Original Article

Effect of hydroalcoholic extract of ginseng on cyclophosphamide detoxification in liver tissue of adult rats

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Abstract

Introduction:
Liver is a vital organ of the human bodies which continuing of life is impossible without it. Cyclophophamide is an anti-cancer drug using in chemotherapy. This study aimed to find an appropriate antioxidant and to examine the effect of ginseng extract on reducing hepatic complications of cyclophosphamide.

Methods and Materials:
We examined 60 adult female rats which were divided into 6 groups of 10 namely, control group which did not receive any drugs, experimental group 1 which daily received 5mg/kg/bw of cyclophosphamide, experimental group 2 which received 2gr/kg/bw of ginseng extraction, and experimental groups 3, 4 and 5 which received 0.5, 1 and 2 gr/kg/bw of hydroalcoholic ginseng extract together with 5mg/kg/bw cyclophosphamide. . Finally, slides were prepared from liver tissue of the rats and studied.

Results:
The cells of control group were normal. Liver cells in experimental group 1 were extremely necrotic. No changes were observed in liver tissue in experimental group 2 Destruction of hepatocytes in the liver cells as well as the sinusoidal congestion and lymphocyte infiltration were observed less in experimental group 3 than in experimental group 1. Likewise, hepatocyte destruction was less in experimental group 4 than in experimental group 1 and 3, and less in experimental groups 5 than in experimental groups 1 and 4.

Conclusions:
Cyclophosphamide destroys liver tissue in a variety of ways including production of active oxygen species, but ginseng extract can reducing such complications to some extent.

Key Words: Cyclophosphamide, Ginseng, Liver, Rat

Introduction

Cyclophosphamide is an anticancer chem-otherapy agent. It is an alkylating drug which forms a bond between two DNA strands, leads to its breakage and inhibits protein and RNA synthesis (1). Although the drug has a proper anticancer effect and reduces tumor size, its additional dose usually leads to weakness in the host defense mechanisms. This weakness usually leads to suppression of immune-logical responses and often develops

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opportunistic infections and sometimes results in cancer relapse (2). Although high doses of cyclophosphamide have such complications, the proper dose increases the immune response in animals and humans (3,4). Treatment with low doses of cyclophosphamide leads to spleen lymphocyte proliferation in animals with cancer, while proliferation is reduced before treatment (6,5). The drug is well absorbed from the gastrointestinal tract and is widely distributed in body tissues. This drug has a hepatic metabolism and is excreted through urine. It inhibits protein synthesis and has a half-life of about 6-4 hours (7).

The liver is one of the key organs for eliminating toxins from the body, and it always deals with many toxins and metabolites. Problems that may arise in the liver are also plentiful and varied. Hepatocytes inactivate different substances such as alcohol, steroids and barbiturates by their smooth endoplasmic reticulum enzymes, oxidation and methylation methods (8).

Ginseng, scientifically known as Panax (from the Greek root meaning medication for any pain), looks like man's body and people believed that it could cure diseases of all parts of the body and strengthen the body. The beneficial effects of ginseng have been confirmed in treating and preventing cardiovascular diseases, diabetes and also relieving mental fatigue and stress. In addition, this drug can improve brain function and improve cognitive abilities in people with aging-induced memory impairment (9). In traditional Chinese medicine, ginseng is mostly used to relieve stress. Ginseng, as an adaptogen, can strengthen body and help body regulate its overall functioning (9,10).

Excessive use of cyclophosphamide, like other anticancer drugs, in rapidly dividing cells induces cytotoxicity such as hepatic damage (11). The pathogenesis of cyclophosphamide toxicity in liver cells is not known, but it seems that increased free oxygen radicals generated by cyclophosphamide metabolites play an important role in its development (12). A study shows that cyclophosphamide causes severe pathological changes such as necrosis and fibrosis in liver tissue (13). Furthermore, cyclophosphamide can cause hepatic damage in rats (14,15).

This study aimed to evaluate the role of hydroalcoholic extract of ginseng on the cyclophosphamide detoxification in liver tissue of adult rats.

