COMBINED THERAPY WITH ANTIOXIDANTS AGAINST CADMIUM INDUCED TESTICULAR DYSFUNCTION IN RABBITS

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Introduction

Cadmium is a very toxic heavy metals and an important environmental pollutant which is present in soil, water, air and food. In the blood and tissues, Cd stimulates the formation of metallothioneins and reactive oxygen species (ROS) thus causing oxidative damage in erythrocytes and in various tissues which results in a loss of membrane functions (Sarker, et al., 1995). The present study examined the effect of Cd exposure, to consider possible free radical involvement in Cd-induced damage tissue. Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen- derived oxidants commonly known as reactive oxygen species (Zikic et al., 1998). The role of oxidative stress in infertility and its impact on reproductive tissues with antioxidant is still in its infancy. Systems of protection against the ravages of which free radicals are capable comprise mixtures of specials compounds whose structure enables them to interact with free radical, rendering them harmless. These are the antioxidants. The process is often called “Quenching” so these protective substances, as well as being called "antioxidant" are also called “free radical quenching agents”. Their role is to “quench” the free radicals before they can harm the delicate structures of cells or tissues and to interrupt any chain reactions that would otherwise cause swathes of cellular damage. Free radicals and ROS are associated with oxidative stress and are likely to play a number of significant and diverse roles in reproduction. Basic research on the involvement of ROS and antioxidants in maintaining normal sperm function is very much warranted. Theoretically, cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. The scavenging potential in gonad and seminal fluid is normally maintained by adequate levels of antioxidants super dismutase (SOD), catalase, glutathione (Shi et al., 1999). This balance can be referred to as oxidative stress status and its assessment may play a critical role in monitoring sperm damage and infertility. SOD has proven a useful probe for studying the participation of free radicals in reaction involving oxygen, since it acts as a defense against oxidative tissue damage by the dismutation of superoxide radicals (Ognjanovic, et al., 2003). Long term exposure to Cd increased lipid peroxidation and caused inhibition of SOD activity indicating oxidative damage in liver, kidney and testes (Patra et al., 1999). The various toxic

effects induced by Cd in biological systems were confirmed by the occurrence of increased lipid peroxidation that is an early and sensitive consequence of Cd exposure. The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system. This defense system includes the enzymes, glutathione peroxidase, glutathione-S-transferase, superoxide diamutase, catalase as well as glutathione, which normally protect against radical toxicity. L-ascorbic acid or vitamin C is an essential component in the diet, and associated with fertility. Luck et al., (1995) reported that vitamin C should be considered as an essential biochemical in the reproductive process and as a potentially significant factoring in the fertility. Vitamin E is the primary components of the antioxidant system of the spermatozoa (Surai et al., 1998), and is one of the major membrane protectants against ROS and lipid peroxidation.

The aim of the present study is to formulate some of antioxidants to provide an excellent combination that is highly effective in improving the testicular function from cadmium induced effects. And in the proper combination, they can perform a wide range of metabolic activities, free radical scavenging and preventive action.

Material and methods

36 mature male, New Zealand White rabbits (2.5 Kg average was raised in the animal house at National Research Center. They divided into 3 groups. The first group served as a control. The second group given cadmium chloride at a dose of 5.16 mg/kg/twice /week (Eltohamy et al., 1996). The third group received Cd as the second group, in addition to antioxidants formula which consisted of Vit. E (200mg/kg tocopherol acetate), Vit C (200 mg/d), Vit A (400mg/d) Zn (40mg/kg zinc sulphate, Merck), Cu (20 mg/d copper sulphate) and Se (0.5 ppm as sodium selenite). The experimental period was 12 weeks.

Blood was collected while sacrificing animals. In erythrocytes, Superoxide dismutase (SOD) was analyzed using kits purchased from randox, UK. Aspartate amino transferase (AST), alanine amino transferase (ALT), measured according to Bergmeyer (1983) and lactate dehydrogenase (LDH), Alkaline and acid phosphatases according to Tietz (1986).using kits purchased from Stanbio Texas. The concentrations of lipid peroxide (LPO) were assayed as thiobarbituric acid reactive substances (TBARS) in the blood. GST activity was measured. In the testes, ALT, AST, ALP, AcP, SOD, GST, LDH, Cd were measured.

Results and discussion

The Cd-induced reproductive dysfunction in the rabbits was the same as those demonstrated in our previous work (Eltohamy et al., 2002). Exposure to Cd increased lipid peroxidation and caused inhibition of SOD activity (Table 1), indicating oxidative damage in the testes (Petra et al., 1999). However, the possible involvement of free radicals leading to oxidative damage to vital organs from Cd was demonstrated (Muller, 1986). Higher susceptibility of testes to oxidative damage was also recorded. Ognjanovic et al. (2003) reported that Cd stimulated reactive oxygen species (ROS) thus causing oxidative damage in various tissues. The present study showed that Cd exposure led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with reduction of the antioxidant system e.g. reduced glutathione (GSH) and the total glutathione peroxidase (GSH-PX). However, by administration of antioxidants, LPO and antioxidant system tend to reach control values, which is contributed by the protective effects of Se on the antioxidant defense system. This defense system includes the enzymes, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase as well as glutathione, which normally protect against free radical toxicity (Sarkar et al., 1997). The present study indicated that antioxidants such as Vit. E, C, GSH, Se, Zn may act synergically preventing lipid peroxidation and cell destruction. The present study would consider some important reasons why we should combine diverse antioxidants in treated Cd –induced effects. When all of the necessary antioxidants spread throughout the body, they engage in a wide spectrum of metabolic activities simultaneously, various antioxidants involved with their own unique activities assist each other in achieving a healthier body. Many antioxidants utilized other antioxidant that have been oxidized or used up while fighting the effects of free radicals. They work synergistically as Vitamin E and selenium interact to provide strong protection against oxidative damage to some organs. While Vitamin C and E produce their effects is far greater than any one individual antioxidant, and protect each other from the onslaught of free radicals. Selenium and Vitamin A work together to help and prevent cancer. Selenium interacts with glutathione, which is a vital component in the production of glutathione peroxidase.

Zinc is a pivotal component of the antioxidant defense network that protects membranes from oxidation. Zinc and copper are involved in cell and tissue growth. Zinc plays an important role in protein synthesis and is intimately involved with copper as cofactors in several important enzyme systems. Zn supplementation induced increases in reactive oxygen n

species. The cellular enzyme super oxide dismutase (SOD) provides physiological defense against superoxide radicals. Since Cu and Zn are necessary for the synthesis of Cu-Zn superoxide dismutase (CuZnSOD). SOD is an efficient suppressor of oxygen radical overproduction by Cd induced toxicity. The present study showed that the levels of SOD in the testes increased and LPO decreased after antioxidant supplementation. This suggests that antioxidant used in the present study has effective antioxidant properties and could well scavenge excess free radicals. Combined therapy with Zn, Cu, Se, vitamin C, and E, were effective in reducing damage to testes in rabbits treated with Cd. The combined antioxidant enhanced the testes'antioxidant/detoxification system, as evidenced by an increase in the level of reduced glutathione in that organ. Decreased in SOD activities indicated the direct role of Cd in inhibiting SOD activities. This might be due to the interaction of tissue Cd with Zn and Cu. The possibility of such Cd interaction with metal moieties has been demonstrated where Cd replace Zn to form Cu-Cd-SOD (Virgili et al., 1999). Chan et al., (1998) demonstrated that Zn is involved in destruction of free radicals through cascading enzyme systems. The present study showed a significant increase in GST activities in the testes of rabbits received Cd. The increment of the activities of AST, ALT, GGT, ALPGST in the serum was mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which indicated liver damage and disruption of normal liver function.

Combined therapy with Zn, Cu, Se, Vit.A, E, C was effective in reducing damage to testicular tissue treated with cadmium. The combined antioxidants enhanced the testicular antioxidant/detoxification system, as evidenced by an increase in the level of reduced glutathione in that organ. They could markedly improve antioxidative vitamin status and enzymatic activities. This study suggested that antioxidants, particularly Zn, Cu SOD, could play a significant role in the reduction of inflammatory responses associated with the cadmium inducing toxicity and exhibited a powerful protective effect against testicular damage and dysfunction. Vitamin E stopped, free radical chain reactions, protected fats from free radical destruction throughout the body, help in producing superoxide dismutas. The conclusion of the present study is that the antioxidant used is effective in combating cell-damaging free radicals, which are known to contribute towards testicular dysfunction. Studies have shown that antioxidants are uniquely different from one another and each have a specific function in the body. They are also synergistic, and will work most effectively when they are used together. These combinations can perform a wide range of metabolic activities, free radical scavenging and preventive actions. The present antioxidant formula used as a blend of

antioxidants specifically designed to assist the body in overcoming a vast array of physiological stressors and to help avert the effects of Cd-induced testicular dysfunctions.

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Table 1 Serum parameters of rabbits received cadmium and cadmium and antioxidants.

Parameters	Control	Cadmium	Cadmium &Antioxidants
AST (U/L)	91.5 ±4.6 [a]	205.9±8.9©	133.2±8.15 [a]
ALT(U/L)	1.6± 0.22 [a]	4.99±1.1©	2.37±0.5 [a]
GGT (U/L)	14.9± 1.3 [a]	25.9±1.7©	15.5± 2.7 [a]
LDH (U/L)	1303±69.9 [a]	1177±40.5©	1225±81 [a]
ALP (U/L)	1732±101 [a]	1800±199 ©	1715±211 [a]
GST((nmol/ml blood x10	0.66±0.04 [a]	0.81±0.04 ©	0.59±0.02 [a]

Different superscripts in row indicates significantly different means at($P < 0.05$).

Table 2 Testicular parameters of rabbits received cadmium and Cadmium and antioxidants.

Parameters	Control	Cadmium	Cadmium and antioxidants
Cd concen. (ug/gm tissue)	0.35±0.04[a]	39.33±6.66©	0.33±0.03[a]
LPO (nmol/gm tissue)	142.66±8.5[a]	403.76±12.3 ©	139.45±7.6[a]
AcP (IU/mg)	566.0±7.7[a]	513.8±8.65©	592.22±32.8[a]
ALP (IU/mg)	988.32±34.8 [a]	867.4±16.6©	122.6±16.8[a]
LDH (IU/mg)	2110±23.3[a]	2860±43.2 ©	1992±36.7[a]
AST (IU/mg)	59.9±2.0[a]	48.9±2.33©	58.6±1.65[a]
ALT (IU/mg)	16.6±4.3[a]	12.7±8.8©	18.4±8.6[a]
SOD (IU/mg)	165.5±22.5[a]	99.8±13.3©	177.0±24.6[a]
GST(IU/mg)	0.35±0.02[a]	3.6±0.9©	0.30±0.9[a]

Different superscripts in row indicates significantly different means at ($P < 0.05$)

Table 3.GSH-Px, GR, activities in red blood cells, GST in the plasma and GSH in whole blood in the three groups.

Parameters	Control	Cadmium	Cadmium and antioxidant
GSH-Px(nmol/mlblood x10)	22.0 ± 3.6 [a]	34.0 ± 7.4 ©	24.0 ± 2.02 [a]
GR (nmol/ml blood X10)	29.7 ± 4.3 [a]	40.0 ± 5.8 ©	31.2 ± 6.4 [a]
GST (nmol/ml bloodX10	34.8 ± 3.2 [a]	41.84 ± 1.97 ©	28.9 ± 3.31[a]
GSH (nmol/mlX10	9.9 ± 0.6 [a]	13.0 ± 1.72 ©	10.2 ± 1.7 [a]

Different superscripts in row indicate significantly different means at($P < 0.05$).