

Cinnamon Bark Extract Improved the Semen Quality of Male Albino Mice

Ahmed Kasim Mahdi

Department of Biology, College of Education for Pure Sciences,
University of Baghdad (akmahdi@hotmail.com)

Abstract

This work was conducted to examine the effect of the aqueous extract of cinnamon bark on some reproductive parameters of male mice. Ten healthy adult males of Swiss albino mice (*Mus musculus* L), their age between 80-100 days and their weight between 28-35 g, were selected, acclimated, then randomly categorized into two groups of five animals each and treated as follows. Group 1: Control mice that received orally by gavage needle 0.1 ml of tap water daily for 30 days. Group 2: Male mice treated orally by gavage needle with 0.1 ml of the aqueous extract of cinnamon bark daily for 30 days. After cervical dislocation both testes and epididymes immediately were dissected out, then the epididymal sperms parameters and the testes histological structures were estimated and the data were statistically analyzed. Cinnamon bark extract caused a highly significant increase ($P < 0.01$) in sperms progressive motility which increased from 71 % in the male mice of the control group to 86 % in the male mice of the treatment group. The treated males had a significant larger ($P < 0.01$) sperms concentration (3.4×10^6 /ml) than the males in the control group (2.1×10^6 / ml). The percentage of vital sperms was significantly increased ($P < 0.05$) from 89.2% in the animals of the control group to 94.4% in the animals of the treated group. There was a highly significant increase ($P < 0.01$) in the percentage of normal sperms in the treated male mice (92.8 %) in comparisons with the untreated (control) male mice (85.7 %). Although, the histological examination of the testes showed no significant differences ($P > 0.05$) between the two groups of male mice, there was an obvious improvement in both of the diameters of seminiferous tubules and the thickness of germinal cell layer in favor of the treated mice. In conclusion, the oral daily treatment of the aqueous extract of cinnamon bark for 30 days improved some significant parameters of semen quality of male mice.

Key words: Cinnamon bark, aqueous extract, semen quality.

مستخلص لحاء القرفة حسن نوعية السائل المنوي في ذكور الفئران البيض

أحمد قاسم مهدي

قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة بغداد (akmahdi@hotmail.com)

خلاصة

صممت هذه التجربة لدراسة تأثير المستخلص المائي للحاء القرفة على بعض المعالم التناسلية في ذكور الفئران. تم اختيار 10 ذكور من الفئران السويسرية البيض (*Mus musculus L*) السليمة والناضجة تراوحت أعمارها بين 80-100 يوم وأوزانها بين 28-35 غم. قسمت إلى مجموعتين وبواقع 5 ذكور في كل مجموعة، مجموعة المقارنة والتي عوملت يوميا ولمدة 30 يوم بـ 0.1 مل من ماء الحنفية بواسطة أنبوبة التغذية القسرية، ومجموعة المعاملة والتي عوملت يوميا ولمدة 30 يوم بـ 0.1 مل من المستخلص المائي للحاء القرفة بواسطة أنبوبة التغذية القسرية. عند انتهاء المعاملة قتل الحيوانات واستخلصت كلا من الخصى والبرابخ ثم قدرت بعض معالم نطاف البرابخ ودرس التركيب النسجي للخصى وحللت النتائج إحصائيا. أدت المعاملة بالمستخلص المائي للحاء القرفة إلى زيادة عالية المعنوية ($P < 0.01$) في النسبة المئوية للحركة التقدمية للنطاف والتي ارتفعت من 71% في ذكور فئران مجموعة السيطرة إلى 86% في ذكور فئران مجموعة المعاملة. كما كان لحيوانات المعاملة تركيز أعلى من النطاف ($3.4 \times 10^6 / \text{ml}$) مقارنة بحيوانات مجموعة السيطرة ($2.1 \times 10^6 / \text{ml}$) ($P < 0.01$). وارتفعت النسبة المئوية للنطاف الحية من 89% في ذكور مجموعة السيطرة إلى 94.4% في ذكور مجموعة المعاملة ($P < 0.05$). كما سجل ارتفاع عالي المعنوية ($P < 0.01$) في النسبة المئوية للنطاف الطبيعية في الذكور المعاملة (92.8%) مقارنة بالذكور غير المعاملة (85.7%). ولم يتضح من دراسة التركيب النسجي وجود فروقات معنوية ($P > 0.05$) بين خصى حيوانات المجموعتين، بالرغم من تحسن اقطار النبيبات المنوية وسمك طبقة الخلايا الجرثومية في خصى حيوانات المعاملة. لقد أدت المعاملة الفموية اليومية ولمدة 30 يوم بالمستخلص المائي للحاء القرفة إلى تحسن بعض المعالم التناسلية المهمة في ذكور الفئران.

