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Original article

Antitumor impact of *Clostridium sporogenes* endospores against diethyl nitrose amine induced hepatocellular carcinoma in rats

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A R T I C L E I N F O

A B S T R A C T

Background: The exploration of bacterial endospores as potential antitumor agents has garnered increased attention in recent years. This study investigates the impact of *Clostridium sporogenes* endospores as modifiers of immune regulation on tumor growth, to refine therapeutic protocols for hepatocellular carcinoma. **Methods**: Hepatocellular carcinoma was induced in adult male albino rats using diethylnitrosamine (DEN) (20mg/kg b.wt/day) orally injected five times a week for 6 consecutive weeks) and subsequently treated with endospores (intravenous injection of endospores once at a dose of 60 million spores/rat), and samples were taken from rats after one month of endospore injection. Multiple parameters, including interferon-gamma (IFN-γ), nuclear factor-κB (NF-κB), tumor necrosis factor-alpha (TNF-α), antioxidant enzymes, malondialdehyde (MDA), and liver function enzymes were assessed. **Results**: Results demonstrated a remarkable suppression of tumor proliferation and the induction of enduring immunity through IFN-γ stimulation. The levels of inflammatory markers associated with tumor promotion (NF-κB and TNF-α) witnessed significant improvement. This was paralleled by enhancements in antioxidant status and a reduction in lipid peroxidation in liver tissues of the endospore-treated rats compared to the untreated rats. Histopathological examinations, along with elevated liver function enzymes, provided additional support for these observed improvements. **Conclusion**: this study offers compelling evidence for the potential of safe concentrations of endospores in modulating immune responses and targeting tumor cells, suggesting their plausible roles as adjuvants to augment cancer therapeutic protocols.

Introduction

Globally, hepatocellular carcinoma (HCC) stands as a prominent contributor to liver-associated mortality. The pathogenesis of HCC is intricately linked to prolonged viral hepatitis infection, non-alcoholic fatty liver dysfunction, and liver cirrhosis [1, 2]. The risk of the disease is further increased by an unhealthy lifestyle that includes drinking alcohol, smoking tobacco, having poor eating habits, and having metabolic diseases including obesity and diabetes mellitus. Moreover, environmental

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pollutants, notably aflatoxin B1 (AFB1), play a substantial role in intensifying the severity of the disease [3]. While surgery remains the most effective treatment for HCC, the 5-year age-standardized relative survival rate is only 18.1%, often compounded by tumor recurrence post-removal [4]. Given the difficulty of early detection of HCC, most of patients are diagnosed with a higher stage of the disease at their initial visits, rendering them ineligible for treatments like radiofrequency ablation (RFA) or hepatectomy. Consequently, HCC stands as the second most common cause of cancer-related death in males over 50, after lung cancer [5]. The clinical efficacy of sorafenib and lenvatinib, the two clinically authorized targeted therapy medicines, offers limited extension of survival time [6]. Hence, there is an urgent need for innovative therapeutic strategies for HCC.

In the treatment of solid tumors, immunotherapy has proven to be both effective and safe, leading to long-term survival and controllable toxicity [7, 8]. Despite the remarkable immunological tolerance of the liver, it can even receive liver transplants and reduce its sensitivity to dietary and bacterial antigens via the portal vein [9]. Together, the immunosuppressive tumor microenvironment in HCC and the liver's tolerogenic nature may hinder the development of anti-tumor immunity against HCC. Given the potential for cancer immunotherapy to provide systemic and long-lasting anti-tumor effects, it may be a compelling therapeutic option for HCC, which has metachronous and multicentric occurrences. The FDA has licensed seven immune checkpoint inhibitors targeting cytotoxic T lymphocyte antigen 4 (CTLA-4) or its ligand, programmed cell death-ligand 1 (PD-L1), for HCC and other cancer types [10, 11]. This approval marks a significant advancement for patients with HCC, offering renewed hope. Additionally, emerging immunotherapeutic approaches, such as chimeric antigen receptor-modified immune cells, adoptive cell therapy, personalized cytokines, and cancer vaccines, hold promise and are on the brink of availability [6, 12].

