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MOLECULAR TOXICOLOGY

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In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats

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Abstract Reactive oxygen species (ROS) may be involved in the toxicity of chlorpyrifos-ethyl (CE) [*O*, *O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate]. We have, therefore, examined the in vivo effects of CE on the rat erythrocyte antioxidant system and evaluated the ameliorating effects of melatonin and a combination of vitamin E and vitamin C on the oxidative damage induced by CE. The experimental groups were: (1) control group, (2) CE-treated group (CE), (3) vitamin E plus vitamin C treatment group (Vit), (4) melatonin-treated group (Mel), (5) vitamin E plus vitamin C plus CE treatment group (Vit + CE), and (6) melatonin plus CE treatment group (Mel + CE). Vitamin E and vitamin C were administered intramuscularly once a day for 6 consecutive days at 150 and 200 mg/kg, respectively, in the Vit and Vit + CE groups. Melatonin was administered intramuscularly at 10 mg/kg per day for 6 consecutive days in the Mel and Mel + CE groups. At the end of the fifth day, the rats of CE, Vit + CE and Mel + CE groups were treated orally with the first of two equal doses of 41 mg/kg CE, the second oral dose being given 21 h later. Blood samples were taken 24 h after the first CE administration. Levels of thiobarbituric acid reactive substance (TBARS), antioxidant defence potential (AOP), and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined in erythrocytes. In comparison with the control group, oral administration of CE significantly ($P < 0.05$) stimulated TBARS activity while significantly ($P < 0.05$) inhibiting AOP and the activities of SOD and CAT. However, GSH-Px activity remained unchanged by CE treatment. Treatment with melatonin and vitamins E plus C significantly ($P < 0.05$) reduced the CE-induced increase of TBARS, and overcame the inhibitory effect of CE on SOD and CAT, but not on AOP. Melatonin treatment significantly ($P < 0.05$) increased only GSH-Px activity, irrespective of the effect of CE. These results suggest that CE treatment increases in vivo lipid peroxidation and decreases antioxidant defence by increasing oxidative stress in erythrocytes of rats, and melatonin and a combination of vitamin E and vitamin C can reduce this lipoperoxidative effect.

Key words Chlorpyrifos-ethyl · Erythrocyte · Lipid peroxidation · Melatonin · Vitamin

Introduction

The widespread use of organophosphorus insecticides (OPIs) has long been shown to exert deleterious effects on living organisms. For instance, the exposure of laboratory animals to OPIs, in particular to chlorpyrifos-ethyl (CE) [*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate], elicits a number of effects including hepatic dysfunction (Gomes et al. 1999), delayed fever (Gordon et al. 1997), ciliotoxicity (Swann et al. 1996), immunological abnormalities (Thrasher et al. 1993; Blakley et al. 1999), embryotoxicity (Muscarella et al. 1984; Muto et al. 1992), genotoxicity (Bagchi et al. 1995; Dam et al. 1998; Roy et al. 1998; Song et al. 1998) and neurochemical and neurobehavioural changes in developing and adult animals (Whitney et al. 1995; Chanda and Pope 1996; Mattsson et al. 1996; Dam et al. 1998; Song et al. 1998; Dam et al. 1999; Hunter et al. 1999). Many reports also indicate that the most commonly used OPIs induce a teratogenic effect in animals (Gupta 1992; Roy et al. 1998).

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Several insecticides have been found to bind extensively to human plasma protein fractions (Datta et al. 1992) and to disturb the biochemical and physiological functions within the erythrocytes, thereby affecting membrane integrity (Agrawal et al. 1991). CE is one of the OPIs commonly used against a wide variety of agricultural pests in Turkey.

Recent findings indicate that toxic manifestations induced by OPIs may be associated with an enhanced production of reactive oxygen species (ROS) (Bagchi et al. 1995). Among ROS, superoxide anions, hydroxyl radicals and hydrogen peroxide enhance the oxidative process and induce lipid peroxidative damage in cell membranes. Hydroxyl radicals were previously proposed as initiators of lipid peroxidation (LPO) through an iron-catalysed Fenton reaction in membranes (Halliwell and Gutteridge 1986). Erythrocytes may be susceptible to oxidative damage due to the presence of haem-iron, polyunsaturated fatty acids (PUFA) and oxygen, which may initiate the reactions that induce oxidative changes in red blood cells. It is possible that CE, during its transportation through the blood stream to the liver, may produce cellular damage in erythrocytes.

The cell has several ways to alleviate the effects of oxidative stress, either by repairing the damage (damaged nucleotides and lipid peroxidation by-products) or by directly diminishing the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants. Enzymatic and non-enzymatic antioxidants have also been shown to scavenge free radicals and ROS.

The enzymatic antioxidants in erythrocytes may counteract oxidative stress. For instance, superoxide dismutase (SOD) catalyses the conversion of super oxide radical ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) while catalase (CAT) or glutathione peroxidase (GSH-Px) converts H_2O_2 to H_2O. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS.

