academicJournals

Vol. 6(2), pp. 20-28, March, 2014
DOI: 10.5897/JCREO2013. 0105
ISSN 2141-2243 ©2014

http://www.academicjournals.org/JCREO

Journal of Cancer Research and Experimental Oncology

*Full Length Research Paper*

# Effects of omega-3 fatty acids against Ehrlich carcinoma-induced hepatic dysfunction

**Ussama Z. Said[1], Neamat H. Ahmed[1]*, Amina M. Medhat[2] and Mustafa M. Mustafa[1]**

[1]National Center for Radiation Research and Technology, Atomic Energy Authority (AEA), Egypt.
[2]Faculty of Science, Ain Shams University, Cairo, Egypt.

Received 31 October, 2013; Accepted 26 January, 2014

**Omega-3 essential fatty acids (ω-3 FATs); found in the highest concentrations in fish oil, claim a plethora of health benefits. The present study aims to evaluate the biological effects of ω-3 FATs supplementation against Ehrlich carcinoma (EC) induced inflammation, oxidative stress, biochemical and histopathological alterations in the liver tissue of albino mouse. ω-3 FATs were orally administered via gavage to mice for a period of 30 consecutive days at a dose of 300 mg/kg body weight. On the 7th day of ω-3 FATs administration, female mice were subcutaneously injected with 0.2 ml of Ehrlich ascite carcinoma for solid tumor induction. The present study revealed that, subcutaneous injection of Ehrlich solid tumor led to hepatic oxidative stress (as significant increase in lipid peroxidation (thiobarbituric acid reactive species, TBARS), concomitant with a significant decrease in glutathione and antioxidant enzymes), systemic inflammation (significant increases in C-reactive protein, tumor necrosis factor-alpha and leukocyte counts) and biochemical alterations (as increase in liver function enzymes)). While in the tumor tissue, significant increase in tumor TBARS content and non significant changes in glutathione and antioxidant enzymes were observed. Histopathological studies showed that EC cells metastasis caused fatty degeneration, enlargement of liver cells nuclei and presence of necrosis. Pretreatment of animals with ω-3 FATs significantly reduced tumor size and markedly improved most of the biochemical parameters associated with the inoculation of EC. It could be concluded that ω-3 FATs administrated to mice, reduce tumor size, inhibit systemic inflammation, improving liver function profile, modulating lipid peroxidation and augmenting antioxidant defense system in EC bearing mice.**

**Key words:** Ehrlich carcinoma (EC), omega-3 fatty acids, liver.

## INTRODUCTION

Malignancy is one of the most serious diseases afflicting mankind today. The cancer burden in developing countries is increasing as a result of increasing ageing and growth population, and adaptation of cancer-associated lifestyle choices including smoking, physical inactivity and 'Westernized diet' (Preetha et al., 2008). Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis (Mantovani, 2005). Chronic inflammation and the metabolic products of phagocytosis are often accompanied by the excessive formation of reactive oxygen and nitrogen species that are potentially damaging to DNA, lipoproteins, and cell membranes (Schottenfeld and Beebe-

*Corresponding author. E-mail: neamathanafi@ymail.com.

Dimmer, 2006). Inflammatory cells also release metabolites of arachidonic acid, or eicosanoids, including prostanoids or prostaglandins and leukotrienes (O'Byrne and Dalgleish, 2001). Animal bearing experimental Ehrlich carcinoma (EC)revealed low activity of antioxidant system (Bhattacharya and Haldar, 2011). In addition, free radicals, particularly oxygen radical, play an important role in the complex course of multistep carcinogenesis (Marnett, 2000). First, an oxidative stress can induce DNA damages that lead to genomic instability and possibly stimulate cancer progression (Oberley, 2002). Secondly, elevated reactive oxygen species (ROS) levels are responsible for constant activation of transcription factors and the progression of the disease (Gupta et al., 1999).

Resistance to conventional anticancer therapies in patients with advanced solid tumors (Patyar et al., 2010); in addition to the side effects that patients experience from conventional chemotherapy, number of studies demonstrated that oils rich in omega-3 essential fatty acids (ω-3 FATs) decrease the tumor weight and metastasis number (Espada et al., 2007). ω-3 FATs; found in the highest concentrations in fish oil, claim a plethora of health benefits. Fish oil-derived ω-3 FATs seem to prevent cancer by influencing the activity of enzymes and proteins related to intracellular signaling and, ultimately, cell proliferation (Bartsch et al., 1999).

These fatty acids are known to have pleiotropic effects, including effects against inflammation (Browning, 2002), platelet aggregation and hyperlipidemia (Hu, 2001).

The main purpose of the current study is to explore the major complications that occur due to experimental EC implantation in female mice, and to explore the potential role of ω-3 essential fatty acids for prevention of tumor progression and disturbances induced in the antioxidants status and metabolic profile.

## MATERIALS AND METHODS

Because Ehrlich ascites carcinoma (EAC) cells were reported to show greater initial growth and total cell count in female than male mice (Vincent and Nicholls, 1967), the present study used female mice as experimental subjects. Adult female Swiss albino mice weighing 22 to 25 g purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. The animals were maintained on a commercial standard pellet diet and tap water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

### ω-3 FATs treatment

ω-3 FATs capsules were purchased from Kirkland Signature Company (Kirkland, Washington, USA). The product is supplied as transparent soft-gelatin capsules filled with light-yellow oil tablets of 300 mg ω-3 FATs (eicosapentaenoic acid (EPA): 180.0 mg/g and docosahexaenoic acid (DHA): 120.0 mg/g). It was suspended in doses of 300 mg/kg body weight/day during 30 successive days(An et al., 2009).

