The effects of hydro alcoholic extract of *Ceratonia siliqua* L. seeds on pituitary – testis hormones and spermatogenesis in rat

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**ABSTRACT**

In traditional medicine, Carob tree has been used as antidiarrheal medicine and to treat digestion diseases. In this research, the possible effects of Carob seeds extract on levels of LH, FSH, testosterone and dihydrotestosterone hormones and testicular tissue as well as improving fertility in male rats were investigated. Fifty adult male rats were divided into five groups of tens as follow: The control group was left on treated, the sham group received only distilled water and experimental groups which received 150,300 and 600 (mg/kg) extract respectively for 31 days. Blood samples were taken to measure LH, FSH, testosterone and dihydrotestosterone concentrations and testicular histological changes was investigated. The consumption of Carob seeds extract caused a significant increase in testosterone and DHT concentrations were as the level of LH declined in experimental groups receiving 150 and 300 (mg/kg) extract and the FSH concentrations did not show any significant changes. Furthermore, sperm density in seminiferous tubules was increased in response to medium and high dosages of the extract. The seed extract of Carob may have some effects on the synthesis of cAMP and the activities of enzymes involved in steroidogenesis in leydig cells thereby leading to an increase in serum concentration of testosterone.

**Key words:** Carob seed, LH, FSH, Testosterone, Dihydrotestosterone, Rat.

**Introduction**

About half century ago, plants were considered as the most important medicinal resources for treating diseases. Fertility disorder and its related ramifications have been identified as one of the most important problems in married life [10]. According to present statistics, about 35% of married infertility is related to men, and the most prevalent reason for men infertility, is their inability to produce sufficient number of intact and active sperms [2,1,22]. Studies indicate that various factors can affect sperm production and contribute to infertility incidence. Among these factors are taking special anti cancer medicines, antibiotics, toxic substances, pesticides, radiation, stress, air pollution and vitamin deficiency. These factors can decrease sperm counts by inducing free radicals and germinal cells oxidation in testicular tissues [1,31]. Previous studies have indicated that infertility incidence in men is on the increase. According to Eskenazib et al., (2005), for reforming semen quality and infertility rate decrease, American, men need to receive vitamin and nutritional supplements [28]. Evidence indicates that usage of medicinal plants could have positive effects on fertility increase, as well as reducing such disorders as hormonal imbalance, sexual weakness, oligospermia, varicocele and prostate inflammation.

Carob or *Ceratonia siliqua* L. is a beautiful tree belonging to Leguminasea family and is about 7 to 12 meters tall. It has compound leaves and its red, yellow or purple flowers has no petal. Its arch pod fruit is bright brown with, 10 to 30 cm long and contain 12 to 16 hard seeds. Carob is native to Mediterranean regions and is found in south of Syria, India and most of Mediterranean areas as well as in California. It grows wildly in shapoor, Fars, Iran [19,44,16].

*Ceratonia siliqua* L. fruits have been used as food sweetener and thickener [38]. According to some researches, its consumption can decrease serum cholesterol and glucose level [12,11,37,45]. In traditional medicine, carob fruits have been used as antidiarrhetic medicine and used for treating digestive diseases [21]. The presence of fiber, tannan and polyphenols in its fruits could lower potential risks of colon cancer [27]. In addition, presence of pectin and lignin in carob could function as regulators of digestive system and could act as a toxin excretor in digested foods [27]. *Ceratonia siliqua* L. pods contain antioxidants and vitamin E, and have anti asthma properties. Its tannans, function

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as antioxidant, tranquilizer, antibacterial and anti allergy and can help to improve bone fractures because the seeds are rich in phosphorus and calcium. Some studies indicate that carob, can help to treat mellitus diabetes and can decrease blood glucose level [37,42]. Since fibers of this plant are complex, containing many substances with various properties, they can improve glucose regulation and lower the risks of diabetes 2 and cardiovascular diseases [18]. Studies on chemical contents of Ceratonia siliqua seed suggests that it contains plenty of fibers, polyphenolic compounds, arachidonic acid, lignin, fats, proteins, carbohydrates, calcium, potassium, and phosphorus[34,30,25,4]. They also contain aspartic acid, glutamic acid, linolenic acid, linoleic acid, vitamin E, betasitosterol, [9] silica, iron and magnesium [44]. These compounds can have different effects on animal bodies. The Carob seeds also considered as a potential source of natural antioxidants [17] and Carob antioxidant activity is related to phenolic compounds (Owen, 2003;Ayaz, 2007)and tannans substances existence [29].

