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Article

Delving into the death signaling pathway of hemp oil and gamma radiation in solid tumor bearing mice

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Abstract

Many studies reported the diverse therapeutic potential of essential oils. They have a crucial role in cancer prevention and treatment. Antioxidant, antimutagenic, and antiproliferative are mechanisms involved. Also, essential oils may enhance immune function and surveillance, induce enzymes, enhance detoxification, and modulate multidrug resistance. Hemp oil, obtained from *Cannabis sativa* L. seeds, is known for its health-enhancing properties and bioactivity. Adult female Swiss albino mice were injected with viable Ehrlich ascites carcinoma cells (2.5×10^6 cells/mouse), and then administered with hemp oil (20 mg/kg) daily for 10 consecutive days pre-, and then 10 days post-exposure to 6 Gy whole-body gamma irradiation. Hemp oil significantly increased Beclin1, VMP1, LC3, cytochrome c, and Bax. More interestingly, Hemp oil showed a significant decrease in Bcl2 and P13k either alone or in combination with γ radiation. Finally, this study documented the possible role of hemp oil in inducing two cell death types, autophagy and apoptosis, as it may be applied as an adjuvant in cancer treatment.

Key words: hemp oil, gamma radiation, Ehrlich ascites carcinoma, cytotoxicity, apoptosis, angiogenesis, histopathology

Introduction

Hemp (*Cannabis sativa* L.) is an annual herbaceous plant found first in Central Asia, and then widely spread around the world (Izzo et al. 2020), well known for its characteristic that produces a class of terpenophenolic compounds named phytocannabinoids chemical (Hanuš et al. 2016). More than 500 different compounds have been characterized in *C. sativa* phytocannabinoids; flavonoids, terpenoids, and fatty acids being the most relevant families (Mikulcová et al. 2017). It was documented that hemp seed oil is worth it due to its nutritional properties in addition to its health benefits, although its fatty acid composition is most often noted, with oil content ranging from 25%–35%.

Likewise, hemp seed oil contains linoleic acid (LA) and α-linolenic acid (LNA). Omega-6 and omega-3 polyunsaturated fatty acids are the key members, respectively. These fatty acids consist of the most desirable contents of the oil, especially due to the ratios in which they exist. The 3:1 ratio of LA to LNA is alleged to be optimal for nutrition (Kiralan et al. 2010). Moreover, the additional presence of gamma-linolenic acid (GLA) in hemp seed oil ultimately makes its nutritional values superior to most comparable seed oils. Cannabinoids (CBD) are present in hemp seed oil as well (Petrovici et al. 2021). Nowadays, CBD show protective effects on several biological mechanisms, including inflammation, immune response, and oxidative stress. Altogether these effects result in inhibitory activity against cancer (Velasco et al. 2016). The full profile of hempseed oil was documented in Farinon et al. (2020). This study aims to investigate the efficiency of hemp oil in controlling tumor growth and managing the adverse effects of radiotherapy by regulating autophagy and apoptosis as types of cell death involved in tumorigenesis.

Materials and methods

Animals

Adult female Swiss albino mice weighing 22–25 g purchased from the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt, were used in this study. Animals were maintained on a commercial standard pellet diet and tap water ad libitum. Animal maintenance and treatments were conducted following the National Institute of Health Guide for Animals, as approved by Institutional Animal Care and Use Committee. All the ethical protocols for animal treatment were followed by the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and supervised by the animal facilities, NCRRT, EAEA (serial No. 20A/20).

Gamma irradiation procedure

Whole-body gamma irradiation of animals was performed using a Canadian 137Cs Gamma Cell-40 at the NCRRT, Cairo, Egypt at a dose rate of 0.61 Gy/min. Mice were exposed to 6 Gy whole-body γ radiation delivered as a single dose.

Tumor transplantation

Ehrlich ascites carcinoma (EAC) cell line was used. The parent line was obtained from the Egyptian National Cancer Institute (NCI), Cairo University. The tumor line was maintained in the experimental female Swiss albino mice by weekly intraperitoneal injection of 2.5 × 105 cells/mouse. EAC cells were counted before intraperitoneal injection using the bright line hemocytometer and dilution was done using physiological sterile saline solution. In our study, 2.5 × 106 cells/mouse were inoculated intramuscularly in the right thigh of female mice (Aldubayan et al. 2019)

Hemp oil

Hemp oil was purchased from Irish Health Oils (250 mL). It contains polyunsaturated fats (LNA (omega 3), LA (omega 6), GLA), monosaturated fats, saturated fats, and CBD.

Experimental design

Eighty mice were divided into eight groups (10 mice/group). (1) Control group (C); animals were injected with saline intramuscularly in the right thigh. (2) EAC group (E); animals were inoculated intramuscularly in the right thigh with 2.5×10^6 cells/mouse viable cells. (3) Radiation group (R); animals were exposed to a single dose of 6 Gy γ radiation. (4) Hemp oil group (H); animals were injected intertumoral with 20 mg/kg hemp oil daily for 20 days. (5) EAC and radiation (ER); animals in this group were injected as per group (2), after 0.8 mm^3 bulb formation (10 days) exposed to γ radiation as per group (3). (6) Hemp and radiation (HR); animals were injected intramuscularly with hemp oil for 10 consecutive days pre- and post-radiation. (7) Hemp and EAC (HE); animals were inoculated intramuscularly with viable EAC after 10 days and the formation of a 0.8 mm^3 tumor bulb, and then mice were injected intratumorally with hemp oil as per group (4). (8) EAC-hemp-radiation (EHR); animals were injected as per group (2), and then after 10 days, mice were injected intratumorally with 20 mg/kg hemp oil for 10 consecutive days, exposed to a single dose of 6 Gy γ radiation, and then injected again with hemp oil for 10 consecutive days starting from the day of radiation exposure.

