E:/Biomedica Vol.24, Jul. – Dec. 2008/Bio-2.Doc (WC) P. 96-98

GINSENG INDUCED FETAL SKELETAL MALFORMATIONS

KHALID, M TAHIR, *AA SHORO.
Department of Anatomy, University of Health Sciences, Lahore; and Liaquat Medical University, Karachi

ABSTRACT
The use of alternative therapies including Herbal medicines is rapidly escalating in both the developed and the developing countries. It is generally believed that 'natural' herbal medicines are better and safer than conventional medicine; herbal medicines are in fact associated with serious toxic effects. The current study was conducted to evaluate the efficacy and safety of Panax Ginseng; it was found that maternal treatment with Panax Ginseng negatively affected the development of skeletal system. Findings in our study suggest that further investigations and monitoring of embryotoxic effects of ginsenosides on human pregnancy are warranted.

INTRODUCTION

The use of herbal medicine is rapidly escalating in the developed as well as the developing countries; amid the various herbal medicines practiced in the world, Ginseng is one of the commonly used and highly researched herby in the world.[1] Panax Ginseng is regarded as a tonic with adaptogenic, stimulant and aphrodisiac properties.[2] It enhances phagocytosis, improves physical and mental performance, increases resistance to exogenous stress factors and affects hypoglycemic activity.[3] The recognized primary active components of Ginseng are a group of 30 different triterpene saponins, also referred to as Ginsenosides, which vary in content and relative proportions among different species of Ginseng. Of numerous Ginsenosides that have been identified six (Rb1, Re, Rc, Rd, Rb2 and Rg1) have been chosen for reference standards for Ginseng products.[2] The mechanism by which the Herbal remedy exerts its affects is most likely through Hypothalamic-Hypophysial-Adrenal axis and through immunostimulation.[4]

It is generally believed that 'natural' herbal medicines are better and safer than conventional also medicine; herbal medicines are, in fact associated with serious toxic effects. Up to 64% of women are reported to take herbal supplements including Ginseng during their pregnancy. Despite wide spread usage of Ginseng during pregnancy, information concerning the potential effects of Ginseng on the developing fetus in vivo are lacking. Various in vitro studies prove that Ginsenosides exert direct teratogenic effects on rat and mouse embryos; there is, however a significant variability in embryotoxic effects of different Ginsenosides.[5-9]

MATERIALS AND METHODS

Thirty albino mice (twenty-four female and six ma-les) 6-8 weeks old were housed in the Research Laboratory of UHS Lahore under controlled conditions. Female mice were left overnight for mating, the pregnancy was confirmed the following morning by the presence of vaginal plug and this was considered as gestational day 0 (zero).[5] Pregnant mice were randomly divided into three groups.

Commercially available Panax Ginseng root powder containing 3% Ginsenosides was obtained from sigma. According to the rule of surface area ratio and an increased metabolic rate observed in albino mice the low dose of Ginseng calculated was 780mg/kg/day, and the high dose of Ginseng calculated was 1560mg/kg/day.

Group 1
0.1ml of distilled water was given orally throughout pregnancy.

Group 2
780 mg/kg/day dissolved in 0.1ml of distilled water was given orally throughout pregnancy.

Group 3
1560 mg/kg/day dissolved in 0.1ml of distilled water was given orally throughout pregnancy.

Skeletal staining
As the protocol for teratological and toxicological studies half of the pups in each litter were examined for skeletal defects; the technique adopted for skeletal staining was a Alcian Blue – Alizarin Red Skeletal Staining method.[10]

Statistical analysis
The statistical analysis was carried out using computer software Statistical package for social sciences (SPSS) Version 15. The difference was regarded statistically significant if the 'p' value was < 0.05.

Observations and Results
In the control group the skeletal elements were well formed with no apparent deformity (Figure 1).

