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Black Garlic Extract and /or Low Doses of Gamma Irradiation Ameliorate Hepatorenal Functions in the Ehrlich Carcinoma Bearing Mice Model

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BLACK garlic is a unique type of garlic that undergoes a specific processing method involving controlled levels of moisture and heat, resulting in a black color. It is known for its potential health benefits, particularly in preventing diseases such as cancer. Additionally, exposure to low doses of gamma radiation has been found to positively impact tumor regression and enhance immune responses. The current study aimed to evaluate the immunomodulatory effects of black garlic extract supplementation and/or low doses of gamma irradiation against Ehrlich carcinoma (EC)-induced hepatorenal complications in albino mice. On the seventh day after tumor inoculation, black garlic extracts (200 mg/kg body weight) were administered orally *via* gavage for 15 consecutive days, while the mice were also subjected to irradiation at 0.25 Gy twice a week for two weeks. The results showed that the implantation of Ehrlich carcinoma in mice increased oxidative stress in the liver and kidney tissues, leading to histopathological alterations. However, pretreatment with black garlic extract and/or low doses of gamma irradiation significantly improved most of the histopathological and biochemical parameters associated with EC inoculation. In conclusion, the administration of black garlic to the mice, either alone or in combination with low doses of gamma radiation, exhibited an antitumor effect. This was reflected by a reduction in tumor size, inhibition of systemic inflammation, improvement in liver and kidney function, modulation of lipid peroxidation, and enhancement of the antioxidant defense system in mice bearing EC.

Key words: Black garlic extracts, Low doses of gamma irradiation, Ehrlich carcinoma, Liver and kidney complications.

Introduction

Black garlic (BG), derived from the fermentation of fresh garlic (*Allium sativum* L.) at high temperatures and humidity, transforms garlic cloves into dark, and chewy, entities with a sweet flavor. The fermentation duration can vary based on cultural practices, manufacturing processes, and intended uses (Ahmed, 2020). Garlic has a rich historical background in food and traditional herbal medicine, dating back to ancient Egypt, with no documented adverse biological effects from acute, chronic, or inhalation studies (Srivastava and Pathak, 2012). The primary active compounds found in garlic are organosulfur compounds (Pratibha et al., 2023). Black garlic has been noted for its therapeutic properties, including antitumor effects and immune system enhancement (Wang and Wang, 2010; Al-Shehri, 2021). Oxidative stress, driven by an

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overproduction of reactive oxygen species (ROS), and the formation of peroxide radicals (Rahgoshai et al., 2018). Several studies suggest that low-dose ionizing radiation (IR) may stimulate the immune system and enhance its efficacy, a phenomenon known as "radiation hormesis" (Zdrojewicz and Belowsha, 2004; Wall et al., 2006; Sahar et al., 2017).

This reproducible hermetic effect is being explored in a variety of fields, including pharmaceuticals, medicine, toxicology, and radiation biology (Calabrese et al., 2012). Research has shown that IR can increase longevity in various organisms (Caratero et al., 1998; Lopez-Martinez and Hahn, 2014), reduce tumor metastasis (Cheda et al., 2004), improve neuronal function in patients with neurological disorders (Cuttler et al., 2016; Cuttler et al., 2014), enhance the condition of patients with type II diabetes (Kojima et al., 2019), and positively impact those suffering from severe Covid-19 (Dhawan et al., 2020). Furthermore, numerous preclinical studies have identified distinct cellular and molecular pathways linked to IRR-induced hormesis in various disease models (Jiang et al., 2017). The present study seeks to assess the biological effects of black garlic extract in conjunction with low doses of gamma irradiation on oxidative stress and explore biochemical and histopathological changes in hepatorenal tissue functions in Ehrlich carcinoma-bearing subjects.

Materials and Methods

The present study utilized female mice as experimental subjects because research has shown that Ehrlich Ascites Carcinoma (EAC) cells exhibit greater initial growth and total cell count in females compared to males (Vincent and Nicholls, 1967). Adult female Swiss albino mice, weighing between 22 and 25 grams, were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines in Cairo. The mice were kept on a standard commercial pellet diet and had access to tap water ad libitum. They were acclimatized to laboratory conditions for seven days prior to the start of the experiment. All procedures outlined in this study were reviewed and approved by the University Animal Ethical Committee.

Radiation facility

Whole-body gamma irradiation of animals was conducted using a Canadian 137Cs Gamma Cell-40 at the National Center for Radiation Research and Technology (NCRRT) in Cairo, Egypt, at a dose rate of 0.61 Gy/min. Mice were exposed to a total dose of 0.25 Gy twice a week for two weeks, with the gamma radiation delivered as a fractionated dose.

