Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 2 (3): 103-109
May - June, 2002

Effect of Fish Oil on Liver Tumorigenesis and Biochemical Perturbations in Toads Treated with 7,12-Dimethylbenz (a) anthracene

[1]Ismail A. Sadek, [2]Kamal S. Ghazaly,
[1]Amina E. Essawy and [3]Atef M. Al-Attar

The effect of fish oil on 7,12-dimethylbenz(a) anthracene (DMBA) – induced liver tumors in toads *Bufo regularis* was investigated. Feeding toads with DMBA 0.5 mg/toad twice weekly for 14 weeks, induced liver tumors (hepatocellular carcinomas) in 32 % of the treated animals. No tumors were detected in toads received DMBA plus 0.1 ml fish oil. Biochemical estimation of several parameters; total lipids, cholesterol, triglycerides, glutamic pyruvic acid transaminase (GPT) and glutamic oxaloacetic acid transaminase (GOT), demonstrated a significant effects of fish oil on the alterations induced by DMBA. The obtained results collectively revealed the anticancer activity of fish oil as well as its pronounced ameliorative effect against the toxic effects of DMBA on the haematochemical parameters in toads *Bufo regularis*.

Key words: Fish oil, toads, hepatocellular carcinomas

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Ismail A. Sadek
Department of Zoology,
Faculty of Science,
Alexandria University,
Alexandria, Egypt

ANSInet
Asian Network for Scientific Information

[1]Department of Zoology, Faculty of Science, [2]Department of Biology, Faculty of Education, Alexandria University, Alexandria, Egypt
[3]Department of Biology, Faculty of Education, El-Taif, Saudi Arabia

Introduction

Marked variations in dietary habits among population of different cultures and life styles have been associated with a risk for the development of cancer. Among the dietary habits, dietary fat has received considerable attention as a risk factor in the etiology of colon and mammary cancer (Carroll and Braden, 1984; Reddy, 1986). Increasing dietary intake of plant polyunsaturated fatty acids rich in the essential fatty acid linoleate, has shown to enhance tumor development in chemically induced mammary carcinogenesis (Ip *et al.*, 1985). Studies in laboratory animals demonstrated that, high fat diets containing corn oil enhanced the chemically induced colon tumors (Holt *et al.*, 1996), mammary tumors (Welsch, 1992 and 1994), Pancreatic tumors (Birt *et al.*, 1990) and liver tumors (Sadek, 1986 ; Sadek and Abdul – Salam, 1994).

Polyunsaturated fatty acids in the form of marine oil, contain a large proportion of n-3 long chain fatty acids, were found to inhibit the growth and metastasis of tumors (Hubbard *et al.*, 1998; Hamid *et al.*, 1999). Epidemiological studies have shown that, the Japanese and Greenland Eskimos have a very low mortality from breast cancer because they consume large amounts of fat derived from marine oils.

As a matter of fact, blood serves as the most convenient indicator of the general condition of the animal body. Haematological and haematochemical parameters give a valuable information on the physiological reactions of animals to carcinogens (Shimokawa *et al.*, 1977; Burt and Brennan, 1980). Blood enzymes such as GOT and GPT play an important role in the metabolic function and have been used in the diagnoses of animal diseases as well as in the detection of tissue damage caused by carcinogenic agents (Casillas and Ames, 1986).

In this aspect, the research work was planned to study the possible inhibitory effects of fish oil on toad's liver tumors induced by 7,12-Dimethylbenz(a) anthracene, which is a well known potent carcinogen in mammals and many animal species (Iversen, 1991). The experiments here were designed also to study the possible role played by the supplementation of fish oil against the toxic effect of DMBA on the haematochemical parameters in the Egyptian toads, *Bufo regularis*, which have been recommended for testing the carcinogens (El-Mofty *et al.*, 1997; Abdel Meguid *et al.*, 1997) and co-carcinogens (Sadek and Abdul-Salam, 1994).

Materials and Methods

Sexually mature male and female toads, *Bufo regularis* weighing approximately 40g each were used. The animals were collected by a regular supplier from El-Nozha District in Alexandria, Egypt. They were kept in a large glass aquaria with small amounts of dechlorinated water that was changed twice daily and were fed with earth worms once every 3 days.

The toads were divided into five groups of 80 toads each. Each toad of the first group (group I) was fed with 0.5 mg DMBA in 0.1 ml olive oil twice a week for 14 weeks. DMBA was purchased from Sigma Chemical Company, ST. Louis, Mo., U.S.A.

Toads of the second group (Group II) were given the same dose of DMBA as in group I, and fed also with fish oil (Sigma Chemical Company, ST. Louis, Mo., U.S.A) at a dose level of 1ml/toad, twice weekly for 14 weeks. Experimental toads of the third and fourth groups (group III and group IV) served as control groups for toads in group I and group II and force fed olive oil 0.1 ml/toad and fish oil 1 ml / toad, respectively. Toads in the fifth group (group V) were untreated and used as controls for all previously mentioned treated groups .

