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Effect of Hydroalcoholic Extract of Cinnamon on the Pituitary-Gonadal Axis in Adult Male Rats under Chemotherapy by Cyclophosphamide

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Abstract

***Background*:** Cyclophosphamide chemotherapy is used as anti cancer drug and weakening immune system for adult widely. As there are inadequate information, so we are to examine possible effect of hydro alcoholic extract of cinnamon on the pituitary - gonadal axis in adult male rats chemotherapy drug by cyclophosphamide.

***Materials and Methods*:** Thirty five male Wistar rats divided into 5 groups of 7 including control, observer, and experiments 1, 2, 3, 4 and 5 respectively. The control group received no treatment while observer group were injected 2.0 mm of distilled water (solvent) intraperitoneally. The experimental mice 1, 2 and 3 were intraperitoneally injected 5 mg/kg cyclophosphamide drug with 50, 100 and 200 mg/kg fed by gavage either cinnamon extract.

***Results*:** The weight of the rats in the experimental groups 1, 2, 3 and 4 were significantly lower than the control group (p=0.01). Right and left testicular weight in experimental groups 2 and 3 than in group 4 was significantly increased in groups 1 and 4 showed a significant decrease compared to the control group (p=0.01). Hormones LH, FSH and testosterone increased significantly in groups 2 and 3 than in group 4 showed the (p=0.05). 5 experimental groups showed significant increase in FSH compared with controls (p=0.05).

***Conclusion*:** This study showed that cyclophosphamide has a destructive effect on testis and secretion of sex hormones in male rats and hence effect of cinnamon improves it to some extent.

Introduction

Today the fight against cancer, especially in fast-growing tumors has been associated with relatively good successes. Cancer treatment due to fundamental limitations faced with many problems. In order to achieve compounds with anti-cancer properties need to a screening test. Meanwhile, research in pursuit of anti-cancer compounds from plant sources gets more prevalent every day [1]. Additional incentive for drug discovery research has created in the field of plant origin by anticancer drugs of plant origin [2]. Cinnamon is a common name and scientific name is Cinnamomum Zeylanicum Nees, a plant which has aromatic and pleasant chemical composition like volatile oils, cinnamomum aldehydes, terpenes, cinnamomum alcohol, and limonene. The therapeutic effects of this herb have anti-spasmodic, carminative, anti-diarrhea, anti-bacterial, anti-parasite and cooling. Also it is useful for the treatment of anorexia, intestinal colic, diarrhea, children colds, flu and especially for colic and flatulence associated with digestive disorders and nausea [3]. Cinnamon is an evergreen tree of the laurel family (Lauraceae) all its part smells fragrant cinnamon. This tree grows in Ceylon and India. The dried bark of the cinnamon tree is used for therapeutic purposes. Skin plant contains 0.5 to 5.2% of in portion of huge aldehydes, acids Synamyk Eugenol and transforms. As well as other compounds such as hydroxy phenyl prvpany aldehyde huge, huge artvmtvksy aldehydes, alcohol and acetate synamyl and terpene compounds found in essential oils. Aldehyde low dose and high dose stimulates the central nervous system and has a soothing effect. Other works include a huge Aldehyde: anti-bacterial and anti-fungal, accelerating the release of catecholamines from the central part of the adrenal gland, papaverine-like effect of poor peripheral blood circulation, lowers blood pressure, bradycardia and increased blood sugar [4].

Cyclophosphamide (with the brand name of endoxana). Cyclophosphamide (CP) is an anticancer drug used in chemotherapy drug is an alkylation and DNA strand break that bond between RNA and protein synthesis and is inhibited [5]. Also this drug for treatment of nephritic syndrome and multiple myelomas have been used [6]. Effects of cyclophosphamide have a lot of nausea and vomiting, hair loss, mouth sores and skin lesions, blood problems, cystitis, cancer, creating amenorrhea, reduced fertility in both sexes (for example azoospermia and oligospermia) [7].

According to the chemotherapy drugs such as cyclophosphamide decreased sexual activity and reproductive and other effects such as weight loss,

reproductive organs. The aim of this study was to investigate the effect of cinnamon extract in reducing the toxicity of cyclophosphamide chemotherapy in hormone levels in male rats' pituitary-gonadal axis.

