

The Influence of Dietary Grapeseed Oil on DMBA-Induced Liver Enzymes Disturbance in the Frog, *Rana ridibunda*

Atef M. Al-Attar

Department of Biology, Teachers College, PO. Box 2375, Dammam 31451, Saudi Arabia

E-mail: atef_a_2000@yahoo.com

Abstract: The current study was designed to determine whether dietary grapeseed oil inhibits liver cytotoxicity induced by 7,12- dimethylbenz(a)anthracene (DMBA) in the frog, *Rana ridibunda*. The experimental animals were divided into five groups and treated for 2 weeks with 7,12- dimethylbenz(a)anthracene, DMBA plus grapeseed oil, grapeseed oil, olive oil and last group was untreated and used as control. Liver enzymes, lactate dehydrogenase, glutamic oxaloacetic acid transaminase, glutamic pyruvic acid transaminase and alkaline phosphatase were chosen to assess liver function. In comparison with control, the administration of DMBA alone significantly elevated the activity of liver lactate dehydrogenase, while the activities of glutamic oxaloacetic acid transaminase, glutamic pyruvic acid transaminase and alkaline phosphatase were declined. Similar results were noted in frogs treated with DMBA plus grapeseed oil. Moreover, it is found that the changes were more pronounced in frogs treated with DMBA plus grapeseed oil than those treated with DMBA. The activities of these enzymes in frogs exposed to grapeseed oil or olive oil were not significantly different from those of controls. These results indicate that grapeseed oil effectively increases DMBA-induced hepatotoxicity in the frogs. Also, the results suggested that grapeseed oil has enhancing effects of DMBA metabolic activation.

Key words: 7,12- dimethylbenz (a) anthracene, grapeseed oil, liver enzymes, *Rana ridibunda*

Introduction

Polycyclic aromatic hydrocarbons (PAH) is a class of organic pollutants that are released into the environment in large quantities, mainly due to human activities. PAH are components of crude and refined petroleum, and coal. Many PAH are quite persistent and some are potent carcinogenic agents. Most PAH in the environment are found during incomplete combustion of organic matter at high temperatures. In addition, many domestic and industrial activities involve pyrosynthesis of PAH. The resulting PAH may be release to the environment in airborne particulates, or in solid or liquid by-products of the pyrolytic process (Neef, 1985). 7,12-Dimethylbenz (a) anthracene (DMBA) is one of polycyclic aromatic hydrocarbons chemical group. DMBA is well known as cytotoxic, carcinogenic, mutagenic and immunosuppressive agent (Smith *et al.*, 1999; Spitsbergen *et al.*, 2000; Miyata *et al.*, 2001; Wijnhoven *et al.*, 2001; Lindhe *et al.*, 2002; Buters *et al.*, 2003). Al-Attar (1998) reported that several haematological and haematochemical parameters were changed in the toad *Bufo regularis* treated with DMBA and found that DMBA-induced hepatocellular carcinoma. Experimental studies showed that DMBA-induced skin, oral, mammary and ovarian tumours (Han *et al.*, 2002; Li *et al.*, 2003; Buters *et al.*, 2003; Suzuki *et al.*, 2003).

Liver is the central organ of metabolism and act as an organ of storage. Many potentially toxic substances are metabolized by cells, especially by the hepatic parenchyma cells. Metabolic action by the hepatic

parenchyma cells has been regarded as an important defense system against toxicants and the transformations involved have been referred to as detoxification. The great susceptibility of liver to damage by chemical agent is presumably a consequence of its primary role in metabolism of foreign substances. The role of liver in metabolic conversion is due to its susceptibility to chemical injury (Zimmerman, 1974). Liver enzymes such as lactate dehydrogenase (LDH), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT) acid phosphatase (ACP) and alkaline phosphatase (ALP) are considered to be biochemical markers for assessing liver function. Epidemiological studies show that dietary factors are the most important environmental risk determinants for human cancers (Doll and Peto, 1981). Goldman and Shields (2003) reported that several lines of evidence indicate that diet and dietary behaviours can contribute to human cancer risk. Many natural food components such as fat, protein, fiber and some minerals and vitamins may influence the incidence of cancer (Appel *et al.*, 1991; O'Neill *et al.*, 1990; Ogawa *et al.*, 1995; Jiang *et al.*, 1996; Yano *et al.*, 2000). Recently, numerous experimental studies have provided evidence that consuming fat diets have an important role in decreasing or increasing tissue carcinogenesis induced by carcinogenic agents (Al-Attar, 1998; Chang *et al.*, 1999; Latham *et al.*, 1999; Z'graggen *et al.*, 2001; Salim *et al.*, 2002; Jelinska *et al.*, 2003). Many investigators have been focused their attention on the effect of dietary

