 % SDS, and the homogenate was incubated at 55 °C overnight with 20 mg/mL proteinase K. Subsequently, RNA was degraded by treatment with RNase at 37 °C for 20 min. The DNA extraction was carried out using 5 M potassium acetate and a chloroform-isoamyl alcohol mixture. Precipitation was

achieved with ice-cold isopropanol at -20 °C, followed by washing with 70 % ethanol-water, air drying, and re-suspending in TRIS-EDTA buffer. The amount of DNA was measured via a nanodrop spectrophotometer. The DNA integrity was measured using electrophoresis with 1 % agarose gel-stained with ethidium bromide (1 %), followed by observation under UV illumination, and documentation by photography.

Histopathological evaluation

Liver samples preserved in formalin were processed into blocks, sectioned, and scored as previously reported [15].

Statistical analysis

The tenth version of GraphPad Prism (CA, USA) was used to analyse the data. Duncan's multiple range test and one-way ANOVA were employed in examining statistical significance at $p < 0.05$.

RESULTS

Organ weight

The results in Figure 1 show that liver and relative liver weights were similar in the entire groups. The lowest values were, however, recorded in DMH-treated group.

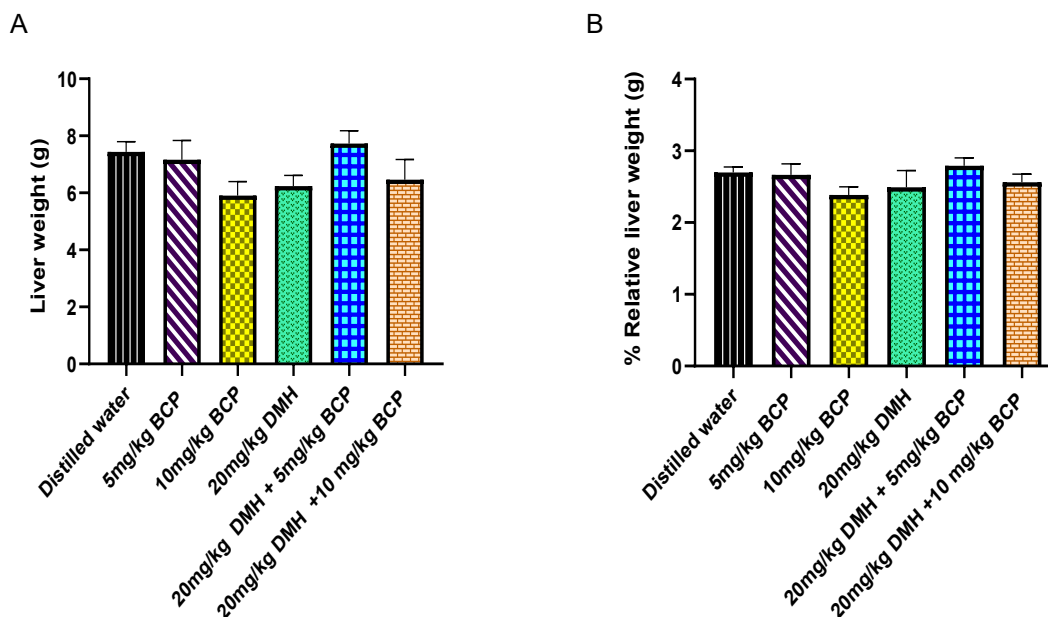


Figure 1: Prevention of DMH-induced reduction of absolute (A) and relative hepatic weight (B) by BCP treatment in rats

Liver function parameters

The serum values of AST, ALT, total protein, and ALB in both test and control cohorts are depicted in Figure 2. Administration of DMH produced significant increases in ALT ($p < 0.01$) and AST ($p < 0.05$) activities in contrast to control values. Supplementation with BCP, however, brought about significant ($p < 0.05$) dose-related decreases in both AST and ALT, contrasted with DMH group. The values obtained were insignificantly different from control. The AST and ALT in rats given either 5 or 10 mg/kg of BCP alone did not differ significantly from control.

Serum total protein and ALB were also similar across all the groups.

Lipid peroxidation and antioxidant enzymes

The impact of DMH and BCP on the peroxidation of hepatic lipid and antioxidant-related enzymes is shown in Figure 3. DMH significantly ($p < 0.01$) elevated MDA levels than control, while BCP co-administration significantly ($p < 0.05$) suppressed MDA in a dose-related fashion. The hepatic MDA concentration in the groups treated with only 5 mg or 10 mg/kg BCP was comparable to control value.