Sampling

Intracardiac blood samples were collected, and serum was separated by centrifugation at 3000 rpm and stored at −80 °C until analysis. Tissue samples were washed with saline and divided into two parts, one of them with a known weight has been homogenized, and then the homogenate was centrifuged; the supernatant was collected for further biochemical and molecular analysis. The other was kept in a 10% formalin solution for histological analysis.

Tumor volume monitoring

The change in tumor volume was measured regularly twice weekly using Vernier calipers and calculated by the following formula:

Tumor volume (mm^3) = $0.52A^2B$, as described by Papadopoulos et al. (1989), where A is the minor tumor axis and B is the major tumor axis.

Table 1. Primer sequences for the genes amplified.

Gene symbol	Primer sequence
VMP1	F: 5′-GGTGCTGAACCAGATGATGA-3′
	R: 5′-GCACCAAAGAAGGTCCAAA-3′
Bax	F: 5′-TTGCTACAGGGTTTCATCCA-3′
	R: 5′-GAGTACCTGAACCGGCATCT-3′
Bcl-2	F: 5′-CGGGAGAACAGGGTATGA-3′
	R: 5′-CAGGCTGGAAGGAGAAGAT-3′
β-Actin	F: 5′-AGAGGGAAATCGTGCGTGAC-3′
	R: 5′-CGATAGTGATGACCTGACCGT-3′

MTT assay

Cytotoxicity assay of hemp oils was made using MTT reagent (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, Germany) according to Bahuguna et al. (2017) with modification. Viable EAC (2.5 × 106) cells were inoculated into 96-well flat bottom plates as control. Hemp oil added at several concentrations 5–20 mg/kg. After 24 h, the supernatants were removed and cells were washed with phosphate buffer saline (PBS, Invitrogen Gibco) and incubated with 300 μL MTT/well solution (0.5 mg MTT/mL) in a 5% Co_2 incubator for 4 h, and then cells were pelleted by centrifugation (15 000 ×*g*) for 5 min. The media were removed, and 500 μL of DMSO was added. Next, the samples were vortexed vigorously and the OD was calculated at 560 nm. The absorbance of EAC cells was considered as 100% viability. Each sample was assayed in triplicate in three independent experiments. Percent growth (%) of viable cells exposed to treatments was calculated as follows: % viable = sample abs/control abs × 100.

Biochemical investigations

The levels of Beclin1 were measured by MyBioSource ELISA kit (Cat No. MBS724152_48) and cytochrome c (Cat No. MCTC0) was measured by the ELISA kit supplied by R&D systems.

Molecular investigation

Determination of vacuole membrane protein 1(VMP1), Bcl-2-associated X protein (Bax), and B cell lymphoma 2 (Bcl-2)-like gene expression was investigated. Total RNA was extracted from tumor tissue using RNeasy mini kit (Qiagen, Cat. No. 74104) according to the manufacturer's instructions. First-strand cDNA (cDNA) synthesis was performed using QuantiTect reverse transcription kit (Qiagen, Cat. No. 205311) according to the manufacturer's instructions using 1 mg RNA as a template. By using the Sequence Detection Software (PE Biosystems, CA, USA), RT-PCR was performed in a thermal cycler step one plus (Applied Biosystems, USA). The oligonucleotides utilized in these experiments are listed in Table 1. The reaction mixture of a total volume of 25 mL consisted of 2× SYBR Green PCR Master Mix (Qiagen, Cat. No. 204 143), 900 nM of each primer, and 2 mL of cDNA. PCR thermal-cycling conditions involved an initial step at 95 °C for 5 min; 40 cycles at 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 20 s. The relative expression of the real-time reverse transcriptase PCR

products was determined by the $2^{-\Delta\Delta Ct}$ method. This method calculates a relative expression to the housekeeping gene using the equation: fold induction $\frac{1}{4}2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ $\frac{1}{4}$ Ct (gene of interest (unknown sample) − Ct housekeeping gene (unknown sample)) − (Ct gene of interest (calibrator sample) − Ct housekeeping gene (calibrator sample)) (Flamand et al. 2008).

Western blot analysis

Western immune blotting analysis of LC3, phosphoinositide 3-kinases (Pi3K), and autophagy-related protein 9 (Atg9) proteins in tumor tissue homogenate were performed. Tumor tissue protein was extracted using TRIzol reagent, and protein concentration was quantified according to Bradford (Kruger 2009). Twenty mg of protein per lane was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 10% acrylamide gels and transferred onto PVDA membranes. Membranes were incubated at room temperature for 2 h with a blocking solution (5% non-fat dried milk in 10 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, and 0.1% Tween 20), and then incubated overnight at −4 °C with a primary antibody toward LC3 or Pi3K, or Atg9 proteins with b-actin as a control. After washing three times in washing buffer (10 mmol/L Tris-HCL, pH 7.5, 100 mmol/L NaCl, and 0.1% Tween 20), the membrane was incubated with the secondary monoclonal antibody conjugated to horseradish peroxidase at room temperature for 2 h, and then membranes were washed four times with the same washing buffer. The membrane was developed and visualized by chemiluminescence using the Invitrogen™ detection kit (Catalog #AHO1202) according to the manufacturer's protocols, and then exposed to X-ray film. Quantification of LC3 or Pi3K, or Atg9 proteins was carried out using a scanning laser densitometer (Biomed Instrument Inc., USA).