Atef M. Al-Attar: Influence of Dietary Grapeseed Oil on DMBA-induced Liver Enzymes Disturbance in Frog

fat on chronic exposure to carcinogens, tissue tumourigenesis and tumour induction. There have been no reports on the effect of grapeseed oil on tumour induction or cytotoxic and carcinogenic agent's action. At present, however, the influence of short-term exposure of DMBA has not yet been fully evaluated. Also, the effect of grapeseed oil on DMBA poisoning has not been established. Grapeseed oil is a polyunsaturated oil that is rich in linoleic acid. Frogs, *Rana ridibunda*, were selected because of its wide availability and suitability as a model for toxicity testing.

The present study was undertaken to evaluate whether grapeseed oil displays chemopreventive role in the frog liver injury induced by DMBA. Liver enzymes (LDH, GOT, GPT, and ALP) were chosen as biochemical indicator in assessing the condition of animals and their responses to the cytotoxic agent, DMBA.

Materials and Methods

Adult male and female frogs (*Rana ridibunda*) weighing 25 ± 3 g were collected from Al-Qatif, Saudi Arabia. The experimental animals were acclimated for 2 weeks in glass aquaria (50 X 50 X 80 cm). The bottoms of these glass aquaria were covered with wet sponges. They maintained on natural day light in an ambient temperature of $24 \pm 1^\circ\text{C}$. Frogs were fed with 0.5 ml of prepared meal (50% total protein) by intragastric intubation, twice daily.

Frogs were divided into five groups of 8 each and treated for 2 weeks as follows:

1. Animals of group 1 were injected into the dorsal lymph sacs with 7,12- dimethylbenz(a)anthracene (DMBA) (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 0.5 mg in 0.1 ml olive oil/frog, twice weekly.
2. Frogs of group 2 were fed with grapeseed oil by intragastric intubation at a dose of 1 ml/frog, twice weekly. After 4 hours, they received DMBA at the same level given in group 1.
3. The experimental animals of group 3 were fed only with grapeseed oil, at the same level given in group 2.
4. Frogs of group 4 were injected into the dorsal lymph sacs with 0.1 ml olive oil, twice weekly.
5. Animals of group 5 were untreated and used as control.

After 2 weeks, all frogs were sacrificed and liver specimens from each group were homogenate. The homogenates were centrifuged at 2000 rpm for 10 minutes. The clear supernatants were used for the estimation of activities of lactate dehydrogenase (LDH), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT) and alkaline phosphatase (ALP). All parameters were analyzed by using BM/Hitachi system 717 Automatic Analyzer and the values were expressed in U/g.

Statistical analysis were performed on paired groups (control group versus treated groups) using the Student's t-test. Also, comparison between DMBA-exposed group and group receiving DMBA plus grapeseed oil was detected. The chosen level of significance is $P \leq 0.05$.

Results

In comparison with control, the administration of DMBA alone for 2 weeks significantly increased the activity of liver LDH, while the activities of GPT, GOT and ALP were decreased in the frog, *Rana ridibunda*, Table 1. Similar results were noted in frogs treated with DMBA plus grapeseed oil. Also, it is found that the changes were more pronounced in frogs treated with DMBA plus grapeseed oil than those subjected to DMBA. The activities of liver enzymes (LDH, GPT, GOT, ALP) in frogs treated with grapeseed oil or olive oil were not significantly different from those of controls.

Discussion

The present study showed that the exposure of *Rana ridibunda* to DMBA alone or DMBA plus grapeseed oil caused significant physiological alterations reflected in an elevation of liver LDH activity, decreasing of GPT, GOT and ALP activities.

