Cibtech Journal of Zoology ISSN: 2319–3883 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjz.htm
2015 Vol. 4 (2) May-August, pp.31-38/Mahmoudi et al.
Research Article

THE EFFECT OF HYDROALCOHOLIC EXTRACT OF CINNAMON ON PITUITARY-GONADAL HORMONE AXIS AND MORPHOMETRIC CHANGE IN MICE TESTIS

Mohammad Niaz Mahmoudi[1], *Sajjad Hejazi[2] and Fatemeh Afshari[3]
[1]*Department of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
[2]*Department of Anatomy, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
[3]*Department of Histology, Tabriz Medical Sciences Branch, Islamic Azad University, Tabriz, Iran*
**Author for Correspondence*

ABSTRACT
The cinnamon extract is effective in healing the created wounds of Vistar rats the plant is also beneficial in nausea and diarrhea treatment and enhances understanding. Based on reviews there are few studies on the effect of cinnamon bark on testis tissue; so, the present study aimed to the study of cinnamon hydro-alcoholic extract effect on mouse testis. Experimental group: received 50 mg/kg alcoholic extract of cinnamon intraperitoneally for 3 weeks (Modaresi *et al.*, 2010). The data was explained as Mean±SEM and T-Test was used to analyze the data of the groups via SPSS software. P was valued less than 0.05 between two groups. The results of the present study are the meaningful increase in spermatocyte number of the treatment group compared with the control group, suggesting the testosterone induction and its effect on spermatogenesis process. The obtained results demonstrate that the hydro-alcoholic effects of cinnamon extract in the experimental group create meaningful differences compared with control group as well as a greater increase in spermatocyte number. The results can approve the cinnamon extract effect on spermatogenesis increase. It can be said that oligospermia and azoospermia in males are two factors leading infertility; so, it can be said that cinnamon can be used to increase fertility in males as a treatment of oligospermia. However, wider researches are recommended in this area.

Keywords: *Cinnamon, Mice Testis, Pituitary-Gonadal Hormone*

INTRODUCTION
Cinnamon is a plant with a scientific name of Cinnamoum zeylanicum belongs to Lauraceous family. The herb has many curative effects important of which is the libido increase. Cinnamon with a scientific name of J.Presl cinnamum verum belongs to Lauraceae family, Cinnamomum genus, and Cinnamoum zeylanicum verum species. Besides its flavoring properties, the plant has other beneficial properties such as anti microbial activity, anti diabetes, prevention the cancer cells proliferation, and effective in cold treatment (Anderson *et al.*, 2004; Peter *et al.*, 2004).
The herb has antioxidant properties because of phenolic and other antioxidant compounds. The most antioxidant compounds of the plant are cinnacassiol, eugenol, camphene, coumarin, cinnamaldehyde, cinnamic asid, and gamma-terpinene. The compounds prevent oxidative reactions and can be obtained by extracting the plant (Murcia *et al.*, 2004; Parthasarathy *et al.*, 2008).
Murica *et al.*, (2004) compared the antioxidant properties of seven spices (cinnamon, anise, ginger, licorice, mint, and vanilla) with common food antioxidants i.e., BHT, BHA, and PG. They found that cinnamon and mint have the higher percentage of prevention against oxidation compared with other spices and food antioxidants. The result was obtained from fat peroxidation. Furthermore, cinnamon is the best counteractive of super oxide radical compared with other spices and analyzed additives (Su *et al.*, 2007). Different parts of the plant have many curative properties, such that using it causes heart, stomach, and intestine strengthening, kidneys improvement and increased libido (Singh *et al.*, 2007). Medical value of the plant is mostly due to its aromatic oil. The main compounds of the extract including cinnamaldehyde, ornol, and safrole have an insulin-like activity and can beneficial in diabetes treatment (Anderson *et al.*, 2004). The plant also used traditionally to treat asthma, eye inflammation, rheumatism, neuralgia, wounds, toothache, flu, fewer, and cold. Using the plant in mentioned cases only has been

popular in traditional medicine and is not supported by laboratory and clinical statistics and analyses except some of which (Mishra *et al.,* 2009). Another traditional use of the plant is the treatment of impotency, cold tempered, and sub-abdominal sexual pain (Donald *et al.,* 2009).