### Tumor transplantation

A cell line of EAC supplied through the courtesy of Dr. Gklien, Amsterdam Holland was maintained in experimental female Swiss albino mice by weekly intraperitoneal injection of $2.5 \times 10^6$ cells per mouse (El-Gawish, 2003). The solid form was done by inoculating $2.5 \times 10^6$ cells per mouse subcutaneously between scapulae in the neck region. After 7 to 8 days from EAC cell inoculation, solid tumor was observed.

### Experimental design

Healthy Swiss albino mice were randomly assigned into four experimental groups (10 mice/group), which were classified as follows: control group (1), the mice in this group were orally administered 1 ml water/mouse daily and served as control group for one months; EC group (2), each mouse in this group was injected subcutaneously (SC) in the neck region with 0.2 ml of EAC which contained $2.5 \times 10^6$ cells for solid tumor induction; ω-3 FATs group (3), the mice in this group were orally treated with omega 3 (300 mg/kg body weight) daily for 30 days from the 1st day of the experiment; ω-3 FATs + EC group (4), the mice in this group were orally treated with ω-3 FATs (300 mg/kg body weight) daily for 30 days, on day 7 each mouse was injected subcutaneous with $2.5 \times 10^6$ EAC cells for solid tumor induction.

### Monitoring of tumor size

The effects of the ω-3 FATs on tumor growth were evaluated through monitoring of tumor growth for each experimental group. Tumors were measured individually using a caliper. Tumor size was determined by the following formula (Jia et al., 2005):

$$\text{Tumor size} = \text{length} \times \text{width}^2 \times 0.52$$

After 24 h of the last dose of ω-3 FATs treatment and 16-h 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 tumor tissue were excised. Parts of the excised liver and tumor tissues were used for the histopathological examination, while the other part was used for the biochemical analysis.

### Biochemical assays

The activities of aspartate and alanine transaminases (AST and ALT) were assayed by the kinetic method using available commercial kits (Spinreact, Spain) according to the method described by Young (2001), while alkaline phosphatase (ALP) activity was assayed depending on the method of Roy et al. (1970) using Biodiagnostic kit. The activities of lactate dehydrogenase (LDH) in serum were assayed by the kinetic method using available commercial kits (Spinreact, Spain) according to the method described by Young (2001). The levels of tumor necrosis factor-alpha in serum were assayed by the standard sandwich enzyme-linked immune-sorbent (ELISA) assay technique using ELISA kit (K0331186, KOMABIOTECH, Seoul, Korea) following the manufacturer's instructions. The levels of Serum C-reactive protein (CRP) were assayed by the latex-agglutination test described by Hanson et al. (1997). The white blood cells (WBCs) count was determined according to the improved Neubauer method using the haemocytometer.

Lipid peroxides, measured as thiobarbituric acid reactive species (TBARs), mainly malondialdehyde (MDA) were measured according warm distilled water and administered via gavages to the mice at

**Table 1.** Effect of ω3 FATs administration on serum tumor necrosis factor alpha (TNFα), reactive protein (CRP) levels and total leucocytes count (TLC) in different animal groups.

| Group | TLC ($10^3$/ml) | TNF-α (Pg/ml) | CRP |
|---|---|---|---|
| Normal control | 7.15±0.11 | 86±4.95 | >6 |
| EC | 18.08±0.47[a] | 223±8.13[a] | 18[a] |
| ω-3 | 7.37±0.19[b] | 71±1.63[b] | >6 |
| ω-3+EC | 12.7±0.44[ab] | 76.75±3.64[b] | >6 |

Each value represents the mean of 6 records ± SE. [a]Significant differences versus normal control group. [b]Significant differences versus Ehrlich carcinoma (EC) bearing animals group.

**Table 2.** Effect of ω3 FATs on liver function enzymes of control or Ehrlich carcinoma (EC) bearing mice.

| Group | AST (U/L) | ALT (U/L) | ALP (U/L) | LDH (U/L) |
|---|---|---|---|---|
| Normal control | 59.0±3.07 | 34.5±2.45 | 78±6.78 | 336±14.75 |
| EC | 160±6.79[a] | 94±4.18[a] | 136±5.28[a] | 490±5.89[a] |
| ω-3 | 63±2.62[b] | 35±2.45[b] | 78±4.58[b] | 355±14.29[b] |
| ω-3+EC | 126±8.14[ab] | 66±5.21[ab] | 105±9.18[ab] | 439±13.44[ab] |

Each value represents the mean of 6 records ± SE. [a]Significant differences versus normal control group. [b]Significant differences versus Ehrlich carcinoma (EC) bearing animals group.

the reported methods of Yoshioka et al. (1979). Reduced glutathione (GSH) were determined according to the method of Beutler et al. (1963). Catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined according to the method of Sinha (1972), Masayasu and Hiroshi (1979) and Gross et al. (1967), respectively.

#### Histopathological studies

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

#### Statistical analyses

All values are presented as mean ± standard error of mean (SEM). 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

EC size monitoring is illustrated as shown in Figure 1. It is clear that the inoculation of 2.5 millions of EC cells in 2 ml physiological saline in the neck region of healthy normal mice produced a tumor with a mean size of 332.8±8.83 $mm^3$ on the 7th day after tumor inoculation (ATI). EC size exceeds 500 $mm^3$ on the 10th day ATI. The increase in size of EC proceeds by days reaching 3300±194.7 $mm^3$ on the 30th days ATI.