Non-enzymatic antioxidants such as vitamin E, vitamin C and melatonin can also act to overcome the oxidative stress, being a part of total antioxidant system. Vitamin E is an important biological free radical scavenger in the cell membrane (Horwitt 1976). This has been shown to provide a protection against superoxides as well as H_2O_2 (Clemens and Waller 1987). It has been also shown that vitamin C, a water-soluble vitamin and known antioxidant, can react with vitamin E radicals to regenerate vitamin E.

Melatonin (*N*-acetyl-5-methoxytryptamine), the chief secretory product of the pineal gland, presumably functions as an exclusive synchroniser of seasonal reproduction, an adjuster of the biological clock, a sleep-inducing agent, and an immune system stimulator. More recently, melatonin was found to act as a free radical scavenger and an antioxidant; exogenous melatonin administration was, therefore, shown to protect DNA, membrane lipids and cytosolic proteins from oxidative damage induced by oxygen-derived free radicals (Reiter 1996).

It has been proposed that xenobiotics may produce ROS leading to LPO (Trush and Kensler 1991). Agrawal et al. (1991) suggested that LPO could be a major molecular cause of tissue injury. The level of thiobarbituric acid reactive substances (TBARS) is a sign of membrane lipid peroxidation resulting from the interaction of ROS and cellular membranes (Mihara and Uchiyama 1978).

An earlier study from our laboratory concentrated on the in vitro effect of CE on LPO and antioxidant enzymes (Gultekin et al. 2000b). The results indicated that increasing CE concentration caused a significant reduction in the activities of SOD and CAT and a significant increase in the level of malondialdehyde and activity of GSH-Px. Addition of exogenous antioxidants (butylated hydroxytoluene and vitamin E) led to no change in SOD activity and MDA level, but increased GSH-Px activity and reduced CAT activity under the influence of CE. It appears that in vitro CE induces LPO in erythrocytes by producing significant changes in the endogenous antioxidant defence mechanism, and the addition of exogenous antioxidants may overcome the LPO induced by CE.

We now propose that in vivo administration of CE may induce oxidative stress, which may be involved in CE toxicity, and the use of melatonin and a combination of vitamin E and vitamin C may reduce CE-induced oxidative stress and CE toxicity.

In order to test the above hypothesis, an experiment was conducted to determine the in vivo effect of CE on antioxidant defence potential (AOP), LPO and on antioxidant enzymes, such as SOD, GSH-Px and CAT in erythrocytes of rats. TBARS was determined as an indicator of the level of LPO. Additionally, some antioxidants such as vitamin E, vitamin C and melatonin were administered to rats to evaluate their protective effects on CE-induced oxidative stress.

Materials and methods

Animals and treatment

Thirty male Wistar albino rats weighing 190–240 g were used as animal subjects in the experiment. The rats were individually caged and fed ad libitum without water restriction. The rats were divided into six groups, each containing five rats. The experimental groups were as follows: (1) control group, (2) CE-treated group (CE), (3) vitamin E plus vitamin C treatment group (Vit), (4) melatonin-treated group (Mel), (5) vitamin E plus vitamin C plus CE treatment group (Vit + CE), and (6) melatonin plus CE treatment group (Mel + CE). Vitamin E (α-tocopherol acetate, Sigma) and vitamin C (sodium-L-ascorbate; Redoxon, Roche) were administered intramuscularly once a day for 6 consecutive days at 150 and 200 mg/kg, respectively, to the Vit and Vit + CE groups. Although there are many routes used for the administration of exogenous antioxidants, intraperitoneal and subcutaneous being mostly preferred, the intramuscular route has also been successfully used (Kennes et al. 1983; Lisoni et al. 1986; Augustin et al. 1992; Lu et al. 1995; Black and Hidiroglou 1996). Melatonin (*N*-acetyl-5-methoxytryptamine; Sigma) was administered intramuscularly at 10 mg/kg per day for 6 consecutive days in the Mel and Mel + CE groups. On each occasion, melatonin, vitamin E and vitamin C were injected with an insulin injector in a volume of 50 μl. At the end of the fifth day, the rats of the CE, Vit + CE and Mel + CE groups were treated

orally with the first of two equal doses of 41 mg/kg body weight CE in corn oil, the second dose being given 21 h later. Equivalent amounts of physiological saline were given both intramuscularly and orally to control rats. Twenty-four hours after the first administration of CE, blood samples were taken from the left ventricle after thoracotomy under anaesthesia (65 mg/kg I.P. sodium pentobarbital), i.e. at the end of the sixth day of the experiment.

We hereby declare that the experiments reported here comply with the current laws and regulations of the Turkish Republic on the care and handling of experimental animals.

Biochemical parameters

Blood samples were centrifuged and plasma was discarded. Erythrocyte packets were prepared by washing erythrocytes three times with cold isotonic saline. Haemoglobin concentration was determined by the cyanmethaemoglobin method from the washed erythrocytes (van Kampen and Zijlstra 1965). The erythrocytes were then stored at –20°C and all measurements were made within a week. The erythrocytes were thawed and the levels of TBARS and AOP and the activities of SOD, GSH-Px and CAT were assessed.

