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The Effect of Vitamin E and Selenium on Cyclophosphamide Detoxification in Hepatic Tissues of Mature Rats

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Abstract

Introduction and Objective: liver is an important body organ without which life is impossible. Cyclophosphamide is an alkylating agent widely used as an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. The present study aimed to find a suitable antioxidant and examine the effect of vitamin E and selenium on reducing the side effects of cyclophosphamide on liver as a vital organ. *Materials and Methods:* in this experiment, 42 mature female rats were divided into 6 groups. The control group did not receive any drug or solvent. The first experimental group received 1 mg / kg-B.W sodium selenite as intraperitoneal injection. The second experimental group received 200 mg / kg-BW 200 vitamin E orally. The third experimental group received 5 mg / kg-BW cyclophosphamide as intraperitoneal injection. The fourth and fifth experimental groups respectively received 5 mg / kg-BW cyclophosphamide as intraperitoneal injection in a daily manner and 1 mg / kg-BW 1 sodium selenite as intraperitoneal injection and 200 mg / kg-BW 200 vitamin E orally for 21 days. The hepatic tissue slides were prepared and examined. *Results:* the hepatic cells were normal in control group. No difference in hepatic tissue was observed in the first and second experimental groups. Cellular necrosis was observed in the third experimental group. Destruction of hepatocytes in hepatic cells was highly observed in the fourth and fifth experimental groups compared to the third experimental group. Furthermore, congested blood sinusoids and lymphocyte infiltration was less observed in the fourth and fifth experimental groups compared to the third experimental group. *Conclusion:* Cyclophosphamide cause damage to hepatic tissues with different mechanisms such as production of reactive oxygen species. Vitamin E and sodium selenite relatively reduce negative effects of cyclophosphamide.

Key words: Vitamin E, Selenium, Cyclophosphamide detoxification, Hepatic tissues

Introduction

Cyclophosphamide (CP) is an anti-cancer drug used in chemotherapy. This drug is an alkylating agent, which binds and breaks the two strands of DNA and inhibits RNA and protein synthesis (1). Although Cyclophosphamide (CP) is an effective anti-cancer drug, it reduces the tumor size. However, additional doses of CP usually weakens the host defense mechanisms. This weakness often inhibits immunological responses and develop opportunistic infections and sometimes leads to recurrence of cancer (2). Nevertheless, high-dose cyclophosphamide has the above side effects. Appropriate dose of CP increases the immune response in animals and humans (3, 4). Treatment with low-dose cyclophosphamide leads to recurrence of spleen lymphocyte proliferation in the animals infected with tumor while tumor proliferation was reduced prior to treatment (5, 6). The drug is well absorbed from the gastrointestinal

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tract and is widely distributed in body tissues. The drug is metabolized in the liver and is excreted through urine. The drug inhibits synthesis of protein. Half-life of this drug is about 6-4 hours (7).
Vitamin E and selenium are antioxidants compounds. Vitamin E is a fat-soluble vitamin involved in free radicals cleanup (8). Selenium is a component of such antioxidant enzymes as glutathione peroxidase, which neutralizes free radicals and eliminates oxidative stress. This element maintains antioxidant substances in the body such as vitamin E and vitamin C and reduces the damage caused by free radicals (9). Excessive intake of cyclophosphamide drug, like other anticancer drugs, leads to cellular toxicity in rapidly dividing cells such, like liver failure (10). Pathogenesis of cyclophosphamide toxicity is not identified in hepatic cells yet. It seems that excess free radicals of oxygen significantly leads to development of cellular toxicity, which was caused by cyclophosphamide metabolites (11). Another study showed that cyclophosphamide causes severe pathological changes such as necrosis and fibrosis in hepatic tissues (12). On the other hand, the rats treated with cyclophosphamide are prone to liver failure (13, 14). The present study aimed to investigate the role of vitamin E and selenium in cyclophosphamide detoxification in hepatic tissues in mature rats.

Materials and Methods

This was an experimental study conducted in winter 2013. In this study, 42 mature female Wistar rats with an average weight of 200 ± 10 g from 2 to 3 months years old were provided from Laboratory Animal Fostering Center in Bandar Abbas University. The laboratory animals were kept in accordance with manual of National Health Institutes. This study was conducted in proper circumstances within 21 days with 25±1ºC controlled temperature and 12 light hours and 12 darkness hours.