Gavages of the experimental animals with ω-3 FATs (0.3 g/kg body weight/day) 7 days before EC tumor cell inoculation, recorded 18.72±1.26 $mm^3$ EC tumor size on the 7th day ATI and reaching 165±10.73 on the 30th days ATI. The percentage of growth inhibition in the 7th days ATI was 94.375% and the percentage of growth inhibition on the 30th days ATI was 95%

### Effect of ω-3 FATs administration to mice on biochemical parameters

Table 1 revealed that subcutaneous injection of EC cells on the back of neck region of female mice produced a significant increase ($P<0.001$) in total leucocytes count (TLC) by 153%, increase serum TNF-α level by 159.3%, and remarked increase in serum CRP level significant in comparison with normal control group values. Treatment of the experimental mice-bearing EC with ω-3 FATs produced a significant decrease in TLC by 29.77%, decrease in serum TNF-α level by 65.58% and great reduction in the CRP level in comparison with EC bearing group.

EC has been found to cause abnormalities in liver function as shown by the significant increase in the activity of serum enzymes. The results demonstrated in Table 2 showed that the EC animal bearing group exhibited a significant increase in serum activity of ALT, AST, ALP, and LDH compared to that of the normal control group. After administration of ω-3 FATs at the dose of 300 mg/kg body weight to EC bearing mice significantly reduced serum activity of liver function enzymes were recorded in comparison with EC control group.

Table 3 showed that EC inoculation; significant increase

**Table 3.** Effect of ω3 FATs on TBARS levels and antioxidant status of liver tissue in control or Ehrlich carcinoma (EC) bearing mice.

| Group | TBARS (umol/g) | GSH (GSH/g) | CAT (umol/min/g) | SOD (ug/mg) |
|---|---|---|---|---|
| NC | 152±17 | 38.80±3.96 | 152.33±6.74 | 5.70±0.46 |
| EC | 180±19[a]* | 32.83±2.13[a†] | 140.17±6.11[a†] | 5.06±0.33[a‡] |
| ω-3 | 135±18[b‡] | 37.93±2.92[b†] | 159.00±5.55[b‡] | 5.58±0.31[b†] |
| ω-3+EC | 154±14[b]* | 36.20±1.77 | 144.50±4.18[a]* | 5.47±0.20[b]* |

Each value represents the mean of 6 records ± SE. [a]Significant differences versus normal control group. [b]Significant differences versus Ehrlich carcinoma (EC) bearing animals group.

**Table 4.** Effect of ω3 FATs on TBARS levels and antioxidant status of EC tumor tissue in mice bearing Ehrlich carcinoma (EC).

| Group | TBARS (umol/g) | GSH (GSH/g) | CAT (umol/min/g) | SOD (ug/mg) |
|---|---|---|---|---|
| EC | 99±1.70 | 18±0.37 | 92±2.48 | 4.08±0.16 |
| ω-3+EC | 127±1.70[b‡] | 17±0.43[b‡] | 84±2.80[b‡] | 4.65±0.26[b‡] |

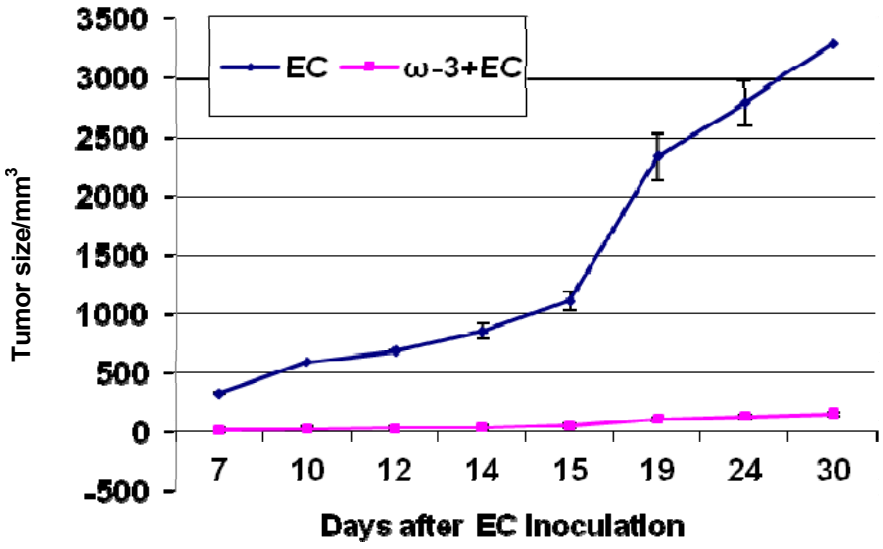


**Figure 1.** Effect of ω3 FATs on tumor size of Ehrlich carcinoma.

increase (P<0.05) in liver TBARS level by 18.4%, meanwhile, a significant decrease in liver GSH, GSH-Px, CAT and SOD content by 15.37, 14.44, 8 and 11.14%, respectively, were observed in comparison with the normal control values (Table 3). Treatment of animals with ω-3 FATs at the dose of 300 mg/kg, increased hepatic antioxidant enzymes compared to that of EC control group. The treatment of EC-bearing mice with ω3 FATs was more effective in restored and corrected the biochemical parameters level (Table 3).

