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The Ameliorative Role of β-Carotene Pretreatment on Diazinon-Induced Enzymological and Histopathological Changes in Wistar Male Rats

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Abstract: The ameliorative activity of β-carotene on diazinon-induced enzymological and histopathological alterations was investigated in Wistar male rats. Forty rats were divided into four equal groups, each of ten. Group one was intraperitoneally administered saline solution, three times weekly for a period of five weeks and served as a control. Rats of group two were intraperitoneally injected with daizinon at a dose of 6.5 mg kg^{-1} body weight (BW) three times weekly for a period of five weeks. Rats of group three were orally supplemented with β-carotene at a dose of 4000 IU kg^{-1} BW and after two hours received diazinon at the same dose given to group two. Rats of group four were administered saline solution at the same dose given to group one and supplemented with β-carotene at the same dose given to group three. At the end of experimental period, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) activities were measured in all groups. Also, histopathological alterations of liver were examined. Results revealed that values of ALT, AST, ALP, CK and LDH were statistically increased in rats treated with diazinon. After five weeks of diazinon treatment, the treated rats revealed advanced liver destruction of architecture along with disarrangement of hepatic strands. Hepatocytes around central vein showed relatively severe apoptosis and necrosis. Also, some inflammatory cells were seen around the necrotic cells. Moreover, an enlargement of the sinusoids and vacuolar formations in hepatocytes and leucocytic infiltrations were noted. The activities of ALT and AST were significantly ($p<0.05$) elevated in rats treated with diazinon plus β-carotene, while the activities of ALP, CK and LDH were statistically unchanged. Also, pretreatment of the rats with β-carotene showed a pronounced protection in diazinon induced liver damage. In conclusion, this study suggested that diazinon-induced enzymological and histopathological changes in rats can be ameliorated by administration of β-carotene.

Key words: Diazinon · β-carotene · Serum enzymes · Liver histology · Rats

INTRODUCTION

Organophosphorous compounds have been widely used since few decades in agriculture for crop protection and pest control, thousands of these compounds have been screened and over one hundred of them have been marketed for these purposes [1-3]. The common use of insecticides in public health and agricultural schedules has caused severe environmental pollution and potential health hazards including severe acute and chronic cases of human and animal poisonings [4-5]. Toxicities of organophosphorous insecticides cause adverse effects on many organs [6]. Systems that could be affected by organophosphorous insecticides are the immune system [7], liver [8], muscles [9], urinary system [10], reproductive system [11], pancreas [12] and hematological system [13]. Diazinon is a nonsystemic organophosphate insecticide widely used in agriculture and for pest control in the environment, which can be highly toxic [14,15]. Diazinon has also been described as genotoxic and mutagenic carcinogenic agents using *in vivo* and *in vitro* models [16,17]. Additionally, several studies have showed that diazinon was capable of inducing physiological and histopathological alterations [18-22].

Beta-carotene (β-carotene) is one of the (nearly) 600 different carotenoids-plant pigments that give red/orange fruits and vegetables their color. It's best known as a precursor to vitamin A (retinol) in the body. The most abundant carotenoids in the diet are β-carotene, α-carotene, lycopene, lutein, β-cryptoxanthin, zeaxanthin

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and astaxanthin. Regular intake of β-carotene is necessary for human beings, because it gets converted into vitamin A when it enters the body. β-carotene is probably best known as one of 50 or so that possesses provitamin A activity [23]. While vitamin A, a fat-soluble nutrient, can be toxic if taken in excess, β-carotene can be safely ingested even in large quantities. This is why many multivitamin supplement formulas rely on β-carotene to supply part- and sometimes all-of their vitamin A activity. β-carotene is an excellent antioxidant and free radical scavenger. A large number of epidemiological studies suggested that the antioxidant nutrients, especially β-carotene, have a protective effect against genetic damage and the development of cancer induced by carcinogenic chemicals [24,25]. Numerous epidemiologic studies have supported the hypothesis that antioxidants could be used as an inexpensive means of prevention and possibly treatment, of cardiovascular diseases [26,27]. Several studies showed that β-carotene attenuated liver [28-30], lung [28], testicular [31] and intestinal [32] injuries induced by different chemical factors. Moreover, several researchers evaluated the anti-diabetic potential of β-carotene in experimental animals [33-35]. However, the effects of dietary β-carotene against diazinon toxicity are not well studied. Thus, the present study was conducted to investigate the effect of dietary supplementation of β-carotene on some physiological parameters and histology of liver in male Wistar rats exposed to diazinon.