LDH is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to Pyruvate with the mediation of NAD⁺ as hydrogen acceptor. Many Carcinogens are known to cause cellular disintegration, mitochondrial damage and anaerobiosis (Ding *et al.*, 1989; Balint *et al.*, 1995; Kostka *et al.*, 1996; Al-Attar, 1998; Chuang *et al.*, 2000; Palacios and Biagianti, 2000; Walter *et al.*, 2000; Wang *et al.*, 2000; Silva *et al.*, 2001). Increased permeability of cell and necrosis are usually characterized by rise in LDH activity (Radhaiah, 1985). The observed DMBA induced increase in the activity of hepatic LDH may be attributed to the enhanced enzyme synthesis. Reports from other laboratories have describe similar situation in different animal species in response to heavy metals and pesticides (Natarajan, 1984; Sastry *et al.*, 1988; Sastry and Shulka, 1994; Sharma and Gopal, 1995, Altuntas *et al.*, 2002). Also, LDH level which indicate the energy demands are met by anaerobic respiration through increase in LDH activity. Moreover, several investigators have been reported that the oxygen consumption and the activities of liver respiratory enzymes (e.g. succinate dehydrogenase, malate dehydrogenase, NAD-isocitrate dehydrogenase) were decreased considerably with an elevation of glucose-6-phosphate dehydrogenase, glyceraldehyde dehydrogenase and/or LDH activities in stressed animals. They suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation (Balavenkatasubbaiah *et al.* 1984, Prasada *et al.*, 1985; Bahskaran, 1988; Rajeswari *et al.*,

Atef M. Al-Attar: Influence of Dietary Grapeseed Oil on DMBA-induced Liver Enzymes Disturbance in Frog

Table 1: Effects of DMBA, DMBA plus grapeseed oil, grapeseed oil and olive oil on the activities of liver enzymes in the frog, *Rana ridibunda*

Treatment	Liver Enzymes (U/g)			
	LDH	GOT	GPT	ALP
Control	115.62±4.29	7.74±0.84	10.61±0.45	7.72±0.62
DMBA	135.79±6.93 ^a	5.33±0.63 ^a	7.40±0.74 ^a	5.93±0.71 ^a
DMBA plus grapeseed oil	155.22±4.74 ^{a,b}	4.09±0.59 ^{a,b}	4.76±0.43 ^{a,b}	4.56±0.65 ^{ab}
Grapeseed oil	113.68±8.42	7.67±0.42	10.59±0.52	7.69±0.64
Olive oil	133.46±5.85	7.38±1.08	10.18±0.24	7.97±0.78

Each value is mean ± S.D. of 5 observations. Significance levels (^aP≤0.05; Student's t-test) shown for difference between control and treated groups. Significance levels (^bP≤ 0.05; Student's t-test) shown for difference between groups exposed to DMBA and DMBA plus grapeseed oil

1989; Gerbracht *et al.*, 1990; Sharma and Gupta, 1990; Reddy *et al.*, 1994; James *et al.*, 1996; Gupta *et al.*, 1997; Vaglio and Landriscina, 1999; Das and Mukherjee, 2000). Moreover, Rady *et al.* (1980) showed that the carcinogenic urethane, dimethylnitrosamine (DMNA), 3-methylcholanthrene (MCA), Benzo(a)pyrene (BP), DMBA and aflatoxin B1 enhanced the activities of glycolytic enzymes (hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase) in mouse lung. Additionally, Sharma (1999) reported that significant decrease in the activity of liver succinate dehydrogenase suggests that anaerobic metabolism was favored over aerobic oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival.

Both the transaminases (GPT, GOT) are important in protein metabolism. As for the decrease in GOT and GPT activities in hepatocytes of DMBA-intoxicated *Rana ridibunda*, in must considered it have also been observed that in different species linear alkylbenzene sulphonate, pesticides, cadmium, lead and mercury intoxications strongly depressed GOT and GPT activities as a consequence of serious cellular structure damage (Vaglio and Landriscina, 1999; Gill *et al.*, 1991a,b; Shakoori *et al.*, 1994; Rahman and Siddiqui, 2003). In addition, the present decrease in frog liver GPT activity may be correlated with the fact that there is deficient conversion of alanine to Pyruvate which enters into Krebs cycle to compensate for energy requirement. GOT is specific for glutamate and "-ketoglutarate but also reacts with nearly all amino acids. The depletion in the activities of GOT and GPT indicates disruption of link between carbohydrate and protein metabolism providing source of keto acids for Krebs cycle and gluconeogenesis (Gupta *et al.*, 1989).