كلمات مفتاحية: لحاء القرفة، المستخلص المائي، نوعيه السائل المنوي.

Introduction

The name cinnamon is derived from the Greek word *kinnámōmon* which may ultimately stem from the Malayan word *kayee manis* meaning sweet wood [1]. In Arabic, it is called *Kerfa*, also best known in colloquial Iraqi as *darseen* maybe it comes from the Persian word *darchini* meaning the Chinese wood.

The cinnamon of commerce is the dried inner bark of a small evergreen tree 10–15 m

tall, belonging to the family Lauraceae, and is native to Sri Lanka, India, Bangladesh, and Nepal. The bark is widely used as a spice because of its distinct odor [2]. Currently, there are two types of cinnamon are cultivated, *Cinnamomum verum*, also known as Ceylon cinnamon, and *Cinnamomum cassia*, also known as Chinese cinnamon [3]

The cinnamon bark is one of the oldest herbal medicines that have been mentioned in Chinese texts as early as 4,000 years ago

[4] eastern and western herbalists used it to treat various health problems. Moreover, modern researches have demonstrated a number of benefits resulting from cinnamon supplementation, most notably hypocholesterolemic [5] hypoglycemic [6] and antioxidant [7] effects.

In male fertility, besides the bark was used by ancient healers to treat impotence and frigidity [3], some recent studies revealed a positive effect from cinnamon supplementation on male's reproductive efficiency [8], [9]

In view of the above, we conducted this work to examine the effect of aqueous extract of cinnamon bark on some reproductive parameters of male albino mice.

Material and Methods

Cinnamon Bark Extract: Cinnamon bark were procured from local market in Baghdad city, ground in a grinder and an amount of 5 g soaked in 100 milliliter of water for one hour then gently heated for 15 minutes, filtered and kept in refrigerator for maximum one week. Each week fresh extract was prepared.

Animals: Ten healthy adult males of Swiss albino mice (*Mus musculus* L), their age between 80-100 day and their weight between 28-35 g, were used in the investigation. Mice were maintained under hygienic conditions in well ventilated room and had free access to maintenance food and water. The animals were kept out for one week prior to the experiment for acclimation in animal house of Department of Biology, College of Education for Pure Sciences, University of Baghdad.

Treatment & Dosage: The animals were randomly categorized into two groups of five animals each and treated as follows. Group 1:

Control mice that received orally by gavage needle 0.1 ml of tap water daily for 30 days. Group 2: Male mice treated orally by gavage needle with 0.1 ml of cinnamon bark extract daily for 30 days.

Sample Collection: After 30 days, all animals were sacrificed by cervical dislocation, and then both testes and epididymes immediately were dissected out and cleared of their adhering tissue. The testes were fixed in formalin 10% for histological analysis. The epididymis was finely minced by anatomical scissors in 1 mL of isotonic saline at 37 °C in a Petri dish. It was completely squashed by a tweezers for 1 min to expel the sperms to the Petri dish.

Assessment of Sperms Parameters: The sperms parameters were assessed according to World Health Organization methods & criteria [10]. The sperms parameters including: progressive motility, sperms concentration, sperms vitality and sperms morphology. Briefly, progressive motility was estimated subjectively at X 400 magnification using a 100-point scale for linear movement. The data were collected from 5 different fields in each sample and expressed in percentage of total cells. Sperm concentration was determined using the standard hemocytometric method. The dilution rate was 1:200 and the concentration was expressed as per ml. Sperms vitality was calculated using eosin nigrosin staining technique, from 2 slides 400 sperms were evaluated at X 400 magnification, the average percentage of vital (unstained) sperms was calculated. Sperms morphology was determined from the same two slides, a total of 600 sperms was examined at × 1000 magnification with oil immersion, the head & tail abnormality of sperms were determined, the average

percentage of normal sperms was calculated.

Histological Examination: For each testis, two slides of 10 μm sections were prepared according to Pease method [11] and stained with H&E [12]. Ten seminiferous tubules (ST) were randomly examined per section, their diameters and germinal cell layer thicknesses (from the basal membrane towards the lumen of the tubule) were measured using an ocular micrometer in a light microscope at X400 magnification, then the mean size of ST and germinal cell layer thickness were calculated [13].