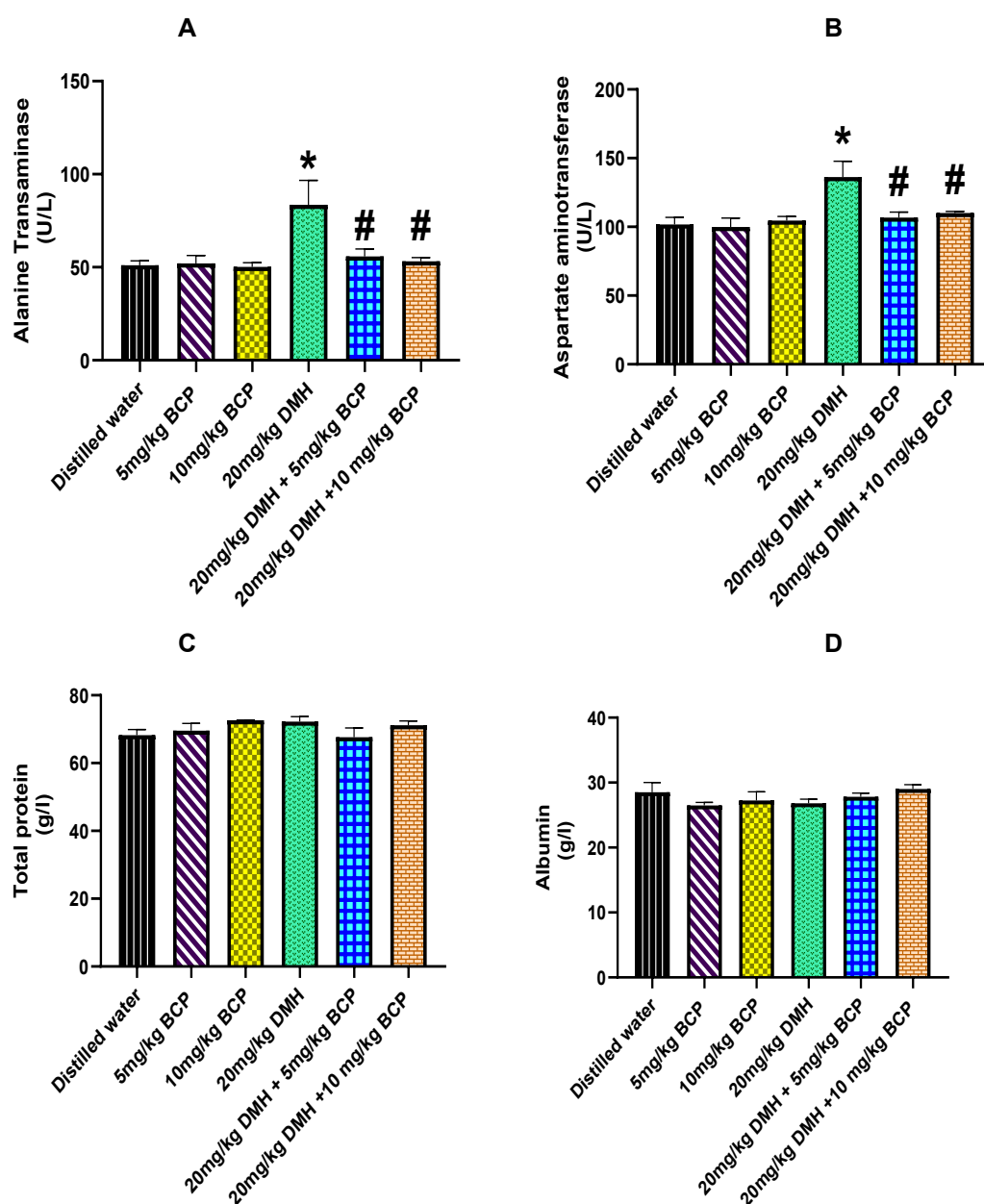


Figure 2: Prevention of DMH-induced alteration of liver function indices by BCP treatment in rats (A) Alanine transaminase, (B) Aspartate aminotransferase, (C) Albumin, (D) Total protein. *Significantly different from control group ($p < 0.05$) except ALT ($p < 0.01$). #Significantly different from DMH-treated group ($p < 0.05$)

DMH treatment decreased catalase activity by 20.5 %, though not significantly, while BCP co-administration restored it. The SOD activity was considerably reduced in DMH group ($p < 0.0001$), but was dose-dependently restored by BCP. The GST activity increased by 39.2 % in DMH group, but BCP treatment reduced it. The DMH treatment notably diminished GPx activity ($p < 0.05$), which BCP also reversed. Antioxidant enzyme activities in BCP-only groups were similar to control values.

