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The effect of Hydroalcoholic Extract of Cinnamon on Mice Testis Tissue

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ABSTRACT: Cinnamon is a plant with a scientific name of *Cinnamoum zeylanicum* belongs to Lauraceae family. The herb has many curative effects important of which is the libido increase. the present study aimed to the study of cinnamon hydro-alcoholic extract effect on mouse testis. The mice received no substance in the control group. The mice of the experimental group received 50 mg/kg alcoholic extract of cinnamon intraperitoneally for 3 weeks. 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 smaller than 0.05 between two groups. The tissue samples were evaluated under the optical microscope. At first, the seminiferous tubules with a similar cross-sectional area were measured under the microscope with a calibrated lens. The studies demonstrated that there is no difference between control and treatment groups in terms of appearance and the tubules distribution. Also, any tissue damage caused by cinnamon administration was not observed. In the samples of cinnamon group the increased density and decreased interstitial spaces of seminiferous tubules were observed. 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.

Key words: Cinnamon, Mice, Testis Tissue

INTRODUCTION

Cinnamon is a plant with a scientific name of *Cinnamoum zeylanicum* belongs to Lauraceae 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 (2004) compared the antioxidant properties of seven spices (cinnamon, anise, ginger, licorice, mint, and vanilla) with common food antioxidants ie., 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, at the present study aimed to the study of cinnamon hydro-alcoholic extract effect on mouse testis.

MATERIAL AND METHODS

8 weeks-old adult male mice weighing 25-30 gr 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, control and Interventional, groups with 15 mice in each group. The mice received no substance in the control group. The mice of the 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).

A. 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).

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 smaller than 0.05 between two groups.

RESULTS

A. The results of tissue changes

The tissue samples were evaluated under the optical microscope (Nikon). At first, the seminiferous tubules with a similar cross-sectional area were measured under the microscope with a calibrated lens. The studies demonstrated that there is no difference between control and treatment groups in terms of appearance and the tubules distribution. Also, any tissue damage caused by cinnamon administration was not observed. In the samples of cinnamon group the increased density and decreased interstitial spaces of seminiferous tubules were observed.

Extension and distribution of blood vessels of interstitial spaces in testicular capsule were the important changes in testis samples of the treatment group. Furthermore, aggregation of leydig cells in interstitial spaces of treatment group samples was different compared with the control group.

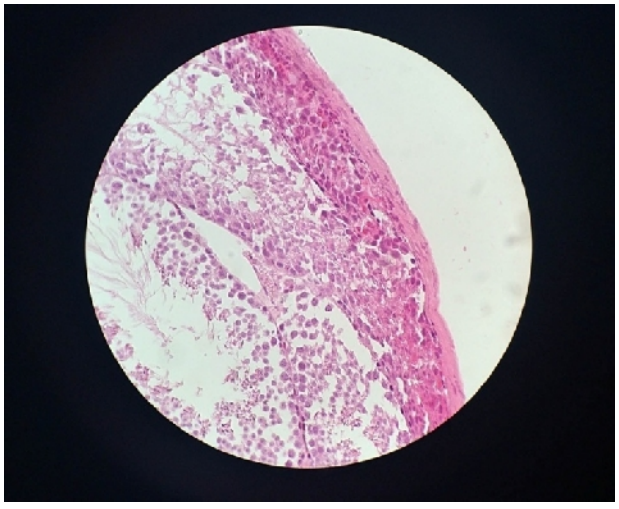

Fig. 1. The microscopic view of testis cross-section at the control group, Observation of normal status in capsule structure and subcapsular space (H&E, 40×).

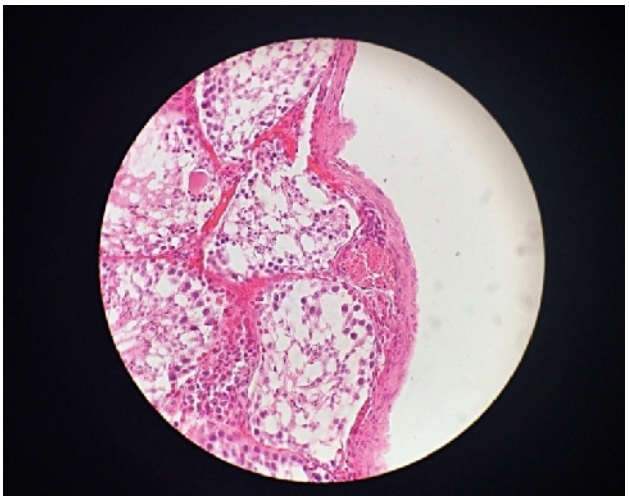

Fig. 2. The microscopic view of testis cross-section at the treatment group, Observation of blood vessels in sub-capsular space and its infiltration into the interstitial spaces (H&E, 40×).