A category of bacteria, known as "oncolytic" or cancer lysing, has demonstrated the ability to selectively target and lyse cancer cells [13]. Beyond this novel capability, oncolytic bacteria offer a more precisely focused approach to cancer therapy with fewer side effects compared to existing conventional methods like chemotherapy or radiation [14]. Among these bacteria is *Clostridium sporogenes (C. sporogenes)*, a rod-shaped, anaerobic bacterium commonly found in soil and the intestines of humans and animals, known for its production of endospores. Notably, *C. sporogenes* is considered safe, designated as a harmless biosafety level 1 organism by the American Type Culture Collection and the UK Advisory Committee on Dangerous Pathogens, classifying it as a harmless hazard group I organism [15]. *Clostridium sporogenes* has been extensively studied in relation to Clostridial-Directed Enzyme Prodrug Therapy (CDEPT). CDEPT includes the use of non-pathogenic Clostridium strains as vehicles for the precise delivery of anti-tumor chemical formulations to solid tumor cells [16, 17]. The development of CDEPT emerged as a response to the imperative to mitigate the often-unpleasant side effects associated with traditional cancer treatments.

Given that *Clostridium* endospores germinate selectively in low oxygen environments, their administration to cancer patients has demonstrated a predilection for targeting hypoxic regions, leading to tumor regression primarily through their oncolytic capabilities [17, 18]. Consequently, spores derived from *Clostridium species* have been extensively investigated as a potential means of introducing prodrug-converting enzymes into hypoxic tumor tissues [17]. This study was conducted to assess the antitumor modality of endospores against diethyl nitrosamine-induced HCC in rats. The focus lies in understanding the effectiveness of these endospores in combatting tumor growth, particularly in the context of a hypoxic tumor environment.

Materials and methods

Chemicals

Reinforced clostridial medium (RCM) (catalog no. CM0149B), Reinforced clostridial agar (RCA) (catalog no. CM0151B), and peptone (catalog no. LP0037B), were purchased from OXOID LIMITED (England). Trypticase soya broth (TSB) (catalog no. VM836259), Sodium chloride (catalog no. K49451104) was purchased from EMD Millipore Corporation (Germany). Ammonium sulfate (catalog no. BP212) was obtained from Thermo Fisher Scientific Inc. (USA). Diethyl nitrosamine (DEN) (catalog no. HY-N7434), and Lysozyme (catalog no. L8120) purchased from Sigma Chem. Co., (St. Louis, USA) and all other

chemicals were of analytical grade and were obtained from standard commercial suppliers.

Clostridium Sporogenes endospore preparation

Clostridium Sporogenes ATCC 19404 (catalog no. 0317) was obtained from American Type Culture Collection (ATCC). *C. Sporogenes was* cultured anaerobically into RCM at 30-35ºC for 3 days. Then, sporulation medium (500 mL) (3% Trypticase, 1% peptone and 1% $(NH4)_2SO_4$) was prepared into screw cap tubes (20 ml of media each tube). Then, 1 ml of grown culture was inoculated into each tube of sporulation medium according to **Perkins** [19] and **Yang *et al.*** [20] with brief modifications. Inoculated tubes were then incubated for 3 days (30-35ºC for 2 days and 40-45ºC for the last day) under strictly anaerobic conditions to encourage endospore formation. Bacterial cells were heated shocked (80°C for 15 min) in water bath and followed by shock in ice cold container according to **Yang *et al.*** [20] with brief modification. Then, *C. Sporogenes* was irradiated with attenuating dose of 2 kGy gamma radiations for endospores production at the National Center for radiation Research and Technology, Cairo, Egypt. This was done using Indian Cobalt-60 gamma chamber 4000 A irradiator at dose rate of 2.5 Krad / h [21].

