

The effects of essential oil of *Lavandula angustifolia* on sperm parameters quality and reproductive hormones in rats exposed to Cadmium

Masoud Hamidi^a, Mojtaba Ziaee^b, Masoud Delashoub^c, Mahdi Marjani^d, Fatemeh Karimitabar^a, Arash Khorami^e, Nayeb Ali Ahmadi^{f*}

^aYoung Researchers and Elite Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran.

^bCardiovascular Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

^cDepartment of Anatomy, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

^dDepartment of Clinical Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran.

^eDepartment of Pharmacology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran.

^fProteomics Research Center, and Department of Medical Laboratory Technology, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Male infertility evaluation is important to recognize an origin and provide treatment if the etiology is correctable. The aim of the present study was to investigate the protective role of *Lavandula angustifolia* in impaired spermatogenesis caused by cadmium chloride in male rats. 36 male Wistar rats were allocated in 6 groups. Cadmium chloride was injected for 3 consecutive days (0.5 mg/kg) in CdCl₂ treated groups. Before CdCl₂ administration, pre-treatment with *L. angustifolia* was conducted for 14 consecutive days. Sex hormones level, malondialdehyde (MDA) and total antioxidant concentration as well as sperm parameters were investigated. Increase in the concentration of MDA and decrease in total antioxidant level were observed following CdCl₂ administration. Accordingly, the sperm viability as well as other sperm characteristics was decreased. In the case of sex hormones, the testosterone and LH levels were decreased and the concentration of FSH was increased (P<0.001). Pre-treatment with *L. angustifolia* significantly decreased the MDA level and increased total antioxidant, LH and testosterone levels. Pre-treatment with *L. angustifolia* also improved semen parameters which were impaired by CdCl₂ administration. Together, these results confirm that *L. angustifolia* pretreatment could protect spermatogenesis in male rats with CdCl₂ administration.

*Corresponding Author: Nayeb Ali-Ahmadi, E-mail: nayebalia@yahoo.com

Introduction

Infertility is one of the major health problems in life, and approximately 30% of infertilities are due to a male factor [1-2]. Additionally no identifiable cause can be found in over 25% of infertile males [3]. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production [4].

Environmental pollutants, including heavy metals are important factors responsible for oxidative stress production. In toxicities by heavy metals, particularly lead, cadmium, arsenic and mercury constitute serious threats to human health [5-6]. Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, and fertilizer industries, and cigarette smoke [7]. With increasing production and utilization of Cd, not only industrial workers but also the general population is exposed to the toxic effects of Cd. This has been found to produce various pathological conditions like hepatic and renal dysfunction, testicular damage, respiratory and nervous system disorders [8-9]. Cd has been reviewed by the International Register of Potentially Toxic Chemicals of the United Nations Environment Program [5]. Varieties of experiments [10-12] have suggested that Cd causes oxidative damage to cells. Cd has been demonstrated to stimulate free radical production.

Cd-induced testicular pathogenicity includes severe hemorrhage, edema, necrosis and atrophy, as well as reduction in counts and motility of sperm and decreased the testosterone concentrations in plasma and testes [9, 13-14]. This toxic metal is also carcinogenic and is deleterious to reproductive process, causing retardation of growth, sterility and embryotoxic effects [15].

Excessive production of free radicals or reactive oxygen species (ROS) can damage sperm, and ROS have been extensively studied [16] as one of the mechanisms of infertility [17]. Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major ROS present in seminal plasma [18].

Moreover, it has been shown that various antioxidants, vitamin A, B, C, and E in diet and antioxidant defense systems protect cells from Cd-induced toxicity [19-20]. Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men. Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important [19].

The use of herbal medicines has recently gained popularity in Europe and the United State [19]. The antioxidant capacity of phenolic compounds, flavonoids, and foods rich in these compounds, has been repeatedly demonstrated in various *in vitro* and *in vivo* systems [19]. World Health Organization (WHO) estimates that more than 80% of the world's population is dependent (wholly or partially) on plant-based drugs [21].