Histopathological examination

The liver was collected from the different experimental groups and routinely processed. The paraffin-embedded blocks were sectioned at 5 micron thickness and stained with hematoxylin and eosin (Bancroft et al. 2012) for histopathological examination by a light microscope (Olympus BX50, Japan).

Statistical analysis

The SPSS (version 15) was used in the data analysis. Data were analyzed with a one-way Analysis of variance (ANOVA) followed by a post-hoc test (LSD alpha) for multiple comparisons. The data were expressed as mean ± standard error (SE). p values <0.05 were considered to be statistically significant.

Results

To reveal the cytotoxicity of hemp oil against EAC, 2.5 × 10^6 viable EAC cells were added to different doses of hemp oil (5–20 mg/kg). EAC cells were considered as the control group with 100% viability. The cytotoxicity of all doses examined is illustrated in Fig. (1). Twenty mg/kg of hemp oil was preferred because it registers the least number of viable cells.

Fig. 1. Percent of cell viability (MTT) of different doses of hemp oil on EAC cells. Each value represents the mean ± SE. Cells were treated with 5–20 mg/mL of hemp oil and incubated with 2.5 × 10^6 viable EAC cells for 24 h.

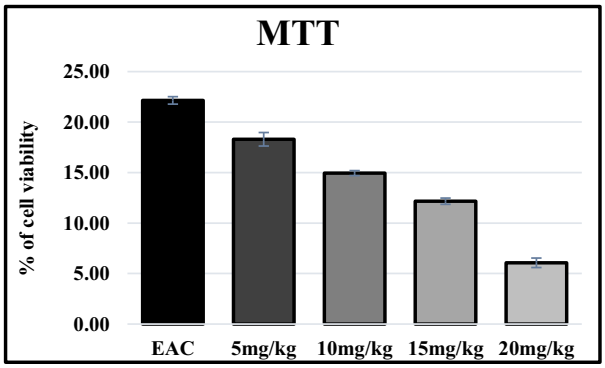
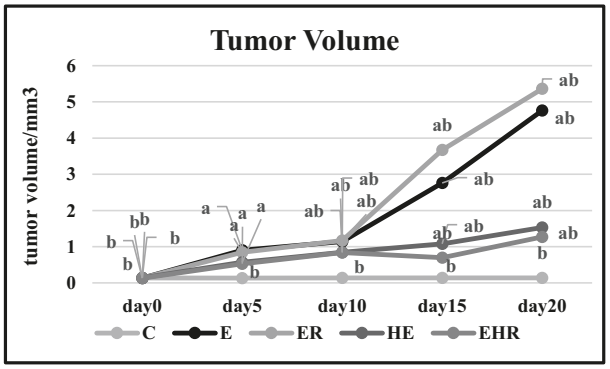


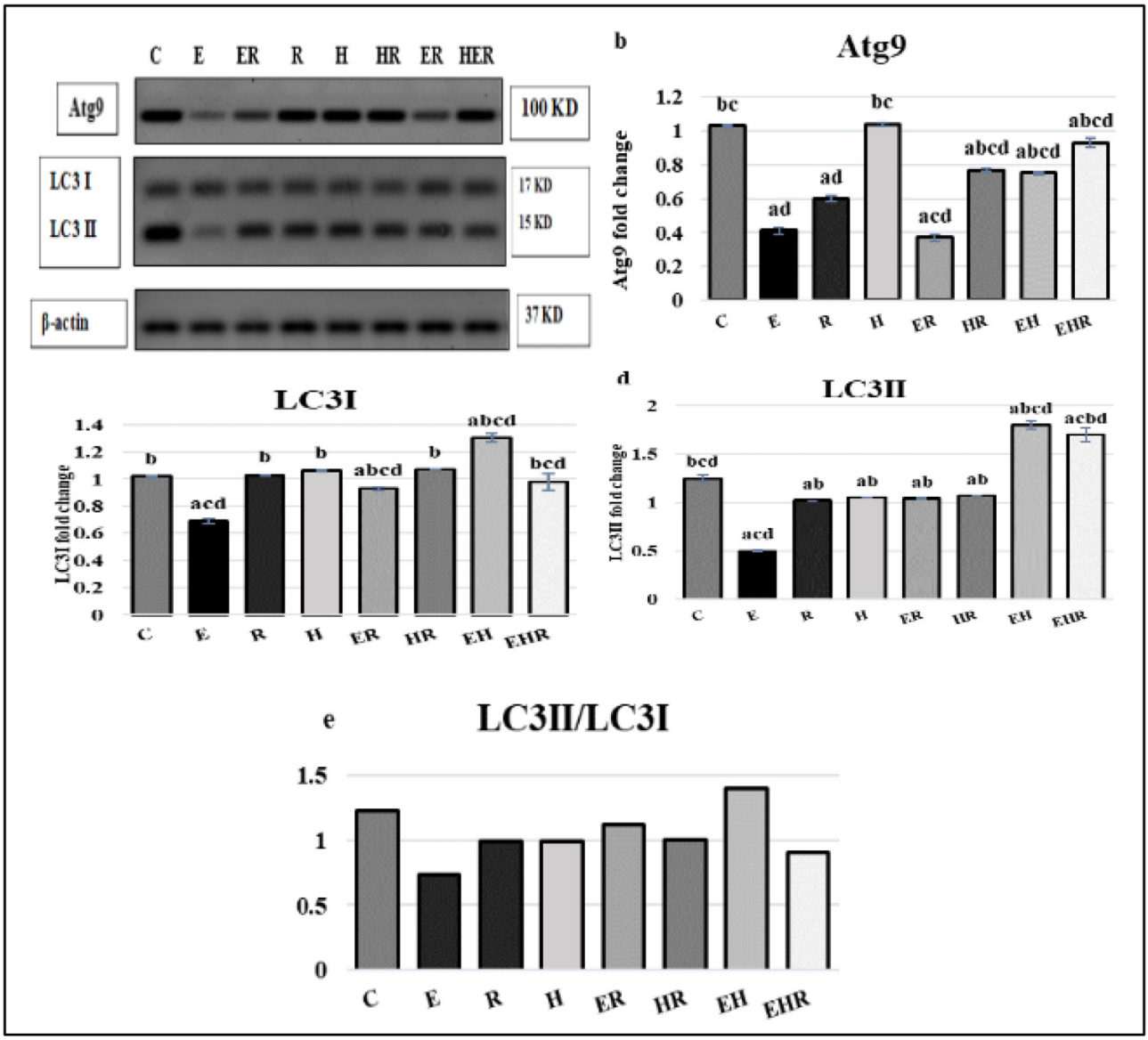
Fig. 2. Effect of hemp oil and (or) γ radiation on tumor size in different time intervals. Each value represents the mean ± SE ($n = 10$). Values with different superscript letters are considered significantly different at $p < 0.05$. a: significant from control and b: significant from EAC.