Histopathological studies: At the end of experimental period (14 weeks) all alive animals were autopsied and all organs were examined by the naked eye. It was noticed that tumors had appeared only in the liver of some DMBA-treated animals. Livers bearing tumors were photographed and removed for fixation . For histological evaluation, the liver tissue was fixed in 10% formalin and embedded in paraffin. Sections 4-5-μm were cut and stained with haematoxylin and eosin.

For electron microscopy, the liver was removed quickly and dropped immediately into F_4G_1 fixative. Small pieces of the liver (1 mm) including tumor nodules were cut within 1-2 min while immersed in the fixative. The fixed samples were postfixed in 1% OsO_4 for 2 h, dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon. Ultrathin sections (50 nm) were cut on an LKB ultratome with a glass knife, double stained with uranyl acetate and lead citrate, and examined with a Jeol 100 CX electron microscope.

The X^2 test (Steel and Torrie, 1960) was used to assess the significant change in tumor incidence.

Biochemical studies: At the termination of the 2^{nd} , 6^{th} , 10^{th} and 14^{th} experimental weeks, blood samples were taken by heart puncture and collected in non heparinized tubes. The blood samples were centrifuged at 8000 r.p.m for 15 minutes.

Serum activities of aspartate aminotransferase (AST or GOT) and alanine aminotransaminase (ALT or GPT) were determined according to the procedures of Reitman and Frankel (1957). Total lipid, cholesterol and triglycerides were measured as described by Zollner and Kirsh (1962), Richmond (1973) and Trinder (1969b), respectively. Data were statistically analyzed using the student's t-test.

Results

Effect of DMBA and DMBA plus fish oil on the histological and subcellular structures of liver cells: Liver tumors were only found in toads (21 out of 66 survivors), which had received DMBA 0.5 mg/toad twice weekly for 14 weeks. No tumors were detected in the liver of toads received DMBA plus fish oil (group II). Also no tumors or other pathological changes were observed in the organs of the control groups (group III, IV and V).

The tumor nodules observed in the liver of DMBA tread toads (Fig. 1) were diagnosed as hepatocellular carcinomas, in which the nodules appeared as localized masses of tumor cells, circumscribed with a fibrous capsule (Fig. 2). The architecture of the fibrous capsule was scantly and mingled with the tumor cells in a diffuse and disorderly manner. The tumor cells exhibited loss of polarity and displayed varying degrees of pleomorphism. The nuclei were pleomorphic and some of them were hyperchromatic. The nuclei are prominent and mitotic divisions of the hepatocytes were abundant (Fig. 3).

The structure of liver in the toads received DMBA plus fish oil showed the normal appearance of liver architecture (Fig. 4).

Electron micrographs of the tumor cells in toads fed DMBA, showed some important criteria of malignancy such as pleomorphic nuclei, irregular nuclear envelop and clefted nucleus (Fig. 5). The most predominant nuclear abnormality was the frequent presence of nuclear pockets (Fig. 6). Some nuclei contained nuclear pseudoinclusions (Fig. 7). These intranuclear inclusions were demarcated by a nuclear envelop and associated heterochromatin mass. As regards cytoplasmic abnormalities the cytoplasm contained disorganized, giant and pleomorphic mitochondria (Fig. 8), cytoplasmic vacuoles (Fig. 6) and autophagosomes (Fig. 9). Pleomorphic fibroblasts and collagen fibers were observed in tumor capsule (Fig. 10).

Electron micrographs of hepatocytes of toads treated with DMBA plus fish oil illustrated the normal appearance of the regular nuclear envelop with distinct nuclear membrane, oval mitochondria with disernible cristae and dense matrix, and normal rough endoplasmic reticulum (Fig. 11).

Effect of DMBA and DMBA plus fish oil on some biochemical parameters: Feeding of DMBA induced a marked elevation ($P \leq 0.05$) in serum GOT, GPT, total lipids, cholesterol and triglycerides, throughout the periods of treatment (Figs.12 and 16).

Administration of DMBA plus fish oil provoked significant increases ($P \leq 0.05$) in the biochemical parameters. However, the magnitude

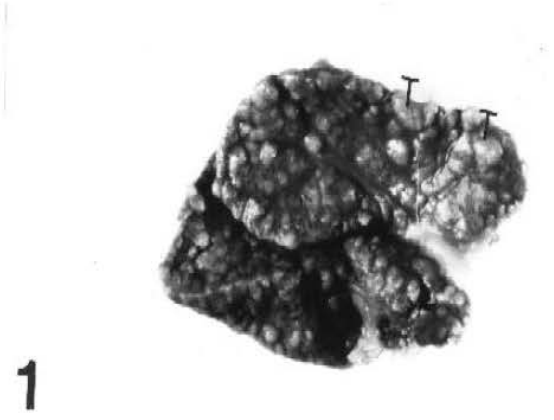

Fig. 1: Liver of a toad treated with DMBA. T, Tumor nodules, (X 3)

