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The Effect of Silymarin on Spermatogenesis Process in Rats

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ABSTRACT

Free radicals and oxidation of germ cells in the testis tissue can reduce sperm levels and cause infertility in men. Silymarinisis a compound with antioxidant effects. So, this study is aimed to evaluate the effects of Silymarinison spermatogenesis, tissue changes of testis and hypothalamic-pituitary-gonadal axis processes in rats. 40 adult male Wistar rats were prepared and divided into 5 groups including control, sham and experimental groups receiving silymarinat. Blood samples wereobtained and serum LH, FSH, GnRH and testosterone levels were measured. Testicular tissue sections were prepared and spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoids, Sertoli and Leydigcells were counted by light microscopy. One-way analysis of variance (one-way ANOVA) and Duncan test at significance level of $p \leq 0.05$ were used to analyze the data. The mean concentrations of FSH, GnRH and LH hormones in experimental groups receiving silymarin at concentration of 150 mg/kg showed a significant increase compared to the control group. Silymarin at concentrations of 100 and 150 mg/kg significantly increased testosterone hormone compared to the control group. Silymarin at concentrations of 100 and 150 mg/kg significantly increased the number of spermatids and spermatozoa cells compared to the control group. Due to the antioxidant property of silymarin, this compound increases the secretion of LH, FSH, GnRH and testosterone and the number of spermatids and spermatozoa cells in rats.

Keywords: Silymarin, Spermatogenesis Process, Rat

INTRODUCTION

Infertility and its related problems have known as one of the major issues in couples' life [1]. The most common cause of infertility in men is their inability to produce a sufficient number of healthy and active sperms [2, 3]. Several factors can affect sperm production that among them can mention to consumption of chemotherapy drugs for cancer, antibiotics, toxins, pesticides, radiations, stress, air pollutions and lack of adequate vitamins. It has been found that the noted factors can reduce sperm levels by production of free radicals and oxidation of germ cells in the testis tissue [4, 5]. Studies have shown that the use of antioxidants can be effective in treatment of infertility in men through reduction of damages caused by free radicals, strengthening the blood-testis barrier and protecting and repairing of sperm DNA [5, 6].

Silymarin is a flavonoid polyphenolic compound extracted from Silybummarianum seeds. Silymarin ingredients contain silybin or silibinin (36.3%), silydianin (5.9%), silychristin (5.7%), taxifolin (1.9%) and other minor compounds [7]. Silybin and silychristin have the highest biological activity among the other ingredients. Numerous studies have been done till now on the therapeutic and biological properties of silymarin. Among its properties can point to anti-inflammatory, antioxidant, anti-cancer and hepatoprotective properties [8, 9]. Antioxidant and protective properties of silymarin have proved in laboratory animals with acute or chronic lesions caused by various medicines or toxins [10]. Silymarin plays its own antioxidant role with scavenging free radical as well as increasing the levels of glutathione peroxidase and superoxide dismutase (SOD) [11].

Some studies have been conducted on the effects of silymarin. Kummer et al. (2003) reported estrogenic effects of silymarin in ovariectomized rats [12]. Oufi et al. (2012) also showed the effectiveness of silybinin (one of the structural isoforms of silymarin) in improvement of testicular indexes of laboratory white mice [13].

So given that some studies performed on silymarin effects on male reproductive system have not been completed yet and mechanism of action of the medicine (in various doses) on hormones of the hypothalamic-pituitary-gonadal axis has not been clearly examinedand due to the antioxidant effects of silymarin, this study is aimed to evaluate the effects of this compound on spermatogenesis process and on changes of testicular tissue and hormones of the hypothalamic-pituitary-gonadal axis in rats.

MATERIALS AND METHODS

A number of 40 healthy male Wistar rats with an average weight of 180-200 g were used in this experimental study. During the experiment, the rats were kept in the condition of 12 hours of light and 12 hours of darkness. Water and compressed food were given to themwithout any restrictions during the periodof the experiment. To adopt with the environment, the rats were kept for a week at animals breeding room in Jahrom University of Medical Sciences. The animals were then divided randomly into 5 groups of 8 each including control and sham groups and experimental groups received various doses of silymarin.

According to previous papers, the injectable concentration of silymarin in quantities of 50, 100 and 150 mg/kg body weight were determined. The lethal dose (LD50) of this compound in rats is 385 mg/kg [14].

Control group: the rats in this group did not receive any treatment during the experiment period (28 days).
Sham group: during the experiment period (28 days), the rats in this group were given distilled water by gavage based on their body weight.
Experimental 1 group: the rats in this group were given 50 mg/kg body weight of silymarin by gavage for 28 days.
Experimental 2 group: the rats in this group were given 100 mg/kg body weight of silymarin by gavage for 28 days.
Experimental 3 group: the rats in this group were given 150 mg/kg body weight of silymarin by gavage for 28 days.

