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Original Research Article

Potential Protective Role of Cinammon Aquaous Extract against Hypercholesterolemia Induced Testicular Damage in Rats

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Abstract: The present study was aimed to investigate the possible protective effect of cinnamon on the testis against high cholesterol diet of adult Wistar rats through biochemical and histopathological examination. Forty rats were used in the current study; they were divided into four groups. Each group contains ten rats group I is the negative control group, which did not receive any treatment, groupII. received cinnamon aqueous extract orally at 200 mg/kg/day dose for 30 days, groupIII is the induction group, which were fed with a high cholesterol diet (rat chow supplemented with 4% cholesterol and 1% cholic acid) for 30 days (positive control group) and group IV, which were fed with high cholesterol diet and received cinnamon aqueous extract for 30 days. At the end of experimental study, Blood samples were collected for biochemical assessment. Rats that received the HCD showed a significant increase in the cholesterol, triglycerides, and low-density lipoprotein levels, and a significant decrease in the high-density lipoprotein level compared with the levels of the control group. These parameters significantly decreased, with the exception of high-density lipoprotein, which increased, in rats that received the HCD and cinnamon compared with those that received only the HCD .The lipid levels showed non significant change in rats that received cinnamon compared with the control group.
Keywords: cinnamon; cholesterol diet, cholesterol, triglycerides level.

INTRODUCTION

Hypercholesterolemia has been recognized as a risk factor for atherosclerosis, and is now emerging as a contributing factor for the progression of renal disease. A high cholesterol diet (HCD) and inadequate physical activity that characterize our modern lifestyle contribute to the development of hypercholesterolemia

Epidemiological data has widely demonstrated that low testosterone concentrations in men are associated with a higher risk of atherosclerosis; however, the detailed relationship between low testosterone and hypercholesterolemia waits to be clarified, although it is well known that cholesterol serves as the major precursor for the synthesis of the sex hormones, including testosterone [1].

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 cinna-maldehyde. In recent years, extensive researches have been made on cinnamon and its components on various organs. Cinnamon can be used to treat diabetes [2], reduced cholesterol and low density lipoprotein (LDL) [3], possess bactericidal activity [4], improve nausea and diarrhea, reduce the release of free radicals in the body and increase the sexual desire [5].

MATERIALS AND METHODS

Preparation of cinnamon aqueous extract

The *C. cassia* aqueous extract was prepared from the air dried powdered cinnamon bark according to Azab *et al.*[6]. The aqueous extract was freshly prepared by soaking 10 g of the grinded bark in 100 ml distilled water at 90 °C for 2 h followed by filtration. The filtrate was dehydrated in oven at 80 °C overnight. The resulting dark reddish brown dry extract was weighed and the dry yield was then calculated.

Experimental Design and Animal Groups

The present study was performed on 40 male Wister rats weighing between 230 and 280 g that were purchased from the animal house in King Fahd Medical Research Center. The rats were housed in stainless

steel cages and maintained in a 12-hour light-dark cycle, with a room temperature of 25±2°C under hygienic conditions. Water was offered and libitum. The rats were randomly divided into four groups:

Group I: served as the control (n=10) and received a standard diet.

Group II:given cinnamon aqueous extract orally at 200 mg/kg/day according to Kim *et al.*[7] and Azab *et al.* [6] for 30 days.

Group III:served as the induction group (n=10) and was fed a high cholesterol diet (rat chow supplemented with 4% cholesterol and 1% cholic acid) according to Thiruchenduran *et al.*[8] for 30 days.

Group IV: served as the treated group and was fed a high cholesterol diet and given cinnamon aqueous extract orally at 200 mg/kg/day according to Kim *et al.*[7] and Azab *et al.* [6] for 30 days.

Lipid Profile Assay

Blood samples were collected under anesthesia through a glass microcapillary tube in retro-orbital region at the end of the experiment for biochemical assessment. Lipid profile (triglycerides, cholesterol, low density lipoprotein and high density lipoprotein levels) were measured. The assay kits for lipid profile were obtained from Randox Laboratories Ltd., Ardmore, Co. Antrim, UK and assessed according to Onyeike *et al.*[9].