Materials and Methods

This study is experimentally and completely random. All the ethics of working with laboratory animals has been observed in this study. Thirty five adult female Wistar rats were purchased, weighing 200g±5 and aged 120 to 100 days from the Razi Serum and Vaccine Research Center, Shiraz. Mice were kept in cages, steel mesh doors and a standard diet (pellet) was used. They were also providing special water bottles. Cage three times a week it was disinfected with 70% alcohol. Ten milliliters of distilled water was added and the wave speed to be settled daily doses of insulin through the needle into the syringe and injected intraperitoneally into the mice. Experimental procedures involving animals were approved by the Medical Science University of Jahrom Animal Care and Use Committee.

Extraction method: extract Cinnamon herb native of Iran was prepared, cinnamon bark or stem of the Faculty of Agriculture, Islamic Azad University, prepared using 24 g of the powder was milled and dried at 20ºC and were dissolved in 96% ethanol medication. The mixture for 24 hours at room temperature (25ºC) was maintained. The combination of using electric mixer for 4 minutes thoroughly mixed and the initial weight recorded on a paper. The weight difference between dry powder remaining on filter paper and the initial value of cinnamon powder must be dissolved. Extraction method with a high content of alcohol is about 20 ml. To remove the alcohol extract from pollution-free medium for 48 h to evaporate excess alcohol and the minimum amount (5 ml) is reached. The extract volume with 9% saline (normal saline) was brought to 150 ml. The mice were randomly divided into 5 groups of 7 are; Control: was maintained at normal without any medication, Sham: 0.2 ml of distilled water as the solvent was injected intraperitoneally per day, Experimental 1: daily 5 mg/kg cyclophosphamide was injected intraperitoneally and 50mg / kg cinnamon extract was orally receive, Experiment 2: daily 5 mg/kg cyclophosphamide was injected intraperitoneally and orally receive cinnamon extract was 100 mg/kg, Experimental 3: daily 5 mg/kg cyclophosphamide was injected intraperitoneally and 200 mg/kg Cinnamon extract was orally received, Experimental 4: daily 5 mg/kg cyclophosphamide was injected intraperitoneally received. Experimental 5: 200 mg/kg daily Cinnamon extract orally received.

After 21 day period, all groups of mice were anesthetized by ether and weighined and blood from the heart were taken by the syringe 5 ml. After separation of serum hormone concentrations of LH, FSH and testosterone were measured by Elisa in the laboratory of Medical Sciences also left and right testicles of mice were removed and weighed, and the left. ANOVA for comparison between treatments (ANOVA) followed by Duncan's test and *t*-test was used for multiple comparisons between groups: $p<0.05$ was considered as significant level. Data analysis and statistical tests were performed using SPSS-17.

Results

Weight of animals in the experimental groups 1, 2, 3 and 4 decreased significantly compared with the control group which are given in table 1 ($p=0.01$). Right and left testicular weight in groups 1 and 4 compared with the control group showed a significant decrease and in groups 2 and 3 than in group 4 was significantly increased ($p=0.01$) (Table 1). LH levels in groups 2 and 3 than in group 4 significantly increased. And four experimental groups decreased significantly compared to the control group. FSH levels have increased in groups 2 and 3 compared to the control group. Also significantly increased in group 4 is also significant increase in group 5 more than in the control group. Sex hormone testosterone has significantly increased in groups 2 and 3 compared to group 4 are. In groups 1 and 4 also showed a significant decrease compared to the control group ($p=0.05$) (Table 2).