TBARS was determined by the double heating method of Draper and Hadley (1990). The method was based on the spectrophotometric measurement of the colour developed during reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling under tap water, the mixture was centrifuged at 1000 *g* for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled under tap water and its absorbance was measured at 532 nm. The concentration of TBARS was calculated using the absorbance coefficient of MDA-TBA complex, 1.56×10^5 cm^{-1} M^{-1}, and expressed in nmol/gHb.

AOP was assessed according to the method of Durak et al. (1998). In this method, the reaction medium was enriched with fish oil. Samples were exposed to superoxide radicals ($O_2^{\bullet -}$) produced by the xanthine/xanthine oxidase (XO) system for 1 h. Fish oil was used for this purpose since it is polyunsaturated oil, which is very sensitive to free radical attack. As is known, when a cell is unable to eliminate free radicals, unsaturated free fatty acids are easily oxidised and subsequently the TBARS concentration can increase. Using this reaction system, it is possible to obtain precise information on the total (enzymatic and non-enzymatic) antioxidant potential of the tissue and cells. For this purpose, TBARS level of the reaction medium was measured before and after $O_2^{\bullet -}$ radical attack. Differences between these two values are inversely proportional to AOP of the erythrocytes.

The analysis procedure was as follows: sample (0.5 ml), xanthine (10 mM, 0.1 ml), XO (0.1 ml), and fish oil (0.01 ml) were mixed together and incubated for 1 h at room temperature (25°C), and then TBARS was measured. A blank sample without any incubation period was also examined. XO (Sigma X-4500, 2.9 mg protein/ml, 1.6 U/mg protein) was prepared by 40-fold dilution with 2 mol/l $(NH_4)_2SO_4$ solution. The final amount of XO in the assay medium reached 0.012 U. Fish oil with a total lipid concentration of 0.706 g/ml was used. The final lipid content of the assay mixture that was prepared by using 0.2 µl fish oil per ml erythrocytes was calculated to be 7.06 mg. Antioxidant potential was calculated from the differences between the TBARS levels of blank sample and other samples studied, and the results were expressed as 1/nmol/gHb.h.

The determination of GSH-Px activity was based on the method of Paglia and Valentine (1967). The principle of the method was as follows: GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide; in the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance of NADPH was measured at 340 nm.

The measurement of SOD was based on the principle in which xanthine reacts with XO to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazon dye. SOD activity is then measured by the degree of inhibition of this reaction (Woolliams et al. 1983).

CAT activity was measured according to the method of Aebi (1984). The principle of the assay is based on the determination of the rate constant (k, per second) of H_2O_2 decomposition. The rate constant was calculated from the following formula: $k = (2.3/\Delta t)\ (a/b) \log (A_1/A_2)$, where A_1 and A_2 are the absorbance values of H_2O_2 at t_1 (time zero) and t_2 (time 15 s), a is the dilution factor, and b is the haemoglobin content of erythrocytes.

An autoanalyser, Olympus AU640 (Japan), was used to determine the activities of SOD and GSH-Px; a spectrophotometer, Shimadzu UV-1601 (Japan), was used to determine the other parameters.

Statistical evaluation

For statistical analyses, the normality was investigated, which showed that some of the values of the parameters did not fit to the normal distribution. Therefore, as stated by Dawson-Saunders and Trapp (1994) in considering the small number of cases, non-parametric the Kruskal-Wallis test and Mann-Whitney U-test were used to compare groups.

Results

The results of the present experiment are shown in the figures. As seen in Fig. 1, TBARS was significantly higher ($P < 0.05$) in the CE group than in all other treatment groups.

In contrast, AOP in CE-treated rats was significantly ($P < 0.05$) lower than that in the control group. There were no significant ($P > 0.05$) effects on AOP of the vitamin and melatonin treatments in the CE group. Similarly, vitamin and melatonin addition in control group did not cause any significant ($P > 0.05$) changes in AOP (Fig. 2).

In comparison to the control group, the activities of SOD and CAT were significantly ($P < 0.05$) lowered by CE treatment (Fig. 3 and 4). There were no significant ($P > 0.05$) effects of vitamin and melatonin treatment in the control group, whereas the use of vitamin and melatonin in CE-treated rats was shown to return the activity levels of SOD and CAT to the that of control group.

There was no significant ($P > 0.05$) effect of CE on GSH-Px activity while GSH-Px activity was higher in the Mel and Mel + CE groups than in the control group (Fig. 5). GSH-Px activity was also found to increase in the Mel + CE and Vit + CE groups, compared with the CE group ($P < 0.05$).