Using the plant prevents organic materials oxidation in the body and causes free radicals decrease due to its strong antioxidant (Skidmore-Roth, 2002). The cinnamon extract is effective in healing the created wounds of vistar rats (Adame *et al.,* 2000). The plant is also beneficial in nausea and diarrhea treatment and enhances understanding. Based on reviews there are few studies on the effect of cinnamon bark on testis tissue; so, the present study aimed to the study of cinnamon hydro-alcoholic extract effect on mouse testis.

MATERIALS AND METHODS

8 weeks-old adult male mice weighing 25-30 g were used in this study. The animals were kept in 12-hour successive periods of darkness and light with sufficient water and food available. Then, the mice were divided randomly into two groups: control group and Interventional group (using cinnamon). It should be mentioned that 15 mice in each group were studied.

Extraction Method

In order to extract, the cinnamon bark was divided into small pieces. The pieces were milled to change into powder. 30 g of the powder was placed into a sterile flask and 30 cc of physiologic serum was added to it.

The flask was left in a cold place for 24 hours. Then, the contains of the flask was mixed with a shaker for 5 min. then, the sample was filtered via Watmann filter and the remained substance in the solution was calculated to determine the cinnamon concentration in the main solution and to prepare the considered dosages. The control group was kept in the similar conditions as the treatment group without any injection. To assure lack of injection effect, the control group received physiologic serum as a daily administration (Modaresi, 2011).

Experimental group: received 50 mg/kg alcoholic extract of cinnamon intraperitoneally for 3 weeks (Modaresi *et al.,* 2010). After the determined period the testis tissue of mice was sampled and weighted with digital scale with a precision of 0.1 g. the testes were placed in 10% formalin to study under the optical microscope and then were stained with H&E method followed by histotechnique stages. Morphometric variables were evaluated with a calibrated lens and numbering the spermatocytes was conducted by eye using microscopic samples with an identical cross-sectional area (10 cross-sections in each sample).

Hormonal Measurement and Analysis

At the end of the treatment period the mice were anesthetized using ether, and then blood sampling was conducted from their left ventricular at amount of 3-4 ml into the laboratorial tubes containing anticoagulant. The collected samples were centrifuged at 3000 rpm for 15 minutes. Then, the samples' serum was separated using sampler and was kept at -20 °c for measurement the LH, FSH, and testosterone concentrations in serum. Hormone measurement was conducted using usual laboratory methods that is, the radioimmunoassay was conducted using hormonal kits (Kavoshgar Co.).

The data was explained as Mean±SEM and T-Test was used to analyze the data of the groups via SPSS software. P was valued less than 0.05 between two groups.

RESULTS AND DISCUSSION

Results

The effect on testosterone hormone

In normal and interventional groups the serum levels were 3.15±0.32 and 4.72±0.57 mg/ml, respectively. The obtained data demonstrated a meaningful difference between two groups ($P<0.05$).

The Effect on LH

In normal and interventional groups the serum levels were 7.5±0.52 and 14.99±1.85 mg/ml, respectively. The obtained data demonstrated a meaningful difference between two groups ($P<0.05$) (Figure 1).

The Effect on FSH

In normal and interventional groups the serum levels were 0.74±1.53 and 1.53±50.9 mg/ml, respectively. The obtained data demonstrated a meaningful difference between two groups (P<0.05).

