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EVALUATION OF PROTECTIVE EFFECT OF OMEGA- 3 FATTY ACIDS AND SELENIUM ON PARAQUAT INTOXICATED RATS

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ABSTRACT

Paraquat (PQ) toxicity involves the generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species. The purpose of this study was to investigate the effect of PQ on biochemical parameters in male rats and possible role of omega 3 fatty acids (ω3) and selenium (Se). The animals were divided randomly into 4 groups and kept at 10 rats per group. The first group was served as a control and administered with corn oil per orally, group 2 was injected with a single dose of PQ (10 mg.kg^{-1} BW, per i.p.) 24 h prior to decapitation, group 3 rats were pretreated per orally with ω3 (20 mg.kg^{-1} BW, three times a week for three weeks) and Se as sodium selenite (200 µg.kg^{-1} BW three times a week for three weeks), group 4 were pretreated per orally with ω3 (20 mg.kg^{-1} BW, three times a week for three weeks) and Se as sodium selenite (200 µg.kg^{-1} BW three times a week for three weeks), prior to injection with a single dose of PQ (10 mg.kg^{-1} BW, per i.p.). Paraquat was found to induce (P<0.001) serum thiobarbituric acid-reactive substances (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities. Treatment with ω3+Se alone increased the activities of SOD and CAT, while it decreased TBARS levels. ω3+Se provided protection against PQ-induced lipid peroxidation and oxidative stress in these rats. Total lipid (TL), cholesterol, triglyceride (TG), and low density lipoprotein (LDL) were significantly (P<0.001) increased while high density lipoprotein (HDL) level was decreased. The administration of ω3+Se alone decreased the levels of lipids and lipoproteins, and neutralized the harmful effects of paraquat. Paraquat increased (P<0.01) serum aminotransferases, phosphatases, lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ-GT). Urea, creatinine uric acid and total bilirubin levels were increased (P<0.01) whilst total protein (TP), albumin, globulin were significantly decreased (P<0.001). The results show that ω3+Se may neutralize changes in biochemical parameters and lipid peroxidation by activation of antioxidant defense system in rats.

Key words: Paraquat; omega-3; selenium; lipid peroxidation; antioxidant

INTRODUCTION

Pesticide poisoning is an important cause of morbidity and mortality in developing countries, although only a quarter of the total world consumption of pesticides is attributed to these regions (Banerjee et al., 1999; Akturk et al., 2006; El-Banna et al., 2008). Every year there are more than three million cases of severe poisoning and 220,000 deaths; the majority of these poisoning and about 99% of the resulting death occur in the Third World (Tinoco and Halperin, 1998; Frasca et al., 2009). Paraquat (PQ: methyl viologen :1,1Ì dimethyl-4,4Ì-bipyridylium ion) is a widely used herbicide that causes lethal intoxication in mammals, probably due to the presence of an active uptake mechanism in epithelial cells that increases the local concentration of the compound; the lungs are the most severely affected organ (Hoet et al., 1995). Because PQ easily undergoes redox cycling with subsequent production of reactive oxygen species (ROS), it has been proposed that PQ causes injury by producing ROS following induction of oxidant stress (Tomita et al., 2005; Samai, et al., 2008).

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Systems that protect the living body from oxidative stress-induced damage include enzymes superoxide dismutase, catalase and glutathione peroxidase which remove ROS; proteins ferritin and transferrin that mask the heavy metal ions; antioxidant system including vitamin E and vitamin C; enzyme repair systems for damaged DNA; and enzyme systems ubiquitin-proteasome that remove damaged proteins (Kikugawa, 2004; Limón-Pacheco and Gonsebatt, 2009).

Antioxidant micronutrients are being widely studied for their alleged beneficial properties in the prevention of human diseases, cancer, arthritis and cardiovascular diseases (Faure et al., 2007). Selenium is a structural component of several enzymes with physiological antioxidant properties, including glutathione peroxidase (GSH-Px) and thioredoxine (Perottoni et al., 2004). A great deal of studies has been carried out on selenium metabolism. Most of the studies used sodium selenite as external selenium source for experimental animals (Shi et al., 2004).

