

Mesodermal Dymorphogenesis of Ginsenosides: An Experimental Study

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ABSTRACT

Background: The versatile and dynamic activities of Panax Ginseng are attributed to its active components. They are readily available over the counter and are known for their effects as an aphrodisiac & health building; in addition, they are given rather generously during pregnancy, as they are considered virtuous for the baby and mother. Despite its easy availability and excess usage, little is known about its effects on the fetus. The current experimental design was focused towards the lack of differentiation and inhibition of cell growth of mesodermal derivatives inflicted by PanaxGingex.

Methods: 18 pregnant albino dams were randomly divided into three groups; Group A was control, Group B was Low dose and Group C was labelled as High dose groups. Tissues (bone, kidney and blood) were selected as derivatives of paraxial, intermediate and lateral plate mesoderm respectively and were used for light microscopic study.

Results and Conclusion: The light microscopic examination demonstrated extensive apoptosis and an escalation of angiogenesis. Both the histological findings were not only statistically significant but was clearly indicative of dymorphogenesis. The results of present study raise a finger towards the un-supervised practice of over the counter preparations especially during the vital antenatal period of development.

Key words: Ginsenosides, embryotoxicity, dymorphogenesis, mesoderm

INTRODUCTION

The antiquity of herbal medicine practice is perhaps as deep-rooted as the civilization itself. With the institution of allopathy, 'Herbalism' went into a swift decline, however, free accessibility and self-proclaimed benefits make them the first line of treatment in a number of developing and developed countries. Ginseng is one of the most frequently consumed and exceedingly investigated herbs^{1, 2}. Chemically active fundamentals entitled Ginsenosides are dispersed in the plant in variable concentrations and due to maximum concentration the root is treasured³. The hypothalamus-pituitary-adrenal axis and immuno-stimulations are the pathways by which the herb yields its effects^{4, 5, 6}. The growing acceptance of these therapies may upsurge the inadvertent use of medicinal herbs particularly in antepartum period, raising the likelihood of antagonistic fetal or neonatal effects^{7,8}. Out of the traditional Chinese remedies used in pregnancy Ginseng was used in 31.7% gestations, the probable indicator for its extensive usage was maternal wellbeing and prevention of

intra-uterine growth retardation⁹. Innumerable in vitro experiments have demonstrated undeviating effects of Ginsenoside on embryos^{10,11,12,13,14}; unconjugated steroids pass the placental membrane without any hindrance and effect the fetal progression and differentiation¹⁵. Ashmaoui et al in 2003 demonstrated numerical & structural chromosomal aberrations associated with Ginseng usage; Ginsenosides had noteworthy teratogenic and morphologic effects on rat embryos especially when the dose concentration was $\geq 30 \mu\text{g/ml}$ ¹⁴.

MATERIALS AND METHODS

18 pregnant dams were randomly divided into three groups with two male albino mice in each group; the animals were kept under controlled settings in the animal house of University of Health Sciences, Lahore. The dams in **Group A** were given 0.1ml of distilled water; **Group B** dams received 780mg/kg/day of Ginseng root powder (Low dose) and **Group C** dams were given 1560mg/kg/day (high dose) of Ginseng root powder. The root

powder was given orally to the experimental animals in 0.1ml of distilled water for 20 days of pregnancy.

Microscopic Examination

The pups were dissected under the dissecting microscope and the organs (liver, kidneys) were attained. The processed tissues were embedded in paraffin and stained with H&E for light microscopic study.

Blood Cell Count of Liver

Regions adjacent to the central vein were chosen for the blood cell count in the liver; the blood cells were counted per mm² at five sites and their mean was taken under X 63 using a 10 x 10 reticule.

Skeletal Staining

To evaluate the skeletal deformities in the pups the procedure embraced was Alcian Blue – Alizarin Red Skeletal Staining technique¹⁶.

Statistical Analysis

Qualitative data was analyzed using chi square test and quantitative data was analyzed using students't' test.

RESULTS

Paraxial mesoderm (Skeletal system)

Axial skeleton evaluation demonstrated disproportionate bone element of the sternum and the ribs. Defect in the lumber vertebrae of animals on high dose was also noted. The transverse processes were not united with the vertebral body; however, vertebral spine was normal (Table 1)

Table 1: Skeletal malformations in experimental groups as compared to control group.

Group (number of embryos)	Embryos with skeletal malformations	Embryos with no skeletal deformities	Degree of freedom	X ²	p - Value
A (52)	00	52	1	16.488	< 0.05
B (47)	05	12	1	20.512	< 0.05
C (43)	04	12			

P value < 0.05 was considered statistically significant

Intermediate Mesoderm (kidneys)

The numbers of glomeruli seen in the experimental groups were markedly reduced as compared to the control group; the reduction in the number was not only statistically significant p< 0.05 but was also dose dependent.

Table 2: Reduction in Number of renal glomeruli in experimental and control groups

Group (number of embryos)	Range per mm ²	Mean ± SE	Value of 't'	p- Value
A (52)	3-6	4.17 ± 0.126	34.785	0.000*
B (47)	1-6	2.27 ± 0.16	14.221	0.000**
C (43)	1-4	1.8 ± 0.133	14.035	0.000***

The visceral and parietal layers exhibited statistically significant signs of cellular degeneration in the experimental groups (p < 0.05: Table 3).