Tumor Transplantation

A cell line of Ehrlich Ascites Carcinoma (EAC) was utilized in this study, courtesy of Dr. Gklien from Amsterdam, Holland. To maintain the tumor line, experimental female Swiss albino mice were given weekly subcutaneous (SC) injections of 2.5 million cells per mouse (El-Gawish, 2003). This work was carried out at the Egyptian National Cancer Institute (NCI) at Cairo University. For the solid form of Ehrlich carcinoma, 2.5 million EAC cells were injected in 2 ml of physiological saline into the neck region of healthy normal mice.

Preparation of Egyptian black garlic extracts (EBG)

According to the previous study of Abd El Wahab et al. (2020), the Egyptian garlic was sourced from a local market in Egypt for the preparation of black garlic. The unpeeled raw garlic bulbs were incubated for 10 days at a temperature between 65 and 70 °C. After this period, 100 grams of black garlic were cut into small pieces and ground with a mortar to form a smooth paste. This garlic paste was then dissolved in 100 ml of distilled water in a conical flask and filtered through gauze to produce an aqueous extract. The filtered solution was stored at 4 °C until it was needed.

Experimental design: The animals were allowed 7 days for adaptation. 50 mice were then randomly distributed into 5 equal groups, 10 mice for each.

The animal groups were recognized as follows:

1. Group (1): Normal control group (N.C): the mice in this group served as controls and were neither treated nor irradiated.
2. Group (2): Ehrlich Carcinoma-bearing group (EC), 10 mice were subcutaneously injected with 0.2ml of 2.5×10^6 /ml/mouse viable EAC cells in the neck region.
3. Group (3): Ehrlich carcinoma black garlic extract-treated group (EC+ Garlic): 10 mice bearing EC were gavage with 200mg/kg bwt black garlic extract for 15 successive days starting from the 7^{th} day after EAC inoculation.
4. Group (4): Ehrlich carcinoma irradiated group (EC+IR): 10 mice bearing EC were Subject to 0.25Gy x2/week for 2 weeks begins on the 7^{th} day after EAC inoculation.

5. Group (5): Ehrlich carcinoma black garlic extract-treated irradiated group (EC+ Garlic +IR): 10 mice bearing EC treated with black garlic extract and subjected to γ – irradiation starting from the 7th day after EAC inoculation.

Monitoring of tumor size

The effects of the black garlic extract on tumor growth were evaluated by monitoring tumor growth for each experimental group. Tumors were measured individually using a caliper. Tumor size was determined by the following formula: Tumor size = length × width^2 × 0.52 (Jia et al., 2005). After 24 hr of the last dose of black garlic extract treatment and 16-hr fasting, animals of each group were sacrificed. Blood samples were collected and serum obtained by centrifugation at 3000 rpm for 10 min for biochemical analysis. Samples of liver and kidney tissue were excised. Parts of the excised liver and kidney tissues were used for the histopathological examination, while the other part was used for the biochemical analysis.

Biochemical assays

serum activities of GPT and GOT and levels of creatinine and urea were measured using colorimetric assay kits, following the manufacturer's instructions (Biodiagnostic, Egypt). The concentrations of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and Caspase-3 in serum were assessed using a standard sandwich enzyme-linked immunosorbent assay (ELISA) technique with an ELISA kit (KOMABIOTECH, Seoul, Korea), adhering to the manufacturer's guidelines. Oxidative damage and antioxidants biomarkers s were measured in liver and kidney tissues homogenate, Lipid peroxides, represented as malondialdehyde (MDA), were quantified according to the methods described by Yoshioka et al. (1979). The levels of reduced glutathione (GSH) were determined following the protocol established by Beutler (1963). On the other hand, Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx) activities were measured using the method described by "Minami and Yoshikawa. (1979) and Gross et al. (1967) respectively.

Histopathological studies

Parts of the excised liver and kidney tissues were fixed in 10% formalin for 48 hr then transferred to 70% ethyl alcohol, processed, and embedded in paraffin blocks. Sections of 5-6 µm thickness were stained with hematoxylin and eosin (H&E) for the histopathology examination.

Statistical analyses

All values are presented as mean ± S.E.M. All groups were compared by one-way analyses of variance (ANOVA) and post hoc multiple comparisons were done with Duncan test in SPSS/ PC software program (version 12.0; SPSS Inc., Chicago, IL, USA) to determine the differences in all parameters. Differences were considered statistically significant at $P \leq 0.05$.

