

# Molecular-based identification of spermatogenesis quality improvement within two weeks induced by combination of intense exercise and cinnamaldehyde in Wistar male rats fed high fat diet

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## ABSTRACT

Exercise, low fat diet, or supplementations alone could show improvement in spermatogenesis quality after long period of times that could reach up to months especially in males who have high access to a high fat diet (HFD), so the current work was generated to induce this improvement in less amount of time, ~ 2 weeks, using intense exercise and cinnamaldehyde in Wistar male rats (WMR) fed an HFD. To initiate the experiment, 40 WMRs (fed an HFD for 4 weeks before the exposure to the experimental variables (EEV) and continued after that) were randomly divided into 4 groups in which a group received intense exercise and cinnamaldehyde (IECd), a group exposed to cinnamaldehyde alone (Cd), another group subjected to intense exercise alone (IE), and a group given the carrier of CD (CrCd). The EEV was lasted for 2 weeks, and the animals were subjected to measuring serum testosterone (ST), sperm counting (SpC) of motile and viable sperms, and inducible nitric oxide synthase (iNOS) gene expression (iNOSge) using real time-polymerase chain reaction (RT-PCR). The levels of the ST and SpC of motile and viable sperms were significantly ( $p < 0.05$ ) increased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of ST and SpC. The iNOSge levels were significantly ( $p < 0.05$ ) decreased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of iNOSge. This study indicates important effects of the intense exercise and cinnamaldehyde when applied together in improving the quality of spermatogenesis in Wistar rats that could be tested in the future on the human model.

**Keywords:** Cinnamaldehyde, high fat diet, intense exercise, spermatogenesis.

## INTRODUCTION

One of the most important conditions that affect the lifestyle of people around the world is infertility, and it places high economic, psychological, and health impacts on people. This may lead to huge stressful life that affects males and females especially within families that look for having babies (1). There are multiple factors that affect the fertility of human males such as environmental factors ranging from exposure to radiation, noisy and hot work environment, exposure to electromagnetic fields, and chemical contaminating substances. This can cause low fertility manifested by decreasing sperm counting (2). Moreover, some cases may have different causes that generate infertility in males such as genetic-based conditions, secondary-induced hypogonadism, obstructions in the seminal tracts, testicular tumors, urogenital congenital defects, and testicular injuries (3). There are some clinical tests should be done before heading to the laboratory methods to identify the presence of infertility. Clinicians may check the spermatic cord for the quality of vas deferens along with the size of testes in which most cases of infertility have small sizes of testes. Moreover, other genital parts should be inspected such as the epididymis to check the caput, corpus, cauda, and any obstructions in the ducts. For more definite diagnosis of infertility,

counting of normal motile sperms should be performed in laboratories. In addition, hormonal tests may be performed to identify the levels of LH, FSH, testosterone, and estradiol (4). Patients with infertility may be subjected to different treatment programs that may take long time periods, could reach months, to show some improvements. Hormonal therapies may need to check the hormonal profiles of patients before starting the treatment; however, such treatments are expensive and sometimes not efficient especially in idiopathic infertility (5,6). New era of treatments involving different lifestyles, healthy diets, and supplementations is promising. While some studies showed no improvements in infertility were noticed (7,8), others provided data of such improvements in the quality of sperms that took months to be induced (9,10). Some studies involved the use of some natural substances to induce improvements in the quality of sperms such as the use of maca products; however, these studies are limited, and such substances need months to show improvements (11). Exercise, low fat diet, or supplementations alone could show improvement in spermatogenesis quality after long period of times that could reach up to months especially in males who have high access to a high fat diet (HFD), so the current work was generated to

induce this improvement in less amount of time, ~ 2 weeks, using intense exercise and cinnamaldehyde in Wistar male rats (WMR) fed an HFD.

## Materials and methods

### Animals and experimental design

This work was done carefully complying with the ethics of animal care. The work included the use of 40 WMRs (6 weeks of age,  $240 \pm 5$  gm, fed an HFD at 21.4% crude fat for 4 weeks before the EEV and continued after that). At 10 weeks of age (the time point for the EEV), the weight was  $430 \pm 10$  gm. The animals were housed in  $22 \pm 2^\circ\text{C}$ , 60% humidity, and 15hrs of light. Food and water were accessed freely by the animals. The animals were randomly divided into 4 groups in which a group received intense exercise and cinnamaldehyde (IECd), a group exposed to cinnamaldehyde alone (Cd), another group subjected to intense exercise alone (IE), and a group given the carrier of CD (CrCd). The EEV was lasted for 2 weeks.