Omega-3 (ω_3) fatty acids are long chain, polyunsaturated fatty acids (PUFA) of plant and marine origin. Because these essential fatty acids (EFAs) cannot be synthesized in the human body, they must be derived from dietary sources. Flax seed, hemp, canola, and walnuts are generally rich sources of ω_3 PUFA alpha-linolenic acid (ALA). Fish provide varying amounts of ω_3 fatty acids in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ALA can be metabolized into the longer chain EPA and DHA. The role played by EFAs in the human body has been the subject of numerous studies, particularly in recent years. The results indicate that ω_3 fatty acids may be of value in the treatment of various medical conditions (Alan et al., 2003).

Recognizing the fact that paraquat is a strong redox agent and contributes to the formation of ROS, attempts have been made to explore pharmacological strategies, which may reduce the formation of these oxidants and/or prevent their toxic effects (Ghazi-Khansari and Mohammadi-Bardbori, 2007; Dinis-Oliveira et al., 2007). The present study was undertaken to determine the potential protective roles of ω3 and selenium against oxidative damage induced by paraquat.

MATERIALS AND METHODS

Chemicals

Paraquat (PQ) (98%) was purchased from Chemical Service (West Chester, PA, USA). Use of PQ was approved by the Animal Care Committee and met all guidelines for its use. Omega 3 oil was purchased from PharmAssure, Inc. Phoenx, AZ, USA. The tested dose of omega 3 fatty acid oil (ω_3) was 20 mg/kg/day. Sodium selenite was purchased from Twin Laboratories Inc. (UTAH, 84003 USA). Thiobabituric acid and all other chemicals were purchased from Sigma Chemical Company (St. Louis, Mo, USA).

Animals

Male albino rats weighting 160 ± 20 g were randomly drawn from the stock colony of National Research Institute, Cairo, Egypt. The animals were housed in universal galvanized wire cages at room temperature (22 ± 2 °C) and under a photoperiod of 12:12 light/dark cycle, 50 ± 5% humidity. The rats were acclimatized for 2 weeks prior to the start of the experiment. Rats were maintained on commercial pellet diet (protein 18%, fat 6%, fibre 6%, carbohydrates 56%, calcium 0.6%, moisture 10% and ash 11%) and water ad libitum. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institutional Animal Ethics Committee. Permission for handling with laboratory animals was obtained from the Institutional Animal Committee. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept at minimum.

Animal treatment and experimental protocol

Adult rat males were grouped randomly into four groups (n=10). The group I - (control) rats received corn oil 24h prior to decapitation; group II rats received a single dose (10 $mg.kg^{-1}$) of PQ i.p. 24h prior to decapitation; group III (ω3+Se) rats received omega3 per orally 20 $mg.kg^{-1}.day^{-1}$ plus Se 200 μg. $kg^{-1}.day^{-1}$ for 21 days); group IV (PQ+ω3+Se) rats received omega3 20 $mg.kg^{-1}.day^{-1}$ plus Se 200 $\mu g.kg^{-1}.day^{-1}$ for 21 days and a single dose (10 $mg.kg^{-1}$) of PQ i.p. 24h prior to decapitation.

Sample collection

The animals were starved overnight for 12h before blood was collected. Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture into sterilized vials. Blood samples were allowed set to clot at 4 °C and centrifuged at 1000 g for 30 min. Then 1000 μl aliquots of serum were placed in microfuge tubes and frozen on dry ice. Labeled bags were placed into freezer at -20 °C until the time of the assay.

