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The Effect of Cinnamon Extract on Spermatogenesis Hormonal Axis of Pituitary Gonad in Mice

Research Article

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ABSTRACT

Cinnamon has many therapeutic effects, such as its impact the increase of sexual ability. This experiment was conducted to determine the effect of cinnamon extract on spermatogenesis and hormonal axis of pituitary gonad in mice. The animals used in this study are male adult mice (weighing about 30-34 g and 9-10 weeks old). Cinnamon was purchased from one of the most valid shops in Jahrom and then powdered. After preparation of cinnamon extract, it was used for injection. The concentration of cinnamon used in this research was 10 and 20 mg/kg BW. The control animals received only drinking water. The sham group received distilled water. The treatment animals received cinnamon extract (10 and 20 mg/kg BW) for two weeks, each day for the amount of 1 mL interaperitoneally. After the injection, the animals were sacrificed. The weights of testes were measursed, and the changes, including the number of Sertoli cells, spermatogonia, spermatid, and Leydig cells were calculated. Also, hormonal concentration changes (LH, FSH and Testosterone) were measured by special hormonal kits. The results revealed significant increase ($P<0.05$) in the number of spermatogonia, spermatocyte, spermatid, Sertoli and Leydig cells in treated animals. Also, the results showed significant increase ($P<0.05$) in the concentration of LH, FSH and Testosterone hormones among treated animals. Due to the increase in LH, FSH and Testosterone hormones and also the increase of the number of Leydig cells, it is concluded that cinnamon may cause an increase in sex cells within seminiferous tubules in mice.

KEY WORDS cinnamon, mice, spermatogenesis, testis, testosterone.

INTRODUCTION

Cinnamon plant belongs to *Luaraceae* family which has many therapeutic effects. One of these important effects is its impact on the increase of sexual ability. The most important components in cinnamon are cinnamomin and cinnamaldehyde. In recent years, extensive researches have been made on cinnamon and its components on various organs. Cinnamon can be used to treat diabetes (Kamath *et al.* 2003; Anderson *et al.* 2004; Shing *et al.* 2007), reduced cholesterol and low density lipoprotein (LDL) (Khan *et al.* 2003), posses bactericidal activity (Nir *et al.* 2000), improve nausea and diarrhea (Skidmore 2002), reduce the release of free radicals in the body and increase the sexual desire (Shagauo and Davidson, 2006).

Cinnamon has been considered to study the sexual power and fertility rates since years ago. Nevertheless, little research has been carried out on its effect on the reproductive system and spermatogenesis. Spermatogenesis process is done within cells of testis seminiferous tubules which produce sperm. Sperm is combined with ovum during fertilization and forms the zygote. Thus, the aim of this study was

to investigate the effect of cinnamon extract on spermatogenesis and hormonal axis of pituitary gonad in mice.

MATERIALS AND METHODS

The animals used in this study were male adult mice (weighing about 30-34 g and 9-10 weeks old), purchased from research institute of vaccination in Shiraz. There were 10 mice in each group (control, sham, treatment I and treatment II groups). During experiment, the temperature was set between 20 and 24 ºC. The animals were exposed to 12 hours darkness and 12 hours light. During the experiment the animals were fed by pellet and drinking water. The cage floor was covered with wood chips and sawdust. Animal cages were cleaned and disinfected twice a week. The animals were kept out for two weeks for adaptation in animal house in Islamic Azad University, Jahrom Branch. Cinnamon was purchased from one of the most valid shops in Jahrom and then powdered. After the preparation of cinnamon extract, it was used for injection. To prepare the cinnamon extract values of 10 and 20 g, cinnamon was weighed on analytical balance, and 100 ml of distilled water was added and gently heated for 30 minutes (about 45 degrees) until extracted out. Then the solution was cleared with filter paper and made ready for injection. To prevent contamination, the extracts were kept in the refrigerator. The control animals received drinking water. The sham group received distilled water. The treated animals received cinnamon extract in concentrations of 10 and 20 mg/kg BW for two weeks, each day 1 mL interaperitoneally. For injection, insulin syringe was used which has relatively good and acceptable sensitivity.

In this study the effect of aqueous extract of cinnamon was examined on the number of sex cells, in seminiferous tubules in mice. After the injection, the animals were sacrificed. The weights of testes were measured, and the changes including the number of Sertoli cells, spermatogonia, spermatid, spermatocyte and Leydig cells were calculated.