The right thigh size of mice was measured five times during the experiment beginning from the EAC inoculation. A significant elevation of tumor volume was detected in the group injected with tumor cells alone or with exposure to 6 Gy gamma radiation compared with the C group. On the other hand, treatment with hemp oil significantly ($p < 0.05$) diminished the tumor volume either in the tumor group alone or when combined with radiation (Fig. 2).

Atg9 (a member of autophagy-related proteins) is the only transmembrane protein involved in the core autophagy machinery and is thought to have a role in autophagosome formation. Western blot examination revealed that Atg9 is highly expressed in hemp oil-administered mice (1.04 ± 0.01). Similarly, Atg9 is extremely demonstrated in mice developing EAC, exposed to γ radiation, and administrated with hemp oil (EHR) (0.93 ± 0.03) compared with E (0.41 ± 0.02); these results are shown in Figs. 3*a* and 3*b*. The expressions of LC3I and II are represented in Figs. 3*a*, 3*c*, and 3*d*, and their expression is diminished in the E group compared with the normal C group. On the other hand, their expressions are increased

Fig. 3. The expression levels of autophagic markers, Atg9, LC3I, and LC3II in different mice groups examined by Western blot assay. Each value represents the mean ± SE ($n = 10$). Values with different superscript letters are considered significantly different at $p < 0.05$. a: significant from control, b: significant from EAC, c: significant from radiation, and d: significant from hemp seed oil.



in mice treated with either HE alone or when combined with γ radiation (EHR) as compared with C and E.

Figure 4*a* represents hemp oil and (or) γ radiation effects on Beclin1 activity in different examined groups. Hemp administration (EH) showed a significant elevation in Beclin1 activity compared with the E group. Also, its activity increased significantly in the EHR group compared with the E, R, and H groups. However, the examination of VMP1gene expression revealed a marked reduction in the group injected with EAC compared with the control. While its expression is elevated as a result of hemp oil treatment either alone or in the combination of radiation compared with the E group (Fig. 4*b*).

The effect of hemp oil and (or) radiation on PI3k protein expression is described in Fig. 5. PI3k is highly expressed in the E group (5.75 ± 0.15) compared with C (1.01 ± .01). On the contrary, its expression is declined in mice treated with hemp oil either alone or in combination; EH (1.9 ± 0.11), HR (1.07 ± 0.01), and EHR (1.3 ± 0.1).

Many apoptotic markers have been investigated to validate the knowledge that autophagy is related to apoptosis. As shown in Fig. 6, Bax gene expression analysis and cytochrome c activity showed a significant elevation after hemp oil treatment when compared with the E group. Furthermore, the combination of H and R showed a significant boost in both Bax expression and cytochrome c activity. BCl2 expression was illustrated in Fig. 6*c*. There was a pronounced elevation in E (6.23 ± 0.13) and R (4.6 ± 0.12) groups compared with C (1.04 ± 0.007) group. On the other hand, a significant decline was documented in H (1.04 ± 0.009), HR (2.37 ± .08), and EHR (3.27 ± 0.16) groups compared with the E group.

Fig. 4. (*a*) Effect of hemp oil and (or) γ radiation on Beclin1(ng/mL). (*b*) Impact of hemp oil and (or) γ radiation on gene expression of VMP1(m-RNA). Each value represents the mean ± SE (n = 6). Values with different superscript letters are considered significantly different at $p < 0.05$. a: significant from control, b: significant from EAC, c: significant from radiation, and d: significant from hemp seed oil.