Table 1. The mean body weight, right and left testicular weight in the treatment groups compared with the control group

Group	Body weight(kg)	Right testis weight(g)	Left testis weight(g)
Control	215.28±6.41	1.84±0.08	1.82±0.077
Sham	213.18±3.80	1.79±0.1	1.78±0.09
Experimental 1	190.36±5.66*	1.54±0.07*	1.59±0.09*
Experimental 2	193.32±4.65*	1.77±0.15**	1.71±0.16**
Experimental 3	198.01±10.88*	1.78±0.01**	1.77±0.02**
Experimental 4	185.28±3.77*	1.51±0.14*	1.53±0.16*
Experimental 5	216±6.48	1.86±0.07	1.85±0.07

Based on the mean values and standard deviation (SD Mean) is presented
Significant difference in average ($p=0.01$)
* indicate significant difference with the control group
** indicates a significant difference with group 4

Table 2. The mean hormones LH, FSH and testosterone treated groups compared with the control group

Group	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (nmol/L)
Control	8.49±0.401	4.99±0.271	7.03±0.033
Sham	7.98±0.557	4.90±0.218	6.87±0.645
Experimental 1	7.80±0.398	3.97±0.244	4.13±0.399*
Experimental 2	0.297±10.01**	4.8±0.519**	5.37±0.262**
Experimental 3	0.487±10.83**	4.83±0.370**	6.01±0.567**
Experimental 4	8.20±0.630	3.37±0.241*	3.26±0.332*
Experimental 5	11.1±0.09*	5.79±0.398	7.1±0.534

Based on the mean values and standard deviation (SD Mean) is presented. Significant difference in average (p=0.05).), * indicate significant difference with the control group, ** indicates a significant difference with group 4

Discussion

Cyclophosphamide is an alkylating agent that has cytotoxic and mutagens cytoastaticy properties. The toxicity characteristic for cells to DNA alkylation the N7 position of guanine and plasticity cross linking between DNA-DNA and DNA-Protein and DNA is single stranded, which ultimately causes performance degradation of nucleic acids and DNA synthesis [8].

Cyclophosphamide is inactive in vitro and in vivo, cytochrome P-450 enzymes in different organs of 4-hydroxy cyclophosphamide is converted to its to tumer aldophosphamide in balance mood. Aldophosphamide spontaneously decomposes to its active metabolite is converted phosphore amid mustard and acroline [9, 10]. Antineoplastic effects of cyclophosphamide is phosphore amid mustard while acroline through interaction [11] to generate oxygen free radicals [12] and the appearance of toxic effects, including cell death, apoptosis, forming Tumor necrosis [13]. Biological compounds with antioxidant properties capable of various cells and tissues, including disorders of the reproductive system against reactive oxygen species and free radicals to protect [14, 15]. Thus, administration of antioxidants during chemotherapy to reduce cyclophosphamide-induced oxidative stress and tissue detoxification is necessary. Testis weight and testicular atrophy due to factors that can interfere with spermatogenesis, including sperm or azoospermia cyclophosphamide is the drug of oligospermia [5].

The reason your testicles atrophy and weight loss in groups 1 and 4.In experimental groups 1, 2 and 3, the weight loss of less than 4 groups appear to be beneficial effects of cinnamon cited. FSH levels decreased in groups 1 and 4 have no sense of FSH to induce spermatogenesis are required. Studies show that reducing the size and weight of the testes and FSH decreased sperm production is reduced and these factors increase with increase in FSH.

Testicular function by FSH and androgens also ideal for luteinizing hormone-induced stimulation of the testicles that can be supported. Removal or neutralization of pituitary gonadotropins in circulation, increases damage to spermatogenic cells [16, 17]. In addition, some findings indicate that low levels of serum testosterone induced apoptosis in testicular germ cells, especially cells are haploid [18]. Since LH and FSH levels in groups 2 and 3 also increased then it can also be applied to the issue by saying that the testicles and cause weight gain.

In groups 1 and 2 than in the control group had significantly lower testosterone levels also decreased testicular weight, which is indicative of induction apoptosis. Also, a lower dose of cinnamon in group 1 than in group 2 and 3 animals injected. And the reduction in group 1 can be due to the effect of cinnamon to improve the dose-dependent induction. Extract at higher doses the effect can be seen more. Significantly lower in group 4 compared with the control group, testicular weight and hormones LH and testosterone has been observed as previous research has shown that cyclophosphamide mechanism of action is not yet fully elliptic in the disturbances; but several studies have shown that cyclophosphamide is able to disrupt the tissue reaction reviver. The biochemical and physiological disorders are the result of excessive oxidative stress is produced [19]. So it is logical that the weight of the testes and sex hormones LH and testosterone significantly lower in group 4 compared to the control group show. Significant changes in testicular weight in groups 5 and hormones LH and FSH hormone testosterone has been observed and only significant increase was observed in past studies that cinnamon has been stated that positive effects on the reproductive system of male sex cells is significantly increased. It also changes the structure and weights of testis extract injection does not cause and can be used to produce medicinal plant can be used to increase fertility in males [20].

