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PROTECTIVE EFFECT OF HERACLEUM PERSICUM ALCOHOLIC EXTRACT ON CYCLOPHOSPHAMIDE-INDUCED GAMETOGENIC DAMAGE IN RATS

F. Dalouchi[1*], S. M. Banan Khojasteh[1], G. Dehghan[1], D. Mohhamadnezhad[2], S. Rostampour[1], N. Farsad[3]

[1]Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
[2] Faculty of medicine, Medical Sciences, University of Tabriz, Tabriz, Iran
[3] Department of Botany, Faculty of Science, Tabriz University, Tabriz, Iran

*Fdalouchi@gmail.com

Abstract

Cyclophosphamide (CP) is used as an anticancer drug in chemotherapy. This drug has some side effects including anorexia, nausea, malfunction of sexual glands, azoospermia and oligospermia. The aim of this study was to evaluate the protective role of *Heracleum persicum* against gametogenesis damages in rats receiving cyclophosphamide. Thirty male Wistar rats (185-195 g) classified in five groups including control group (receiving 0.2 ml/day distilled water), second group (receiving 0.5 gr/kg/day intra-peritoneal cyclophosphamide), and three other groups (receiving same dose of cyclophosphamide intra-peritoneal with different doses of oral alcoholic extract of *Heracleum persicum*). The experiment lasted 21 days. At the end of experiment, the testes of rats were examined for histological and morphometric changes. The results showed that cyclophosphamide significantly reduced sperm counts, SI and TDI indices compared with the control group ($P < 0.01$). However, groups who received the extract of *Heracleum persicum* in association with cyclophosphamide represented significantly improved parameters **(**$P < 0.01$**).**

Key words: Cyclophosphamide, *Heracleum persicum*, gametogenesis, rats.

Introduction

Mammalian spermatogenesis is an ordered and well-defined process occurring in seminiferous tubules of the testis. It is characterized by mitotical spermatogonia divisions producing spermatocytes that proceed through meiosis to form a population of haploid cells (spermatids) over a period of several weeks. Spermiogenesis is the haploid phase of spermatogenesis transforming spermatids into spermatozoa (1). There are some disorders in spermatogenesis located in the preservations or the types of methods used for treatment (2). Impairment in spermatogenesis as well as disorders in the sperm production and function are among the most common causes of male infertility. Trauma or anatomical defects in the reproductive system or the use of certain drugs to treat disease lead to impaired sperm production and ultimately to impotency in men. (2). Infertility is one of the major health problems in human life. Furthermore, approximately 30% of infertilities are due to a male factor. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Other factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have also harmful effects on spermatogenesis and sperm normal production. Several studies have reported that antioxidants and vitamins A, B, C, and E contained in diets can protect the DNA of sperms from free radicals and thus increase blood testis barrier stability(3).

By definition, cancer is a disease in which cells are abnormally divided and multiplied and they form tumors. Tumors are known as hard lumps or ulcerated lesions that appear on surface of skin and muscles. Both benign and malignant anatomically masses in forms of ulcers or abscesses that arise between the muscle and bone and hard obstructions that arise in the internal organs (4). Serious disorders, including benign and malignant tumors, stem from internal injuries, emotional trauma, invasion of pathogenic factors-such as heat, cold, dampness, dryness, or the accumulation of toxins- that are often due to improper digestion and poor elimination of metabolic wastes. Besides, general therapeutic measures, such as chemotherapy, are used to remove obstruction and produce new fresh blood (4). Chemotherapy prevents the division and proliferation of cancer cells and causes damage to this process. Chemotherapy drugs are transported through blood to cancerous cells and all the organs.

These drugs have many destructive side effects on healthy cells. Chemotherapy and radiation therapy are associated with changes in the male genital tract (5). Due to their intercalating with DNA causing irreversible lesion, the drugs used in chemotherapy contain highly toxic neoplastic agents that interfere with the cellular division (6). The high frequency of cellular divisions that occurs in the cells of the seminiferous epithelium makes the testis a highly vulnerable organ to the chemotherapeutical drugs(6). Cis-diamminedichloroplatinum (II) or cisplatin (CIS) is a highly effective anti-neoplastic DNA alkylating agent used to treat many types of solid tumors, including testicular, ovarian, breast, lung, bladder, head and neck. Furthermore, severe testicular damage characterized by germ cell apoptosis, Leydig cell dysfunction and testicular steroidogenic disorder are effectively cured by this measure (6,7) The use of medicinal plants has recently been proposed to reduce the unfavorable effects of various therapeutic procedures. In addition, many researches have been done in this regard. A wide range of different biological activities, including antioxidant, anti-bacterial, anti-coagulation, vasodilation, anti-inflammatory and anti-cancer medicinal plants have been identified, some of which reduce the adverse effects of chemotherapy. Identification of the effects of medicinal plants on tissue and organs is a developing research domain (3,8).

