# Curcumin modulates the level of oxidative status through the up-regulation of Follicle-Stimulating Hormone in the testes of lead-exposed mice

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**ABSTRACT**

*The level of Follicle-Stimulating Hormone (FSH) is one of the main factors involved in infertility. Nowadays, exposure to lead is considered as a strong factor induces infertility. In the present study, for the first time, we studied the effects of curcumin, an herbal compound, on the expression of FSH in lead pre-exposed mice using enzyme-linked immunosorbent assay (ELISA). FSH is in turn strongly correlated with INHBB and both together play a pivotal role in fertility. Furthermore, we were seeking to determine if there is any relationship between the level of FSH and the presence of lead and how curcumin may exert a synergistic effect. In addition, we further studied the oxidative stress caused by lead. Therefore, we measured the level of lipid peroxidation, and reactive oxygen species in curcumin-treated, lead-treated, curcumin/lead co-treated, and non-treated mice. According to our data, curcumin remarkably increased the level of serum FSH and reduced the negative effects of lead in oxidative status of lead-treated mice. Consequently, curcumin would be considered as a potential reagent for therapeutic purposes related to fertility.*

**Keywords**: FSH, Curcumin, Lead-treated mice, Oxidative stress

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**INTRODUCTION**

Infertility is considered as a disease of human reproductive systems defined by World Health Organization as failure to get a clinical pregnancy after one year or more trying of regular unprotected sexual relationship [1, 2]. Infertility is a global public health concern affecting 15% of couples, half of which are attributed to a male factor [3, 4]. The most frequent causes of male infertility are most likely associated with failure in spermatogenesis [5, 6]. Several factors affect the process of spermatogenesis, one of which is the level of follicle-stimulating hormone (FSH).

FSH is a member of the glycoprotein hormone family, which acts through specific G-protein-coupled receptors (GPCRs) on the surfaces of target cells. FSH is secreted from the pituitary and contributes to gonadal function and regulation of reproduction in mammals [7]. FSH, in females, interacts with those receptors expressed on granulosa cells and proceeds the maturation of ovarian follicles [8, 9]. However, FSH in males proliferates Sertoli cell in testes and involves in spermatogenesis process [8]. The level of FSH in serum is in part regulated by INHBB secreted from Sertoli cells, through a negative feedback loop on the anterior pituitary. Inhibin is a heterodimer glycoprotein hormone and a member of the TGF-β family, composed of two different subunits (α and either $\beta_{An}$ or $\beta_B$) linked together by disulfide bonds. INHBB is the most accessible and an appropriate endocrine marker of spermatogenesis in subfertile men and is the principal circulating form generated in the fetal and adult males, whereas INHBA is undetectable. Serum level of INHBB hereupon could conceivably be applied to directly study testicular function, and in combination with FSH measurement, it could be used as an indirect index for evaluation of reproductive hormones regulation [10].

Exposure to heavy metals such as lead due to the occupational and environmental condition is considered as one of the major health problem worldwide, especially in developing countries. [11, 12]. Several studies have reported the adverse effects of lead exposure on reproduction and abnormalities of spermatogenesis [13]. Recently in 2009, Hsieh et al. found that spermatogenesis in male workers with a long-term exposure to lead was indirectly affected [14]. According to the harmful effects of heavy metals such as lead, finding a solution to retreat the patients and also to prevent the progression of the adverse effects is of great interest.