ALP is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs, and hydrolase and transphosphorylase in function. Decrease in ALP activity may be taken as index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963). Inhibition of ALP reflects alterations in protein synthesis and

uncoupling of oxidative phosphorylation (Verma *et al.*, 1984) . The decrease in ALP by stressors probably indicates an altered transport of phosphate (Engstrom, 1964) and an inhibitory effect on the cell growth and proliferation (Goldfischer *et al.*, 1964). The inhibitions of liver ALP activities were demonstrated in animals exposed to different heavy metals, pesticides and sewage (Ram and Sathyanesan, 1985; Sastry and Shubhadra, 1985; Rajan, 1990; Shakoori *et al.*, 1994; Sharma, 1999; Rahman *et al.*, 2000).

The present data indicate that the administration of DMBA plus grapeseed oil results in more marked hepatotoxic effects than observed in group treated with DMBA only. Dietary intake of the n-6 fatty acid linoleic acid has a strong metastasis and growth - promoting effect on many rodent tumour and human tumour xenografts grown in immunodeficient rodents (De Vries and van Noorden, 1992; Weisburger, 1997; Sauer *et al.*, 2000). The mechanism by which grapeseed oil increased DMBA toxicity is unknown. Rogers (1983) reported that mechanisms proposed for enhancement of carcinogenesis by dietary fat include alteration of endocrine balance and stimulation of cell division or changes in differentiation in the organs (e.g. mammary gland). Dommels *et al.* (2000) stated that gap junctional intercellular communication, which modulates cell growth and differentiation, may play an important role in tumour growth. They found that cell incubation with linoleic acid inhibited gap junctional intercellular communication and cytotoxicity probably mediated by lipid peroxidation products. Also, Dommels *et al.* (2003) investigated the role of the enzyme cyclooxygenase and its prostaglandin product in n-6 and n-3 polyunsaturated fatty acid-mediated effects on cellular proliferation of two human colorectal carcinoma cell lines. They found that cells incubation with linoleic acid increased cell proliferation. They suggested that growth inhibitory and cytotoxicity effects of polyunsaturated fatty acids are due to peroxidation products that are generated during lipid peroxidation and cyclooxygenase activity. Collectively, the results of this study demonstrate that dietary grapeseed oil increases hepatotoxicity induced by DMBA. Although the exact mechanisms are not unknown, it can be

Atef M. Al-Attar: Influence of Dietary Grapeseed Oil on DMBA-induced Liver Enzymes Disturbance in Frog

explained by its ability to change the metabolic activation of DMBA. Additional investigations are required to understand (1) the basic mechanisms of grapeseed oil action with carcinogenic agent and (2) the exact role of grapeseed oil in human diets, consumption rates and health.

Acknowledgments

The author expresses his appreciation to the staff of Biochemical Laboratory, Dammam Central Hospital. I thank Mr. Ahmad Awad for his skillful technical assistance.