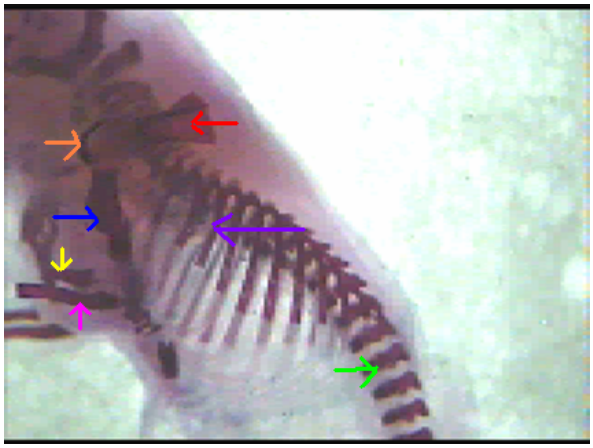

Figure 1: *Photograph of fetal skeleton from Control group stained with Alcian blue and Alizarin red, showing ossified parts of upper limb. Ossified parts of scapula (red arrow), humerus (blue arrow), ulna (yellow arrow), radius (pink arrow), ribs (lilac arrow), vertebrae (green arrow) and clavicle (orange arrow) are evident.*

Mal-union of the sternaebrae was, however, observed in two fetuses, one in each of the treated groups. Both the fetuses had asymmetric bone element of the sternum and the ribs as compared to the control group (Figure 2).

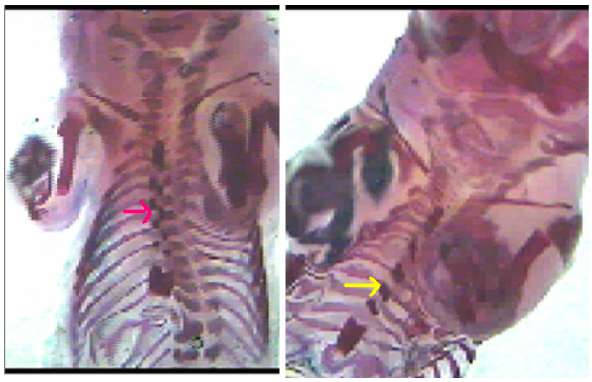

Figure 2: *Photograph of skeleton of low dose treated groups, stained with Alcian blue and Alizarin red, showing malformation of sternum (pink arrow) compared to normal ossification of sternum (yellow arrow) from the control group.*

Group	Fetuses with skeletal malformations	Fetuses with no skeletal deformities
Control (52)	00	52*
Low dose (47)	05	12
High dose (43)	04	12

Defects in the lumbar vertebrae were also seen in one fetus of high dose treated group. Mal union of the transverse processes with the vertebral body was additionally evident; however, no malformation of the vertebral spine was seen (Table 1, Figure 3).

Figure in parenthesis shows number of fetuses in each group. * P< 0.05.

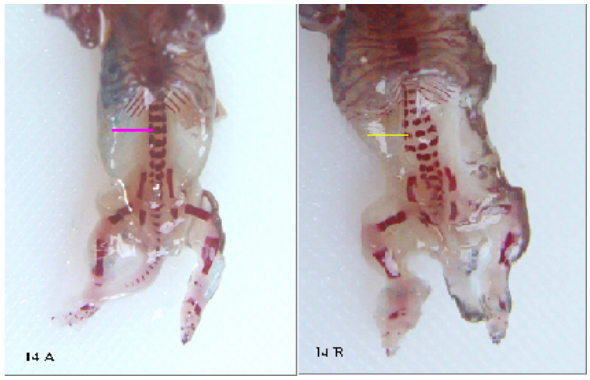

Figure 3: *Photograph of the fetal skeleton stained with Alcian blue and Alizarin red showing mal union of lumbar vertebra in high dose treated group as c ompared to the control.*

DISCUSSION

In previous studies Panax Ginseng has been used in the prevention of IUGR,[11] teratogenic effects of hexavalent chromium and hyperthermia.[12,13] Ginseng with its structural similarities with the steroids may contain an endocrine-like active substance affecting neonate development.[14] It is an established fact that bone development and growth is affected by estrogen.[15,16] Ginsenosides Rb1 can mediate its activities through activation of estrogen receptors –α and β[14] and Ginsenoside Rg1 can mediate its activities through activation of estrogen receptors; the activation of Ginseng saponins is probably the basis of skeletal malformations observed in fetuses in treated groups. The skeletal malformations were pronounced in the high dose treated group as compared to the low dose treated or the control group .The findings in the current project were in accordance with the findings of Ashmaoui et al 2003. They found that 5.4% of the fetuses were negatively affected by Ginseng; the limbs were more involved than the axial skeleton.[9] The involvement of the appendicular skeleton is, however, contradictory to our findings in which axial skeleton deformities were marked.