Endospores were purified by high-speed centrifugation (12,850 xg for 10 min at 4°C) and multiple washes by deionized water. To remove any leftover vegetative cells and enforce endospore release, endospore pellets were resuspended in PBS (1x) supplemented with lysozyme (500 µg/ml) and incubated for 2 hours at 37°C followed by ultrasonication for 5 min. Finally, endospores were extensively washed by deionized water [22- 24].

Endospore cultivability and calculation

The purified spores were suspended in 10 ml of saline solution followed by serial dilution till 10^{-8} dilution. Then, each dilution were added into petri dishes followed by RCA media and incubated for 3 days at 30-35ºC under anaerobic condition. Colonies were counted and the average number was designated as colony forming units (CFU) per volume of original sample. The cultivability was determined as the percentage of spores capable to germinate to vegetative cells.

Animals

The male adult Swiss albino rats weighing (120-150g) used in this study were obtained from the breeding unit of the National Centre for Radiation Research and Technology. Rats were subjected to acclimatization and maintaining on a standard commercial pellet diet and water for one week.

Ethics approval statement

Experimental rats were handled by following the recommendations of the National Institute of Health (NIH No 85:23, revised 1996) for the care and use of laboratory animals and under the regulations of the Ethical Committee of the NCRRT, Atomic Energy Authority, Cairo, Egypt

Induction of liver cancer

Hepatocellular carcinoma was induced by orally administration of (20mg/kg b.wt/day) Diethyl nitrosamine (DEN) dissolved in 0.9% saline five times per week for six consecutive weeks according to **Balamurugan *et al.*** [25].

Endospore injection

Endospores were intravenously IV administered once at a dose level of (60 million spores/rat) according to **Möse *et al.*** [18] with a brief modification.

Experimental design

Rats were categorized into 4 equal groups of 10 rats each as a follow:

Group 1 (Control): Normal healthy control animals.

Group 2 (Endospore only): Normal healthy animals received Endospore for one dose.

Group 3 (Untreated HCC): Animals received diethyl nitrosamine (DEN) orally for six weeks.

Group 4 (Endospore Treated HCC): Animals received (DEN) and then treated with Endospore.

Sampling and preparation of blood and tissue

Rats were anesthetized with urethane at the end of experiment (after one month of intravenous injection of endospores). Blood samples were gathered by cardiac perforation, permitted to coagulate at ambient temperature, and then centrifuged at 4000 rpm for 15 min using a centrifuge (Hettich Universal 32A, Germany). The collected sera were further separated and kept at − 80 °C until use. Liver tissues were excised. A portion was then washed in ice-cold saline solution and homogenates were prepared for biochemical estimation, while the remaining part was used for histopathological investigation.

Biochemical assay

As a marker of oxidative stress, the levels of lipid peroxide and Malondialdehyde (MDA) in liver tissue were measured. A colorimetric kit (Cat no. MD2529, Bio-Diagnostic, Giza, Egypt) was

used to measure the MDA levels [26]. At 95°C, thiobarbituric acid (TBA) and MDA interacted in an acidic solution to produce a pinkish reactive product with a wavelength of 534 nm. Superoxide dismutase (SOD) activity was carried out using a diagnostic kit (Cat no.SD2521, Bio-Diagnostic, Giza, Egypt) according to **Nishlkimi et al.** [27]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye. Percent inhibition and SOD activity was calculated by measuring the change in absorbance at 560 nm. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were carried out according to the colorimetric method described by **Reitman et al.** [28] using spectrum diagnostic kit purchased from Egyptian company for biotechnology (Cat no. 264 and 260 001&002, respectively). The amino group is enzymatically transferred by ALT present in the sample from L-alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. For AST, the amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate. AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm.

Enzyme linked immune sorbent assay

Rat-specific ELISA kits (R&D Systems) were used to measure the levels of inflammatory mediators, tumor necrosis factor-α (TNF-α), and NF-κB in enzyme-linked immunosorbent assays using an ELISA microplate reader (DV990 BV 416; Gio.DE VITA and CO., Rome, Italy) in accordance to the manufacturer's instructions.