MATERIALS AND METHODS

Animals and Experimental Design: Forty health male Wistar rats weighing 175 to 196 g were housed in standard plastic cages in a room at 25±1°C with normal 12-hour light-dark cycles. Rats were fed chow diet and tap water *ad libitum* for one week before the experiment. After one week of acclimation, rats were randomly divided into four equal groups (n=10 each). Group I was intraperitoneally administered saline solution, three times weekly for a period of five weeks and served as a control. Rats of group II were intraperitoneally injected with daizinon at a dose of 6.5 mg kg^{-1} body weight, BW, [36], three times weekly for a period of five weeks. Rats of group III were orally supplemented with β-carotene at a dose of 4000 IU kg^{-1} BW and after two hours received diazinon at the same dose given to group II, three times weekly for a period of five weeks. Rats of group IV were administered saline solution at the same dose given to group I and supplemented with β-carotene at the same dose given to group III, three times weekly for a period of five weeks. At the end of experimental period, rats were anaesthetized with diethyl ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2000 rpm for 20 minutes and the clear supernatants sera were obtained for biochemical measurements. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) were measured using an automatic analyzer (Reflotron® Plus System, Roche, Germany). Serum lactate dehydrogenase (LDH) was estimated using Automated Clinical Chemistry Analysis System, Dimension® type RXL Max (Dade Behring Delaware, DE 19714, U.S.A.). Also, rats were dissected, liver tissues were quickly isolated from each group, fixed in 10% buffered formalin, sectioned and stained with haematoxylin and eosin (H&E) for histological examination.

Statistical Analysis: Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. Statistical Package for Social Sciences (SPSS for windows, version 12.0) was used for the statistical evaluation. Significance level was set at $P<0.05$.

RESULTS

Serum activities values of ALT, AST, ALP, CK and LDH in control, diazinon, diazinon plus β-carotene and β-carotene treated rats are shown in Table 1. These biochemical parameters were statistically increased in rats treated with diazinon compared with control, diazinon plus β-carotene and β-carotene treated rats. In comparison with control values, the activities of ALT and AST were significantly elevated in rats treated with diazinon plus β-carotene. The activities of ALP, CK and LDH were statistically unchanged in diazinon plus β-carotene treated rats. Insignificant changes in the activities of ALT, AST, ALP, CK and LDH of were noted in rats supplemented with only β-carotene.

Liver histopathological results were depicted in Fig. 1. Fig. A and D showed the normal structure in control and β-carotene-treated rats, respectively. The liver sections of normal control and β-carotene-treated rats showed normal hepatocytes with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. After five weeks of diazinon treatment (group II), the treated rats revealed advanced changes in liver architecture along with disarrangement

Table 1: The values of serum ALT, AST, ALP, CK and LDH in control, diazinon, diazinon plus β-carotene and β-carotene treated rats

	Treatment			
Parameters	Control	Diazinon	Diazinon + β-Carotene	β-carotene
ALT (UL^{-1})	44.50±1.69	74.67±2.25[ab]	53.67±2.46[a]	46.00±2.130
AST(UL^{-1})	72.83±2.86	112.67±4.19[ab]	92.17±4.92[a]	78.83±7.139
ALP (UL^{-1})	430.17±2.26	527.33±5.95[ab]	448.83±7.37	418.83±10.70
CK (UL^{-1})	228.83±3.14	301.83±8.69[ab]	231.33±7.69	230.78±2.920
LDH (UL^{-1})	414.17±9.05	814.66±10.25[ab]	438.14±6.75	412.33±10.18

The values were expressed as mean±standard error (SE) of six rats from each group. The values were statistically significant at $P<0.05$. Statistical significance was compared within the groups as follows: Diazinon, diazinon plus β-carotene and β-carotene treated rats were compared with control rats (a). Diazinon treated rats were compared with diazinon plus β-carotene and β-carotene treated rats (b)