Results

Chemical composition of Egyptian black garlic extracts

The chemical composition of Egyptian black garlic extracts was published in the previous study of Abd El Wahab et al. (2020)

Role of black garlic extract and low doses of γ- irradiation either alone or combined on tumor tissue of mice bearing Ehrlich Carcinoma (EC).

1- EC size monitoring

As shown in Fig. 1, it is clear that the inoculation of 2.5 million EC cells in 2 ml physiological saline in the neck region of healthy normal mice produced a tumour with a mean size of 1136.4 mm^3 after 3 weeks of tumour inoculation (ATI). Gavages of the experimental animals with black garlic extract (200 mg/kg/ day) 7 days after EC inoculation caused a delay of EC size recorded 215.2 mm^3 on the 14th day ATI ($P<0.001$) and reaching 316.4 on the 21st days ATI ($P<0.001$) compared to EC group.

Exposure of the tumour-bearing animals to 0.25 Gy x2/ week for two weeks of γ-radiation begins on the 7th and ends after the 21st ($P<0.001$) day of tumor inoculation. The tumour size exceeds 414 mm^3. Treatments of tumour-bearing mice with black garlic extract and γ-irradiation caused a pronounced delay in EC progression. The tumor size was recorded 227 mm^3 on the 21st day of ATI ($P<0.001$).

2. Anti-inflammatory Markers and Caspase-3

Table 1 revealed that results of EC bearing group of Casp-3, and IL-6, TNF-α compared to control level. Treatment of the experimental mice-bearing EC with black garlic extract produced a very highly significant decrease in the TNF-α and IL-6 levels and a very highly significant increase in the Caspase-3 level against EC-bearing group. Meanwhile, whole-body γ- irradiation of experimental mice bearing EC revealed a very highly significant increase in the Caspase-3 and a significant decrease in the TNF-α level compared to the EC-bearing group. Treatment of experimental mice bearing EC with black garlic extract and exposed to 0.25 Gy twice a week for 2 weeks induced very high significant decrease in the TNF-α level and a very highly significant increase in the Caspase-3 level compared to EC –bearing group.

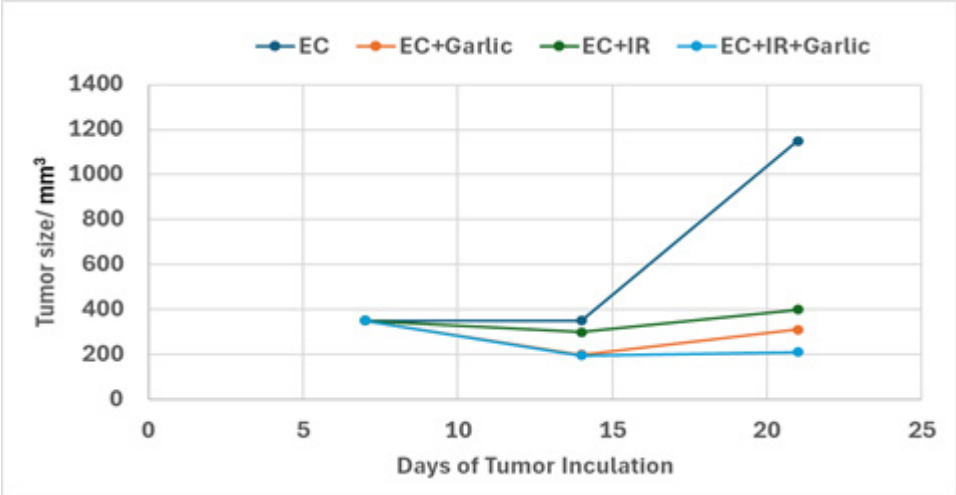

Fig. 1. Effect of black garlic extract and/or low doses γ-irradiation on tumor size of Ehrlich carcinoma.

TABLE 1. Effect of black garlic extract and/or low doses of γ-irradiation on serum levels of IL-6 (pg/ml), TNF-α (pg/ml) and caspase-3 (ng/ml) in different groups in mice bearing EC

parameters Groups	Caspase-3	IL-6	TNF-Alpha
control	29.5±4 [b]	34.2±4.4[b]	45.2±3.9 [b]
EC	58.4±5.5 [a b]	89.5±5.8 [a]	111.4±9.0 [a]
EC+Garlic	73.2±5.4 [a]	46.2±6.8 [a b]	56±4.3 [a b]
EC+IR	64.2±3.9 [a b]	54.2±5.6[ab]	73.2±3.5[ab]
EC+IR+Garlic	68.4±6.6 [a b]	49.2±4.6[ab]	94.2±3.2 [a b]

Each value represents the mean of 10 records ± SE. [a]significant difference versus normal control group bSignificantdifferences versus Ehrlich carcinoma (EC) bearing animalsgroup.