Ether was used to anaesthetize the animals and then the blood samples were obtained from their hearts and were centrifuged, concentration of FSH, LH and Testost-erone hormones were measured with immunoradiometeric assay. The concentration of LH, FSH and Testosterone hormones in blood sample was measured by special LH and FSH hormones kits (Iran Teb Pishtaz Company, Iran) using ELISA (Enzyme-Linked Immu-noSorbant Assay) method. Also, the concentration of Testosterone hormone was measured by special testoster-one kit (DRG Company, Germany) using ELISA method. Furthermore, testis was isolated and weighted. Then testis was washed with normal saline and transferred to Bovin fixative and later was embedded in paraffin. After sectioning and staining, histological studies were worked out by light microscope. The results were analyzed using SPSS statistical software, T-test, Duncan method and one way analytical variance (twice replicate for one treatment) were performed. Analysis and mean comparison was carried out ($P<0.05$).

RESULTS AND DISCUSSION

The results of testis weight showed no significant change in the treated groups compared to control and sham groups (Table 1).

Table 1 The results of cinnamon extract on testis weight (X±SE) in control, sham and treated groups*

Observations	Testis weight (g)	N
Control	0.13±0.01^{b}	10
Sham	0.15±0.03^{ab}	10
Treatment I	0.16±0.03^{a}	10
Treatment II	0.17±0.02^{a}	10

*The means that have at least one common letter, do not have significant difference ($P>0.05$).

The number of spermatogonia, spermatocyte, sperma-tid, Sertoli and Leydig cells showed significant increase ($P<0.05$) in the treated compared to control and sham groups (Tables 2, 3, 4, 5 and 6). The amount of FSH, LH and Testosterone hormones were significantly increased in treated group compared sham and control groups ($P<0.05$) (Table 7, 8 and 9).

Table 2 The results of cinnamon extract on mean of spermaogonia number (X±SE) in control, sham and treated groups*

Observations	Mean of spermatogonia	N
Control	35.25±5.38^{c}	10
Sham	28.54±1.22^{d}	10
Treatment I	44.12±5.25^{b}	10
Treatment II	53.34±6.26^{a}	10

*The means that have at least one common letter, do not have significant difference ($P>0.05$).

As it can be noted from figures 1 and 2 there is no difference in the structure of seminiferous tubules, however, the number of sex cells has increased in treated group.

Along with scientific advances, researchers have recognized the effects of various compounds on reproductive system. Cinnamon (*Cinnamomum zeylanicum*) a medicinal plant belongs to *Luaraceae* family. This plant has many therapeutic effects. One of its most important effects is its impact on the increase of sexual ability. The most important components in cinnamon are cinnamomin and cinnamaldehyde (Shagauo and Dvidson, 2006).

The effects of cinnamom have not yet been fully identified on reproductive system. This study concentrated on the effect of cinnamon extract on spermatogenesis and hormonal axis of pituitary gonad in mice.

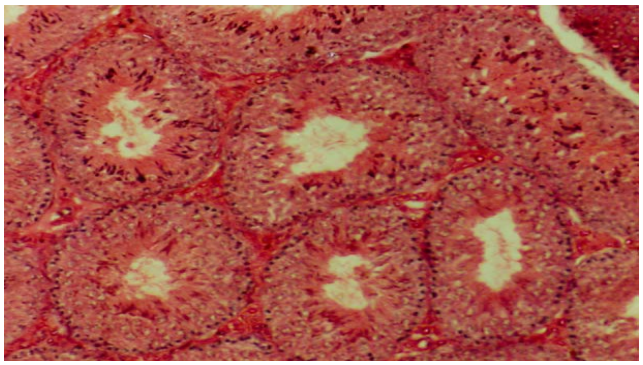

Figure 1 Transverse section of seminiferous tubules in control group. Magnification: ×400, Staining: Hematoxylin-eosin

Table 3 The results of cinnamon extract on mean of spermatocyte number (X±SE) in control, sham and treated groups

Observations	Mean of spermatocyte	N
Control	40.00±2.56^{c}	10
Sham	38.00±3.40^{c}	10
Treatment I	51.00±2.15^{b}	10
Treatment II	68.00±1.35^{a}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

Results of cinnamon extract in the treatment group revealed that cell number of spermatogonia, spermaticyte and spermatid were significantly increased compared to the control group (Tables 2, 3 and 4).

