Original Article

Ginseng Conduces Degeneration of Differentiating Tissues in Developing Albino Mice

Degeneration of differentiating tissues

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ABSTRACT

Objective: The current study was conducted to evaluate its effects on developing skin, heart and intestines, derivatives of ectoderm, mesoderm and endoderm respectively, when given during pregnancy to albino mice.
Study Design: Experimental Randomized controlled trial study.
Place and Duration of Study: This study was conducted at Anatomy Department, University of Health Sciences, Lahore from Jan 2011 to July 2011.
Materials and Methods: Twelve pregnant albino mice were divided into two groups of 6 each; group A was given distill water, Human therapeutic dose (HTD) (780mg/kg/day) was dissolved in 0.1ml of distilled water and was administered to the animals of group B for entire length of pregnancy. Fetuses were delivered and dissected on 18th day of gestation. Tissue samples comprising, skin, heart and small intestine derivatives of ectoderm, mesoderm and endoderm respectively, were removed and processed for light microscopic study.
Results: In the current study, the difference between dead and alive fetuses, when compared between groups was found to be statistically significant (p value < 0.05). In addition, the histological examination of the above tissues revealed extensive cell death resulting into loss of normal architecture of skin, heart and small intestine. Cells showed pyknotic nuclei and scanty cytoplasm, indicating process of apoptosis, which when compared between groups was found to be statistically significant ($p<0.05$).
Conclusion: It is suggested, therefore, that further investigations and monitoring of additional tissues for the effects of Ginsenosides during pregnancy are warranted.
Key Words: Ginseng, Fetus, Apoptosis, Differentiation

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INTRODUCTION

Herbal medicines are generally believed to be far better than the medicines prepared conventionally; those derived from ginseng are commonly used during pregnancy also and is being evaluated from the point of view of its safety. Extensive research is being conducted in the field of herbal medicines from the point of view of their safety and toxicity[1,2]; it contains the maximum number of active constituents which have extensive pharmacological effects, based on specific mechanism of actions[3]. The Ginseng root resembles human body, the plant is conventionally considered to be panacea for all ailments of the body[4].

Nearly 10% of women in Asian countries had been using ginseng during pregnancy and on the premise that it is helpful for maintaining healthy mother and developing being[5].

Triterpene saponins are active ingredients of Ginseng under the name of ginsenosides[2]; six different types had been identified as (Rb1, Re, Rc, Rd, Rb2 and Rg1) [6]. By initiating different mechanisms concomitantly and produce different effects in the same tissue which is due to diversity in their structure; an overall complex picture of result is thereby created[7]. This is stipulated to be operated through the hypothalamus-pituitary-adrenal axis and through stimulated through immune system [8].

Numerous beneficial effects of Ginseng had been reported; little information is available however, about its toxic adverse effects and much less is available about its teratogenicity [9]. Various in vitro studies had indicated that Ginsenoside exerted direct toxic effects on the development of rat embryos [10, 11]. It is postulated that un-conjugated steroid hormones cross the placental barrier rather freely [12], and active endocrine like substance in ginseng may be responsible for its effect on neonatal development. [9].

A case report was found in the literature that reported a potential link between Panax ginseng used by a pregnant woman and the death and androgenization of her fetus[13].

There is a gap in the basic clinical information as to the indications for use and the safety of herbs used by women during pregnancy. On this basis, we have tried to see the histological effects of administration of Ginseng to pregnant dams on developing tissues of fetuses to substantiate the earlier observations. Further studies are warranted to evaluate the embryotoxic effects of ginsenosides.

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MATERIALS AND METHODS

Sixteen albino mice (twelve female and four males) 6-8 weeks of age were obtained from the National Institute of Health, Islamabad; The animals were maintained in the animal house of University of Health Sciences, Lahore; the temperature of the environment was controlled at 22±0.5ºC and humidity was set at 50 ± 10%. Light and dark periods were for 12 hours each. Breeding was accomplished by placing one male with three females overnight for 12 hours. Gestational day 0 (zero) was confirmed upon seeing the vaginal plug.[3]. Experimental animals were randomly divided into three groups; each containing, six female and two male mice. Sigma was source for obtaining Panax Ginseng root powder containing 3 % Ginsenosides.

Grouping

Group A: Animals were given 0.1ml distilled water for the entire period of pregnancy.

Group B: Distil water, 0.1 ml was used as a solvent for HTD (780mg/kg/day) for the complete duration of pregnancy

Microscopic Examination: The pregnant mice were killed on the 18th day of pregnancy and the fetuses were taken out. The fetuses were dissected to remove the tissues pieces from skin, heart and small intestine for histological preparation. The tissues were fixed in 10% formalin for 48 hours. They were processed, embedded in paraffin wax and stained with haematoxylin and eosin for light microscopic examination.

