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ORIGINAL ARTICLE

Effect of Cinnamon Bark Oil on Leydig Cell Count and Morphology along with Serum Testosterone Levels in Rats after Cadmium Induced Testicular Toxicity

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ABSTRACT

Cadmium is a toxic heavy metal that causes number of health issues.

Purpose: To demonstrate the effect of cinnamon bark oil on Leydig cell count and morphology with measurement of serum testosterone levels.

Study Design: Experimental study.

Methodology: Healthy male wistar rats (n=30) were taken and divided into 3 groups with n= 10. Group A functioned as control. Cadmium chloride was administered to the rats to induce testicular toxicity in group B. Group C was the treatment group. Animals were euthanized on day 15. Leydig cell count and morphology was done after haematoxylin and eosin staining of the testicular tissue sections. Serum testosterone level was done by ELISA.

Statistical analysis: Data analyzed by SPSS 22.0v.

Results: Cadmium chloride was observed to significantly reduce leydig cell counts and serum testosterone levels in group B. No significant effect was observed on the morphology of the leydig cells. Cinnamon bark oil significantly improved the leydig cell count. Serum testosterone levels were observed to increase after the cinnamon bark oil administration. **Conclusion:** This study clearly showed that cinnamon bark oil has protective effect on the cadmium induced testicular toxicity.

Key Words: Enzyme Linked Immuno-sorbent Assay, High Density Lipoprotein, Cinnamon Bark Oil and Optical Density.

INTRODUCTION

Cadmium (Cd) is a toxic heavy metal thus causing multiple health issues. In near past, its uses has changed that include mainly electroplating of metals, in pigments and stabilizers for plastics. Almost its consumption (55%) is by cadmium-nickel battery manufacture with assumption that its use will expand in future. Nowadays it is a vital part of modern technology having countless benefits in electronics, communications, power generation and aerospace industries[1].

Literature review revealed that elemental Cadmium and its compounds produce harmful effects on human health causing cancer. Its major sources include contaminated drinking water, food supplies, industrial pollutants and tobacco or cigarette smoking. Its estimated half-life among humans is >20 years. Its pathogenesis include accumulation in tissues like lung, liver, kidney, cardiovascular and reproductive system thus damage them.

Cadmium causes severe damage to ovaries and testes, which are highly sensitive to Cadmium toxicity. It has been demonstrated in previous studies that Cadmium exposure increased oxidative stress producing imbalance of hormones controlling the reproductive system. It also increased production of pro-apoptotic proteins. Cadmium toxicity is reported to lead to decreased sperm motility and low plasma testosterone levels in experimental animals. Cadmium damage the male reproductive organs by causing testicular degeneration and seminiferous tubule damage[2].

For centuries, Cinnamon has been in use for treating ailments as a herbal medicine. Literatutre review revealed that it has almost 250 species being scattered globally in the form of trees.[3] Its major constituents include cinnam-aldehyde and *trans*-cinnam-aldehyde. They are present in the essential oils thus adding fragrance and biological activities to them[4].

Its essential oil holds antimicrobial properties thus used as a preservative in food industry.[4] It also plays vital role in treatment of type-II diabetes, hyperlipidemias as revealed by studies.[5,6]. Thus investigations showed the potential of cinnamon for its use as a natural oral agent having both hypoglycemic and hypo-lipidemic effects.

In previous studies it was observed that cinnamon bark extract improved glucose metabolism and lipid profile in diabetic rats[7]. It has been reported previously that ethanolic extract of cinnamon bark improved reproductive organ weight and sperm quality[8]. Oral administration of cinnamon extract elevated the serum testosterone level, improved sperm motility and alleviated testicular degenerative changes in diabetic rats. Researchers described the improvement in fertility parameters to antioxidant property of cinnamon[9].

However, there is no reported study to document the effect of cinnamon bark oil on Cadmium induced testicular toxicity. The present investigations were therefore planned

to evaluate the effect of cinnamon bark oil on Cadmium induced testicular toxicity in albino rats.

OBJECTIVE

To demonstrate the effect of cinnamon bark oil on Leydig cell count and morphology with measurement of serum testosterone levels.

METHODOLOGY

The experimental study was carried out at the Experimental and Research Laboratories of University of Health Sciences, Lahore after obtaining approval from Ethical Committee of UHS for research. Healthy male wistar rats (n=30) were taken, 6-8 weeks of age and weighing 200-220 gm and were divided into 3 groups. Group A functioned as control. Cadmium chloride was administered to the rats to induce testicular toxicity in group B. Group C was the treatment group.

Group-A: Rats were given 1ml/100gm of distilled water and 0.5ml/100gm of olive oil by oral gavage, daily for 14 days.

Group-B: Rats were given 1.5mg/100gm of $CdCl_2$ dissolved in 100ml of distilled water and 0.5ml/100gm of olive oil by oral gavage daily for 14 days[10].

Group-C: Rats were given 1.5mg/100gm of $CdCl_2$ dissolved in 100ml of distilled water and 100mg/kg of Cinnamon bark oil (CBO) in 10ml of olive oil by oral gavage daily for 14days. The dose of CBO was given 2hrs after Cadmium.

