GINSENG INDUCED GROSS MALFORMATIONS IN ALBINO MICE IN VIVO

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It is generally believed that herbal medicines are safer to use during pregnancy than the conventional. Herbal therapies have been recently reported to be associated with toxic effects in vitro, including teratogenic. There is paucity of evidence regarding effects of Geinseng on developing concepts in experimental animals. The present work was, therefore, designed using albino mice; these were given Panax ginseng root powder throughout pregnancy, the dams were sacrificed on 18th day of gestation and foetuses were delivered. Gross malformations were evident in the treated groups and were found to be statistically significant (P< 0.05) and dose dependent, being more pronounced in the high dose treated group as compared to the low dose treated or the control group. Previous studies have shown that different monomers of ginsenosides have teratogenic effects in vitro; we adjoin by concluding that ginsenosides present in the commercially available Ginseng products have teratogenic effects in vivo as well.

The usage of alternative medicine, including the herbal medicine, is rapidly increasing in both the developed and the developing countries. Ginseng is regarded as a tonic with adaptogenic stimulant with aphrodisiac properties[1].

Ginseng is the common name of two species of 'Panax' of the family Araliaceae. Harding in 1972 described four varieties of Panax Ginseng, the Asian species and three varieties of Panax quinquefolius, the American species [2]. The name 'Panax' is derived from the Greek words Pan (all) and Akos (healing) or panacea whilst 'Ginseng' means 'man root' due to the shape of the root[3].

The recognized primary active components of ginseng are a group of 30 different triterpene saponins, also referred to as ginsenosides; these vary in content and relative proportion among different species of Ginseng[4]. All parts of the plant contain pharmacologically active constituents, it is the root that is highly regarded, making Ginseng one of the most popular and expensive herbs in the world[3].

Up to 64% of women are reported taking Herbal supplements, including Ginseng during their pregnancy[5]. It is generally believed that 'natural' herbal medicines are better and safer than the conventional treatment; recent reports have appeared indicating that herbal medicines are associated with serious toxic effects[6]. Despite wide spread usage of Ginseng during pregnancy, information concerning the potential effects of Ginseng on the developing foetus in vivo are lacking. Various in vitro studies have shown that Ginsenoside exerts direct teratogenic effects on rat and mouse embryos and a significant variability has been reported in embryotoxic effects of different Ginsenosides.[6-10]

MATERIALS AND METHODS

Panax ginseng root powder:
Commercially available Panax Ginseng root powder containing 3% Ginsenosides was obtained from Sigma.

Dosage
The dosage of Ginseng was calculated by determining:

1. Maximum tolerated dose[11].
2. Human therapeutic dose[12].

According to the rule of surface area ratio and an increased metabolic rate observed in albino mice, the human therapeutic dose of Ginseng was calculated to be 780 mg/kg/day, and the maximum tolerated dose of Ginseng was 1560 mg/kg/day.

Animals
Thirty albino mice (twenty-four females and six males) 6-8 weeks old were procured from the National Institute of Health, Islamabad. The Mice were housed in the Research Laboratory of University of health sciences, Lahore, under controlled conditions of temperature (22 ± 0.5 °C, humidity (50 ± 10%) and 12 hours light and dark cycles; they were fed on solid diet and tap water ad libitum.

Grouping
Female mice were left overnight for mating, the pregnancy was confirmed the following morning by the presence of vaginal plug; this was considered as gestational day zero[6]. Pregnant mice were randomly divided into three groups; each group contained eight female and two male mice.

Group 1
In group 1 (Control), 0.1 ml of distilled water was additionally given orally throughout pregnancy.

Group 2
In group 2 (Low dose treated group) the human therapeutic dose of Ginseng (780 mg/kg/day) dissolved in 0.1ml of distilled water was given orally throughout pregnancy.

Group 3
In group 3 (high dose treated group) the maximum tolerated dose of Ginseng (1560 mg/kg/day) dissolved in 0.1ml of distilled water was given orally throughout pregnancy.