Discussion

TBARS

Recent findings indicate that toxic manifestations induced by OPIs may be associated with the enhanced

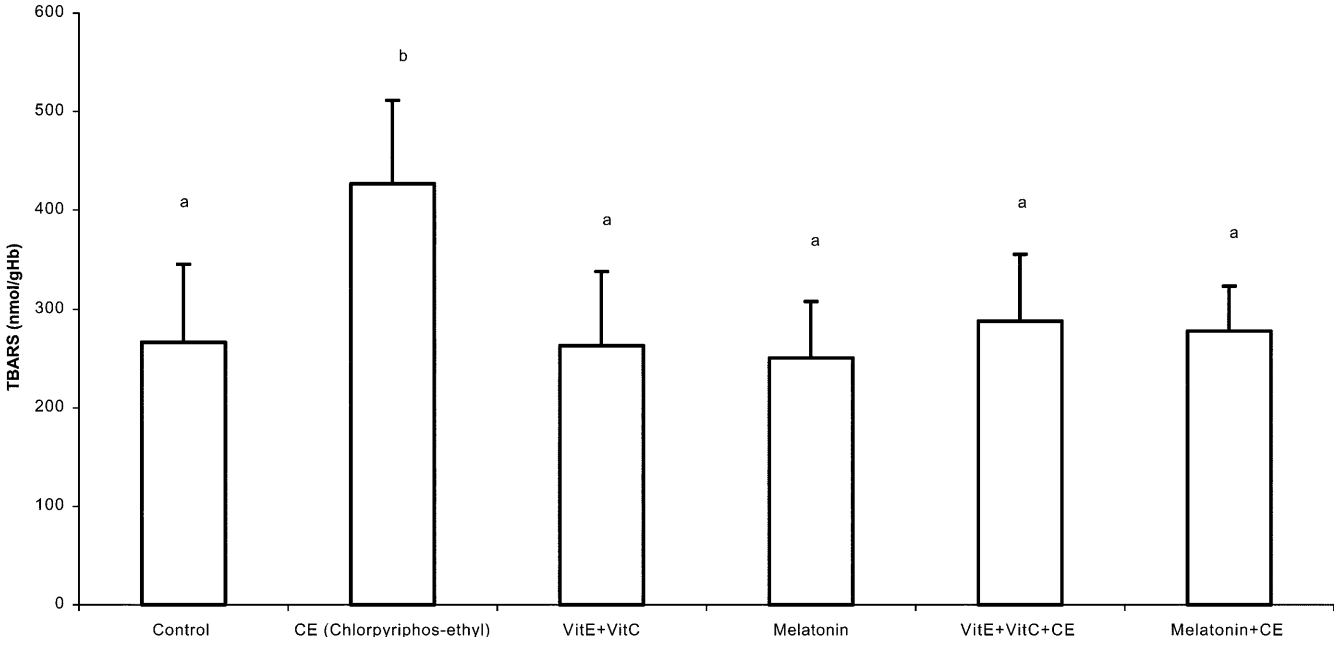


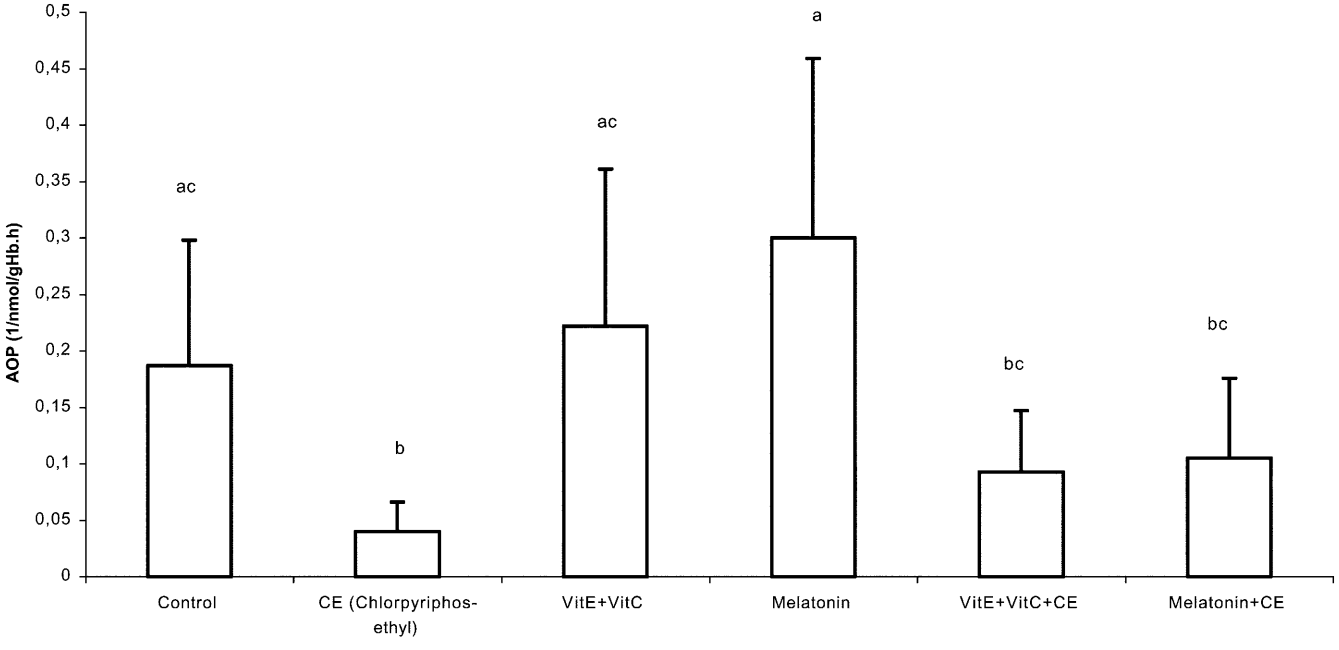
Fig. 1 Thiobarbituric acid reactive substance (TBARS) levels in rat erythrocytes of the treatment groups. Bars represent the group means +SD. [a,b] Different superscripts indicate significant differences between groups ($P < 0.05$, Mann-Whitney U-test)