Table 1: Comparison of mean of serum levels associated with testicle in control and exprimental groups.Dissimilar letters in each vertical column indicate a significant difference Mean+SD (P<0.05)

Parameters	Groups Control group	Experimental group
Testosterone (ng/ml)	3.15±0.32[a]	4.72 ± 0.57[b]
LH (mlU/ml)	4.5 ± 0.52[a]	14.99 ± 1.85[b]
FSH (mlU/ml)	0.74 ± 0.64[a]	1.53 ± 0.19[b]

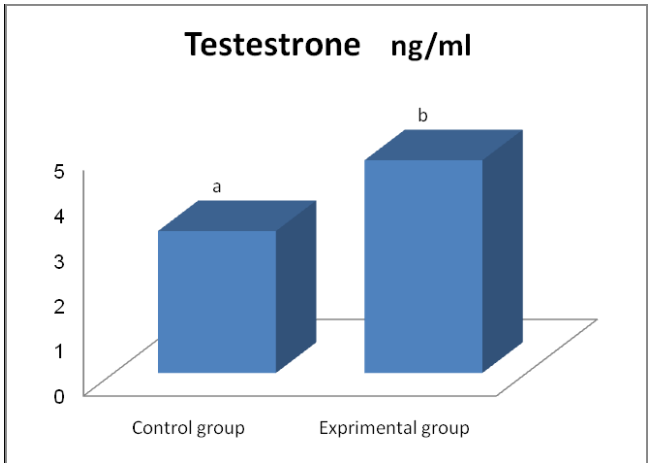

Diagram 1: Comparison the Mean ± SD of the serum testosterone level between two, control and experimental, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P>0.05)

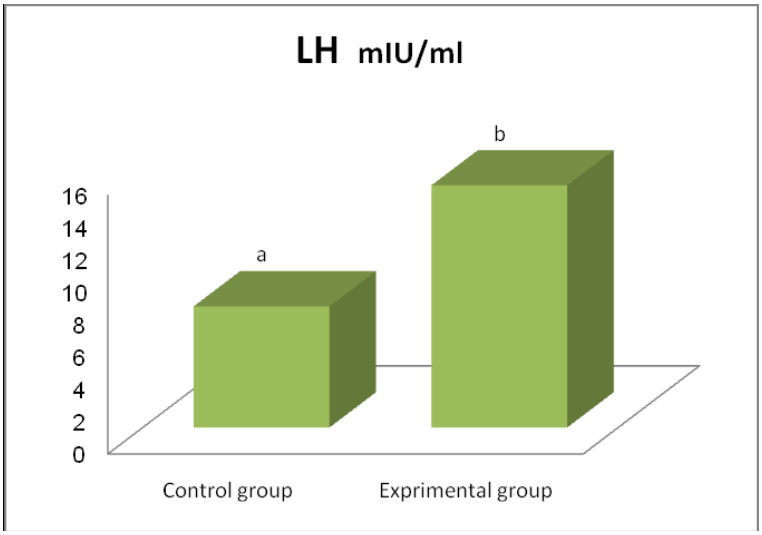

Diagram 2: Comparison the Mean±SD of the serum LH level between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P>0.05)

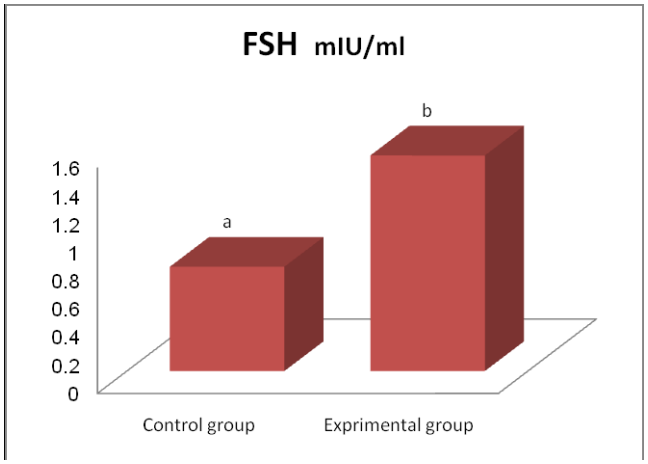

Diagram 3: Comparison the Mean±SD of the serum FSH level between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P<0.05)

Morphometric Results

Effect on Spermatocyte Numbers

The average spermatocyte numbers of control and experimental groups was 66.07±3.78 and 78.8±4.12 in each scope. The obtained results showed a meaningful difference between the two groups (P<0.05).

