The protective role of lycopene against oxidative damage in the liver, heart and kidney tissues of mice exposed to CoCl₂

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Abstract
Heavy metals are harmful to both the environment and human health. One of these heavy metals is cobalt. Lycopene is a potent antioxidant. The purpose of this study was to investigate the effects of lycopene on change of lipid peroxidation in liver, kidney and heart of experimentally exposed mice with cobalt (Co). Experimental protocol: For this purpose, 30 Swiss Albino male mice of 3-4 months of age and weight ranging from 45 to 50 g were used. Mice were subdivided into 3 groups including control, cobalt and cobalt lycopene (combined). The control group mice were given 3 mg/kg/day saline (by intramuscular injection) and 10 mg/kg/day saline (orally) for 30 days in order to achieve equality with administration to the mice in the experimental group. At the end of this process, malondialdehyde (MDA), glutathione (GSH), vitamin-E and β-carotene were analyzed in the prepared homogenates. According to findings; Increase in liver MDA levels in cobalt group was significant (p<0.01). Additionally, it was found out that cobalt toxicities increased the level of MDA in the heart most (p<0.001). The level of GSH in the tissue of liver, kidney and heart of the cobalt group were lower than control and combined group (p<0.001). However, the kidney and the liver vitamin E level of the both control and combined group were very lower according to control group (p<0.001), but not important in liver (p>0.05). It was observed that liver and kidney β-carotene level in cobalt group was lower than control and combined group. This decrease is statistically significant in the kidney (p<0.05), but this decrease is statistically insignificant in the liver (p>0.05). As a result, increasing the antioxidant levels of GSH, vitamin E and β-carotene together with lycopene application may play an important role in preventing the negative effects of lycopene on free radicals (MDA, etc.) caused by acute cobalt oxidation.

Keywords
Mice, Cobalt Toxicities, Lycopene, Oxidative Stress

INTRODUCTION
In recent years, industrial activities resulting from the use of high amounts of toxic and heavy metals in agriculture and the emergence of chemical and environmental pollution have threatened living organisms in many areas. Automobile exhaust gases and mineral oils, dusts of mines, industrial and industrial activities such as electronic and iron-steel production, wastes from the environment, pesticides and insecticides used in agriculture and stockbreeding, leaks from natural resources or areas where toxic substances are stored can be given as examples of environmental pollutants (Kaya et al., 1998). Heavy metals can induce oxidative stress in the organism and cause the formation of various reactive oxygen species such as O₂⁻, OH⁻, NO, H₂O₂. These materials may affect the lipid peroxidation (Neckardien et al., 1991; Leon et al., 1998), the deterioration of the antioxidant defense system, the synthesis of proinflammatory cytokines, the structure of proteins and the oxidation of nucleic acids and the DNA repair mechanism (Van den Brooke et al., 1998). Cobalt, one of these heavy metals, is a very strong toxic metal that contributes to the production of free oxygen radicals. Also, it has been proven by some experiments that it is a H₂O₂ producer that can be transformed into important...
free radical sources by fenton reactions (Neckardien et al., 1991).

Some studies have carry out that cobalt causes cardiomyopathy, thyroid complications, hematological changes, and damage to the immunological system in humans and its isotopes cause cancer in some organs (Horowitz et al., 1988; Leon et al., 1998). Lycopene is an important antioxidant for reducing the damage caused by free radicals resulting from oxidative stress in the organism for various reasons. Lycopene; β-carotene is a strong antioxidant due to the openness of the acyclic ring at the end of the hydrocarbon chain. Lycopene has been shown to have important effects such as anti-inflammatory and anticancer (Levy et al., 1995; Rao and Agarval, 1999). Lycopene also binds to growth hormone receptors uncontrolled in cancer cells, stimulating the return of cells to their normal state. Additionally, it has been found to be effective in the formation of anti-inflammatory responses by activating defense mechanisms against infectious agents (Rao and Agarval, 1999). The aim of this study was to investigate the effects of lycopene on change of lipid peroxidation in liver, kidney and heart of experimentally exposed mice with cobalt (Co).