At the end of the experiment each animal was given ether anesthesia. Sterilized instruments were used for dissection. A vertical midline incision on skin was given from chin to pubic symphysis. The incision was extended laterally at the midpoint on each side and the skin and muscles were reflected. The sternum was bissected and retracted laterally to expose the heart. Cardiac puncture was performed to obtain 3ml of blood for calculating testosterone level. The blood was transferred to the vacutainer that was centrifuged in the machine (EBA-20 Heittich), at the speed of 3000 rotations per minute, for 10 minutes. The cleared serum was collected with the help of a micropipette in a sterile Eppendorf for testing of testosterone levels at a later date. Serum testosterone levels were assessed by ELISA using a commercial kit purchased from Elabscience[11]. Eosin and haematoxylin stained sections were examined under X10 and X40 magnifications for observing the morphology of leydig as normal, degenerated or hyperplastic[10].

STATISTICAL ANALYSIS: Data analyzed by SPSS 22.0v. Quantitative variables were presented as means ± SD while qualitative variables were presented by frequency and percentage (%). Results were tabulated and statistical analysis and comparisons among various groups carried out by applying Chi Square test. P-value <0.05 was taken as significant.

RESULTS

In group A, the Leydig cell count/mm^2 ranged between 15.80-16.80 with a mean of 16.20±0.30/mm^2; in group B, the Leydig cell count ranged between 12.83-13.96 with mean of 13.42±0.43/mm^2 and in group C, the Leydig cell count ranged between 13.26-14.46 with a mean of 14.51±1.25/mm^2 were presented in table-1.

Table-1: Comparison of Leydig Cell Count among Enrolled Groups as Mean ± SD

Parameter	Group-A Mean ± SD	Group-B Mean ± SD	Group-C Mean ± SD
Mean Leydig cell count(per mm^2)	16.20±0.30	13.42±0.43$^{\#\#\#}$	14.51±1.25*

*Statistically significant (P-value<0.05), # Highly significant (P-value<0.001)

The histological features of Leydig cells were comparable between all groups with insignificant P-value as shown in table-2.

Table-2: Comparison of Percentage Leydig Cell Parameters among Enrolled Groups

Leydig cell parameters	Percentage			
	Group A n = 10	Group B n = 10	Group C n = 10	Total 30
Normal	10(100%)	10(100%)	10(100%)	30(100)
Degenerated	0(0.00%)	0(0.0%)	0(0.0%)	0(0.0%)
Hyperplastic	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

Testosterone levels were reduced in the group B with the P value < 0.01. Cinnamon bark oil administration in the group C significantly increased the levels with the P value < 0.001 (table3).

Table-3: Comparison of Mean Values of Serum Testosterone Levels among Groups

Serum testosterone levels (ng/ml)	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD
	4.66±1.49	1.39±0.75$^{\#\#}$	3.50±1.52***

Statistically significant (P-value<0.05), # Highly significant (P-value<0.001)

DISCUSSION

Male infertility is one of the important problems nowadays and affects almost about 10% of couples in the society. Cadmium is an environmental toxin that adversely affects all the organs especially testicles. It disrupts the free radicle scavenging systems and induces apoptotic cell death in male reproductive cells especially the leydig cells. Cadmium treated rats are reported in literature with low testosterone levels[12]. In one study it was observed that the testicular weight and testosterone levels were significantly reduced after cadmium administration[13]. Cadmium is responsible for histopathological changes like

degeneration, necrosis, disorganisation, desquamation, reduction in germinal cells, spermatogenic arrest[10]. Hence, it is widely accepted to induce testicular toxicity. Cadmium induced apoptotic cell death in the leydig cells is via ROS/JNK signalling pathway[14]. These findings were consistent with our study as the mean number of Leydig cells was significantly decreased in $CdCl_2$ treated rats in group B.

Cinnamon bark oil is reported to possess antioxidant, anti-inflammatory and anti-tumorigenic effects. It is also reported to preserve and restore the sperm health and fertility[13]. In previous experiment, it was seen that after the administration of cinnamon powder to male healthy rats, there was an improvement in the sperm quality parameters and serum testosterone levels. These effects were associated with increased level of glutathione , superoxide dismutase and catalase[15].

Cinnamon bark oil was able to ameliorate this cell damage as is evident from the preservation of the leydig cell count in the treatment group of our study (Table-1). Leydig cells produce testosterone. Due to the preservation of leydig cell counts in the treatment group there was a consequent preservation of the serum testosterone levels seen in the current study (Table-3).

Drugs and chemical that are reported to ameliorate the testicular toxicity demonstrate normalisation of the serum testosterone levels. Herbal blend of cinnamon, ginger and clove was observed to ameliorate testicular damage and conserved the architecture. It was also observed to restore the reproductive function[15]. These results are consistent with our study.

However, in this study no significant effects were seen on the morphology of the leydig cells after the administration of cadmium or cinnamon bark oil. During our experiment we administered our drugs for only 14 days. This duration of time was may be not sufficient to produce the morphological changes in the leydig cells. In most of the studies the effect of cadmium is studied after a longer duration of administration i.e 28 days[12] or after administering a very high single dose of 10mg/kg/bw[13].

Further studies are needed to elucidate the molecular pathways that are responsible for these effects of cinnamon bark oil. Techniques like western blotting could have been used to check for the expression of antioxidant enzymes and pro apoptotic proteins. Administering cinnamon bark oil for longer period of time should be done to check for its subacute, subchronic and chronic toxicity.

Limitations: Our limitations include small sample size with limited financial and human resources. No genetic workup was done.

CONCLUSION

This study clearly showed that cinnamon bark oil has protective effect on the cadmium induced testicular toxicity hence can be employed as treatment tool against its toxicity.

Author's Contribution: AM &HM : Conceptualized the study, analyzed the data, and formulated the initial draft.
AA : Contributed to the histomorphological evaluation.
ZSJ & TL: Contributed to the analysis of data and proofread the draft.

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