Trace metals

The effect of DMH on trace metals in the presence and absence of either of the two doses of BCP is shown in Figure 4. The DMH administration caused significant decreases in hepatic calcium ($p < 0.05$), copper ($p < 0.05$), and zinc ($p < 0.01$) concentrations when contrasted to their corresponding concentrations in control. In contrast, co-administration of either of the two doses of BCP increased the hepatic concentration of the metals to near-control values. While a dose-dependent augmentation of calcium and copper was noted in the BCP co-treatment group, the hepatic elevation of zinc was not dose-dependent.

Although statistical significance was not reached, DMH treatment resulted in a 32.8 % decrease in selenium liver concentration compared to the value recorded for control. Concurrent treatment with 5 and 10 mg/kg BCP, however, returned the selenium concentration to near-control values. Relative to control, the group that only received DMH showed a 39.8 % rise in hepatic iron content. Simultaneous administration of 5 and 10 mg/kg BCP reversed the hepatic iron concentration to a similar value to control. The levels of manganese and magnesium in the liver were comparable among all groups.

DNA Fragmentation

The extent of DNA fragmentation in treated and control rats after administration of DMH and BCP is shown in Figure 5. Bands I, II, and III depict DNA obtained from control and BCP at 5 and 10 mg/kg, respectively, with some degree of shearing. The extent of shearing was escalated in band IV, which contained DNA isolated from DMH-treated group. Additionally, DNA fragmentation was also noticeable in band IV. Bands V and VI depict DNA isolated from the 5 and 10 mg/kg co-treatment groups, which displayed de-escalated shearing compared to DMH-treated groups. Additionally, DNA degradation did not occur in either band.

Histopathological findings

The effect of DMH on liver histology in the presence and absence of either of the two doses of BCP is shown in Figure 6. Photomicrographs of liver sections from control and rats administered BCP alone at both 5 and 10 mg/kg (A – C) revealed normal histological features, including clearly defined hepatocytes with distinct oval shapes, prominent round nuclei, abundant cytoplasm, and preserved hepatic parenchyma. In contrast, liver tissues from DMH-treated rats (D and E) displayed significant architectural disruption, characterized by portal inflammation (arrows) and focal hepatocellular necrosis. Notably, co-administration of BCP at 5 and 10 mg/kg (F and H) effectively attenuated these histopathological alterations, preserving the structural integrity of hepatocytes.

DISCUSSION

The liver plays a crucial role in biotransforming xenobiotics, including DMH, which induces colon cancer and hepatic injury in rodents. The hepatoprotective potential of BCP, a naturally occurring sesquiterpene cannabinoid, was studied in a DMH-induced rat model. The findings suggest that BCP mitigates DMH-induced liver dysfunction, lipid peroxidation, antioxidant depletion, trace metal imbalance, and DNA damage.

Elevated serum AST and ALT levels indicate hepatic damage, as seen in DMH-treated group. These enzymes, biomarkers of liver injury, are released upon hepatocyte membrane disruption. Similar findings were reported in hydrazine-exposed aircraft technicians and DMH-injected rats [4]. Co-treatment with BCP reduced AST and ALT levels, suggesting its hepatoprotective effect via membrane stabilization.

Lipid peroxidation and antioxidant markers further highlight BCP's protective role. DMH bioactivation generates reactive species that damage hepatocellular macromolecules. Consistent with previous studies [4], increased MDA levels and depletion of catalase, SOD, and GPx were observed, exacerbating oxidative stress [16]. The increased GST activity in DMH-treated rats likely represents an adaptive response to detoxify reactive intermediates. The BCP co-treatment normalized MDA and antioxidant enzyme activities, indicating its ability to counter oxidative stress, as also reported in aflatoxin B1-treated rats [5]. The BCP's structure allows radical scavenging, and its activation of CB2 receptors has been linked to reduced hepatic oxidative stress and inflammation.

