

The Effect of Ginseng Extract on Reducing the Toxicity of Cyclophosphamide in the Adult Rat Kidney

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Abstract: Since kidney disease is one of the biggest problems of the world population, so more study and attention to kidney mechanism may have important effect on human health. Cyclophosphamide is known as an anti-cancer drug used in chemotherapy which has complications in kidney. No previous study has been done on ginseng extract as an anti-oxidant to identify its effect. The aim of this study was to evaluate the influence of ginseng as an anti-oxidant on nephrotoxicity of cyclophosphamide complications; we examined 60 adult female rats which were from Vistar race. We classified our samples into 6 groups namely; control group which did not receive any drug, experimental group 1 which received daily 5 mg/kg/bw of cyclophosphamide, experimental group 2 which received 2 g/kg/bw of hydro alcoholic ginseng extraction. In addition, experimental group 3,4 and 5 which have received 0.5, 1 and 2 g/kg/bw of hydro alcoholic ginseng extract together with 5mg/kg/bw cyclophosphamide. Gavaj method was used for ginseng, and cyclophosphamide used by interapertioneal technique for 21 days in the experimental groups. ANOVA (one-way analysis of variance) has been used to analyze the results by SPSS (version 15) and $p < 0.05$ was expected as significant results. We have identified a significant decrease in weight of kidney among all samples. Also, the density of uric-acid showed a significant increase in experimental group one. In addition, density of blood nitrogen showed a significant increase in experimental groups 1, 3, 4 and 5 and the density of keratin was significantly higher in experimental groups 1, 3 and 5 in comparison of the control group. Diagonal of malpighian corpuscle and glomerular showed a significant decrease in groups 1, 3, 4 and 5, and diagonal of bowman's capsule showed a significant increase in experimental group 2. According to our results, it has been identified that cyclophosphamide has adverse effects on kidney tissue but ginseng extraction reduced the complications of cyclophosphamide. Although, we recommend using higher doses of ginseng.

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Introduction:

Chemical substances are increasing daily, but some effects including self-immunity resistance phenomena created by using medicaments willfully and continuously for a long time or sometimes stopping their use led to side effects may be more dangerous the disease. Although nowadays the synthetic medicaments have been developed due to their successful effects they have side effects and are expensive in a way that the researchers have studied their side effects on different parts of the body. Considering nowadays renal diseases are of the most important problems in the communities' precise attention to their function and structure may play an important role in people's personal health (Molitoris et al, 2002).

Cyclophosphamide is an anti-neoplastic (anti-carcinoma) medicament converted to an active alkylation metabolite with effects similar to moostin

(anti-carcinoma). Cyclophosphamide is well absorbed by the digestive system and distributed vastly in the body tissues and fluids. The medicament passes blood-brain barrier and converted to active metabolites in the liver and finally excreted by the kidneys (Shahraz et al, 2004) (Figure 1).

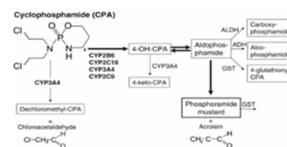


Figure 1: Metabolites derived from cyclophosphamide

Cyclophosphamide killing cell quality is often due to binding between two NDA molecular fibers and broken DNA and RNA as well as protein synthesis control This creates lateral bonds between the arms and bases' unnatural coupling and breaks DNA.

If cyclophosphamide is used with doxorubicin and danorubisin which have toxic effects on the heart, it may increase the effects (Shahraz et al, 2004). Also if cyclophosphamide is used with anti-coagulation medicaments, it may increase eatable anti-coagulation activity and decrease their activity, too (Shahraz et al, 2004). Cyclophosphamide usage may lead to side effects such as hemorrhage and coagulation in kidney, tubular necrosis, blood uric acid increases potassium increases and sodium decreases in blood (Shahraz et al, 2004), nephron glomeruli tubes' inflammation, cavity and cleavage, decreased lysosome enzyme activities and protein increase in kidney (Abraham et al, 2007; Aiko et al, 2008).