Effect on Leydig Cell Number

The average leydig cell number of control and experimental groups was 23.8±2.54 and 32.73±2.01 in each scope. The obtained results showed a meaningful difference between the two groups (P<0.05).

Effect on Seminiferous Diameter

The average seminiferous diameter of control and treatment groups was 62.07±1.75 and 64.47±3.33 µm. The obtained results showed a meaningful difference between the two groups (P<0.05).

Effect on the Seminiferous Epithelial Thickness

The average seminiferous epithelial thickness of control and treatment groups was 16.6±10.8 and 16.67±1.67 µm. The obtained results showed no meaningful difference between the two groups (P>0.05).

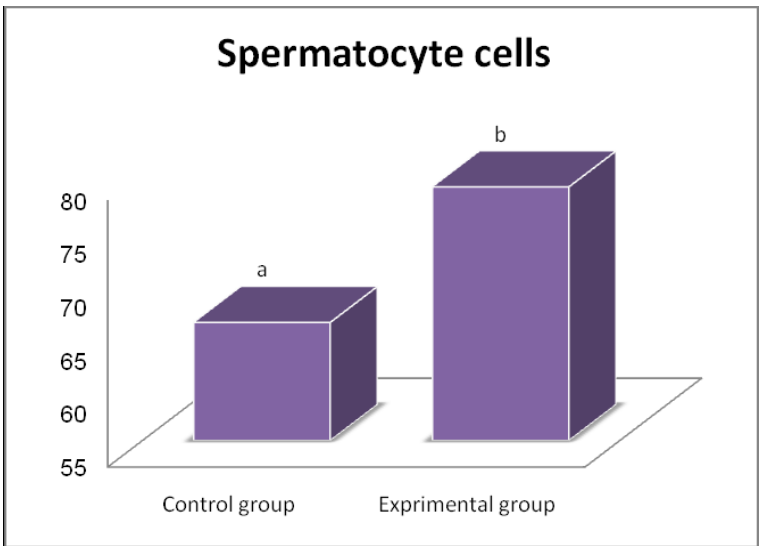

Diagram 4: Comparison the Mean±SD of spermatocyte number between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P<0.05)

Table 2: Comparison of mean on Histomorphometric parameters in control and exprimental groups.Dissimilar letters in each vertical column indicate a significant difference Mean+SD (P<0.05)

Parameters \ Group	Control group	Experimental group
Spermatocyte	66.07 ± 3.78[a]	78.8 ± 4.12[b]
Leydig	23.8 ± 2.54[a]	32.73 ± 2.01[b]
Seminiferous diameter (µm)	62.07 ± 1.75[a]	64.47 ± 3.33[b]
Seminiterons epithelial diameter (µm)	16.6 ± 10.8[a]	16.67 ± 1.67[a]

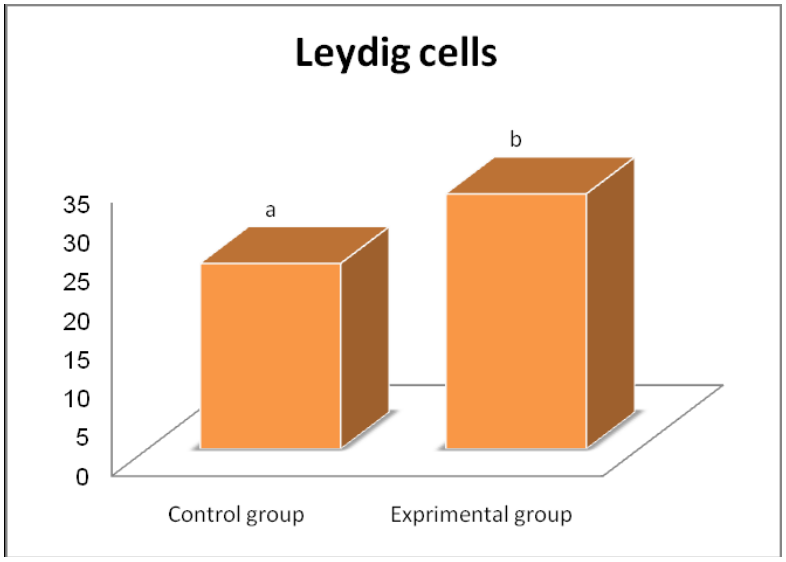

Diagram 5: Comparison the Mean±SD of leydig cell number between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P<0.05)