For example, Citrus fruits, such as oranges, contain components that have antioxidant potential and this factor has a useful effect on spermatogenesis and sperm parameters (3). It is known that Croton Cajucara prevents testicular damage in rats undergoing chemotherapy (6). Ginkgo Biloba (GB) has been used in traditional Chinese medicine for about 5000 years. Due to its antioxidant features, this herbal drug has been widely used in many situations. Studies show that GB could be involved in reducing toxicity in rats experiencing chemotherapy drugs such as CIS (7). *Heracleum persicum* is a plant of Umbelliferae family and it is scientifically named as *Heracleum persicum*. It is a perennial plant of the parsley dark that grows in high mountain areas, especially in Azerbaijan, Fars, Ramsar, Hamedan, Tehran, Damavand and other regions. This plant has thick roots and complete cut leaves. Persian's *Heracleum persicum* is "Angdan". However, some people call it "Anjudan". *Heracleum persicum* has two types. One type is an aromatic one that is named as *Heracleum persicum* and Golapar.

The second kind is a funky plant that is called anghoze, angozhd, fat fingers, infected Haltit and black Haltit. The chemical compounds of *Heracleum persicum* contain oil and this oil is volatized in the form of powder so that foods can be aromatized. The plant of *Heracleum persicum* has many medicinal features; it can fortify the stomach and other digestive organs as well as repulsive gastrointestinal bloating. Furthermore, it can increase the gastric secretion and excretion of toxins in the body and milk of women. Finally, it is a strong microbicide, disinfectant, and diuretic material. Small amounts of these plants may have effective medicinal effects on the reproductive system (8,9).

Having regarded the traditional medicine, the present study aimed to investigate the effect of *Heracleum persicum* in preventing adverse effects of chemotherapy on spermatogenesis in rats.

Materials and Methods

Animals

Thirty adult male Wistar rats (weighing 185 to 195 g) were provided from Tabriz Medical University. All the rats were healthy and not infected with virus or bacteria and the keeping place of them had natural light and darkness. They were sufficiently provided with food and water except in experimentation time.

Chemicals

Flowers of *Heracleum persicum* growing wild in Iran, were collected in May 2013 from Babol (North of Iran). The aerial parts of the plant were gently washed in tap water and completely dried under room temperature (25±2˚C) for 2 weeks protected from direct heat or sunlight. Preparation of *Heracleum persicum* methanol extract (HPME).The powdered plant material (1000 g) was extracted with methanol (MtOH) (80%), at room temperature (RT) overnight. The extraction was repeated three-times and the solvent was evaporated in vacuum, and dried extracts were stored at 4˚C until use (10).

Treatment of animals

The 30 rats were divided into 5 groups randomly as:

The first group (control) received daily 0.2ml of distilled water as drug solvent and injected intra-peritoneally.

The second group) the first experimental group(received daily 5mg/kg/day intra-peritoneal injections of cyclophosphamide and 0.5gr/kg/day ethanol extract of *Heracleum persicum* orally.

The third group) the second experimental group(received daily 5mg/kg/day intra-peritoneal injections of cyclophosphamide and 1gr/kg/day ethanol extract of *Heracleum persicum* orally.

The fourth group) the third experimental group(received daily 5mg/kg/day intra-peritoneal injections of cyclophosphamide and 2gr/kg/day ethanol extract of *Heracleum persicum* orally.

The fifth group) the fourth experimental groups(received 5mg/kg/day intra-peritoneal injections of cyclophosphamide.

