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Studying the Effect of Vitamin E and Selenium on Liver Enzymes in Chemotherapy Rat with Cyclophosphamide

Mozhdeh Khanalizadeh and Mahmood Najafian*

Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom Iran.

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Cyclophosphamide is a drug of alkylating group and used widely as an anticancer drug. Vitamin E and selenium have important role in protecting the body by their antioxidant factor. The aim of this study is investigating the effect of selenium and vitamin E on liver enzymes in cyclophosphamide chemotherapy rats. The experimented animals were a total of 42 were Wistar rats which randomly divided into 7 equal groups. CO group did not receive any drug or solvent. SA Group daily received 0.2 ml of physiological saline as drug solvent intraperitoneally. SN group received 1 mg/kg-B.W of *sodium selenite* intraperitoneally. VE group received 200 mg/kg-BW of vitamin E gavage feeding. CP group received 5 mg/kg-BW of cyclophosphamide intraperitoneally. CP-SN and CP-VE groups once a day received 5 mg/kg-BW of cyclophosphamide intraperitoneally and respectively 1 mg/kg-B.W of *sodium selenite and* 200mg/kg-BW of vitamin E gavage feeding for 21 days. Then the rats were bled and serum was separated. Then, activities of ALT, AST, ALP enzymes were evaluated by the kinetics method and photometer. The results showed that the activities of measured enzymes in SA, SN and VE groups have not significant different compared to the CO group but the CP group has increased significant and in groups of CP-VE and CP-SN has a decreased significant compared to the CP group. According to the results from studies of liver enzymes we can conclude that cyclophosphamide with increasing active oxygen types, increased the liver enzymes' activity in serum. Vitamin E and sodium selenite also have antioxidant factor and somewhat reduces the negative effects of cyclophosphamide. So, the use of vitamin E and sodium selenite is recommended for reducing cyclophosphamide toxicity in cancer patients.

Key words: vitamin E, sodium selenite, cyclophosphamide, liver enzymes

Cyclophosphamide is an anti-neoplastic drug and in the body becomes an active metabolite of alkylating with similar effects of Mustin. Cyclophosphamide with the DNA alkylating like other alkylating factors of a group, binds alkyl (C_nH_{2n+1}) to DNA molecules and by this procedure prevent DNA replication. Cyclophosphamide is well absorbed from the digestive system and is widely distributed in the tissues of and body and fluids and also crosses the blood-brain barrier. This drug in the liver turns to active metabolites and eventually excreted through the kidneys[1-2]. We can refer to nausea, vomiting, diarrhea, weight loss, colitis, liver toxicity, marrow suppression, acute myeloid leukemia, hemorrhagic cystitis, blood in urine(hematuria), neoplasm, amenorrhea, azoospermia, impotence, ovarian fibrosis, alopecia , dermatitis, nail bed pigmentation, transverse nail ridges (Beau's lines), interstitial pulmonary fibrosis, embolism, pulmonary edema, headache and dizziness to mention another effects of using cyclophosphamide[1-2]. Cyclophosphamide is a potent alkylating drug that acts mainly because of two DNA molecule connections, break of the DNA, also RNA and inhibit protein synthesis[3].Vitamin E by