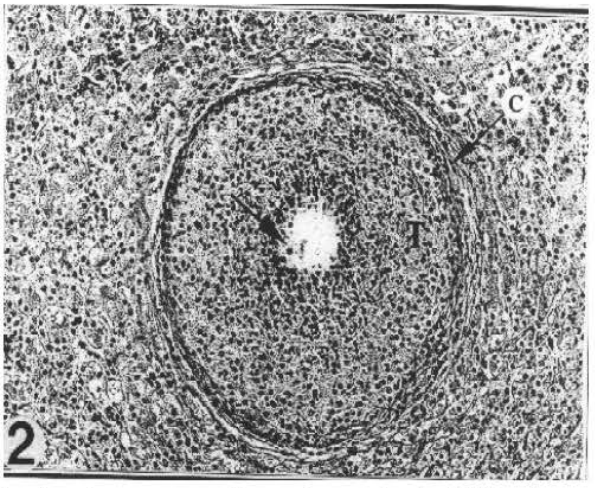

Fig. 2: Liver section of a toad treated with DMBA, showing tumor nodule (T) surrounded by fibrous capsule (C). Not the necrosis of central area of tumor nodule (arrow), (X 130)

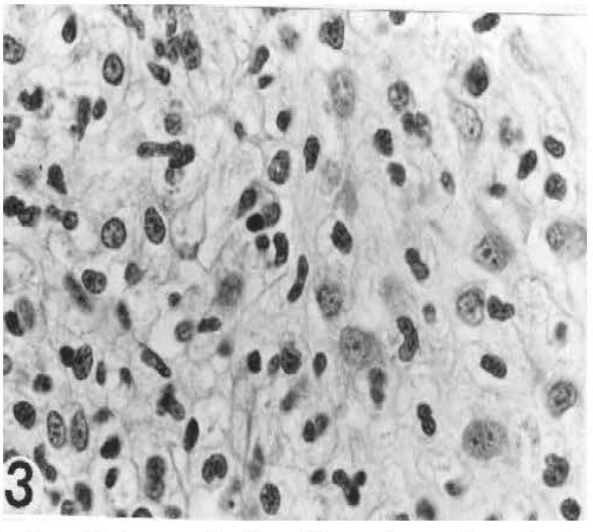

Fig. 3: Higher magnification of the previous section, showing pleomorphic nuclei with mitotic figures (X 1160)

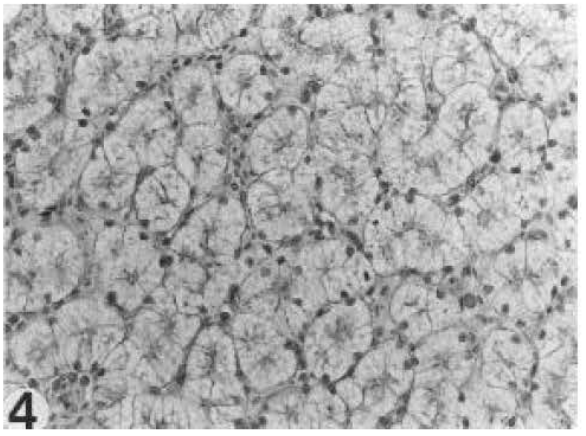

Fig. 4: Liver section of a toad received DMBA plus fish oil, demonstrating the normal liver architecture. The hepatic acini are nearly circular in out line and the hepatocytes contain an eccentric basophilic nucleus. (X 500)

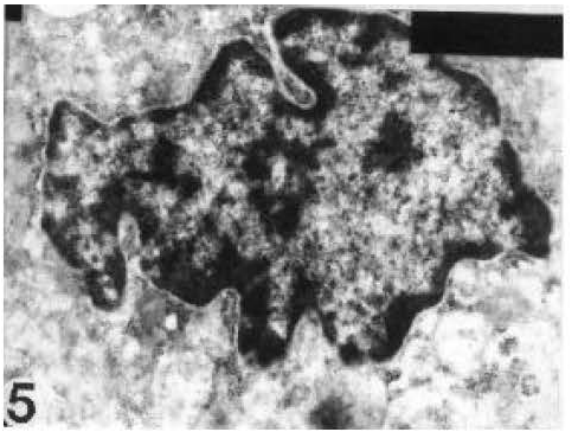

Fig. 5: Electron micrograph of hepatocyte of a toad treated with DMBA, showing disorganized nucleus with irregular nuclear envelop. Note the mostly absence of mitochondria and rough endoplasmic reticulum (X 16000)