Materials and methods

In this experimental study, 60 adult female Wistar rats were studied with a mean weight of 185-195 grams and the age of 2-3 months procured from Laboratory Animal Breeding of Islamic Azad University, Jahrom Branch. Laboratory animals were housed according to the guidelines of the National Institute of Health. This study was conducted within 21 days in good conditions with controlled temperature 25± 1 °C under a 12 hr light/dark cycle.

Preparation of different doses of cyclophosphamide

A 50 mg cyclophophomide tablet was powdered and dissolved in 10 ml of distilled water and was injected intraperitoneally into each rat daily per kg of body weight with a syringe and insulin needle. It should be noted that oral cyclophosphamide does not completely dissolve in distilled water and forms a suspension.

Ginseng extraction method

To prepare ginseng extract, first the root of the plant is powdered and then the extract is prepared using percolation method (16). To do so, dry powder is poured into cylindrical part of percolator that is filled up to two-thirds with alcohol 80% and the rest with water. When the first solution exits the end valve, the valve is shut and the extract is collected after 24 hours. Then the extract collected is dried at 30-40 °C in a sterile environment (16).

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Animals were randomly divided into six groups based on weight range as follows:
Control group: No drug was injected into rats in this group.
Experimental group 1: They received cyclophosphamide daily 5 mg/kg of body weight.
Experimental group 2: They received hydroalcoholic extract of ginseng daily 2 g/kg of body weight.
Experimental group 3: They received cyclophosphamide daily 5 mg/kg of body weight and hydroalcoholic extract of ginseng daily 0.5 g/kg of body weight.
Experimental group 4: They received cyclophosphamide daily 5 mg/kg of body weight and hydroalcoholic extract of ginseng daily 1 g/kg of body weight.
Experimental group 5: They received cyclophosphamide daily 5 mg/kg of body weight and hydroalcoholic extract of ginseng daily 2 g/kg of body weight.
Cyclophosphamide was injected intraperitoneally and ginseng extract was administered orally to rats. On the 22nd day, the rats were anesthetized by diethyl ether and their livers were completely removed by surgery. Then liver tissues were placed in containers containing formaldehyde 10% for 21 days for preparing five micron cross-sections. After preparing tissue slides, they were stained by hematoxylin and eosin method and inflammatory parameters were studied. Then, slides photomicrograph was prepared by binocular optical microscope (Labomed × 400, England) and the camera (UV 100, Korea) and data were analyzed by computer (China). In each group, liver tissues were studied in terms of histological changes such as hyperemia, cell necrosis, lymphocytic infiltration, mononuclear accumulation, hydropic swelling and cell necrosis. Pathological changes in each group were reported compared to control group.

Results

This study showed that liver cells in the control group which did not receive any drug were normal (Figure 1). In the experimental group 1, hydropic swelling of liver cells and cell necrosis were observed to a large extent (Figure 2). In the experimental group 2, no change was observed in the liver tissues and hepatocyte cells were normal (Figure 3). Liver cell damage in the experimental group 3 was less than that in the experimental group 1 and cytoplasm was darker than that in the control group. Congested blood sinusoids and lymphocytic infiltration were less observed in this group compared to experimental group 1 (Figure 4). Also, hepatocyte damage in the experimental group 4 was less than that in the experimental groups 1 and 3 (Figure 5). In the experimental group 5, hepatocyte damage was less than that in the experimental groups 1 and 4 (Figure 6). Granular liver cells were also observed in experimental groups 3, 4 and 5.

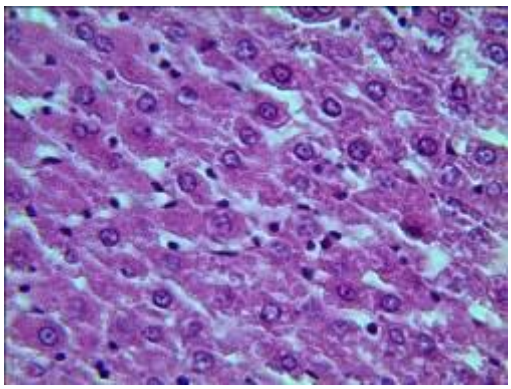

Figure 1: Photomicrograph of liver tissues in the control group (×400) (H & E staining). Liver cells are normal.