Assay systems

Serum thiobarbituric acid reactive substances (TBARS) were measured at 532 ηm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 cm^{-1} $mole^{-1}$ was used for calculation (Tapel and Zalkin, 1959). Superoxide dismutase (SOD) (EC. 1.15.1.1) activity was measured in serum according to Flohe and Otting

(1984). Catalase (EC. 1.11.1.6) activity in serum was assayed by monitoring the decomposition of H_2O_2 at 240 nm as described by Aebi (1984). The activity of acetylcholinesterase (AChE) (EC 3.1.1.7) was measured according to the method of Ellman et al. (1961) using commercial kit obtained from Quimica Clinica Aplicada S.A. Serum alanine aminotranseferase (ALT; EC 2.6.1.2) and aspartate aminotranseferase (AST; EC 2.6.1.1) activities were determined using commercial kits obtained from Bio M'erieux, France. The principle reaction of the colorimetric determination of AST or ALTactivity is based on the reaction of aspartate or alanine with α-ketoglutarate to form oxaloacetate or pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenylphosphate as a substrate (Rosalki and Foo, 1993). Acid phosphatase activity (ACP; EC 3.1.3.2) was determined according to Rudolph (1967) using commercial kits obtained from Bio ADWIC, Egypt. Serum lactate dehydrogenase (LDH; EC 1.1.1.27) was determined according to the method of Friedman and Young (1997).

Serum samples were analyzed for total protein (TP) by the Biuret method according to Armstrong and Carr (1964). Albumin concentration was determined by the method of Doumas et al. (1971). Globulin concentration was determined as the difference between total protein and albumin. Serum total bilirubin was measured using the method of Walters and Gerade (1970). Also, low density lipoprotein (LDL) cholesterol was measured according to the method of Assmann et al. (1984), high density lipoprotein (HDL) cholesterol was measured according to the method of Burstein et al. (1970). Cholesterol and triglycerides (TG) were measured according to the method Carr et al. (1993), and serum gamma glutamyl transferase (γ−GT) was measured according to the method of Szasz (1969), using commercial kits obtained from BioSystems Co., Spain. Total lipids were measured according to the method of Knight et al. (1972) using commercial kits obtained from Bio ADWIC, Egypt. Serum creatinine was measured according to the method of Mitchell (1973), uric acid was estimated according to the method of Morgenstern et al. (1966), using commercial kits obtained from Biocon® Diagnostik, Marienhagen, Germany. Urea was measured according to the method of Patton and Crouch (1977) using commercial kit obtained from Diamond Co., Egypt.

Statistical procedures

Data are expressed as the means ± S.E.M. The data were analyzed using a one-way analysis of variance and the statistical significance between specific means was determined using the Newman-Keuls multiple range test.

RESULTS

Rats administered with ω3+Se showed no signs of toxicity. On the other hand immediate behavioural changes were observed in the paraquat-intoxicated rats, two hours after an i.p. dose administration of 10 mg/kg of body weight, leading to tremors and convulsions. Omega3+Se *per se* had no effect on serum levels of TBARS while compared with control (Table 1). Paraquat treated rats accounted for a significant (P<0.001) increase of lipid peroxidation in serum as evidenced by the enhanced TBARS levels in serum compared with those of control (Table 1). With ω3+Se pretreatment in paraquat treated rats, a dramatic reduction (P<0.001) in TBARS levels was noted compared to paraquat treated group.

SOD activity in ω3+Se treated rats (group 3) significantly (P<0.01) increased as compared to control (Table 1). Treatment with paraquat (group 2) showed significant (P<0.001) increase in serum SOD activity as compared to control. ω3+Se pretreated rats treated with paraquat (group 4) showed significant (P<0.01) decrease in serum SOD activity compared to paraquat-treated group (group 2).

Omega3+Se -administered rats (group 3) showed serum CAT activity comparable to control (Table 1).