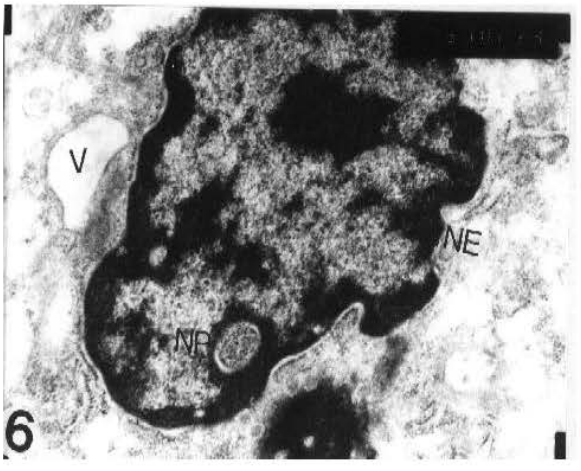

Fig. 6: Electron micrograph of hepatocyte of a toad treated with DMBA, illustrating irregular nuclear envelop (NE), nuclear pocket (NP) and vacuolated cytoplasm(V). (X 16000)

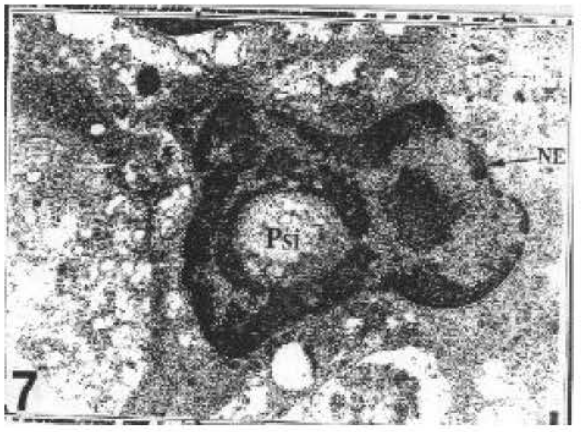

Fig. 7: Electron micrograph of hepatocyte of toad treated with DMBA, demonstrating pseudoinclusion (Psi) inside the nucleus. (X 10000)

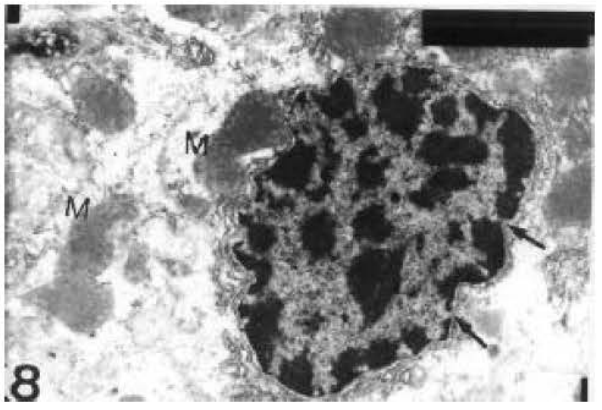

Fig. 8 Electron micrograph of hepatocyte of a toad treated with DMBA, showing irregular nuclear envelope with numerous nuclear pores (arrows), disorganized mitochondria (M) and loss of ribosomes from rough endoplasmic reticulum (X 10000)

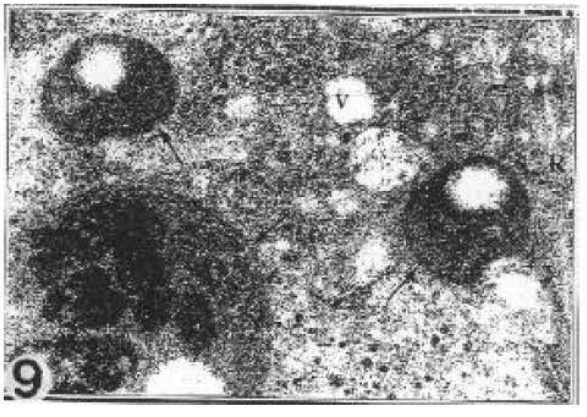

Fig. 9: Electron micrograph of hepatocyte of a toad treated with DMBA, illustrating autophagosomes (arrows), vacuolated cytoplasm and numerous free ribosomes (R). (X 16000)

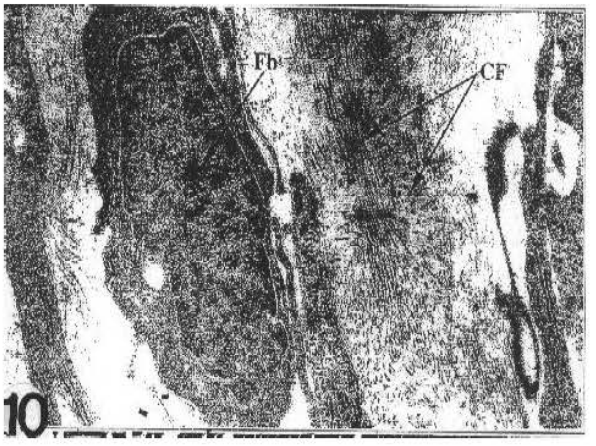

Fig. 10: Electron micrograph of liver tumor capsule of a toad treated with DMBA, demonstrating fibroblast (Fb) and collagen fibres (CF). (X 10000)

