ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Role of Nigella Sativa oil in alleviating increased reproductive hormones and some inflammatory mediators caused by cadmium toxicity in rat testes

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Manuscript Info

Manuscript History:

Received: 25 July 2014
Final Accepted: 26 August 2014
Published Online: September 2014

Key words:
cadmium testicular toxicity, male reproductive hormones, inflammatory markers, Nigella Sativa oil, rats.

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Abstract

Background: Environmental pollution with heavy metals represents global problem. One of these heavy metals is the cadmium chloride that emits from many industries such as cement plants and it also comes from smoke, batteries and others. Exposure to cadmium affects most of the body's organs. Testes seem to be greatly affected by cadmium since it has a unique vascular system. **Objective:** Therefore the present study aimed to evaluate the protective effect of Nigella Sativa oil against cadmium-induced testicular toxicity in rats. **Design:** Thirty male adult albino rats were used in this study. They were equally divided into three groups; group I "control group", group II "cadmium treated group" and group III "cadmium and Nigella Sativa oil treated group". **Results**: Administration of cadmium chloride (5 mg/kg body weight for four successive weeks) in rats resulted in significantly increased serum levels of testosterone, LH and FSH. It also led to significant increase in the testicular tissue homogenate contents of TNF-α and IL-1β. Administration of Nigella Sativa oil (2 ml/kg body weight) with cadmium chloride (5 mg/kg body weight) for four successive weeks in rats succeeded in ameliorating these cadmium induced changes.

Conclusion: It can be concluded that cadmium chloride testicular toxicity in rats led to disturbance of serum levels of male reproductive hormones and increased testicular contents of inflammatory markers. The use of Nigella Sativa oil succeeded in ameliorating these changes.

Introduction

Cadmium is a heavy metal and a major environmental toxicant. Its elemental form occurs naturally in the earth's crust. It is commonly found in combination with other element such as oxygen (cadmium oxide), sulfur (cadmium sulfate), chloride (cadmium chloride), and carbon (cadmium carbonate). The general population is exposed to cadmium via contaminants found in drinking water and food (WHO, 2000; ATSDR, 2008), while occupational exposure to cadmium usually takes place during mining or manufacturing of batteries and pigments that utilize cadmium. Industrial activities, such as smelting and refining of metals, and municipal waste incineration also release cadmium to the atmosphere as cadmium oxide, chloride or sulfide. Cadmium is ranked the 7th toxicant in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry (ATSDR, 2007).

This heavy metal has the potential to affect reproduction and development in many different ways, and at every stage of reproductive process (Thompson and Bannigan, 2008). Testes seem to be greatly affected by cadmium since it has a unique vascular system (Santos et al., 2004 & 2005).

Nigella Sativa is an annual herbaceous plant, sometimes known as black seed, black cumin or habit EL-Baraka. It has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of various diseases for over 2000 years. In recent years, the pharmacological investigations confirmed that most of the therapeutic properties of this plant are due to thymoquinone which is major active component of Nigella sativa oil (Gali-Muhtasib et al., 2006). Previous data suggest that the seeds oil, and thymoquinone, exhibited spermioprotective effect against testes damage.

Since exposure to cadmium is greatly increased all over the world especially in industrial areas, this leads to increased cadmium toxicity that affects most of the body's organs. No available studies concerning the protective effect of Nigella Sativa oil against cadmium induced rat testicular toxicity. So as a new trial, the present study aimed to examine the ameliorating effects of Nigella Sativa oil on cadmium chloride induced testicular toxicity in rat.

Materials and Methods

Chemicals

Cadmium chloride (99.0%) was purchased from Loba Chemie Company (India). Nigella Sativa oil was purchased from El-Captain Company (Cap-pharm, Egypt) for extracting nutriment oils, natural herbs and cosmetics.

