

MASTER'S THESIS

The impact of rhizoma chuanxiong in fetal bone development

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ABSTRACT

Background and purpose:

Rhizoma Chuanxiong (CX), the dry rhizome of *Ligusticum chuanxiong* Hort., is a commonly used Chinese herbal medicine to treat gynecological diseases. So far, more than 60 chemical components have been identified from CX such as volatile oils (ligustilide, etc.), phenolic acids (ferulic acid, etc.) and alkaloids (chuanxiongzine, etc.). These components in CX are the basis of its wide pharmacodynamic actions including estrogen-like, progesterone-like and anti-coagulant/anti-platelet effects.

In our recent survey based on previous published clinical trials, CX was ranked as one of the top 20 herbs commonly used for anti-miscarriages amongst Chinese pregnant women. However, CX should be used with caution during pregnancy as its property of “invigorating blood circulation and removing blood stagnation”. Despite its wide applications, the safe dosage of CX in pregnant women remains unclear with no records found in the current Chinese Pharmacopoeia or other guidelines. Thus, verification regarding the impacts of CX preparations and its components in embryonic development is urgently required.

In view of the limited experimental evidence that is currently available to assess the safety of CX, this project aims to (1) identify the general impacts of CX aqueous extract in maternal function and fetal development with an *in vivo* mouse model; and to (2) investigate the adverse impacts and underlying mechanisms of CX aqueous extract in fetal bone development with a biomarker assay and metabolomics analysis.

Method:

Referred to the guidelines of WHO, the Chinese Pharmacopoeia and the Hong Kong Chinese Materia Medica Standards, CX aqueous extract was prepared, and its reference marker (ligustilide and ferulic acid) were quantitatively

authenticated by HPLC analysis. LC/MS fingerprint analysis was performed for the quality control purposes. In addition, pesticide residues and heavy metals found in CX aqueous extract were examined using GC-MS and ICP-MS analysis.

In the Segment II study as per FDA and OECD guidelines, pregnant mice were randomly assigned into 6 groups (n=18 per group): i.e. mice were orally administrated with distilled water as the negative controls (Group 1); or CX aqueous extract of 2, 16, 24 and 32 g/kg/day respectively from the gestation day (GD)6 to 16(Group 2, 3, 4 and 5); or vitamin A (200,000 IU) on GD7, 9 and 11 as the positive controls (Group 6). All mice were sacrificed to assess maternal and fetal parameters on the GD18.

In the mechanistic study, the expressions of biomarkers related to fetal bone development including PICP, ICTP, B-ALP, BGP, Gdf-5, BMPs, BMP-6, BMP-8, BMP-11, IL-4, IL-4r, IL-10 and IL-10r in fetal tissue samples of the Group 1 and 5 (32 g/kg/day, n=18) were measured using ELISA analyses on GD16. Meanwhile, the metabolites of two-group samples were also analyzed by the UHD Accurate-Mass Q-TOF LC/MS, and profiling data was further analyzed by specific software.

During statistical analysis, measurement data from G1, 2, 3 4 and 5 groups were analyzed using one-way ANOVA(SPSS software, version 16.0). LSD test in Post hoc method was applied to compare differences between every two groups. Pearson's χ^2 -test was used to analyze category data from G1, 2, 3, 4 and 5 groups, and Fisher's exact test was applied to compare differences between different groups. The student t-test was also used to compare differences between G1 and G6 groups in animal studies as well as G1 and G5 groups using ELISA or metabolomics results. An intra-group difference with a *p*-value less than 0.05 was considered as statistical significant.

Result:

(1) There was no statistical significant difference in maternal and fetal parameters found between the Group 1 and 2 ($p > 0.05$). However, the maternal body weight (BW), gravid uterine weight, corrected BW change, live fetus/litter, mean fetal BW in the Group 4 and 5 were significantly lower than those in the Group 1 ($p < 0.05$); while the resorption site/litter, post-implantation loss/litter and percentage of abnormal skeletal variation were significantly higher than those in the Group 1 ($p < 0.05$).

(2) The expression of PICP, osteocalcin, BMPs, BMP-6, BMP-11, IL-4 and IL-10 receptor in fetal tissue sample of Group 5 were significantly lower than Group 1 ($p < 0.05$). The metabolites between two-group samples were significantly different (explained 48.74% of the variance), and two targeted components AA and PGE₂ were significantly decreased in the Group 5 when compared to Group 1 ($p < 0.001$). It indicated that CX aqueous extract might down-regulate above biomarkers and metabolites during fetal osteogenesis.

Conclusion:

CX aqueous extract at a low dosage of 2 g/kg/day (equals to the daily dosage of human adults) did not cause adverse effect in pregnant mice, and it suggested that this dosage of CX preparations should be safe for pregnant women. Our data demonstrated that high dosage and long-term use of CX aqueous extract might result in embryonic toxicities including fetal bone malformations for the first time. As the CX aqueous extract in this study was not contaminated by pesticide residues and heavy metals, the adverse impacts of CX aqueous extract should be considered as a result of its intrinsic components in the herb. Furthermore, CX aqueous extract might significantly down-regulate biomarkers related to bone formation and metabolism during osteogenesis. It is therefore valuable to establish a practical approach to systematically assess the safety of CX and other herbal medicines.

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