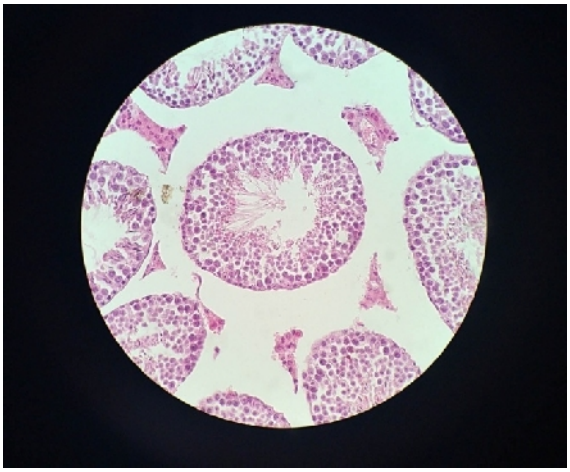

Fig. 3. The microscopic view of testis cross-section at the control group, Observation of leydig cell mass in interstitial spaces and blood vessels distribution (H&E, 40×).

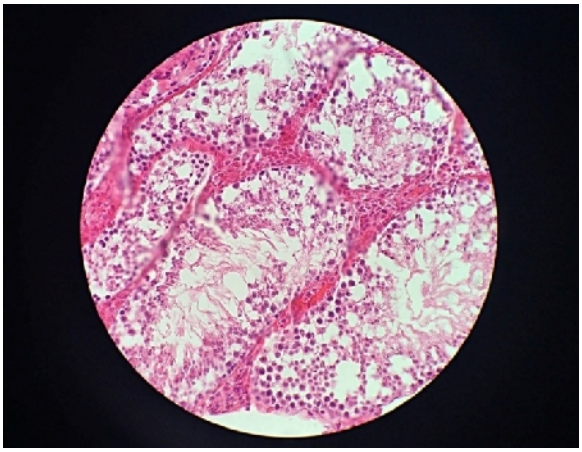

Fig. 4. The microscopic view of testis cross-section at the treatment group, Observation of leydig cell density in interstitial spaces and blood vessels distribution (H&E, 40×).

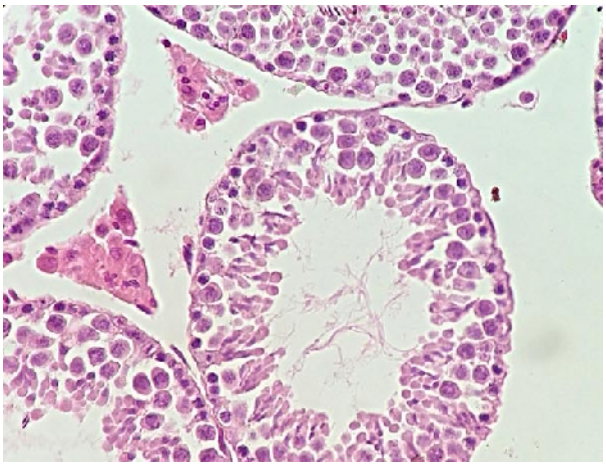

Fig. 5. The microscopic view of testis cross-section at the control group, Observation of leydig cell population in interstitial spaces (H&E, 100×).

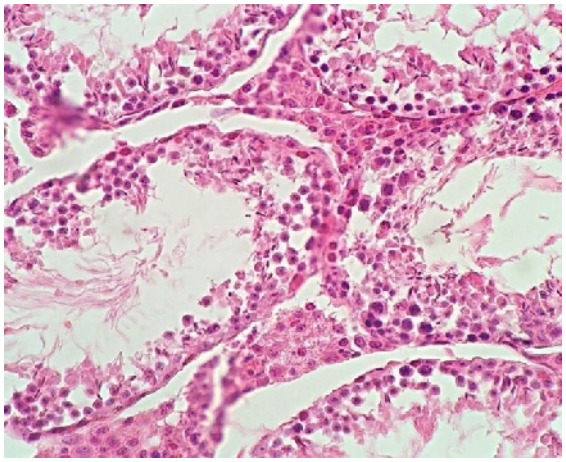

Fig. 6. The microscopic view of testis cross-section at the treatment group, Observation of leydig cell population in interstitial spaces (H&E, 100×).

B. Morphometric results

Effect on testis weight. The average testis weight of control and treatment groups was 0.073±0.04 and 0.077±0.08. The obtained results showed no meaningful difference between the two groups.

