The Study of Chinese Herbal Medicine in Embryonic Development of Mice

TIAN Xiao Ying

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Principal Supervisor: Dr. XU Min

Hong Kong Baptist University

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ABSTRACT

Congenital defect is a severe health and social problem that results in a large expenditure of health care resources. Although herbal therapy has been recognized as a unique way to treat pregnant disorders since ancient times, there is insufficient research evidence on its foetal and maternal impacts. It is hazardous for pregnant women and their offspring as some herbs may be associated with embryotoxicity, teratogenicity and maternal toxicity that include all severity levels and organ systems.

In this study, commonly used Chinese herbs for treating and preventing miscarriages were systematically reviewed and analyzed based on published clinical trials; the effects on embryonic development of four selected herbs with different properties (hot or cold) and functions (promoting blood circulation or stopping bleeding) were examined in mouse studies following internationally recognized guidelines; meanwhile, the integrated effects of two herbs with opposite features or functions were investigated; and the possible reasons and mechanisms of herbal effects or interactions were also inspected by HPLC (such as pesticide residue and heavy metal analyses) and transmission electron microscope techniques (such as apoptosis).

In view of that the most popular herbal dosage-form in clinics was the herbal decoction, aqueous herbal extracts of *Psoralea corylifolia* (BGZ, 补骨脂) with hot property, *Scutellaria baicalensis* (HQ, 黄芩) with cold property, *Ligusticum chuanxiong* (CX, 川芎) with the function of warming blood and promoting circulation, and *Boehmeria nivea* (ZMG, 苦麻根) with the function of cooling blood and stopping bleeding were prepared and quantitatively authenticated by HPLC. For testing each herbal extract, pregnant mice were randomly assigned into 5 groups in each experiment and treated respectively with distilled water as negative controls, or herbal extract of low-, middle-, high-dose, or vitamin A (a well known teratogen) of 200,000 IU/kg/day as positive controls. In addition, paired herbs i.e. BGZ plus HQ or CX plus ZMG were applied in further mouse experiments to explore herb-herb interactions.

In the study of BGZ, results showed that (1) the rates of post-implantation loss in positive control (vitamin A) and high-dose (8 g/kg/day) groups were higher than those in other 3 groups ($P<0.001$ and $P<0.01$); and the rate was also higher in middle-dose (4 g/kg/day) group than low-dose (2 g/kg/day) and negative control groups ($P<0.05$); (2) the external- and skeletal-malformed foetus rates in positive control group were higher
than other 4 groups ($P<0.001$), whereas it was similar among other 4 groups; (3) the maternal liver weight in high-dose group was higher than those in negative control group or low- and middle-dose groups ($P<0.05$). It indicated that BGZ aqueous extract at middle- or high-dose (about 3.6-7.2 times of human daily dose) might have foetal and maternal adverse effects.

In the study of CX, results showed that (1) the rate of post-implantation loss in positive control group was higher than that in any other groups ($P<0.001$); moreover it was higher in middle- (16 g/kg/day) and high-dose (24 g/kg/day) groups than those in negative control and low-dose (2 g/kg/day) groups ($P<0.01$); (2) the rate of external malformed foetus in positive control group was higher than that in any other groups ($P<0.001$); whereas there was no significant difference among other 4 groups; (3) the rate of skeletal malformed foetus in positive control group was higher than that in any other groups ($P<0.001$); moreover it was higher in high-dose group than those in negative control, low- and middle-dose groups ($P<0.01$); (4) the maternal relative body weight gain in middle- and high-dose groups were lower than those in negative control and low-dose groups ($P<0.05$ and $P<0.01$). It indicated that CX aqueous extract at middle-dose (about 14.4 times of human daily dose) or above might have foetal and maternal adverse effects.

