The effect of hydroalcoholic extract of cinnamon on sex hormone changes caused by lead poisoning in female rats

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Abstract

Introduction: Lead is one of the toxic heavy metals. This study investigated the antioxidant effect of cinnamon on lead poisoning in reproductive system in order to find a solution for the people exposed to lead.

Materials and Methods: Forty-two Wistar rats were divided into 7 groups of 6 each: one control group which received no treatment, sham group 1 (distilled water with alcohol for 14 days), sham group 2 (0.6 g/l lead acetate daily for 14 days), control group 3 (0.4 g/kg cinnamon extract daily for 14 days), experimental group 1, 2, 3 (0.6 g/l lead acetate in addition to respectively 0.1, 0.2, 0.5 g/kg cinnamon extract for 14 days). At the end of the study, estrogen and progesterone concentrations were measured by ELISA.

Results: The results showed that the concentration of estrogen decreased in sham group 2 and increased in sham group 3 significantly compared to the control group (p<0.05). The concentration of progesterone decreased in sham group 2 and increased in sham group 3 significantly compared to the control group (p<0.05).

Comparing experimental groups of 1, 2 and 3 with one another revealed that group 3 experienced a higher increase in estrogen and progesterone compared with groups 1 and 2.

Conclusion: The results of this study indicated that cinnamon extract with its antioxidant properties reduces the side effects of lead on mean concentrations of sex hormones in female Wistar rats in a dose-dependent manner.

Keywords: Cinnamon, Lead, Estrogen, Progesterone, Rat

Introduction

Human is exposed to chemicals and heavy metals such as lead frequently every day. Lead is a toxic heavy metal with many effects on biological organs of living organisms, including human and causes various risks (1). Saturnism or lead poisoning is one of such complications which was first described by Hippocrates (2). Since then, numerous researchers have addressed it and studied its effects and complications from various aspects. One of the properties of this toxic substance is its accumulation in the body, such that it accumulates in tissues, organs, and
The effect of hydroalcoholic extract of biological fluids of exposed people over time and exerts its harmful effects. Its activity is chronic and it has a wide range of complications (3). Lead not only affects the hematopoietic system, teeth, kidneys, gastrointestinal tract and respiratory tract and disrupts their functioning, but also affects the reproductive system and impairs fertility (4). The severity of complications has drawn researchers’ attention and a lot of studies have been conducted in this area.

A study on abnormalities and their effects on the health of the reproductive system examined the role of lead and its effects such as decreased male fertility, and suggested that fertility declines in men exposed to lead (5). This could be the case about the female reproductive system, too, and it can be stated that lead reduces fertility in both sexes (6).

Infertility is one of the complex issues of medical sciences, which can be treated in many cases (7). Moreover, man has long tried to solve the problem using medicinal plants and have sought the treatment of any type of pain or illness in nature. Cinnamon (Cinnamomum zeylanicum) is a medicinal plant with multiple applications. Different parts of the plant including its bark have many therapeutic properties (8). Cinnamon is one of the oldest medicinal plants. In traditional medicine, it is used as an important drug strengthening the heart, stomach, and intestines, and improving kidney function and increasing sexual power (9). It is also used for treating nausea and diarrhea and enhancing the power of understanding (9, 10). Cinnamon has strong antioxidant properties and prevents oxidation of organic matter and reduces free radicals in the body (11).

Since no studies have yet been conducted on the therapeutic effects of cinnamon extract on the eliminating the toxic effect of lead on reproductive system of the females, and due to the importance of fertility and the role of the reproductive tract in the survival of generations on the one hand, and the increased exposure of human being to heavy metals on the other hand, the present study was conducted to evaluate the therapeutic effects of cinnamon extract on the eliminating the toxic effects of lead on sex hormones of rats.