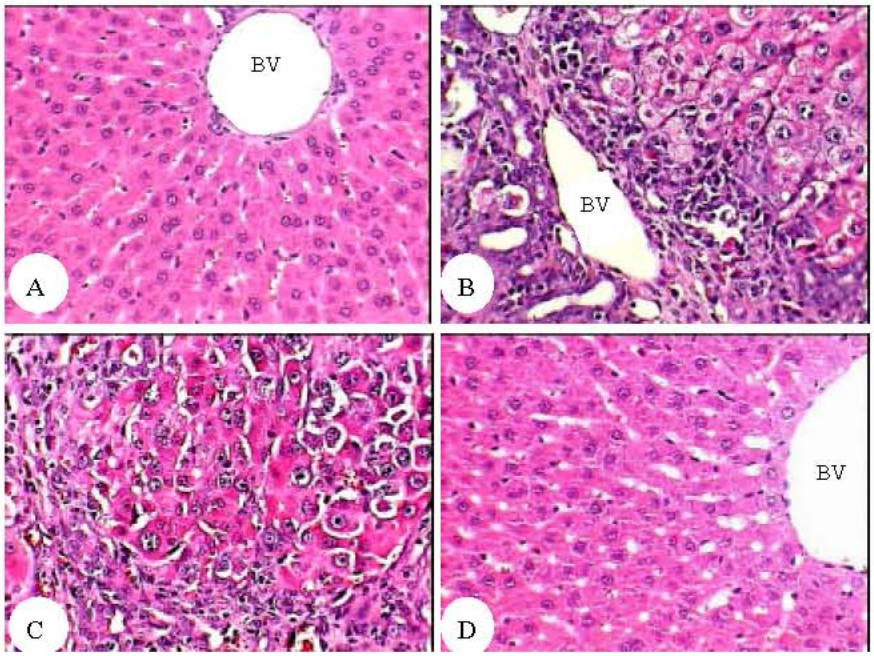

Fig. 1: Liver micrographs of control (A), daizinon (B), diazinon plus β-carotene (C) and β-carotene (D) treated rats. Original magnificationX400.

of hepatic strands. Hepatocytes around central vein showed relatively a high number of apoptotic and necrotic cells. Some inflammatory cells also seen around the necrotic cells. Moreover, an enlargement of the sinusoids and vacuole formations in hepatocytes and leucocytic infiltrations were noted (Fig. B). Pretreatment of the rats with β-carotene showed mild protection in diazinon induced liver damage. Also, some apoptotic cells were seen and necrotic cells were observed very rarely in diazinon plus β-carotene treated rats (group III).

DISCUSSION

A number of chemicals have been reported to cause severe physiological and histological disturbances, which sometimes becomes difficult to manage by medical therapies. It is important to search for compounds that can be used for better management of the physiological and histological alterations. The present study was designed to evaluate whether pretreatment with β-carotene would have a protective effect on diazinon induced physiological disturbances and liver histological injury. Treating rats with diazinon induced significant increases of serum ALT, AST, ALP, CK and LDH. Damage of hepatocytes is reflected by an elevation in the levels of hepato specific enzymes (ALT, AST and ALP), these are cytoplasmic in lection and are released in to circulation after cellular damage [37]. The present increases of serum ALT, AST and ALP in rats exposed to diazinon could be taken as an index of liver damage.

Liver histopathological examination also confirms these findings. However, several studies showed that theses enzymes were increased in human and experimental animals subjected to different pesticides including diazinon [19, 21, 38-43]. Additionally, the present histopathological changes of liver in rats treated with diazinon are similar with those showed by several authors in rats exposed to different pesticides [44-48].