Experimental animals and design

Thirty male adult albino rats weighing (120 ±150 g) were obtained from the animal house, Helwan, Egypt. They were kept under suitable conditions for one week for adaptation. They were maintained in stainless steel cages in a well-ventilated animal house at normal temperature (22 ± 5 ℃) under 12:12 h light dark cycle. They were fed with standard laboratory diet and given water ad libitum. The rats were maintained under standard conditions in an animal house as per the guidelines of Beni-Suef University Committee for the Purpose of Control and Supervision on Experiments on Animals. The rats were randomly divided into three equal groups (10 rats each). Group I (control group) was orally administered distilled water by stomach tube daily for four successive weeks. Group II (cadmium treated group) was orally administered cadmium chloride at a dose of 5 mg /kg body weight (EL- Demerdash et al., 2004) dissolved in distilled water by stomach tube daily for four successive weeks. Group III (cadmium plus Nigella sativa oil treated group) was orally administered the same previous dose of cadmium chloride and then Nigella sativa oil at a dose of 2 ml/ kg body weight (Mohamadin et al., 2010) by stomach tube daily for four successive weeks.

Sampling and biochemical analyses

Rats were sacrificed after 4 weeks and blood samples were collected in clean dry centrifuge tubes. They were left for 20 minutes at room temperature to clot. They were centrifuged at 1000 X g for separation of blood serum. The serum samples were separated in Eppendorf tubes and stored at − 20°C until used for the biochemical assays. Serum levels of testosterone, LH and FSH were measured by using enzyme-linked immunosorbent assay (ELISA) kits from Kamiya Biomedical Company (Washington, USA) following the instructions of the manufacturer.

The testes were removed and dissected free from the surrounding fat and connective tissue. 0.5 g of each testis was homogenized in 5 ml of phosphate buffered saline (pH 7.4), centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was used for determination of TNF-alpha and IL-1β. TNF-alpha was measured by rat TNF-α Quantikine ELISA kit (R&D Systems, Inc. USA) according to instructions of the manufacturer. IL-1β was measured by ELISA kit from Kamiya Biomedical Company (Washington, USA) following the instructions of the manufacturer.

Statistical analysis

The values are expressed as mean ± SEM. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test using Graph Pad Instate software (version 3). Differences were considered significant at $P<0.05$.

Results

Effect of Nigella Sativa oil on changes in serum levels of reproductive hormones induced by cadmium chloride toxicity in rat.

Measurement of the serum levels of each of testosterone, LH and FSH in the rats of the three tested groups, revealed significant elevation of their levels in serum of the rats of cadmium treated group compared to those of the rats of control group. Coadministration of nigella sativa oil and cadmium chloride significantly decreased their levels in the NSO & Cd treated group as compared to those of Cd treated group as obviously seen in table 1 and the percentage of improvement is illustrated in fig. 1.

Table (1): Effect of Nigella Sativa oil on serum levels of testosterone, LH and FSH in rats exposed to cadmium chloride toxicity.

	Testosterone (pg/ml) "% change from control group"	LH (pg/ml) "% change from control group"	FSH (ng/ml) "% change from control group"
Group I (Control group)	117±11.6 "0"	138.39±9.1 "0"	2.31±0.45 "0"
Group II (Cd treated group	616.88±69.29*** "427.24"	1126.48±288.69* "713.90"	13.24±2.93* "473.16"
Group III (NSO & Cd treated group)	285.23±15.54[a] "122.89"	309.15±13.76[b] "123.39"	6.35±0.89 "174.89"

Cd means cadmium, NSO means Nigella Sativa Oil

***significantly different at $p < 0.001$ from group I and * significantly different at $p < 0.05$ from group I.

[a] significantly different at $p < 0.01$ from group II.

[b] significantly different at $p < 0.05$ from group II.