Statistical Analysis: The Statistical Analysis System (SAS) in completely randomized design (CRD) [14] was used to analyze the data. Differences among treatment means were compared for statistical significance, using *t* test.

Results

Cinnamon bark extract caused a highly significant increase ($P < 0.01$) in sperm progressive motility which increased from 71 % in the male mice of the control group to 86 % in the male mice of the treatment group Fig.1.

The treated males had a significant larger ($P < 0.01$) sperms concentration ($3.4 \times 10^6 / \text{ml}$) than the males in the control group ($2.1 \times 10^6 / \text{ml}$) Fig.2

The percentage of vital sperms was significantly increased ($P < 0.05$) from 89.2% in the animals of the control group to 94.4% in the animals of the treated group Fig.3

Also, there was a highly significant increase ($P < 0.01$) in the percentage of normal sperms in the treated male mice (92.8 %) in comparisons with the untreated (control) male mice (85.7 %) (Fig. 4).

Despite the histological examination of the testes showed no significant differences ($P > 0.05$) between the two groups of male mice, there was an obvious improvement in both of the diameters of seminiferous tubules and the thickness of germinal cell layer in favor of the treated mice (Figs.5).

The seminiferous tubule diameter was 181.2 μm & 144.7 μm in the treated and untreated male mice respectively. Fig.6.

The thickness of germinal cell layer was 64.1 μm in treated male mice and 50.2 μm in the untreated (control) male mice Fig.7.

Discussion

Nutraicetical is a foodstuff that provides health benefits in addition to its basic nutritional value [15] and cinnamon has many bioactivities as well as rich in carbohydrate and essential amino acids [16] hence it can promote both health and reproduction.

In tradition Chinese medicine cinnamon was used as appetizer [17] and considered as a warming herb that can improve blood pelvic flow and prescribed to fortify yang (masculine force according to Chinese philosophy) [18]

One of its remarkable bioactivity is that cinnamon could act as an insulin mimetic. The aqueous extract of cinnamon increased glucose metabolism roughly 20-fold in-vitro in the epididymal fat cells [19]. The most active compound methyl hydroxy chalcone polymer (MHCP) increased insulin sensitivity by activating key enzymes that stimulate insulin receptors, while inhibiting enzyme that deactivate them [20]. In addition to that, cinnamon contains a number of antioxidants compounds which can effectively reduce oxidative stress by scavenging reactive oxygen species (ROS) [21]

It is clearly from our study results that cinnamon has positive effects on the quality of male mice semen. The daily treatment for 30 days increased ($P < 0.01$) the sperms progressive motility from 71% in the untreated animal to 86% in the treated animals, the sperm motility is regarded as a manifestation of sperm functional competence [22] and related to pregnancy rates [23]. Furthermore, the treatment increased ($P < 0.01$) the sperms concentration from 2.1×10^6 / ml in the untreated males to 3.4×10^6 /ml in the treated males, the sperm concentration are related to both time to pregnancy [24] and pregnancy rates [23] and are predictors of conception [25]. These findings can be explained on the basis of cinnamon versatile bioactivities, cinnamon can increase the concentration of FSH, LH and testesone hormones [9] either by its direct effect [8] or probably because its effective mimetic of insulin. The insulin regulates the male hypothalamic-pituitary-gonadal axis and is essential for fertility [26]. The functions of FSH, LH and testesone hormones are well documented in male reproductive system.

The significant improvement in the percentage of vital sperms which raised from 89.2% in the control group to 94.4 % in the treatment group, and the percentage of normal sperm which increased from 85.7% in the control group to 92.8 % in the treatment group may be explained in the light of the antioxidants properties of cinnamon which can improve semen quality. Oxidative stress is considered as a major factor in the aetiology of male infertility [27] because both spermatogenesis [28] and Leydig cell steroidogenesis [29] are vulnerable to oxidative stress caused by ROS. The natural antioxidants can protect DNA and other molecules from cell damage induced by

