Effects of valerian consumption during pregnancy on cortical volume and the levels of zinc and copper in the brain tissue of mouse fetus

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OBJECTIVE: The aim of the present study was to determine the effects of valerian (Valeriana officinalis) consumption in pregnancy on cortical volume and the levels of zinc and copper, two essential elements that affect brain development and function, in the brain tissues of mouse fetuses.

METHODS: Pregnant female mice were treated with either saline or 1.2 g/kg body weight valerian extract intraperitoneally daily on gestation days (GD) 7 to 17. On GD 20, mice were sacrificed and their fetuses were collected. Fetal brains were dissected, weighed and processed for histological analysis. The volume of cerebral cortex was estimated by the Cavalieri principle. The levels of zinc and copper in the brain tissues were measured by atomic absorption spectroscopy.

RESULTS: The results indicated that valerian consumption in pregnancy had no significant effect on brain weight, cerebral cortex volume and copper level in fetal brain. However, it significantly decreased the level of zinc in the brain (P<0.05).

CONCLUSION: Using valerian during midgestation do not have an adverse effect on cerebral cortex; however, it caused a significant decrease in zinc level in the fetal brain. This suggests that valerian use should be limited during pregnancy.

KEYWORDS: Valeriana; prenatal injuries; zinc; copper; stereotaxic techniques; mice

Valerian (Valeriana officinalis) is a perennial plant native to the temperate areas of Americas, Europe and Asia[11]. Valerian is used as a hypnotic, sedative, anxiolytic, anticonvulsant and antidepressant drug[25]. The rhizomes of V. officinalis contain several components with demonstrable...
pharmacological activities. These include the essential oil and its sesquiterpenoids (valeric acid), epoxy iridoid esters (valeropatrate, valrate, didralvrate), amino acids (arginine, γ-aminobutyric acid (GABA), glutamine, tyrosine), and alkaloids[4]. It also has been reported that the ethanolic and aqueous extracts of valerian root could inhibit GABA reuptake[7,8]. Valerian is traditionally contraindicated in pregnancy, but there are no studies to support this warning.

Zinc and copper are essential for normal brain development. Several studies have shown that zinc deficiency in lactating mothers leads to neuroanatomical malformations and functional abnormalities in suckling offsprings[6,10]. The effects of maternal zinc deficiency on postnatal development of the rat cerebellar cortex have been investigated by Dvergesten et al[11,12]. The number of granule cells was sharply reduced, Purkinje cell maturation was impaired, and differentiation of basket and stellate cell dendrites was reduced. In other studies, adult rats showed significant learning and memory deficits if their mothers were mildly or severely zinc-deficient during late pregnancy and lactation[13,14]. Copper is also essential for proper brain development, particularly the cerebellum. It functions as a cofactor for enzymes including mitochondrial cytochrome c oxidase. In humans, deficiency of copper leads to severe mental retardation and could slow brain development especially in cerebellum[15,16]. In rats, copper deficiency in the cerebellum exhibited blunted development including a reduction in myelination and synaptogenesis as well as motor function disturbances[17,18].

The present study was designed to evaluate the effects of valerian consumption in pregnancy on the volume of cerebral cortex and levels of zinc and copper in fetal brain of mice.

1 Materials and methods

1.1 Valerian preparation The valerian preparation used in the present study was donated by Gol Ghatreh-Toos Pharmaceutical Company ( Mashhad, Iran). The supplied extract contained 6.2% valerian.

1.2 Experimental protocol Adult female bulb/C mice ((25±5) g, 10 to 12 weeks old) bred in the Animal House Facility Unit, Faculty of Medicine, Mashhad University of Medical Sciences, Iran, were used. Animals were housed as 3 to 4 mice per cage in a temperature-controlled colony room under a 12:12 light-dark cycle. Food and water were available ad libitum. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by the Mashhad University of Medical Sciences, Iran.

Female mice were mated with one male mouse overnight. The presence of vaginal plaque at the next morning was taken as evidence of mating. This day was designated as gestation day (GD) 0. The pregnant mice were randomly assigned into two groups. Control group (n = 15): animals received 0.5 mL normal saline intraperitoneally once a day on GD7 to GD17. Valerian group (n = 15): animals were treated with 1.2 g/kg valerian extract intraperitoneally once a day on GD7 to GD17.