**MATERIALS AND METHODS**

**Animals and Experimental Design**

In this study, 30 Swiss Albino male mice of 3-4 months of age and weight ranging from 45 to 50 g were used. Before the study, mice were allowed to adapt to the environment in 15 days. Mice were divided into 3 groups (each group including 10 mice). The groups were named as control (placebo), cobalt and cobalt+lycopene (combined). Mice were fed by following ad libitum procedure. In this study, 5 (five) mice cages were used. The cages were kept in a setting of 12 hours of dark/LIGHT room with temperature of 22±2°C. Also, bottles with droppers at the tip of the special section of the cages were placed in such a way that the mice could continuously drink water. The cages were cleaned regularly every day. The control group mice were given 3 mg/kg/day saline (by intramuscular injection) and 10 mg/kg/day saline (orally) for 30 days in order to achieve equality with administration to the mice in the experimental group.

The mice in the cobalt group were given 3 mg/kg/day Cobalt chloride (CoCl$_2$) (Merck, USA) intramuscularly (IM) corresponding to LD$_{50}$ as indicated by Singh and Junnarkar (1991). In the combined group (Cobalt+Lycopene) (n=10), mice received 3 mg/kg/day of CoCl$_2$ intramuscularly for one month and 10mg/kg/day lycopene (Sigma, USA) as reported by Jonker et al. (2003) was dissolved in 2 ml of corn oil and given orally with gastric gavage.

**Organ Sampling and Biochemical Analysis**

After the mice were anesthetized with ether, 2-3 ml of blood was taken from their hearts by heparinized injector (Nevparin 5.000 IU ml). Death of the mice after this application is provided. After the death of the mice, the abdominal cavities were opened. Liver, kidney and heart were washed with cold deionized water and stored for 1 month at -86°C. The preparation of homogenates was performed as determined by the methods for each parameter. Malondialdehyde (MDA) was determined in the tissues according to the method described by Placer et al. (1966). In order to determine the vitamin E and β-carotene levels, the spectrophotometric method described by Kayden et al. (1973) was used. To determine the GSH levels, Sedlak and Lindsay (1968) defined spectrophotometric method were used.

**Statistical Analysis**

The significance of the difference between the groups was done by ANOVA test (SPSS Inc. Chicago Version: 16.0 USA). For figure drawing, Sigma Plot (Systat Software Inc. Version 10 Canada) was used. p<0.05 was considered statistically significant.

**RESULTS**

Liver GSH a level of all groups of mice exposed to cobalt toxicity is shown in figure I. Kidney GSH levels is pointed out in figure II. Heart GSH levels is indicated in figure III. A liver MDA levels is expressed in figure IV. A renal MDA level is figured in figure V. Heart MDA levels is shown in figure VI. A liver vitamin E level is represented in figure VII. A kidney vitamin E level is described in out in figure IX. Renal β-carotene a level is shown figure VIII. Liver β-carotene level is pointed in figure X.
When the GSH levels of the cobalt group were compared with the control group, there was a significant decrease in the GSH levels of cobalt group. This decrease was statistically significant (p<0.001). There was also a decrease in renal GSH levels of the combined group compared to the control group but this decrease was not statistically significant (p>0.05) (Figure 2). The heart GSH levels of the cobalt group were lower than the control and combined group. This decrease was statistically significant (p<0.001). However, the decrease in the heart GSH levels of the combined group was not significant compared to the control groups (p>0.05) (Figure 3). MDA levels of the cobalt group were compared with the control and combined group. Liver MDA levels in cobalt group were higher than control and combined group. It was statistically significant (p<0.01). The increase in liver MDA levels in cobalt group was not statistically significant compared to the control group (p>0.05) (Figure 4).
Renal MDA levels were compared with each other separately. As a result; Renal MDA levels in experimental groups increased compared to the control group. This increase was insignificant in the combined group and statistically significant in the cobalt group (p<0.01) (Figure 5). When heart MDA levels of the experimental groups were compared with controls, heart MDA levels of the experimental groups were found to be significantly higher. Although this increase was statistically significant for both groups, the significance was found to be p<0.001 (Figure 6).