Interferon gamma (IFN-γ) level in liver tissue homogenate of each group was assayed using Rat specific (IFN-γ) ELISA kit purchased from Cloud-Clone Corp. (CCC, USA), (Cat no. SEA049Ra). The microplate provided in this kit has been pre-coated with an antibody specific to IFN-γ. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to IFN-γ. Next, Avidin conjugated to horseradish peroxidase (HRP) was added to each well and incubated. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm $\pm$ 10nm. The concentration of IFN-γ in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Histopathological examination

Rats from various groups had autopsies, and the liver tissues were collected. The liver samples were preserved in 10% formalin saline for a whole day. After that, they were cleaned with tap water and serial dilutions of alcohol (methyl, ethyl, and 100% ethyl) were applied to dehydrate them. Following that, the specimens were cleaned in xylene and immersed in paraffin for 24 hours at 56°C in a hot air oven. The tissue blocks' paraffin wax was created, and a slide microtome was used to segment the tissue blocks at a thickness of 4 mm. In accordance with **Banchroft et al.** [29], the resultant tissue sections were then collected on glass slides, de-paraffinized, and stained with hematoxylin and eosin (H&E) stain for routine examination using a light electric microscope.

Statistical analysis

Statistical analysis was accomplished by one-way analysis of variance (ANOVA) then followed by Tukey–Kramer multiple comparison tests. Graph Pad prism 8 was used for statistical analysis (Graph Pad Software Inc, San Diego, California, USA). Data were expressed as mean values $\pm$ standard error of the mean (SEM) and differences between values are considered significance at $p < 0.05$.

Results

Effect of *C. sporogenes* endospore on oxidative stress

Malondialdehyde level is commonly known marker of oxidative stress. Untreated HCC rats showed significant increase in MDA when compared with control normal rats. Treatment of HCC rats with endospores showed significant decrease of MDA levels. **Figure 1** represents MDA levels in liver tissues in different groups. Superoxide dismutase (SOD) is one of the antioxidant proteins. SOD catalyzes the dismutation of superoxide anion to H_2O_2, which is subsequently detoxified to oxygen and water by catalase or GSH peroxidase. Our results showed significant decrease in SOD activity in untreated HCC rats when compared with control normal rats. Treatment with endospores showed significant increase of SOD activity (**Figure 2).**

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

When the liver is damaged, ALT and AST are released into the bloodstream and levels increase, these enzymes reflect liver function status. Activity of these enzymes in serum of different groups were recorded and showed significant increase in untreated HCC group when compared with normal control rats. Both of ALT and AST activities showed significant decrease after endospores injection. **Figure 3** and **Figure 4** represent ALT and AST activity in the serum of different groups, respectively.

Results of Immunological Parameters

Nuclear factor kappa B (NF-κB) play essential roles in the regulation of cell death, inflammation and wound healing which make it an important modulator of hepatic disease progression. NF-κB is a potential link between chronic liver injury, fibrosis and hepatocellular carcinoma. Our data indicated that NF-κB level in liver tissue showed significant increase in untreated HCC group when compared with control normal rats. On the other hand, endospore treatment showed significant decrease in NF-κB level when compared to untreated group. **Figure 5** represents NF-κB level in the liver tissue of different groups.

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells. TNF-α levels showed a significant increase in untreated HCC group when compared with control normal rats. Treatment with endospore showed significant decrease in its level when compared to untreated cancer group (**Figure 6**).

Interferon gamma (IFN-γ) is a cytokine that provides protection against diseases by acting directly on target cells or through activation of the host immune system. IFN-γ levels in liver tissues homogenate showed significant increase in untreated HCC group when compared with control normal rats. Treatment with endospore showed significant decrease in IFN-γ levels when compared to untreated group (**Figure 7**).

