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Effect of soybean on male reproductive physiology in mice

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Abstract: **Aims: Soybean is a member of (Fabaceae),it is 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 & treatment of some cancers such as Colon ,Prostate & breast . Methods: In this research a total of male mice with 30-35 gram weight were bought from Razi Institute in Iran .At first samples were kept under adaptation condition for tow weeks & then randomly grouped in to 4 experimental sections.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 collected & serum was stored for hormonal analysis . Result: Result were consider with SPSS software & results compared with control group.In 20% group the level of testosterone have meaningful decrease in comparison with control group, but in 50% group the level of testosterone have meaningful increase .level of LH in 30% & 50% group have a meaningful increase but no significant differences were observed in FSH & 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 & 50 percent soy diet had negative effect on male reproductive system in mice.**

Keyword: Soybean ,Phytoestrogen , reproductive physiology , Mice

I. INTRODUCTION

Soya or Chinese beans is an annual plant belong in to Leguminose and being from Fabaceae family. It is planted mostly in order to produce oil and common flour. Squeezed seed is used in cooking. Soy bean has been eaten for centuries in Asia and specially in china, where it is used with rice as main course. Soy bean include minerals, protein ,vitamins and different kinds of carbohydrates. 60% of total dry weight is fat and protein that means 40-67/36% protein & 17-20%fat.Since soy protein is full of vitamins B1 and B2, Pantotenic acid, Colin, Niacin and all necessary amino acids for the body ,it is considered an invaluable source of nutrient for human beings (8).Another important thing which can be found in soy and is the main reason that today the world has a tendency toward it, is those compounds called phytoestrogenes. Phytoestrogens are vegetable derivations of estrogen (with diphenolic structure) that can be found in fruits, vegetables. Phytoestrogens contain all the physiological and physiochemical characteristics of estrogens (6, 15).The main isoflavon of soy is Genistein. According to resent research , hormone replacement therapy (HRT) in menopausal women causes the increase in the risk of breast and uterus .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 (17). The reported effects of soy on cases like cancer ,osteoporosis and heart disease have caused an increasing interest in soy use among people. More over in 1999 , food and drug ministry in USA pointed to the effect of soy in the decrease of cholesterol. Furthermore , isoflavonoids in soy of high interest. The genistein in soy makes vessels wider. Though increasing the release of Nitric Oxide and as a result 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 (4,9). In some studies done on the harmonic effects of soy use on men, positive a negative effects of it compete with each other. Right when positive effects of estrogenic compounds in soy on the cure of prostate cancer was 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 soy bean)can effect 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 (3,20,27) andlower testosterone(28).Researches done by Perry in 2007 ,which were conducted on macao monkeys rejected any connection between using different doses of phytoestrogen with those characteristics contributing to sperm quality (22). The purpose of this research is investigating the role of phytoestrogenes in soy seeds , on the function of reproductive organ in male mice.

II. MATERIALS AND METHOD

For the purpose of the present study , 32 male mice, type Balb/C weighing about 30±5 grams were used. The sample was studied for a month in laboratory ,with the temperature of 25±3, natural light period, sterilized cages while enough food and water was available for the animals. The

experimental groups were divided as it is explained below: 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 count 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 ,50% of which was 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 given food. The food used for feeding the mice was grounded in to powder using electronic mixer and then the food 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 plate and was made available to the used by animals. The experiment lasted 9 weeks for each animal. After 9 weeks their blood was tested to investigate the level of FSH ,LH and testosterone hormones .Testis and epididym tubules of each experimental and control group was fixed in 10% formalin carefully and was made ready for preparing tissue section.

III. STATISTICAL ANALYSIS

In this research the data was analyzed 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%.

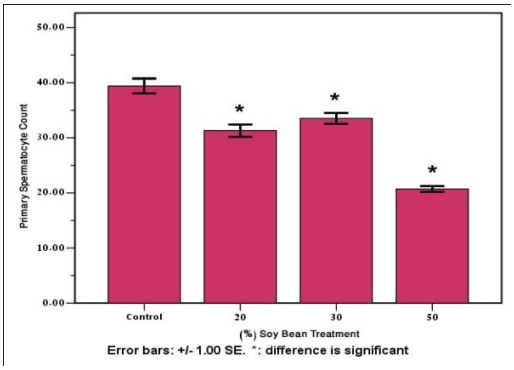
IV. RESULTS

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

- Results of investigating the weight of testies: 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 made clear that there is no significant difference between them.