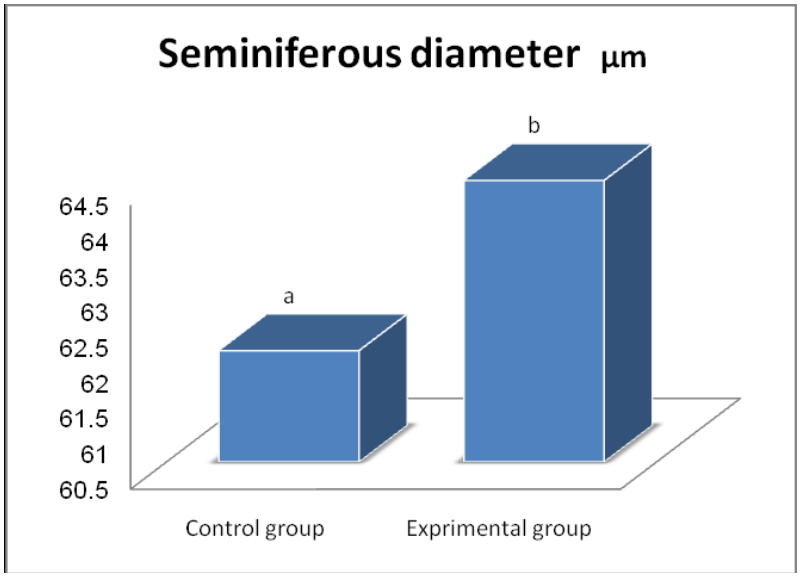

Diagram 6: Comparison the Mean±SD of the seminiferous diameter between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P<0.05)

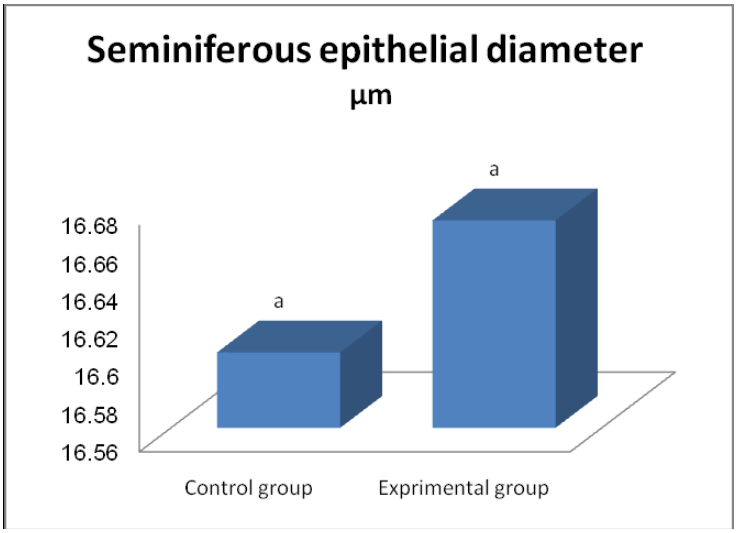

Diagram 6: Comparison the Mean±SD of the seminiferous thickness between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P<0.05)

Discussion

The results of the present study are the meaningful increase in spermatocyte number of the treatment group compared with the control group, suggesting the testosterone induction and its effect on spermatogenesis process.

In the present study the leydig cell number of the treatment group had a meaningful increase compared with the control group, suggesting its hormonal induction that led to increased testosterone level and its effect on other testicular structures. It can be concluded that the cinnamon extract has increased leydig cells besides spermatocytes that are responsible for male sexual hormone, testosterone, and secretion to induce spermatogenesis.

Also, it can be concluded that one of the possible mechanisms of the extract effect on testis tissue is the increase of leydig cells and consequently increase the male sexual hormones and increased blood circulation in testis tissue via angiogenesis. Both of the cases cause increased sperms via affecting the sertoli cells that controls spermatogenesis process.