production of ROS. The interaction of haemoglobin with redox drugs or xenobiotics is a source of production of radicals in erythrocytes (French et al. 1978; Winterbourn et al. 1978), giving rise to superoxide radicals, hydrogen peroxide and in some cases peroxy radicals, and inducing membrane LPO and haemolysis (Clemens et al. 1984).

OPIs, such as phosphomidon, trichlorfon and dichlorvos have been reported to induce oxidative stress as shown by enhanced MDA production (Naqvi and Hasan 1992; Yamano and Morita 1992). It was recently shown that agrochemicals increased lipid peroxidation in vitro in human erythrocytes (Gultekin et al. 2000a). In our previous study, CE caused an in vitro increased LPO in human erythrocytes and adding exogenous antioxidants overcame CE-induced LPO (Gultekin et al. 2000b).

The results of the present study showed that CE caused a significant increase in TBARS formation, and this clearly demonstrates that following

Fig. 2 Antioxidant potential (AOP) levels in rat erythrocytes of the treatment groups. Bars represent the group means +SD. [a,b,c] Different superscripts indicate significant differences between groups ($P < 0.05$, Mann-Whitney U-test)



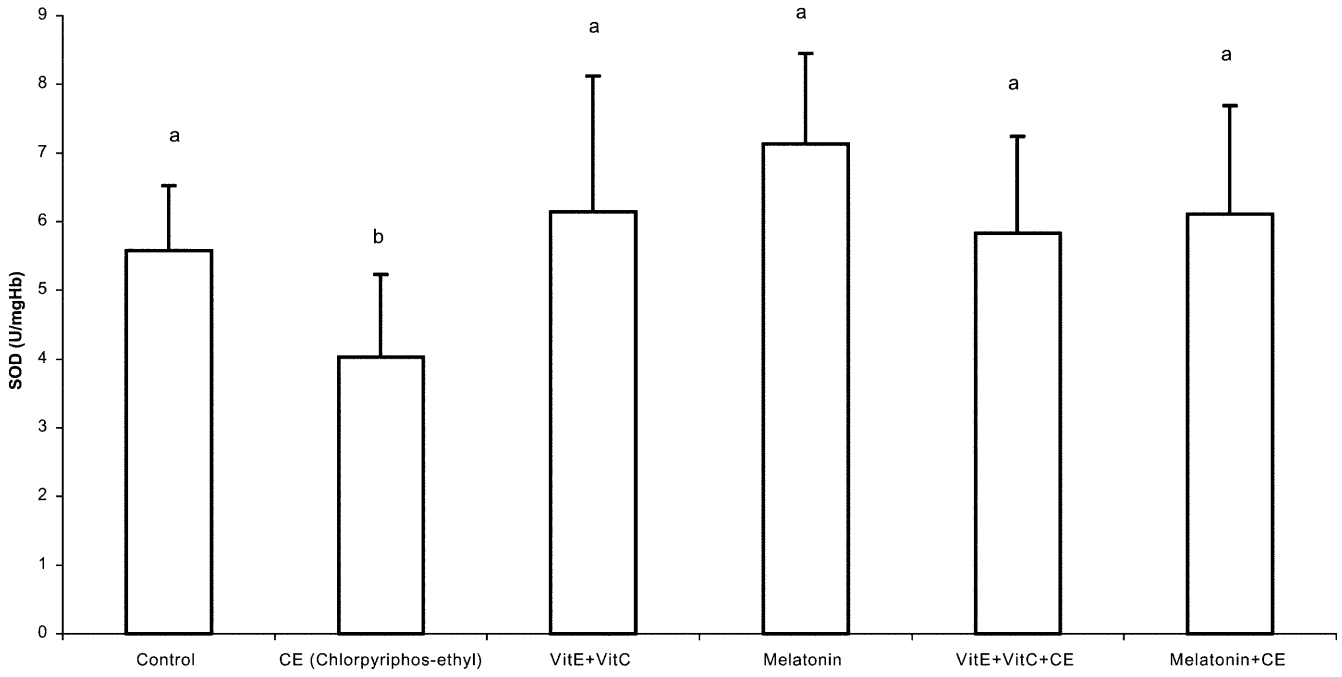
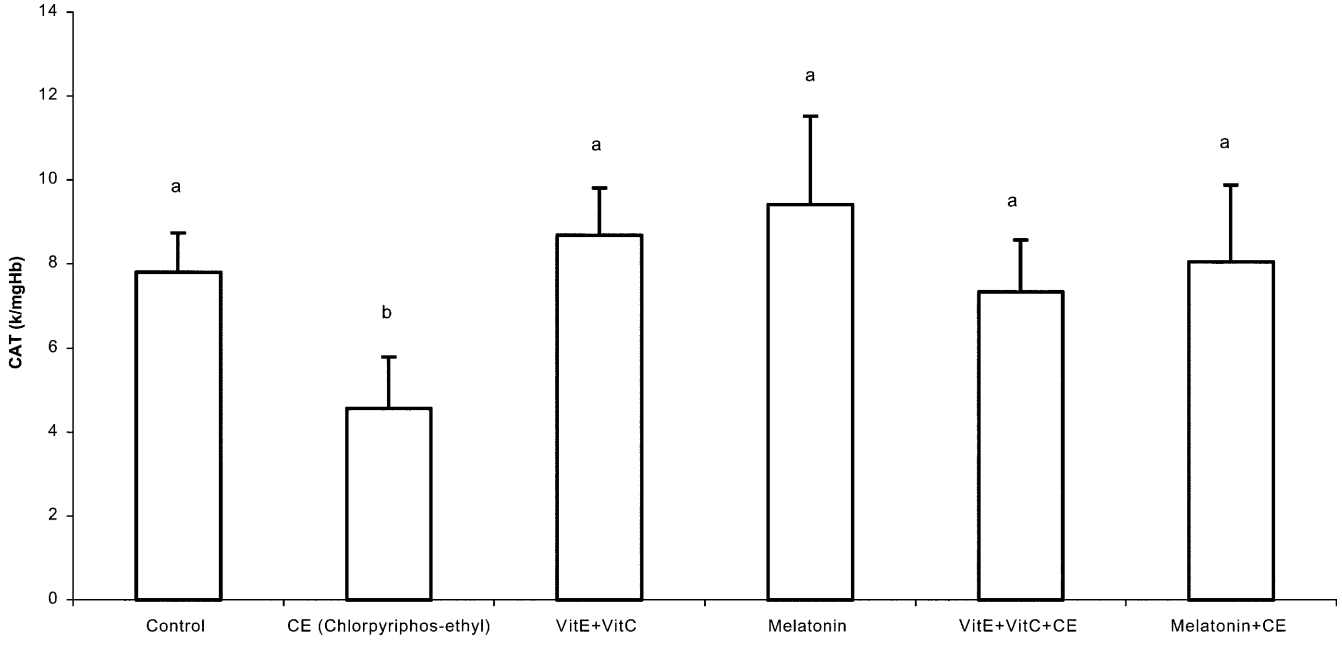