At the end of the test (29^{th} day) and after weighing, the rats were anesthetized by diethyl etherandblood samples were directly taken from their hearts using a 5 cc syringe. The blood serum was then collected using centrifuge device (adjusted at 3000 rpm for 15 min) and kept at -20 ˚C. Enzyme-linked immunosorbent assay (ELISA) kits for rats were used to measure LH, FSH, GnRH and testosterone levels. At the end of the experiment period, the testes were removed and tissue sections obtained. The sections were then stained with Hematoxylin and Eosin stain and counting and checking the cells were done using light microscopy and with the help of special neobar and graticule lams.

The related data to each group were recorded and analyzed using SPSS and one-way analysis of variance (one-way ANOVA) test. $P<0.05$ was considered as the significance level. Data in the section of Results were calculated and compared as Mean$\pm$SEM.

RESULTS

The average concentration of FSH, GnRH and LH hormones in the experimental 3 group showed a significant increase at level of $p\leq0.05$ compared to the control and the sham groups (Table 1). It was found in comparison of groups received silymarin with each other that the concentration of 150 mg/kg had more effect in increased LH, FSH and GnRH levels compare to the other concentrations (Table 1).

The average concentration of testosterone hormone in the experimental 2 and 3 groups showed a significant increase at level of $p\leq0.05$ compared to the control and the sham groups (Table 1). Compared with all groups received silymarin with each other, it was found that the concentration of 150 mg/kg had more effects in increased testosterone levels than the other concentrations (Table 1).

The number of spermatogonia cells and primary and secondary spermatocytes in each group did not have a significant difference compared to the control group (Table 2). The average number of spermatid and spermatozoid cells in the experimental 2 and 3 groups showed a significant increase at level of $p\leq0.05$ compared to the control and the sham groups (Table 2).Compared with the groups received silymarin with each other, it was found that the concentration of 150 mg/kg had more effects in increased number of spermatid and spermatozoid cells than the other concentrations (Table 2).

The number of Sertoli and interstitial cells in all the experimental groups did not show a significant difference compared to the control group (Table 2).

Table 1: The mean serum LH, FSH, GnRH and testosterone levels in different groups of the experiment

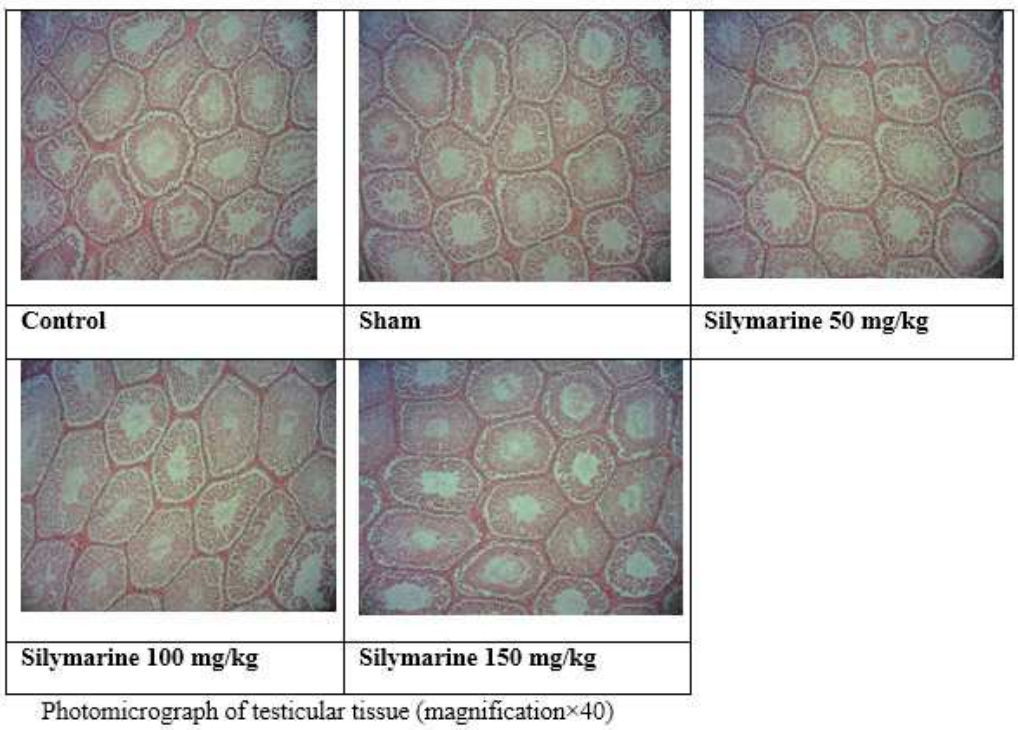
Groups Variables	Control	Sham	Silymarin (50 mg/kg)	Silymarin (100 mg/kg)	Silymarin (150 mg/kg)
GnRH (ng)	64.4±1.02a	65.2±1.98a	67±1.51a	69±2.34a	79±1.53b
FSH (ng)	9.34±0.83a	9.36±0.24a	9.86±0.93a	10.64±1.17ab	11.56±1.51b
LH (ng)	4.48±0.274a	4.48±0.222a	4.98±0.420a	5.38±0.493ab	6.40±0.628b
Testosterone (ng)	1.33±0.36a	1.35±0.025a	1.42±0.029a	1.56±0.038b	1.74±0.028c

According to Duncan test the means available in each row (Mean±SEM), which at least have one letter in common, do not have significant difference with each other at level of 5% of Duncan test.