Materials and Methods
This randomized experimental study was conducted by observing ethical principles of working with laboratory animals. A total of 42 adult female Wistar rats weighing approximately 185-195 g and aged 2-3 months were procured from laboratory animal breeding center of the Islamic Azad University of Jahrom, Iran. Animals were fed with rat feed and tap water. Animals were kept under a 12:12 h light-dark cycle during all tests. The mice were randomly divided into 7 groups of 8 animals as follows:

- The control group: Kept in normal conditions without receiving any medication
- The sham group 1: Kept in similar conditions to the control group plus 2.0 ml of distilled water and alcohol intake per day
- The sham group 2: They received 0.6 g/l of lead per day orally.
- The sham group 3: They received 0.4 g/kg of cinnamon extract for 14 days orally.
- The intervention group 1: They received 0.1 g/kg of oral cinnamon extract and 0.6 g/l of lead per day.
- The intervention group 2: They received 0.2 g/kg of oral cinnamon extract and 0.6 g/l of lead per day.
- The intervention group 3: They received 0.4 g/kg of oral cinnamon extract and 0.6 g/l of lead per day.

The amount of cinnamon given to the animals was 1 ml.

Lead administration method
An amount of 0.6 g/l of lead acetate powder (Merck, Germany) was weighed and dissolved in one liter of distilled water...
daily. Then it was orally given to the rats in two weeks.

Cinnamon administration method
The cinnamon bark was ground by a mill and 500 g of the powder was dissolved in 100 ml of 96% ethanol. The resulting mixture was stored for 24 hours at room temperature (25 °C). Then, the mixture was mixed using an electric mixer for 4 minutes and then filtered through a Whatman paper, the initial weight of which was recorded in advance. The paper and the powder residue on it were dried in an oven at 50 °C for 1.5 hours. Comparing the difference of the dried powder remaining on the filter paper and the initial amount of cinnamon, the amount of dissolved powder was obtained. The obtained extract contained a large amount of alcohol (~20 ml). In order to remove the extra alcohol, the extract was stored in a clean environment for 48 hours in order that the excess alcohol evaporates and reaches its minimum amount (5 ml). The volume of the extract was increased to 150 ml using normal saline 9% serum to obtain the maximum dose of the extract. The extract was given to the groups at certain doses every day (11).

At the end of the study, the rats were anesthetized by ether after weighing and 5 cc blood was taken from their hearts by syringe. After separation of blood serum, the concentrations of estrogen and progesterone hormones were measured by ELISA method in the laboratory of Jahrom University of Medical Sciences. The treatments were compared using one-way ANOVA. Then t-test and Duncan's test were used for multiple comparisons among the groups. The statistical significance level was considered as P<0.05. Data was analyzed in statistical analysis software SPSS 16.

Results
According to Figure 1, estrogen levels decreased significantly in the sham group 2 and increased significantly in the sham group 3 compared to the control group (P<0.05). The intervention groups 1, 2 and 3 showed a significant increase compared to the sham group 2 (P<0.05). All three doses of cinnamon showed a significantly increased estrogen compared to the groups receiving lead, while the intervention groups showed a significant decrease compared with the control group. The comparison of intervention groups with one another revealed that the 0.4 concentration of cinnamon hydroalcoholic extract had better effects than other concentrations and increased the estrogen level more in comparison to the lead groups.

According to Figure 2, progesterone levels decreased significantly in the sham group 2 and increased significantly in the sham group 3 compared to the control group (P<0.05). Progesterone levels in the intervention group 3 increased significantly compared to the sham group 2 at 5%. Cinnamon groups at doses of 0.2 and 0.4 had an insignificant decrease in progesterone levels compared to the control group. The comparison of intervention groups with the lead group showed that the intervention group 3 had a significant increase in serum levels of progesterone compared to the lead group.
According to Duncan’s test, if there is at least one common letter in each group, the groups are not significantly different.

Table 1: The comparison of evaluated parameters in different treatment groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Quality</th>
<th>Progesterone (ng/dl)</th>
<th>Estradiol (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>b/b</td>
<td>13.96± 0.21</td>
<td>b 100.11±2.44</td>
</tr>
<tr>
<td>Distilled water and alcohol (Sham 1)</td>
<td>bc</td>
<td>13.07±0.81</td>
<td>b 103.32±7.72</td>
</tr>
<tr>
<td>Lead (Sham 2)</td>
<td>d</td>
<td>7.49±1.013</td>
<td>e 14.57±1.01</td>
</tr>
<tr>
<td>Cinnamon (Sham 3)</td>
<td>a</td>
<td>17.63±0.529</td>
<td>a 123.87±1.63</td>
</tr>
<tr>
<td>Lead + 0.1 Cinnamon (Intervention 1)</td>
<td>cd</td>
<td>9.64±0.211</td>
<td>d 21.2±0.88</td>
</tr>
<tr>
<td>Lead + 0.2 Cinnamon (Intervention 2)</td>
<td>bcd</td>
<td>10.91±0.681</td>
<td>d 23.2±5.82</td>
</tr>
<tr>
<td>Lead + 0.5 Cinnamon (Intervention 3)</td>
<td>bc</td>
<td>11.26±0.505</td>
<td>c 41.73±3.21</td>
</tr>
</tbody>
</table>