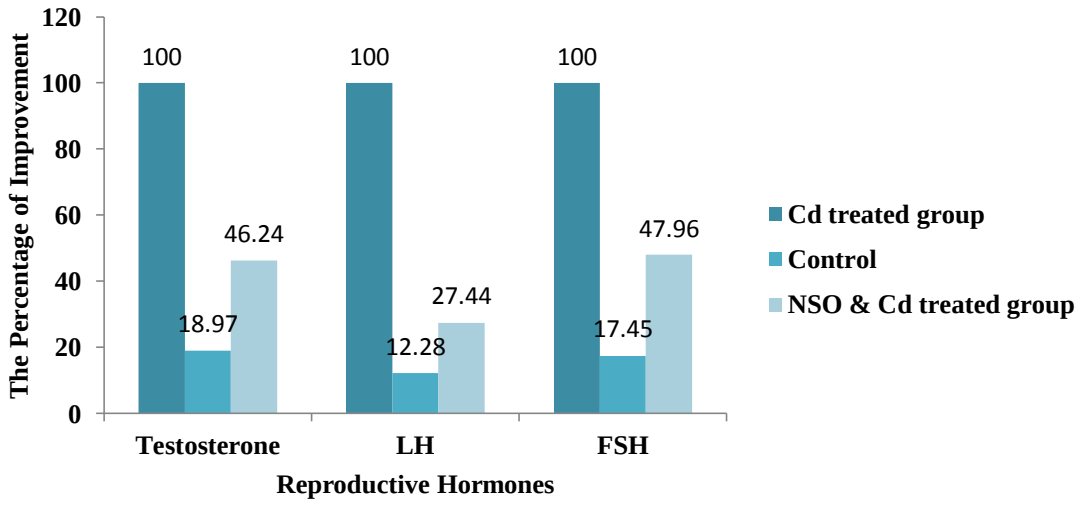

Fig.(1): The percentage of improvement of serum levels of testosterone, LH and FSH in rats group treated with Nigella Sativa oil compared to cadmium chloride treated group.

Effect of Nigella Sativa oil on changes in testicular homogenate contents of some inflammatory mediators induced by cadmium chloride toxicity in rats

Measurement of testicular homogenate contents of TNF-α and IL-1β of the rats of the three tested rat groups showed a significant increase in these two cytokines in Cd treated group compared to those of the control group. On the other hand, administration of Nigella Sativa Oil with cadmium chloride led to significant decrease of these two cytokines in NSO & Cd treated group in comparison with those of Cd treated group as clearly seen in table 2 and the percentage of improvement is illustrated fig. 2.

Table (2): Effect of Nigella Sativa oil on testicular TNF-α and IL-1β levels in rats exposed to cadmium chloride induced toxicity.

	TNF-α (pg/mg tissue)	IL-1β (pg/mg tissue)
Group I (Control group)	28.8 ± 3.93 **"0"**	28.93 ± 1.55 **"0"**
Group II (Cd treated group	101.87 ± 9.25*** "253.72"	88.63 ± 10.02*** "206.36"
Group III (NSO & Cd treated group)	68.53 ± 2.37** [a] "137.95"	51.67 ± 5.68 [a] "78.60"

Cd means cadmium, NSO means Nigella Sativa Oil

***significantly different at $p < 0.001$ from group I and ** significantly different at $p < 0.01$ from group I.

[a] significantly different at $p < 0.05$ from group II.