Preparation and Injection Methods

Distilled water was added to powdered cyclophosphamide drug to prepare a suspension. Five mg / kg / B.W. 5 were injected intraperitoneally using an insulin syringe in a daily manner for 21 days. The control group did not receive any drug or solvent.
The first experimental group received 1 mg / kg-BW sodium selenite as intraperitoneal injection.
The second experimental group received 200 mg / kg-BW vitamin E orally.
The third experimental group received 5 mg / kg-BW cyclophosphamide as intraperitoneal injection. The fourth and fifth experimental groups respectively received 5 mg / kg-BW cyclophosphamide as intraperitoneal injection in a daily manner and 1 mg / kg-BW sodium selenite as intraperitoneal injection and 200 mg / kg-BW vitamin E orally for 21 days.
All cyclophosphamide and sodium selenite drugs were injected intraperitoneally while vitamin E was administered orally to animals. The rats were anesthetized by diethyl ether at the 22th day. After complete anesthesia, the livers of rats were surgically removed. The hepatic tissues were placed in the plates containing 10% formalin stabilizer for 21 days to prepare 5-micron tissue sections. The tissue slides were prepared. Then, the tissue specimens were stained with hematoxylin-adenosine. Then, inflammatory parameters were evaluated. Then, the slides were examined with binocular optical microscope using the 400 × Labomed model (made in England) and a computer (made in China) for data analysis. A UV 100 Camera (made in Korea) was used to prepare photomicrographs. In each group, the hepatic tissues were examined in terms of changes in congestion, cellular necrosis, infiltration of lymphocytes, mononuclear accumulation and swelling. Pathological changes in each group were reported in comparison to controls.

Results

The study showed that the control group (receiving no medication) had normal hepatic cells (Figure 1). No changes in hepatic tissues were observed in the first and second experimental groups and hepatocytes cells were normal (Figure 2 and 3). Largely significant hydropic swelling of hepatic cells and cellular necrosis were observed in the third experimental group (Figure 4). Destruction of hepatocytes in the hepatic cells was less observed in the fourth and fifth experimental groups compared to the third experimental group. Congested blood sinusoids and lymphocyte infiltration was less observed in the fourth and fifth experimental groups compared to the third experimental group (Figures 5 and 6). The granularity of hepatic cells was observed in the fourth and fifth experimental groups.

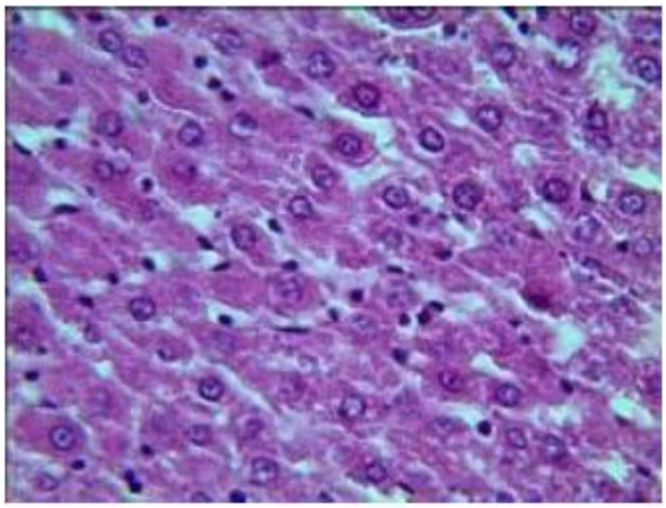

Figure 1: photomicrograph of hepatic tissues in the control group (x400) (hematoxylin-eosin stain) Hepatic cells were normal

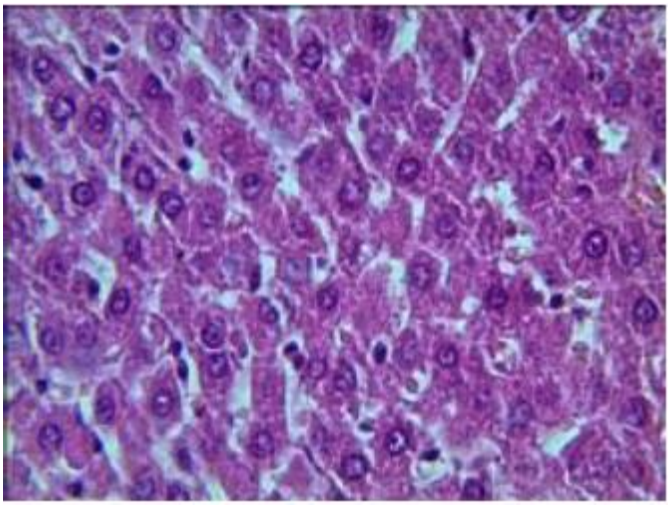

Figure 2: photomicrograph of hepatic tissues in the control group (x400) (hematoxylin-eosin stain) No changes were observed in hepatic tissues and hepatocytes cells were normal.