Tissue processing for light microscopy

Rats from each group were sacrificed after 30 days. The testes were extracted, weighed and put in Bouin's fixative. After cutting the testes vertically in two halves, the largest testicular diameter (TD) was measured. The tissue was prepared through paraffin technique. 5um thick sections were stained with H&E stain [10].

Statistical analysis

Statistical Data analysis were carried out using the statistical package for the social sciences (SPSS) for windows version 16. For nonparametric data, analysis of variance (ANOVA) and the KruskalWallis test, followed by a thoc test (based on Dunn's procedure), was used to analyse each pair of groups and there by avoid the multiple-comparison effect. For the parametric data, the different groups were compared using ANOVA (f test), followed by Bonferrroni'sposthoc test. A P value less than 0.05 was considered significant.

RESULTS

Total Cholesterol Assay

Rats that received the HCD showed a significant increase in the cholesterol, triglycerides, and low-density lipoprotein levels, and a significant decrease in the high-density lipoprotein level compared with the levels of the control group. These parameters significantly decreased, with the exception of high-density lipoprotein, which increased, in rats that received the HCD and cinnamon compared with those that received only the HCD (Table 1). The lipid levels showed non significant change in rats that received cinnamon compared with the control group.

Table 1: Effects of cinnamon, HCD and HCD+ Cinnamon on rat's cholesterol, triglyceride, LDL and HDL levels compared to negative control group

Group	Triglyceride (mg/100ml)	Cholesterol (mg/100ml)	LDL (mg/100ml)	HDL (mg/100ml)
Group I (control)	36.93 ± 0.24	65.52 ± 1.24	35.8 ± 2.1	19.13 ± 0.30
GroupII (cinnamon)	32.07 ± 0.32	63.34 ± 2.01#	34.1± 2.4	18.30 ± 0.24
Group III (HCD)	70.89 ± 0.05*†	110.42 ± 1.18*†	43.2 ±1.0*†	14.67 ± 0.56*†
GroupIV (HCD+cinnamon)	39.77 ± 0.32#	66.14 ± 1.02#	36.8 ± 2.1#	21.13± 0.30#

Values are represented as mean ± SD (n=6) Significance was considered P<0.05

* Significant change compared to control untreated (group I)

Significant change compared to HCD group (group III)

†Significant change compared to HCD treated with cinnamon group (group IV).

Histological changes

(Control Group)

Light microscopic examination of the testis stained by H & E showed the testicular parenchyma was composed of seminiferous tubules separated by interstitial tissue and enclosed within a capsule that had two layers. In the outer layer; the tunica albuginea was composed of collagenous C.T., in the inner layer; the tunica vasculosa was athin loose areolar tissue rich in fine blood vessels.

Each seminiferous tubules had an outer capsule or tunica propria of fibrous C.T., flattened fibroblasts (which closely invested the tubules) and a single continuous layer of squamous shaped cells, the myoidcells,a thin homogenous basement membrane, which separated the vascular supply outside from the germ cells within, and a lining of complex stratified

epithelium. This epithelium consisted of two tyoes of cells, the supporting somatic cells (sertoli cells) and the germ cells (spermatogenic cells) as shown in Fig 1.

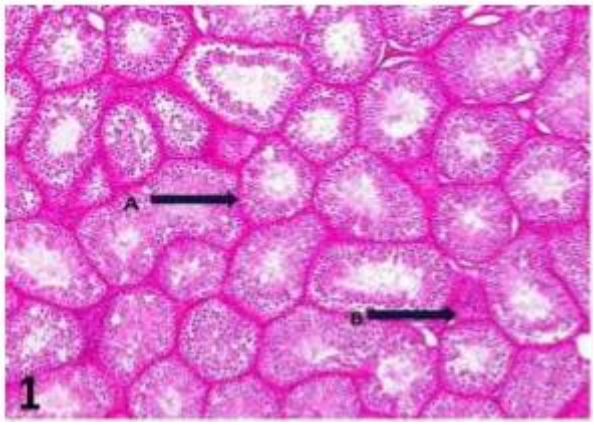

Fig-1: A photomicrograph of a section in the testis of control adult rat showing seminiferous tubules (A) separated byinterstitial tissue (B) (H&Ex200)

Sertoli cells had the distinction of being the only non-germinal cells within the wall of the seminiferous tubules. They were tall irregular columnar cells, which extended from the basement membrane. The cell borders were usually indistinguishable in routine stained preparations.