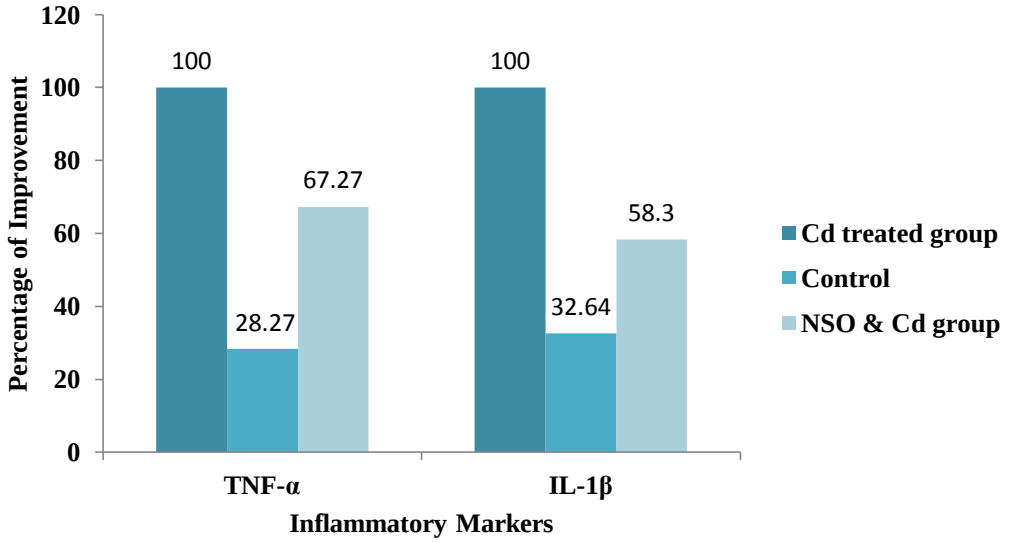

Fig.(2): The percentage improvement of testicular TNF-α and IL-1β levels in rats group treated with Nigella Sativa oil compared to cadmium chloride treated group.

Discussion

Exposure to Cadmium reduces male fertility in both human beings and rodents (Benoff et al., 2000), as low as 1 to 2 mg/kg body weight exposure can cause testicular damage without pathological changes to other organs. In male rodents, it is well established that cadmium is a known endocrine disruptor by affecting the synthesis and/or regulation of several hormones. It significantly alters the circulating levels of several hormones (e.g., testosterone, LH, FSH) (Lafuente et al., 2004).

Previous studies demonstrating that steroidogenic disruption in Leydig cells is likely to be an initial target of cadmium toxicity as an endocrine modulator. Cadmium also decreased steroidogenic acute regulatory protein (StAR), LH receptor and cAMP levels in the testis (Gunnarsson et al., 2007). Cadmium can also modify hormone levels by affecting the hypothalamic–pituitary–testicular axis in different aspects, not only via its effects on Leydig cells, but also through its effect on the circadian pattern release of noradrenaline, a regulator of hypothalamus hormone secretion, which resulted in changes in the daily pattern of plasma testosterone and LH levels (Lafuente et al., 2004). In addition, plasma levels of pituitary hormones (e.g., LH, FSH, prolactin, ACTH) were also changed after cadmium exposure (Lafuente et al., 2003).That result agreed with our results which revealed that cadmium toxicity induced significant increase in the serum levels of testosterone, LH and FSH. Coadministration of Nigella Sativa oil with cadmium succeeded in ameliorating the cadmium-induced elevation of the serum levels of these hormones (Table 1 & Fig. 1).

Cadmium is one of the inflammation-related xenobiotic and its exposure to the tissues is often accompanied with infiltration of inflammatory cells. TNF-α, a cytokine produced by activated macrophages in response to pathogens and other injurious stimuli, is a necessary and sufficient mediator of local and systemic inflammation (Tracey, 2002). TNF-α amplifies and prolongs the inflammatory response by activating other cells to release both cytokines such as IL-1, and mediators such as eicosanoids, nitric oxide and reactive oxygen species, which promote further inflammation and tissue injury (Tracey, 2002). Our results revealed an increased in the testicular tissue homogenate contents of both inflammatory markers, TNF-α and IL-1β. The results of previous studies, come in agreement with the present study. They demonstrated that cadmium toxicity was associated with alteration in inflammatory markers as evident by significant increase in testicular homogenate levels of TNF-α and IL-4 (Al-Azemi et al., 2010).
The anti-inflammatory activity of black cumin (*Nigella sativa)* and its main component thymoquinone is well known (Salem, 2005). Thymoquinone as a novel inhibitor of proinflammatory pathways provides a promising strategy that combines anti-inflammatory and proapoptotic mode of action (Chehl et al., 2009). Tekeoglu et al. (2006) detected thymoquinone anti-inflammatory effects on experimentally-induced arthritis by methotrexate in rats as decreased levels of TNF-α and IL-1β in circulation were observed. That was achieved in our study as elevated testicular homogenate levels of TNF-α and IL-1β in cadmium chloride group was significantly reduced due to administration of Nigella Sativa oil (Table 2 & Fig. 2).

Conclusion:

It can be concluded that cadmium chloride testicular toxicity in rats led to increase of serum levels of rats' male reproductive hormones and increased testicular contents of inflammatory markers (TNF-α and IL-1β). The Coadministration of Nigella Sativa oil with cadmium succeeded in ameliorating these changes.

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