References

- Al-Attar, A.M., 1998. Physiological studies on the effect of fish oil on liver tumour induced by DMBA in the Egyptian toad. Ph.D. thesis. Alexandria University. Egypt.
- Altuntas, I., N. Delibas, M. Demirci, I. Kilinc and N. Tamer, 2002. The effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins E and C. Arch. Toxicol., 76: 470-473.
- Appel, H.J., G. Rovers and R.A. Woutersen, 1991. Inhibitory effects of micronutrients on pancreatic carcinogenesis in azaserone-treated rats. Carcinogenesis, 12: 2157-2161.
- Bahskaran, R., 1988. Effect of DDT and methyl parathion on the mitochondrial respiration, SDH and ATPase activity of an air breathing fish *Channa striatus*. Environ. Ecol., 6: 198-203.
- Balavenkatasubbaiah, M., A.U. Rani, K. Geethanjali, K.R. Purushotham and R. Ramamurthi, 1984. Effect of Cupric chloride on oxidative metabolism in the freshwater teleost, *Tilapia mossambicus*. Ecotoxicol. Environ. Saf., 8: 289-293.
- Balint, T., T. Szegletes, Z.S. Szegletes, K. Halasy and J. Nemcsok, 1995. Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and pyrethroid deltamethrin. Aquat. Toxicol., 33: 279-295.
- Buters, J., L. Quintanilla-Martinez, W. Schober, V.J. Soballa, J. Hintermair, T. Wolff, F.J. Gonzalez and H. Greim, 2003. CYP1B1 determines susceptibility to low doses of 7,12-dimethylbenz[a]anthracene-induced ovarian cancers in mice: correlation of CYP1B1-mediated DNA adducts with carcinogenicity. Carcinogenesis, 24: 327-334.
- Chang, W.C.L., R.S. Chapkin and J.R. Lupton, 1999. Fish oil blocks azoxymethan-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. J. Nutr., 128: 491-497.
- Chuang, S.E., A.L. Chen, J.K. Lin and M.L. Kuo, 2000. Inhibition by curcumin of diethylnitrosamine-induced hepatic hyperplasia, inflammation, cellular gene products and cell-cycle-related proteins in rats. Food. Chem. Toxicol., 38: 991-995.
- Das, B.K. and S.C. Mukherjee, 2000. Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham.). Toxicol. Lett., 114:11-8.
- De Vries, C.E. and C.J. Van Noorden, 1992. Effects of dietary fatty acid composition on tumor growth and metastasis. Anticancer Res., 12: 1513-1522.
- Ding, J.L., P.L. Hee and T.J. Lam, 1989. Differential susceptibility of a fish, tilapia *Oreochromis mossambicus* (Teleostei, Cichlidae) to hepatocarcinogenesis by diethylnitrosamine and methylazoxymethanol acetate. Carcinogenesis, 10: 493-499.
- Doll, R. and R. Peto, 1981. The causes of cancer: quantitative estimates of avoidable risk of cancer in the United States today. J. Nat. Cancer. Inst., 66: 1193.
- Dommels, Y.E., G.M. Alink, J.P. Linssen and B. Van Ommen, 2002. Effects of n-6 and n-3 polyunsaturated fatty acids on gap junctional intercellular communication during spontaneous differentiation of the human colon adenocarcinoma cell line Caco-2. Nutr. Cancer, 42: 125-130.
- Dommels, Y.E., M.M.G. Haring, N.G.M. Kestera, G.M. Alink, P.J. Van Bladeren and B. Van Ommen, 2003. The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE2 synthesis and cytotoxicity in human colorectal carcinoma cell lines. Carcinogenesis, 24: 385-392.
- Engstrom, L., 1964. Studies on bovine liver alkaline phosphatase, phosphate incorporation. Biochem. Biophys. Acta., 92: 71.
- Gerbracht, U., C. Einig, D. Oesterle, E. Dem, B. Schlatterer and E. Eigenbrodt, 1990. Di(2-ethylhexyl)phthalate alters carbohydrate enzyme activities and foci incidence in rat liver. Carcinogenesis, 11: 2111-2115.
- Gill, T.S., H. Tewari and J. Pand, 1991a. *In vivo* and *in vitro* effects of cadmium on selected enzymes in different organs of *Barbus conchonioides* Ham. (Rosy barb). Comp. Biochem. Physiol., 100: 501-505.
- Gill, T.S., H. Tewari, J. Pand and S. Lal, 1991b. *In vivo* tissue enzyme activities in the rosy barb (*Barbus conchonioides* Hamilton) experimentally exposed to lead. Bull. Environ. Contam. Toxicol., 47: 939-946.
- Goldfischer, S., E. Essner and A.B. Novikoff, 1964. The localization of phosphatase activities at level of ultrastructure. J. Histochem. Cytochem., 12: 72-95.
- Goldman, R. and P.G. Shields, 2003. Supplement: Biomarkers of nutritional exposure and nutritional status food mutagens. J. Nutr., 133: 965S-973S.
- Gupta, A.K., A. Anand, K. Ravindar and Rajana, 1997. Enzymological study on the effect of aldrin on a freshwater teleost fish, *Notopetrus notopetrus*. J. Nat. Conserv., 9: 9-12.