Treatment of the experimental mice-bearing solid EC with ω3 FATs produce significant increase (P<0.05) in tumor TBARS and SOD content by 28.28 and 13.97%, respectively, against EC-bearing group. Meanwhile, a significant decrease in tumor GSH, GSH-PX and CAT content was observed by 5.68, 17.65 and 8.57%, respectively in comparison with EC-bearing group (Table 4).

## Histopathological changes in EC tumor tissues and liver as effected by ω-3 FATs treatment.

### *EC tumor tissue*

Histopathological examination of the EC tumor under

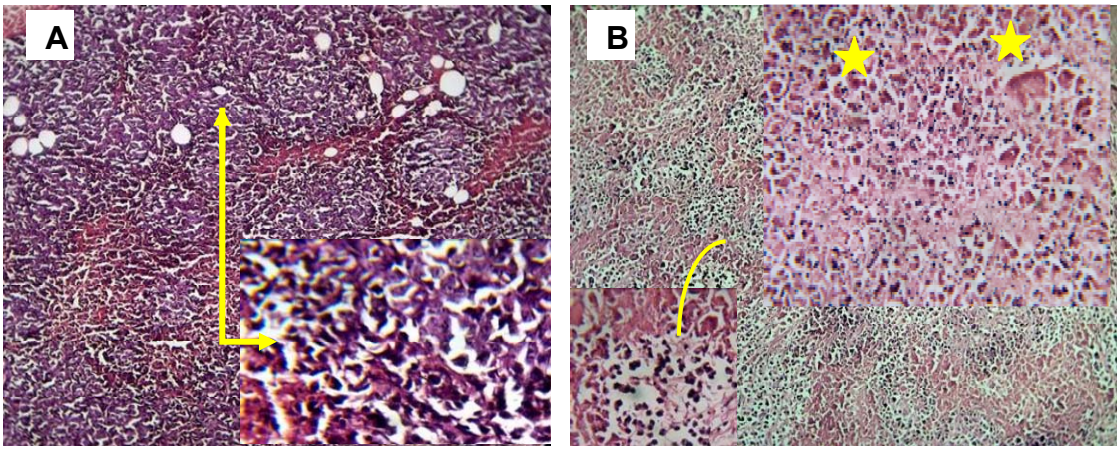


**Figure 2.** Photomicrograph represents subcutaneous solid Ehrlich carcinoma in mice.
(A): Control solid Ehrlich carcinoma. (B): Solid Ehrlich carcinoma in mice treated by ω3 FATs. (H & E x100-Encircled parts x400).

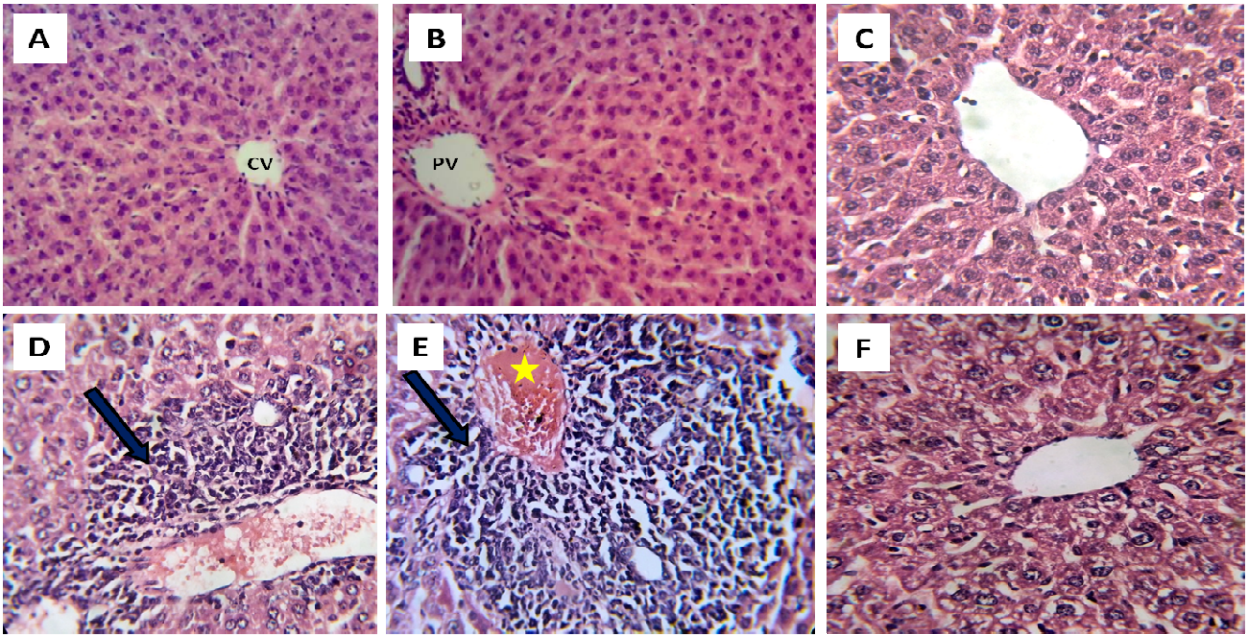


**Figure 3.** Photographs of sections in liver of mice.
A, B: Control sections showing the normal appearance of hepatocytes and the central vein (CV) in A and portal vein (PV) in B. C: Normal appearance in ω-3 treated group. D, E: Liver sections of mice bearing EC represented accumulation of EC cells (blocked arrow) around a congested portal blood vessel (star) with completely haemolysed RBCs. F: Liver tissue represents great disappearance of metastatic ECs in ω3 FATs treated group. (H&E stain × 400).