Table 2: The mean number of seminiferous, Leydig and Sertoli cells in different groups of the experiment

Groups Variables	Control	Sham	Silymarin (50 mg/kg)	Silymarin (100 mg/kg)	Silymarin (150 mg/kg)
Spermatogonia cells	62.6±2.01a	61.4±2.35a	64.20±1.77a	62±3.30a	62.4±3.07a
Primary spermatocytes	61.8±1.01a	61.4±2.44a	65.2±1.74a	65.8±1.52a	66.2±1.52a
Secondary spermatocytes	112±2.77a	113.8±1.39a	123.6±1.69a	115±6.62a	123.8±3.78a
Spermatids	97.8±1.98a	95.2±4.14a	106.2±2.95ab	116.6±4.70b	135.4±4.16c
Spermatozoids	111.8±2.49a	110.8±3.02a	120.8±5.16ab	146.2±3.99b	163.8±1.74c
Sertoli cells	19.8±1.06a	19.8±1.24a	20.6±0.87a	22.2±0.37a	20.6±1.63a
Leydig cells	8.8±0.37a	8.8±0.66a	8.8±0.66a	9.4±0.40a	9.2±0.58a

According to Duncan test the means available in each row (Mean±SEM), which at least have one letter in common, do not have significant difference with each other at level of 5% of Duncan test.



Photomicrograph of testicular tissue (magnification×40)

DISCUSSION

Hypothalamic gonadotropin releasing hormone (GnRH) by influencing on the anterior pituitary gland increases the secretion of FSH and LH and thus stimulates the secretion of testosterone. Based on the results obtained in this study, simultaneous increase in serum levels of testosterone and LH, FSH and GnRH indicated the influence of silymarin on hypothalamus-pituitary-testis axis. Hypothalamus-pituitary-testis axis is affected by positive and negative control factors. Norepinephrine is one of the factors influencing the axis [15, 16]. It has been shown that silymarin have increased the concentration of norepinephrine, serotonin and dopamine in certain areas of the brain of laboratory white mice [17]. It seems in the current study that increase of gonadotropin hormones from pituitary gland is related to increased release of norepinephrine by silymarin. Norepinephrine by increasing the synthesis of

nitric oxide will increase releasing of GnRH from hypothalamus and LH and FSH hormones from pituitary gland [15, 16].

In addition to central effects of silymarin on hypothalamus-pituitary-testis axis, increased testosterone levels in the current study can also be related to synthesis and metabolism of this hormone. Silymarin is counted as a potent inhibitor of aromatase enzyme [13]. Aromatase catalyzes the conversion of testosterone to estrogen. By inhibiting this enzyme, the serum levels of testosterone increase [13].

In this study a significant increase of the mean number of spermatid and spermatozoid cells in the groups receiving dose of100 and 150 mg/kg of silymarin was observed compared to the control group. Oufi et al. (2012) in their investigation onsilybin (one of the structural isoforms of silymarin) effects on testicular tissue of laboratory white mice showed that this flavonoid is able to improve the testicular parameters such as the diameter of the primary spermatocytes and spermatids, the motility of sperm and the percentage of live sperms. It is also able to increase the levels of testosterone secretion [13].

There is a direct relationship between serum concentration of LH and FSH hormones and the number of spermatogenic cells. FSH receptors are present on the surface of Sertoli cells and by binding to these receptors, FSH activates adenylyl cyclase enzyme and increase cAMP levels and as a result activates protein kinase C (PKC) in the cytosol [18]. The catalytic subunit is then activated and enters to the nucleus and activates there the transcription of ABP gene. FSH increases synthesis and secretion of ABP (androgen binding protein) and thus the concentration of testosterone in the seminiferous tubules is provided for normal process ofspermatogenesis[19]. By binding to Leydig cells, LH also increases the secretion of testosterone [20]. Testosterone is also known as the survival factor of spermatogenesis process [21]; so, an increase in sperm density and count is detected due to the enhancement of the level of the mentioned hormones by silymarin.

Polyunsaturated fatty acids contain a major part of fatty acids constructing the mammalian sperm membrane. Therefore, the sperm membrane is so sensitive to damages caused by oxygen free radicals which are generated by lipid peroxidation [22]. Since free radicals produced in daily reactions of the body are more effective in reducing the count and motility of sperm,one of probable mechanisms of silymarineffectson enhancement of sperm count may be caused by the antioxidant properties of it.

Studies results show that antioxidants in male reproductive system reduce oxidative stress in testis and increase the activity of Leydig cells and so resulting in increase of secretion of testosterone and improvement of spermatogenesis (sperm generation) process [23, 24]. The additive or synergistic effect of silymarin on the content of glutathione peroxidase has been approved in the various tissues of rats [25, 26]. Glutathione peroxidase (GPX) which plays a special role in protection of sperms and ductus deferens in testis tissue and epididymis is one of the major antioxidants [27].

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