In vitro studies have revealed significant teratogenic and morphologic effects especially after maternal treatment with Panax Ginseng.[5-8] These findings are in synchronization with our findings in which the gross malformations were pronoun-

ced in the high dose treated group as compared to low dose treated or the control group.

The unguarded rise in use of herbal medicines raises a question as to how safe are these preparations for the unborn fetus. Our society has no problem in procuring these remedies as these are considered safer and better than most of the conventional or allopathic medicines. If we adopt a causal attitude to the potential embryotoxic effects of ginsenosides, it might induce some kind of severe consequence in humans.

ACKNOWLEDGEMENTS

The authors gratefully acknowledges the V.C. of U.H.S for providing funds and lab. Facilities.

REFERENCES

1. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pahrmacol 1999; 58: 1685- 93.
2. Kitts DD, Hu C. Efficacy and safety of ginseng. Public Health Nutrition 2000; 3 (4A): 473-85.
3. Kiefer D, Pantuso T. Panax Ginseng. Am Fam Physician 2003; 688: 1539-42.
4. Kim D, Moon Y, Jung J, Min S, Son B, Suh H, Song D. Effects of ginseng saponin administered intraperitoneally on the hypothalamo-pituitary-adrenal axis in mice. Neuroscience Letters 2003; 343: 62-6.
5. Chan LY, Chiu PY, and Lau TK. An in-vitro study of ginsenoside Rb1-induced teratogenicity using a whole rat embryo culture model. Hum Reprod. 2003; 18 (10): 2166-8.
6. Chan LY, Chiu PY, Lau TK. Embryotoxicity study of ginsenoside Rc and Re in in-vitro rat whole embryo culture. Reproductive Toxicology 2004; 19 (1): 131–4.
7. Liu P, Yin H, Xu Y, Zhang Z, Chen K, Li Y. Effects of ginsenoside Rg1 on post implantation rat and mouse embryos cultured in vitro. Toxicology in Vitro 2006; 20: 234–8.
8. Liu P, Xu Y, Yin H, Wang J, Chen K, Li Y. Developmental toxicity research of ginsenoside Rb1 using a whole mouse embryo culture model; Birth Defects Res B Dev Reprod Toxicol.2005 Apr; 74 (2): 207-9.
9. Ashmaoui HME, Girgis SM, Raouf AE. Evaluation of the potential mutagenic effects of ginseng on maternally treated postimplanted mouse fetuses. The Egyptian Journal of Hospital Medicine 2003; 13: 57-65.
10. Erdodan D, Kadiodlu D, Peker T. Visualisation of the fetal skeletal system by double staining with alizarin red and alcian blue. Gazi Medical Journal 1995; 6: 55-8.
11. Zhang WY, Teng H, Zeng Y. Ginseng Saponin treatment for intrauterine growth retardation. Zhonghua Yi XUE Za Zhi, 1994; 74 (10): 608-10
12. Helian LI, Bowei W, Dan Z, Liying H. Protective effects of vitamin B12, ginseng saponin, and folic acid against murine fetal deformities caused by hyperthermia. Chinese Medical Journal 2003; 116 (11): 1776- 8.
13. Elsaieed EM, Nada SA. Teratogenicity of hexavalent chromium in rats and the beneficial role of ginseng. Bull. Environ. Contam. Toxicol. 2002; 68: 361-8.
14. Awang DV. Maternal use of ginseng and neonatal adrogenization. JAMA 1991; 266 (3): 363.
15. Vidal O, Lindberg MK, Hollberg K, Baylink DJ, Andersson G, Lubahn DB et al. Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. Proc Natl Acad Sci U S A. 2000; 97 (10): 5474-9.
16. Lindberg MK, Alatalo SL, Halleen JM, Mohan S, Gustafsson JA, Ohlsson C. Estrogen receptor specificity in the regulation of the skeleton in female mice. J Endocrinol. 2001 Nov; 171 (2): 229-36.