In the past few years, many researchers targeted various disorders and diseases and tried to find herbal compounds for therapeutic purposes [15-17]. Active ingredients in herbal compounds usually have a biological balance and therefore do not accumulate in the body and have fewer side effects. Curcumin (diferuloylmethane), an active component and yellow pigment extracted from the rhizoma of turmeric (*Curcuma longa*) considerably prevents the generation of reactive oxygen species (ROS) and regulates the level free radicals in normal concentration. Curcumin has antioxidant, antiproliferative, anti-carcinogenic and anti-tumor properties in a variety of cell lines and animals [18-21]. Considering these features curcumin is a suitable and potential candidate to treat infertility. Furthermore, curcumin triggers apoptosis in contaminated cells. Apoptosis automatically occurs when cells undergo stresses or lesions such as DNA damage, disruption in transduction of survival signal, nutrients, or oxygen [22, 23]. Therefore, here we studied the improving effects of curcumin on the expression of INHBB, the level of FSH and how curcumin changes the oxidative status in lead-contaminated male NMRI mice.

## EXPERIMENTAL SECTION

### Chemicals and Reagents
Lead acetate, curcumin and corn oil were purchased from Sigma-Aldrich, Germany.

### Animals
Totally 40 male NMRI mice, at 6-8 weeks age (Laboratory Animals Production Complex, Pasteur Institute of Iran, Tehran, Iran) were used in this study in full accordance with ethical guidelines for using laboratory animals. The mice were kept in cages with the ambient temperature of $(24\pm2)°C$, relative humidity of $(60\pm10)\%$, and in 12-hour light-dark cycle. Free access to food in the form of dry pellets and water was allowed. Before the beginning of the experiment, all mice received basal diet for 10 days for adaptation and to ensure normal growth and behavior.

### Animal protocol
Mice were divided into four groups, each contained 10 mice. The first group (group I) served as control and received the vehicle, corn oil only (5 ml/kg body weight). Mice of the second group (group II) received lead acetate dissolved in drinking water at the dose of 25mg/L with 3 ml of 5% acetic acid per liter to keep the lead salt in solution. Mice of the third group (group III) received curcumin dissolved in corn oil at a dose of 15 mg/5 ml/kg body weight for two months by gastric gavages. Mice of the fourth group (group IV) received a combination of lead acetate and curcumin at the same dose and route used for groups II and III. At the end of the experimental period, animals were left night fasted and at the next day they were euthanized following protocols and ethical procedures then testes were removed from the body.

### Serum FSH measurement
FSH in serum was measured by enzyme-linked immunosorbent assay (ELISA) kits (USCN Life Science Inc., Wuhan, China) according to the manufacturer's protocols. Here, both the serum samples and the standards were incubated in 96-well plates at $37°C$. The plates were washed using washing buffer and re-incubated at $37°C$, after the addition of substrate solution and stopping buffer. Using plate reader (Perkin Elmer, Massachusetts, USA.) absorption was measured at 450 nm and concentration of serum FSH was calculated from the standard curves.

### Studies on oxidative status: lipid peroxidation and ROS generation
The testes of animals were removed after they were put to sleep with ketamine injection of 100 mg/kg m.c. and dislocation of cervical vertebrae, and used for further investigation. They were weighed and frozen at $−80°C$. For performing biochemical tests, the samples were thawed, washed with 0.9% NaCl plus EDTA, homogenized in ice-cold 20 mM Tris-HCl Buffet (pH = 7.4), and centrifuged for 10 min at 4°C and 14600 g. The supernatants were used for lipid peroxidation assays. To measure the level of markers of lipid peroxidation – malondialdehyde + 4hydroxynonenal (MDA + 4HDA), Lipid Peroxidation Assay Kit (Calbiochem) was used. ROS generation was measured in all the four groups using Abcam's Cellular Reactive Oxygen Species Detection Assay Kit (Cambridge, UK) according to the manufacturer.

**Statistical analysis**
Comparisons between groups were made using unpaired Tukey's HSD posthoc test. Results were considered statistically significant if the $P < 0.05$. Where appropriate the data were also analyzed by one-way ANOVA.