Statistical analysis: The statistical analysis was done using computer IBM SPSS Statistics version 22. Data were present in frequency and percentage. The significance between two groups was calculated Chi-square test and Fisher's exact test. The difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS

Litter size: The total available litter size was 97, of which 52 belonged to group A and 45 were from group B. In the latter group 12 fetuses were dead and the difference between the alive fetuses of the two groups was statistically significant ($p < 0.05$).

Histological Features: Histological examination of skin, heart and small intestine from the treated group revealed focal degeneration; loss of tissue architecture and apoptosis. The difference between tissue degeneration in the treated group when compared to the control group was statistically significant ($p < 0.05$) (Table 2).

Skin: Histological examination of fetal skin from the control group revealed a well organized epidermis with distinct layers of closely packed cells. It was characterized with prominent stratum basal and corneum Fig1.(I).

The histological sections of skin from group B, focal areas of degeneration in epidermis were observed along with the rudimentary buds of hair follicles in the dermis Fig. 1. (II).

Heart: On histological examination, the longitudinal section of heart from the control group showed well organized myocardial architecture. Cardiac muscle fibers show regular branching pattern with well formed oval and central nuclei Fig. 2. (I).

The myocardium in group B showed myofibrillar disarray, multifocal apoptosis in various stages; the nuclei were pyknotic and cytoplasm was scanty Fig. 2. (II).

Small Intestine: Histological examination of small intestine from the control group showed closely packed villi covered with simple columnar epithelium overlying well developed lamina propria and muscularis externa Fig. 3. (I).

In group B, the villi lacked normal histological architecture due to disrupted epithelium, submucosa and muscularis externa Fig. 3. (II).

Table No.1: Comparison between litter size from groups A & B.

Parameter	Control Group (n=52) n (%)	Treated Group(n=45) n (%)	*p*-value
Dead fetuses n (%)	0 (0%)	12 (26.7%)	<0.001*

*p≤ 0.05 is statistically significant

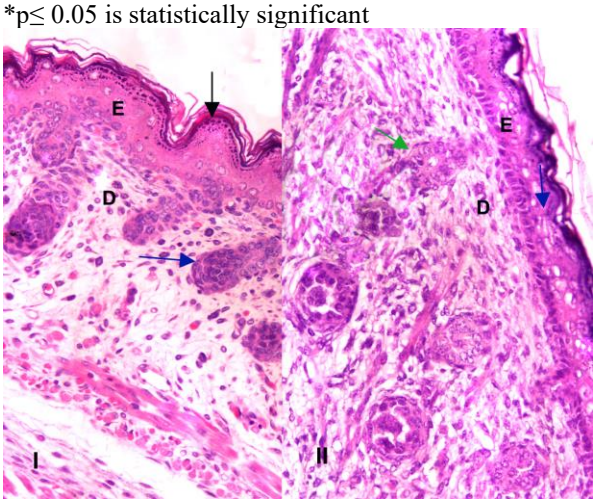

Fig.1. (I); Photomicrograph of fetal skin from control group showing well defined epidermis (E) with prominent stratum basal and stratum corneum (black arrow). Dermis (D) shows numerous hair follicles(blue arrow) at different stages of differentiation. H and E stain X 200.

(II); Photomicrograph of fetal skin from treated group shows significant epidermis (E) with large intercellular spaces(blue arrow). Dermis (D) shows hair follicles comprising a few premature buds(green arrow). H and E stain X 200.

Table No.2: Comparison of occurrence of apoptosis in skin, heart and small intestine from groups A & B.

Tissues	Group A(52) n (%)	Group B(45) n (%)	*p*-value
Skin	0 (0%)	10 (22.2%)	< 0.001
Heart	0 (0%)	15 (33.3%)	< 0.001
Small intestine	0 (0%)	18 (39.1%)	< 0.001

*p≤ 0.05 is statistically significant

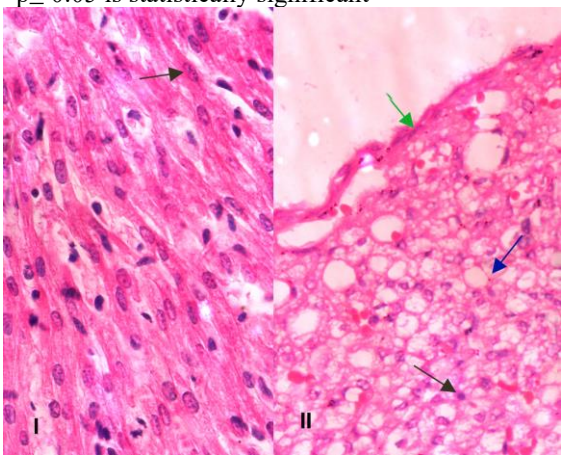

Figure No. 2. (I); Photomicrograph of fetal heart from group A:myocardial cells show regular branching pattern and have centrally located nucleus(black arrow). H and E stain X 400.