1.3 Histological procedures On the last day of pregnancy (GD20), the pregnant mice were anesthetized by chloroform and their pups were collected after abdominal incision. Fetal brains were dissected carefully using a stereomicroscope (Olympus SZ30, Japan). Then, the brains were fixed in 10% formalin and dehydrated in a gradient of ethanol, embedded in paraffin and cut into 10 μm serial coronal sections. After deparaffinization, the brain sections were stained with hematoxylin-eosin and examined by a light microscope (Zeiss, Germany).

1.4 Stereological study The volume of cerebral cortex was estimated by the Cavalieri principle[20]. In the Cavalieri principle, the object under study is cut into a series of parallel plane sections at a constant distance apart. The surface areas of the cut sections are estimated and multiplied by the mean section thickness to provide a volume of the examined object. The cut surface area of each section is estimated with point counting grids (PCG). The PCG, which has sets of points at specific densities on a transparent sheet, is randomly

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superimposed on sections, and the numbers of points that cover the region of interest are counted. Finally, the volume of the object \( V \) is estimated with the following equation: \( V = t \times (a/p) \times [\Sigma P - 1/2P_{\text{max}}] \), where \( t \) is the section thickness, \( a/p \) represents the area of each point on the point counting grid (corrected with the reduction ratio of the printed films), \( \Sigma P \) is the total number of the points hitting the section's cut surface, and \( 1/2P_{\text{max}} \) is half of the maximal number of points counted on the largest section of the examined subject\([11]\).

1.5 Zinc and copper measurements The concentrations of zinc and copper in the cerebral cortex were measured by atomic absorption spectrophotometry (Perkin-Elmer 3030, USA) according to Parker et al\([12]\). Metal levels were expressed as microgram per gram tissue.

1.6 Statistical analysis Results were expressed as mean±standard deviation. Data were analyzed by independent sample \( t \)-test using SPSS Version 13.0 software. The differences were considered significant when \( P<0.05 \).

2 Results

2.1 Effects of valerian on the number of mouse fetuses There was no significant difference in the number of fetuses born between the valerian group (9.0±2.4) and the control group (9.8±1.9).

2.2 Effects of valerian on brain weight and cortical volume in mouse fetuses Considering the fetal brain weight and the volume of cerebral cortex, as shown in Table 1, there were no significant differences between the two groups. Figure 1 presents the histological results of brain sections stained with hematoxylin-eosin and examined by a light microscope.

### Table 1 Effects of valerian on brain weight and cortical volume in fetal mouse (Mean±standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Brain weight (mg)</th>
<th>Cortical volume (mm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>146.57±7.00</td>
<td>2.41±0.25</td>
</tr>
<tr>
<td>Valerian</td>
<td>15</td>
<td>132.78±9.60</td>
<td>2.36±0.22</td>
</tr>
</tbody>
</table>

2.3 Effects of valerian on the levels of zinc and copper in fetal mouse brain The level of zinc in fetuses of the valerian group was significantly lower than that of the control group \( (P<0.05) \). However, there was no significant difference in copper level between the valerian group and the control group (Table 2).

### Table 2 Effects of valerian on levels of zinc and copper in fetal mouse brain (Mean±standard deviation, \( \mu g/g \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Zinc level</th>
<th>Copper level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>35.80±2.34</td>
<td>4.10±0.75</td>
</tr>
<tr>
<td>Valerian</td>
<td>15</td>
<td>31.43±2.48*</td>
<td>3.17±1.30</td>
</tr>
</tbody>
</table>

\* \( P<0.05 \), vs control group.

3 Discussion

In vitro studies have shown that valeropiates such as valtrate and didrovaltrate, have significant cytotoxic, mutagenic and carcinogenic activities\([25,26]\). In contrast to in vitro studies, overt toxicity has not been demonstrated by in vivo studies. Oral administration of valeropiates (12 and 24 mg/kg per day) to rats for 30 d resulted in no change in the average length of the estrous cycle, the number of estrous phases and the fertility index\([27]\). Also, incidence of external and internal malformations in fetus did not increase. In a human study, oral administration of 2.5 g per day valerian (range 0.5 to 12 g per day) for 4 years to 23 patients resulted in no clinical evidence of acute hepatitis in 12 of these 23 patients\([28]\). Tortarolo et al\([26]\) also found that while valeropiates were cytotoxic to mouse bone marrow early progenitor cells in their in vitro study, no significant changes were observed with oral administration, even at doses as high as 1 500 mg/kg. More recently, Yao et al\([29]\)
reported that valerian at dose of 2.79 g/kg per day on either GD 1 to GD 8 or GD 8 to GD 15 had no adverse effects on fertility or fetal development. Poor absorption and distribution of valepotriates in oral administration has been proposed as a possible explanation to explain this discrepancy between results from in vivo and in vitro studies[31]. However, there is no pharmacokinetic data for valepotriates to support this hypothesis.