Increase in LH and FSH hormones administered in doses of cinnamon that these changes can be observed in the experimental groups 2 and 3. Sato et al. conducted similar experiments in the present study showed. The increase in LH and FSH hormones that caused by compounds in the stem bark of cinnamon, which was driven by the effect on the hypothalamic-pituitary-testicle, causing an increase in the hormone. The centerpiece of its influence and control various positive and negative feedback is such that it is nitric oxide. The active molecule increases the secretion of hormones, gonadotropins and LH, can enhance sperm motility and induce erection [21].

Secretion of neurotransmitter nor epinephrine, which is influenced by various factors such as the activation and synthesis of nitric oxide and nitric oxide, which in turn stimulates the secretion of LHRH and LH is increasing

[22]. It contains ingredients such as cinnamon huge aldehydes, tannins and [4] and a huge aldehyde directly or indirectly increases nitric oxide synthesis of LH and FSH hormone levels will increase, so the cinnamon is well seen in the present study, however, a significant increase significant compared with group 4. Groups 2 and 3 also significantly increased compared to controls are Cinnamon is the better part of the same sign as described. Previous investigations shown that cyclophosphamide cause biochemical and tissue changes of testis [23]. Thence this subject can another reason for testosterone decrease in 4 group, because testosterone secreted from testis laydig cells [24].

In a previous review stated that the Delta cadine increased secretion of LH and LH cinnamon in turn is a direct effect of increasing testosterone synthesis [25]. The lower dose of cinnamon is a significant reduction in the amount of testosterone secreted Huron can be observed is the destructive effects of cyclophosphamide [26]. Another study also stated that cinnamon extract alone increased hormones, LH, FSH, and testosterone is [20] which agrees with the study of the changes in LH and testosterone hormone is meaningless.