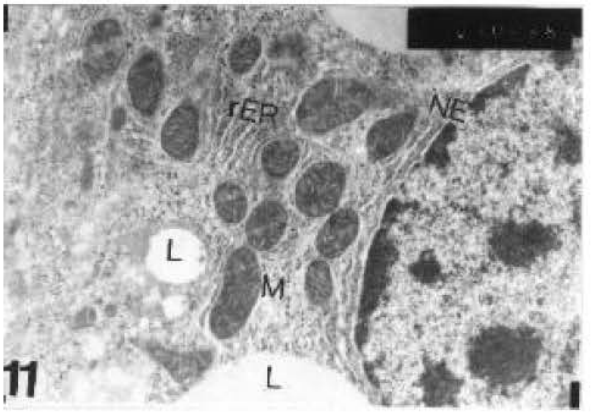

Fig. 11: Electron micrograph of hepatocyte of a toad received DMBA plus fish oil, illustrating regular nuclear envelop (NE) with distinct nuclear membrane, normal mitochondria (M) with numerous cristae, normal rough endoplasmic reticulum (rER) and lipid droplets (L). (X 10000)

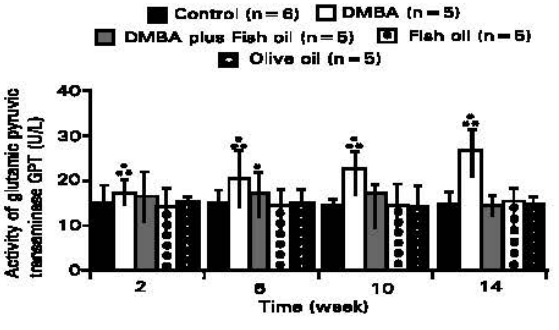

Fig.12: Effect of DMBA, DMBA plus fish oil,fish oil and olive oil on the activity of glutamic pyruvic acid transaminase (GPT) in serum of the toad, *Bufo regularis* treated for 14 weeks

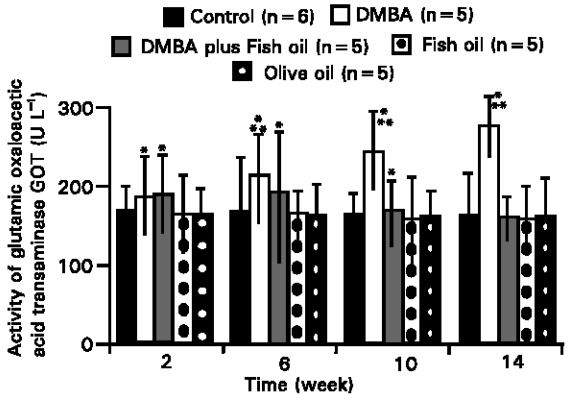

Fig.13: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the activity of glutamic oxaloacetic acid transaminase (GOT) in serum of the toad, *Bufo regularis* treated for 14 weeks

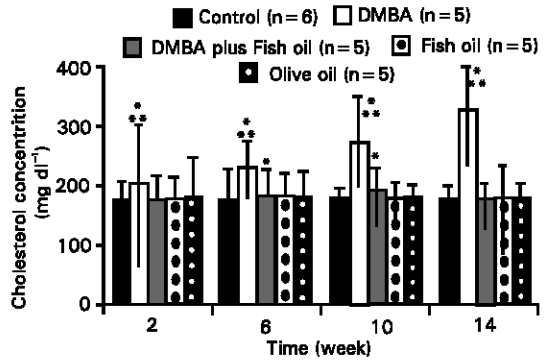

Fig. 14: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the serum level of cholesterol of the toad *Bufo regularis* treated for 14 weeks

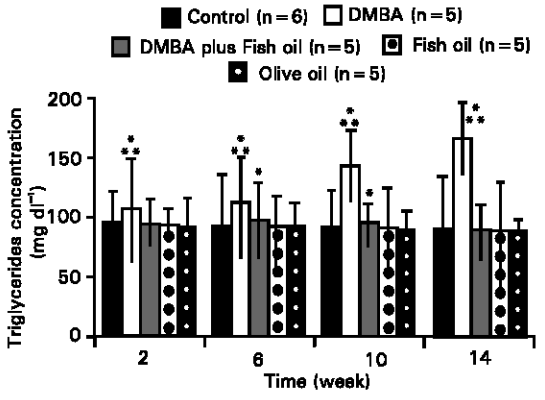

Fig. 15: Effect of DMBA, DMBA plus fish oil,fish oil and olive oil on the serum level of triglycerides of the toad *Bufo regularis* treated for 14 weeks

of changes was smaller (Figs. 12-16). The activity of GOT was elevated at 2nd, 6th and 10th week of treatment. The level of total lipids, cholesterol and triglycerides were increased at 6th and 10th weeks. The activity of GPT was elevated only at the 6th week of administration. Treatment the toads with both fish oil and olive oil did not alter any of the biochemical parameters in comparison with the untreated animals.