* To whom all correspondence should be addressed.
E-mail: M.Najafian@jia.ac.ir

the formula of $C_{29}H_{50}O_2$ is a heterocyclic compound and derived from the chrome core. Chrome core is obtained by connecting a benzene ring and an oxygen-containing heterocyclic ring called Piran. Tocopherol in the benzene ring has three roots of methyl radical, a hydroxyl factor; in Piran ring have a methyl root and a chain of 16 carbons. In addition to alpha-tocopherol, other similar compounds exist in natural which their chemical structure with alpha-tocopherol differ in the number and location of the methyl root of benzene ring[4]. Vitamin E in 1920 known as a fat-soluble factor and anti-abortion and its scientific name is tocopherol. This vitamin is studied, especially about animals such as rats. Experiences have shown that if rats take an artificial diet without fat, disturbances appears in the reproduction of the animal. Evans 2 showed that these disturbances are caused by a lack of fat-soluble vitamin other than vitamin A and vitamin D[4]. After absorption in the intestine, vitamin E is transmitted to blood through shilomicrons and then transmitted to the liver through the debris of shilomicrons. Then, by VLDL transmitted to other tissues of the body. Due to lipophilic of vitamin E, it is accumulated in cell membranes, fat deposits and other lipoproteins of blood circulation. Original location to store this vitamin is fat tissues. This vitamin as a natural antioxidant removes free radicals and molecular oxygen and prevents the peroxidation of unsaturated fatty acids of the membrane. Vitamin E and vitamin C associated with each other because of antioxidant factor. Vitamin C by oxidizing materials revives oxidized vitamin E by oxidized materials. Vitamin E because of antioxidant effect could be effective in treating inflammatory diseases and also cancer prevention[5]. Selenium is a trace element substance that exists in few amounts in the nature. But this little amount play extra key role in the health of living organisms such as human. Selenium has a positive effect on in many physiological and normal processes of the body such as growth hormone, thyroid and the immune system. This factor plays its role by antioxidant factors by the peroxides glutathione enzyme[6]. Scientific evidence shows the help of selenium in reducing the risk of many types of cancer (including prostate cancer, liver cancer, lung cancer, etc.). Also cause the chemotherapy effects in these patients. Selenium can boost the immune system and increase the body's resistance against many diseases. Selenium helps to protect the important antioxidant of the body such as vitamin C and vitamin E and reduce the trauma caused by free radicals[7-9]. According to the mentioned subjects and daily increase of cancer, using of antioxidants materials may be a great help for these patients to reduce the effects of the cyclophosphamide drug. Because no research has done on the effects of selenium and vitamin E on liver effects reduction and cyclophosphamide; so the main objective of the present study is the mentioned issue.

Methodology

This study was conducted in vitro and completely random. All ethic principle of laboratory animals' use is considered in this research. A total of 42 adult female Wistar rats weighing 190 ± 10gram and the age of 75 days which provided from the University Of Medical Sciences Of Bandar Abbas. Rats in Animal House of University of Medical Sciences Of Bandar Abbas putted for 2 weeks in vitro, in 21± 2 °C and a cycle of 12 hours of light and 12 hours of darkness. Rats were kept in cages with metal mesh door and eat standard food. Also, the water was available to them in special glass bottle. Soluble Vitamin E in 1 CC vial provided from drugstore and this drug concentration was 100 mg / ml and the needed amount gavages according to the weight of each rat. Soluble sodium selenite provided in 1 CC vial and the concentration of the drug was 500IU / ml and the needed amount due to the weight of each rat was purred in insulin syringe and injected to the animal. Used cyclophosphamide pills were 5 mg. A pill for every 5 rats after crushing in a mortar and ground in an electric mill turned to a volume of 1 ml by physiological saline and injected intraperitoneally to 5 rates . This means that each rat were injected 0.2 ml of cyclophosphamide at each dose.

Cyclophosphamide and sodium selenite by insulin syringe by intraperitoneally injection and vitamin E gavage feeding during 21 days gives to the rates. Injection and gavage did every day at 9 am. The studied rates were putted into 7 groups of 6 rates as follows:

1) CO: Rats in this group kept normally and without any medication.
2) SA: Rats in this group received 0.2 ml saline as a drug solvent.
3) SN: Rats in this group received 1 mg / kg-B.W of sodium selenite.

4) VE: Rats in this group received 200mg / kg-B.W of vitamin E.
5) CP: Rats in this group received 5mg / kg-B.W of cyclophosphamide.
6) CP-SN: Rats in this group received 5 mg / kg-B.W of cyclophosphamide and 1 mg / kg-B.W of sodium selenite.
7) CP-VE: Rats in this group received 5 mg / kg-B.W of cyclophosphamide and 200 mg / kg-B.W of vitamin E.