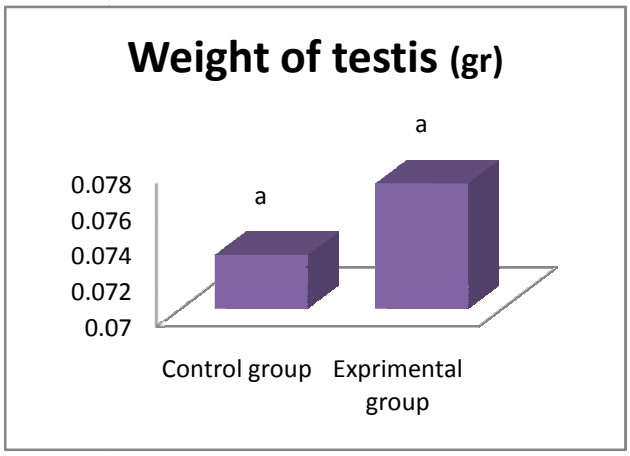

Fig. 7. Comparison the Mean ±SD of testicular weight between two, control and treatment, groups.

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.

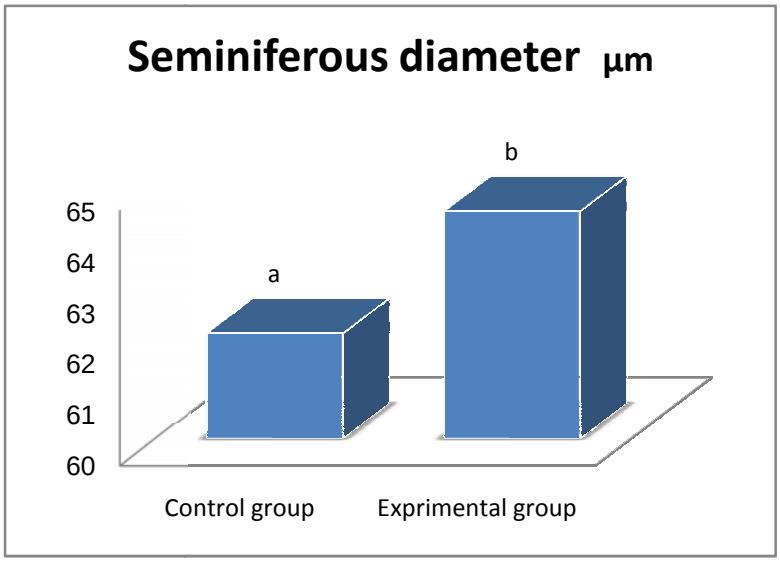

Fig. 8. Comparison the Mean±SD of seminiferous diameter between two, control and treatment, groups. The different letters show a meaningful difference of mean among groups (P<0.05)

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.

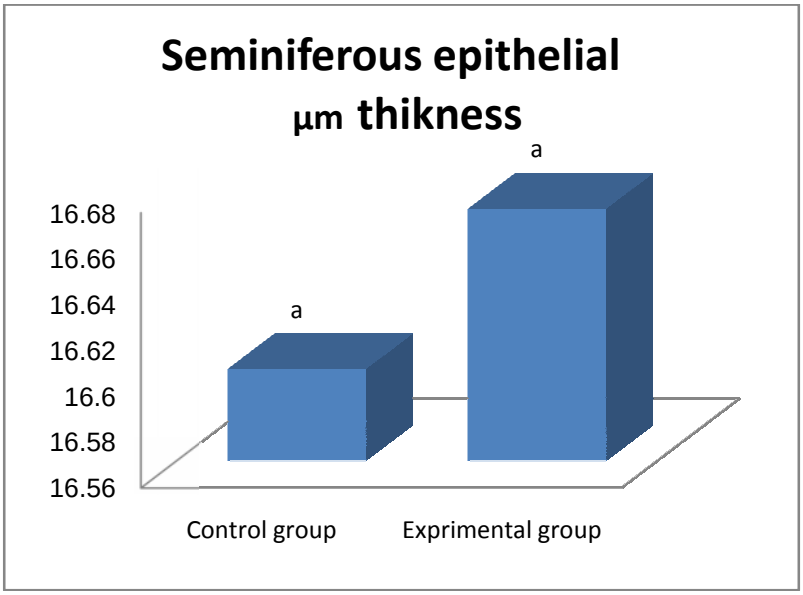

Fig. 9. Comparison the Mean±SD of seminiferous thickness between two, control and treatment, groups. (P>0.05).

Effect on spermatocyte numbers. The average spermatocyte numbers of control and treatment 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.