Liver and Kidney Function Tests

Activities of SGOT, and SGPT

In Table 2 liver functions (SGOT, and SGPT) and kidney functions (Creatinine and urea) levels were represented. The control group revealed that the mean value of serum SGOT and SGPT were 340±25.5 and 129±8.2, respectively. The subcutaneous injection of Ehrlich carcinoma cells to mice produced a significant increase ($P<0.05$) in SGOT and SGPT, activities (512±37 and 195±10.9, respectively) in comparison with control levels (340±25.5). Treatment of the experimental mice-bearing solid Ehrlich with black garlic extract revealed a significant decrease in serum SGOT and SGPT, activities by 258±31.8 and168±5.8 in comparison with the EC group. Whole-body γ-irradiation of experimental mice-bearing solid Ehrlich revealed a significant decrease in serum SGOT and SGPT activities in comparison with EC-bearing mice.

Treatment of experimental mice-bearing solid Ehrlich with black garlic extract and exposed to whole body γ-radiation revealed a significant decrease in the SGOT and SGPT activities by 310±30 and 175±10.8 respectively in comparison with the corresponding EC-bearing mice values.

Levels of Creatinine and Urea

The control group revealed that the mean value of serum creatinine and urea were 0.30±0.04 and 37±4.0, respectively. The subcutaneous injection of Ehrlich carcinoma cells on the back of neck region of experimental mice produced a significant increase ($P<0.05$) in serum creatinine and urea, levels (0.45±0.03 and 51±6.8 respectively) in comparison with control levels.

TABLE 2. Effects of black garlic extract and/or low doses of γ-radiation on liver enzymes (SGOT and SGPT) and kidney function markers (creatinine and urea) in serum of different groups in mice.

Parameters / Groups	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg/dl)	Urea (mg/dl)
Control	340±25.5[b]	129±8.2[b]	0.30±0.04[b]	37±4.0[b]
EC	512±37[a]	195±10.9[a]	0.45±0.03[a]	51±6.8[a]
EC+Garlic	258±31.8[ab]	168±5.8[ab]	0.35±0.035[b]	38±2.4[b]
EC+IR	450±40.6[ab]	180±7.8[ab]	0.35±0.04[b]	48±5.0
EC+IR+Garlic	310±30.9[b]	175±10.8[ab]	0.4±0.034[a]	46±4.8

Values are presented as means ± SE of 10 observations.
aSignificant difference versus the normal control group.
bSignificant differences versus Ehrlichcarcinoma (EC) bearing animals group.

Treatment of the experimental mice-bearing solid Ehrlich with black garlic extract revealed a significant decrease in serum creatinine and urea, levels by 0.35±0.035 and 38±2.4 in comparison with the EC group. Whole-body γ-irradiation of experimental mice-bearing solid Ehrlich revealed a significant decrease in serum creatinine and urea levels in comparison with EC-bearing mice. At the same time treatment of experimental mice-bearing solid Ehrlich with black garlic extract and exposed to whole body γ-radiation revealed a development in serum creatinine and urea levels in comparison with the corresponding EC-bearing mice values.

The oxidative stress and the antioxidant status

The oxidative stress and the antioxidant status of liver tissue in experimental female mice bearing Ehrlich carcinoma treated with black garlic extract and/or low doses of γ-radiation are represented in Table 3. Experimental female mice bearing Ehrlich carcinoma recorded a significant increase ($P<0.05$) in liver MDA level by 19.5±1.4, meanwhile, a significant decrease in SOD, GSH, GSH-Px, content (14.5±0.8, 24.5±3.4 and 14.5±2.5 respectively) were observed in comparison with the normal control values. Treatment of experimental animals with black garlic extract at the dose of 200 mg/kg, decreased oxidative stress (MDA level) and increased hepatic antioxidant enzymes compared to that of EC control group. The treatment of EC-bearing mice with black garlic extract was more effective in restoring and correcting the level of the biochemical parameter. Either treatment of female mice bearing Ehrlich carcinoma with low doses of γ-radiation and/or black garlic extract have a role in modulating of their hepatic antioxidant status when comparing with EC bearing group.

TABLE 3. Effect of black garlic extract and/or low dose of γ-radiation on MDA levels and antioxidant status of liver tissue in Ehrlich carcinoma (EC) bearing mice.