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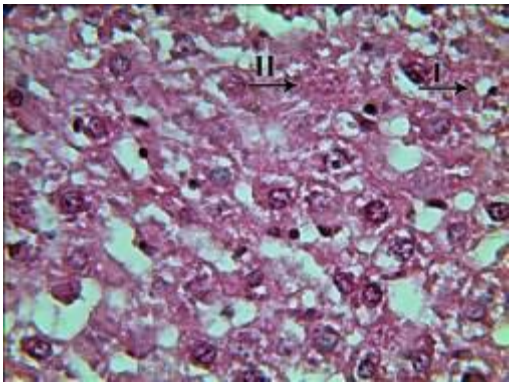

Figure 2: Photomicrograph of liver tissues in the experimental group 1 (×400) (H & E staining). Hydropic swelling of hepatocytes (I) and cell necrosis (II) were observed to large extent.

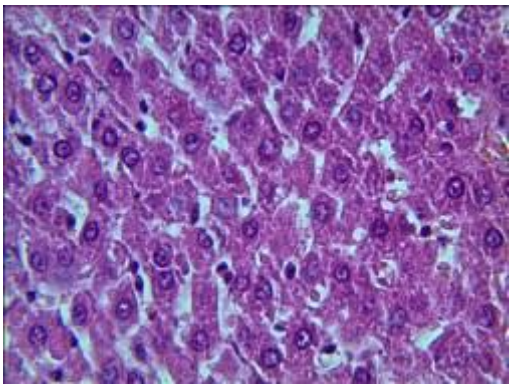

Figure 3: Photomicrograph of liver tissues in the experimental group 2 (×400) (H & E staining). No changes were observed in liver tissues and hepatocyte cells are normal.

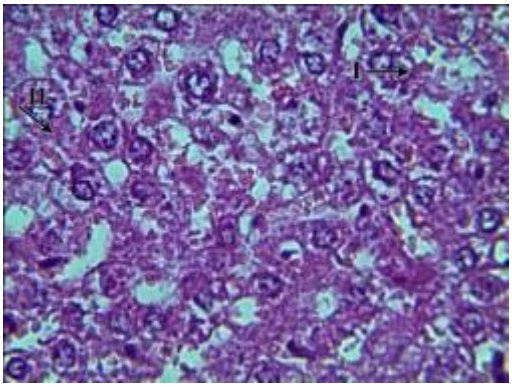

Figure 4: Photomicrograph of liver tissues in the experimental group 3 (×400) (H & E staining). Hepatocyte congestion (I) is less than that in the experimental group 1 and granular cytoplasm of hepatocytes (II) can be seen in the liver tissue.

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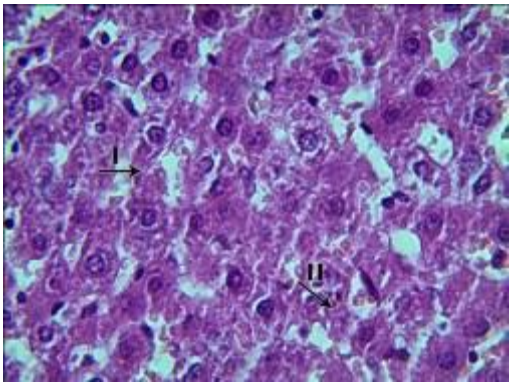

Figure 5: Photomicrograph of liver tissues in the experimental group 4 (×400) (H & E staining). Congestion and necrosis of hepatocytes (I) are lower than those in the experimental groups 1 and 3 and granular liver cells (II) can be seen.