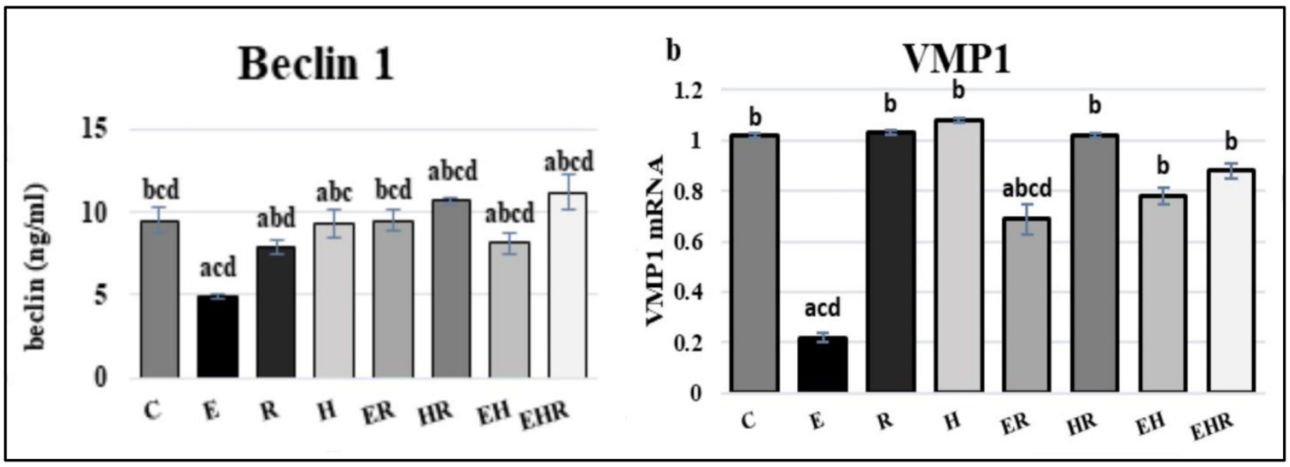
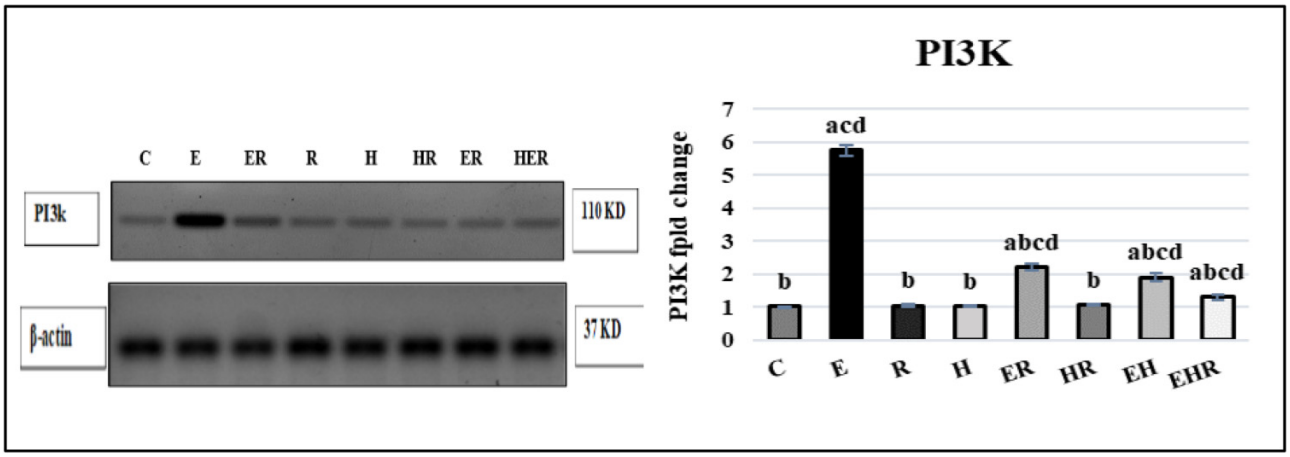


Fig. 5. Protein expression of autophagic markers, PI3k in different mice groups examined by Western blot assay. Each value represents the mean ± SE ($n = 10$). Values with different superscript letters are considered significantly different at $p < 0.05$. a: significant from control, b: significant from EAC, c: significant from radiation, and d: significant from hemp seed oil.