Lavandula angustifolia (Lamiaceae), locally known as 'Osto khuddous', is cultivated throughout Europe as well as in different parts of Iran [22]. The fresh and dried flowers of this plant are frequently used in traditional Mediterranean cuisine as an additive [23]. In recent years *L. angustifolia* flowers exhibit such various biological and pharmacological activities as anti-tumour, anti-inflammatory, antihistaminic, antidiabetic, and antimicrobial activity and modulating the central nervous system [24-25].

The present study was aimed at investigating the possible beneficial effects of *Lavandula angustifolia* essential oil as a source of natural antioxidants to aid the sperm parameters and modulate follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and oxidative stress in rats.

Materials and methods

Preparation of plant extracts

Aerial parts of *Lavandula angustifoli* were purchased by a botanist from a traditional store. The dried aerial parts of the plant (100 g) were subjected to hydrodistillation by using a Clevenger apparatus for 3 h. The oils were dried over

anhydrous Na₂SO₄ and stored in seal vials in dark place at 4 °C.

Experimental animals

Adult Wistar albino male rats (n=36) were included in the present study. The rats were 8 weeks old and weighing 250-270 g each. All rats were housed in individual cages, 6 rats per cage, and given standard diet and tap water ad libitum. They were obtained from animal facility of Pasture Institute of Iran. Male rats were housed in temperature controlled rooms (25 °C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care.

The rats were divided into six experimental groups, each consisting of 6 animals and were treated as follows: Group 1 – control rats (treated intraperitoneally (i.p.) with (0.3 mL) isotonic saline for 3 days) ; Group 2- sham (received 20 mg/kg *Lavandula* essential oil for two weeks); Group 3 – CdCl₂ (received 0.5mg CdCl₂/kg body weight in 0.3 ml isotonic saline for three days, i.p.); Group 4- *Lavandula* + CdCl₂ (received 5 mg/kg *Lavandula* essential oil for two weeks and in last three days 0.5mg CdCl₂/kg body weight); Group 5 and 6 – *Lavandula* + CdCl₂ (received *Lavandula* 10 and 20 mg/kg body weight followed by CdCl₂ administration as previously described. The number of injections synchronized in different group with saline administration. Body weight daily intake of food and water were determined several times per week throughout the study.

Surgical procedure

In fifteenth day, the Pentobarbital sodium (40 mg/kg) was administered for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. The intestines were shifted over to the left and located the widest part of the posterior vena cava. A 25 gauge needle was inserted into the vein and the blood draw slowly. The blood was centrifuged at 2200 rpm for 10 min at 10 °C and the separated plasma

was stored at –80 °C for subsequent analysis. Thereafter testis and epididymis in control and experimental groups were immediately removed [17].

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin. After 5 min incubation at 37 °C (with 5% CO₂), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method [26]. Both quantitative sperm motility (percentage of motile spermatozoa) and qualitative sperm motility (motility grade; from 0 to 4) were assessed [18, 27].

Total Serum FSH, LH total testosterone hormone measurements

Serum concentration of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA) (Izotop Company Ltd., Budapest, Hungary). Rat FSH / LH kits obtained from Isotope Company, Budapest, Hungary, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2ng/ml and 0.14ng/ml for FSH and LH respectively. Serum concentration of total testosterone was measured by using a double antibody RIA kit from immunotech Beckman Coulter Company-USA. The sensitivities of hormone detected per assay tube were 0.025ng/ml.

Total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentration measurement in serum

A TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute-China. According to this method, the antioxidant defense system, which consists of enzymatic and non-

enzymatic antioxidants, is able to reduce Fe³⁺ to Fe²⁺. TAC was measured by the reaction of phenanthroline and Fe²⁺ using a spectrophotometer at 520 nm. At 37 °C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of serum. Radical damage was determined by specifically measuring malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product. Concentration of MDA was measured in serum and testis homogenates using a method prescribed previously [28]. Testicular tissue was removed and homogenized in a Teflon-glass homogenizer with a buffer containing 1.5 % potassium chloride to obtain 1:10 (w/v) whole homogenate. The lipid peroxide was measured spectrophotometrically at 532 nm and expressed as nano mole MDA per mL of serum or gram of testis tissue.

Statistical analysis

One way ANOVA followed by Tukey posttest was used for post hoc comparisons between the treatment and control groups using SPSS for

Windows software (version 17). Data were presented as Mean ± SEM. The P-values < 0.05 was considered as significant level during this study.