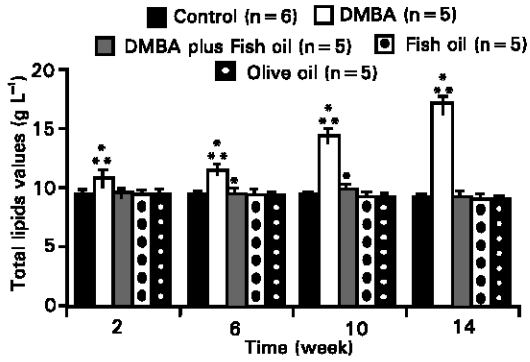

Fig. 16: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the serum level of total lipid of the toad *Bufo regularis* treated for 14 weeks

* Statistically significance of the toad group receiving DMBA, DMBA plus fish oil, fish oil and olive oil at $p \leq 0.05$, different from the control

** Statistically significance of the toad group receiving DMBA, alone at $p \leq 0.05$, different from the toad group receiving DMBA plus fish oil

Discussion

The results obtained in this research work revealed that, treating the toads with fish oil significantly prevented the incidence of liver tumors induced by DMBA. These results are in agreement with the work of Reddy *et al.* (1991), who reported that, incidences of colon tumor were significantly reduced in rats fed the high fish oil diet as compared with those fed the high corn oil diet. Jiang *et al.* (1997) showed that dietary fish oil inhibited Sprague-Dawley rat colon tumorigenesis. Also, Singh *et al.* (1997) found that the high fish oil diet inhibited azoxymethane (AOM) – induced rat colon tumorigenesis compared with high corn oil diet.

The literature supports an association of chronic inflammation with the development of tumors. The colon contains a specialized lymphocyte population that may influence various stages of colon carcinogenesis. Results of a study carried out by Kuratko (2000) indicated that fish oil (high in n-3 fatty acids) slows the inflammatory response in the colon as compared to corn oil (high in n-6 fatty acids). Moreover, polyunsaturated fatty acids in fish oil have been shown to inhibit the growth and metastasis of breast tumors (Hubbard *et al.*, 1998).

The precise mechanism by which fish oil inhibits toad liver tumor is not known. However, it has been found that, fish oil may inhibit prostaglandin synthesis. Rao and Reddy (1993) demonstrated that the high fat fish oil and high fat olive oil inhibited rat colon carcinogenesis and significantly suppressed the prostaglandin level in plasma, liver and colon mucosa compared with high fat corn oil. Large number of human cancers and experimentally induced cancers in animals contain and/or produce large quantities of prostaglandins, particularly E series (Appel and Woutersen, 1994; Kargman *et al.*, 1995).

The biosynthesis of prostaglandins through the cyclooxygenase system and hydroxy fatty acids via lipoxygenase pathway from arachidonic acid exert a variety of biological activities. Several studies have shown that cyclooxygenase metabolites, particularly prostaglandins of type –2 series, modulate cell proliferation, tumor growth, and immune responses (Marnett, 1992; Smith, 1992), whereas lipoxygenase metabolites can influence various biological responses including chemotoxic responses, hormone secretion, ion transport, tumor cell adhesion, stimulation of tumor cell spreading, and regulation of tumor cell metastatic potential (Honn and Tong, 1992; Timer *et al.*, 1992).

Hamid *et al.* (1999) demonstrated the presence of two immunoreactive isoforms of cyclooxygenase (COX-1 and –2), and the modulating effects of n-3 fatty acids on their expression, in N-nitrosomethylurea (NMU) – induced rat mammary tumors. They

concluded that, high fish oil diet (rich in n-3 fatty acids) significantly suppressed both COX-1 and COX-2 protein levels when compared to the high corn oil diet.

It can not excluded the possibility that prostaglandins exert an inhibitory effect on natural killer (NK) cells; components of the defense system (Brunda *et al.*, 1980; Goodwin and Ceuppens, 1983). Prostaglandin E_2 is known to suppress a variety of immune responses including T-cell functions (Fulton and Levy, 1980), cytokine production (Renz *et al.*, 1988) and macrophage / natural killer cell-mediated cytotoxication (Young *et al.*, 1986). Natural killer cells are considered to be a possible first line of defense in the host's antitumor immuno-surveillance (Herberman, 1983). Clearly, amphibians have a well developed immune system that consists of major cellular and humoral components (Cooper, 1976). In addition, frogs possess cytotoxic cells which appear functionally similar to mammalian killer cells (Ghoneum *et al.*, 1990).