After the study and during 21 days ,the rats of all groups dissection and blood taken from their heart by 5 CC syringe and after separating blood serum, the enzyme activity of AST , ALT and ALP measured by kinetics method and photometer in the laboratory. For comparing the treatments; one-sided variance analyses (ANOVA) and followed by Duncan's test was used for multiple comparisons between different groups. The significant level were considered ($p<0.05$). Data analysis and statistical tests were performed by using SPSS version 18.

RESULTS

Figure 1 shows ALT enzyme activity by cyclophosphamide treatment and different doses of vitamin E and sodium selenite. SA, SN and VE groups have no significant difference compared to the CO group. In the CP group has significant increase compared to the CO group. In CP-SN group enzyme activity decreased compared to the CP group but this decrease is not significant compared to the CP group. In CP-VE group the decline is more but this decline is still not significant compared to the CP group. Sodium selenite and vitamin E somewhat have reduced damaging effect of cyclophosphamide.

Figure 2 shows activity changes of AST enzyme because of treatment with cyclophosphamide and different doses of vitamin E and sodium selenite and as can be seen in the chart, SA, SN and VE groups have not significant difference compared to the CO group . In CP group significant difference is observed compared to

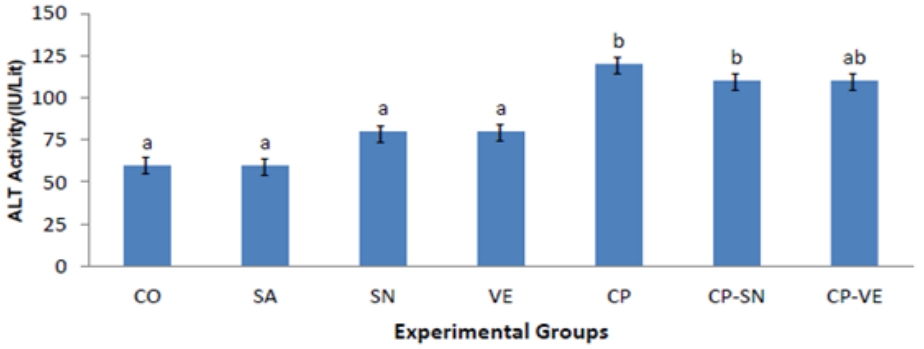

Fig. 1. Activity changes of ALT because of treatment with cyclophosphamide, sodium selenite and vitamin E Columns that contain at least one common letter have not significant difference with each other

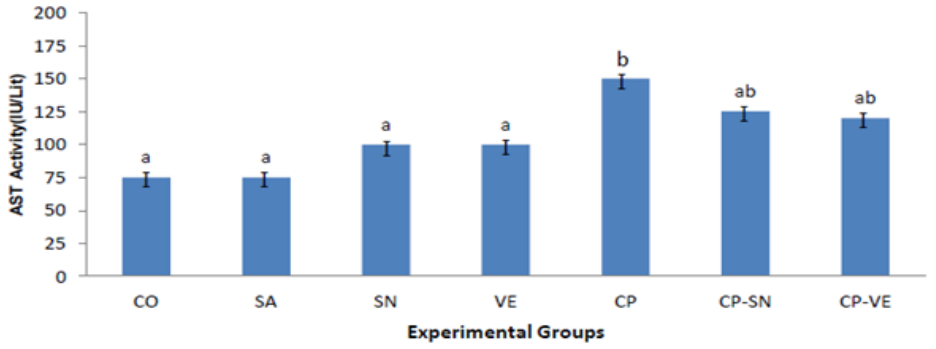

Fig. 2. Activity changes of AST because of treatment with cyclophosphamide, sodium selenite and vitamin E Columns that contain at least one common letter have not significant difference with each other

the CO group. In CP-SN and CP-VE groups, the enzyme activity decreased compared to the CP group but this decrease is not significant compared to the CP group. Sodium selenite and vitamin E somewhat have reduced damaging effect of cyclophosphamide.