It was also revealed in this study that cinnamon administration caused meaningful increase in seminiferous diameter and seminiferous epithelial thickness that can be attributed to meaningful increase in testosterone hormonal level.

In this regard, it is pointed out in a study that cinnamon use causes testosterone, FSH, and LH increase. The increase can be resulted of compounds available in cinnamon bark that affect the hypothalamus-pituitary axis and cause the mentioned hormones increase. Probably, the cinnamon extract can increase testosterone synthesis via LH secretion increase or a direct effect (Modaresi *et al.*, 2010).

It has been reported in the study that delta- cadinene in cinnamon acts as testosterone increasing factor (Braun, 2005).

Based on free radicals theory (Lindi *et al.*, 2005), imbalance among peroxidants and anti oxidants consequently caused oxidative damages in cell processes and decreased steroidogenesis in leydig cells (Lindi *et al.*, 2005).

It has been concluded in a study that the libido increase is one of the important effects of cinnamon (Mirheidar, 2004; Shah, 1998).

Conducted researches indicate the presence of anti oxidant compounds in cinnamon (Onderoglu *et al.*, 1999). Researchers attribute the anti oxidant effect of cinnamon to eugenol and methyl. Oral administration of hydroxy chalcon eugenol (MHCP) causes normal activity of glutathione peroxidase and increased cell- restored glutathione (Van Kampen & Zijlstra, 1985).

Delta-cadinene of cinnamon can also act as increasing factor of testosterone and increase its synthesis directly (Braun and Cohen, 2005).

One of the effective factor on this axis is acid nitric; this active molecule increases the secretion of gonadotropins and LH hormone, as well as the highest sperm motility and inducing erection in men (Sato, 2000; Gonzalez, 2001). The secretion of the neurotransmitter increases by the effect of different factors such as Norepinephrine. It increases the LH secretion by the activation of Oxide nitric synthesis to induce LH releasing hormone (Parvizi, 1982). Studies demonstrate that the secretion of Norepinephrine increase under the effect of cinnamaldehyde (the main component of cinnamon). Cinnamaldehyde makes the connection between calcium ion and the membrane and therefore releasing the annular AMP and increasing the Norepinephrine secretion (Chin, 2000). The hormone Leptin causes increased FSH via nourological oxide nitric synthesis (Kosier-Korzeka, 2000). Other researches show that delta-cadinene in cinnamon acts as increasing factor of testosterone (Braun and Cohen, 2005). In a study conducted by Shah in 1998 it was proved that oral administration of cinnamon caused increased spermatogenesis in rats (Shah, 1998). According to the explanations provided, the reason of increased LH and FSH level can be contributed to the direct or indirect affect of cinnamon components especially cinnamaldehyde on increased syntheses of oxide nitric. In the present study the testosterone hormone increased in 50 mg/kg dosages which were probably associated to the cinnamon components. Maybe, cinnamon extract can increase testosterone synthesis via direct effect or by increasing the LH secretion.

Conclusion

The obtained results demonstrate that the hydro-alcoholic effects of cinnamon extract in the experimental group create meaningful differences compared with control group as well as a greater increase in spermatocyte number. The results can approve the cinnamon extract effect on spermatogenesis increase. It can be said that oligospermia and azoospermia in males are two factors leading infertility; so, it can be said that cinnamon can be used to increase fertility in males as a treatment of oligospermia. However, wider researches are recommended in this area.

REFERENCES

Adame J and Adame H (2000). Parte 1. In: *Plantas curativas del noreste mexicano*, edited by Adame J and Adame H, Monterrey: Ediciones Castillo 135.

Anderson RA and Broadhurst CL (2004). Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *Journal of Agricultural and Food Chemistry* **52** 65-70.

Braun L and Cohen M (2005). *Herbs and Supplement An Evidence-based Guide, Sydney*, 3rd edition (New York, Elsevier Mosby) 808.