Results and discussion

Sperm count, viability, motility and abnormality

It is demonstrated that cadmium exposure produces reactive oxygen species (ROS) which leads to the endogenous antioxidants consumption and cytotoxicity. As revealed in Table 1 the percentage of motile sperm, motility grade and sperm count were reduced from 58±2.9, 3.3±0.18 and 59±4.7 in control group to 37 ±3.4, 2±0.16 and 34±3.3 in cadmium chloride exposed animals ($p < 0.001$). Furthermore, the results showed that there was significant increase in the mean percentage of morphologically abnormal sperms in cadmium administered animals (from 4.5±0.9 to 11±1.7). However, pretreatment with *Lavandula angustifolia* essential oil with 10mg/kg and 20mg/kg body weight doses attenuated the destructive effect of cadmium chloride. We showed that dose 20mg/kg *Lavandula angustifolia* essential oil significantly improved viability, motility, motility grade and abnormality ($p < 0.001$).

Table 1. The protective effect of *Lavandula angustifolia* essential oil on sperm parameters.

	Count (million/mL)	Viability (%)	Motility (%)	Motility grade	Abnormality
Control	59± 4.7	68±4.7	58±2.9	3.3± 0.18	4.5± 0.9
Sham	63±3.4	69±4.5	60±4.3	3.3± 0.15	5± 0.6
Cd	34± 3.3***	42±3.7***	37±3.4***	2± 0.16***	11± 1.7***
Cd+ Lav 5mg	41±4	50±3.7	48±3.8#	2.4± 0.22	8± 1.1
Cd+ Lav 10mg	49±4.2 #	61±4.4##	57±3.4##	2.9± 0.21##	6± 1.4##
Cd+ Lav 20mg	60.5±4.9###	66±3.6###	62±3.8###	3.2± 0.19###	4.7± 1###

Values are the mean ± SEM (n = 6); *** $p < 0.001$ from respective control value, # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ as compared with cadmium chloride treated group using one way ANOVA with Tukey *post-hoc* test.

Serum total testosterone, LH and FSH hormones measurement

Cadmium chloride administration had significant effect on the sex hormone profile. We showed that serum testosterone and LH levels significantly ($p < 0.001$) decreased (from 1.3±0.12 and 1.4±0.13 to

0.78±0.06, 0.8±0.07 respectively) compare to the control group (Fig. 1). Pretreatment with *Lavandula angustifolia* essential oil especially with higher dose (20 mg/kg) attenuated the toxic effect of cadmium chloride and increased these two hormones to normal levels ($p < 0.001$). Serum level of FSH significantly increased in cadmium

administered rats ($p < 0.001$) compare to the control group (Fig. 2). The reduction of testosterone and increase of FSH level may be caused by direct toxic effect of cadmium chloride on Leydig cells where produce testosterone.

Reduction of testosterone in serum reduces its negative feedback on FSH which increases FSH secretion. Administration of 20mg/kg *L. angustifolia* essential oil considerably ($p < 0.001$) could improve serum FSH level.

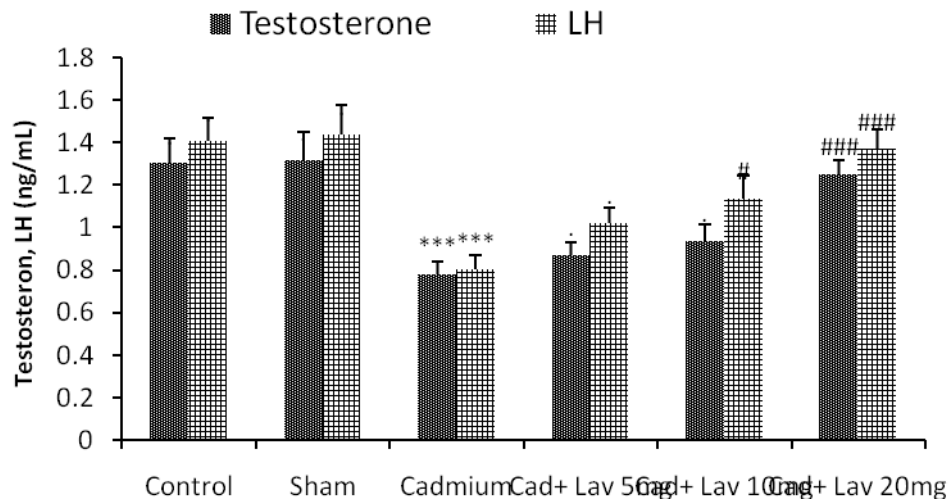


Fig. 1. The protective effect of *Lavandula angustifolia* essential oil on serum LH and total testosterone level.