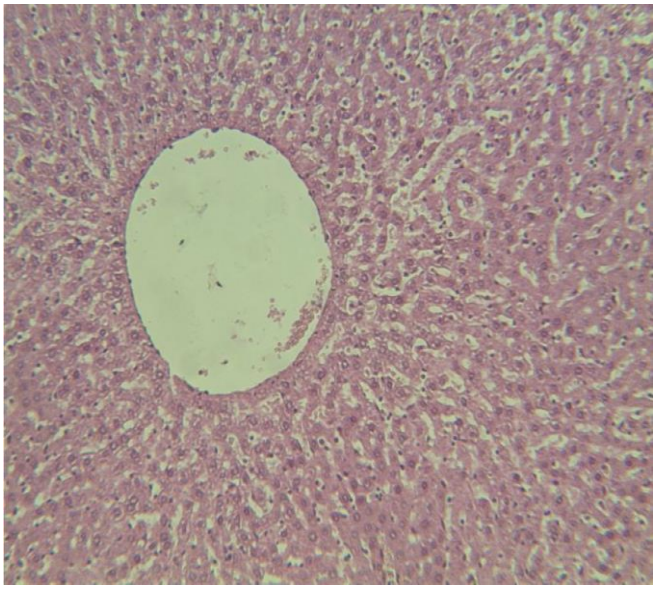

Figure 3: photomicrograph of hepatic tissues in the control group (x100) (hematoxylin-eosin stain) No changes were observed in hepatic tissues and hepatocytes cells were normal.

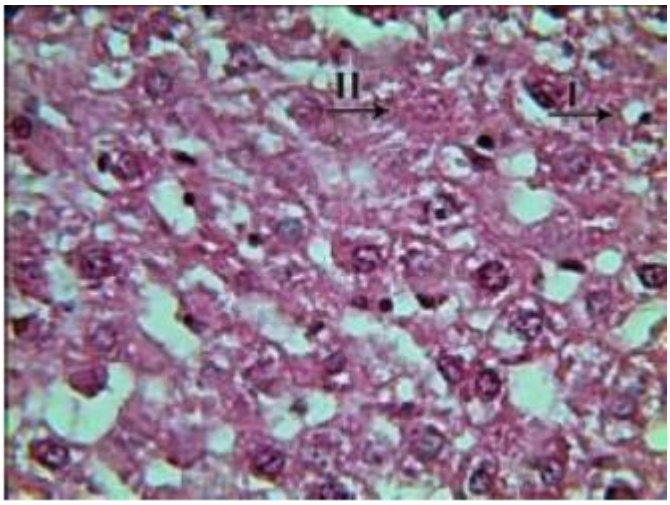

Figure 4: photomicrograph of hepatic tissues in the control group (x400) (hematoxylin-eosin stain) Hydropic swelling of hepatic cells (I) and cellular necrosis (II) were significantly observed.

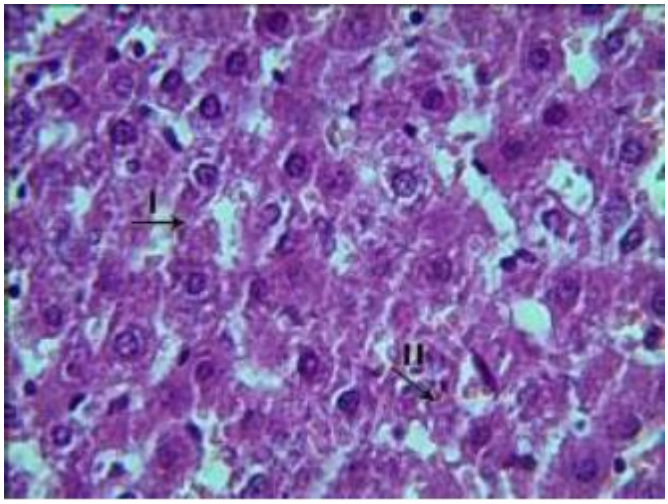

Figure 5: photomicrograph of hepatic tissues in the control group (x400) (hematoxylin-eosin stain) Congested blood sinusoids and necrosis of hepatocytes (I) was less observed compared to the third experimental. Granularity of hepatic cells was also observed (II).