Table 1: Effects of omega 3 (ω_3) fatty acids and selenium (Se) on thiobarbituric acid reactive substances (TBARS) levels, and superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase (AChE) activities in serum from paraquat treated rats

Parameter	Control	Paeaquat	ω_3+Se	Paraquat + ω_3+Se
TBARS (nmol/ml)	2.422 ± 0.214	3.99 ± 0.215 ^{z}	1.961 ± 0.215 $^{x, c}$	2.891 ± 0.11 $^{y, c}$
SOD (U/ml)	1.531 ± 0.171	3.44 ± 0.253 ^{z}	2.14 ± 0.115 $^{y, b}$	2.32 ± 0.351 $^{z, c}$
Catalase*	498.1 ± 29.14	695.0 ± 11.3 ^{z}	597.6 ± 31.57 $^{z, c}$	634 ± 29.9 $^{z, a}$
AChE μmol/min/ml	3.858 ± 0.154	3.171 ± 0.212 ^{y}	3.336 ± 0.251	3.623 ± 0.091 ^{b}

Values are expressed as Means ± SEM; n= 10 for each treatment group. ^{x} p<0.05, ^{y} p<0.01, ^{z} p<0.001 compared with control respectively; ^{a} p<0.05, ^{b} p<0.01, ^{c} p<0.001 as compared between paraquat and paraquat+ω_3+Se respectively. * μmoles of H_2O_2 decomposed/min/mg protein

Treatment with paraqaut (group 2) showed significant (P<0.001) increase in serum CAT activity as compared to control. Omega3+Se -pre administered rats treated with paraquat (group 4) showed decrease in serum CAT compared to paraquat-treated group (group 2).

AChE activity in serum was significantly (P<0.01) inhibited in paraquat treated rats (group 2) as compared to control (Table 1). Omega3+Se -administered rats treated with paraquat showed high recovery (group 4).

Data represented in Table 2 show that i.p. treatment with paraquat caused significant (P<0.001) increase in serum total lipid, cholesterol, triglycerides and LDL concentrations, whilst HDL levels were lower compared to control animals (Table 2). Omega3+Se alone caused significant (P<0.05) decrease in lipid profile compared to control. Omega3+Se Se administered rats treated with paraquat showed reduction in the serum lipid level caused by paraquat. Results also showed significant increase in serum HDL of animals pre administered with ω3+Se prior paraquat treatment compared to paraquat treated group (Table 2).

Treatment with paraquat significantly (P<0.01) increased the activities of AST, ALT, AIP, AcP, LDH, γ-GT and total bilirubin in serum compared to control animals (Table 3). The present study demonstrates that treatment with ω3+Se alone did not cause any significant change in the enzyme activities in serum and alleviated the toxicity of paraquat. Omega3+Se in combination with paraquat alleviated its negative effect on the activities of the above measured enzymes.

Data presented in Table 3 show that treatment with paraquat caused significant (P<0.001) decrease in serum TP, albumin and globulin, and increase (P<0.01) in total bilirubin compared to control. Treatment with ω3+Se

Table 2: Effects of omega 3 (ω_3) fatty acids and selenium (Se) on lipid and lipoprotein profiles in serum from paraquat treated rats

Parameter	Control	Paeaquat	ω_3+Se	Paraquat + ω_3+Se
TL (g/dl)	2.82 ± 0.051	3.926 ± 0.0345 ^{z}	2.145 ± .010$^{z, b}$	3.124 ± 0.06
Cho (mg/dl)	66.99 ± 2.68	168.2 ± 8.28^{z}	64.6 ± 5.49	124.8 ± 13.48^{c}
TG (mg/dl)	98.80 ± 2.63	213.0 ± 19.6^{z}	65.4 ± 4.174^{Z}	164.0 ± 15.92^{y}
HDL (mg/dl)	48.34 ± 1.195	29.25 ± 3.024^{z}	59.35 ± 1.92 $^{z, c}$	40.12 ± 5.160^{b}
LDL (mg/dl)	44.235 ± 2.25	58.231 ± 3.11^{z}	35.134 ± 2.30^{z,c}	49.22 ± 1.88^{c}

Values are expressed as Means ± SEM; n= 10 for each treatment group. TL, total lipids; Cho, cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol. ^{x} p<0.05, ^{y} p<0.01, ^{z} p<0.001 as compared with control respectively; ^{a} p<0.05, ^{b} p<0.01, ^{c} p<0.001 as compared between paraquat and paraquat+ω_3+Se, respectively

Table 3: Effects of omega 3 (ω_3) fatty acids and selenium (Se) on enzymes in serum from paraquat treated rats