Atef M. Al-Attar: Influence of Dietary Grapeseed Oil on DMBA-induced Liver Enzymes Disturbance in Frog

- Gupta, B.N., A.K. Mathur, C. Agarwal and A. Sing, 1989. *In vitro* effect of linear alkylbenzene sulphonate (LAS) on some enzyme in liver and gill of the teleost *Channa punctatus*. Bull. Environ. Contam. Toxicol., 42: 375-381.
- Han, B.S., K. Fukamachi, N. Takasuka, T. Ohnishi, M. Maeda, T. Yamasaki and H. Tsuda, 2002. Inhibitory effects of 17-estradiol and 4-n-octylphenol on 7,12-dimethylbenz[a]anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. Carcinogenesis, 23: 1209-1215.
- James, R., K. Sampath and S. Alagurathinam, 1996. Effects of lead on respiratory enzyme activity, glycogen and blood sugar level in the teleost *Oreochromis mossambicus* (Peters) during accumulation and depuration. Asian Fish. Sci., 9: 87-100.
- Jelinska, M., A. Tokarz, R. Oledzka and A. Czorniuk-Sliwa, 2003. Effect of dietary linseed, evening primrose or fish oil on fatty acid and prostaglandin E(2) contents in the rat livers and 7,12-dimethylbenz[a]anthracene-induced tumours. Biochem. Biophys. Acta, 637: 193-199.
- Jiang, Y.H., J.R. Lupton, W.C. Chang, C.A. Jolly, H.M. Aukema and R.C. Chapkin, 1996. Dietary fat and fiber differentially alter intracellular second messengers during tumor development in rat colon. Carcinogenesis, 17: 1227-1233.
- Kostka, G., J. Kope-Szlezak and D. Palut, 1996. Early hepatic changes induced in rats by two hepatocarcinogenic organohalogen pesticides: bromopropylate and DDT. Carcinogenesis, 17: 407-412.
- Latham, P., E.K. Lund and I.T. Johnson, 1999. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduce mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis, 20: 645-650.
- Li N., X. Chen, J. Liao, G. Yang, S. Wang, Y. Josephson, H. Chi, J. Chen, M.T. Huang and C.S. Yang, 2003. Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters by tea and curcumin. Carcinogenesis, 23: 1307-1313.
- Lindhe, R., L. Granberg and I. Brandt, 2002. Target cells for cytochrome P450-catalysed irreversible binding of 7,12-dimethylbenz[a]anthracene (DMBA) in rodent adrenal glands. Arch. Toxicol., 76: 460-466.
- Miyata, M., M. Furukawa, K. Takahashi, F.J. Gonzales and Y. Yamazoe, 2001. Mechanism of 7,12-Dimethylbenz[a]anthracene-induced immunotoxicity: Role of metabolic activation at the target organ. Jpn. J. Pharmacol., 86: 302-309.
- Natarajan, G.M., 1984. Effect of sublethal concentration of metasystax on selected oxidative enzymes tissue respiration and hematology of the freshwater air breathing fish *Channa striatus*. Pestic. Biochem. Physiol., 21: 194-198.
- Neef, J.M., 1985. Polycyclic aromatic hydrocarbons (PAH's). In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamental of Aquatic Toxicology., Hemisphere Publ. Corp., pp: 416-454.
- Ogawa, T., S. Higashi, Y. Kawarada and R. Mizumoto, 1995. Role of reactive oxygen is synthetic estrogen induction of hepatocellular carcinomas in rats and preventive effect of vitamins. Carcinogenesis., 16: 831-836.
- O'Neill, I.K., S. Bingham, A.C. Povey, I. Brouet and J.C. Bereziat, 1990. Modulating effects in human diets of dietary fiber and beef, and of time and dose on the reactive microcapsule trapping of benzo[a]pyrene metabolites in the rat gastrointestinal tract. Carcinogenesis, 11: 599-607.
- Onikienko, F.A., 1963. Enzymatic changes from early stages of intoxication with small doses of chloro-organic insecticides. Giginea. I. Fiziol. Truda. Proizy. Toksilol. Klinika (Kietcv: Gos IZ.Med. Lit. UKHSSR) 77.
- Palacios, S. and R. Biagianti, 2000. Biochemical and (ultra) structural hepatic perturbation of *Brachydanio rerio* (Teleostei, Cyperinide) exposed to two sublethal concentrations of copper sulfate. Aquat. Toxicol., 50: 109-124.
- Prasada, R.K.S., S.I.K. Ahammad and R.K.V. Ramana, 1985. Methyl parathion (O-O-dimethyl O-4-nitrophenyl thiophosphate) effect on whole-body and tissue respiration in the teleost, *Tilapia mossambicus* (Peters). Ecotoxicol. Environ. Saf., 9:339-345.
- Radhaiah, V., 1985. Effect of heptachlor (OC) on kidney of a fresh water fish, *Tilapia mossambicus* (Peters), M. Phil. Thesis, S.V. University, Tirupati, India.
- Rady, A., I. Arany, F. Bojan and P. Kertai, 1980. Effect of carcinogenic and non-carcinogenic chemicals on the activities of four glycolytic enzymes in mouse lung. Chem. Biol. Interact., 31: 209-213.
- Rahman, M.F. and M.K. Siddiqui, 2003. Biochemical enzyme activity in different tissues of rats exposed to a novel phosphorothionate (RPR-V). J. Environ. Sci. Health, 38: 59-71.
- Rahman, M.F., M.K. Siddiqui and K. Jamil, 2000. Acid and alkaline phosphatase activities in a novel phosphorothionate (RPR-11) treated male and female rats. Evidence of dose and time-dependent response. Drug Chem. Toxicol., 23: 497-509.
- Rajan, M.R., 1990. Acid and alkaline phosphatase activity in different tissues of *Labeo rohita* (Hamilton) in relation to sublethal concentration of domestic sewage. J. Nat. Conserv., 2: 121-131.
- Rajeswari, K., S. Janardan, G.M. Reddy, S.N. Rafi and S.N. Reddy, 1989. Impact of thiodon on the metabolic pathway of the fish *Tilapia mossambicus*. Environ. Ecol., 7: 863-866.