Results of histopathological examination

The microscopic examination of control normal rats' liver revealed normal histological hepatic lobular structure, regular arranged hepatic cords separated by sinusoids that lined by Kupffer cells (**Figure 8**). The liver of group that was injected by endospore only showed mild vacuolization of hepatocellular cytoplasm of random distribution presumably to be microvesicular steatosis.

The untreated HCC liver tissues showed severe histopathological alterations with disruption of lobular structure and formation of multiple nodules in hepatic parenchyma with diffuse vacuolization of hepatocytes. The nodular structures were either hepatocellular adenoma with dysplasia or low-grade HCC of eosinophilic and clear cell types. The treatment with endospores significantly reduced the hepatic histopathological lesions score, improved the grade of dysplasia, and reduced the number of hepatocellular carcinomas, and fibrosis grades. Also, inflammation and the grade of biliary reaction were decreased significantly with mild hyperplasia of bile ducts.

Figure 1. Effect of *Clostridium Sporogenes* endospores on Malondialdehyde (MDA) levels in different animal groups. C: control, E: endospores, D: DEN injected rats (untreated HCC), D+E: rat injected with DEN and treated by endospores. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).
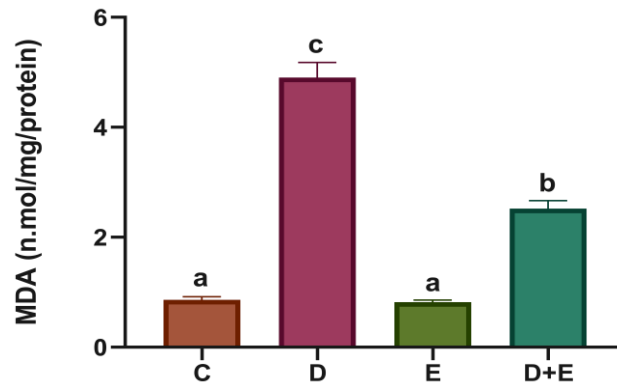

Figure 2. Effect of *Clostridium Sporogenes* endospore on superoxide dismutase (SOD) activity. C: control, E: endospores, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).
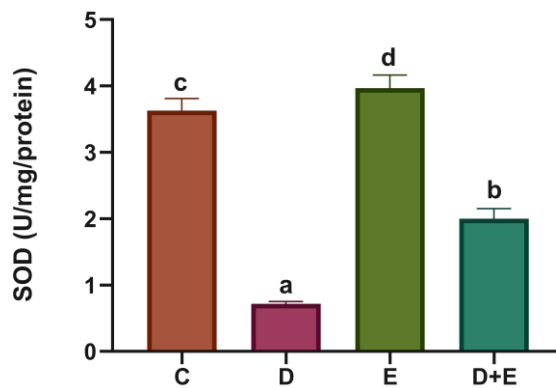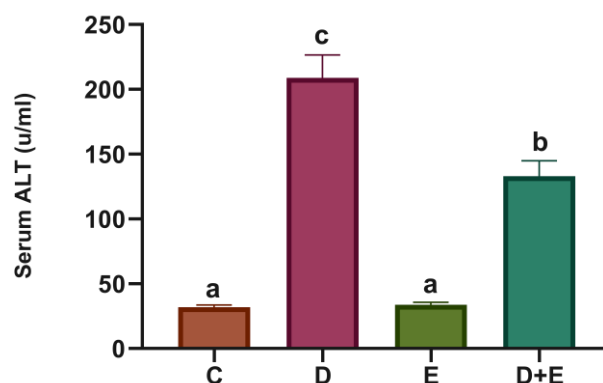

Figure 3 Effect of *Clostridium Sporogenes* endospores on alanine aminotransferase (ALT) activity in different rat groups. C: control, E: endospore, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).