Fig. 3 Superoxide dismutase (SOD) activities in rat erythrocytes of the treatment groups. Bars represent the group means +SD. [a,b] Different superscripts show significant differences between groups ($P < 0.05$, Mann-Whitney U-test)

treatment with CE, lipid peroxidation significantly increases in erythrocytes. Additionally, the treatment with melatonin and vitamins C and E led to a significant decrease in TBARS formation, as shown in the Vit+CE and Mel+CE groups compared with the CE group. The result of our previous in vitro study (Gultekin et al. 2000b) confirms the effect of antioxidant vitamins in decreasing TBARS, as also seen in the present case.

Melatonin was reported to act as an antioxidant in the membrane and cytosol because of its lipophilic and hydrophilic character (Shida et al. 1994). It is also well documented that the treatment of the animals with melatonin (10 mg/kg) totally prevented the accumulation of the paraquat- or $FeSO_4$-related lipid peroxidation (Melchiori et al. 1995; Reiter et al. 1997). It is most likely that the use of melatonin at a low dose (10 mg/kg) was even more effective in reducing TBARS formation

Fig. 4 Catalase (CAT) activities in erythrocytes of the treatment groups. Bars represent the group means +SD. [a,b] Different superscripts show significant differences between groups ($P < 0.05$, Mann-Whitney U-test)