## RESULTS AND DISCUSSION

**Serum FSH measurement**
Serum FSH was measured as nano gram per milliliter. In control group mice serum FSH was measured 162 ng.mL$^{-1}$ and a significant increase was observed in other groups. In curcumin treated mice serum FSH was at the highest level (1053 ng.mL$^{-1}$) and this increase were 405 and 664.2 ng.mL$^{-1}$ for lead-treated and lead/curcumin co-treated mice (Fig. 1). To further demonstrate the relationship between the level of INHBB and FSH and their relative changes compare to control group in different treatment we inserted a chart to show the changes (fig. 2).
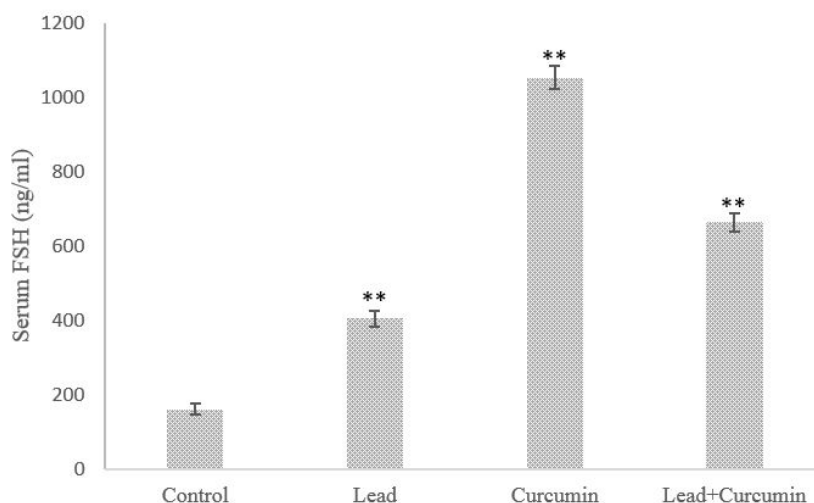


**Figure.1. Serum FSH measurement in four different groups of mice as described in Material and Method (P<0.01\*\*)**
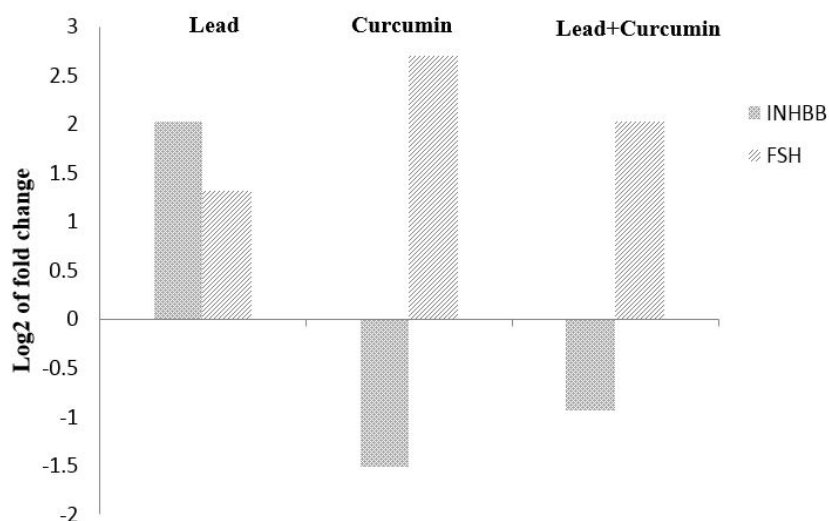


**Figure.2. Comparison of fold changes in serum FSH and INHBB as log2 of fold change**

**Oxidative status**
As illustrated in Figure 3, mice exposed to lead have 1.6 fold increase in the level of lipid peroxidation markers ($P < 0.001$) and in the presence of curcumin this increase is just 1.1 fold in comparison with control group. Furthermore, in mice treated with curcumin a small decrease is observed in lipid peroxidation. As shown in figure 4, comparing to control group ROS generation increased 3.7 fold when cells treated with lead, and co-treatment with curcumin decrease this amount to 2.4 fold increase. Treatment only with curcumin has no significant effect on the overall ROS generation.