Figure 4 Effect of *Clostridium Sporogenes* endospores on aspartate aminotransferase (AST) activity in different rat groups. C: control, E: endospore, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).
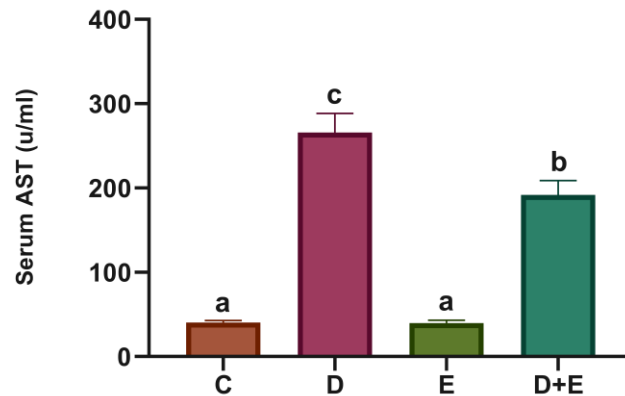

Figure 5 Anti-inflammatory effect of *Clostridium Sporogenes* endospores in NF-κB level in hepatocellular carcinoma in rats. C: control, E: endospores, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).
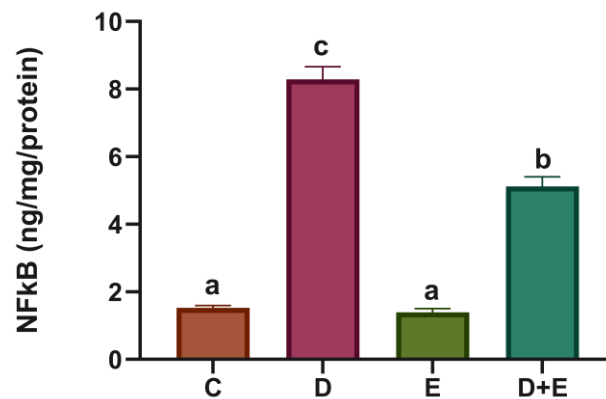