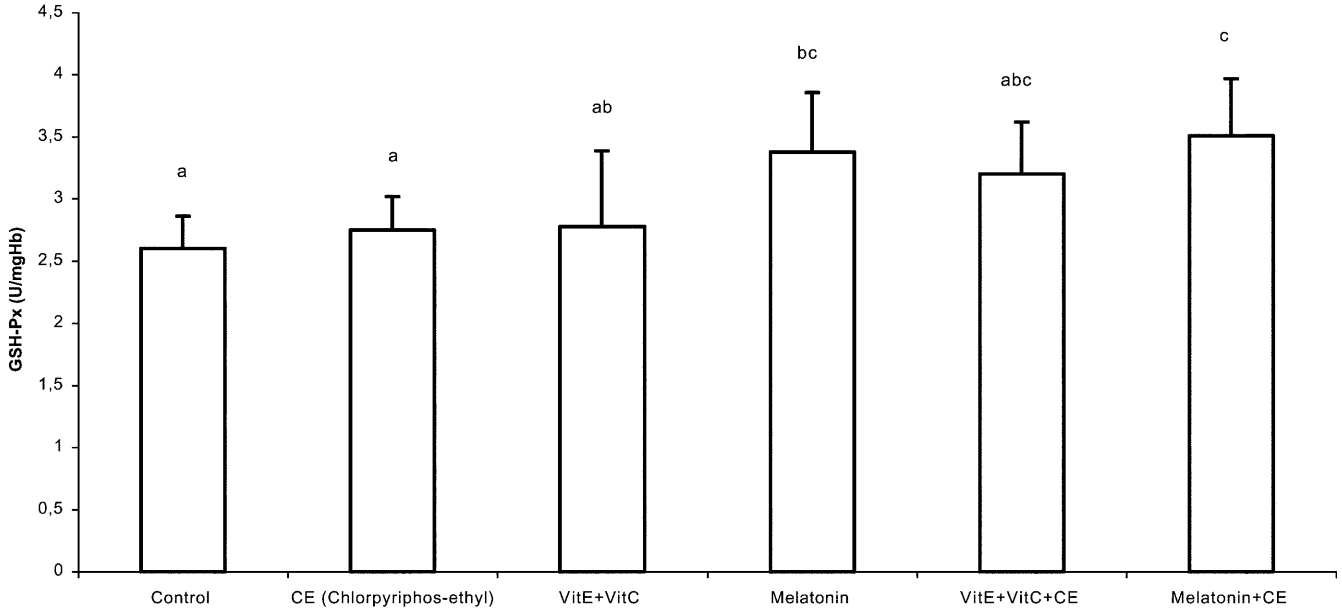


Fig. 5 Glutathione peroxidase (GSH-Px) activities in erythrocytes of the treatment groups. Bars represent the group means + SD. [a,b,c] Different superscripts indicate significant differences between groups ($P < 0.05$, Mann-Whitney U-test)

than the high doses of vitamin E and vitamin C in combination (150 and 200 mg/kg, respectively). Melatonin is reported to be approximately twice as effective as the water soluble vitamin E, i.e., Trolox, in neutralizing the peroxyl radical (Pieri et al. 1994).

How melatonin greatly inhibits TBARS formation even at low doses compared with other antioxidant vitamins can be explained by at least two mechanisms. The compound itself may scavenge free radicals and/or inhibit their formation, and additionally the compound can act by up-regulating endogenous antioxidant defences.

Many reports suggest that melatonin may act in both ways (Pierrefiche et al. 1993; Abe et al. 1994; Tan et al. 1994; Melchiori et al. 1995). For example, it can directly and rapidly scavenge hydoxyl and peroxyl radicals (Tan et al. 1993; Pieri et al. 1994), effectively neutralise singlet oxygen (Cagnoli et al. 1995) and it can also scavenge hypochlorus acid (HOCl) (Chan 1996). On the other hand, melatonin does not react directly with the superoxide anion radical, although the indolyl cation radical, which is generated from melatonin when it donates an electron, may do so (Hardeland et al. 1993). In both the cerebellum and the hypothalamus, melatonin reduces nitric oxide synthase activity and thereby limits nitric oxide production (Pozo et al. 1994; Bettahi et al. 1996). The antioxidant enzyme activity of GSH-Px in some tissues such as brain (Barlow-Walden et al. 1995), liver and lung (Reiter 1993) and in the harderian gland (Kabuto et al. 1998) was stimulated by melatonin. Melatonin can also stimulate the synthesis of SOD and of GSH-Px (Antolin et al. 1996) and the activity of glucose-6-phosphate dehyrogenase in some tissues (Pierrefiche and Laborit 1995).

These results may indicate that treating rats with CE causes lipoperoxidative damage in erythrocytes, and the treatment of antioxidants may prevent this cell damage.

SOD activity

It has been reported that OPIs, such as phosphomidon, trichlorfon and dichlorvos, caused inhibition of SOD activity (Naqvi and Hasan 1992; Yamano and Morita 1992). However, Maiti and Kar (1997) showed that dimethoate increased hepatic SOD activity. It was also shown that in vitro SOD activity was increased by phosphomidon in human erythrocyte, whereas it was decreased in plasma (Datta et al. 1992).

In one of our in vitro experiments (Gultekin et al. 2000b), SOD activity was significantly reduced only by a high dose of CE administration to erythrocytes, suggesting that at low- and medium-dose CE did not have any direct inhibitory effect on SOD activity. The in vitro increase of ROS by CE was, therefore, attributed to the in vitro decrease in SOD activity. In this study, an increase in TBARS was presumably associated with increased ROS, confirming the fact that the singlet oxygen and peroxyl radicals inhibited the activity of SOD induced by CE (Escobar et al. 1996). SOD activity was significantly increased by exogenous antioxidants (melatonin or vitamins C and E) only under the inhibitory effect of CE, perhaps due to the likelihood that the antioxidants scavenge free radicals, preventing SOD from being inactivated by ROS.