Chin-Chuan TSAI, I-Min LIU and Juei-Tang CHENG (2000). Stimulatory effect of trans cinnamaldehyde on norepinephrine secration in cultured pheochromacytoma (PC 12) cell. *Science Press* **21**(12) 1174-8.

Donald G and Barceloux MD (2009). Cinnamon (*Cinnamomum* Species). *Disease-A-Month* **55** (6) 327-335.

González LC, Pinilla L, Tena-Sempere M, Bellido C and Aguilar E (2001). Effects of systemic blockade of nitric oxide synthases on pulsatile lh, prolactin, and gh secretion in adult male rats. *Hormone Research* **55** 229-35.

Kosior-Korzecka U and Bobowiec R (2006). Leptin effect on nitric oxide and GnRH-induced FSH secretion from ovine pituitary cells in vitro. *Journal of Physiology and Pharmacology* **57**(4) 637-47.

Lindi L, Haolin C, Michael A, Trush MD, Show M, Anway D and Barry Zirkin R (2005). Aging and the Brown Norway Rat Leydig Cell Antioxidant Defense System. *Journal of Andrology* **22** 32-37.

Mirheidar H (2004). Plant knowledge; *Application of Plants in Prevention and Remedy of Diseases* (Tehran, Islamic Culture Press) 323-328.

Mishra AK, Mishra A, Kehri HK, Sharma B and Pandey AK (2009). Inhibitory activity of Indian spice plant Cinnamomumzeylanicumextracts against Alternariasolaniand Curvularialunata, the pathogenic dematiaceous moulds. *Annals of Clinical Microbiology and Antimicrobials* **8** 9.

Modaresi M (2011). The Effect of Cinnamon Extract on Serum Proteins Levels of Male Balb/c MiceArmaghane-danesh. *Yasuj University of Medical Sciences Journal* **16**(5) 444-452.
Modaresi M, Messripour M and Rajaei R (2010). Effect of cinnamon extract on the number of spermatocyte and spermatozoa cells in mice. *Iranian Journal of Medicinal and Aromatic Plants* **26**(1) 83-90.
Murcia MA, Egea I, Romojaro F, Parras P, Jimenez AM and Martinez-Tome M (2004). Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. *Journal of Agricultural and Food Chemistry* **52** 1872-81.
Onderoglu S, Sozer S, Mine-Erbil K, Ortac R and Lermioglu F (1999). The evolution of long-term effects of cinnamon bark and olive leaf on toxicity induced by streptozotocin administration to rats. *Journal of Pharmacy and Pharmacology* **51** 1305- 1312.
Parvizi N and Ellendorff F (1982). Further evidence on dual effects of norepinephrine on LH secretion. *Neuro Endocrinology* **35**(1) 48-55.
Parthasarathy VA, Chempakam B and Zachariah TJ (2008). *Chemistry of Spices* (Cambridge: Biddles Ltd, King's Lynn) 124-139.
Peter KV (2001). *Handbook of Herbs and Spices* (Cambridge: Woodhead Ltd, Abington Hall) 159-168.
Sato Y Tsukamamoto (2000). Effects of nitric oxide stimulation on the brain. *Drugs Today* **36**(2-3) 83.
Shah AH, AL-Shareef AH and Ageel AM (1998). Qureshi S. Toxicity studies in mice of common spices Cinnamomum zeylanicum bark and piper lonum fruits. *Plant Food for Human Nutrition* **52** 231-9.
Singh G, Maurya S, Deampona MP, Delampasona MP, Can C and Cesar AN (2007). A cmparision of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oil. *Food and Chemical Toxicology* **45**(9) 1650-61.
Skidmore-Roth L (2002). *Handbook of Herbs and Natural Supplements*, 2nd edition (St Louis, Mosey) 38.
Su L, Yin JJ, Charles D, Zhou K, Moore J and Yu L (2007). Total phenolic contents chelating capacities and radicalscavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry* **100** 990-7.
Van Kampen EJ and Zijlstra WG (1985). Determination of hemoglobin and it's derivatives. *Clinical Chemistry* **8** 1414.