Figure 6. Anti-inflammatory effect of *Clostridium Sporogenes* endospores in TNF-α level in hepatocellular carcinoma in rats. C: control, E: endospore, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at ($p<0.05$).
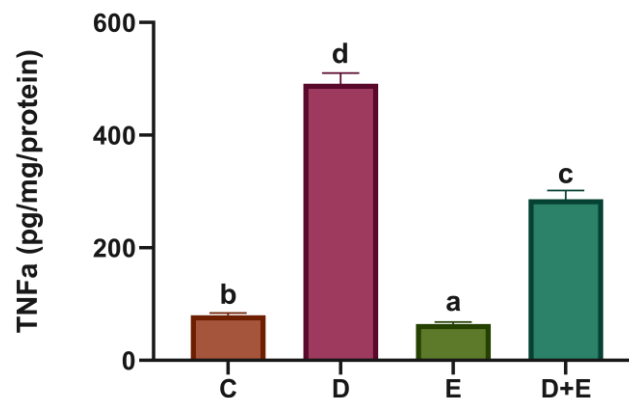
Figure 7. immune stimulating effect of *Clostridium Sporogenes* endospores in IFN-γ level in hepatocellular carcinoma in rats. C: control, E: endospores, D: DEN injected rat, D+E: rat injected with DEN and treated by endospore. Each value represents the mean ± SE (n = 5). Values with different superscript letter were significantly different at (p<0.05).
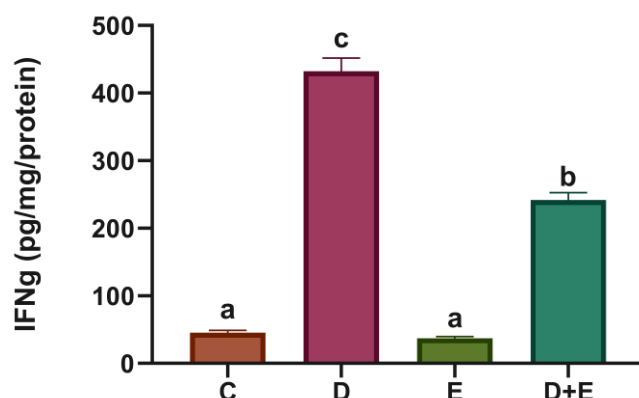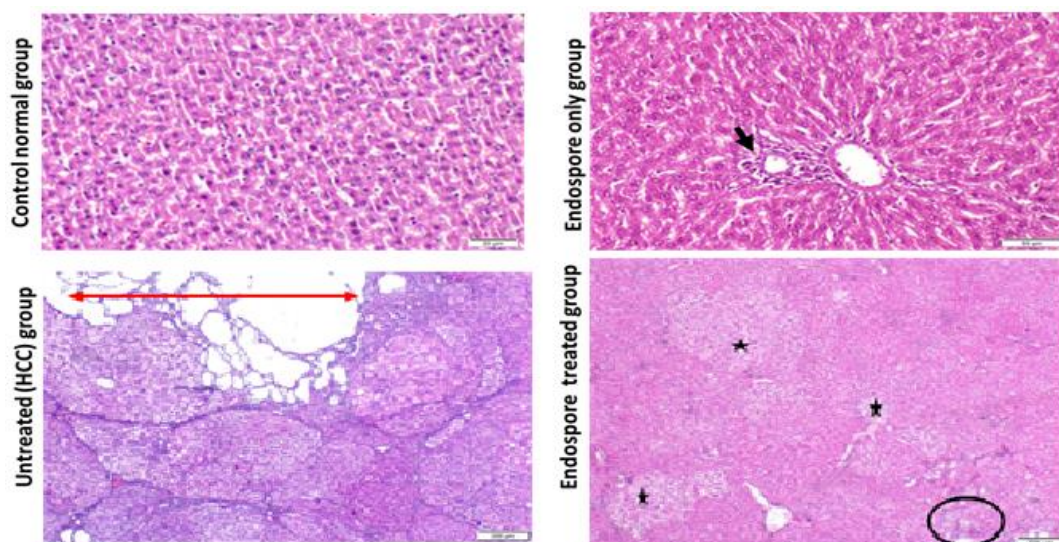

Figure 8. Photomicrographs of rat liver sections stained with haematoxylin and eosin. (H&E x200).

Discussion

Immunotherapy appears to have a wide range of effectiveness, which is most likely dependent on intrinsic tumor features unaffected by treatments mediated by oncolytic bacteria [30]. Owing to the immune system's critical role in the development of cancer, numerous studies have been conducted to create tumor-driven immune therapies, which aim to boost the antitumor immune response and eventually eradicate the neoplasm beforehand. One reported application of bacterial endospores in anticancer therapy is the reactivation of immune responses [17]. In an attempt to improve cancer therapeutic protocols, this study was undertaken to evaluate the antitumor effect of *Clostridium sporogenes* endospore against hepatocellular carcinoma induced in male rats.

According to earlier studies on DEN-induced hepatocellular carcinoma, oxidative damage and ROS formations led to the development of carcinogenesis and were associated with reduced antioxidant enzyme activity, inflammation, and antioxidant depletion [31, 32]. The development of injury at the tissue and cell level is linked to the pathogenesis of liver tissue cancer and the prevalence of oxidative stress indicators in serum. Serum biomarker data corroborated the study's findings, which showed that giving rats DEN elevated the liver damage markers ALT and AST. Similar results were reported by **You et al.** [33] and **Ahmed et al.** [34]. In this investigation, our data showed that stimulation of liver with DEN leads to tissue damage as was established by the elevated levels of ALT and AST. The liver releases cytokines in response to stimulation from DEN. Hepatocyte

membrane injury led to changes in cellular and mitochondrial membrane permeability, which in turn caused an increase in ALT enzyme activity. This is consistent with **Elleithi et al.** [35] findings, which demonstrated a substantial increase in ALT activity in rats with DEN-induced HCC as compared to normal control rats. The degree of hepatic damage indicated by a lower serum ALT level was lessened by endospore therapy.