(II); Photomicrograph of fetal heart from group B: showing epicardium lined by simple squamous epithelium(green arrow), cardiac muscle cells with pyknotic(black arrow), scanty cytoplasm(blue arrow); branching pattern is not discernible. H and E stain X 400.

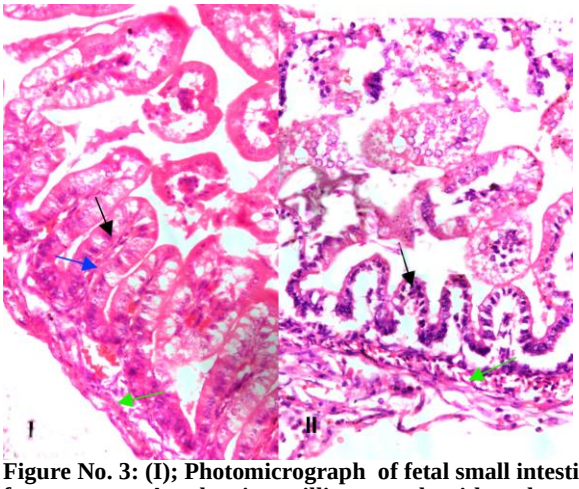

Figure No. 3: (I); Photomicrograph of fetal small intestine from group A: showing villi covered with columnar epithelium(black arrow), a core of lamina propria(blue arrow) surrounded by a layer of muscularis externa(green arrow). H and E stain X 200.

(II); Photomicrograph of fetal small intestine from group B: showing degenerative changes; surface epithelium was damaged(black arrow), crypts were disorganized, lamina propria and muscularis externa(green arrow) were also disrupted. H and E stain X 200.

DISCUSSION

The World Health Organization estimates that 65 - 80% of the world's population uses traditional medicine as their main health care system. Alternative therapies are becoming important in the health profession nowadays and therefore, these are being extensively investigated for their safety and efficacy [12].

Ginsenosides have been shown to have a variety of beneficial effects including anti-inflammatory, and anticancer effects [2]. Ginseng has been reported to have wide variety of biological activities including immunomodulatory effects, anti-inflammatory, antioxidant and antitumour activity[2,6].

In the present study, we investigated the effects of ginseng on histological structure of fetal tissues involving skin, heart and small intestine, derivatives of three germ layers ectoderm, mesoderm and endoderm respectively. Ginseng at Human therapeutic dose (HTD) 780mg/kg/day, resulted in high mortality rate on account of extensive tissue damage done by it; the difference of alive and dead fetuses was found to be significant between groups A and B. Our findings were consistent with another study in which ginsenoside Rg1 at different concentrations during period of organogenesis was found to be embryotoxic in both rats and mouse; the heart was among affected organs[14].

Evident in the sections of the skin, heart and small intestine were loci of degeneration and apoptosis; these loci were statistically significant in treated group as compared with the control group. Histological examination revealed loss of normal tissue architecture; pyknotic nuclei, and disruption of cell membranes indicating process of degeneration going in the tissues as a result of Ginsenosides. These findings are supported by earlier studies in which Ginseng was reported to possess cytotoxic activity; the basis for its anticancer activity[15]. Ginseng probably arrests the growth of progenitor cells resulting in increased foci of degeneration and apoptosis.

Many Ginsenosides are reported to possess toxic and growth inhibitory effects on cell against tumor cells, while others had been shown to induce differentiation and inhibit metastasis[1]. Rh2 Ginsenoside had shown to inhibit growth and stop cell division at the G1 stage[16]. Cell death induced by gensinoside Rh2 is mediated in part by the caspase-dependent apoptosis and in part by caspase-independent paraptosis, a type of cell death that is characterized by accumulation of cytoplasmic vacuoles[17]. Structural similarities of Ginsenosides with steroids conduce them to traverse across cell membranes freely, and produce cytotoxicity. It has been stated that steroid hormones bind with nuclear receptors and are said to affect primarily the mRNA transcription leading to protein synthesis and causing cell death [18].

Any toxic insult during development to proliferating and differentiating cells shall lead to their death and

degeneration; Compound K is a Ginseng metabolite formed by intestinal bacteria after oral administration of Ginseng extract in humans and rats [19]. It has been speculated that intestine absorb compound K, the major protopanaxadiol saponin which readily penetrates the mitochondria and causes activation of caspase 9 [17]. Caspase are a family of proteins, activated in the initial phases of apoptosis and breakdown or cleave key cellular substrates which are needed for normal cellular function. They also maintain structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. The caspases also promote other enzymes such as DNAse, which are responsible for the cleavage of DNA in the nucleus [20].

CONCLUSION

Our study has demonstrated that Ginsenosides present in the commercially available Ginseng products exert direct on mouse embryos. Until more is known about the effects of Ginsenosides in women of reproductive age, we suggest that Ginseng may be sparingly used for various diseases during pregnancy.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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