Follicle-stimulating hormone (FSH) is a key factor in mammalian reproduction. FSH binds to G-protein coupled receptor on the surface of target cells and stimulates testicular and ovarian functions. FSH is a glycoprotein hormone and acts as disulphide-rich heterodimers composed of two non-covalently connected subunits 4. In different species, the α-chain is common but β-chains is responsible for functional specificity 4. There is a strong connection between the level of FSH and INHBB, both of which play important roles in fertilization.

INHBB is a heterodimer glycoprotein synthesized and secreted by Sertoli cells in the male testis. [24]. The subunits of inhibin appear to be regulated by FSH both in vivo and *in vitro*, as FSH receptors stimulate cAMP production, which suggests that INHBB subunits may be regulated through signals dependent on protein kinase A [25, 26]. In our previous research, we showed that the level of INHBB mRNA increased in mice treated with lead and intriguingly the expression of INHBB decreased in both groups of treated with curcumin and co-treated with curcumin/lead. Therefore, it is possible that curcumin exerts an adverse effect against the effect of lead in increasing the expression of INHBB. According to our finding, curcumin is able to affect the expression of INHBB 4.1, 2.86 and 1.91 in protein level respectively in lead-treated, curcumin-treated and curcumin/lead co-treated mice.
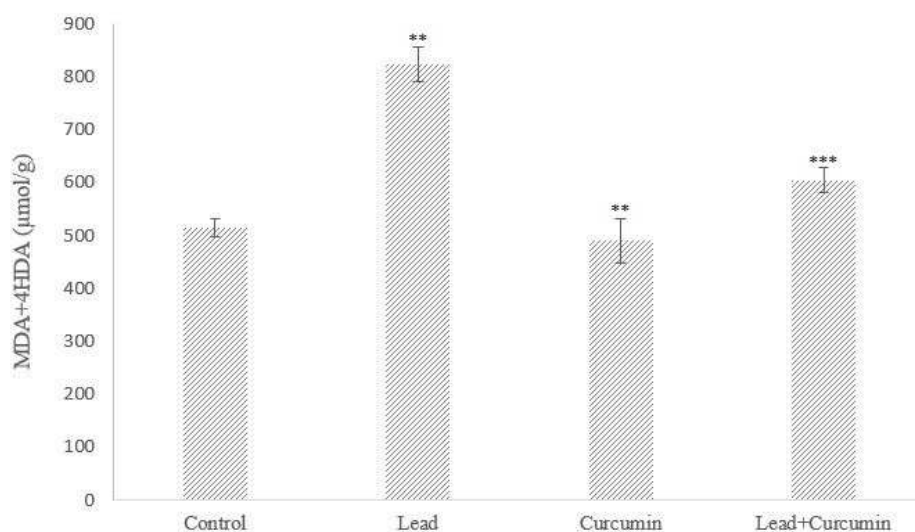


**Figure. 3. The concentration of lipid peroxidation markers in testicular homogenates of four groups of mice (P<0.01**, P<0.001***)**



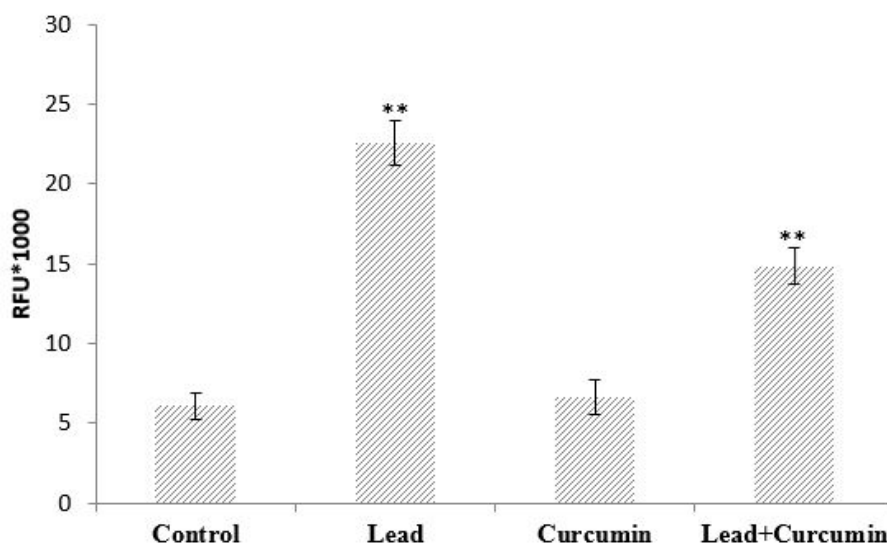**Figure 4. ROS generation in four groups of mice (P<0.01**)**