Liver Vitamin E levels of the experimental groups were lower than the control group. However, this decrease was not statistically significant for any group (p>0.05) (Figure 7). There was a significant decrease in renal vitamin E levels of the experimental groups compared to the control group. This decrease was also significant in both experimental groups and it was determined as p<0.001. The difference between the experimental groups was not significant (p>0.05) (Figure 8).
Liver β-carotene levels in experimental groups were slightly lower than controls but this decrease statistically insignificant (p>0.05) (Figure 9). There was a significant decrease in renal β-carotene levels in cobalt group compared to control and combined group. This decrease was statistically significant compared to control group (p<0.05) but it was insignificant compared to combine group (p>0.05) (Figure 10). At the end of the experiment the weight of the mice was compared; no significant difference was observed in the live weight of the combined group compared to the control group; It was found that approximately 40% of the weight loss occurred in the cobalt group compared to the controls and the difference between compared and control group was statistically significant (p <0.001). The decrease in the combined group was statistically insignificant.

**DISCUSSION AND CONCLUSION**

Cobalt is one of the toxic industrial and environmental heavy metals. Acute and chronic toxicity of cobalt in various organs has been histologically demonstrated by many investigators (Barborik and Dusek., 1972, Horowitz et al., 1988). Experimental and environmental exposure to this heavy metal; Cardiovascular diseases such as cardiomyopathy, hepatic damage, loss of renal function and anemia occur (Horowitz et al., 1988; Neckardien et al., 1991; Bucher et al., 1999). The toxic effects of cobalt on various cells are usually nucleus membrane damage, chromatin condensation, damage to mitochondria crystals and end cell death (Kasprzak et al., 1994). It is possible to prevent these toxic effects of cobalt through antioxidant systems (Christova et al., 2002).

Therefore, it has been suggested that administration of antioxidant agents for protective purposes against oxidative stress caused by cobalt may be effective (Christova et al., 2002; Shirivastava et al., 2008). Lycopene is a powerful antioxidant, protects cells against oxidative damage, but the research in this area is inadequate. Conducted research (Gonzales et al., 2005; Ewing and Maines, 1993) reported that c.t. has an effect on weight loss. Our findings are supported by these studies. When we examine the live weights of all groups in our study; no significant difference was observed in the live weight of the combined group compared to the controls. The cobalt group had a live weight loss of about 40% compared to the controls. However, Bucher et al. (1999) did not find different weight loss in mice and rats exposed to different concentrations of cobalt sulfate for
long periods of time. Decrease in weight might be the result of c.t. C.t leads to increase in free radical metabolism, cell damage and changes in the activities of catabolic defenses enzymes. It is possible to explain that the insignificant live weight loss in the combined group is due to lycopene. C.t. has been shown to cause oxidative stress in tissues by causing peroxidation in lipids and also disrupting antioxidant balance (Nackerdien et al., 1991; Gonzales et al., 2005). It is pointed out in many studies that c.t. increased MDA levels and decreased antioxidant levels (Neckardien et al., 1991; Gonzales et al., 2005; Osinsky et al., 2004).