Ginseng is a plant whose name derived from the Greek word (Panax), meaning 'Curing all diseases' (Abraham et al, 2007). Ginseng is an aromatic pharmaceutical plant belonging to Panax genus (Lisa et al, 2001) and is common in traditional medicine in Asia (Merrily et al, 2000). The Ginseng's modern usage began in the 19th century when an article published by a priest. It has been used to improve memory, decrease stress, cure disability, feebleness and other disorders due to fatigue and aging and prolong life since several centuries ago (Abraham et al, 2007). It has been confirmed as a preventive factor and it increases mental and physical abilities and plays an important role in recovering from weak state, disability or when concentration lost during convalescence (Merrily et al, 2000). The virtues are due to the root. The root contains triterpene saponin, necessary oils, alkaloids, amino glycosides, poly-acetylene, poly-saccharide, peptidoglycan, nitrogen compounds, fat acids, carbohydrates and phenol compounds (Paranagama et al, 2001). The most important active element of the root is the ginsenosides (saponins) which have triterpene quality and the pharmacological activities of the plant are due to it (Katzowng et al, 1999). The ginseng's antioxidant virtues are due to salicylate and anile acids in it so it may be used to prevent wearing. Besides, ginseng has some compounds similar to hormones decreasing blood cholesterol (Scott et al, 2001).

This test is conducted to examine the effect of ginseng hydro-alcoholic extract on kidney tissue to identify possible anti-oxidant compounds and their effect on cyclophosphamide and the medicaments used in chemotherapy.

Materials and Methods:

In this study, we have used 60 female adult mice of the Vistar race prepared from Shiraz Medical Science University Animal Room and kept in Islamic Azad University of Jahrom Animal Room in order to adapt with new environment for two weeks. Their age was between 2 to 3 months and their mean weight was about 200 g. They ate compressed food prevented from

animal food producing factories. Their room temperature was about 20 to 22 ° C; they had 12 hours light as well as 12 hours darkness every day. The relative humidity of air was between 40% to 60%.

The ginseng root which was confirmed by the specialists of agriculture department was used in the study. The cyclophosphamide which was used for this study was 50 g cyclophosphamide tablet. About cyclophosphamide, intra peritoneal injections were used with the help of reset chamber and the ginseng was used as oral eaten.

Animal Groups:

The mice to be tested were divided randomly into six separated groups as follows:

1- Control group including ten mice having food and water daily for 21 days of the test without receiving any medicament.

2- Experimental group 1 including 10 mice injected 5 mg/kg weight cyclophosphamide into the peritoneum daily.

3 - Experimental group 2 including 10 mice eaten 200 g/kg weight ginseng hydro-alcoholic extract daily.

4 - Experimental group 3 including 10 mice injected 5 mg/kg weight cyclophosphamide into the peritoneum and eaten 50 g/kg weight ginseng hydro-alcoholic extract daily.

5 - Experimental group 4 including 10 mice injected 5 mg/kg weight cyclophosphamide into the peritoneum and eaten 100 g/kg weight ginseng hydro-alcoholic extract daily.

6- Experimental group 5 including 10 mice injected 5 mg/kg weight cyclophosphamide into the peritoneum and eaten 200 g/kg weight ginseng hydro-alcoholic extract daily.

In each group the kidney tissue was examined in relation to changes such as blood congestion, kidney lobules congestion and channel diameter, the rate of blood urea nitrogen factors' changes, creatinine and uric acid.

Ginseng Extraction:

Roots have prepared in powder form and percolation technique was used for extraction. Dry powder added to the cylindrical section of the percolator and then two-third of it was filled up with 80% alcohol and the rest was filled up with water. The machine tap was closed immediately after the first exit of solution and then it has opened after 24 hours so that the extraction could exit. Then, we dried the extraction in a germfree environment with 30 to 40 °C temperatures.