Parameter	Control	Paeaquat	ω_3+Se	Paraquat + ω_3+Se
TP (mg/dl)	8.224 ± 0.201	4.499 ± 0.655^{z}	7.812 ± 0.662	6.666 ± 0.212^{c}
Albumin (mg/dl)	5.404 ± 0.143	2.652 ± 0.394^{z}	5.384 ± 0.1650	3.720 ± 0.515^{b}
Globulin (g/dl)	2.98 ± 0.108	1.847 ± 0.25^{z}	2.588 ± 0.125	2.924 ± 0.104^{b}
AST (U/L)	118.40 ± 9.66	170.6 ± 13.80^{z}	134.4 ± 4.314	137.81 ± 6.58^{b}
ALT (U/L)	42.40 ± 1.496	61.20 ± 3.31^{z}	35.40 ± 6.31	44.40± 2.65^{c}
ALP (U/L)	74.64 ± 11.64	143.4 ± 22.66^{z}	51.40 ± 2.24^{x}	95.40 ± 15.06^{a}
AcP (U/L)	35.10 ± 3.39	49.87 ± 8.12^{y}	30.81 ± 1.95	32.51 ± 0.92^{b}
LDH (U/L)	571.1 ± 21.3	750.1 ± 16.83^{z}	468.5 ± 29.15 ^{c}	555.3 ± 41.1^{c}
γ-GT (U/L)	2.541 ± 0.543	5.01 ± 0.12^{z}	2.114 ± 0.41 ^{c}	2.812 ± 0.31^{c}
Bilirubin (mg/dl)	0.510 ± 0.02	0.636 ± 0.028^{y}	0.50 ± 0.043	0.538 ± 0.030^{c}

Values are expressed as Means ± SEM; n = 10 for each treatment group. TP, total protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AcP, acid phosphatase; LDH, lactate dehydrogenase; γ GT, gamma glutamyl transferase. ^{x} p<0.05, ^{y} p<0.01, ^{z} p<0.001 as compared with control respectively; ^{a} p<0.05, ^{b} p<0.01, ^{c} p<0.001 as compared between paraquat and paraquat+ω_3+Se, respectively

Table 4: Effects of omega 3 (ω_3) fatty acids and selenium (Se) on urea, creatinine and uric acid levels in serum from paraquat treated rats

Parameter	Control	Paeaquat	ω_3+Se	Paraquat + ω_3+Se
Urea (mg/dl)	29.31 ± 2.45	38.84 ± 1.33^{y}	22.32 ± 2.63^{x}	28.02 ± 1.92^{c}
Creatinine (g/dl)	0.045 ± 0.014	0.160 ± 0.008^{z}	0.029 ± 0.01	0.099 ± 0.029^{b}
Uric acid (mg/dl)	3.58 ± 0.26	4.724 ± 0.11^{z}	3.52 ± 0.28	4.124 ± 0.127^{b}

Values are expressed as Mean ± SEM; n= 10 for each treatment group. ω_3, omega-3 fatty acids; Se, selenium; x $p<0.05$, y $p<0.01$, z $p<0.001$ as compared with control; a $p<0.05$, b $p<0.01$, c $p<0.001$ as compared between paraquat and paraquat+ω_3+Se, respectively

alone significantly increased serum TP, albumin and globulin and decreased billirubin content. The presence of ω3+Se with paraquat counteracted its hazardous effects.

Paraquat intoxicated rats showed a constellation of disorders in renal function witnessed by increased urine output and changes in creatinine, urea, and uric acid levels (Table 4). Compared to the controls, creatinine levels were higher ($P<0.01$) in serum of paraquat intoxicated rats. Pretreatment of rats with ω3+Se significantly ($P<0.001$) reduced creatinine concentration compared to paraquat treated animals. Urea levels in treated rats were higher in serum and lower in antioxidant challenged groups compared to the controls. Uric acid levels were higher in serum of paraquat treated rats and kept at normal levels in ω3+Se pretreated intoxicated rats.