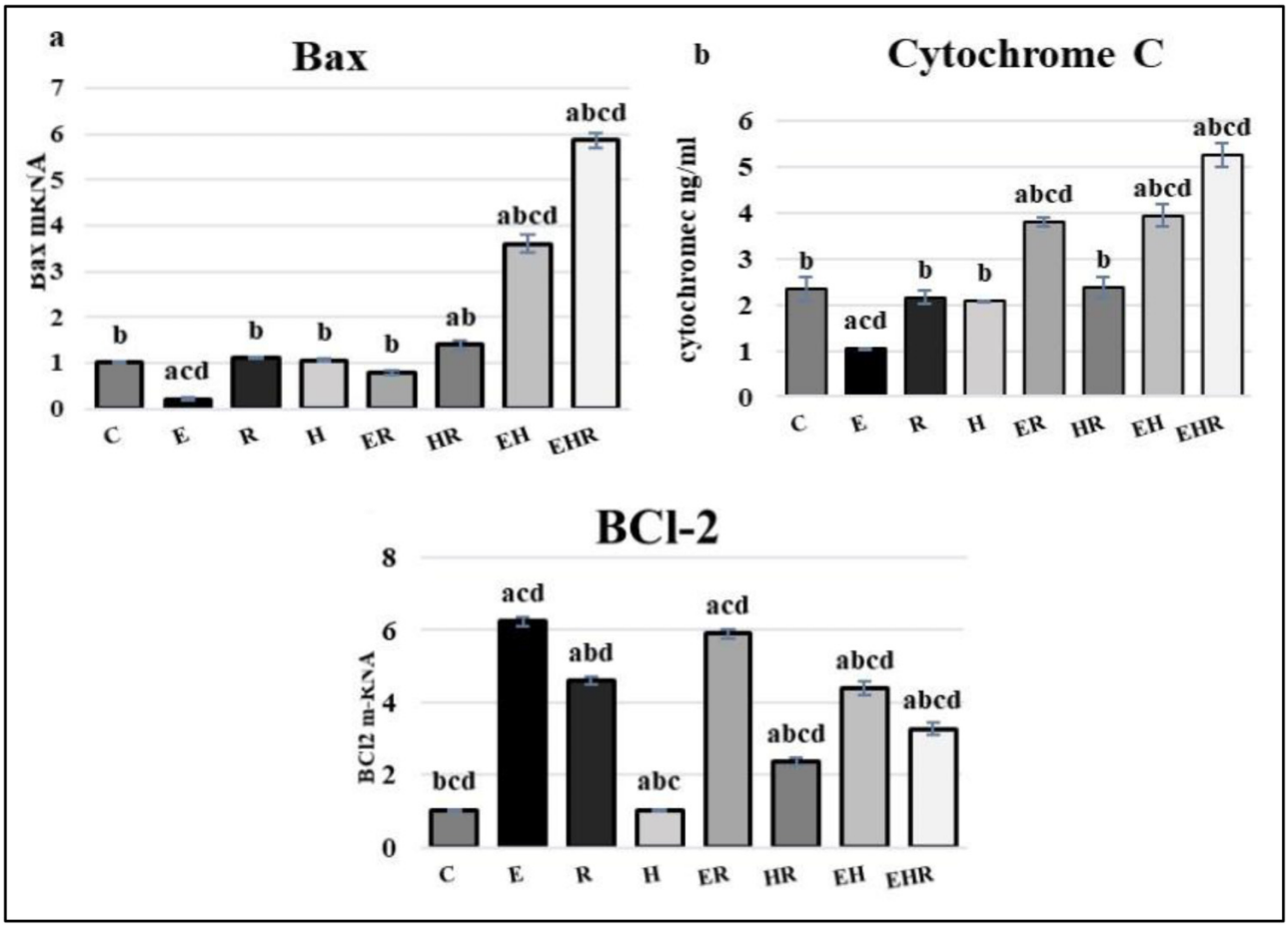
Histopathological findings

The liver tissue of the negative control group revealed standard histological architecture, including central vein, blood sinusoids, hepatic cells, and Kupffer cells (Fig. 7*a*). The EAC group showed a massive tumor and inflammatory cell infiltration, parenchymatous degeneration, and focal necrosis in the hepatocytes (Fig. 7*b*). Histological structure of the liver in the ER mice reported some ameliorations in the hepatic structure, including leukocytic infiltration and focal cytoplasmic vacuolar degeneration (Fig. 7*c*). Gamma radiation-treated mice (R) showed minimal histological changes with focal cytoplasmic vacuolar degeneration (Fig. 7*d*). Both H and HR groups recorded normal histological architecture with no histopathological changes (Fig. 7*e* and 7*f*). EAC tumor mice treated with hemp oil (HE) showed ameliorations in the hepatic structure, together with little tumor and leucocytic cell infiltration (Fig. 7*g*). EAC tumor mice treated with both hemp oil and radiation (EHR) revealed complete recovery of hepatic tissue with no histopathological alterations that appeared as healthy as the control negative group (Fig. 7*h*).

Discussion

Autophagy (self-eating) is a conserved cellular degradation process (Li et al. 2020). It is generally a non-selective response of the body to pressures, which is of great importance under physiological and pathological conditions (Cao et al. 2021). Using gamma radiation is considered one of the classical modalities in cancer treatment. There is growing evidence highlighting the fundamental role of reactive oxygen species (ROS) production in inducing autophagy and cell death in many cancer cell types. Inhibition of ROS production by ROS scavengers blocks autophagy and cell death in many cancer types (Liang et al. 2015). Phytocannabinoids play a vital role in