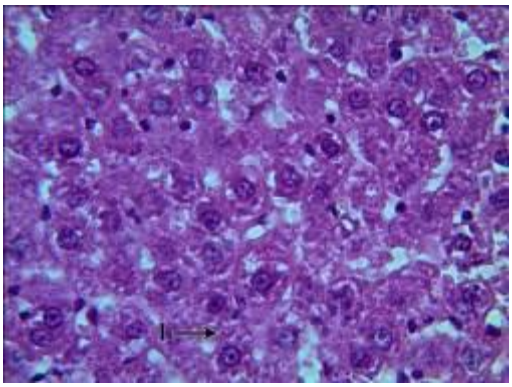

Figure 6: Photomicrograph of liver tissues in the experimental group 5 (×400) (H & E staining). Compared to the experimental groups 1 and 4, less hepatocyte damage can be seen in liver cells and granular liver cells (II) are also observed.

Discussion

Regarding the development of traditional medicine over thousands of years and its important role in people's health, it seems that evolutionary experience of traditional medicine for advanced drug discoveries has a concept beyond providing new chemical constituents (17). Today, finding new formulations, especially of plant origin, is a therapeutic strategy for diseases such as cancer. Using ginseng as an herbal medicine to reduce complications of cyclophosphamide, whose possible complications have been partly proved in the liver, is very important and finding a suitable antioxidant as its replacement is necessary. This study was conducted for this purpose.

Among compounds found in plants, many studies have been conducted on the pharmacological properties of essential oils from herbs. Many physiological properties of plants are associated with their essential oils which are used as medicine or a part of medicine (18). Of the most important antioxidant compounds of ginseng, phenolic compounds and saponins can be noted that prevent oxidative reactions (19). Antioxidant property of this plant occurs through stimulating the activity of antioxidant enzymes such as glutathione and superoxide dismutase (20,21,22), which could eliminate superoxides. According to photomicrographs prepared in this study, there are hydropic swelling and liver cell necrosis in groups receiving cyclopho-sphamide due to increased reactive oxygen species and ginseng with antioxidant properties reduce the complications. Also, reduction in liver tissue damage is observed in groups receiving both ginseng

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and cyclophosphamide and in a group receiving only ginseng indicating beneficial effects of this plant extract.

Several parameters were evaluated in the study of histopathologic changes in the liver in different groups. In this study, extensive destructive changes and necrosis of lobular center and hemorrhage were created by cyclophosphamide. The occurrence of destructive changes and necrosis around the central small vein can also occur as a result of exposure to toxins (23), so the liver histopathology findings in this study shows direct and obvious toxic effects of cyclophosphamide. Reviews show that in physiological conditions, there is a balance between the formation of oxidizing species and their removal by antioxidant compounds. Oxidative stress occurs when the balance is disrupted by excessive production of oxygen free radicals or weak antioxidant defense system (24). The ability of cyclophosphamide has been reported in the production of free radicals, lipid peroxidation and oxidative stress in big laboratory rats (25). Studies show that cyclophosphamide is an alkylating agent and applies its apoptosis effect by forming a connection between two DNA strands, breaking the DNA and inhibiting protein synthesis. This alkylating drug forms reactive molecules which alkylate nucleophilic group on DNA especially 7-N guanylate and create lateral links between bases and abnormal coupling of arms and break the DNA molecule and reduce meiosis divisions (26).

According to the results of this study, administration of cyclophosphamide cause liver damage. Some results of this study are consistent with the findings of some other research. Senthilkumar et al. showed that cyclophosphamide causes severe tissue damages such as necrosis, hemorrhage and hepatic fibrosis in rats (13). On the other hand, in patients who used cyclophosphamide, impaired hepatic function and toxicity have been reported (27).

Liver tissue is the first place of drug microsomal activity. Cyclophosphamide hepatic activity generates toxic metabolites and therefore destroys liver cells and ducts (13). KhyatNori in his research stated that toxic metabolites production by liver microsomal enzymes activity may cause damage to liver tissues (28), which is consistent with the results of this study. So, it is recommended that ginseng be used for people who need to use cyclophosphamide.

Conclusion

In this study, although cyclophosphamide as an alkylating agent had damaging effects on the liver tissues of female rats by disrupting the function of cells and producing free radicals, ginseng with its antioxidant properties and effective substances reduces complications due to using cyclophosphamide. Therefore, to reduce complications of using cyclophosphamide, ginseng root extract can be used.

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