Table 3: Cellular degeneration/apoptosis seen in visceral and parietal layers of renal glomeruli in experimental and control groups

Group (number of embryos)	Fetuses with malformations	Fetuses with no malformations	df	X ²	p- Value
A (52)	00	52	1	12.30	< 0.05*
B (47)	10	37	1	9.87	< 0.05*
C (43)	20	19			

The medullary and the cortical collecting ducts demonstrated dose dependent, statistically significant ($p < 0.05$) degeneration & cell death (Table 4).

Table 4: Medullary & cortical collecting duct degeneration & cell death in experimental and control groups

Group (number of embryos)	Fetuses with tubular degeneration	Fetuses with no tubular degeneration	df	X ²	p- value
A (52)	00	52	1	18.04	< 0.05*
B (47)	14	33	1	9.87	< 0.05**
C (43)	20	19			

Angiogenesis

The mesodermal lineage cells witnessed in histological section of the fetal liver were Neutrophils, Eosinophils, Lymphocytes & Erythrocytes, the change in the number of cells detected is summarized in Table 5

Table 5: comparison of Mesodermal lineage cells in liver

Group (number of embryos)	Neutrophils (Range) per mm ²		Eosinophils (Range) per mm ²		Lymphocytes (Range) per mm ²		Erythrocytes (Range) per mm ²	
		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE
A (52)	0 – 3	1.00 ± 0.122	0 – 2	0.49 ± 0.11	3 – 15	5.79 ± 0.39	0 – 18	3.59 ± 0.5962
B (47)	0 – 4	1.04 ± 0.132	0 – 2	0.77 ± 0.10	7 – 17	13.29 ± 0.39	4 – 26	17.17 ± 0.746
C (43)	0 – 4	2.05 ± 0.19	0 – 2	0.82 ± 0.10	6 – 25	15.38 ± 0.79	5 – 51	21.44 ± 1.62 °

There was a statistically significant change in the number of blood cells witnessed in the experimental groups in comparison with control group. The most imperative increase was in the Erythrocytes count observed in the experimental groups as compared to control group.

DISCUSSION

In several nations, herbal medicines such as extracts of Ginseng are employed on the market as supplements, which are easily accessible over the counter without any supportive scientific evidence¹⁷. The fetuses in the treated groups showed arrested growth and gross malformations; anomalies of the skeletal system and gross structure were remarkably prominent in the group receiving high dose (Table 1). The findings were statistically significant ($p < 0.05$) and corroborate with the findings of Ashmaoui et al¹⁴. Previous experimental studies have shown noteworthy morphological and teratogenic effects in fetuses of mothers treated with herb extract^{11,12,13,14}. Chan et al in 2003 conducted an experiment to evaluate the effects of Ginsenoside Rb1 on a whole rat embryo culture; they established that reduction in somite number & Crown-Rump Length was pronounced when the

concentration of Ginsenosides was Increased¹¹; the findings are in harmony with our work, indicating embryotoxicity. Degenerated pale areas in the sub-cortical region of the renal interstitium with scattered nuclei showed signs of decay; the cells did not exhibit any distinctive cell boundaries; changes seen in the light microscopic pictures were probably the result of apoptosis triggered by Ginseng saponins. Numerous Ginsenosides have demonstrated cytotoxic and delayed differentiation against tumor cells, while a couple of others have shown complete contradictory effects^{1,18}. Propagation of the G1 stage of cell cycle is constant feature of ginsenoside Rh2¹⁹. Active components of ginseng bear the same organization as that of steroid hormones, owing to which are able to move across the placental membrane without any hindrance, arresting cell division and

differentiation. As is the case with the steroids, they can adhere with the nuclear membrane receptors hampering the mRNA transcription leading to defective protein synthesis²⁰. The features of cellular disintegration & death seen in cortical & medullary collecting ducts possibly resulted on account of elevated NO production triggered by Ginsenosides; a major functional component of Ginseng root is known to elevate levels of NO₂ and NO₃^{21,22}. Evident in microscopic sections, was an abundance of blood cells in the histological sections of liver. The treated groups manifested an increase in blood cell count with the amount of lymphocytes and erythrocytes more pronounced in the histological preparations); the increase in lymphocyte number, observed in the treated groups, was statistically significant (p < 0.05: Table 15). The number of the lymphocytes increased with the increasing dose of the Ginsenosides which are known to differentially modulate lymphocyte proliferation²³; the proliferation of lymphocytes in Ginsenosides treated animals earlier reported is in accord with our findings in which the number of lymphocytes was significantly increased in the treated groups. Migration, proliferation and tube formation of Human umbilical vein endothelial cells (HUVEC) was stimulated by Rg1, which also promoted angiogenesis^{24,25}. The escalation in the number of blood cells particularly erythrocytes evident in the liver sections was most probably due to this property of ginseng.

CONCLUSION

Embryotoxic effects of Ginsenosides in experimental model by implication may be considered to induce some kind of malformations in humans, such as low birth weight. We adjoin with previous researchers in saying that caution should be harvested during usage of over the counter medications including Ginseng products during antenatal period. Results in our experiment propose that auxiliary research and observations during human development and growth are mandatory.

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