Intraperitoneal Injection:

For injection, amount of doses was needed depend on the animal weight (mg per kg) was taken by syringe. One leg of the animal was taken and its head was a bit lower, so the organs were become in lower level. Then, interapertioneal injection was used. The

injections were at 9 in the morning, during of 21 days for the experimental groups, but the control group received no injection.

Taking Out The Kidneys:

The stomach was cut and the kidneys were taken by scalpel and forceps from the lipid tissues around the kidney and Fallopian tube was isolated and then all kidneys were measured and washed with physiology syringe. Then each one of them was put into a bottle of 10% containing formaldehyde. All of them were sent to histology section, Shiraz MRI hospital to provide lamella. The slides provided from different sections made possible to study the kidney tissues. In each slide the kidney weight, glomeruli diameter bowman capsule, far bent tube diameter and near bent tube diameter were measured by the Dino capture software.

Analysis:

The findings were analyzed by SPSS and the Duncan test was taken into consideration in the significant level of 1% to 5%.

Results:

We have evaluated and analyzed the animal weights together with both left and right kidney weights. Also we analyzed the cortex diagonal, medulla diagonal, diagonal of maplighian corpuscle, glomerular, kidney capsule, proximal tubular, distal tubular and loop. Also, we have investigated the amount of blood uric acid, blood urea nitrogen and keratin factors.

Statistical analysis showed that there is no significant increase or decrease in both left and right kidney weights. Also, no significant increase or decrease has been identified in diameter of renal cortical (cortex), the diameter of the central part (medulla), and capsule, proximal, distal tubular and loop. Statistical analysis showed that there is a significant decrease in the animal body weight in experimental groups 1, 3 and 4, together with a significant increase in experimental group 2 in comparison to control group. The results of malpighian corpuscle diagonal showed that there is a significant decrease in experimental groups 1 and 3 in comparison to control and experimental groups 2. Also, statistical analysis showed that there is a significant decrease in the size of glomerular in experimental group 1.

We have analyzed the amount of blood uric acid which statistical analysis showed that there is a significant increase in experimental group 1 in comparison to groups 2, 3, 4 and 5. It has been identified that there is a significant increase of blood urea nitrogen in experimental groups 1, 3, 4 and 5 in comparison to control group and experimental group 2 as well as a significant decrease in experimental groups 2, 3, 4 and 5 in comparison to group 1. Statistical analysis showed that there is a significant increase in

the amount of keratin in experimental groups 1, 3 and 5 in comparison to control group.

The kidney tissue was quite naturally in the control group which did not receive any cyclophosphamide or ginseng (Figure 2).

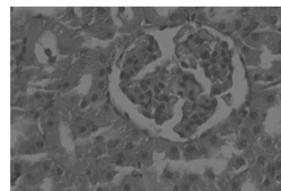


Figure 2: Photomicrograph, Cortex (control group), (hematoxylin eosin staining x400)

The changes which have happened in kidney tissue of experimental group 1 which has received cyclophosphamide shows that cyclophosphamide may severe destruction of tissue. Figure 3(A); shows the destruction of renal tract and glomerular clearly.

Figure 3(B); shows that ginseng is not destructing to the kidney tissue as it is clear that glomerular and cortex are quite natural. Changes of kidney tissue in groups 3 which have received cyclophosphamide together with the minimum dose of ginseng shows tissue destruction especially in glomerular together with vascular and destruction of renal tracts (Figure 3(C)). In addition, we were witness that tissue destruction significantly decreased in group 4 which have received cyclophosphamide and average dose of ginseng. Glomerular destruction decreased and there is just a few hyperemia (Figure 3(D)). Finally, we identified low destruction of kidney tissue in experimental group 5 which have received cyclophosphamide together with high dose of ginseng in comparison to other groups (Figure 3(E)).