DISCUSSION

Consumption of some ω−3 polyunsaturated fatty acids such as eicosapentaenoic (C20:5 n−3, EPA) and docosahexaenoic (C22:6 n−3, DHA) acids from fish oil has shown a preventive action against cardiovascular diseases. Food and Drug Administration (FDA) announced a qualified health claim for the use of EPA and DHA in conventional foods and dietary supplements (FDA, 2006). These fatty acids reduce both overall mortality related to infarction and sudden death in patients with coronary heart disease (Bucher et al., 2002) by inhibition of pro-inflammatory eicosanoid formation. Other mechanisms include decrease in plasma very low-density lipoproteins (VLDL) and TG, and production of both larger and less atherogenic LDL (Frenoux et al., 2001, Hudert et al., 2006, Song et al., 2000 and Carrapeiro et al., 2007).

The behavioural changes observed in the paraquat-intoxicated rats in the present study is in accordance with that of Suntres et al. (2002) in which administration of a single dose of paraquat (25 mg/kg) to rats resulted in severe neurological disturbances 1-2 h after i.p injection. This emphasizes the role of paraquat in inducing neurotoxicity. Other changes were hyperactivity and restlessness with an indifferent attitude towards food intake and water consumption. Later on, 24-hrs post-paraquat treatment, respiratory difficulties were observed, in accordance with the paraquat-intoxication mechanism in the lung, and the lung being the target organ of this xenobiotic. These observations are in-agreement with observations reported by Suntres (2002).

Lipid peroxidation (LPO) has been suggested as one of the molecular mechanisms involved in organochlorine, carbamate or organophosphorous pesticides and paraquat. Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Ahmed et al., 2000). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction and hence the question of whether oxidative stress is a major cause of injury remains equivocal. In this study the effects of of ω3+Se administration by paraquat in regards to LPO induction was invesigated. To our knowledge there is no information concerning to protective action of ω3+Se on oxidative injury induced by paraquat. Paraquat caused an increase in TBARS levels, indicating an increase in lipid peroxidation. This finding is supported by another report of increase in lipid peroxidation in the lung and serum by paraquat (Mustafa et al., 2002). Oral administration of ω3+Se was able to prevent the increase in TBARS levels due to paraquat toxicity. The role of nutrients in modulating paraquat toxicity in experimental animals has also been investigated, but not as extensively as for antioxidants. The injection of fish oils (cod liver and menhaden oils) reduced paraquat lethality (Fritz et al., 1994). The protective effect conferred by this oil is not clear, but it does not appear to be due to their vitamin E content or due to alteration in the absorption or distribution of paraquat (Fritz et al., 1994). It has been demonstrated that an increase in monosaturated fatty acids or a reduction in polyunsaturated fatty acids in lipid membranes decreases the susceptibility of membranes to oxidant attack (Suntres (2002).

Under normal physiological conditions, a delicate balance exists between the rate of formation of H_2O_2 via dismutation of O^{-2} by SOD activity and the rate of removal of H_2O_2 by CAT and glutathione peroxidase. Therefore any impairment in this pathway will affect the activities

of other enzymes in the cascade (Kono and Fridovich, 1992). Results from this study suggest that pretreatment with ω3+Se exerts an antioxidative effect by decreasing lipid peroxidation, and maintaining normal levels of SOD and CAT activities. Higher levels of antioxidant enzymes have been well correlated with increased lipid peroxidation after paraquat administration. The activity of peroxide metabolism enzymes and TBARS was studied in mice, significant increases in SOD and GSH-Px were observed at 15 or 18 h after paraquat injection (Takenaka and Goto, 1994).

