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Original Research Paper

Effect of Soybean on Levels of LH, FSH and Testosterone Hormones and Testis in Adult Male Mice

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ABSTRACT

Soybean is a member of Fabaceae and a species of legume native to east Asia. It is an annual plant that has been used in China for 5000 years as a food and a component of drugs. Soy contains significant amount of all the essential amino acids for humans and so is a good source of protein. Soy has an important role in improvement and treatment of some cancers such as colon, prostate and breast. A total of male mice with 30-35 g weight were bought from Razi Institute in Iran. At first the samples were kept under adaptation condition for two weeks and then randomly grouped into 4 experimental sections as: 1-Control group were feed with soy-free basic diet. 2-Nutrition containing 20% soy diet. 3-Nutrition containing 30% soy diet. 4-Nutrition containing 50% soy diet. At the end of 8 weeks of treatment blood was collected and serum stored for hormonal analysis. Results were computed with SPSS software and compared with control group. In 20% group the level of testosterone has meaningful decrease in comparison with control group, but in 50% group the level of testosterone has meaningful increase. Levels of LH in 30% and 50% groups have a meaningful increase but no significant difference was observed in FSH and weight of testicles. The number of sperms in all of the treatments have a meaningful decrease. The result of this research indicated that the 20, 30 and 50 percent soy diet had negative effect on male reproductive system in mice.

INTRODUCTION

Soya or Chinese bean is an annual legume plant belonging to Family Fabaceae. It is planted mostly for producing oil and common flour. Squeezed seeds are used in cooking. Soybean has been eaten for centuries in Asia and especially in China, where it is used with rice as main course. Soybean include minerals, protein, vitamins and different kinds of carbohydrates. 60% of total dry weight is fat and protein that means 40-67% protein and 17-20% fat. Since soy protein is full of vitamins B1 and B2, pantothenic acid, colin, niacin and all necessary amino acids for the body, it is considered an invaluable source of nutrients for human beings (Evans 1995). Another important thing which can be found in soy and is the main reason that today the world is after it, is the presence of those compounds called phytoestrogenes. Phytoestrogens are vegetable derivations of estrogen (with diphenolic structure) which can be found in fruits and vegetables. Phytoestrogens contain all the physiological and physico-chemical characteristics of estrogens (Crouse 1999, Lichtenseion 2002). The main isoflavon of soy is Genistein. According to recent researches, hormone replacement therapy (HRT) in menopausal women causes the increase in the risk of breast and uterus cancers. Using pseudo-estrogene compounds, especially phytoestrogenes, which can be abundantly found in soya products such as soy milk and soy protein, not only satisfies body needs of estrogen in menopausal women but also has no side effects and can decrease the risk of cancer in them (Malloy 2001). The reported effects of soy on cancer, osteoporosis and heart disease have resulted in an increasing interest in soy use among people. Moreover in 1999, Food and Drug Ministry in USA pointed out the effect of soy in the decrease of cholesterol. Furthermore, isoflavonoids in soy are of high interest. The genistein in soy makes vessels wider. Through increasing the release of nitric oxide improves the flexibility of vessels and decrease blood pressure. All these happenings in the end have positive effects on heart and cause the decrease in systolic and diastolic pressure in heart (Borradail 2002, Foy 2001). In some studies done on the harmonic effects of soy use on men, positive and negative effects of it compete with each other. When positive effects of estrogenic compounds in soy on the cure of prostate cancer were found, some other researches conducted reported that environmental estrogen (use of those compounds containing phytoestrogen) decrease sperm quality in men. It has been made clear through experiments that having a diet rich in estrogenic compounds like seeds containing phytoestrogens such as soybean, can affect hypothalamic-pituitary-gonadal axis in men exactly in the same way as diethylstilbestrol compounds do. In some other researches in rodents, exposure to phytoestrogens in uterus or during early post-natal life through diet or subcutaneous injection results in multiple reproductive abnormalities during adult life, including decreased testicular weight or size decrease spermatogenesis, and therefore, the decreased of total

sperm count in puberty (Atanassova 2000, Mitchell 2001, West 2005) and lower testosterone (Wisnieswki 2003). Researches done by Perry on macao monkeys rejected any connection between using different doses of phytoestrogen with those characteristics contributing to sperm quality (Perry et al. 2007). The purpose of this research is investigating the role of phytoestrogenes in soy seeds on the function of reproductive organ in male mice.