Values are the mean \pm SEM (n = 6); *** $p < 0.001$ from respective control value, # $p < 0.05$, ### $p < 0.001$ as compared with cadmium chloride treated group using one way ANOVA with Tukey *post-hoc* test.

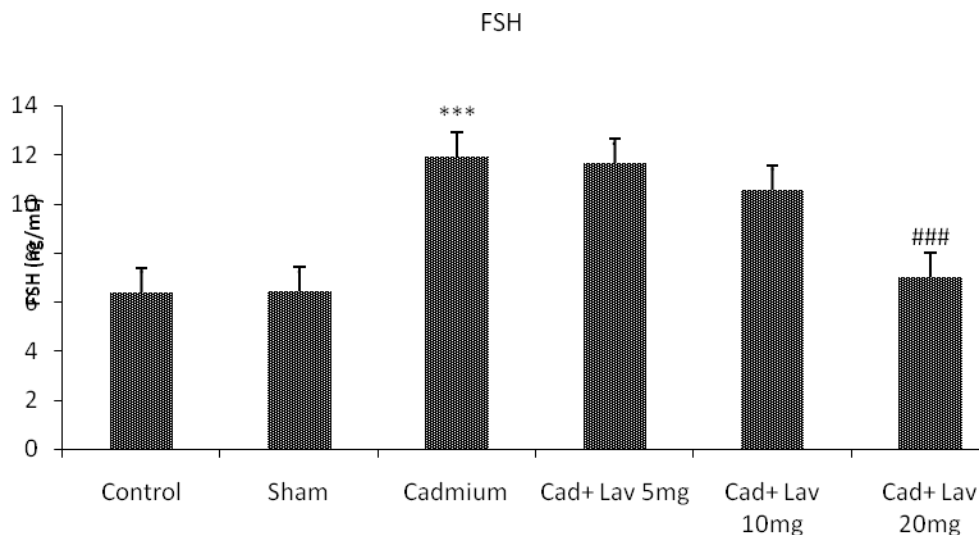


Fig. 2. The protective effect of *Lavandula angustifolia* essential oil on serum FSH level.

Values are the mean \pm SEM (n = 6); *** $p < 0.001$ from respective control value, ### $p < 0.001$ as compared with cadmium chloride treated group using one way ANOVA with Tukey *post-hoc* test.