The nucleus was ovoid pale stained with finely dispersed chromatin granules and usually contained one or more prominent nucleoli which really distinguished sertoli cells from the spermatogenic cells within the seminiferous tubules. The position of the nuclei varied from the basement membrane.

The spermatogenic cells lay between the sertoli cells in an orderly manner and from four to six or eight layers occupied the space between membrane and the lumen. Directly on the basement membrane were the primitive germ cells or spermatogonia, which were spherical or cuboidal cells, their nuclei were spherical and rich in chromatin. These cells gave rise to primary spermatocytes.

Primary spermatocytes were the largest germ cells seen within the tubules, lying next to spermatogonia. They had large vesicular nuclei. Each cell gave rise by division to two secondary spermatocytes. Almost as soon as formed, each secondary spermatocytes divided to form two spermatids and therefore were seldomly seen.

The spermatids were the daughter cells of the secondary spermatocytes. They adjoined the lumen of the seminiferous tubules and were easily recognized by their small sizes and their location. They were first, small with spherical nuclei but soon became elongated and their nuclei took up apposition at the proximal end of the cell. They did not further divide but they profound changes in their structure gave rise to mature spermatozoa.

The interstitial tissue was found between the seminiferous tubules. It contained some collagenous fibers, blood vessels and several cell types including fibroblasts, macrophages and the specific interstitial cells of leydig, which were a marked feature of this tissue. The interstitial cells Leydig were large cells in which the eosinophilic cytoplasmic were clearly outlined and often appeared vacuolated because of the dissolved lipid droplets. The nuclei contained coarse chromatin granules and distinct Nucleoli (Fig., 2).

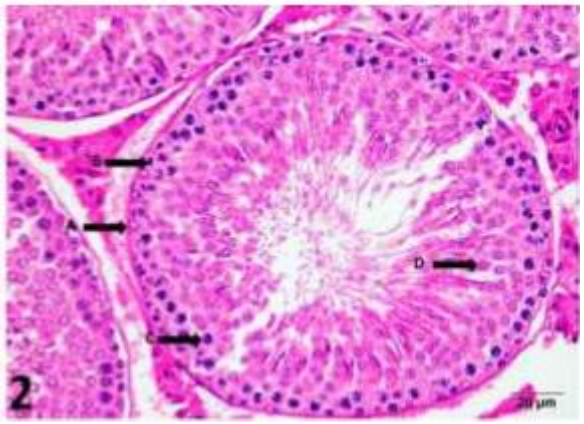

Fig-2: A photomicrograph of a section in the testis of control adult rat showing Sertoli cells (A), spermatogonia (B), primary spermatocytes (C), spermatids (D) .scale bar

Cinnamon Group

Light microscopic examination of the testis stained by H & E showed no changes in group II compared to group Irats (Fig., 3).

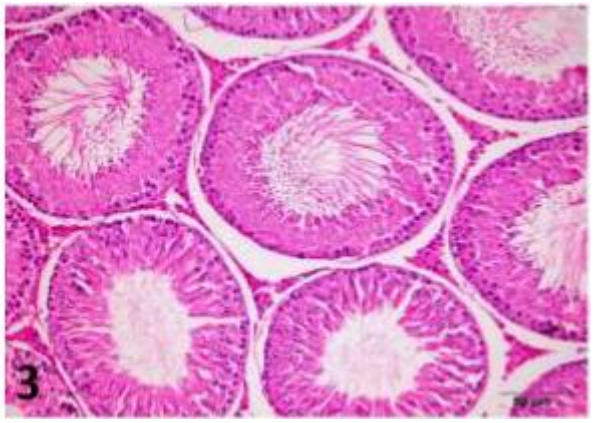

Fig-3: A photomicrograph of a section in the testis of a rat given cinnamon aqueous extract for 30 days showing no changes compared to control group (H&Ex200)

Hypercholesterolemia Group

Light microscopic examination of the testis stained by H & E showeddisorganized seminiferous tubules lined with vacuolated germinal epithelium and contained desquamated germ cells with darkly stained nuclei (Fig., 4). In addition, showed many seminiferous tubules with wide lumina and irregular outline (Fig., 5). Some time they form multicellular aggregates with multiple nuclei in a single mass of cytoplasm these aggregates resembled abnormal multinucleate giant cells . There was widening of the interstitial spaces because of shrinkage of the seminiferous tubules (Fig., 6).