MATERIALS AND METHODS

For the purpose of the present study, 32 male mice, type Balb/C weighing about 3±5 g were used. The sample was studied for a month in laboratory at the temperature of 25±3°C, natural light period, sterilized cages with enough food and water available for the animals. The experimental groups were divided as: (1) Control group: In order to achieve basic concentration of FSH, LH and testosterone hormones and also in order to observe and investigate the section of testis natural tissue and counting the sperms, the control group was kept in identical situation to the experimental groups but did not receive a diet containing soy within the period of experiment. (2) Experimental group 1: In this group 8 mice received a diet, 20% of which was soy protein. (3) Experimental group 2: In this group 8 mice received a diet, 30% of which was soy protein. (4) Experimental group 3: In this group 8 mice received a diet with 50% soy protein.

Since the base of the experiment was feeding the mice with soy protein as food, the amount of food eaten by the animals during a day was calculated for a week through calculating the difference in weight of the given food. The food used for feeding the mice was ground into powder using electric mixer and then was mixed with 20, 30 and 50% soy based on the dose required for each experimental group. The food was then made again in the form of paste and was made available to be used by animals. The experiment lasted 9 weeks for each animal. After 9 weeks the blood was tested to investigate the level of FSH, LH and testosterone hormones. Testis and epididymis tubules of each experimental and control group were fixed in 10% formalin carefully and made ready for preparing tissue section.

Statistical analysis: The data were analysed using SPSS statistic software and the mean of the data gathered by the result of the experiments was compared using one-way variance analysis and Duncan test with the safety level being higher than 95%.

RESULTS

The results of the study are depicted in Figs. 1-4.

Investigation on the sections of tissues: After preparing tissue sections, they were studied using optic microscope. No change of tissue was found in the sections studied.

Investigations on the weight of testes: After calculating the average weight of mice's testis among the experimental and the control group using Duncan test with safety level being over 95%, it was found that there was no significant difference between them.

Investigations on the number of primary spermatocytes: The investigation and counting the primary spermatocyte taken from tissue sections and comparing the number of spermatocyte in experimental and control groups revealed that the average number of spermatocytes in the experimental groups receiving 20% , 30% and 50% soy in their diet, is significantly less than that of control group.

Investigations on the spermatozoid number: The comparison of the number of spermatozoid (million/cm²) in the experimental and control group revealed that the average number of sperms in the experimental group is significantly less than that of control group.

The results obtained by hormone tests: The investigation of FSH levels in serum of the experimental groups and the control group based on (mL U/mL) proved that there is no significant difference between them. Comparison of the level of LH hormone in serum (mL U/mL) of the mice in the experimental and control groups reveal that the average level of LH hormone in the experimental groups 2 and 3 that received a diet containing 30% and 50% soya respectively, is significantly higher than the level of LH hormone in the control group. The level of LH hormone in experimental group (1) with 20% soy in their daily diet was not significantly different from that of control group.

Investigation of the level of testosterone hormone: By investigating the level of testosterone hormone (mL U/mL) in serum of the mice in the experimental and control groups, it was found that the average level of this hormone in the first experimental group with 20% soy in the diet is significantly less than that of the control group, while the level of testosterone hormone in experimental group 3 that received 50% soy in their daily diet showed a significant increase compared to the control group.

DISCUSSION

In spite of the fact that soybean has positive effects on the cure of diseases like osteoporosis, vessel blockage, heart disease, blood-pressure, as well as having positive effects on the control of blood lipid level, cure and control of the development of prostate, breast and clone cancers, there are some worries about negative effects of using soy in men diet due to its bad effects on reproductive system. There are controversial studies, which pit the results of the previous stud-