In recent decades, human resort to medicinal plants, because chemical medicines have side effects and complications. Twenty-First century is called the century of returning back to nature. Many attempts have been made to exploit medicinal plants. Considering the limited number of studies on the effects of carob seed extract on male reproductive activities, this study has made an attempt to investigate the probable effect of hydro - alcoholic extract of carob seed on the rate of FSH, LH, testosterone and dihydrotestosterone hormones and testis histological changes. The possible results could be useful in fertility and family planning.

Materials And Methods

Experimental Animals:

In this experimental study, 50 adult male wistar rats, each weighing about 210 ± 10 g and 2.5 - 3 months old were used. Animals were divided into 5 groups of 10 as follow: The control group was left on treated, the sham group received only distilled water and experimental groups which received 150,300 and 600 mg/kgbw extract respectively for 31 days. These groups were kept in separate cages at 22 ± 2°C. Water and dry food from Fars birds nutrition stock company were available to rats. The polycarbonate cages were sterilized twice a week and debris of wood was changed every two days. The experiment was started one week after the accommodation of animals in the lab environment.

Extract Preparations:

Ripen fruits of carob tree were collected around Kazeroun in the middle of spring 2010. Pods were crushed, seeds were separated, washed, dried and grinded until a fine powder was obtained. The powder was solved a 50/50 ratio of water and %96 alcohol for 72 hours during which was shaken several times, then it was filtered and hydro – alcoholic extract was obtained. It was dried in oven at 40°C and a concentrated brown extract was obtained [23,15].

Hormones measurement and tissue study:

At the end of experimental period, rats were weighed, and blood samples were taken from heart under light anesthetic. Blood samples were kept in the lab conditions for 20 minutes and were centrifuged for 15 minutes at 2000 RPM and collected and frozen at −20°C to be used for hormone measurement. The concentration of FSH, LH, testosterone and dihydrotestosterone were measured using RIA. Testis were also removed, washed, dried, weighed, fixed in 10% formalin, sectioned and got ready for microscopic examination. Data were analyzed by ANOVA and complementary Tukey test. The P values was sat at P<0/05.

Results:

Statistical studies and comparison of LH, FSH, testosterone and dihydrotestosterone hormones were made among experimental, control and sham groups, following consumption of hydro – alcoholic extract of Ceratonia siliqua seeds and the results are shown in table 1.

Table 1: The comparison of mean serum concentrations of testosterone, LH, FSH, DHT hormones among groups receiving different dosages of hydro – alcoholic extract of carob seeds (P ≤ 0/05). Values are based on X ± SEM and asterisk shows significant difference among groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LH (IU/L)</th>
<th>FSH (mIU/L)</th>
<th>Testosterone (ng/ml)</th>
<th>DHT (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.25 ± 0.03</td>
<td>17.97 ± 1.65</td>
<td>1.024 ± 0.21</td>
<td>0.211 ± 0.007</td>
</tr>
<tr>
<td>Sham</td>
<td>0.24 ± 0.02</td>
<td>17.97 ± 1.77</td>
<td>1.141 ± 0.19</td>
<td>0.212 ± 0.005</td>
</tr>
<tr>
<td>150</td>
<td></td>
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</tbody>
</table>
Hormonal measurements indicate a significant increase in serum testosterone level in groups receiving medium and maximum dosages of the seed extract in comparison to control and sham groups (P < 0.05) while it shows no significant difference in group receiving minimum dosage (Table 1). In contrast, the serum LH concentration declined significantly in groups taking medium and maximum dosages of extract and showing no meaningful difference in group receiving minimum doses (Table 1). At the same time, serum FSH levels did not confirm any significant differences among various groups (Table 1). Also dihydrotestosterone levels increased significantly only in group receiving maximum dosage (Table 1).

According to photomicrograph and comparison of seminiferous tubule cross sections among experimental, control and sham groups, there was no tissue changes in spermatogonium, primary spermatocyte and other cells in terms of size, numbers, cytoplasm characteristics, nucleus and their staining. However, the results of histology indicate that consumption of medium and maximum dosages of seed extract can result in sperm density raise [Figures 1,2,3,4].
Discussion:

Many studies are performed on the use of medicinal plants for treating sexual impotent and according to traditional medicine, it is possible to use herbs for improving fertility. Hydro – alcoholic seed extract of Ceratonia siliqua contains such compounds as phytosterols, and can cause a decrease in total cholesterol , LDL and triglycerides, through an increase in lipoproteins and hepato lipases as well as reducing HMG – COA reductase activity [20].