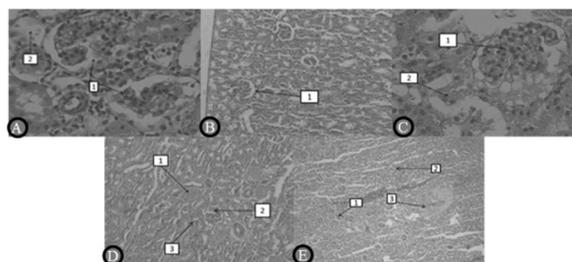


Figure 3:

- (A) Changes related to experimental group 1; 1) Glomeruli shrink 2) Destruction of renal tubules (hematoxylin eosin staining x400)
 (B) Tissue changes related to experimental group 2; 1) Normal renal glomeruli and ducts (hematoxylin eosin staining x100)
 (C) Tissue changes related to experimental group 3; 1) Glomerular damage 2) Hyperemia (hematoxylin eosin staining x400)
 (D) Tissue changes related to experimental group 4; 1) Hyperemia 2) Glomerular damage 3) Tract damage (hematoxylin eosin staining x100)
 (E) Tissue changes related to experimental group 5; 1) Glomerular 2) Renal tract 3) Hyperemia (hematoxylin eosin staining x40)

Discussion:

Same as all biological fields concerning human the main instrument to examine the remedial mechanisms and guidelines is an animal. Nowadays traditional medicine and herbal medicaments are very common, but ginseng has been interested less when it has been used to cure many diseases since more than 4,000 years ago (Attel et al, 2002). Currently, sciences are going towards herbal medicaments so using them beside the chemical ones may be useful to treat some diseases (Akhyam et al, 2010). Cyclophosphamide has been mentioned as an essential and important element in many pharmaceutical compounds. It is used as anti-carcinoma drug and weakens the body immunity system specially control the refusal of transplanted organs of the body (Faghihi 2004; Bokser et al, 1990). The cyclophosphamide is used to treat patients and tested conducted in the lab. Animals followed by unwanted effects such as genital organ side effects. Notwithstanding useful results of the drug against carcinoma it has had harmful effects on the liver and kidneys (Deleve 2003).

Our study findings indicate the body weight decreased significantly in experimental groups 1, 3 and 4 compared to the control one and experimental group 2 had significant increase compared to the control one. So cyclophosphamide with harmful effects had decreased the weight in the groups 1, 3 and 4, but ginseng compensated it somehow by its antioxidant qualities. By virtue of studies it became clear that cyclophosphamide had toxic effects on the genital organs, liver and kidneys and created proteins (Shahraz 2004; Diasio et al, 1996). Previous studies showed low dose and long use of cyclophosphamide in mice may decrease their weight and genital organs (Subush et al, 2007). So decreased weight in the groups receiving cyclophosphamide is in consistent with previous studies and indicates negative effects of cyclophosphamide. In a Sampa study in 2001 no significant weight change or decrease was reported in the mice because of cyclophosphamide that may be due to the test period and subject and sexual of samples. In 1991 Kim showed that cyclophosphamide induces toxicity in the immunity system of the animals to the substance. By virtue of the reports the toxicity decreases because of ginseng (Gayton et al, 1996). In the present study the groups which have received cyclophosphamide and high dose of ginseng, showed increases of weight which indicates improvement. In the present study the glomeruli diameter decreased significantly in the groups which have received cyclophosphamide indicating negative effects of it. Also the malpighian corpuscle diameter decreased in the groups which have received cyclophosphamide. Cyclophosphamide is an essentially pharmaceutical with high toxicity and side effects such as amenorrhea