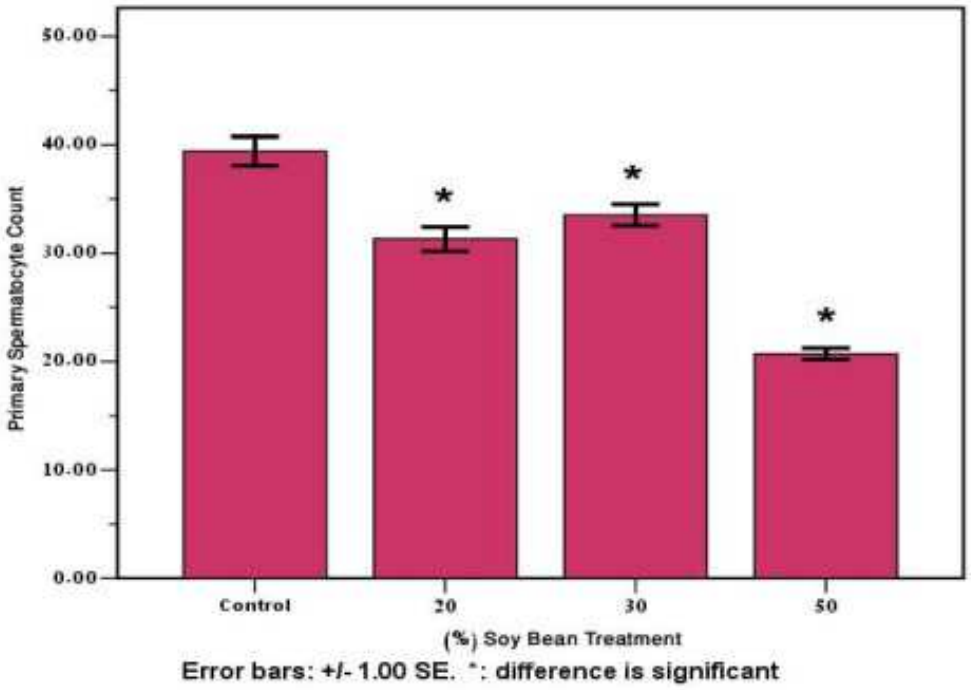

Fig. 1: The results of the primary spermatocyte number in experimental and control groups.

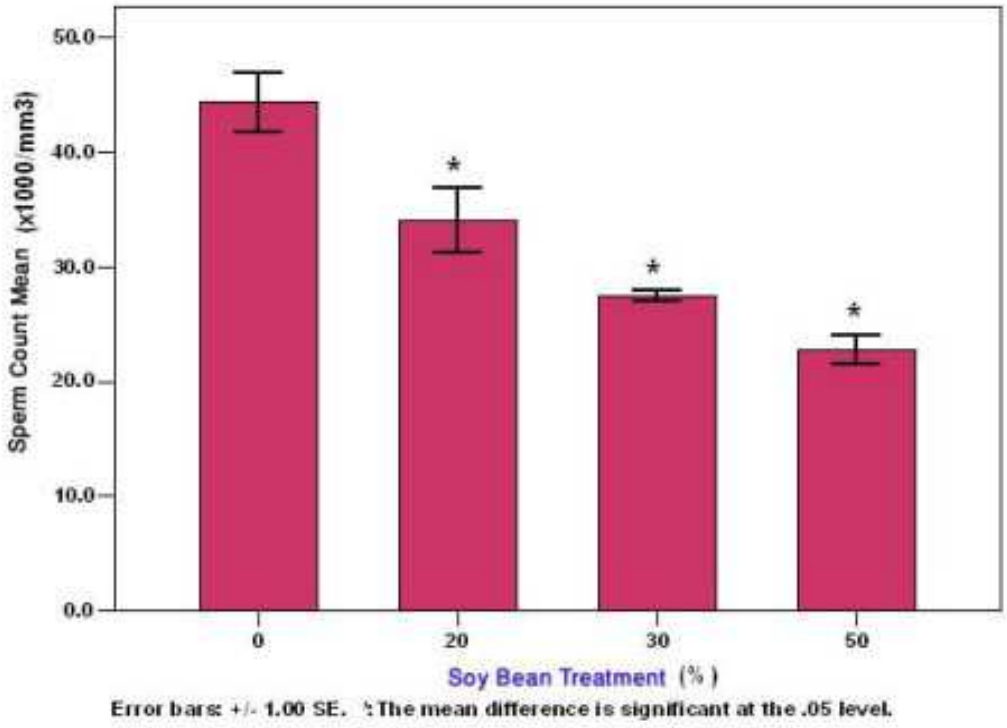

Fig. 2 : The results of the spermatozoid nomber in experimental and control groups