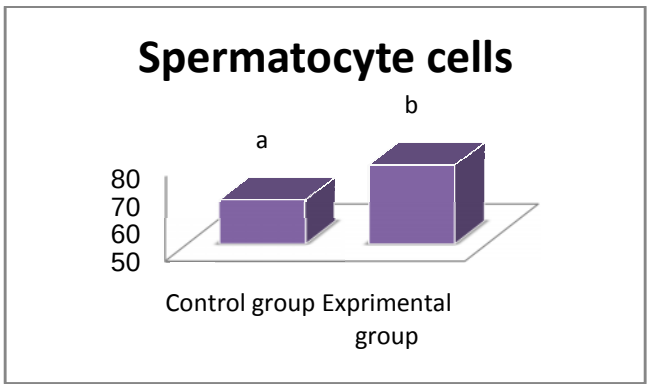

Fig. 10. Comparison the Mean±SD of spermatocyte number between two, control and treatment, groups. The different letters show a meaningful difference of mean among groups (P<0.05).

Effect on leydig cell number. The average leydig cell number of control and treatment 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.

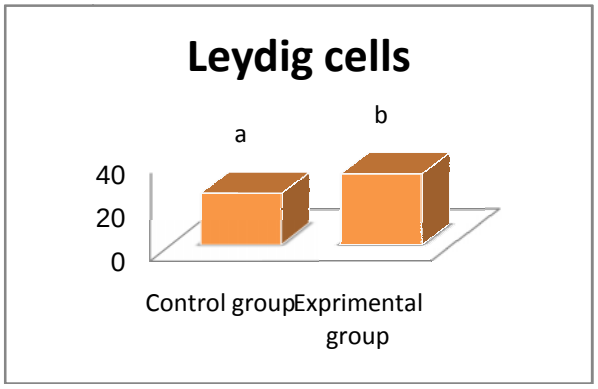

Fig. 11. Comparison the Mean±SD of leydig cell number between two, control and treatment, groups. The different letters show a meaningful difference of mean among groups (P<0.05).

Effect on the testicular capsule thickness. The average testicular capsule thickness of control and treatment groups was 5±0.75 and 5.13±1.77 µm. The obtained results showed no meaningful difference between the two groups.

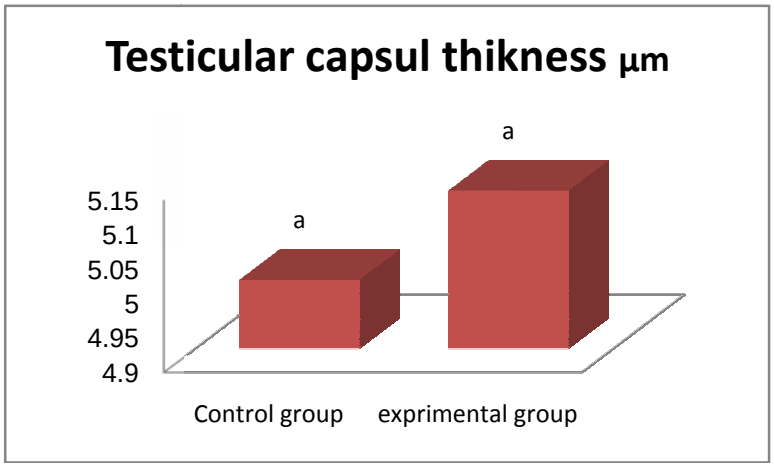

Fig. 12. Comparison the Mean±SD of testicular capsul thickness between two, control and treatment, groups. Dissimilar letters between groups indicate a significant difference of mean between groups (P>0.05).

DISCUSSION

In the conducted studies by Shah, 1998 and Mirheidar, 2004 it has been concluded that a libido increase is one of the important effects of cinnamon.

The obtained results of the present study indicated that injection of cinnamon hydro-alcoholic extracts can affect testis weight meaningfully compared with the control group, suggesting hormonal level induction in the animal's body. The result of the present study conformed to the results obtained from a study on male mice treated with cinnamon extract. In that study, the hydro-alcoholic effects of cinnamon have been reported in increased weight as well as an increased movement and number of animal's sperms (Shah *et. al.,* 1998). This revealed that cinnamon has no toxic effect on sperms in long-term treatment of mice.

Another result of the present study is 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). It was seemed from other results of the study that cinnamon administration has no degenerative or deformation effect on testicular capsule or seminiferous tubules. 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). Free radicals cause damage of other molecules such as biologic membrane fatty acids and their oxidation. As a result, fluidity, structure, and function of the membrane are endangered (Halliwell & Gutterridge, 1989). Antioxidant compounds are able to protect cell membranes against damages (Rice Evans & Eurdon, 1994). 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). In a study the administration of glutathione for 8 weeks caused the improvement of sperm number, mobility, and natural morphology (Lenzi *et al.,* 1994). So, it is possible that cinnamon causes live sperm number increase via strengthening the anti oxidant defensive system. Delta-cadinene of cinnamon can also act as increasing factor of testosterone and increase its synthesis directly (Braun and cohen 2005).

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.

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