Twenty one days after the treatment, rats were anesthetized by chloroform and their abdomens were opened and the testes were placed in 10% formalin for histological supplying. Having prepared the tissue samples, the microtome sections were measured and the diameter of each section was determined as 5 microns. Then, stained tissue slides were prepared with hematoxylin and Eosin. The prepared slides were examined via using an optical microscope with different magnifications.

Statistical analysis

The parameters considered in this study were spermatid, sperm construction index (SI) and tubular differentiation index (TDI) respectively. Data analysis was performed using software SPSS.19. Furthermore, Duncan's method was used in order to compare the means. Analysis of variance date showed that there were significant differences among the studied groups (P <0/01).

Results

Table.1 shows the spermatid count, SI and TDI parameters. Analysis of variances indicates that there are significant differences among different groups (P <0/01).

Discussion

Cyclophosphamide is an anticancer drug which is popular under brand names of “Cytoxan, neosar, Procytax”. It is part of the antineoblastic alkaline drug groups that, along with alkelilasion RNA and DNA, inhibit the enzymes to convert amino acids to proteins. As such, they can also cause DNA strands bind (11,12). The side effects of this drug can include a decrease in gonadal function, isospermia, oligospermia, as well as eventual reduced spermatogenesis (13,14). Cyclophosphamide also affects the DNA molecule, inhibits proliferation of

germ cells and reduces the number of sperm cells, spermatid, primary and secondary spermatocytes. Therefore, it reduces testis weights. Reduce in the spermatid, SI and TDI are signs indicators of disorder in spermatogenesis incurred by using cyclophosphamide (15,16).

Heracleum persicum contains copper. Furthermore, there are some chemical compositions within this substance including Hexyl butyrate, Octyl acetate, hexyl methylbutanoate, and hexyl isobutyrate. Natuarally speaking, *Heracleum persicum* is a warm substance and it can fortify the stomach and other digestive organs as well as repulsive gastrointestinal bloating. Furthermore, it can increase the gastric secretion and excretion of toxins in the body and milk of women. Finally, it is a strong microbicide, disinfectant, and diuretic material (17). *Heracleum persicum* is a plant that contains chemicals including protein, fiber, non-revive and to revive sugar, three types of coumarin, a family of organic camphor compounds, phytosterol and 1 and 2 dimethoxyl 4-methyl benzene. These compounds possess anti-mitotic properties, estrogenic, anti-cancer, anti-diabetic, anti-obesity and anti-mutant characteristics (8).

There are six furanocoumarins and flavonoids within the fruits contain *Heracleum persicum* (18). Some isolated furanocoumarin have antioxidant functions (19) and they reduce free radicals (20). Therefore, it is suggested that furanocoumarin has a protective effect against lipid peroxidation (21). Antioxidants are involved in the neutralization process of free radicals and reactive oxygen species (ROS) (20, 22).

Reactive oxygen species (ROS) are molecules that are highly disruptive to cellular function and have free radicals. ROS are produced in the testis as a usual physiological event; the modifications of the synthesis of ROS motivate the oxidation and DNA damage to cells (23,21). Excessive ROS productions that exceed the critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress. Therefore, ROS production can be used as a marker of oxidative stress in seminal fluid and is correlated with male infertility. It is suggested that abnormal sperm morphology combined with elevated ROS production may serve as a useful indicator of potential damage to sperm DNA (24). The sperm plasma membrane has polyunsaturated fatty acids, which are sensitive to peroxidative damage.

The lipid peroxidation demolishes the formation of the lipid in the membranes of spermatozoa.

Hence, it reduces the sperm's motility and results in defects to membrane integrity. Obviously, the structure of the lipid matrix in the membranes of spermatozoa is destroyed by peroxidation of sperm lipids and it is connected with the fast loss of intracellular ATP. This in turn can result in axonemal damage, decrease in sperm viability and increase in mid-piece morphological defects. Furthermore, it can even completely inhibit spermatogenesis in severe cases. Antioxidants are compounds that contribute to the control of ROS and lipid peroxidation (23).

Sperm motility in experimental groups that received cyclophosphamide along with *Heracleum persicum* increased which could be attributed to an increase in antioxidant capacity and protective effect of *Heracleum persicum*.