According to the study, we can say cyclophosphamide cause harmful effects on the secretion of sex hormones in male rats is Cinnamon and improve the effects due to the effects of higher doses of cinnamon can be expressed Cinnamon effect is dose-dependent manner, and these effects may be less generalized to humans Of course, in this case how much use and extracts and cyclophosphamide is also very important during the test period and so care must be taken in generalizing these results to humans, and experiments need to be done.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Sharififar F, Moshefi MH, Dehghan GR and Alishahi F. Various essential oils and plant extracts for cytotoxicity cinnamon and ginger spice Baby brine shrimp toxicity test. J Med Herb 2010; 8(2): 110- 119.
2. Cellini L, Di Campli E, Masulli M, et al. Inhibition of Helicobacter pylori by garlic extract (Allium Sativum). FEMS Immunol Med Microbiol 1996; 13(4): 273-277.
3. Sheykh N, Safari MR, Araghchiyan M and Zeraati F. Effect of cinnamon, sumac and pepper on Glykh reaction to albumin in vitro. J Med Herb 2003; 2(7): 13.
4. Moattar F, Shams-Ardakani MR. Civil works of Hakim Seyed Esmaeil Jorjani]. Proceedings of the congress of honouring Hakim Seyed Esmaeil Jarjani. Tehran: Iran ACAD Med Sci 2002; 17-9.
5. Johari H, Sharifi E, Ansari N, et al. Hydro alcoholic ginger extract on body weight, testicular weight and spermatogenesis in male rats receiving chemotherapy drug cyclophosphamide. J Med Sci 2009; 17(5): 365- 374.
6. DeLeve L D. Cellular target of cyclophosphamide toxicity in the murine liver: Role of glutathione and site of metabolic activation. Hepat 2003; 24(4): 830-837.
7. Sampa G, Monomohon M, Ujjal B, et al. Effect of human chorionic gonadotrophin co administration on ovarian steroidogenic and folliculogenic activities in cyclophosphamide treated albino rats. Reprod Toxicol 2001; 15(2): 221-225.
8. Hemminki K, Kallama S. Reactions of nitrogen mustards with DNA. IARC Sci Publ 1986; (78): 55-70.
9. Hengstler JG, Fuchs J, Tanner B, et al. Analysis of DNA singlestrand breaks in human venous blood: A technique which does not require isolation of white blood cells. Environ Mol Mutagen 1997; 29(1): 58-62.
10. Qiu J, Hales BF, Robaire B. Damage to rat spermatozoal DNA after chronic cyclophosphamide exposure. Biol Reprod 1995; 53(6): 1465-73.
11. Arumugam N, Sivakumar V, Thanislass J and Devaraj H. Effects of acrolein on rat liver antioxidant defense system. Indian J Exp Biol 1997; 35(12): 1373-4.
12. Mythili Y, Sudharsan PT, Selvakumar E and Varalakshmi P. Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced oxidative cardiac injury. Chem Biol Interact 2004; 151(1): 13-9.
13. Kern JC, Kehrer JP. Acrolein-induced cell death: A caspase-influenced decision between apoptosis and oncosis/necrosis. Chem Biol Interact 2002; 139(1): 79-95.
14. Das UB, Mallick M, Debnath JM and Ghosh D. Protective effect of ascorbic acid on cyclophosphamide induced testicular gametogenic and androgenic disorders in male rats. Asian J Androl 2002; 4(3): 201-7.
15. Ghosh D, Das UB, Misro M. Protective role of alpha-tocopherol-succinate (provitamin-E) in cyclophosphamide induced testicular gametogenic andsteroidogenic disorders: A correlative approach to oxidative stress. Free Radic Res 2002; 36(11): 1209-18.
16. Troiano L, Fustini M, Lovato E, et al. Apoptosis and spermatogenesis: Evidence from an in vivo model of testosterone withdrawal in the adult rat. Biochem Biophys Res Commun 1994; 202(3): 1315-1321.
17. Johnson Y, Speakman H. Study of energy hemostasis and spermatogenesis in the mamales. Endoc 2010; 175: 369-382.
18. Dunkel L, Perheentupa J, Apter D. Kinetics of the steroidogenic response to single versus repeated doses of human chorionic gonadotropin in boys in prepuberty and early puberty. Pediatr Res 1985; 19(1): 1-4.
19. Hosseini A, Zare S, Ghaderi-pakdel F and Ahmadi A. Evaluation of antioxidant effect of plant extracts and vitamin E Jynsyng on fertility in male laboratory rats raised to seek long-term treatment with cyclophosphamide. J Repro Infer 2010; 11(4): 227- 237.
20. Modaressi M, Mesripor M, Rajae RA. Effect of hydroalcoholic extract of cinnamon on the number of spermatocyte cells and spermatozoa in mice. Armaghan-e-Danesh 2010; 14(1): 83-90.
21. Sato Y, Tsukamoto T. Effects of nitric oxide stimulation on the brain. Dru Tod (Barc) 2000; 36(2-3): 83-92 .

22. Parvizi N, Ellendorff F. Further evidence on dual effects of norepinephrine on LH secretion. Neuroendoc 1982; 35(1): 48-5.
23. Reichman BS, Green KB. Breast cancer in young women: Effect of chemotherapy on ovarian function, fertility, and birth defects. J Natl Cancer Inst Monogr 1994; (16): 125-9.
24. Radcliffe J. Cryptorchidism: A prospective study of 7500 consecutive male births, 1984-8. Hospital Cryptorchidism Study Group. Arch Dis Child 1992; 67: 892-899.
25. Braun L, Cohen M. Herbs and supplement an evidence-based Guide. 1st ed. Sydney: Else Press; 2007: 271.
26. Namasivayam E, Tzeon-Jye C, Woan-Fang T and Sin-Tak C. Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxic 2006; 222(1-2): 60- 70.

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