Table 4 The results of cinnamon extract on mean of spermatid number (X±SE) in control, sham and treated groups

Observations	Mean of spermatid	N
Control	138.32±2.52^{c}	10
Sham	120.32±5.53^{d}	10
Treatment I	158.25±2.33^{a}	10
Treatment II	153.52±4.54^{b}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

Therefore, as spermatogonia cells increase, the other cells such as spermatocyte and spermatid increase too. spermatogonia cells are divided to spermatocyte cells with mitosis division and this cells population is able to be divided to spermatid. Research conducted on these cells show that they are capable of regular divisions and will eventually become sperm. Therefore, increase in the number of spermatogonia cells will lead to generation of other sex cells.

Table 5 The results of cinnamon extract on mean of Sertoli cells number (X±SE) in control, sham and treated groups*

Observations	Mean of Sertoli	N
Control	5.12±1.36^{d}	10
Sham	7.51±2.38^{c}	10
Treatment I	12.16±1.45^{b}	10
Treatment II	16.23±1.28^{a}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

The results of this study showed that concentration of LH, FSH and Testosterone hormones have been increased significantly (Tables 7, 8 and 9). This effect could be due to the presence of compounds in cinnamon which affect the hypothalamus-pituitary axis and has thus increased concentrations of these hormones. Parivzi and Ellendorff (1982) showed that cinnamaldehyde extracted of cinnamon increase norepinephrine and this hormone can increase the release of nitric oxide. Cinnamaldehyde release cAMP with connecting calcium in cell membrane and cause increase in norepinephrine secretion. Norepinephrine increase LH secretion with activation of nitric oxide. Nitric oxide affects hypothalamus axis and release gonadotropin hormone (GnRH). Gonadotorpin hormones increase secretion of other hormones such as LH and FSH of pituitary gland. LH hormone affects Leydig cells and this cells release Testosterone hormone. Testosterone is the most important hormone in sex cells proliferation (Parivzi and Ellen-dorff, 1982; Sato and Tsakanmamoto, 2000).

Table 6 The results of cinnamon extract on mean of Leydig cells number (X±SE) in control, sham and treated groups

Observations	Leydig cells number	N
Control	44.00±1.22^{c}	10
Sham	42.25±1.51^{d}	10
Treatment I	51.14±1.25^{b}	10
Treatment II	57.25±1.45^{a}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

Therefore, increased concentration of hormones LH, FSH and Testosterone in serum in the treatment groups appear to increase GnRH (Norman and Way, 1998). Also, Kosior and Bobowiec (2006) proposed that leptin hormone increase FSH secretion with intermediation of nitric oxide synthesis

(Kosior and Bobowiec, 2006). Leptin hormone stimulates increasing of androgenic hormones secretion such as Testosterone and this hormone proliferates sex cells (Xia and Richard, 2006). Studies also indicate that delta-cadenin compound extracted from cinnamon can increase the concentration of Testosterone (Parivzi and Ellendorff, 1982; Cembridge, 1984; Sato and Tsukanmamoto, 2000). Researchers have shown that Leydig cells release in addition to the production of Testosterone, hormones and other factors such as oxytocin, B-endorphins, prostaglandins, and other steroids. Leydig cells release Testosterone. Reports show that Leydig cells target various factors including vasopersin and interleukin (Keys and Anderson, 1995; Lee *et al*. 2004; Parvinen *et al*. 2007; Kander and Hsueh, 2009).

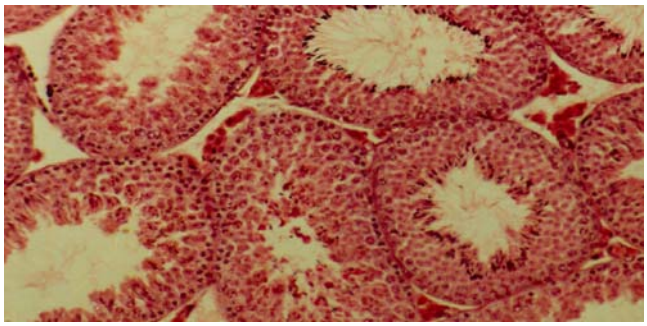

Figure 2 Transverse section of seminiferous tubules in treatment. Magnification: ×400, Staining: Hematoxylin-eosin