Fig. 3 shows activity changes of ALP enzyme because of treatment with cyclophosphamide and different doses of vitamin E and sodium selenite and as can be seen in the chart, SA, SN and VE groups have not significant difference compared to the CO group . In CP group significant difference is observed compared to the CO group. In CP-SN and CP-VE groups, the enzyme activity decreased compared to the CP group but this decrease is not significant compared to the CP group.

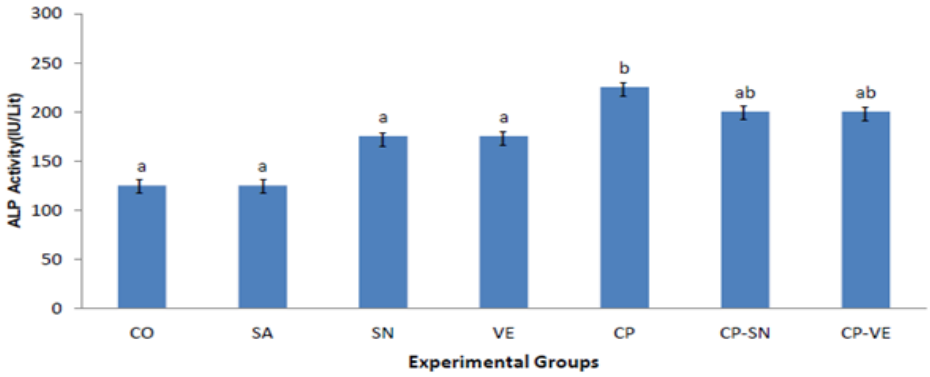

Fig. 3. Activity changes of ALP because of treatment with cyclophosphamide, sodium selenite and vitamin E Columns that contain at least one common letter have not significant difference with each other

DISCUSSION

In this study, the effect of vitamin E and selenium on liver and tissue changes of liver in cyclophosphamide chemotherapy rats were studied. Liver damage caused by cyclophosphamide as a very important lesion has been studied by numerous scientists. In this study, the effect of cyclophosphamide and vitamin E and selenium on enzymes of alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were investigated and the results showed a significant increase in enzyme activity in CP group compared to the CO group and caused by tissue damage by the use of cyclophosphamide and in CP-SN group that received sodium selenite with cyclophosphamide and partly because of selenite consumption ,the damaging effect of cyclophosphamide somewhat reduced. In CP-VE group which received vitamin E with cyclophosphamide; because of vitamin E, recovery rate and damage reduction is more than CP-SN group .Free radicals of cyclophosphamide by hepatocyte membrane damage caused by the increase of the enzymes' activity of ALT, AST and ALP and this factor enters the bloodstream within the cytosol cell enzymes. The activity increasing of ALT, AST and ALP enzymes represent the amount and type of damages to the liver[10]. In groups that In addition to cyclophosphamide received vitamin E and selenium, showed a significant decrease about the activity of these enzymes compared to the group treated with cyclophosphamide. Again normal enzymes activity by vitamin E and selenium is clear evidence on the liver protective effects of vitamin E and selenium. Cyclophosphamide with damaging liver cells cause significant changes in the studied factors in cyclophosphamide rats group compared to the control and observer groups, but vitamin E and selenium in addition to reduce changes of cyclophosphamide treatment , make the state of the biochemical factors to near normal status. Because (AST) in all the body tissues and is not considered a specific test of liver; the recent study of this kind can be justified by the existence of AST in all body tissues, hence is not regarded as a specific test of liver test. Serum AST levels may be increased in liver disease of all animal species. In explaining the measurement results of AST, we must be certain about health heart and body muscle because the heart and muscle diseases will increase serum AST levels. In ruminants, AST increasing