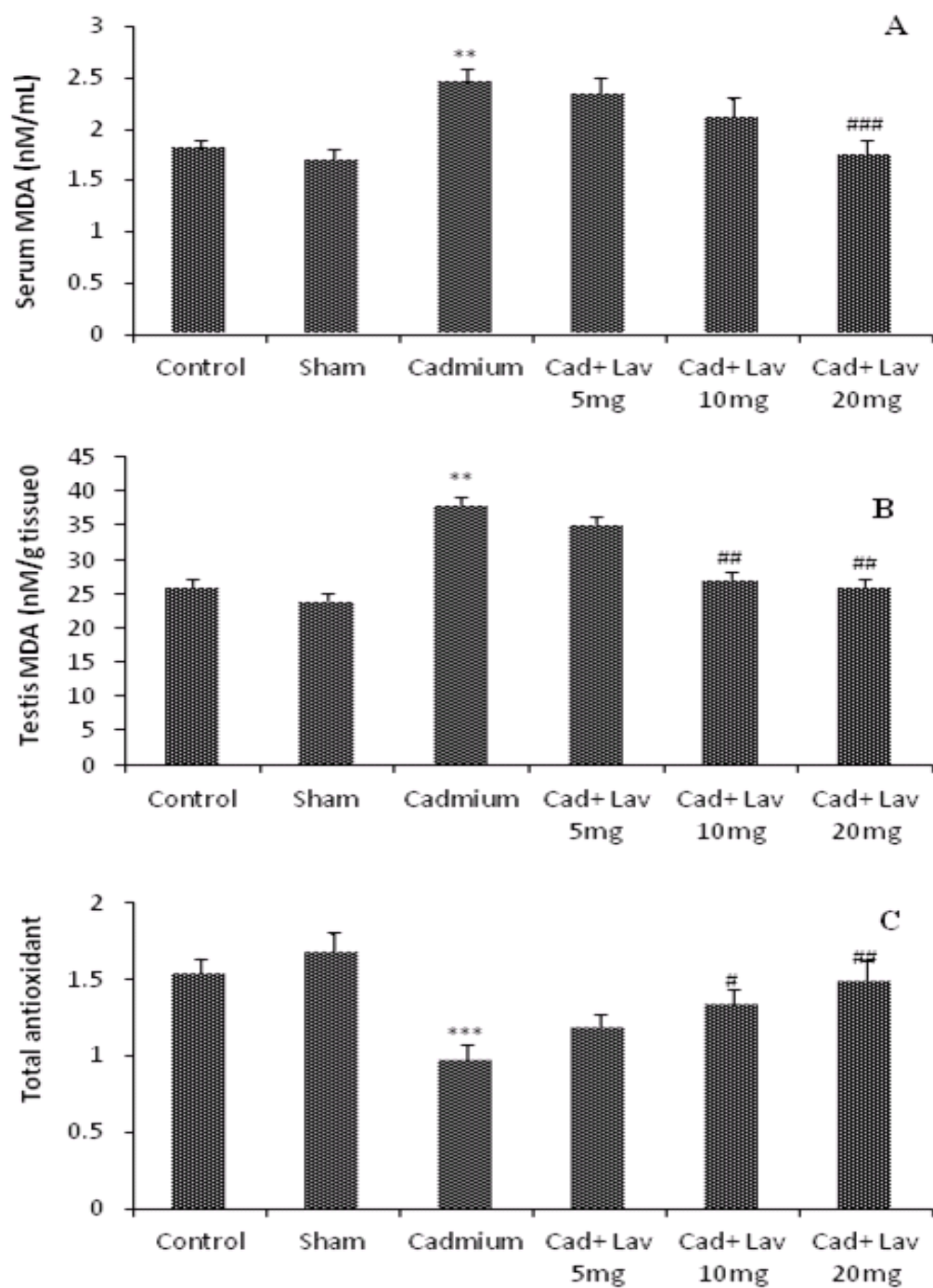


Fig. 3. The effect of *Lavandula angustifolia* essential oil on serum total antioxidant capacity and serum and testis tissue MDA level.

Values are the mean \pm SEM (n = 6); ** $p < 0.01$, *** $p < 0.001$ from respective control value, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ as compared with cadmium chloride treated group using one way ANOVA with Tukey *post-hoc* test.

Total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentration measurement in serum

In tissues with continuous cell division, endocrine function and spermatogenesis such as testis, epididymis and seminal vesicle high demand of energy is required and oxidative stress induced by cadmium chloride administration can cause cell damage and DNA mutilation. Fig. 3 shows the effect of cadmium chloride toxicities as well as *L. angustifolia* administration effects on the MDA concentration and total antioxidant status in serum and testis tissue. The mean concentration of malondialdehyde (MDA) level in serum and testis tissue was significantly ($P < 0.01$) increased from 1.81 ± 0.08 and 26 ± 2.3 to 2.46 ± 0.013 , 38 ± 3 in cadmium chloride administered animals, respectively. Pretreatment with high dose (20mg/kg) *L. angustifolia* considerably ($p < 0.01$) decreased serum and testis tissue MDA level. Besides, diminished level of total antioxidant induced by cadmium chloride significantly ($p < 0.01$) recovered using 10mg/kg and 20mg/kg of *L. angustifolia* essential oil.

Conclusions

According to the mentioned data, we can conclude that *Lavandula angustifolia* pretreatment could attenuate the toxic effect of cadmium on sperm parameters in male rats. The improvement in sperm characteristics maybe related to antioxidant activity of *L. angustifolia*.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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