Table 1 shows the average number of spermatids in groups 1, 2, 3 and 4 compared to the control group. Experimental group 4 received only cyclophosphamide-induced activation of cyclophosphamide metabolites and the effect of this drug on DNA resulted in reduced meiotic division. The number of spermatid cells derived from primary and secondary spermatocytes dividing cells decreased. This fact has been affirmed by some books on pharmacology (25,26). The observed reductions in experimental groups 1, 2, 3 and 4 are related to the metabolites resulting from cyclophosphamide. Thus, it can be argued that, in addition to cyclophosphamide, experimental groups 1, 2 and 3 received *Heracleum persicum*. Therefore, increase in the dose of *Heracleum persicum* decreased the number of cells spermatids. Finally, the numbers of cells spermatids in experimental group 3, which received the maximum dose of *Heracleum persicum*, are significantly lower than the control group. However, they are close to this group.

This relative increase of spermatid's cells can be understood due to the above-mentioned active components in *Heracleum persicum*. As mentioned before, these components are antioxidants and can eliminate free radicals and metabolites body so they can decrease the damaging effects of chemical drugs such as cyclophosphamide.

The extract of *Heracleum persicum* acts as the receiver of free radicals and thus protects cells from oxidative stress induced by cyclophosphamide use (27).

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Table 1. Results of spermatid count, SI and TDI parameters. Different letters shows significant difference among groups (P< 0.01).

Group	Spermatid count	SI	TDI
1	152.61±0.56a	88.67±0.4a	87.02±0.49a
2	74.8±0.37d	49.24±0.52d	64.62±0.34c
3	89.77±0.65c	52.58±1.1c	67.06±0.26b
4	96.24±0.38b	55.86±0.36b	67.58±0.35b
5	71.54±0.81e	17.94±0.46e	17.79±0.42d

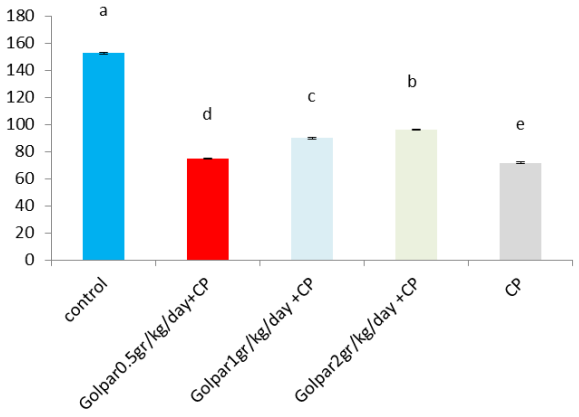

Fig. 1. Spermatid count results, Different letters show significant difference among groups (P< 0.01).

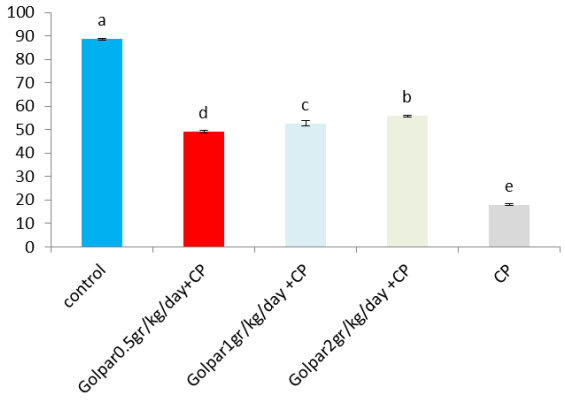

Fig. 2 SI results, Different letters show significant difference among groups (P< 0.01).

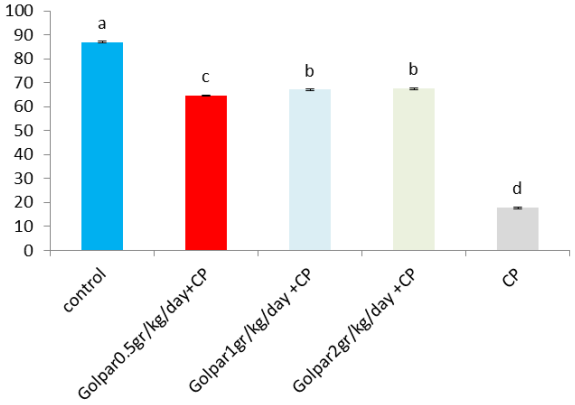

Fig. 3. TDI results, different letters show significant difference among groups (P< 0.01).