Atef M. Al-Attar: Influence of Dietary Grapeseed Oil on DMBA-induced Liver Enzymes Disturbance in Frog

- Ram, R.N. and A.G. Sathyasesan, 1985. Mercuric chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa punctatus*. *Environ. Ecol.*, 3: 265-268.
- Reddy, D.S., V.V. Ghanathay, S.L.N. Reddy and K. Shankariah, 1994. Hepatotoxic effects of hexachlorocyclohexane on carbohydrate metabolism of a freshwater fish *Channa punctatus* (Bloch). *Bull. Environ. Contam. Toxicol.*, 53: 733-739.
- Rogers, A.E., 1983. Influence of dietary content of lipids and lipotropic nutrients on chemical carcinogenesis in rats. *Cancer Res.*, 43: 2477-2484.
- Salim, E.I., H. Wanibuchi, K. Morimura, T. Murai, S. Makino, T. Nomura and S. Fukushima, 2002. Induction of tumors in the colon and liver of the immunodeficient (SCID) mouse by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-modulation by long-chain fatty acids. *Carcinogenesis*, 23: 1519-1529.
- Sastry, K.V. and K. Shubhadra, 1985. *In vivo* effects of cadmium on some enzyme activities in tissues of the freshwater catfish, *Heteropneustes fossilis*. *Environ. Res.*, 36: 32-45.
- Sastry, K.V. and V. Shulka, 1994. Acute and chronic toxic effects of cadmium on some haematological, biochemical and enzymological parameters in the fresh water teleost fish *Channa punctatus*. *Acta Hydrochem. Hydrobiol.*, 22: 171-176.
- Sastry, K., A.A. Siddiqui and M. Samuel, 1988. Acute and chronic toxic effects of carbamate pesticide sevin on some haematological, biochemical and enzymatic parameters in the fresh water teleost fish *Channa punctatus*. *Acta Hydrochem. Hydrobiol.*, 16: 625-631.
- Sauer, L.H., R.T. Dauchy and D.E. Blasko, 2000. Mechanism for the antitumor and anticachectic effects of n-3 fatty acids. *Cancer Res.*, 60: 5289-5295.
- Shakoori, A.R., M.J. Iqbal, A.L. Mghal and S.S. Ali, 1994. Biochemical changes induced by inorganic mercury on the blood, liver and muscles of freshwater Chinese grass carp, *Ctenopharyngodon idella*. *J. Ecotoxicol. Environ. Monit.*, 4: 81-92.
- Sharma, B., 1999. Effect of carbaryl on some biochemical constituents of the blood and liver of *Clarias batrachus*, a fresh-water teleost. *J. Toxicol. Sci.*, 24: 157-164.
- Sharma, B. and K. Gopal, 1995. Changes in lactic acid content and activity of lactate dehydrogenase in *Clarias batrachus*, exposed to carbaryl. *Toxicol. Environ. Chem.*, 47: 89-95.
- Sharma, D. and R.C. Gupta, 1990. Effect of dyes on succinic dehydrogenase activity in a few tissues of *Colisa fasciatus*. *J. Nat. Conserv.*, 2: 115-119.
- Silva, V.M., C. Chen, G.E. Hennig, H.E. Whiteley and J.E. Manautou, 2001. Changes in susceptibility to acetaminophen-induced liver injury by the organic anion indocyanine green. *Food . Chem. Toxicol.*, 39: 271-278.