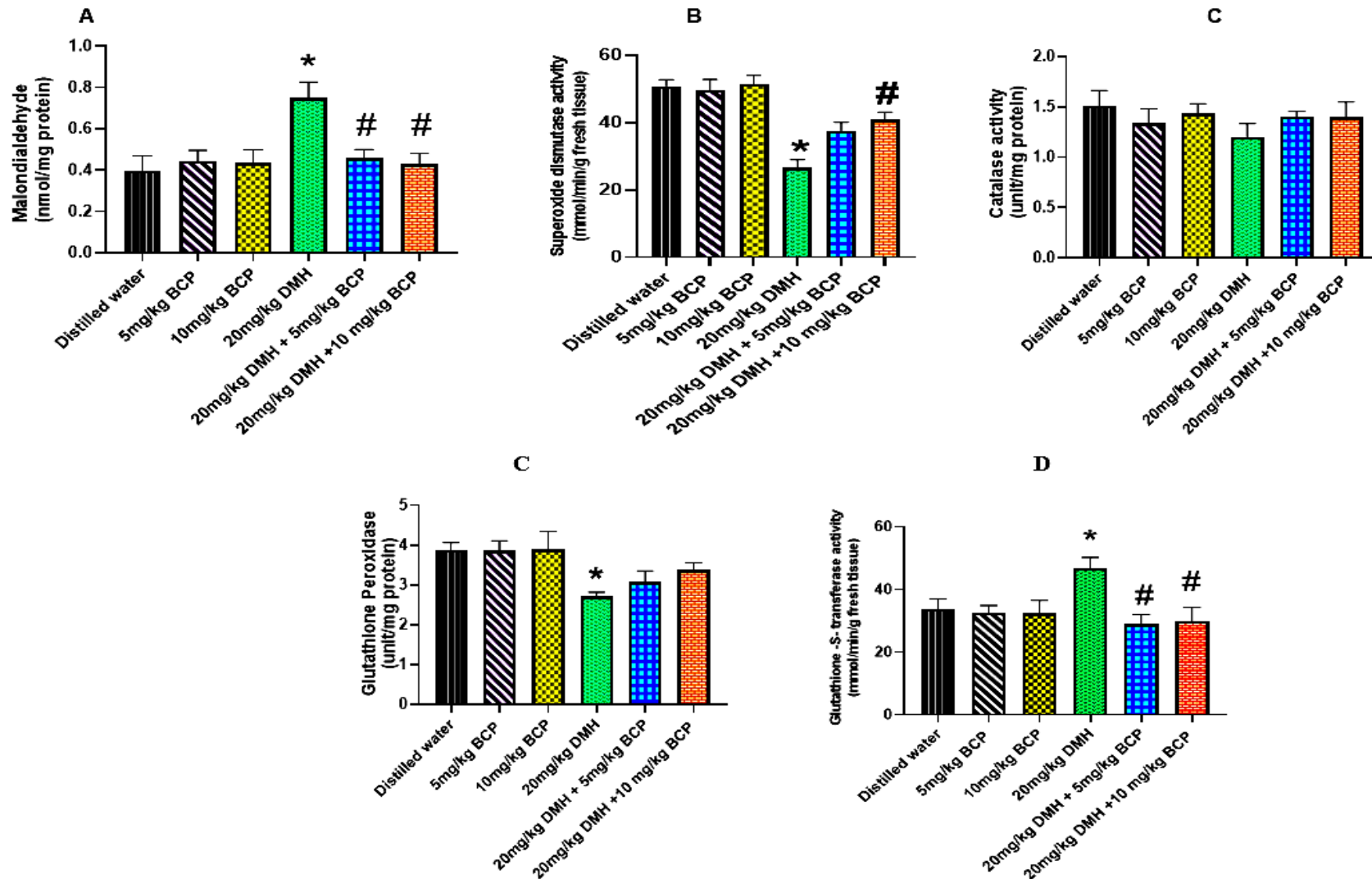


Figure 3: Prevention of DMH-induced oxidative stress by BCP treatment in rats. (A) Malondialdehyde, (B) Superoxide dismutase activity, (C) Catalase activity, (D) Glutathione peroxidase, (E) Glutathione-S-transferase activity. *Significantly different from control ($p < 0.05$) except SOD ($p < 0.0001$). #Significantly different from DMH-treated group at $p < 0.05$