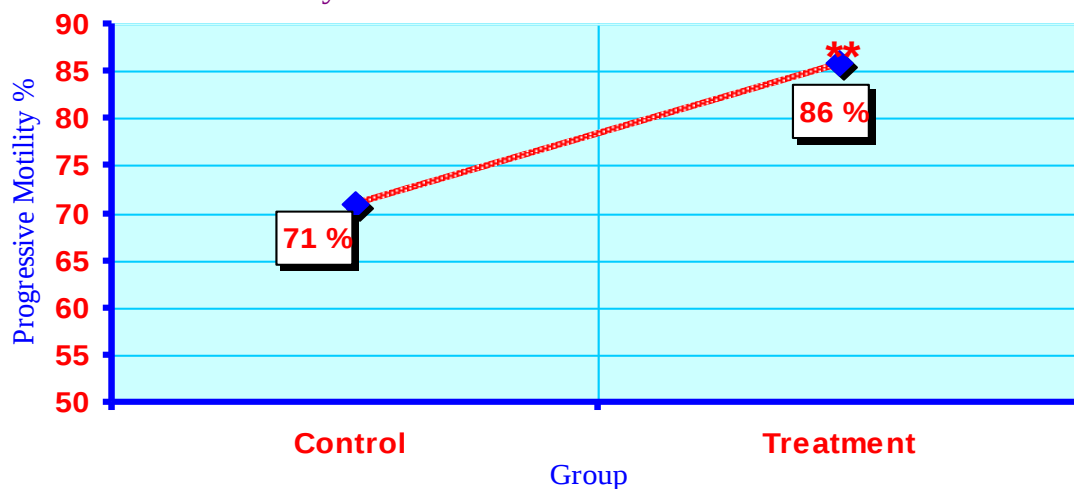
oxidation and can improve sperm quality and increase reproductive efficiency of males [30]

Our research results are in agreement with the results of Havez [8] and the results of Hemayatkah Jahromi *et al* (9). More research are needed to elucidate the mechanism of cinnamon action because until recently, little was known about mechanisms involved in its biological effects [31]

Conclusion

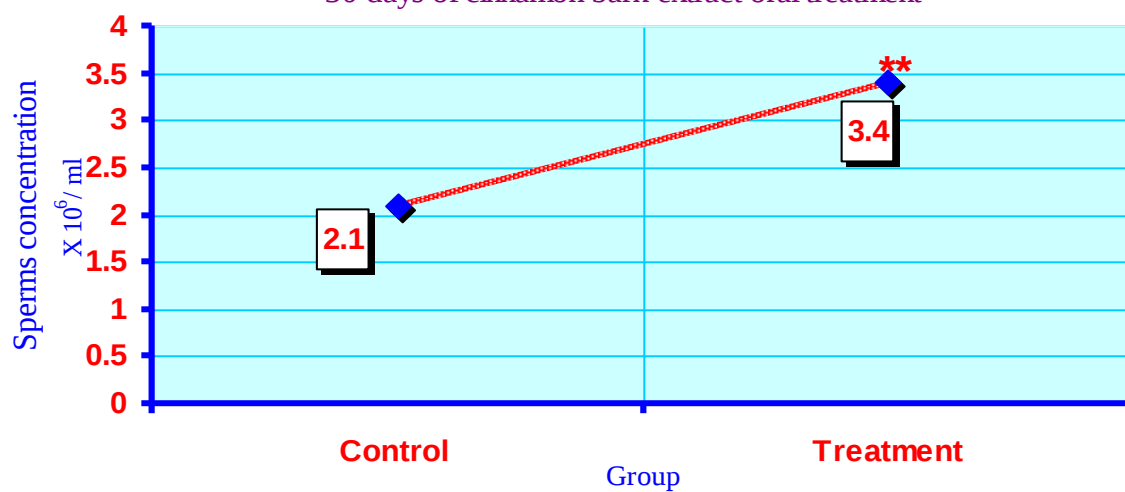
The oral daily treatment of the aqueous extract of cinnamon bark for 30 days improved some significant parameters of semen quality of male mice.

Fig1:The sperms progressive motility of male mice after 30 days of cinnamon bark extract oral treatment