Light microscope showed compactness and aggregation of the tumor tissue cells spread subcutaneously within the soft tissues in the neck region. EC tumor showed groups of large, round and polygonal cells, with pleomorphic shapes, hyperchromatic nuclei and binucleation. Several degrees of cellular and nuclear pleomorphism were seen (Figure 2A).

Treatment of female mice bearing EC tumor by ω-3 FATs recorded great destruction of tumor tissue represented by the appearance of dead (arc) and necrotic cells (star) (Figure 2B).

### *Liver tissue*

Normal histological pattern of the control liver of young mice is as shown in Figure 3A and B. 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. Treatment of female mice with ω-3 FATs represented a normal appearance of liver tissue section (Figure 3C).

The liver sections of mice bearing EC showed

accumulation of EC cells around congested portal blood vessels with completely haemolysed red blood cells (RBCs) in the portal vein (Figure 3D and E). Treatment of the experimental mice-bearing solid EC with ω-3 FATs revealed great disappearance of metastatic EC cells (Figure 3F) from the liver tissue. Some hydropic degeneration in hepatocytes cytoplasm and increase in Kupffer cells were also detected.

## DISCUSSION

Two major limitations are known to negatively affect the utilization of anticancer agents for therapy; firstly, the lack of selectivity for tumor cells and the second cytotoxicity of these substances, especially when applied in high doses. Implantation of EC tumor into female Swiss albino mice has been proved to induce oxidative stress provoking oxidative damage, organ dysfunction and metabolic disturbances. Oxidative stress is a main mediator in ROS-induce liver dysfunction and has been implicated in many pathological conditions, including, DNA damage, tumor promotion and cancer. Cytotoxic ROS were identified in tumor cells and are possibly associated with depletion of antioxidant enzymes (Yamamoto et al., 2003). Some polyunsaturated fatty acids (PUFAs) exert a selective cytotoxic or anti-proliferative effect on tumor cells rather than on normal cells (Judé et al., 2006).

The results obtained in the present study showed that, the serum levels of ALT, AST, ALP and LDH increased in mice bearing-EC. The elevation in liver enzymes of mice treated with EC has already been reported in earlier studies and is still consistent with more recent results (Sakr et al., 2011). Most experimental solid tumors have elevated levels of ROS and consequently oxidative stress (Maeda and Akaike, 1998). Oxidative stress has been suggested to represent an important contributory factor to liver injury, and to enhanced morbidity related to liver hypo-function (Siddique et al., 2004). Therefore, accumulation of ROS and GSH depletion in tumor cells could induce unsaturated fatty acid to undertake lipid oxidation, thereby disordering the transport and storage of $Ca^{2+}$ in mitochondria, endoplasmic reticulum and cell membrane, increasing the content of plasmic $Ca^{2+}$, and ultimately causing death of cell. ALT, AST, ALP and LDH are then released into the blood (Hua and Ya-Wei, 2005; Wallace, 2007).

LDH activity was inhibited in tumor group due to increase in inflammatory cells which caused reduction in protein level (Saad-Hossne et al., 2003) or due to hepatotoxicity (Kalapos et al., 1993) or the other possible reason for elevated levels of LDH may be due to higher glycolysis in cancerous conditions (Al-Jasabi et al., 2013).

Treatment with ω-3 FATs caused improvement in which ameliorating hepatic enzyme levels which related to the free radical scavenging activity, so ω-3 FATs protect liver membrane from free radicals and decrease lipid peroxidation level, as a result of that, LDH was prevented from releasing outside the cell (Schmidt et al., 2005).

The results of the current study indicated that treatment of the experimental mice-bearing EC with ω-3 FATs produced significant increase in tumor TBARS and SOD content associated with a significant decrease in tumor GSH, GSH-PX and CAT content in comparison with EC-bearing group. In the present work, the increase of TBARS level in tumour tissue might be attributed to the deficiency of antioxidant defence mechanisms or probably due to the generation of ROS and altered redox statuses which are common biochemical aspects in tumor cells. ROS can react with the PUFAs of lipid membranes and induce lipid peroxidation. Earlier studies observed increased lipid peroxidation and decreased antioxidant levels in the cancer patients (Manju et al., 2002).

Significant elevation in SOD in tumor group may be correlated to the oxidative stress in response to the continual generation of free radicals by the increase of tumor load (Fahim et al., 2003). The significant decrease in GSH level in tumor tissue may be due to lack of amino acids which is used in the making of GSH (Deepa and Varalakshmi, 2003).

Long chain ω-3 PUFAs, are highly susceptible to lipid peroxidation (LPO), because of their double bonds. Hence, their incorporation into phospholipids of cellular membranes may sensitize cells to ROS and thereby induce oxidative stress in tumour cells (Sawyer and Field, 2010).

The results of the current study revealed that subcutaneous transplantation of EC cells on the back of neck region of experimental mice produced a significant increase in liver TBARS level associated with a significant decrease in liver GSH, GSH-Px, CAT and SOD content in comparison with the normal control values. The results obtained support previous findings that EAC has been suggested to cause the generation of ROS resulting in oxidative stress, alter the antioxidant defense system in tissues and cellular injury, which may be one of the factors in the etiology of cancer (Bansal et al., 2005).