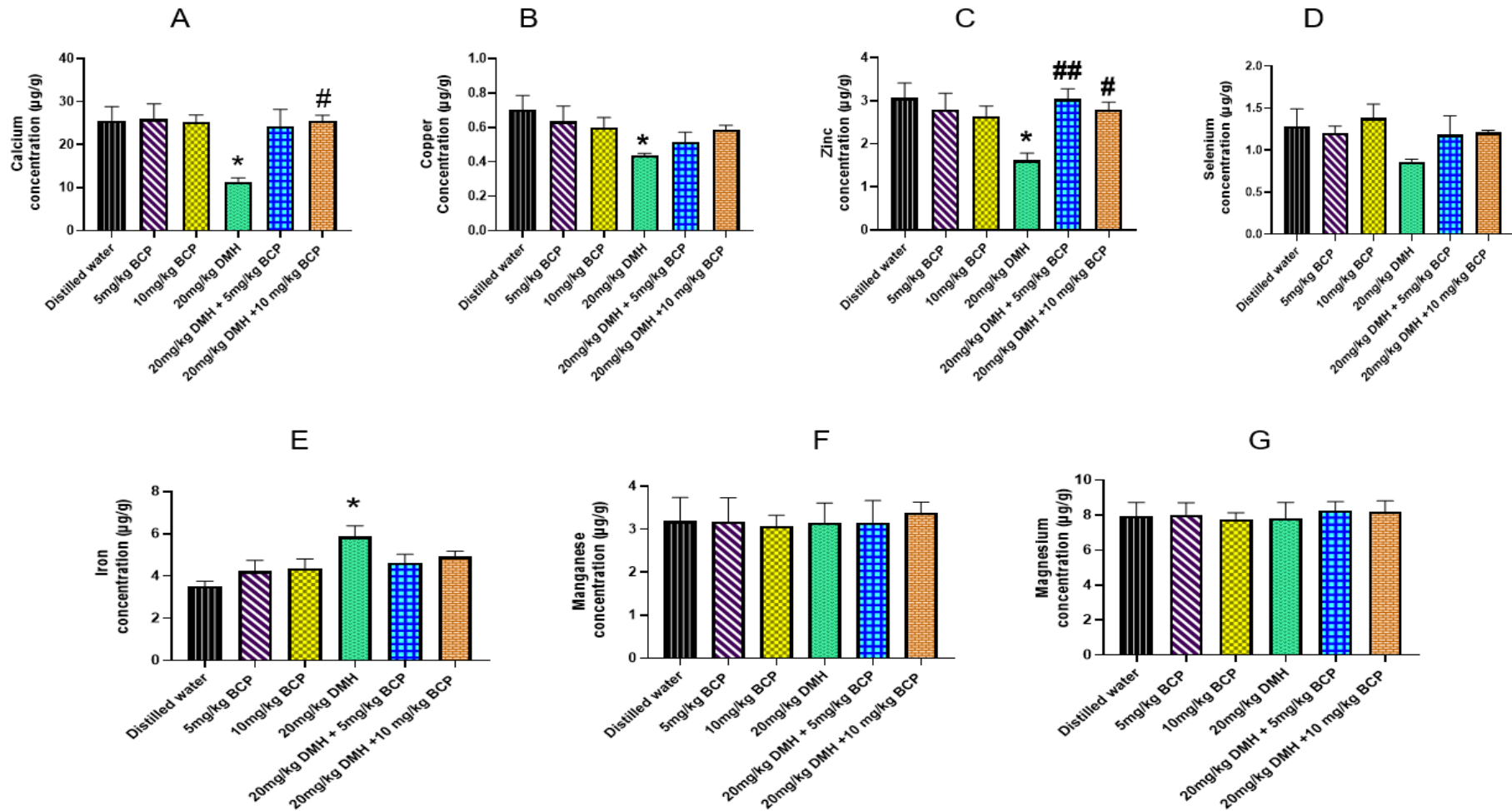


Figure 4: Prevention of DMH-induced hepatic biological metal perturbation by BCP treatment in rats. *Significantly different from control ($p < 0.05$), except for zinc ($p < 0.01$). #Significantly different from DMH-treated group at $p < 0.05$. ##Significantly different from DMH-treated group at $p < 0.01$