### Intense exercise

Five-days directly before the EEV, the rats were subjected to a small size treadmill, at 5° inclination, for familiarization to direction of running and staying away from the electrical stimulus grid, 0.5mA. The protocols for familiarization and IE, overload in intensity, were followed from (12). The IE continued for 2 weeks.

### Cinnamaldehyde exposure

In the case of Cd supplying, 0.9 mg/kg bwt of Cd was given orally every other to the IECd and the Cd groups. After the experiment time was done, the animals from all groups were sacrificed. Serum testosterone (ST) was measured using ELISA kits

(RapidBio Lab, CA) specific for rats and following the company protocol. Sperm counting in the epididymis (SpC) and inducible nitric oxide synthase (iNOS) gene expression (iNOSge) using real time-polymerase chain reaction (RT-PCR)

### Real time-PCR

RT-PCR was used to measure iNOSge using methods from (13). The total RNA from the testes was extracted using Biozol kit (Bioflux, Japan) and using the kit protocol. The process of reverse transcription was performed using 0.2  $\mu\text{g}$  of the total RNA in the SuperScript® First-Strand Synthesis System for RT-PCR (ThermoFisher, USA). The kit used for the RT-PCR master mix was Rotor-Gene Probe PCR Kit (QIAGEN, Germany) and following the kit protocol. The primers were followed from (13-15).

### Statistical analysis

Graphpad prism v7.0 was used to analyze data. Mean  $\pm$  SE was used to show data. One-way ANOVA was performed.  $P < 0.05$  was used.

### Results

The levels of the ST, figure 1, and SpC of motile and viable sperms, figure 2, were significantly ( $p < 0.0001$ ) increased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of ST and SpC. The iNOSge levels, figure 3, were significantly ( $p < 0.0001$ ) decreased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of iNOSge.

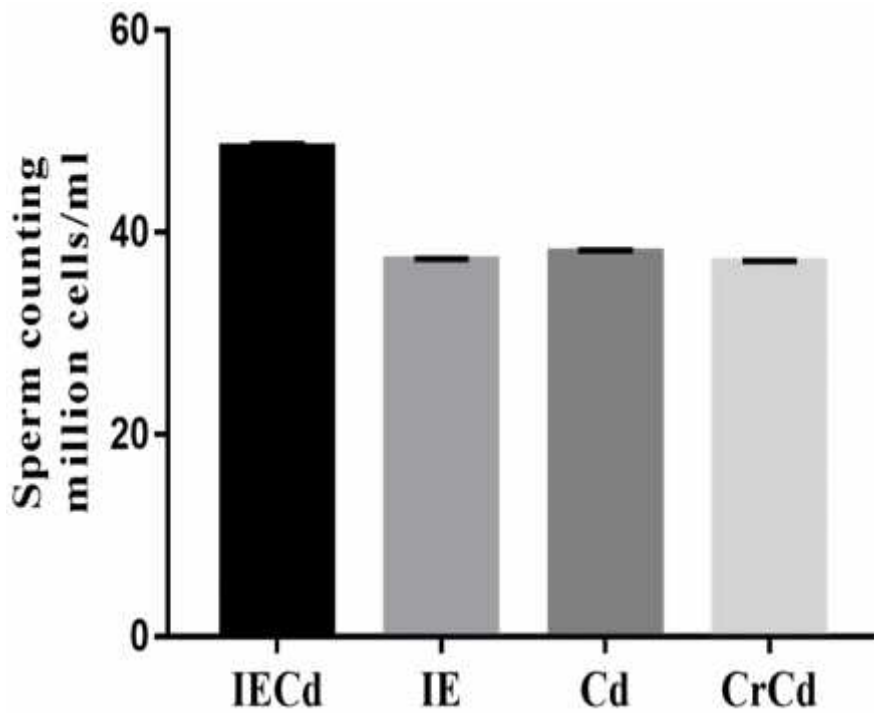


Figure 1: Serum testosterone levels. The levels were significantly ( $p < 0.0001$ ) increased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of ST.

