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

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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 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±1^{o}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*.,

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 ([a]$P \leq 0.05$; Student's t-test) shown for difference between control and treated groups. Significance levels ([b]$P \leq 0.05$; 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 alkalylbenzene 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 xengrafts 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 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.

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