Moreover, the inhibition of antioxidants enzyme activity and a reduction in glutathione level as a result of tumor growth was reported (Gupta et al., 2004). This phenomenon could be attributed to the exhaustion of these antioxidants especially glutathione and glutathione containing enzymes in the detoxification of free radicals and peroxides, generated due to the presence of the EC tumor. The decrease in liver SOD activity in EC bearing mice is probably due to loss of $Mn^{2+}$ containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver (Sun et al., 1999). A small amount of CAT in tumor cells was reported (Sun et al., 1999).

Most experimental solid tumors have elevated levels of ROS (Maeda and Akaike, 1998). Thus, in the present study, a significant expected elevation of free radicals in

the tumor group was observed. It has been suggested that ω-3 FATs may stimulate the production of ROS generating cells, such as activated macrophages and T lymphocytes (Avogadri et al., 2008). The prominent antioxidant activity of ω-3 FATs was extensively documented in different experimental situations; it inhibit hepatocellular carcinoma cell growth through blocking β-catenin and cyclooxygenase-2 (Kyu et al., 2009).

In the present study, the presence of EC tumor in female Swiss albino mice was accompanied by a systemic inflammation as manifested by a significant increases in C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and leukocyte counts (leukocytosis) reflecting systemic inflammation in the liver. All of these could come from the tumor C-reactive protein (CRP) is an exquisitely sensitive systemic marker of inflammation and liver tissue damage (Kerner et al., 2005). The significant increase in the level of serum CRP might be attributed to EC-induced oxidative damage and increases in inflammatory activity (Hayashi et al., 2001).

Etiologically, leukocytosis is a primary pathological condition affecting the white blood cells, but it frequently arises as a reaction to infection, chronic inflammation and cancer (Ruka et al., 2001). The increase in total leucocytes count (leukocytosis) observed in the present study could be due to induction of solid tumor in mice which was accompanied by marked cellular, molecular, and biochemical changes. Leukocytosis which observed in the current study might be attributed to the decreased significantly number of lymphocytes, or due to lipid peroxidation (Tibaldi et al., 2008).

Significant increases in proinflammatory cytokines (as TNF-α), in the present study might be attributed to the fact that Ehrlich solid tumors can cause a series of deleterious side-effects, including oxidative stress that may triggers lipid peroxidation which in turn initiates release of malondialdehyde and binds to hepatocyte proteins initiating a potentially harmful immune response and stimulate neutrophil chemotaxis or activates transcriptional factor NFκB which in turn increases the production of proinflammatory cytokines as TNF-α, promoting hepatocyte injury and apoptosis and hepatic stellate cell activation (Duvnjak et al., 2007).

In the present study supplementation of mice with ω-3 FATs has significantly ameliorated serum systemic inflammatory level. This could be attributed to the role of ω-3 FATs in EC tumor regression and minimizing EC-induced oxidative injury as well as to its anti-inflammatory effect (Raso et al., 2002). Clinical studies have reported that fish oil supplementation has beneficial effects in rheumatoid arthritis, inflammatory bowel disease, and among some asthmatics, supporting the idea that the ω-3 FATs are anti-inflammatory and immunomodulatory (Philip, 2001). The first mechanism whereby ω-3 FATs can reduce inflammation is by producing metabolites which exert potent anti-inflammatory effects, including resolvins, docosatrienes and protectins (Bazan, 2007; Dyall, 2010). A second mechanism by which ω-3 FATs can reduce inflammation is by lowering the level of omega-6 (ω-6) PUFAs in the brain that can stimulate pro-inflammatory eicosanoid metabolites including prostaglandins, leukotrienes and thromboxanes (Farooqui et al., 2007).

In the present study, treatment of tumor-bearing mice with ω-3 FATs exerted a marked effect in the retardation of tumor growth as compared to tumor bearing mice group. These observations are consistent with the previous findings by Majumder et al. (2006). The increase of tumor size in EC might be attributed to EC-induced oxidative stress (Dwivedi et al., 2007) or due to activation of activate nuclear factor (NF-κB)-induces tumor growth and metastasis and reduces cytokines-induced apoptosis (Takada et al., 2006).

Experimental studies suggested a beneficial role of ω-3 FATs in health, owing to their antioxidant properties, and their ability to modulate the activity of various enzymes (Depasis et al., 2002). ω-3 FATs, especially the eicosapentanenoic acid (EPA) and docosahexanoic acid (DHA), were shown to exert cancer-protective activity. On the other hand, the selectivity of DHA and other PUFAs) on tumor cells has been a worthwhile goal of research. In this context, *in vitro* cell culture studies have led to a consensus that some PUFAs exert a selective cytotoxic or anti-proliferative effect on tumor cells rather than on normal cells (Judé et al., 2006).

Evidence increasingly suggests that ω-3 FATs, particularly eicosapentaenoic acid (20:5n−3) (EPA) and docosahexaenoic acid (22:6n−3) (DHA) are protective against cancer, and the data is the strongest for breast and colon cancer. These protective effects are mediated by a variety of different mechanisms, including the incorporation of ω-3 FATs into cell membranes, which changes membrane fluidity, may affect the association of proteins within cell membranes (Wang et al., 2000), and/or may initiate different signal-transduction processes. ω-3 FATs have also been shown to decrease cell proliferation and/or increase apoptosis during the tumorigenesis process and modulating angiogenesis (Szymczak et al., 2008).