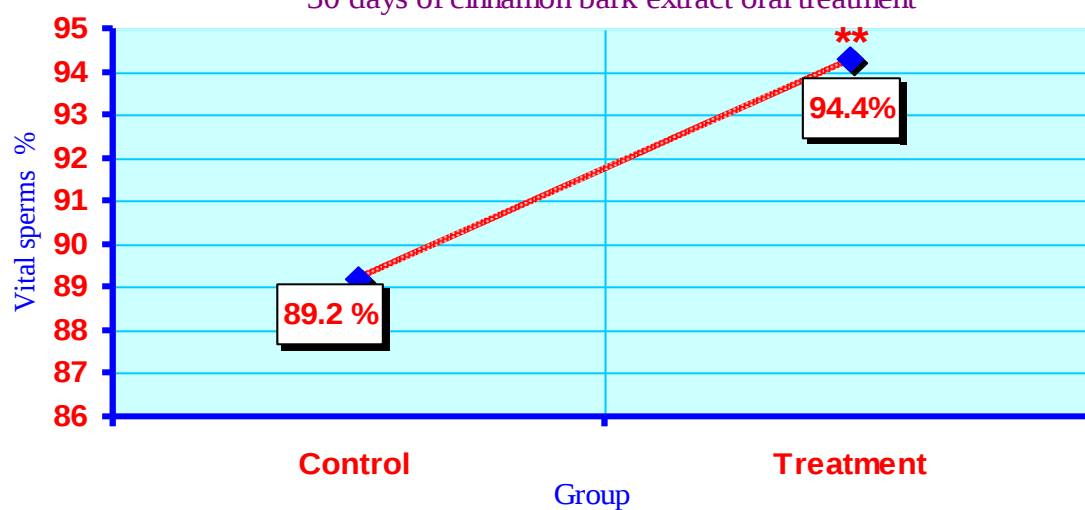
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Fig 2: The sperms concentration of male mice after 30 days of cinnamon bark extract oral treatment



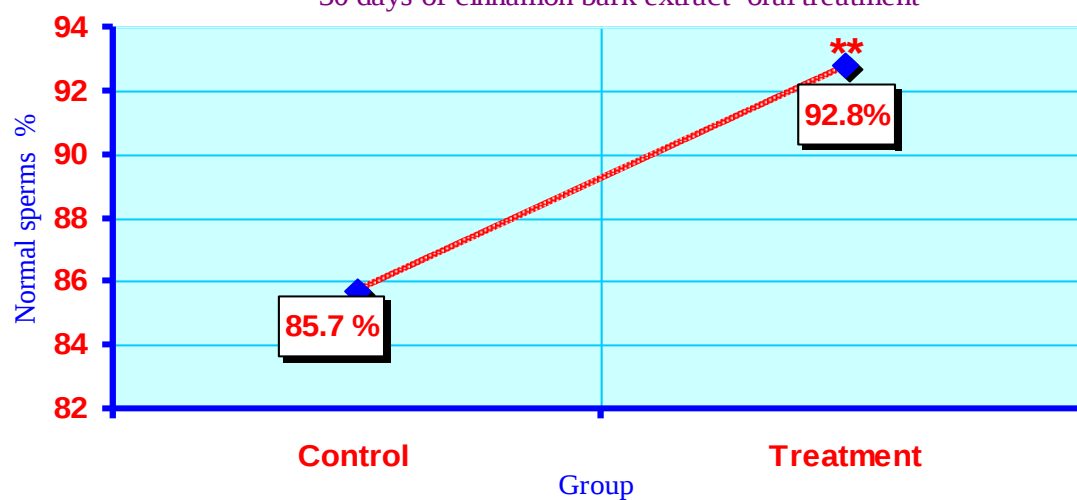
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Fig 3: The vital sperms percentage of male mice after 30 days of cinnamon bark extract oral treatment



**** Differ from the control significantly ($P < 0.05$)**

Fig 4: The normal sperms percentage of male mice after 30 days of cinnamon bark extract oral treatment