One of the most valid and reliable methods for assessing muscular damage is to check for increases in blood serum levels of CK. CK catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP) to form creatine phosphate, the major storage form of high energy phosphate required by muscle [49,50]. The present elevation of serum CK level in diazinon-treated rats may be due to the damage of cardiac muscle tissues. However, several investigators showed that serum CK levels were increased in human and animals exposed to diazinon and other pesticides [41, 51-54]. LDH is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide $(NAD)^+$ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favours the reverse reaction, namely the reduction of pyruvate to lactate. Activity of LDH is present in almost all cells of the body and is found only in the cytoplasm of the cell [55,56]. Generally, high concentrations of LDH are found in the liver, heart, erythrocytes, skeletal muscles and kidneys. Consequently, diseases affecting those organs, such as renal infarction, myocardial infarction and haemolysis, have been reported to be associated with significant elevations in total serum LDH activity. Such elevations have been widely applied as diagnostic indices for kidney, liver, heart and red blood cell dysfunction [57-59]. Additionally, high serum LDH activity has also been reported in a variety of cancers [60]. The present increased of LDH value could be attributed to the lesion caused by diazinon in vital organs of rats. These results are consistent with several previous experimental studies [38,42,43].

From the present study, It is obviously that the pretreatment of rats with β-carotene attenuated the highly increases of serum ALT and AST induced by diazinon administration. Also, the present results showed that there is no significant difference in serum ALP, CK and LDH values in rats treated with diazinon plus β-carotene compared with control rats. These results proved that β-carotene produced a protective role against diazinon toxicity. The mechanism by which β-carotene alleviates the severe effects of diazinon exposure to rats is unknown. Generally, β-carotene is located in between the lipid bilayers of tissue cells. It lies parallel with the membrane surface deep within the hydrophobic core of the two layers [61]. It is a lipid soluble antioxidant that aids in the reduction of lipid peroxidation within cells [62]. β-carotene has the ability to quench some free radical reactions within membrane systems. This process entails the inactivation of electronically excited molecules such as singlet oxygen (O_2), generated from lipid peroxidation of cell membranes. In singlet oxygen, the peripheral electron is excited to an orbital above that which it usually occupies. Singlet oxygen can damage cells unless removed by antioxidants. β-carotene can also react with peroxyl radicals that are involved in the oxidation of lipids, [63] thus contributing to the defense of tissue cells. This ability to quench free radicals and other reactive oxygen species (ROS) can be attributed to the conjugated double bonds within the chemical structure of carotenoids. β-carotene reacts with singlet oxygen by transferring excitation energy from the oxygen so that it may return to its ground state, without any chemical change to the β-carotene. el-Demerdash *et al.* [64] investigated the role of alpha-tocopherol (vitamin E), β-carotene and/or their combination as antioxidants against the toxicity of fenvalerate, an insecticide, on blood hematology, free radicals, biochemical parameters and semen quality in male rats. They found that that fenvalerate significantly induced free radicals in plasma and brain and insignificantly in liver and testes. While, vitamin E, β-carotene alone and/or in combination decreased the levels of free radicals in plasma, liver, testes and brain. The activities of glutathione S-transferase (liver), ALP (plasma and liver), AST (plasma, liver and testes) and ALT (plasma and liver) were significantly increased due to fenvalerate administration. The activity of acetylcholinesterase was significantly decreased in brain and plasma, while plasma glucose, urea, creatinine and bilirubin concentrations were significantly increased in rats treated with fenvalerate. Also, their results showed significant alterations in plasma proteins, hematological parameters, body weight and relative weights of organs. Sperm concentration and motility percentage were significantly decreased, while, dead and abnormal sperm increased in rats exposed to fenvalerate. Vitamin E, β-carotene alone and/or in combination did not cause any changes in the investigated parameters, but improved semen quality and minimized the toxic effect of fenvalerate. Finally they suggested that the obtained

results demonstrated the beneficial influences of vitamin E, beta-carotene alone and/or in combination in reducing the harmful effects of fenvalerate. Lin *et al.* [65] examined the effects of β-carotene on antioxidant status in rats with chronic alcohol consumption and they stated that β-carotene supplementation can prevent ethanol-induced liver damage and increase glutathione (GSH) concentrations in erythrocytes and the liver.

In conclusion, this study confirmed that β-carotene plays a protective role against diazinon induced enzymological and histopathological changes. Further studies are needed to pursue the mechanism action of β-carotene against diazinon toxicity.

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