Statistical analysis
The statistical analysis was carried out using computer software Statistical package for social sciences (SPSS). The arithmetic mean of observations was calculated; standard error of mean values was calculated and the significance between two means was calculated by the paired sample t test in SPSS. The difference was regarded statistically significant if the 'p' value was < 0.05.

The significance between the two groups was calculated by chi-square test. The test was applied using SPSS; the difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS

Litter size
There was a noticeable reduction in the number of foetuses in the treated groups. In the low dose treated group the litter size was 47 in the high dose treated group the litter size was 43 as compared to a litter size of 52 in the control group. The reduction in the foetal litter size was, however, statistically in-significant p > 0.05 (Table 1).

Table 1: *Comparison of litter size of control, low dose treated and high dose treated groups.*

Group	Foetuses	Mean ± SE in numbers
Control (8)	52	6.50 ± 1.11
Low dose (8)	47	5.87 ± 0.39
High dose (8)	43	5.38 ± 0.68

Crown-rump length and weight
The average Crown rump length (CR length) of the foetuses in the group treated with low dose of Ginseng was 2. 40 cm and of the foetuses receiving high dose of Ginseng was 2.10 cm as compared to 2.80 cm seen in the control group. The reduction in the CR length of the foetuses in treated groups compared with the CR length of foetuses in control group was significant p < .05 (Table 2)

Table 2: *Comparison of length of foetuses of control, low dose treated group and high dose treated group.*

Group	Range in cm	Mean ± SE in cm
Control (52)	2.5-4.0	2.80 ± 0.470
Low dose (47)	2.0-2.8	2.4 ± 0.035
High dose (43)	1.3-2.7	2.10 ± 0.057

There was an obvious reduction in the average weight of the foetuses, the weight of the foetuses in the low dose treated group was 1.27 gm and the average weight of the fetuses in the high dose treated group was 1.12 gm as compared to 1.64 gm seen in control group. The decrease in the weight of the foetuses in treated groups compared with the weight of foetuses in control group was significant p < 0.05 (Table 3).

Table 3: *Comparison of weight of foetuses of control, low dose treated group and high dose treated group.*

Group	Range in gm	Mean ± SE in gm
Control (52)	1.11 – 2.99	1.66 ± 0.069
Low dose (47)	1.04 – 1.90	1.27 ± 0.040
High dose (43)	0.6 – 1.53	1.12 ± 0.04

Gross features
Gross malformations were observed in the treated groups, these were, however, pronounced in the high dose treated group. In the low dose treated group, two foetuses were observed to have limb deformities; the fore limb was neither distinguishable into three parts nor had prominent interdigital clefts. All other morphological parameters were the same as were observed in the control group (Figure 1 next page).

One dam which was taking maximum tolerated dose of Ginseng throughout pregnancy did not show any weight gain for continuous three days from day 14; she was sacrificed on the 17th day of gestation instead of the usual 18th day in fear of foetal resorption. Three foetuses were delivered, two were partially resorbed; there was no evidence of sustained development. (Figures 2 and 3).

The third foetus had severe malformations as observed under the dissecting microscope. The face and upper limbs were grossly malformed, and there was a complete absence of the lower limbs (Figure 4).

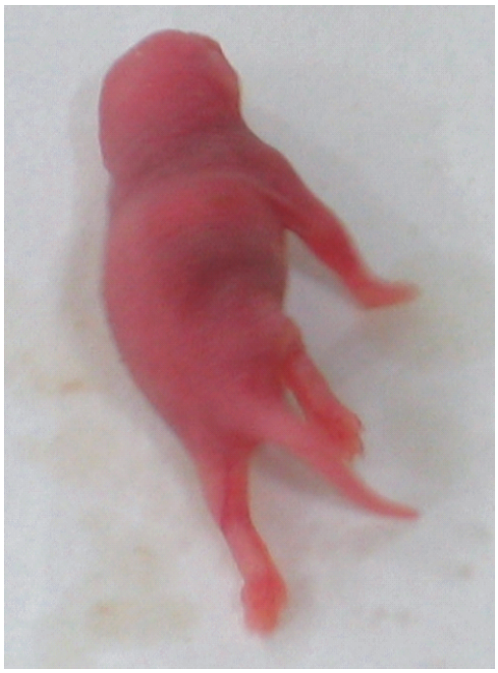

Fig. 1: Foetus of low dose treated group with malformed fore limb; the foetus was unable to stand on its limbs and showed an abnormal extension of the hind limb when forced to stand.