In consistent with in vivo studies, the present study showed that 11 d of intraperitoneal injection at a dose of 1.2 g/kg per day valerian extract to pregnant rats on GD 7 to GD 17 (as early fetal period and organogenesis) had no adverse effects on fetal brain, since fetal brain weight and fetal cerebral cortex volume did not significantly change. On the other hand, valerian extract injection significantly decreased zinc level in the fetal brain compared with the control, but it had no significant effect on copper level in the fetal brain.

One negative consequence of zinc deficiency can be an increase in apoptosis in the fetus. It has been shown that cell death was increased in peri-implantation embryos that were cultured in low zinc medium[32]. Also, post-implantation embryos from zinc-deficient dams have shown increased apoptosis in the somites, pharyngeal arches, otic placode, optic vesicle, forelimb buds, and neural tube, compared to the controls[13,34]. Even a few days of zinc deficiency during midgestation is sufficient to produce cell death in the posterior dorsal midline, indicating that neural crest cells may be particularly sensitive to the adverse effects of zinc deficiency[35]. Zinc deficiency could have a long-term impact on brain health by alternating the finely tuned processes of neurogenesis, neuronal migration, differentiation, and apoptosis, which involve the developmental shaping of the nervous system[36].

In our study, in spite of the decrease in the zinc levels in the cerebral cortex, the cortical volume did not change. This in part may be due to a low degree of zinc deficiency and its onset time. It has been indicated that active neuroblast multiplication coincides with the commencement of brain growth spurt which in rat it begins at birth and is over at postnatal day 25[37]. In our study the brain was removed on GD 20 prior to brain growth spurt; therefore, zinc deficiency (for a short period) did not have enough time to induce reductional impact especially in a windows of time just prior to active neuroblast multiplication.

Zinc deficiency induces apoptotic cell death through the intrinsic pathway, which involves cytochrome c release from mitochondria. Cytochrome c can then activate the caspase cascade via its interaction into the apoptosome complex leading to activation of caspase-9 followed by caspase-3, with subsequent DNA fragmentation and inevitable cell death[38]. Zinc has also been shown to have a direct inhibitory effect on caspase-3 enzyme activity such that a lack of zinc can lead to its activation[39,40] and increased apoptosis.

To our best of knowledge, this is the first study, reporting that using valerian on midgestation can change zinc homeostasis in the fetal brain and lead to zinc deficiency in the fetus. Animal studies have also demonstrated that zinc deficiency during prenatal development could increase the risk of numerous neurobehavioral defects, such as learning, attention and memory defects[41,42].

4 Conclusion

Although, using valerian during midgestation did not decrease cortical volume, it caused a significant decrease in zinc level in the fetal brain. This suggests that valerian use should be limited during pregnancy.

5 Acknowledgements

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6 Competing interests

The authors declare that there are no conflicts of interest.

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孕期小鼠腹腔注射缬草提取物对胎鼠脑皮质体积及脑组织内锌和铜水平的影响

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目的：探讨孕期小鼠腹腔注射缬草提取物对胎鼠脑皮质容量及脑组织内锌和铜水平的影响。
方法：孕期雌性小鼠于怀孕第7～17天每日腹腔注射生理盐水或1.2 g/kg 体重质量的缬草提取物，分别作为对照组和实验组。怀孕第20天，处死母鼠并取出胚胎。对胎鼠的脑组织脱水，称取质量并进行形态学观察。根据卡万帕里原则测量胎鼠脑皮质的体积，并使用原子吸收光谱测试法测量胎鼠脑组织中锌和铜的水平。
结果：孕期小鼠腹腔注射缬草提取物对胎鼠的大脑质量、脑皮质的体积及脑组织中铜的水平没有影响；然而实验组与对照组相比，胎鼠脑组织中锌的水平明显降低（P<0.05）。
结论：虽然孕期小鼠腹腔注射缬草提取物对胎鼠没有明显影响，只是降低了胎鼠脑组织中锌的水平，但孕期使用缬草仍应引起注意。
关键词：缬草属；妊娠毒性；锌；铜；立体定位技术；小鼠