AChE activity in the serum of paraquat treated rats reduced significantly as compared to control. Moreover, the activity of γ-GT enhanced significantly. The inhibition of AChE resulted in the accumulation of acetylcholine (ACh), which may cause stimulation of lymphocytes and elevated concentration of cellular cGMP, increased lymphocyte motility and cytotoxicity (Malik and Summer, 1982). Since AChE and γ-GT are both membrane bound enzymes; γ-GT could interact with amino acid neurotransmitter, ACh which may be removed from the binding with AChE and may result in decreased activity of AChE (Szelenyi et al., 1987). The biological significance of γ-GT-dependent lipid peroxidation *in vivo* might be multifold. Varying levels of γ-GT activity can be detected in erythrocytes and lymphocytes. It is conceivable that the pro-oxidant effects of γ-GT activity are normally balanced by its established role in favouring the cellular uptake of precursors for GSH re-synthesis, thus allowing the reconstitution of cellular antioxidant defense (Banerjee et al., 1999).

HDL may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion. Also, high level of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. In addition, HDL could protect LDL against oxidation *in vivo* because the lipids in HDL are preferentially oxidized before those in LDL (Stocker et al., 1992).

Injection of paraquat to rats revealed a significant hepatic damage as observed from the elevation of hepatospecific enzyme activities, as well as severe alteration in different liver parameters. Activities of AST, ALT, ALP and AcP in serum were significantly higher in paraquat intoxicated rats. Pretreatment with ω3+Se provides significant protection against paraquat. A significant rise in ALT and AST might be taken as an index of liver damage. The reversal of increased transaminases returned to normal by ω3+Se supplementation with healing of hepatic parenchyma and regeneration of hepatocytes.

Lactate dehydrogenase (LDH) is a cellular enzyme that contributes to carbohydrate metabolism. Specifically, it catalyzes the reversible oxidation of lactate to pyruvate. LDH is present in almost all-metabolizing cells, especially in the liver, heart, kidneys, skeletal muscle, brain, and erythrocytes. It is released during tissue injury and increases in its reported value usually indicate cellular death and leakage of the enzyme from the cell (McFarland, 1994). Lactic dehydrogenase activity was enhanced due to paraquat treatment in the present study. These results indicated that membrane dysfunction by paraquat is ascribed to iron-catalysed reaction of extracellularly increased hydrogen peroxide. The augmentation of LDH can be used as an indicator of cellular damage and cytotoxicity of pesticides (Bagchi et al., 1995). When endogenous antioxidant defense capabilities are exceeded by the oxidant flux, tissue injury occurs. Pretreatment of paraquat intoxicated rats with ω3+Se was able to reduce the elevation of serum LDH. This process can be intercepted pharmacologically at different levels with agents that scavenge reactive oxygen metabolites, block their generation or enhance endogenous antioxidant capabilities (Attia and Nasr, H.M. 2009). Selenium is a structural component of several enzymes with physiological antioxidant properties, including GSH-Px and thioredoxine (Perottoni et al., 2004).

The reduction in serum protein due to paraquat treatment, particularly albumin, could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein depression may be due to loss of protein either by reducing protein synthesis or increasing proteolytic activity or degradation (Yeragi et al., 2003). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of paraquat on liver cells as confirmed by the increase in the activities of serum AST and ALT.

Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (Garba et al., 2007). Therefore, the significant increases in urea and creatinine levels noted in this study are classical signs of adverse affects of paraquat administration on kidney. Kidney dysfunction and nephrotoxicity induced by paraquat in the present investigation are mediated through oxidative stress. The rise in kidney biochemical parameters could be attributed to toxic effect of this insecticide on kidney and may result from vasoconstriction, tubulointerstitial and renal injury (Garba et al., 2007).

A report indicated that additive and synergistic interactions may occur with combinations of dietary antioxidants against free radical inducing damage in cells and tissues (Hercberg et al., 1998). The present sudy demonstrates that administration of ω-3 fatty acids and selenium to rats modulates the antioxidant enzymes in a manner that favours the reduction of lipid peroxidation and suggests a possible adaptive mechanism to counteract

oxidative stress situation. Hence such studies on oxidative/antioxidant status during a free radical challenge can be used as an index of protection against the development of lipid peroxidation in experimental animals for assessment for dietary and therapeutic purposes. In conclusion, this study in rats suggests that ω-3 fatty acids and selenium may have preventive actions against unwanted free radicals generated from paraquat exposure to mammals.

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