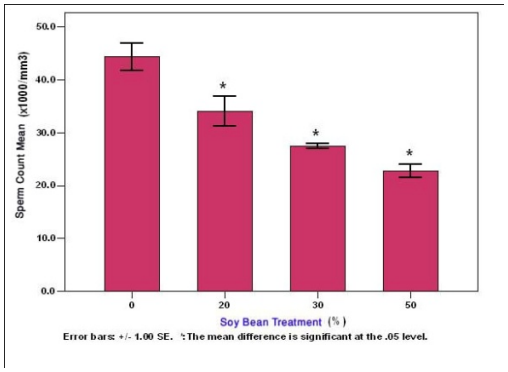
- Investigating the number of primary spermatocyte: The investigation and counting the primary spermatocyte taken from tissue sections and comparing the number of spermatocyte in experimental and control groups revealed that the average number of spermatocytes in the experimental groups (received 20%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

CHART 1 : THE RESULTS OF THE PRIMARY SPERMATOCYTE NUMBER IN EXPERIMENTAL AND CONTROL GROUPS



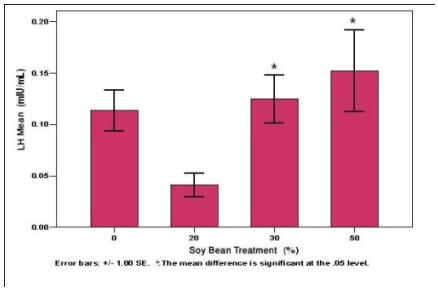
Investigating the spermatozoid number: The comparison of the number of spermatozoid (1million/cm²)in the experimental and control group revealed that the average number of sperms in the experimental group (received 20% , 30% and 50% soy in their diet) is significantly less than that of control group.

CHART 2 : THE RESULTS OF THE SPERMATOZOID NOMBER IN EXPERIMENTAL AND CONTROL GROUPS



The results obtained by hormones tests: The investigating of FSH levels in serum of the experiment groups and the control group based on(mLU/ml)proved that there is no significant difference between the average of the experimental and the control group. Comparison of the level of LH hormone in serum(mLU/ml) of the mice in the experimental and control groups reveal that the average level of LH hormone in the experimental group 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. Although 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.

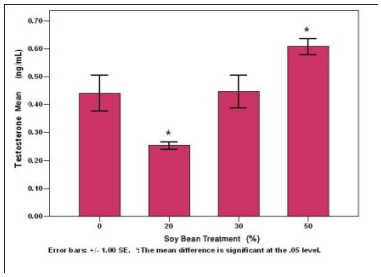
CHART3 : THE RESULTS OF LH IN EXPERIMENTAL AND CONTROL GROUPS



Investigation of the level of testosterone hormone:

By investigating the level of testosterone hormone (mLU/ml) in serum of the mice in the experimental and control group ,it was found that the average level of this hormone in the first experimental group with 20% soy in their diet is significantly less than that of 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.

CHART4 : THE RESULTS OFTESTOSTERON IN EXPERIMENTAL AND CONTROL GROUPS



Discussion :

In spite of the fact that soy bean have 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 that has caused an increasing tendency in the world in using this grain in their daily diet, there are also some worries about negative effect of using soy in men diet, due to the bad effect of it on their reproductive system .There are controversial studies in the respect each of which pits the results of the previous study under question and adds to the doubts & uncertainties. Most of 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(1 , 2). If this material is entered in body through the common way, which is by eating, the results are much more reliable(3). 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 1,2,3 is 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. Since in this study ,the level of FSH in the two experimental groups received 20% & 30% soy in their diet showed a decrease , although not significant –compared to the control group and 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 & finally the decrease in ABP level (Androgen- Binding-Protein),therefore testosterone hormone , which is an important factor in spermatogenesis can not be guided in to seminiferous tubules as much as before which makes spermatogenesis modified. Moreover having an ability to attach estrogen receptor α (Erα) , Phytoestrogens decreased reproductive ability of male users of isoflavonoids compounds in soy bean.(5 , 11 , 14 , 18, 19 , 25). This happens because Phytoestrogens can have both agonist & 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 &therefore the membrane of testes cells ,through making agonist effects on estrogen decrease the amount of it .when there is a decrease in spermatogenesis ,therefore there is a need for increasing the secretion of testosterone hormone & hypothalamus is stimulated & secrets more GnRh hormone which finally cause an increase in LH through anterior hypothesis Testosterone hormone as well as LH hormone showed a significant decrease in experimental group 1(which received 20%soy in their diet) compared to the control group. In contrast , the amount of this hormone in experimental group 2& experimental group 3 (which received 30%& 50%soy in their diet)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 research it was shown that small doses of phytoestrogens in experimental group1decrease Testosterone level & big doses of it in experimental group 3 increases 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 over weight or due to some genetical problems, produce less sperms than normal situations should take more precaution using estrogenic compounds such as soya, since soy compounds decrease the expression of estrogen receptors on testes tissue & occupy the active site of these receptors & have a negative role on hypothalamic– pituitary–gonadal axis .

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