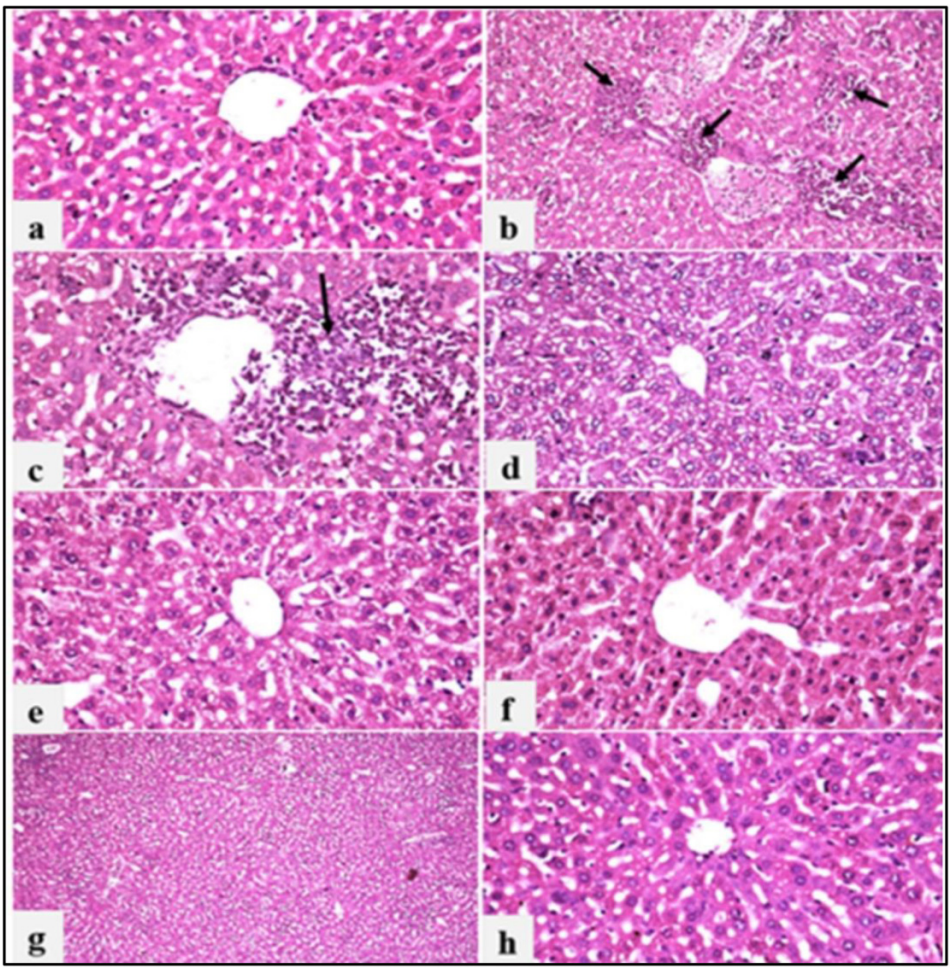
Fig. 6. Hemp oil efficacy on different examined groups. (*a*) Bax gene expression (mRNA). (*b*) Cytochrome c level. (*c*) BCl2 gene expression (mRNA). Each value represents the mean ± SE ($n = 10$). Values with different superscript letters are considered significantly different at $p < 0.05$. a: significant from control, b: significant from EAC, c: significant from radiation, and d: significant from hemp seed oil.



different physiological and pathological processes; they can control tumor growth through the regulation of biological responses strictly related to the carcinogenesis process, such as oxidative stress and apoptosis (Afrin et al. 2020). This study aims to establish the pro-autophagic and pro-apoptotic activity of hemp oil and (or) gamma radiation against EAC-bearing mice.

MTT cytotoxicity assay was carried out in vitro to examine the hemp oil cytotoxicity and measure hemp oil's appropriate dose. Many investigations established a cytotoxic role of CBD in several types of cancer (Ivanov et al. 2020). The tumor cell death caused by hemp oil might be owing to the loss of mitochondrial function, which is one of the trademarks of the apoptotic pathway (Hassan et al. 2016). Atg9 is a crucial regulator of autophagy induction (Staiano and Zappa 2019). It is a membrane protein, thought to be incorporated into the lipid bilayers supply needed for autophagosome formation. In this work, a significant elevation in Atg9 protein expression has been detected in mice treated with hemp oil alone (EH) or combined with gamma radiation (EHR) in EAC-bearing mice. Also, the same results have been detected in Beclin1 activity. Beclin1 (autophagy protein gene) was one of the earliest mammalian autophagy proteins discovered (Li et al. 2020). It functions as a tumor suppressor gene, so it has been deleted in many types of cancer (Vega-Rubín-de-Celis 2020). In this study, the results showed that Beclin1 was declined in the E group compared with the C. On the other hand, its level was increased in H, HR, and EHR groups, which may specify the hemp oil rule in autophagy enhancement. As mentioned earlier, hemp oil is rich with cannabidiol, which exhibits a robust antineoplastic effect in many cancer types (Cerino et al. 2021). Similarly, Liput et al. (2021) mentioned the function of LA (one of the precursors of endogenously produced CBD) in the suppression of cancer cell growth by persuading ROS production and mitochondrial damage. The antiapoptotic protein Bcl2 is known to prevent autophagy by binding with Beclin1 (Liang et al. 2019). Furthermore, the result revealed its mRNA expression was increased in the E group and decreased in the EHR group. More interestingly, Beclin1 binds Bcl2 to form a complex, which prevents autophagy; so to trigger autophagy, Beclin1 must release BCL-2. Many former studies revealed that low expression of Beclin1 and high level of expression of BCL-2 was associated with advanced clinical stage at diagnosis and poor prognosis in cancers (Vega-Rubín-de-Celis 2020). PI3K also has a vital role in autophagy regulation besides its function as a cancer progression key (Yang et

Fig. 7. Photomicrographs of mice liver from different experimental groups stained with hematoxylin and eosin showing, (*a*) control negative group with normal liver of no histopathological changes (×400), (*b*) EAC group with multifocal infiltration of tumor cells and leukocytes (arrows), together with focal necrotic areas (×200), (*c*) ER group with little infiltration of tumor cells and leukocytes (arrow), together with focal cytoplasmic vacuolar degeneration (×400), (*d*) R group with focal cytoplasmic vacuolar degeneration (×400), (*e*) H group with normal liver of no histopathological changes (×400), (*f*) HR group with normal liver of no histopathological changes (×400), (*g*) HE group with ameliorations in the hepatic structure, together with little tumor and leukocytic cells infiltration (×200), and (*h*) EHR group with normal liver of no histopathological changes (×400).



al. 2019). As Lee et al. (2021) study documented, Beclin1 offers a stage for the recruitment and beginning of the P13K class III complex formation. After Bcl-2 dissociation from the Beclin1–Bcl2 complex, the Beclin-1/class III PI3K complex was formed and autophagy is induced. Interestingly, PI3K works as a tumor promotor key in this study; so its expression is elevated in the E group, while decreased in groups treated with hemp oil (Urasaki et al. 2020).