Parameters / Groups	MDA mmol/gm	SOD µ/mg	GSH GSH/gm	GSH-PX gm GSH/min/gm
control	6.5±0.6[b]	25.1±0.8[b]	43.2±2.8[b]	35±3.9[b]
EC	19.5±1.4[a]	14.5±0.8[a]	24.5±3.4[a]	14.5±2.5[b]
EC+Garlic	8.6±0.5[ab]	21.3±2.3[ab]	29.5±4.1[a]	21.3±4.3[ab]
EC+IR	14.5±0.8[ab]	15.4±1.3[ab]	39.5±4.1[ab]	24.5±4.1[ab]
EC+IR+Garlic	10.6±0.9[ab]	18.2±1.7[ab]	37.1±2.7[ab]	29.5±4.2[ab]

Values are expressed as Means of 10 records ± standard Error (M±SE).
asignificant difference versus the normal control group.
bSignificant differences versus Ehrlichcarcinoma (EC) bearing animals group.

The oxidative stress and the antioxidant status of kidney tissue in experimental female mice bearing Ehrlich carcinoma treated with black garlic extract and/or low doses of γ-radiation are represented in Table 4. Experimental female mice bearing Ehrlich carcinoma recorded a significant increase ($P<0.05$) in kidney MDA level (13.2±0.6), and a significant decrease in SOD, GSH, GSH-Px, content (14.5±1.7, 19.6±3.3 and 10.2±2.1 respectively) were observed in comparison with the normal control values (23.3±1.6., 37.5±4.8 and 29.7±4.2 respectively). Treatment of experimental animals with black garlic extract at the dose of 200 mg/kg, decreased oxidative stress (MDA level) and increased renal antioxidant enzymes compared to that of EC- bearing group. The treatment of EC-bearing mice with black garlic extract was more effective in restoring and correcting the level of the biochemical parameter. Either treatment of female mice bearing Ehrlich carcinoma with low doses of γ-radiation and/or black garlic extract have a role in modulating of their hepatic antioxidant status when comparing with EC bearing group.

TABLE 4. Effect of black garlic extract and/or low dose of γ-radiation on MDA levels and antioxidant status of kidney tissue in Ehrlich carcinoma (EC) bearing mice.

Parameters / Groups	MDA mmol/gm	SOD µ/mg	GSH GSH/gm	GSH-PX gm GSH/min/gm
Control	4.9±0.5[b]	23.3±1.6[b]	37.5±4.8[b]	29.7±4.2[b]
EC	13.2±0.6[a]	14.5±1.7[a]	19.6±3.3[a]	10.2±2.1[a]
EC+Garlic	7.8±0.5[ab]	19.8±3.0[ab]	29.6±3.3[ab]	15.6±2.8[ab]
EC+IR	6.5±0.95[ab]	20.4±2.3[ab]	24.2±1.4[ab]	19.2±2.3[ab]
EC+IR+Garlic	9.2±0.6[ab]	17.9±3.7[ab]	22.4±3.7[ab]	23.4±2.8[ab]

Values are expressed as Means of 10 records ± standard Error (M±SE).
asignificant difference versus the normal control group.
bSignificant differences versus Ehrlichcarcinoma (EC) bearing animals group.

Histopathological changes in liver tissue in Ehrlich carcinoma-bearing mice treated with black garlic extract and/or low doses of gamma irradiation

The normal histological pattern of the control liver of young mice is as shown in Fig. 2A and 2B. Normal central vein (CV) is surrounded by radiating cords of hepatocytes with prominent Kupffer cells. Normal sinusoidal spaces, branches of the hepatic portal veins (PV), and branches of the hepatic arteries and bile ducts could also be noticed. The liver sections of mice bearing EC carcinoma showed an accumulation of EC cells around congested portal blood vessels with completely hemolyzed red blood cells (RBCs) in the portal vein (Fig. 2C and 2D). Treatment of the experimental mice-bearing solid Eh with black garlic extract and /or low doses of gamma irradiation revealed great disappearance of metastatic EC cells (Fig. 3) from the liver tissue. Some hydropic degeneration in hepatocytes cytoplasm and an increase in Kupffer cells were also detected (Fig. 3 E, 3F, 3J, 3H, 3L and 3M).

Histopathological changes in Kidney tissue in Ehrlich carcinoma bearing mice treated with black garlic extract and/or gamma irradiation

Kidney sections derived either from the control group showed the circular areas of the renal Malpighian corpuscle (Fig. 4A). The kidney sections of mice bearing EC carcinoma showed accumulation of EC cells around the Malpighian corpuscle and the convoluted tubules (Fig. 4B). Treatment of the experimental mice-bearing solid EC with black garlic extract and /or low doses of gamma irradiation revealed great disappearance of metastatic EC cells (Fig. 3C, 4D, and 4E) from the kidney tissue.