In our study, although we observed an increase in MDA levels in all tissues of the cobalt group, the most significant increase was in heart tissue (p <0.001). It is reported in some studies that rats exposed to cobalt sulfate toxicity more than 13 weeks are tend to cardiomyopathy (Bucher et al., 1999; National Toxicology Program., 1991). Likewise, Kerfoot et al. (1975) found severe ventricular disorders in the electrocardiograms of pigs exposed to a toxic dose administration of cobalt sulfate of 0.1mg/kg for 5 hours a day, 5 days a week for 3 months. This increase in tissue MDA levels can be contributed to the increase due to c.t. However, reduction of MDA levels in the combined group is related to lycopene, which has a reductive effect on lipid peroxidation products caused by a toxic substance such as cobalt (Figures IV, V, VI). It is known that many agents have high affinity for thiol groups such as GSH. GSH protects cells from oxidative damage by reacting with free radicals and peroxides caused by many toxic agents such as cobalt in cells. Formation of oxidative stress occurs due to various reasons such as lipid peroxidation (Gonzales et al., 2005; Christov A et al., 2002). However, there are limited studies on the discussing the relationship between c.t. and GSH (Shirivastava et al., 2007; Gonzales, 2005; Olivieri et al., 2001). But, a study conducted reported decrease in GSH levels due to lipid peroxidation (Tania et al., 2003). Similarly, in our results, some researchers as stated (Shirivastava et al., 2007; Gonzales 2005), significant decreases in GSH levels were detected due to c.t. (Figure I, II, III). However, this decrease was less in the combined group compared to controls.

According to these data, significant reduction in GSH level might be the result of formation of oxidative damage. Vitamins play a very important role in the conservation of cellular balance in the organism. Although all vitamins are equally important in supporting the living characteristics of living things, β-carotene, which is a chain-breaker antioxidant that can protect cells against the harmful effects of peroxides, and β-carotene, which is the precursor of vitamin A, is considered as important vitamins due to being a powerful singlet oxygen trap. They are particularly important in maintaining the cellular balance against increased free radicals in many cases that are stressing organism such as heavy metal intoxications, infectious diseases, cancer, all kinds of trauma, pregnancy. Although the effects of vitamin E and β-carotene on heavy metal toxicity have been reported in the literature search, there has limited information about protecting the cells against toxic agents by β-carotene. In addition to this, there is no information about the effect of free radical formation on tissue vitamin E and β-carotene levels. In a study conducted in rats pointed out that vitamin E administration against Co, Pb and Hg induced kidney tubular cell degeneration could be protective (Hanafy and Soltan, 2004).

In another study, the vitamin E reserve of the organism was increased and thus the prevalence of lipid peroxidation could be prevented and consequently the size of the oxidative tissue damage could be reduced (Asar et al., 2004). Similarly, in another study, it was observed that the intake and distribution of cadmium was restricted in rats liver and kidneys by decreasing the cadmium toxicity of vitamin E through antioxidative mechanisms (Yin et al., 1999; Beytut, 2002). In our study, significant decreases in renal vitamin E and levels of combined and cobalt group compared to controls were determined. This reduction can be explained by the fact that cobalt, hydroxyl radicals and superoxide anions produce reactive species such as \( \text{H}_2\text{O}_2 \) in metal-oxygen complexes and biological systems, and oxidative damage is caused by oxidative damage by decreasing the antioxidant defense due to oxidative damage. While the liver vitamin E and β-carotene levels of the cobalt group were compared with the control and combined group, this decrease was statistically insignificant.
as seen in our study (Figure VII and IX). In this study, decrease in liver vitamin E and β-carotene levels represented formation of oxidative damage. Despite of oxidative stress, liver vitamin E and β-carotene levels is higher than kidney. These reasons may be due to better vitamin E and β-carotene in liver reserves.

Conclusions
Cobalt toxicity caused live weight loss in mice. It was observed that it increased MDA levels in tissues. At the same time, this toxicity decreases GSH, vitamin E and β-carotene levels. However, lycopene supplementation decreased the MDA levels of the combined group, while GSH increased the vitamin E and β-carotene levels and live weight loss was found to prevent. In conclusion, it was shown that lipid peroxidation in liver, kidney and heart was increased by cobalt toxicity. The most significant increase in lipid peroxidation was observed in the heart. Lycopene supplementation had a reductive effect on lipid peroxide products and decreased tissues damage caused. We believe that all of obtained data will be the basis for further studies in the future.

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