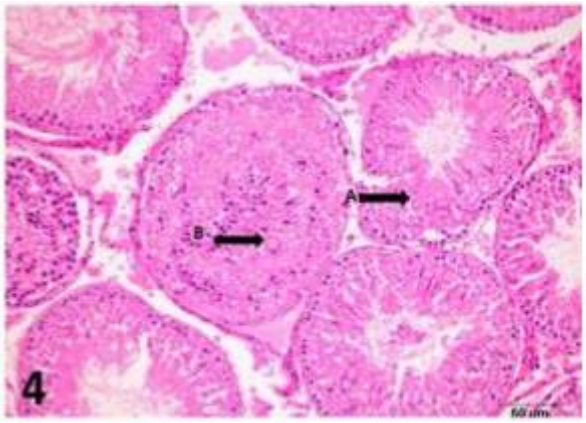

Fig-4: A photomicrograph of a section in the testis of a rat fed a high cholesterol diet for 30 days showing disorganized seminiferous tubules lined with vacuolated germinal epithelium (A) and contained desquamated germ cells with darkly stained nuclei (B) (H&Ex200)

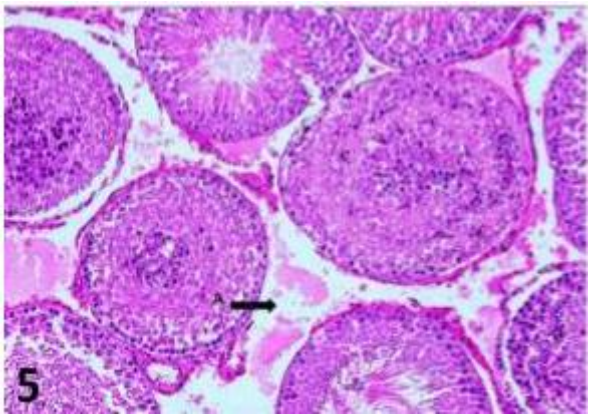

Fig-5: A photomicrograph of a section in the testis of a rat fed a high cholesterol diet for 30 days showing many seminiferous tubules with irregular outline (A). (H&Ex200)

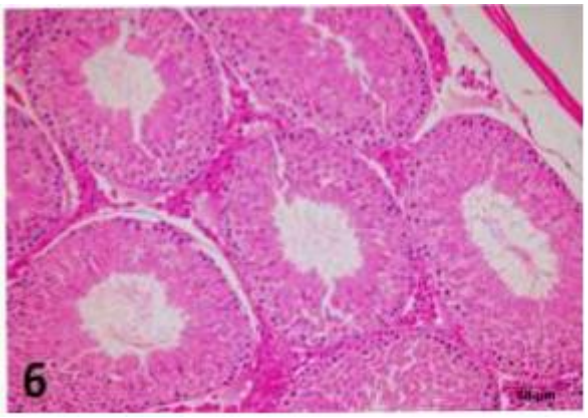

Fig-6: Aphotomicrograph of a section in the testis of a rat fed a high cholesterol diet for 30 days showing widening of the interstitial spaces because of shrinkage of the seminiferous tubules (H&Ex200)

Cinnamon and Hypercholesterolemia Group

Light microscopic examination of the testis stained by H & E showed the testicular tissue had nearly normal histological architecture. The cellular contents of the seminiferous tubules, germinal epithelium and sertoli cells, all were of normal shape and arrangement.

DISCUSSION

In the present study, we investigated the protective effect of cinnamon on the testis against high cholesterol diet of adult Wistar rats through biochemical and histopathological examination.