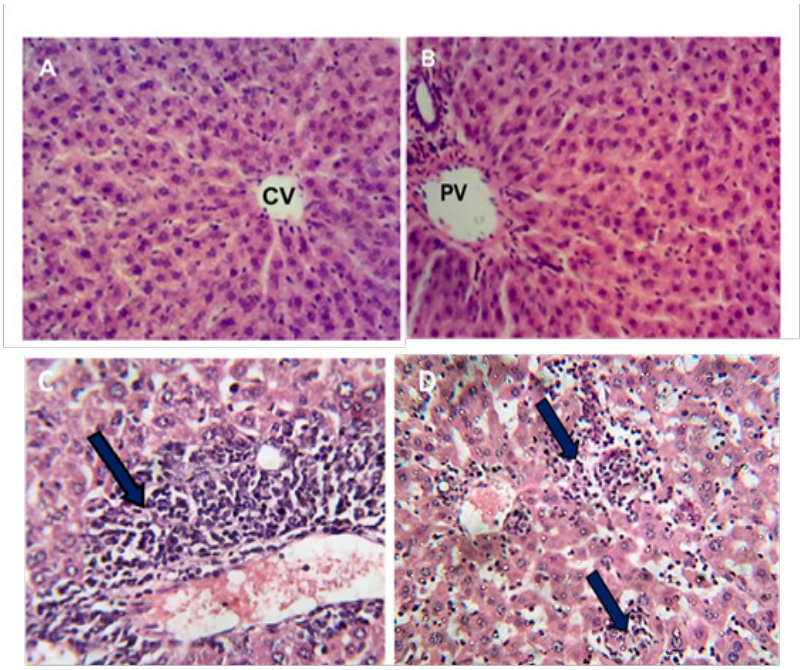

Fig. 2. Photographs of sections in the liver of mice. A, B: Control sections showing the normal appearance of hepatocytes and the central vein (CV) in A and portal vein (PV) in B. C, D: Liver sections of mice bearing EC represented the accumulation of EC cells (blacked arrow) around a congested portal blood vessel with completely haemolysed RBCs (H&E stain X 400).

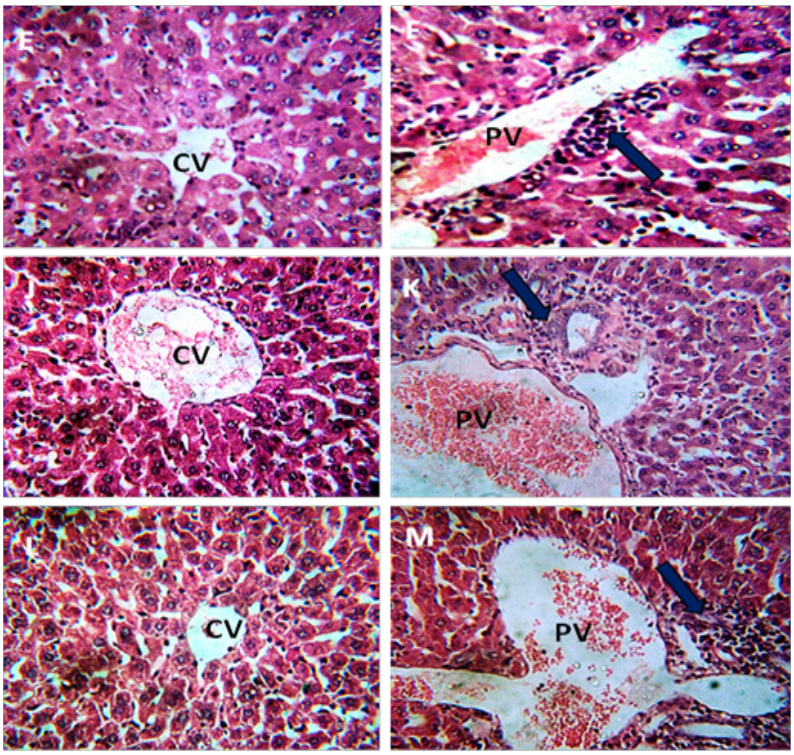

Fig. 3. Photographs of sections in the liver of mice bearing Ehrlich carcinoma (ECs). H: Liver tissue represents great disappearance of metastatic ECs in the garlic extract-treated group. I, J: Liver tissue represents some appearance of metastatic ECs (blacked arrow) around the portal blood vessel (PV) in the irradiated group. K: Widened CV (↔) and disappearance of metastatic EC carcinoma cells were observed in the liver of garlic extract and irradiation treated group. (H&E stain X 400).