- Smith, D.A., G.G. Schuring, S.A. Smith and S.D. Holladay, 1999. Inhibited cytotoxic leukocyte activity in tilapia (*Oreochromis niloticus*) following exposure to immunotoxic chemicals. *Int. J. Toxicol.*, 18: 167-172.
- Spitsbergen, J.M., H.W. Tsai, A. Reddy, T. Miller, D. Arbogast, J.D. Hendricks and G.S. Bailey, 2000. Neoplasia in zebrafish (*Danio rerio*) treated with 7,12-dimethylbenz[a]anthracene by two exposure routes at different developmental stages. *Toxicol. Pathol.*, 28: 705-715.
- Suzuki, J.S., N. Nishimura, B. Zhang, Y. Nakatsuru, S. Kobayashi, M. Satoh and C. Tohyama, 2003. Metallothionein deficiency enhances skin carcinogenesis induced by 7,12-dimethylbenz [a] anthracene and 12-O-tetradecanoylphorbol-13-acetate in Metallothionein-null mice. *Carcinogenesis*, 24: 1123-1132.
- Vaglio, A. and C. Landriscina, 1999. Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. *Ecotoxicol. Environ. Saf.*, 43: 111-116.
- Verma, S.R., M. Saxen and I.P. Tonk, 1984. The influence of Idet 20 on the biochemical composition and enzymes in the liver *Clarias batrachus*. *Environ. Poll.*, 33: 245-255.
- Walter, G.L., P.D. Jones and J.P. Giesy, 2000. Pathologic alterations in adult rainbow trout, *Oncorhynchus mykiss*, exposed to dietary 2,3,8-tetrachlorodibenzo-p-dioxin. *Aquat. Toxicol.*, 50: 287-299.
- Wang, C.J., J.M. Wang, W.L. Lin, C.Y. Chu, F.P. Chou and T.H. Tseng, 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food. Chem. Toxicol.*, 38: 411-416.
- Weisburger, J.H., 1997. Dietary fat and risk of chronic disease: mechanistic insight from experimental studies. *J. Am. Assoc.*, 97: 16-23.
- Wijnhoven, S.W.P., H.J.M. Kool, L.H.F. Mullenders, R. Slater, A.A. Van Zeeland and H. Vrieling, 2001. DMBA-induced toxic and mutagenic response vary dramatically between NER-deficient *Xpa*, *Xpc* and *Csb* mice. *Carcinogenesis*, 22: 1099-1106.
- Yano, T., S. Yajima, K. Hagiwara, I. Kumadaki, Y. Yano, S. Otani, M. Uchida and T. Ichikawa, 2000. Vitamin E inhibits cell proliferation and the activation of extracellular signal-regulated kinase during the promotion of lung tumorigenesis irrespective of antioxidative effect. *Carcinogenesis*, 21: 2129-2133.
- Z'graggen, K., A.L. Warshaw, J. Werner, F. Graeme-Cook, R.E. Jimenez and C. Fernandez-Del Castillo, 2001. Promoting effect of a high-fat/high-protein diet in DMBA-induced ductal pancreatic cancer in rats. *Ann. Surg.*, 233: 688-695.
- Zimmerman, H.J., 1974. Serum enzyme measurement in experiment hepatotoxicity. In: Elikan, M., Eschar, J., Zimmerman, H.J. (Eds.), *International symposium on hepatotoxicity*. Academic Press, New York.