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## REFERENCES

An WS, Kim H J, Cho KH, Vaziri ND (2009). Omega-3 fatty acid supplementation attenuates oxidative stress, inflammation, and tubulointerstitial fibrosis in the remnant kidney. Am. J. Physiol. Renal Physiol. 297(4):895-903.

Avogadri F, Mittal D, Saccheri F, Sarrafiore M, Ciocca M, Larghi P, Orecchia R. and Rescigno M. (2008). "Intra-Tumoral Salmonella Typhimurium Induces a Systemic Anti-Tumor Immune Response That is Directed by Low-Dose Radiation to Treat Distal Disease," Eur. J. Immunol. 38(7):1937-1947.

Bansal AK, Bansal M, Soni G, Bhatnagar D (2005). Protective role of Vitamin E pre -treatment on N-nitrosodiethylamine induced oxidative

stress in rat liver. Chemico-biol. interact. 156(2):101-111.
Bartsch H, Nair J, Owen RW (1999). Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis 20(12):2209-2218.
Bazan NG (2007). Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Curr. Opin. Clin. Nutr. Metab. Care. 10(2):136-141.
Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61:882.
Bhattacharya S, Haldar PK (2011). Trichosanthes dioica root extract induces tumor proliferation and attenuation of antioxidant system in albino mice bearing Ehrlich ascites carcinoma. Interdiscip. Toxicol. 4(4):184-190.
Browning LM (2003). n-3 polyunsaturated fatty acids, inflammation, and obesity-related disease. Proc. Nutr. Soc. 62:447-453.
Deepa PR, Varalakshmi P (2003). Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem. Biol. Interact.146:201-210.
Duvnjak M, Lerotić I, Baršić N, Tomašić V, Virović Jukić L, Velagić V (2007). Pathogenesis and management issues for non-alcoholic fatty liver disease. World J. Gastroenterol. 13(34):4539-4550.
Dwivedi S, Sharma A, Patrick B, Sharma R, Awasthi YC (2007). Role of 4-hydroxynonenal and its metabolites in signaling. Redox Rep. 12:4-10.
Dyall SC, Michael GJ, Michael-Titus AT (2010). Omega-3 fatty acids reverse age-related decreases in nuclear receptors and increase neurogenesis in old rats. J. Neurosci. Res. 88(10):2091-2102.
El-Gawish M (2003). Antitumor activity of inositol hexaphosphate (Phytic acid) in mice loaded with solid tumor. Egypt. J. Biomed. Sci. 11:106-121.
Espada CE, Berra MA, Martinez MJ, Eynard AR, Pasqualini ME (2007). Effect of Chia oil (Salvia hispanica) rich in ω-3 fatty acids on the eicosanoid release, apoptosis and T-lymphocyte tumor infiltration in a marine mammary gland adenocarcinoma. Prostaglandins Leukot. Essent. Fatty Acids 77:21-28.
Fahim FA, Esmat AY, Mady EA, Amin MA (1997). Serum LDH and ALP isozyme activities in mice bearing solid Ehrlich carcinoma and/or treated with maximum tolerated dose (MTD) of aloin. Dis. Markers 13:183-193.
Farooqui AA, Horrocks LA, Farooqui T (2007). Modulation of inflammation in brain: a matter of fat. J. Neurochem. 101(3):577-599.
Gross RT, Bracci R, Rudolph N, Schroeder E, Kochen JA (1967). Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. Blood 29(4):481-493.
Gupta A, Rosenberger SF, Bowden GT (1999). Increased ROS levels contribute to elevated transcription factor and MAP kinase activities in malignantly progressed mouse keratinocyte cell lines. Carcinogen 20(11):2063-2073.
Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi MLM (2004). Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascites carcinoma in Swiss albino mice. J. Pharmacol. Sci. 94(2):177-184.
Hayashi I, Ohotsuki M, Suzuki I, Watanabe T (2001). Effects of oral administration of *Echinacea purpurea* (American herb) on incidence of spontaneous leukemia caused by recombinant leukemia viruses in AKR/J mice. Nihon Rinsho Meneki Gakkai Kaishi 24:10-20.
Kerner A, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, Levy Y, Brook GJ, Aronson D (2005). Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 25(1):193-197.
Hanson LO (1997). Estimation of C-reactive proteins. Curr. Opin. Infect. Dis. 10:196-201.
Hu FB (2001). The balance between ω-6 and ω-3 fatty acids and the risk of coronary heart disease. Nutrition 17(9):741-742.
Jia LJ, Xu HM, Ma DY, Hu QG, Huang XF, Jiang WH, Li SF, Jia KZ, Huang QL, Hua ZC (2005). Enhanced therapeutic effect by combination of tumor-targeting Salmonella and endostatin in murine melanoma model. Cancer Biol. Ther. 4(8):840-845.
Judé S, Roger S, Martel E, Besson P, Richard S, Bougnoux P, Le Guennec JY (2006). Dietary long-chain omega-3 fatty acids of marine origin: a comparison of their protective effects on coronary heart disease and breast cancers. Progress Biophys. Mol. Biol. 90(1):299-325.
Kalapos MP, Littauer A, qde Groot H (1993). Has reactive oxygen a role in methylglyoxal toxicity? A study on cultured rat hepatocytes. Arch. Toxicol. 67(5):369-372.