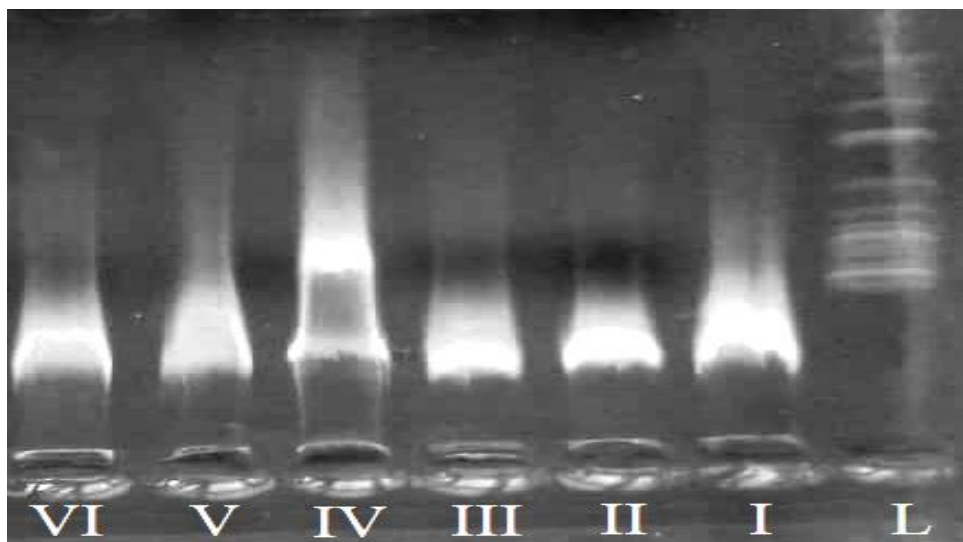


Figure 5: DNA fragmentation in the liver of test and control groups. Band L: DNA marker, Band I: control, Band II: 5 mg/kg BCP, Band III: 10 mg/kg BCP, Band IV: DMH, Band V: 5 mg/kg BCP + DMH, and Band VI: 10 mg/kg BCP