Moreover, the obtained data displayed a significant elevation in the lipid peroxidation markers associated with depletion in antioxidant enzyme activity such as SOD. According to **Elsonbaty et al.** [36], the antioxidant enzymes are regulators of antioxidants and are in charge of eliminating ROS and other free radicals from tissues and cells. Rats given DEN alone showed higher MDA forms, lower SOD levels, and decreased enzymatic antioxidant activity. Endospores significantly decreased lipid peroxidation levels, protecting liver tissue morphology from DEN-induced oxidative damage [37].

Our results showed a significant increase in the concentration of IFN-γ followed by significant increase in TNF-α and NF-κB concentration in DEN-induced HCC rats compared to normal. Hepatocytes released large amounts of the pro-inflammatory cytokines IFN-γ and IL-6 in response to DEN treatment, which demonstrated the magnitude of liver injury. Hepatocytes express the transmembrane IFN-γ receptor more when they are injured or inflamed in the liver, which likely makes them more sensitive to IFN-γ stimulation [36]. While preventive and therapeutic administrations of endospore revealed a significant hepatic improvement that encounters hepatic toxicity. Endospore triggers a heavy polarization of Th1 responses in its host. It induces NK cells and T cells to produce IFN-γ, thereby increasing cytotoxic cell activity and encouraging an anti-tumor immune response [38]. Tumor growth is inhibited by the enhanced inflammatory response in the tumor microenvironment brought on by the production of Th1 cytokines and the activation of a T cell response. TNF-α can make the vascular lining of tumor cells more permeable, which makes it possible for drugs to enter the cells and for immune and blood cells to infiltrate, causing hemorrhagic necrosis. Tumor cell death is facilitated by the activation of a JNK signaling pathway by TNF-α [39, 40].

On the other hand, our findings demonstrated a noteworthy increase in NF-κβ following the induction of liver cancer using DEN. Key proinflammatory cascade inducers, such as NF-κβ and STAT-3 transcription factors, when activated, increase angiogenesis, invasion, metastasis, apoptotic evasion, and cell proliferation—all well-known cancers [41]. The activation of NF-kβ pathways, which is also an underlying prognosis of hepatocellular carcinoma, is triggered by the translocation of p-p65 into the nucleus [42]. Increased p65 expressions in untreated liver tissues indicate a role for the inflammatory response in the pathogenesis of hepatocellular cancer.

Clostridial endospores are used in bacterial-based cancer therapy, which provides a selective advantage in overcoming necrosis and hypoxia. Since *Clostridium species* are absolutely anaerobic, they can only colonize oxygen-free environments. When injected systematically, spores proliferate in the hypoxic/necrotic regions of solid tumors [43]. Currently, there are several unique cancer therapy techniques that employ this characteristic of Clostridium growth to deliver treatments directly to the solid tumor location by using Clostridium as a vector. As spores, clostridial vectors can be given in a safe manner, and several preclinical trials have shown how well they work to deliver and secrete therapeutic proteins [44].

Clostridium sporogenes has been thoroughly investigated as a prodrug-converting enzyme delivery vector. It was also shown that *C. sporogenes* germinates only in necrotic tissues, and that injecting genetically modified *C. sporogenes* spores with the prodrug PR-104 intravenously inhibits the formation of tumors [17]. Notably, *C. sporogenes* possesses the ability to produce methionine γ-lyase (MGL), an enzyme capable of catalysing the γ-elimination of L-methionine (MET). As MET plays a pivotal role in the growth of malignant tumour cells, MGL has been investigated for its potential anticancer properties [45].

Finally it can be concluded that, *C. sporogenes* endospores revealed a potency to trigger immunity that can target tumor cells. Further studies are required to explore the mechanistic aspects of anti-apoptotic and anti angiogenic effect of endospores.

Conflicting interests

The Authors declare that there is no conflict of interest.

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