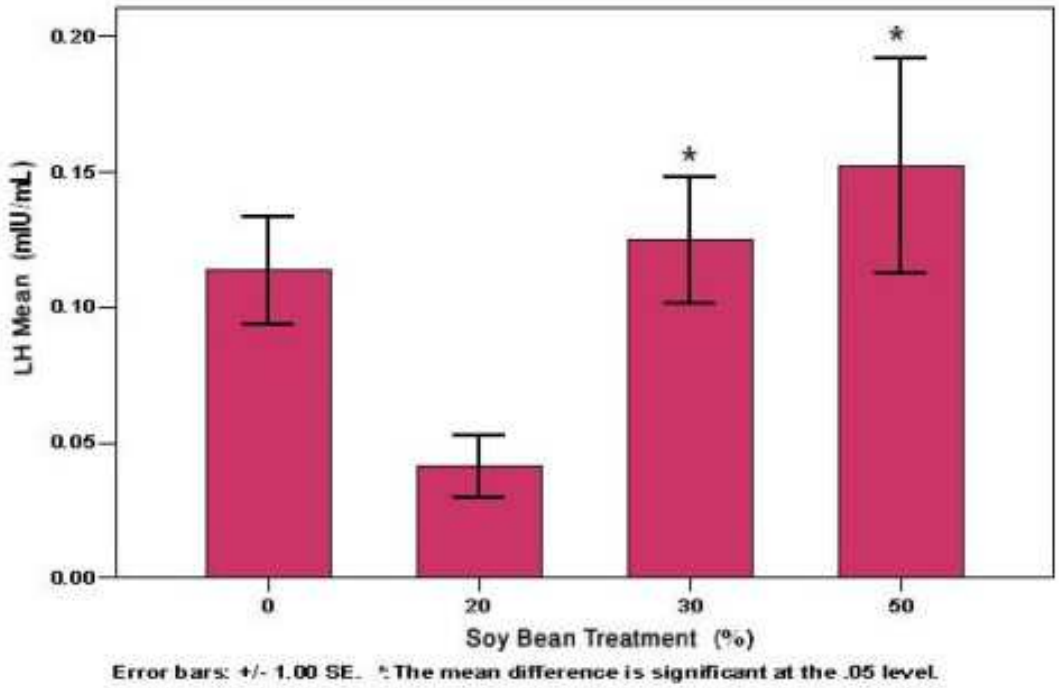

Fig. 3: The results of lh in experimental and control groups,

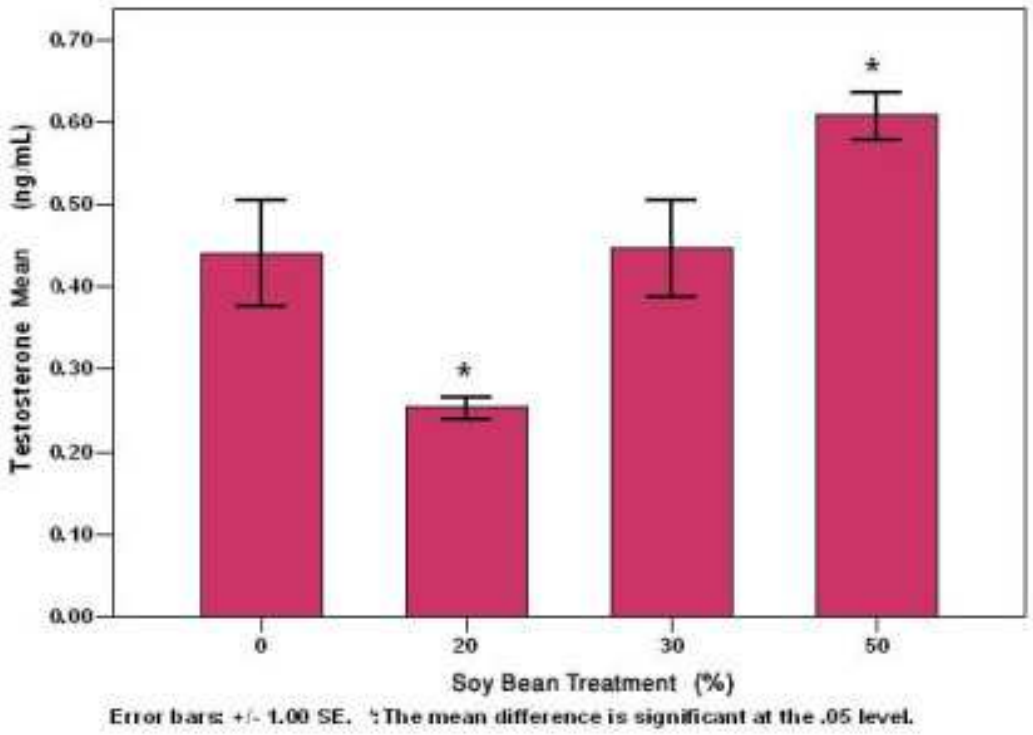

Fig. 4: The results of testosteron in experimental and control groups.

ies under question and add to the doubts and uncertainties. Most of the genistein is conjugated when it is being absorbed by intestine. This happens before genistein enters blood circulation system. The rest of this happens in liver when genistein is passing blood circulation system (Andlauer 2000). If this material is entered in body through the common way by eating, the results are much more reliable (Atanassova 2000). The comparison of tissue sections prepared from testicle tissue of the mice in this study revealed that the small changes in the tissue of the experimental groups 1, 2 and 3 are not significantly different from the control group expect when it is related to the dose of primary spermatocytes level. In this case it was significantly less than that of the control group. One of the other major changes seen in this study is a significant decrease in sperm count in experimental groups 1, 2 and 3. This decrease has a reverse correlation with the used dose. In this study, the level of FSH in the two experimental groups receiving 20% and 30% soy in their diet showed a decrease, although not significant as compared to the control group. Since FSH is one of the important factors contributing to spermatogenesis process, it seems that the decrease in FSH hormone, especially in the second experimental group (receiving 30% soy) has caused the decrease in adenylate cyclase enzyme stimulation, which itself leads to the decrease in cAMP level and finally the decrease in ABP level (Androgen-Binding-Protein). Therefore, testosterone hormone , which is an important factor in spermatogenesis can not be guided into seminiferous tubules as much as before making the spermatogenesis modified. Moreover, having an ability to attach estrogen receptor α (Erα), phytoestrogens decreased reproductive ability of male users of isoflavonoid