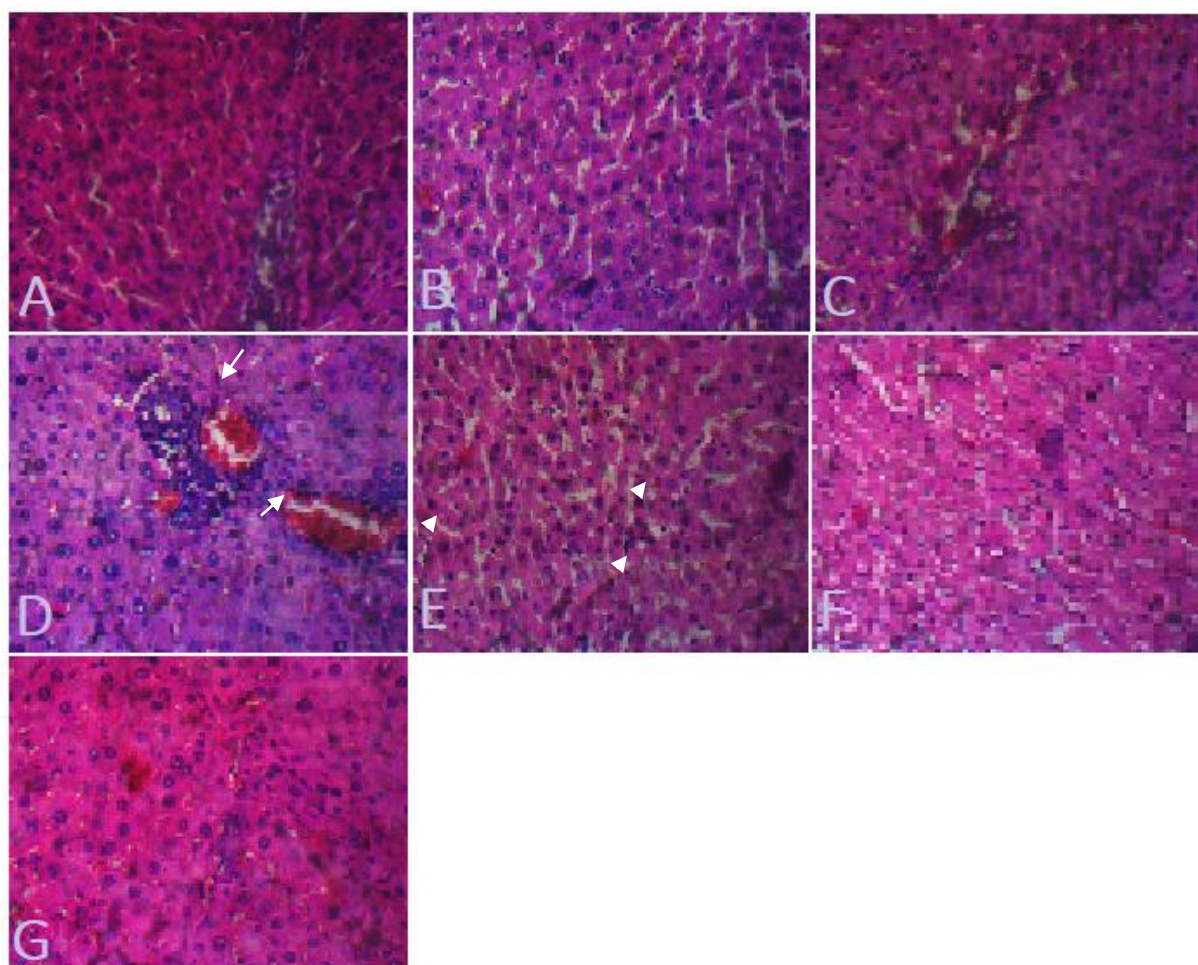


Figure 6: Prevention of DMH-induced hepatic lesion by BCP treatment in rats. Liver histological section (H&E) 100X. Liver section of Control (A), 5 mg/kg BCP (B), and 10 mg/kg BCP (C) with normal histo-architecture. Portal inflammation (arrow) and random hepatocellular necrosis (arrowhead) in DMH-treated group (D&E). Normal hepatic architecture was found in DMH + 5 mg/kg BCP (F) and DMH + 10 mg/kg BCP (G)

Lowered hepatic calcium, selenium, zinc, and copper levels seen in DMH group could result in metabolic dysfunction such as oxidative stress and hepatic injury. Selenium deficiency negatively impacts seleno-proteins like GPX, while zinc deficiency alters cellular redox status and compromises redox-sensitive transcription factors. Reduction of hepatic copper interferes with metabolic processes, including DNA and membrane integrity, energy metabolism, and antioxidant defense.

Cuproenzymes, such as superoxide dismutase, are the most severely affected. Copper depletion also leads to iron overload, which fosters oxidative stress and hepatic lesions via Fenton chemistry. However, BCP enhanced calcium, selenium, zinc, and copper levels while reducing iron, likely through its iron-chelating properties.

DMH-induced oxidative stress and metal imbalance contribute to DNA damage and cell death. Increased DNA fragmentation in DMH-treated rats aligns with prior studies, as DMH forms adducts and induces strand breaks [17]. BCP significantly reduced DNA fragmentation, highlighting its protective role against DMH toxicity. Histopathological analysis confirmed DMH-induced hepatocellular necrosis and portal inflammation, consistent with previous findings [3]. In contrast, BCP preserved liver integrity, reinforcing its hepatoprotective efficacy, as also demonstrated in models of hepatic disease [5].

This study is limited by the absence of direct oxidant measurements and the exclusive focus on enzymatic antioxidants. Further research is needed to explore BCP's effects on non-enzymic antioxidants and elucidate its molecular protective mechanisms against DMH-induced hepatic damage.

CONCLUSION

The data indicate that β -caryophyllene, a natural sesquiterpene cannabinoid, protects the liver from 1,2-dimethyl hydrazine-induced damage by modifying lipid peroxidation, antioxidant systems, biological metal homeostasis, and cell death. The results could be beneficial for clinical practice in preventing liver disease because β -caryophyllene is a permitted dietary additive.

DECLARATIONS

Acknowledgment/Funding

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Ethical approval

The national ethics committee at FMC-A authorized the research protocol employed in the study (FMCA/470/HREC/01/2023/12).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The research was planned and developed with inputs from every author. Reagent preparation, data gathering, and analysis were done by Kazeem Akinwumi, Adegbola Oluwatosin, Eleyowo Oluwole, Odesola Kafilat, Adebayo Eniola, Juliet Nwabueni and Adepoju Aminat. The manuscript's initial draft was written by Kazeem Akinwumi and Aminat Adepoju. The final draft was approved by every author following feedback on previous iterations.

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