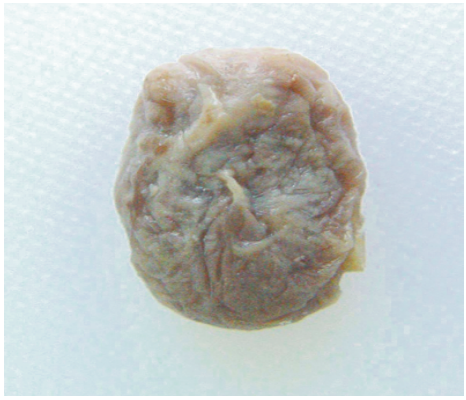

Fig. 2; *Photograph of partially resorbed foetus.*

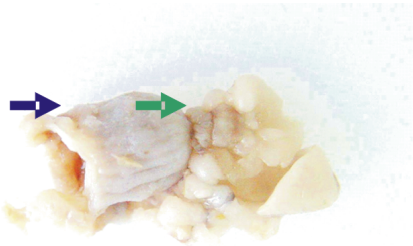

Fig. 3: *Photograph of a resorbed fetus (blue arrow) in the uterine horn in high dose treated group, also evident is left Fallopian tube (green arrow).*

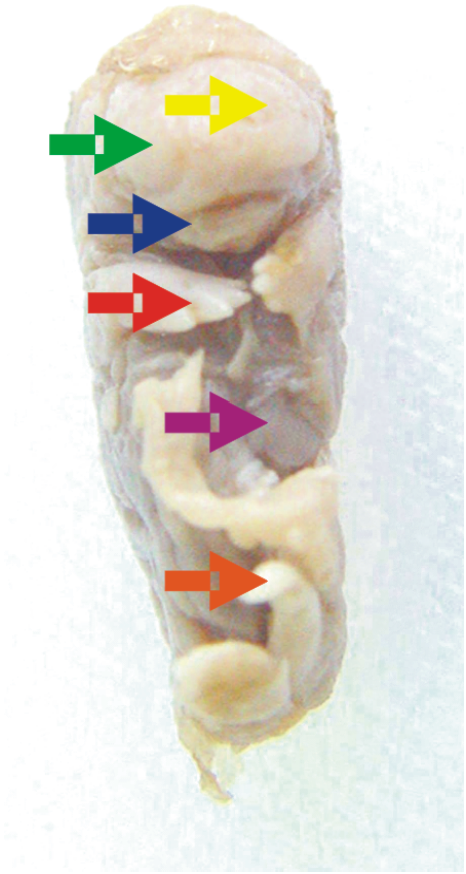

Figure 4: *Photograph of malformed foetus in the high dose treated group. The upper limbs were short and the fore paws showed three inter-digital clefts (red arrow); the face was grossly anomalous with malformed external nares (yellow arrow), orbital margins (green arrow), oral fissure (blue arrow). The tail was short and cranially directed (orange arrow), and the thoracic cavity was flattened (pink arrow).*

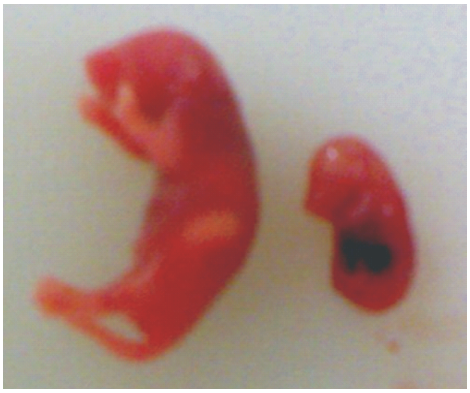

Fig. 5: *Photograph of growth retarded foetus of low dose treated group as compared with the foetus of control group.*

In a litter of low dose treated group one partially malformed foetus with a CR length of 1.3 cm and a weight of 0.6 gm was also observed in a litter of eight foetuses (Figure 5). The gross abnormallities observed in the treat-ed groups were statistically significant P <0.05 as compared to the control group (Table 4).