is a good sign of necrosis of the parenchymal cells of the Liver[11-13].Vozarova in 2002 found that liver dysfunction which is identified by elevated levels of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes. Aminotransferase is an introducer of the health of the liver cells, ALT is found basically in the liver, but AST is also found in other tissues, so is considered as less specific markers of liver[1]). Alkaline phosphatase was the first serum enzymes which their clinical value recognized and in 1920 they found that the enzyme increased with liver disease and bone[15]. Alkaline phosphatase serum level naturally is higher in young animals than in older animals; in adult, alkaline phosphatase mainly stems from the liver. While in children growing, bone cells are the most important source of this enzyme[15]. The amount of this enzyme increases in diseases such as liver and bile ducts, development of liver lesions, lesions caused by bone osteoclasts, during bone repair, gastrointestinal lesions such as malabsorption, necrosis of lungs and kidneys, spleen infarction and hyperparathyroidism[16,17]. Alkaline phosphatase along the bile ducts, increases hepatitis, cirrhosis and accumulation of fatty deposits in the liver[11-13].

CONCLUSIONS

According to the results of the liver enzyme study; it can be concluded that cyclophosphamide increasing serum liver enzymes with increasing active oxygen types. Vitamin E and selenium also have antioxidant factor and somewhat reduces the negative effects of cyclophosphamide. Therefore; using vitamin E and selenium is recommended in order to reduce the liver toxicity of cyclophosphamide in cancer patients.

REFERENCES

1. Shahrzad, Saeed.; Ghaziani, Tahereh., Comprehensive Textbook of Iran's official drugs 1383. Ziba Publications, 1383 .
2. Heshmati, M., Culture of Iran's generic drugs. 1387; 258-300.
3. Katzong, Bertram, Niyayesh M, Modares Mousavi F, Fathollahi A, Basic Clinical Pharmacology, Arjmand Edition, 1378; 372-378. (Persian).
4. Galesic K, Antiatherosclerotic effects antihypertensive drugs lijec Vjesn . 2006; **128** (11-12): 341-50 Reviewz.
5. Singh u, Devaraj S, Jialal Vitamin E, oxitative stress, and inflammation Anno Rew nutr 2005; 25: 151-74 Reviw.
6. Kaur R, Kaur K. Effects of dietary selenium on mor-phology of testis and cauda epididymis in rats. *Indian J Pharmacol*. 2000; **44**(3)265-72.
7. Schwatrz S. Essentiality and metabolic function of Selenium. *Med Clin North Am.* 1976;60:745-58.
8. Burk RF, Hill KE, Motley AK. Selenoprotein meta-bolism and function: evidence for more than one function for selenoprotein *P. J Nutr.* 2003; **133**: 151-78.
9. Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. *J Androl*. 2005; **26**(6) 654-60.
10. Ahmad A, Pillai KK, Najmi AK, Pal SN. Evaluation of hepatoprotective potential of Jigrine post-treatment against thioacetamide induced hepatic damage. *J Ethnopharmacol*. 2002; **79**(1): 35-41.
11. Karimi, Nasser. Farhang Nia, Mohammad., The main points of clinical diagnosis of diseases of cattle, Compilation A. H Anderson. University Press of Khuzestan. 1373; 156-155.
12. Arjomand, Mohsen- Mahmoud, Eshagh Hosseini., Harrison Principles of Internal Medicine 2005 "Diseases of the liver and biliary tract", Nasle Farda Publications, 2005; 134 63.
13. Rakhshan, Mohammad. Salahi, Mehrdad., The most complete source of diagnostic and laboratory tests, Iliya Publications, 1378.
14. Vozarova B, Stefan N, Lindsay R.S, Saremia A, Pratly R e and Bogardus C., High Alanin Aminotransferase Isassociated with Decreased Hepatic Insulin sensitivity and predicts the Development of type 2 diabets. Diabets; 2002; **51**: 95.
15. Kaneko JJ. Clinical biochemistry of domestic animals, 4th ed 1989; 374-385, 143-160.
16. Cles EH. Veterinary clinical pathology, 4th ed 1986; 130-150.
17. Blood D.C. and Radostits OM. Veterinary medicine, 7th ed. Billier and tindal., London. 1989; 228-298, 1320.