depending on the amount of used dose. Toxicity may create cystic hemorrhagic results in urinary mucus and carcinoma in bladder (Takada et al, 2001). Also intravenous injection of cyclophosphamide leads to complete insufficiency of kidney or death in the group with high keratin with prognosis (Vera et al, 1999). Each disorder in urine flow and its latest results are known as uropathy blockage. Complete urine blockage is important in relation to effects on the kidneys and related treatments and prevention, but the exact mechanisms of kidney changes are not clear and it is interested by researchers (Klahr et al, 1999; Klahr et al, 1994). The researchers found that increased pressure in the blocked part increases the reactive pressure and damages kidney parenchyma and leads to different disorders such as oxidative equilibrium, energy metabolism, homodynamic and executive function of kidneys (Vaughan et al, 2004). Hypersomnia may be with glomeruli vein damage, high blood pressure and perfusion decrease in kidney and may create local fibrosis (Kang et al, 2002; Nagahama et al, 2004; Sanchez et al, 2005). Also, it may due to cyclophosphamide disorder recovering reactions in tissues. Chemical and physiological disorders are created by excessive oxidative stress (Kulmatycki et al, 2002).

In present study glomeruli and malpighian corpuscle diameters decreased in the groups which have received cyclophosphamide indicates disorder in the kidneys, but the effects were less in groups which did not receive ginseng perhaps due to extract's antioxidant properties. By virtue of the examinations concerning antioxidant extract effects of ginseng on other organs it was found that ginseng has protective and remedial effects against the disorders due to 2, 3, 7, 8 tetra chloride di benzoic-p-dioxin. Previous findings indicate that ginseng may decrease the toxic effect of cyclophosphamide in the genital organ (Kumar et al, 1993). Ginseng extract may destroy the tumors sensible and insensible to natural killer cells by spleen of cell reproduction and natural killer cell activation. A composition known as G511 of ginseng extract is increasing immunity reactions of direct effect on immunity system and increases T helper lymphocyte rates (Bastianotto et al, 2000; Helms et al, 2004).

Keratin concentration in experimental groups 1, 3, 4 and 5 showed significant increases compared to control group and experimental group 2. Also the experimental groups 2, 3, 4 and 5 showed significant decrease compared to experimental group 1 indicating cyclophosphamide increases nitrogen concentration in blood urea which means negative effects due to kidney dysfunction and such factor decrease in the groups receiving ginseng indicates that ginseng was able to decrease keratin concentration and compensate

somehow the cyclophosphamide effect by substances including ginsenoidea with remedial effect and salicylate and anilic acids with antioxidant effect . Ginseng decreases other parameters such as renal markers and serum lipid which are significant in statistical viewpoint. It was stated in previous studies that keratin has significant increases in sick and decreases in under treatment groups, respectively (Xie et al, 2004). In 2007 some researchers reported that ginseng was ineffective in uremia (Yoon et al, 2007). Also by virtue of a study concerning rabbits the keratin changes were examined and indicated that there were less increase in keratin concentration in early study and considerable and significant increase after 8th week (Yoon et al., 2007). Previously, it was stated that the keratin rate depends on the weight and volume of muscular mass and observed more in men than the women. Also keratin increased in groups which has received cyclophosphamide indicates the latter's harmful effects in tissues' destruction.

In relation to uric acid concentration in present study the experimental group 1 had significant increase compared to the control one and had a significant decrease in the experimental groups 2, 3, 4 and 5 compared to the experimental group 1. Considering cyclophosphamide damages and glomeruli changes and malpighian corpuscle diameter it is possible to relate the uric acid increase to kidney dysfunction to excrete the acid and its concentration increased in blood while uric acid concentration decreased in the recipient ginseng groups which indicates positive effects of ginseng extract mentioned before. In present study the tissue changes indicate the damage to the kidneys by cyclophosphamide and ginseng extract decreased somehow such harmful effects.

Conclusion:

In present study cyclophosphamide is known as alkylation factor destroying tissue and crating free radicals in renal cells. Also, increases blood urea nitrogen, uric acid and keratin secretion. As an appropriate antioxidant ginseng extract decreased somehow the cyclophosphamide side effects, but it is recommended to study more about ginseng use.

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