Table 4: *Comparison of gross foetal abnormalities of control, low dose and high dose treated groups.*

Group	Fetuses with gross malformaions	Fetuses with no gross malformations
Control (52)	00	00*
Low dose (47)	05	42**
High dose (43)	04	39***

DISCUSSION

Study of embryos recovered from the mothers of the treated groups with the dissecting microscope, revealed that they showed changes upon treating them with the herbal remedy; the effect on the CR length (Table 2) and weight (Table 3) were statistically significant (p<0.05). The difference in weight and CR length compared between the low dose treated and high dose treated groups was also significant (p < 0.05), implying thereby that the effect on the CR length and weight was dose dependent.

The findings in the current investigation were in accordance with the findings of Ashmaoui et al 2003; they conducted an experimental study in Egypt to evaluate the potential mutagenic effects of Ginseng on maternally treated post implanted mouse foetuses in 2003 and found that 5.4% of the foetuses were grossly abnormal; the limbs were more involved than the axial skeleton[10]. The involvement of the appendicular skeleton is, however, contradictory to our findings; on the contrary we found axial skeleton deformities were marked. A small but significant decrease in foetal body weights was also reported by Ashmaoui et al, it being more marked in the group receiving high dose of Panax Ginseng. This finding is comparable to our that reduction of weight and CR length is dose dependant.

In vitro studies on foetuses revealed significant teratogenic and morphologic effects, especially with high concentration of Panax Ginseng.[6-9] Chan et al in 2003 concluded that reduction in total morphological scores, somite number, crown rump length and scores of some individual features were pronounced when the concentration of ginsenosides was increased[6]. In another study conducted to evaluate the effects of ginsenosides *Rc and Re* on rat and mouse foetuses cultured in vitro concluded that ginsenoside Re induced severe developmental delay with significant reduction in morphological score of all systems[7]. GRb_1 was reported to negatively affect the total morphological score from the concentration of 30µg/ml[9]. GRg1 and GRb1 exert embryotoxicity both in rats and mice[8]. These findings are similar to our observations in which we found that gross malformations were dose dependent and pronounced in the high dose treated group as compared to low dose treated or the control group (Table 4).

Contrary to our findings in which the CR length and weight progressively decreased with increase of dose of the drug in treated groups, a study conducted in 1994 reported that intra uterine growth retardation (IUGR) was prevented by using Ginseng Saponins[11]. In previous studies it had been reported that Panax Ginseng effectively prevented the teratogenic effects of hexavalent chromium and hyperthermia, p value being < 0.05[12,13]; the investigators, however, failed to mention the type of Ginseng saponin or concentration of ginsenosides in the preparation used in their studies.

Ginsenosides (except Ro) belong to a family of steroids named steroidal saponins. Ginseng, with its structural similarities to the steroids may contain an endocrine-like active substance which affects neonate development[14]. Further, it had also been reported that bone development and growth is affected by estrogen[15]. Ginsenosides Rb1 from Panax Ginseng activates estrogen receptors –α and β and ginsenosides Rg1 can mediate its activities through activation of estrogen receptors[16]; the activation of the estrogenic receptors by Ginseng saponins could possibly explain the basis of dwarfism observed in foetuses of the treated groups.

In a study conducted in Egypt[10], numerical aberrations and structural deformities of chromosomes were shown on chromosomal analysis of foetuses produced by the dams treated with ginseng during gestation; the gross malformations apparent in the treated groups could possibly be produced by chromosomal changes.

It is **Concluded** that previous studies have shown that different monomers of ginsenosides have teratogenic effects in vitro[6-10]; we adjoin by concluding that ginsenosides present in the comercially available Ginseng products have teratogenic effects in vivo. Although results from animal teratogenicity may not reflect the circumstances in human, findings in our study suggest that further investigations and monitoring of possible embryo toxic effects of ginsenosides in human are warranted.

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