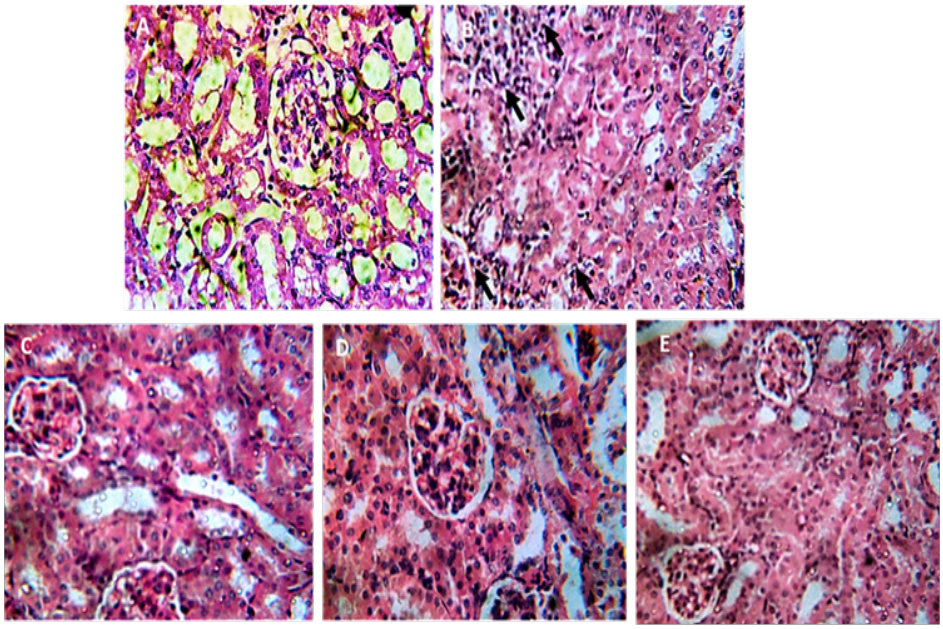

Fig. 4. Histopathology of the kidney. Light micrographs of the mice kidney sections from the different treatment groups. (A) Control kidney mouse section showing normal renal cortex and glomerular tufts, (B) infiltration of Ehrlich carcinoma cells surrounding the distorted glomeruli and tubules. The disappearance of metastatic EC carcinoma cells was observed in the kidney sections of the garlic extract and/or irradiation treated group (C, D, E) (H&E X 400).

Discussion

Cancer remains one of the leading causes of death worldwide, with its incidence steadily increasing and further rises anticipated in the coming years (Bray et al., 2018). The primary treatment options for cancer include surgery, radiation, and chemotherapy. However, chemotherapy is often linked to cancer recurrence, the development of drug resistance, and severe side effects (Talib and AbuKhader, 2013). In this study, the inoculation of 2.5 million EAC cells into the neck region of healthy mice led to a tumor size exceeding a mean of 1136.4 mm^3 after three weeks. A solid tumor is characterized as an irregular tissue mass that lacks cysts or liquid and is primarily composed of epithelial cells (Anand et al., 2011). As tumors grow, the production of reactive oxygen species (ROS) can influence other organs in the body. ROS can activate nuclear factor-κB (NF-κB) and initiate the phosphorylation of its inhibitor, IκB. This activation allows NF-κB to move into the cell nucleus, where it binds to DNA and regulates the transcription of various target genes, including inducible nitric oxide synthase, cyclooxygenase II, and cytokines, which can lead to cell damage (Aschner et al., 2007). Notably, in tumor cells, cytokines can stimulate NF-κB, which protects these cells from TNF-α-induced apoptosis (Pöppelmann et al., 2005). The activation of NF-κB in cancer cells regulates the expression of genes that promote cell proliferation, inhibit apoptosis, and enhance invasion. Consequently, NF-κB activation contributes to tumor growth and metastasis while diminishing apoptosis induced by cytokines (Takada et al., 2006). Inflammation is a fundamental response of the body to infection and injury, playing a crucial role in the wound healing process by facilitating the deposition of extracellular matrix and fibrosis in the liver. There is significant evidence indicating that proinflammatory cytokines, particularly TNF and IL-6, are vital in the progression of steatohepatitis (Tilg, 2010). Tumor necrosis factor-alpha (TNF-α), a 17 kilodalton cytokine produced by monocytes, macrophages, natural killer cells, and certain T lymphocyte subsets (Goeddel et al., 1986), is involved in both promoting and inhibiting tumor development. It can originate from tumor cells or inflammatory cells within the tumor microenvironment. The role of TNF-α in chronic inflammation and its tumor-promoting properties are well-documented (Mocellin et al., 2005); it plays a pivotal role in the initiation, proliferation, angiogenesis, and metastasis of various cancers.