Lead, like other metalloids such as arsenic, is a toxic element [27].In human bodies, it interferes with heme synthesis, disrupts calcium homeostasis, increases reactive oxygen species (ROS), and concentrates INHBB in serum [28]. ROS production stimulates the secretion of FSH which inhibits the generation of ROS [29]. Subsequently, the increased amounts of FSH stimulate the secretion of INHBB by binding to receptors on Sertoli cells. INHBB regulates the

secretion of FSH from pituitary through negative feedback, while FSH inversely stimulates the secretion of INHBB [30]. As FSH is involved in the spermatogenesis, any increase or decrease in the level of FSH may adversely affect this process. In the current study, we measured the level of serum FSH because of its association in infertility and its direct effect on INHBB. According to data presented in figure 1, the level of FSH increased in all groups compare to control groups. However, curcumin significantly increased the level of FSH in serum, while lead had the least effect. In co-treated mice, FSH increased but not as much as those treated with curcumin. As shown in figure 2, in the presence of heavy metal (lead) the level of both FSH and INHBB increases which seems to be compatible with negative feedback regulation of these two hormones [31]. When mice treated with lead, INHBB expression increases which in turn increases the level of FSH although theoretically there may be a decrease in the level of FSH due to negative feedback regulation. It seems that in an oxidative stress INHBB increases and also increases the secretion of FSH. Then after, elevated FSH acts on INHBB expression to decline its synthesis and secretion, which conveys that at the first step of regulation the levels of FSH and INHBB are directly proportional and then the relationship is adversely proportional. In curcumin treated mice, due to the effect of curcumin on decreasing the ROS generation, INHBB increases which adversely regulate the secretion of FSH. In curcumin/lead co-treated mice, curcumin decreases INHBB and increase the level of FSH is the result. In other words, curcumin in co-treated mice not only increases the level of FSH but also significantly decrease the level of elevated INHBB in lead treated mice. This shows that curcumin can attenuate the oxidative status of the cells as will further discuss later.

Mammalian testes and spermatozoa contain polyunsaturated fatty acids that are the reason for sperm fluidity, although it makes them vulnerable to oxidative stress. As a result, ROS may induce disturbance in the structure and function of spermatozoa. Furthermore, these polyunsaturated fatty acids are the most important substrates for lipid peroxidation [32]. Therefore, we measured the level of lipid peroxidation by measuring MDA and 4-HAD. As shown in figure 3, the level of lipid peroxidation is almost at a similar level in the control group and that of treated with curcumin. In other words, curcumin does not change the whole pattern of lipid peroxidation in testes tissue although small insignificant decrease is observed. In the other side, lipid peroxidation is at maximum in lead-treated mice and curcumin somehow neutralizes this increase but is unable to keep it at the same level as a control group. Heavy metals such as lead cause oxidative stress due to the generation of the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH•) [33]. As illustrated in figure 4, in lead-treated mice, ROS generation increased while in the curcumin-treated group the level of ROS is at a similar level as a control group. In co-treated group, ROS is 2-fold greater than that of control mice but it is obvious that curcumin has the potential to decrease ROS generation caused by lead.

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