compounds in soy bean (Branham 2002, Harris 2002, Kuiper 1998, Matthews 2000, Miksicek 1994, Song 1999). This happens because phytoestrogens can have both agonist and antagonist effect by taking the used dose, the number of estrogen receptor in the tissue under study and the kind of tissue in to account. Soy protein probably has effects on hypothalamic-pituitary-gonadal axis. Phytoestrogenes in small dose indirectly occupy the estrogen receptor α on compounds, and cause their secondary effect on spermatogenesis and therefore, the membrane of testes cells, through making agonist effects on estrogen decrease the amount of it .When there is a decrease in spermatogenesis, there is a need for increasing the secretion of testosterone hormone and hypothalamus is stimulated and secrets more GnRh hormone which finally causes an increase in LH through anterior hypothesis. Testosterone hormone as well as LH hormone showed a significant decrease in experimental group 1 (received 20% soy in their diet) compared to the control group. In contrast, the amount of this hormone in experimental groups 2 and 3 (received 30% and 50% soy) showed an increase. Although this increase was not significant in group 2, the third experiment group had a significant increase in this hormone, compared to the control group. In this study it was shown that small doses of phytoestrogens in experimental group1 decrease testosterone level and high doses of it in experimental group 3 increases the testosterone level. The decrease in the level of serum testosterone can be justified in this way. So it seems reasonable that those men who suffer from metabolic syndrome or those who are overweight or due to some genetical problems, produce less sperms than normal situation should take more precaution using estrogenic compounds such as soya, since soy compounds decrease the expression of estrogen receptors on testes tissue and occupy the active site of these receptors and have a negative role on hypothalamic-pituitary-gonadal axis.

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