Hypercholesterolemia is known to have detrimental effects on male reproductive function. In previous studies, hypercholesterolemia has been connected with testicular dysfunction in male patients [11,23,24]. Further conclusions have been derived from investigations on hypercholesterolemic rats. Researchers found that sperm functionality and maturation, Leydig and Sertoli cells functions, and spermatogenesis were impaired following diet-induced hypercholesterolemia in animals [12-16]. In the present study, seminiferous tubules degeneration and subsequently impaired spermatogenesis was noted in hypercholesterolemic rats. This finding is consistent with that of the investigation by Shalaby and colleagues [17].

Results from serum lipid, cholesterol and lipoprotein status of HCD rats showed increased triglyceride, cholesterol serum, low-density lipoprotein (LDL), and decreased in the high-density lipoprotein concentration (HDL) compared to the three other groups these results are in accordance with Thiruchenduran *et al.*[8] in rats, Shimamoto and Sofikitis [13] in rabbits. The mentioned levels could affect the male rat fertility. The increased level of cholesterol in HCD rats (group III) indicated that, there

is an abnormal uptake of cholesterol from the provided HCD diet. The administration of cinnamon extract with the HCD diet resulted in reduction of cholesterol absorption as reflected by serum cholesterol levels. These results are in accordance with Martínez-Martos *et al.*[18] their study concluded that, hypercholesterolemic diet produced a significant increase in serum total cholesterol level compared to negative control rats. Several authors have also described an impaired testicular function with hypercholesterolemia. Thus, Tanaka *et al*[15] have investigated the effects of hypercholesterolemia on different parameters of testicular function, including serum-circulating testosterone in Sprague–Dawley rats, using a standard chow containing 2% cholesterol. They found, after 4 weeks of treatment, that serum cholesterol was significantly higher (206% that of controls), and serum testosterone was significantly lower (49% that of controls), and suggested that hypercholesterolemia is an independent risk factor for testicular dysfunction. In the same way, Feng *et al.*[19] have investigated the effects of diet-induced hypercholesterolemia on testosteronelevels and other parameters, in C57BL/6 mice, using a standard chow containing 15% fat, 1.25% cholesterol and 0.5% sodium cholate. After 8 weeks, they found also a significant increase of serum cholesterol (307% that of controls)and tryglicerides, and lower serum testosterone levels (8% that of controls).

Light microscopic examination of HCD group stained with H & E showed disorganized and shrinking seminiferous tubules lined with vacuolated germinal epithelium and contained desquamated germ cells with darkly stained nuclei, in addition to multiple nuclei in a single mass of cytoplasm, these aggregates resembled abnormal multinucleate giant cells . On the other hand, cinnamon treated group and HCD-cinnamon co-treated group showed testis as normal as negative control group testes, with seminiferous tubules have layers of spermatogenic cells undergoing successful spermatogenesis giving rise to mature.

All these changes revealed by light microscopic examination using H&E staining and anti Ki-67 antibody labeling in addition to the differences of testicular diameter and testicular weight might lead to the reduction of HCD male fertility because of the reduction of normal germ cells undergoing spermatogenesis, hence the reduction of sperm number and/ or sperm motility. The previous explanation is in agreement with Shimamoto and Sofikitis [13]. They reported that, that hypercholesterolemia has a detrimental effect on Leydig cell function, spermatogenesis, the epididymal sperm maturation process, and the overall sperm fertilizing capacity.

Our results revealed that, rats co-treated with HCD and cinnamon extract exhibited testicular structure and functioning seminiferous tubules similar to those of the control group. Altogether, it seems that cinnamon extract has a protective effect on the testes. It reverted the adverse effect produced by HCD by reducing the cholesterol transport. There are several investigations proposed that, the administration of cinnamon to mice positively affected the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased and plasma triglycerides were reduced [7]. Another study by Rahman *et al.*,[20] found a reduction in the total cholesterol, triglycerides, and low-density lipoproteins in rats administered cinnamon powder (15%) for 35 days. Additionally, cinnamon oils reduced the cholesterol levels in broiler chickens [21]. All those beneficial effects might due to antioxidant and cholesterol and lipid-lowering activity of cinnamon extract as previously described by Rao and Gan[22], they reported that, cinnamon has an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering activities.

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