In addition to the previously discussed anticancer activity of fish oil, this study showed its effect in reducing the DMBA induced changes in the haematochemical parameters. Insuring to our results, Castillo *et al.* (1999 and 2000) demonstrated that, dietary fish oil reduces cholesterol and triglycerides in chick plasma. Hyperlipidaemia, hypercholesterolaemia and hypertriglyceridaemia are previously known to be associated with liver tumors in rat (Rao *et al.*, 1997) and human (Gholson and Bacon, 1993). Results obtained by Tarao *et al.* (1997) demonstrated an intimate relationship between liver tumorigenesis and the elevation in human blood activities of GPT and/or GOT.

In conclusion, data revealed that the anticancer activities of fish oil as well as its pronounced ameliorative effect against the toxic effects of DMBA on the haematochemical parameters. These results are in agreement with the previous studies in supporting the hypothesis that fish oil possesses chemopreventive properties and validate its potential usefulness in the field of cancer prevention.

References

Abdel-Meguid, N., M. El-Mofty, I. Sadek, A. Essawy and E. Abdel-Aleem, 1997. Ultrastructural criteria that prove the similarities between amphibia and human tumors. Oncology, 54: 528-263.

Appel, M. and R. Woutersen, 1994. Modulation of growth and cell turnover of preneoplastic lesions and of prostaglandin levels in rat pancreas by dietary fish oil. Carcinogenesis. 15: 2107-2112.

Birt, D., A. Julius, E. Dwork, T. Hanna, L. White and P. Pour, 1990. Comparison of the effects of beef tallow and corn oil on pancreatic carcinogenesis in the hamster model. Carcinogenesis (Lond.). 11: 745-748.

Brunda, M., R. Herberman and H. Holden, 1980. Inhibition of murine natural killer cell activity by prostaglandins, J. Immunol., 124: 2682-2687.

Burt, M. and M. Brennan, 1980. Incidence of hypercalcemia and malignant neoplasm. Arch. Surg., 115: 704-707.

Carroll, K. and L. Braden, 1984. Dietary fat and mammary carcinogenesis. Nutr. Cancer, 6: 254-259.

Casillas, E., W. Ames, 1986. Hepatotoxic effects of CCl_4 on English sole (*Parophrys vetulus*): Possible indicators of liver dysfunction. Comp. Biochem. Physiol., 84C: 397-400.

Castillo, M., F. Amalik, A. Linares, E. Garcia – Peregrin, 2000. Fish oil reduces cholesterol and arachidonic acid levels in plasma and lipoproteins from hypercholesterlemic chicks. Mol. Cell Biochem., 210: 121-130.

Castillo, M., F. Amalik, A. Linares and E. Garcia – Peregrin, 1999. Dietary fish oil reduces cholesterol and archidonic acid levels in chick plasma and very low density lipoprotein. Mol. Cell Biochem., 200: 59-67.

Cooper, E., 1976. Immunity mechanisms. In: Lofts B. (ed.). Physiology of amphibia. Academic Press. New York, 3: 164-272.

El-Mofty, M., N. Abdel Meguid, I. Sadek, A. Essawy and E. Abdel Aleem, 1997. The use of toads (*Bufo regularis*) in a new biological assay for screening chemicals or drugs which induce leukaemia in man. Oncology Reports, 4: 657-660.

Fulton, A. and J. Levy, 1980. The possible role of prostaglandins in mediating immune suppression by non-specific T suppressor cells. Cell Immunol., 52: 29-37.

Gholson, F. and B. Bacon, 1993. Essentials of clinical hepatology. Mosby Year Books. St. Louis. Baltimore. Boston. Chicago. London. Philadelphia. Syndney. Torronto, 131-132.

Ghoneum, M., E. Cooper, I. Sadek, 1990. Variability of natural killer cell activity in anuran amphibians. Dev. Comp. Immunol., 14: 359-365.

Goodwin, J. and J. Ceuppens, 1983. Regulation of immune response by prostaglandin. J. Clin. Immunol., 3: 295-315.

Hamid, R., J. Singh, B. Reddy and L. Cohen, 1999. Inhibition of dietary menhaden oil of cyclooxygenase-1 and −2 in N-nitrosomethylurea – induced rat mammary tumors: Int J. Oncol., 14: 523-528.

Herberman, R., 1983. Natural Killer cells and tumor immunity. Surv. Immun. Res., 2: 306-308.

Holt, P., A. Mokuolu, P. Distler, T. Liu and B. Reddy, 1996. Regional distribution of carcinogen-induced colonic neoplasia in the rat. Nutr. Cancer, 25: 129-135.

Honn, K. and D. Tong, 1992. Adhesion molecules and cancer cell interaction with endothelium and subendothelial matrix. Cancer Metastasis Rev., 11: 353-375.

Hubbard, N., D. Lim and K. Erickson, 1998. Alteration of murine mammary tumorigenesis by dietary enrichment with n-3 fatty acids in fish oil. Cancer Lett., 124: 1-7.