Therefore, increased concentration of hormones LH, FSH and Testosterone in serum in the treatment groups appear to increase GnRH (Norman and Way, 1998). Also, Kosior and Bobowiec (2006) proposed that leptin hormone increase FSH secretion with intermediation of nitric oxide synthesis (Kosior and Bobowiec, 2006). Leptin hormone stimulates increasing of androgenic hormones secretion such as Testosterone and this hormone proliferates sex cells (Xia and Richard, 2006). Studies also indicate that delta-cadenin compound extracted from cinnamon can increase the concentration of Testosterone (Parivzi and Ellendorff, 1982; Cembridge, 1984; Sato and Tsukanmamoto, 2000). Researchers have shown that Leydig cells release in addition to the production of Testosterone, hormones and other factors such as oxytocin, B-endorphins, prostaglandins, and other steroids. Leydig cells release Testosterone. Reports show that Leydig cells target various factors including vasopersin and interleukin (Keys and Anderson, 1995; Lee *et al*. 2004; Parvinen *et al*. 2007; Kander and Hsueh, 2009).

Reports indicate that spermatogenesis depends on cells to cells interactions such as Sertoli cells and Leydig cells interactions (Sharp, 2009).

Vinkovsky *et al*. (2003) showed that Leydig cells increase the activity and secretion of cellular factors (oxytocin secretion) thus leading to spermatocyte formation. The most important factor in the spermat-ogenesis is Testosterone hormone. Also, Vinkovsky showed that testosterone hormone is naturally required in production of sex cells. Studies indicate that secretory function of seminiferous tubules controls the number of Leydig cells and their differentiation (Vinkovsky *et al*. 2003).

Table 7 The of cinnamon extract on mean of FSH concentration (X±SE) in control, sham and treated groups*

Observations	FSH concentration (mlU/mL)	N
Control	0.20±0.01^{d}	10
Sham	0.40±0.02^{c}	10
Treatment I	0.80±0.02^{b}	10
Treatment II	0.90±0.01^{a}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

Table 8 The results of cinnamon extract on mean of LH concentration (X±SE) in control, sham and treated groups*

Observations	LH concentration (mlU/mL)	N
Control	0.20±0.01^{c}	10
Sham	0.20±0.01^{c}	10
Treatment I	0.46±0.05^{a}	10
Treatment II	0.30±0.02^{b}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

Vinkovsky *et al* (2003) showed that Leydig cells increase the activity and secretion of cellular factors (oxytocin secretion) thus leading to spermatocyte formation. The most important factor in the spermatogenesis is Testosterone hormone. Also, Vinkovsky *et al* (2003) showed that Testosterone hormone is naturally required in production of sex cells. Studies indicate that secretory function of seminiferous tubules controls the number of Leydig cells and their differentiation (Vinkovsky *et al*. 2003).

Table 9 The results of cinnamon extract on mean of testosterone concentration (X±SE) in control, sham and treated groups*

Observations	Testosterone concentration (mlU/mL)	N
Control	0.30±0.01^{c}	10
Sham	0.20±0.01^{d}	10
Treatment I	0.50±0.03^{b}	10
Treatment II	0.58±0.01^{a}	10

*The means that have at least one common letter, do not have significant difference (P>0.05).

It has also been suggested that sex cells determine the sensitivity of Sertoli cells to Leydig cells for secretion of androgenic hormones. Evidences indicate that injection of LH and hCG (human choreionic gonadotropin hormone)

hormones cause changes in testicular blood flow. The response to hCG depends on the activation of Leydig cells. These cells control blood flow in testicular blood (Bernard *et al*. 2005).

In treated groups significant increase (P<0.05) were observed in the number of Leydig cells (Table 6). Also, the number of spermatogonia and spermatid cells showed significant increase (P<0.05) in treated group com-pared to control group (Tables 2 and 3). According to research conducted it is shown that there is a very close relationship between the performance of Leydig cells and sex cells of seminiferous tubules and also according to the fact that differentiation of Leydig cells are controlled by seminiferous tubules, hence, it must be accepted that a significant increase in generating sex cells in seminiferous tubules increase the performance of Leydig cells (Sharp, 2009).

Due to the increase in LH, FSH and Testosterone hormones and increased activity of Leydig cells it can be concluded that cinnamon may cause increase of sex cells in mice. The researches done by Shagauo and Davidson (2006) also show that cinnamon is capable of releasing LH hormone by affecting hypothalamus axis and increasing the secretion rate of GnRH hormone. Also, they prop-osed that GnRH cause proliferation of sex cells by incre-asing the Leydig cell activities in adult rats. However, further research is required to throw some more lights on the subject.

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