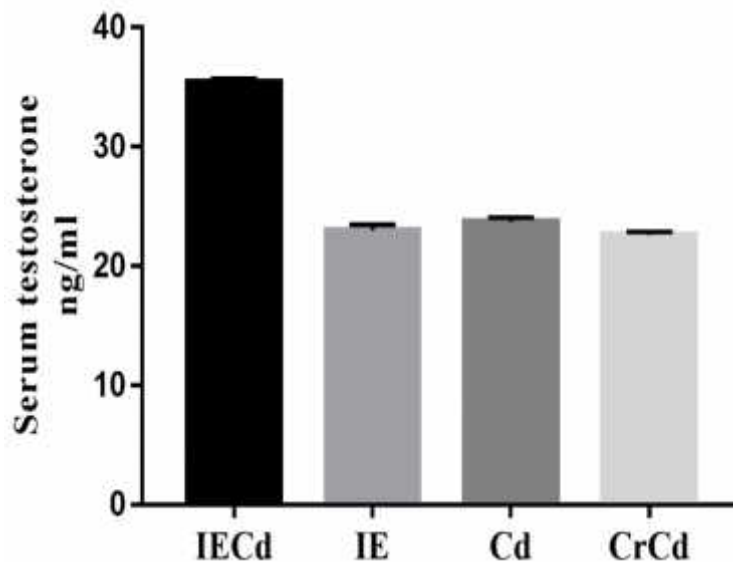
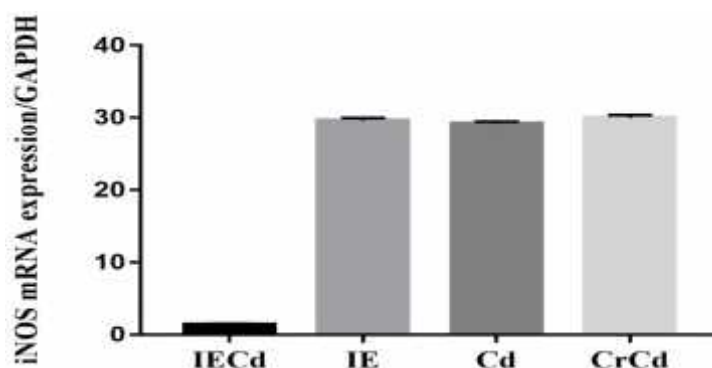


Figure 2: Sperm counting levels. The levels were significantly ( $p < 0.0001$ ) increased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of SpC.



**Figure 3: Gene expression of iNOS gene. The levels were significantly ( $p < 0.0001$ ) decreased in the IECd group when compared with those in the Cd, IE, and CrCd groups. No differences ( $p > 0.05$ ) were noticed between Cd, IE, and CrCd groups when tested for the levels of iNOSge.**

### Discussion

One of the most important conditions that affect the lifestyle of people around the world is infertility, and it places high economic, psychological, and health impacts on people. This may lead to huge stressful life that affects males and females especially within families that look for having babies (1). Studies showed variable degrees of treatments when using hormonal or supplemental therapies; however, these treatments may have limited effects and need long periods of time to improve infertility (16–18). In order to overcome these obstacles of treatments, replacements and new methods of treatments should be tested and followed. Here, the combination of IE and Cd revealed significant effects on improving the levels of serum testosterone and counting of motile and viable sperms in the rats after 6 weeks of feeding on an HFD. Some studies proved the effects of exercise alone on these levels, but these studies needed sometimes months to show such improvements (19). Some studies revealed increase these levels when used natural supplementation alone such as maca and Cinnamomum zeylanicum; however these supplements needed long periods of times that could reach up to months (20,21). In contrast to those studies, the current study revealed improvement in these factors in less than 2 weeks when used both IE and Cd together. Increasing the levels of gene expression of the iNOS gene were significantly lower in the IECd group than those in the other groups indicating synergistic effects of intense exercise and cinnamaldehyde in decreasing the levels of iNOS products. HFDs were shown to elevate the levels of iNOS gene expression and its products (22). iNOS was linked to decreasing testosterone production via inhibiting cholesterol

conversion into pregnenolone via the suppression to the CYP17A1 enzyme (23,24). IE and Cd worked together to reduce the effects of the HFD on generating more activity of iNOS and lead to produce more testosterone from the testes. This study indicates important effects of the intense exercise and cinnamaldehyde when applied together in improving the quality of spermatogenesis in Wistar rats that could be tested in the future on the human model.

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