IP, C., C. Carter, M. IP, 1985. Requirement of essential fatty acid for mammary tumorigenesis in the rat. Cancer Res., 45: 1997-2001.

Iversen, O., 1991. The skin tumorigenic and carcinogenic effects of different doses, numbers of dose fraction and concentrations of 7,12-dimethylbenz(a) – anthracene in acetone applied on hairless mouse epidermis. Possible implications for human carcinogensis. Carcinogenesis, 12: 493-502.

Jiang, Y., J. Lupton, R. Chapkin, 1997. Dietary fish oil blocks carcinogen - induced down- regulation of colonic protein kinase C isozymes. Carcinogenesis, 18: 351-357.

Kargman, S., G. O'Neill, P. Vickers, 1995. Expression of prostaglandin G/H synthetase-1 and-2 protein in human colon cancer. Cancer Res., 55: 2556-2559.

Kuratko, C., 2000. Proliferation of colonic lymphocytes in response to inflammatory cytokines is lower in mice fed fish oil than in mice fed corn oil. Cancer Lett., 148: 27-32.

Marnett, L., 1992. Aspirin and Potential role of prostaglandins in colon cancer. Cancer Res., 52: 5575 – 5589.

Rao, C. and B. Reddy, 1993. Modulating effect of amount and types of dietary fat on ornithine decarboxylase, tyrosine protein kinase and prostaglandins production during colon carcinogenesis in male F344 rats. Carcinogensis. 14: 1327-1333.

Rao, K., M. Elm, R. Kelly, 1997. Hepatic hyperplasia and cancer in rats: Metabolic alterations associated with cell growth. Gastroenterol., 113: 238-248.

Reddy, B., 1986. Diet and Colon Cancer: evidence from human and animal model studies. In: Reddy B.S. and Cohen L.H. (eds.) Diet, nutrition and Cancer: Acritical evaluation. FL: CRC Press. Boca Raton., 47-65.

Reddy, B., C. Burill and J. Rigotty, 1991. Effect of diets high in n-3 and n-6 fatty acids on initiation and postinitiation stages of colon carcingenesis. Cancer Res., 51: 487-491.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination on of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.

Renz, H., J. Gong and A. Schmidt, *et al.*, 1988. Release of tumor necrosis factor - α from macrophage. J. Immunol., 141: 2388-2393.

Richmond, W., 1973. Determination of blood cholesterol. Clin. Chem., 19: 1350.

Sadek, I., 1986. The Egyptian toad as a senstive model to show the effect of corn oil on liver tumor induced by DMBA. Nutr. Res., 6: 333- 335.

Sadek, I. and F. Abdul-Salam, 1994. Effect of diallyl sulfide on toad liver tumor induced by 7,12 dimethylbenz (a) anthracene. Nutr. Res., 14: 1513-1521.

Shimokawa, Y., K., Okuda., Y. Kubo, 1977. Serum glutamic oxaloacetic transaminase / glutamic pyruvic transaminase ratios in hepatocellular carcinomas. Cancer Res., 57: 253-258.

Singh, J., R. Hamid and B. Reddy, 1997. Dietary fat and colon cancer: modulating effect of types and amount of dietary fat on ras - P21 function during promotion and progression stages of colon cancer. Cancer. Res., 57: 253-258.

Smith, W., 1992. Prostanoid biosynthesis and mechanism of action. Am. J. Physiol., 263: F181 – F191.

Steel, R. and J. Torrie, 1960. Principles and procedures of statistics. McGraw-Hill. New York, USA.

Tarao, K., S. Takemiya, S. Tamai, Y. Sugimasa, S. Ohkawa, M. Akaike, H. Tanabe, N. Shimizu, M. Uoshida and A. Kakita, 1997. Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatoctomized patients with hepatitic C virus – associated cirrhosis and HCC. Cancer, 79: 688–694.

Timer, J., Y. Chen, B. Liu., R. Bazer, J. Taylor and K. Honn, 1992. The Lipoxygenase metabolite 12(5) – HETE Promotes and IIb B_3 intergrin – mediated tumor – cell spreading on fibronection. Int. J. Cancer, 52: 594–603.

Trinder, P., 1969b. Determination of blood triglycerides. Ann. Clin. Biochem., 6: 24

Welsch, C., 1992. Relationship between dietary fat and experimental mammary tumorigenesis: A review and critique. Cancer Res., 52: 2040–2048.

Welsch, C., 1994. Interelationship between dietary lipids and calories and experimental mammary gland tumorigenesis. Cancer, 74: 1055-1062.

Young, M., E. Wheeler and M. Newby, 1986. Macrophage-mediated suppression of natural killer cell activity in mice bearing lewis lung carcinoma. J. Natl. Cancer Inst., 76: 750-754.

Zollner, N., K. Kirsh, 1962. Micro-determination of lipids by sulfophosphovaniline reaction. Z. Ges. Exp. Med., 135:545-560.

MS received 22th April, 2002; accepted 25th May, 2002