Results of this study are indicative of Carob ability to make changes in pituitary- testis hormonal axis and these changes can affect on male reproductive activities.Reports indicate that carob seeds are rich in phytosterol. These compounds are structural analogues of cholesterol and can prevent from many cancers (such as breast and prostate cancers) [5]. According to our results, daily consumption of 300,600 (mg/kg) hydro – alcoholic seed extract of Ceratonia siliqua could elevate serum testosterone concentration. It seems that the increase in testosterone levels by higher dosages of extract is due to its direct effect on leydig cells and on testosterone biosynthesis. These effects are probably performed through stimulating PGE2 synthesis. Furthermore, carob seeds contain gammalinolenic acids and alpha linolenic acid that can be transformed into dihomogamma linolenic acid and then into arachidonic acid which is the precursor of all prostaglandins, like PGE2.

In addition, these seeds contain own arachidonic acid [7]. It appears that arachidonic acid plays a major role in testis steroidogenesis. Studies indicate that this organic acid and its metabolite (PGE2) can increase the production of cyclic adenosine monophosphate (cAMP), thereby elevating cholesterol side chain break-down, and stimulating testosterone production [38]. Some studies indicate that arachidonic acid and it’s metabolite (PGE2) stimulate prostate growth which is more influenced by testosterone. Eicosanoids are so involved in steroidogenesis thus, insufficient use of alphalinolenic acid and abnormal production of eicosanoids can lead to infertility [14].

Another compound found in carob seeds is aspartic acid [9] which can function as a second messenger in leydig cells through increasing synthesis of cAMP and testosterone release. In addition, this fatty acid along with a second messenger production in pituitary gland give rise to synthesis and release of LH; thereby elevating the concentration of testosterone [13].

Similarly, treatment with hydro – alcoholic extract of Ceratonia siliqua seeds in high dosages induces LH decrease. It seems that this decline in hormone level is the result of negative feed back control of testosterone secretion which has a direct effect on hypothalamus and reduction of GnRH. At last, LH secretion is balanced by anterior part of pituitary. Therefore, testosterone, has a direct negative and weak feedback effect on anterior of pituitary and this feedback lowers LH secretion [36]. Considering the results of FSH concentration, indicated that there was no significant changes in experimental groups receiving extract compared to control and sham groups. Feedback mechanisms on FSH are not exerted just by testis steroids, but also by inhibit, activin and folistatin. These factors have central effects on GnRH to regulate FSH concentration, and perhaps lack of any significant change in FSH level is the result of adjusting effects of these factors.

Presence of vitamin E in carob seeds has been demonstrated [9]. One of the most important chemical properties of vitamin E is its high sensitivity to oxidants and this vitamin has been used as an antioxidant in pharmaceutical industry. In addition, the lack of vitamin E in some mammals; for example rats, brings about animal sterilization. Some researches have indicated that vitamin E is helpful in decreasing cadmium effects on testicular tissues and on spermatogenesis [41]. Moreover some studies have been performed on the usefulness of vitamin E and the other antioxidant such as glutathione and co-enzyme Q10 in treating male infertility brought about stresses, environment pollution and malnutrition. The results of these studies are indicative of its helpfulness and can preserve cell's nuclear DNA [33,24,32,3]. Similarly, therapeutics effects of vitamin E on testis has shown that as an antioxidant, it could regenerate seminiferous tubules after being damaged by ozone gas; it also reduces the destructive effects of this gas on the tissues [24]. Based on the above studies, one can infer a safe role for vitamin E in comparison to vitamin C (an effective antioxidant element) in testicular tissues against exterior toxic elements. Sperms are very sensitive to ROS injected into semen by leukocytes and ROS can cause infertility in men [22]. Furthermore, according to some Studies, usage of antioxidants and vitamin B,E and C can lower infertility through reducing damages caused by free radicals, sperms DNA regeneration and preservation, and through strengthening blood-testis barrier [2,8,26]. In present study, the experimental groups receiving high doses of extract show increase in sperm density within seminiferous tubules, which probably the result of a boost in testosterone synthesis and the presence of antioxidants and vitamin E.

Conclusion:

Based on the results of this study, the use of high dosages hydro – alcoholic extract of Ceratonia siliqua seeds probably have some influences on cAMP production and activity of enzymes involved in rat’s testicular steroidogenesis and can increase testosterone synthesis. Some LH effects on leydig cells are effected by the extract content, modifying.
the release rate of this hormone from anterior of pituitary. Similarly, consumption of extract in high doses increases sperm density in seminiferous tubules. Finally, it should be mentioned that more studies are necessary in this field.

References