**** Differ from the control significantly ($P < 0.01$)**

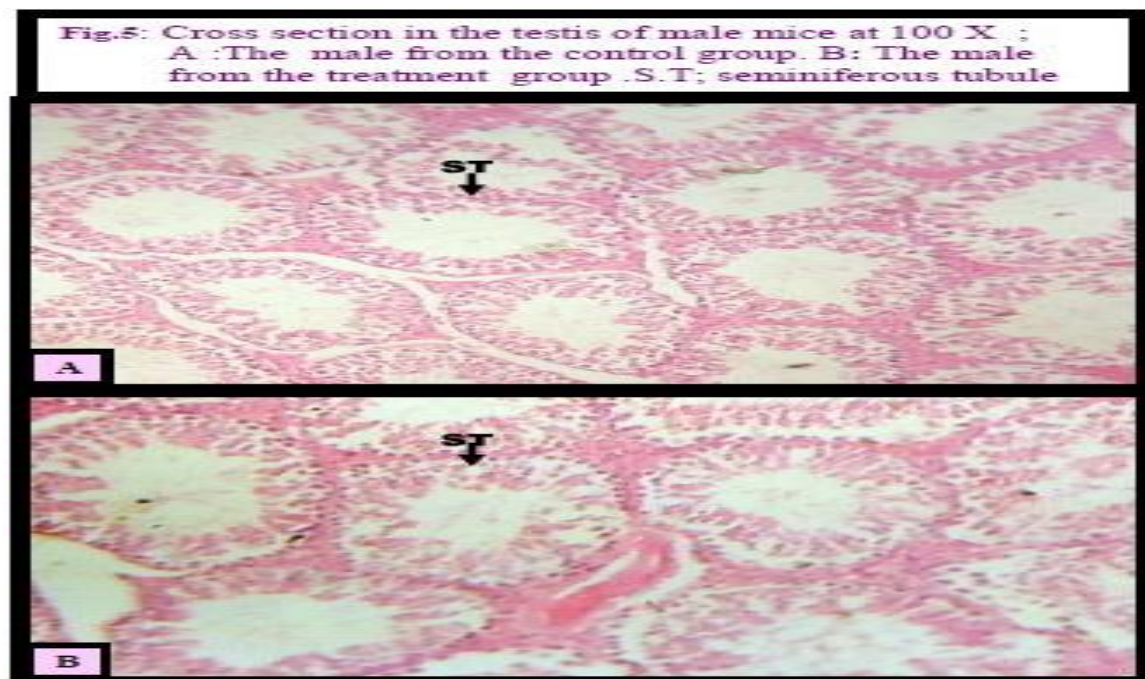


Fig 6 : The seminiferous tubules diameters of male mice testes after 30 days of cinnamon bark extract oral treatment

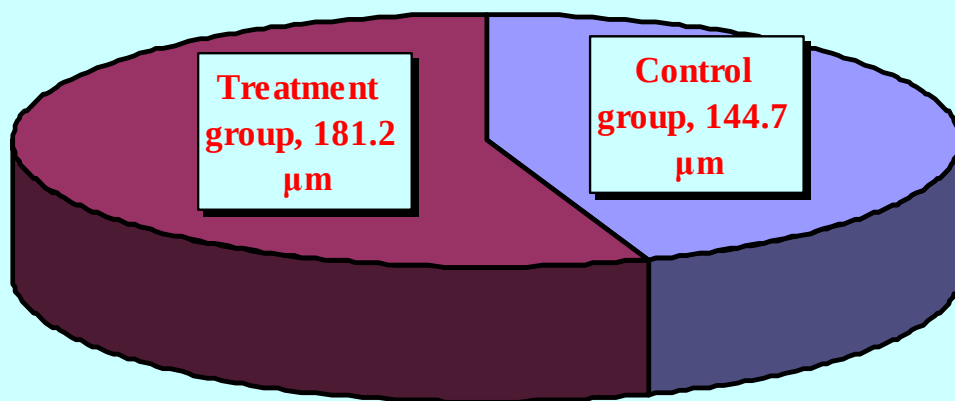
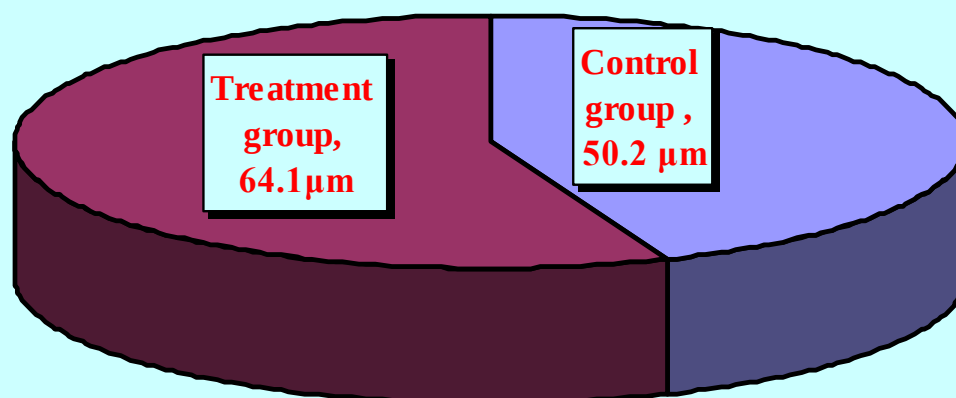


Fig 7 : The germinal cell layers thickness of male mice testes after 30 days of cinnamon bark extract oral treatment



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