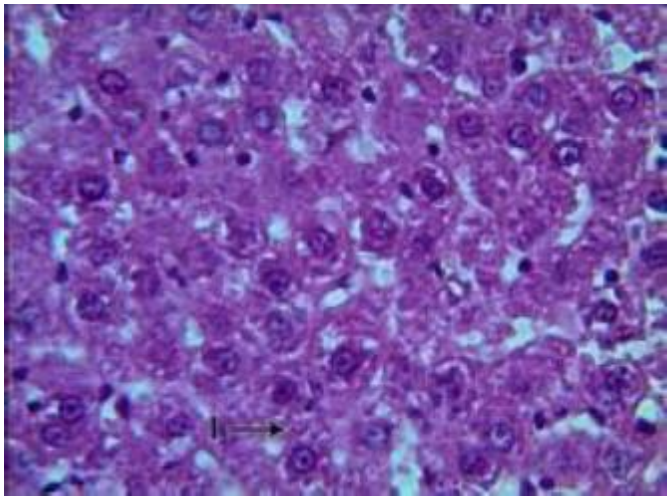

Figure 6: photomicrograph of hepatic tissues in the control group (x400) (hematoxylin-eosin stain) destruction of hepatocytes in liver cells was less observed in comparison to the third experimental group. The granularity of liver cells (I) was observed.

Discussion

Cyclophosphamide is an anti-cancer drug (15), which is well absorbed from the gastrointestinal tract and passes the blood-brain barrier and converts to active metabolites in the liver and is eventually excreted through the kidneys (16). Anti-neoplastic effects of cyclophosphamide is due to phosphoramide mustard. Acrolein produces free radicals of oxygen with involvement by interfering with antioxidant defense system in the tissues. Acrolein is responsible for such toxic effects as cell death, apoptosis, emergence of multiple tumors and necrosis (19-17). Alpha-Tocopherol or vitamin E can prevent the damaging effects of ROS on body tissues due to the fat-soluble capability (20). A tocopherol molecule as a chain breaking antioxidant can inhibit two lipid peroxide radicals and eventually two potential peroxidation chain

reactions (21). Selenium is a component of such antioxidant enzymes as glutathione peroxidase, which neutralizes free radicals and eliminates oxidative stress (22). In the present study, the photomicrographs showed hydropic swelling and cellular necrosis in the groups receiving cyclophosphamide. Cyclophosphamide may increase reactive oxygen species and cause damage to the hepatic tissue. Therefore, vitamin E and selenium with antioxidant properties reduced the side effects of cyclophosphamide. It was also observed that the groups receiving cyclophosphamide + vitamin E, selenium + cyclophosphamide and selenium + vitamin E less suffered from destruction of hepatic tissue, which showed beneficial effects of antioxidant compounds.

Different parameters were studied in investigation of histopathologic changes in the liver in different groups. In this study, extensive destructive changes and necrosis around lobular center and hemorrhage were caused by cyclophosphamide. Destructive changes and necrosis around the central venule may be due to exposure to toxins (23). Therefore, hepatic histopathology findings reflected the direct and significant toxic effects of cyclophosphamide in this study. Other studies showed a balance between formation of oxidative species and elimination of these species by antioxidant compounds in physiological conditions. Oxidative stress occurs when the balance is disrupted by excessive production of free oxygen radicals and weakness in antioxidant defense system (24). It is reported that cyclophosphamide is involved in formation of free radicals, lipid peroxidation and oxidative stress in rats (25). Research has shown that cyclophosphamide is an alkylating agent, which binds the double-stranded DNA molecule, breaks the DNA stand and inhibits protein synthesis during apoptosis. The alkylating agent produces reactive molecules, which alkylates nucleophilic group on DNA strand, especially 7-N Guanine, which leads to lateral bonds between base pairs and abnormal bonds between the base pairs, which breaks the DNA stand and reduce the meiotic division (26).

As shown earlier, administration of cyclophosphamide causes liver failure. Some results of this study are consistent with reports of some academics. Shentil Kumar *et al.* showed that cyclophosphamide causes severe tissue damage such as necrosis, hemorrhage and fibrosis in the rat livers (13). On the other hand, liver dysfunction and toxicity has been reported in the patients taking cyclophosphamide (27). Hepatic tissue is the preliminary place of microsomal drug activity. Hepatic cyclophosphamide activity generates toxic metabolites and damages hepatic cells and channels (13). Khayat Nouri stated that formation of toxic metabolites by hepatic microsomal enzyme activity damages hepatic tissues (28). These results were consistent with those obtained in the present study. Thereby, it is recommended that vitamin E and selenium be prescribed for the patients who should use the cyclophosphamide drug.

Conclusion

In this study, cyclophosphamide as an alkylating agent caused cellular dysfunction and excess oxygen species damaged hepatic tissue. However, vitamin E and selenium with antioxidant properties relatively reduced the negative effects of cyclophosphamide. Thereby, it is recommended that vitamin E and selenium be prescribed for cancer patients to reduce the risk of toxicity due to cyclophosphamide.

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