In the studies of HQ and ZMG, the data in positive control groups were the same as above studies; meanwhile, there was no difference in the rates of post-implantation loss, external- and skeletal-malformed foetus, and the maternal relative body weight gain among negative control, low- (2 g/kg/day), middle- (8 g/kg/day) and high-dose (32 g/kg/day) groups. However, the maternal relative liver weight and kidney weight in high-dose group of HQ were significantly higher than those in negative control group, low- and middle-dose groups ($P<0.05$). It indicated that HQ aqueous extract at high-dose (about 28.8 times of human daily dose) or above might have maternal adverse effects.

In the study of BGZ plus HQ, results showed that (1) the rates of post-implantation loss in BGZ 8 g/kg/day and 12 g/kg/day groups were significantly higher than that in negative control group ($P<0.01$) as expected as the previous study; whereas in BGZ 8 g/kg/day plus HQ 8 g/kg/day and BGZ 12 g/kg/day plus HQ 12 g/kg/day groups, they were not significantly different from that in negative control group ($P>0.05$); (2) maternal relative liver weight in BGZ 8 g/kg/day and 12 g/kg/day groups were significantly higher than that in negative control group ($P<0.01$) as expected as the
previous study; whereas in BGZ 8 g/kg/day plus HQ 8 g/kg/day and BGZ 12 g/kg/day plus HQ 12 g/kg/day groups, they were not different from that in negative control group ($P>0.05$); (3) there was no significant difference in developmental and maternal toxicity in HQ 8 g/kg/day and 12 g/kg/day groups compared with negative control group. It indicated that at the same dose level, combining hot property herb BGZ with cold property herb HQ might be safer than administering BGZ only.

In the study of CX plus ZMG, results showed that (1) the rates of post-implantation loss in CX 16 g/kg/day and 24 g/kg/day groups were significantly higher than that in negative control group ($P<0.01$) as expected as the previous study; whereas in CX 16 g/kg/day plus ZMG 16 g/kg/day and CX 24 g/kg/day plus ZMG 24 g/kg/day groups, they were not significantly different from that in negative control group ($P>0.05$); (2) the rate of foetal skeletal malformations in CX 24 g/kg/day group was significantly higher than that in any other group ($P<0.01$) as expected as the previous study; whereas there was no statistical difference among negative control, CX 16 g/kg/day, CX 16 g/kg/day plus ZMG 16 g/kg/day and CX 24 g/kg/day plus ZMG 24 g/kg/day groups ($P>0.05$); (3) the maternal relative body weight gain in CX 16 g/kg/day and 24 g/kg/day groups were significantly lower than those in negative control group ($P<0.05$ and $P<0.01$) as expected as the previous study; however, the relative body weight gain in CX 16 g/kg/day plus ZMG 16 g/kg/day and CX 24 g/kg/day plus ZMG 24 g/kg/day groups were not significantly different from those in negative control group ($P>0.05$); (4) there was no significant difference in developmental and maternal toxicity in ZMG 16 g/kg/day and 24 g/kg/day groups compared with negative control group. It indicated that ZMG with the function of cooling blood and stopping bleeding might decrease foetal and maternal adverse effects caused by CX, a herb with the function of warming blood and promoting circulation.

The pesticide residue and heavy metal in BGZ and CX herbal samples were analyzed with HPLC, and results documented that foetal and maternal adverse effects showed in the above mouse experiments were not caused by environmental contaminations of herbal materials due to some toxic factors. Although no abnormal change in morphologic examinations of maternal liver, kidney and heart was found in all in vivo studies, a significant increase of apoptosis after treatments with vitamin A or high-dose BGZ extract was observed in mouse embryos under transmission electron microscope. It hinted that abnormal apoptosis might play an important role in embryotoxicity related to herbal treatments.
In conclusion, some commonly used herbs for treating and preventing miscarriages might have foetal and maternal adverse effects, and more toxicological studies and clinical data should be valuable to further identify their safety for pregnant women; it must be cautious to prescribe herbs with some properties and functions such as herbs with hot property and promoting blood circulation during pregnancy, although integrating herbs with different properties or functions according to the theories and experiences of traditional Chinese medicine may increase their safety clinically; and it is necessary to further expound underlying mechanisms of herbal effects and herb-herb interactions with modern scientific techniques.
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