In this study, female Swiss albino mice exhibited systemic inflammation, as indicated by elevated serum levels of TNF-α. The observed rise in this proinflammatory cytokine may be linked to the adverse effects caused by Ehrlich carcinoma (EC), including oxidative stress. This oxidative stress can initiate lipid peroxidation, leading to the release of malondialdehyde, which combines with hepatocyte proteins and triggers a harmful immune response. This process stimulates neutrophil chemotaxis and activates the transcription factor NF-κB, further escalating the production of proinflammatory cytokines (Duvnjak et al., 2007).

Caspases, which are aspartate-directed cysteine proteases, play a significant role in the initiation and execution of necrosis, apoptosis, and inflammation. Their dysfunction can lead to tumor growth and various autoimmune diseases (Ghavami et al., 2009). Upon activation, caspases cleave cellular substrates, resulting in the characteristic morphological changes associated with apoptosis (Zhang et al., 2000; Savill and Fadok, 2000).

The implantation of Ehrlich carcinoma tumors in female Swiss albino mice has been shown to induce oxidative stress, leading to oxidative damage, organ dysfunction, and metabolic disturbances. Oxidative stress is a key mediator of reactive oxygen species (ROS)-induced hepatorenal dysfunction and has been implicated in numerous pathological conditions, including tumor promotion and cancer.

Previous studies have indicated that cancer can develop in any organ at any time, characterized by a malignant transformation that disrupts normal cell-cycle regulation, resulting in uncontrolled proliferation and lack of differentiation. Excessive damage and toxicity from EC have been observed in the liver and kidneys (Maha et al., 2022). Histopathological examination of the liver showed a buildup of EC cells around congested portal blood vessels in the cancer group, along with significant inflammation. The migration of Ehrlich carcinoma cells into the liver parenchyma can contribute to liver carcinoma and cellular degeneration (Islam et al., 2014).

Common liver enzymes, SGOT and SGPT, are reliable indicators of liver injury (McLellan et al., 2003). When liver injury occurs for any reason, these enzymes are released into the bloodstream (Sushmita et al., 2012). The marked increase in SGOT and SGPT levels in the Ehrlich group may result from hepatocellular damage caused by the inoculation of EACs. The presence of tumors can significantly disrupt the activities of vital organs, particularly the liver, regardless of the tumor's location (De Wys, 1982).

Furthermore, our findings indicate that the induction of Ehrlich carcinoma disrupted kidney function, evidenced by elevated serum levels of urea and creatinine, likely due to renal tissue injury induced by the tumor. This aligns with Hussein's (2003) findings that tumor presence increased serum creatinine levels in mice. Salem et al. (2011) also reported elevated serum urea and creatinine levels in female mice following exposure to Ehrlich carcinoma. These results corroborate those of Abd El-Wahab and Fouda (2009) and Badr et al. (2016), who documented glomerulus atrophy, degeneration of renal tubules, leukocyte infiltration, and the presence of protein casts within the renal tubules. Additionally, Salem et al. (2011) demonstrated that Ehrlich carcinoma induced damage in both the renal tubules and glomeruli in the cortex and medulla. Abd El-Wahab and Fouda (2009) and Chakraborty et al. (2007) noted infiltration of internal organs by EC cells, resulting in clusters of inflammatory cells.

Many researchers have suggested that ROS and oxidative stress play critical roles in cancer pathogenesis (Jenner, 2003). Oxidative damage affects both membrane lipid bilayer fluidity and permeability, potentially leading to lipid peroxidation (Pandey and Mishra, 2003). The level of malondialdehyde, a product of lipid peroxidation, increased in the cancer group, likely due to heightened lipid peroxidation caused by EAC. The antioxidant glutathione, a potent inhibitor of neoplastic processes, is predominantly found in high concentrations in the liver. It can be concluded that treatment with garlic alone/or in combination with low dose of gamma radiation might ameliorate the hepato-renal dysfunctions ensured associated with Ehrlich carcinoma solid tumor by improving the biochemical and histopathological investigations, however the amelioration in EC group treated with garlic was more significant than EC and IRR group compared with EC. Also, it is recommended to use black garlic as adjuvant agent parallel with chemotherapeutic drugs.

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