Herein, the radiation impact was illustrated in ER group; Beclin1 level was elevated compared with the E group. As found in Hu et al. (2016), ROS accumulation can initiate autophagy by many pathways; among them is disruption of BeCN1–BCl2 interaction. In agreement with this study, Cerino et al. (2021) showed that CBD displayed its mode of action by inducing ROS production and desensitization of transient receptor potential cation channel subfamily.

A member (TRPA1) possesses a critical role in maintaining cellular homeostasis in response to oxidative stress. Furthermore, the obtained results showed the VMP1 mRNA expression (an ER transmembrane protein). VMP1 has a vital role in the enhancement of BECN1–BCL2 complex dissociation that leads to autophagy activation (Folkerts et al. 2019). Hemp oil showed a significant role in VMP1 mRNA expression, and the exact effect was revealed by gamma radiation as ROS accumulation was present in HR and EHR groups. Conversely, its expression is significantly reduced in E mice.

Microtubule-associated protein light chain 3 (LC3) is one of the main proteins involved in autophagosome formation (Mizushima and Yoshimori 2007). Post-translation, during the autophagosomal membrane formation, the LC3 preform is sliced by ATG4 family proteins to form LC3-I, and then conjugated to phosphatidylethanolamine (PE) to form LC3-II (Yoshii et al. 2017). In this study, LC3-II/LC3-I ratio was detected, LC3I protein expression decreased and LC3-II increased in the hemp oil and EHR groups compared with the C and the E group. These results are in agreement with Marafon et al. (2020), who stated that LC3II/LC3I ratio was detected throughout a short starvation period, so the amount of LC3-I declines

and that of LC3-II rises. LC3-II reflects autophagosomes and autophagy-related structure number and the number of autophagosomes rises with nutrient starvation, therefore the amount of LC3-II also increases (Yoshii et al. 2017). Koay et al. (2014) proved that all the CBD delivered significant LC3-II formation, which leads to autophagy development. In such a manner, high dose gamma radiation can inhibit the autophagy process by increasing LC3I and decreasing LC3II, and the ratio is decreased (R and HR groups). However, several reports establish that various drugs combined with radiation induce autophagic cell death before apoptosis. Hence, autophagy made by radiation also works as a pro-death mechanism (Hu et al. 2016).

In a state of starvation, the cancer cells choose to produce ROS, the vital process that affects autophagosome formation. ROS accumulation in cancer cells can trigger oxidative stress, and then the autophagy pathway is activated. If the autophagy is unable to improve from this oxidation state, they will have to face the fate of death, and apoptosis will start (Gao et al. 2020). Radiation-induced autophagy has a bidirectional effect on cell fate choice; whether cells survive or die depends on the severity and duration of this phenomenon (Hu et al. 2016). The reason for autophagy and apoptosis crosstalk can be drawn that Bcl-2 family subclasses members (Bak, Bax, and BH-3 only) are found in the endoplasmic reticulum and regulate both caspase-dependent apoptosis and autophagy (Das et al. 2021). ROS are generally produced endogenously by mitochondrial damage or exogenously in cells exposed to oxidative stressors; the mitochondria can release cytochrome c, which activates caspases (Gao et al. 2020). So, the data of this study revealed a significant rise in Bax mRNA expression and cytochrome c levels in treated mice compared with the E group; on the contrary, there is a pronounced decrease in BCL2 in the same groups. Moreover, Bcl2 inhibits autophagy and apoptosis in the E group compared with control mice. On the other hand, mice exposed to radiation showed high mRNA expression of BCL2 (ER) compared with the treated group; this may be attributed to the role of gamma radiation in ROS production. Moreover, Ivanov et al. (2020) documented that CBD treatment can activate apoptotic death in glioma cells by both the intrinsic and extrinsic death pathways, whereas γ irradiation enhances apoptotic and non-apoptotic cell death. These obtained results are supported by the histopathological investigations, which recorded nearly ordinary histological architecture in the HR group and amelioration in ER, HE, and EHR groups compared with E mice.

This study has shown that hemp oil can induce apoptosis in mice who developed solid tumors (EAC) by enhancing Bax and cytochrome c and diminishing Bcl2. Similarly, it can activate autophagy via boosting Atg9 autophagic protein, VMP1 and LC31. Additionally, there is a synergetic effect when using hemp oil combined with γ radiation (as radiotherapy) in the activation of both cell death modalities. Further investigations are needed to fully understand the role of radiation and hemp oil in autophagy and apoptosis activation, which may offer a new era in radiotherapy and adjuvant treatment against aggressive cancers.

Accordingly, this study revealed the influence of hemp oil on the mutuel balance between autophagy and apoptosis in Ehrlich-bearing solid carcinoma. However, deeper investigation is revealed to indicate the crosstalk and the switch between autophagy and apoptosis under certain conditions, which is considered a limitation of this study that will be addressed in further study.

Conclusion

This study has shown that hemp oil can induce apoptosis in mice who developed solid tumors (EAC) by enhancing Bax and cytochrome c and diminishing Bcl2. Similarly, it can activate autophagy via boosting Atg9 autophagic protein, VMP1 and LC31. Additionally, there is a synergetic effect when using hemp oil combined with γ radiation (as radiotherapy) in the activation of both cell death modalities. Further investigations are needed to fully understand the role of radiation and hemp oil in autophagy and apoptosis activation, which may offer a new era in radiotherapy and adjuvant treatment against aggressive cancers.

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Notes

All the ethical protocols for animal treatment were followed by the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and supervised by the animal facilities, NCRRT, EAEA (serial No. 20A/20).

Data availability

Data are available as request.

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