GSH-Px activity

It has been reported that OPIs, such as phosphomidon, trichlorfon and dichlorvos caused a decrease in gluta-

thione peroxidase activity (Naqvi and Hasan 1992; Yamano and Morita 1992). Administration of a pesticide mixture including chlorpyrifos reduced the activities of GSH-Px in rat liver; the reduced enzyme activity was highly dependent on the increased dose of pesticide mixture (Lodowici et al. 1994). In our previous study, GSH-Px activity decreased in human erythrocytes during in vitro incubation with CE (Gultekin et al. 2000b). In the present experiment, CE caused no significant changes in GSH-Px activity. However, it has been shown in a previous study that CE activated a purified and commercially available GSH-Px, while superoxide radicals caused an inhibition of GSH-Px activity (Gultekin et al. 2000b). Blum and Fridovich (1985) also showed that superoxide radicals could inhibit the activity of GSH-Px. In the present case, increased ROS may be the reason for the fact that GSH-Px activity, as expected, did not increase. Supporting this idea, it has been reported that GSH-Px is more susceptible to oxyradical inactivation than SOD and CAT (Li et al. 1994). However, while GSH-Px is activated by CE, it is inhibited by CE-inducing ROS, giving an overall insignificant change in GSH-Px activity.

It has been shown that besides scavenging the highly toxic free radicals, melatonin also stimulates the antioxidant enzyme activity of GSH-Px in brain (Barlow-Walden et al. 1995), liver and lung (Reiter 1993) and in the harderian gland (Kabuto et al. 1998). In the current experiment, melatonin caused a significant increase in GSH-Px activity whereas vitamins C and E caused no significant increase. That melatonin causes an increased GSH-Px activity in erythrocytes has contributed to the findings of previous studies. The increase in GSH-Px activity caused by melatonin may result either directly from the activating effect of melatonin on GSH-Px or indirectly from decreasing the inhibitory effect of ROS on GSH-Px by scavenging free radicals.

CAT activity

In the present experiment, treating rats with CE decreased CAT activity. The degree of CAT inhibition was greater than that of SOD (see Figs. 3 and 4). We previously showed that CE in vitro inhibited CAT activity (Gultekin et al. 2000b). It has been also seen that CAT activity was inhibited by free radicals such as singlet oxygen and superoxide and peroxyl radicals (Kono and Fridovich 1982; Escobar et al. 1996). Therefore, CAT may be inhibited by both CE itself and by an increase in ROS induced by CE. These may be the causes of higher inactivation of CAT.

In comparison with the CE group, CAT activity significantly increased both in the Mel + CE and Vit + CE groups. The increase in CAT activity by antioxidants could result from a decreased inhibitory effect of ROS scavenged by antioxidants.

Antioxidant defence potential (AOP)

AOP comprises enzymatic and non-enzymatic antioxidants. It was recently shown that agrochemicals decreased AOP in human erythrocytes in vitro (Gultekin et al. 2000a). In the present case, AOP was decreased by CE, indicating that the total antioxidant defence mechanism failed to protect the erythrocytes from lipoperoxidative damage caused by CE. The decrease in SOD and CAT activities may result in a decrease in AOP. The treatment with CE plus melatonin and with CE plus vitamins C and E caused significant increases in individual antioxidant enzymes (except GSH-Px in the Vit + CE group), but not at all in AOP. The latter may be due to a possible inactivation of non-enzymatic antioxidants by CE.

Overall evaluation

In the present experiment, treating rats with CE decreased SOD and CAT activities. It is possible that since CAT activity was inhibited by CE, H_2O_2 will accumulate more and will be oxidised to H_2O less. Thus, increased H_2O_2 is able to cause SOD inhibition, thereby ultimately increasing superoxide radicals. The increased superoxide radicals will inhibit both CAT and GSH-Px so that the H_2O_2 will accumulate greatly in the reaction media, inducing inhibition of SOD and increased superoxide radicals. Finally, the inhibition of SOD, CAT and GSH-Px will gradually increase. GSH-Px did not change under the influence of CE in the current study, whereas the results of the previous study revealed that GSH-Px was either indirectly inhibited by CE inducing ROS in one experiment or directly activated by CE itself in another experiment (Gultekin et al. 2000b). Therefore, the overall effect of CE can be said to be incomplete inhibition of GSH-Px activity.

SOD and CAT activities significantly increased by adding vitamin E plus vitamin C and melatonin to the CE group. On the other hand, only the addition of melatonin to both the control and CE groups caused a significant increase in GSH-Px activity. The present results indicate that CE causes significant inhibition of SOD and CAT activities. Administration of exogenous antioxidants together with the CE increases the activities of endogenous antioxidant enzymes, preventing a possible involvement of ROS in the inhibition of those enzymes.

These results suggest that treating rats with CE increases lipid peroxidation in vivo and decreases AOP by increasing oxidative stress in erythrocytes. Furthermore, the administration of melatonin and vitamin E and vitamin C in combination can reduce lipid peroxidation caused by CE.

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