Kyu L, Chang H, Yifan D, Miaoda S, Tong WL, Han C, Dai Y, Shen M, Wu T (2009). Omega-3 polyunsaturated fatty acids inhibit hepatocellular carcinoma cell growth through blocking β-catenin and cyclooxygenase-2. Mol. Cancer Ther. 8(11):3046-3055.
Manju V, Kalaivani SJ, Nalini N (2002). Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case-control study. Clin. Biochem. 35(8):621-625.
Maeda H, Akaike T (1998). "Nitric Oxide and Oxygen Radicals in Infection, Inflammation, and Cancer." Biochem. Mosc. 63(7):854-865.
Majumder S, Dutta P, Mookerjee A, Choudhuri SK (2006). The role of a novel copper complex in overcoming doxorubicin resistance in Ehrlich ascites carcinoma cells in vivo. Chemico-Biol. Interact. 159(2):90-103.
Mantovani A (2005). Cancer: inflammation by remote control. Nature 435(7043):752-753.
Marnett LJ (2000). Oxyradicals and DNA damage. Carcinoma 21:361-370.
Masayasu M, Hiroshi Y (1979). A simplified assay method of superoxide dismutase activity for clinical use. Clin. Chim. Acta. 92(3):337-342.
Oberley TD (2002). Oxidative damage and cancer. Am. J. Pathol. 160(2):403.
O'Byrne KJ, Dalgleish AG (2001). Chronic immune activation and inflammation as the cause of malignancy. Br. J. Cancer. 85:473-483.
Patyar S, Joshi R, Byrav DP, Prakash A, Medhi B, Das BK (2010). Review Bacteria in cancer therapy: a novel experimental strategy. J. Biomed. Sci. 17(1):21-30.
Philip M, Rowley DA, Schreiber H (2004). Inflammation as a tumor promoter in cancer induction. Semin. Cancer Biol.14:433-439.
Preetha A, Ajaikumar BK, Chitra S, Kuzhuvelil BH, Sheeja TT, Oiki SL, Bokyung S, Bharat BA (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm. Res. 25(9):2097-2116.
Raso GM, Pacilio M, Carlo G, Esposito E, Pinto L, Meli R (2002). In-vivo and in-vitro anti-inflammatory effect of *Echinacea purpurea* and Hypericum perforatum. J. Pharm. Pharmacol. 54(10):1379-1383.
Roy AV (1970). Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin. Chem. 16(5):431.
Ruka W, Rutkowski P, Kaminska J, Rysinska A, Steffen J (2001). Alterations of routine blood tests in adult patients with soft tissue sarcomas: Relationships to cytokine serum levels and prognostic significance. Ann. Oncol. 12:1423-1432.
Sakr Mahran HA, Lamfon HA (2011). "Protective Effect of Ginger (*Zingiber officinale*) on Adriamycin-Induced Hepatotoxicity in Albino Rats." J. Med. Plant Res. 5(1):133-140.
Sawyer MB, Field CJ (2010). Possible mechanisms of ω-3 PUFA anti-tumor action. In: Calviello G, Serini S (eds.) Dietary omega-3 polyunsaturated fatty acids and cancer. Diet Cancer 1:3-38.
Schottenfeld D, Beebe-Dimmer J (2006). Chronic Inflammation: A Common and Important Factor in the Pathogenesis of Neoplasia. CA Cancer J. Clin. 56:69-83.
Sinha AK (1972). Colorimetric assay of catalase. Analyt. Biochem. 47(2):389-394.
Sun SY, Li W, Yue P, Lippman SM, Hong WK, Lotan R (1999). Mediation of N-(4-hydoxyphenyl) retinamide-induced apoptosis in human cancer cells by different mechanisms. Cancer Res. 59(10):2493-2498.
Szymczak M, Murray M, Petrovic N (2008). "Modulation of angiogenesis by ω-3 polyunsaturated fatty acids is mediated by cyclooxygenases". Blood 111(7):3514-3521.
Takada Y, Ichikawa H, Badmaev V, Aggarwal BB (2006). Acetyl-11-keto-β-boswellic acid potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis by suppressing NF-κB and NF-κB-regulated gene expression. J. Immunol. 176(5):3127-3140.
Tibaldi C, Vasile E, Bernardini I, Orlandini C, Andreuccetti M, Falcone A (2008). Baseline elevated leukocyte count in peripheral blood is associated with poor survival in patients with advanced non-small cell lung cancer: a prognostic model. J. Cancer Res. Clin. Oncol.

134:1143-1149.
Vincent PC, Nicholls A (1967). Comparison of the growth of the Ehrlich ascites tumor in male and female mice. Cancer Res. 27(6 Part 1):1058-1065.
Wang M, Liu YE, Ni J, Aygun B, Goldberg ID, Shi YE (2000). Induction of mammary differentiation by mammary-derived growth inhibitorrelated gene that interacts with an omega-3 fatty acid on growth inhibition of breast cancer cells. Cancer Res. 60:6482-6487.
Yamamoto T, Hsu S, Lewis J, Wataha J, Dickinson D, Singh B, Schuster G (2003). Green tea polyphenol causes differential oxidative environments in tumor versus normal epithelial cells. J. Pharmacol. Experiment Ther. 307(1):230-236.
Yoshioka T, Kawada K, Shimada T, Mori M (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am. J. Obstet. Gynecol. 135(3):372-376.
Young DS, Friedman RB (2001). Effects of Disease on Clinical Laboratory Tests, 4th edition. American Association for Clinical Chemists Press, Washington DC.