The means in each column that at least have one letter in common do not have a significant difference at of 5% level in Duncan’s test.
Discussion

The results showed that estrogen levels in sham group 2, intervention groups 1, 2 and 3 had a significant decrease at the 5% level compared to the control group. The progesterone levels in sham group 2 and intervention group 1 had a significant decrease at the 5% level compared to the control group.

Studies on the effects of lead on changes of sex hormones in male rats suggested that lead decreased the serum level of testosterone by affecting Leydig cells (12). Lead also decreased testosterone in male rats by producing free radicals and damaging the Leydig cells (13).

It is possible that lead damaged ovary tissue and reduced estrogen and progesterone levels through free radicals. Various studies suggested that lead had damaging effects on the ovaries and reproductive tract morphology such that it reduced the secretion of progesterone in luteal cells at low doses and it inhibited progesterone completely at high doses (14).

Other studies reported that lead destroyed GABA receptors - receptors mediating LH secretion by the pituitary gland - and reduce the LH secretion (13). They also indicated that lead reduced the pituitary gland weight and thus LH and FSH hormones (13). LH has a direct effect on the estrogen and progesterone change such that the reduction of LH reduces estrogen and progesterone levels (15). Thus, estrogen and progesterone changes in this study might be due to the increased production of reactive oxygen species and the effect on the hypothalamic-pituitary-gonadal axis.

Other studies also suggested that pituitary-gonadal axis is influenced by various factors such as nitric oxide. Nitric oxide, in turn, stimulates LHRH and increases LH (16). Nitric oxide is a highly unstable compound that acts as a secondary messenger molecule in the majority of organs (17). Studies suggest that lead suppresses the secretion of nitric oxide (18). Lutein cells also increased the secretion of estrogen in small quantities and progesterone in large quantities. It is likely that lead reduced the secretion of nitric oxide in this study, which reduced LH. Decreased LH led to the reduced performance of ovarian tissue and reduced growth of ovarian follicles as well as lutein cells and thus reduced estrogen and progesterone.

The results showed that the levels of estrogen in the sham group 3 (the group receiving cinnamon extract) had increased significantly compared to the control group at 5% level. The intervention groups 1, 2 and 3 showed a significant increase in estrogen levels compared to the sham group 2 (the group receiving lead) at 5% level.

Progesterone level in the intervention group 3 had a significant increase compared to the control group. Also, progesterone level in the intervention group 3 had a significant increase compared to the sham group 2.

It is suggested that cinnamon affects the pituitary-gonadal axis and increases the secretion of gonadotropin hormone mediated by an increase in the concentration of nitric oxide as well as norepinephrine. Norepinephrine increases the secretion of nitric oxide and nitric oxide, in turn, increases the secretion of LH-releasing gonadotropin. Increased LH secretion will also lead to sex hormones secretion (7). Also, studies have suggested that the Delta-cadinene in cinnamon increases the secretion of LH which directly increases the synthesis of estrogen and progesterone (15). It is likely that cinnamon extract affected pituitary-gonadal axis and increased female sex hormones in this study.

Other studies have also stated that the sex hormone levels have a direct relationship with increasing doses, as higher doses are associated with more sex hormones (7). In the present study, higher doses led to more increased levels of sex hormones, too.
Conclusion
Considering the above, it can be stated that lead decreased LH by affecting the hypothalamic-pituitary-gonadal axis and then indirectly decreased the secretion of estrogen and progesterone. However, cinnamon extract increased nitric oxide release and subsequently the secretion of gonadotropin, resulting in an increase in estrogen and progesterone. Cinnamon extract might be appropriate to increase fertility in treating people exposed to lead, and in order to verify and complete these results, further studies are recommended.

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Conflict of interest
The authors declare no conflicts of interest.

References: