PANAX GINSENG, ITS EFFECTS ON DEVELOPMENT OF HAIR FOLLICLES – AN EXPERIMENTAL STUDY

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ABSTRACT

Introduction: The use of Herbal medicines in developed and developing countries is rapidly increasing on the presumption that they are better and safer than conventional medicines. It is now being reported that herbal preparations have far reaching effects on body systems especially in the developmental processes. The current study was conducted to evaluate the effect of Panax ginseng on developing hair follicle of albino mice.

Materials and Methods: Thirty mice, 6 – 8 weeks old weighing 30 – 35 gm were housed together for mating and pregnancy was confirmed by vaginal plug. The fetuses were delivered and dissected on the 19th day and the fetuses were delivered, microscopic slides of the hair follicles small pieces of skin were removed and processed for to be examined under light microscope.

Results and Conclusion: The pregnant dams were treated with Panax Ginseng and the litter obtained showed an increase in the number of hair follicles. Histological sections showed signs of haemorrhages and tissue degeneration. Although, embryo – toxicity in experimental model of animals may not reflect the situations in humans, further investigation and monitoring of the adverse effects of the Panax ginseng during pregnancy is warranted in humans.

Key words: Hair follicles, Embryotoxicity, Panax ginseng.

INTRODUCTION

Panax ginseng is regarded as a tonic with adaptogenic, stimulting and aphrodisiac properties,[1] it has extensive pharmacological effects and specific.[2] It enhances phagocytosis, improves physical and mental performance, increases resistance to exogenous stress factors and affects glycemia.[3] The recognized primary active components of Panax ginseng are a group of 30 different triterpene saponins, also referred as ginsenosides, which vary in content and relative proportions among different species of Ginseng. Based on the dammarane structure, more than forty ginsenosides have been identified and one ginsenoside Ro, is derived from Olenoic acid.[4] The dammarane saponins are derivatives of either protopanaxadiol or protopanaxatriol. Commonly available ginseng extracts usually contain a mixture of ginsenosides.[5] Of numerous ginsenosides that have been identified six (Rb_1, Re, Rc, Rd, Rb_2 and Rg_1) have been chosen as a standard reference for ginseng products.[4] The concentration of individual ginsenoside depends on its source, part of the plant and time of year it was harvested.[6] Recent studies have also identified an acidic polysaccharide, Ginsen, which is reported to have immunostimulatory activity. Other constituents present in the root extract are volatile oils, anti-oxidants, polysaccharides, fatty acids, vitamins, heavy metals and polyacetylenes.[4]

The mechanism by which the Herbal remedy exerts its affects is most likely through Hypothalamo – Hypophysio – Adrenal axis and through immuno-stimulation.[7] Various ginsenosides produce different effects, through variable mechanisms; these create an overall complex pharmacological picture.[6] It is generally believed that 'natural' herbal medicines are safer than those used conventionally; however, serious toxic effects have been reported to be associated with the former.[8] Despite, nearly 64% of women are reported to take herbal supplements, including, ginseng during their pregnancy. In spite of wide spread usage of ginseng during pregnancy, information concerning the side effects of ginseng on the developing conceptus in vivo are lacking. Various in vitro studies have shown that ginsenosides exert direct teratogenic effects on rat and mouse embryos; there is, however, a significant variability in embryotoxic effects of different ginsenosides.[5,9-13] Most of the investigators have used a standardized ginseng extract of 200 mg per day.[4] The Maximum Tolerated Dose (MTD) of ginseng as described by the European Committee for Herbal medicines is 2–9 gm/day.[14]

MATERIALS AND METHODS

Thirty albino mice (twenty – four female and six males) 6 – 8 weeks old and 30 – 35 gm weight were procured from the National Institute of Health, Islamabad. All the animals were examined thoroughly

and weighed before the commencement of the experiment. The Mice were housed in the Research Laboratory of University of Health Sciences, Lahore under controlled conditions of temperature 23 ± 2°C, humidity 55 ± 5 and light and dark cycle of 12 hours each. 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). Pregnant mice were randomly divided into three groups; each group contained eight female and two male mice. Each group was further divided into two subgroups comprising of four female mice and one male mouse each.

Commercially available Panax ginseng root powder containing 3% Ginsenosides was obtained from sigma. The dosage of Ginseng was determined by:

- Maximum tolerated dose (MTD).
- Human therapeutic dose.

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

Group 1
In group 1 (Control group), 0.1ml of distilled water was 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 0.1 ml 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 difference was regarded statistically significant if the 'p' value was ≤ 0.05.

RESULTS AND OBSERVATIONS

Hair follicles and hair are present over almost the entire body; these are absent only from the sides and palmer surfaces of the hands and feet, the lips, and the region around urogenital orifices. The hair follicle is responsible for the production and growth of hair; coloration of hair is attributable to the content and type of melanin contained in the hair. The hair follicle is divided into three segments, infundibulum extending from the surface opening of the follicle to the level of level of the opening of its sebaceous gland, it is part of the pilosebaceous canal that is used as a route for the discharge of sebum; isthimus extending from the infundibulum to the level of insertion of arrector pili muscle. The inferior segment is the growing follicle is of nearly uniform diameter except at its base where it expands to form the bulb; the base of the bulb is invaginated by a tuft of vascularized loose connective tissue called a dermal papilla (Figure 1).

There was an increase in the number of hair follicles per mm² in the treated groups as compared to in the control Table 1.

Table 1: *Comparison of number of hair follicles of fetuses of control, low dose treated and high dose treated groups.*

Group	*Range per mm²*	*Mean ±SE in cm*
Control (52)	3 – 4	3.5*
Low dose (47)	4 – 6	5**
High dose (43)	5 – 8	6.5***

Figure in parenthesis indicate total number of animals in each group.
*: P value < 0.05 compared with control group
**: P Value < 0.05 compared with control group
***: P Value < 0.05 low doses compared with high dose.

Table 2: *Comparison of RBC infiltration in hair follicles of fetuses of control, low dose and high dose treated groups; values were compared using chi-square test.*

G	*RBC infiltration*	*No infiltration*	*X^2*	*P value*
A (52)	00	52	15.10	< 0.05*
B (47)	12	35	32.22	< 0.05**
C (43)	17	22	-	-

Figure in parenthesis indicate total number of fetuses in each group. (*Control Vs low dose treated group;** Control Vs High dose treated group)

In the histological sections of treated groups read blood cell infiltration was noticed, which was especially evident in the high dose treated group as compared to the low dose treated group or the control group (Table 2, Figure 2, 3).

Also evident in the histological sections of treated groups are the signs of degeneration which were especially marked in the high dose treated group as compared to the low dose treated group or the con-

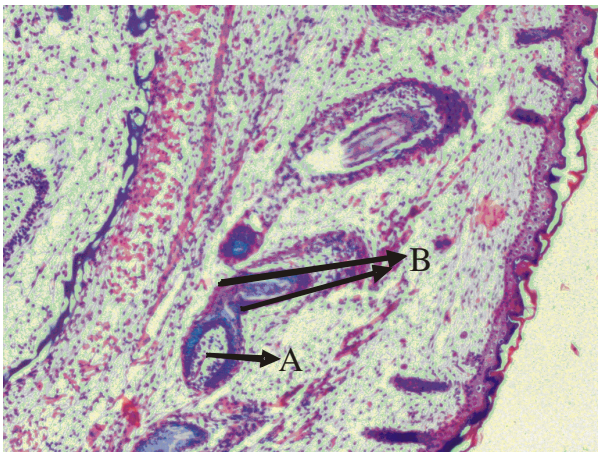

Fig. 1: *Photomicrograph of fetal hair follicles from the control group showing dermal papilla (A), inner and outer root sheaths (B). X 400 H&E stain. The control group.*

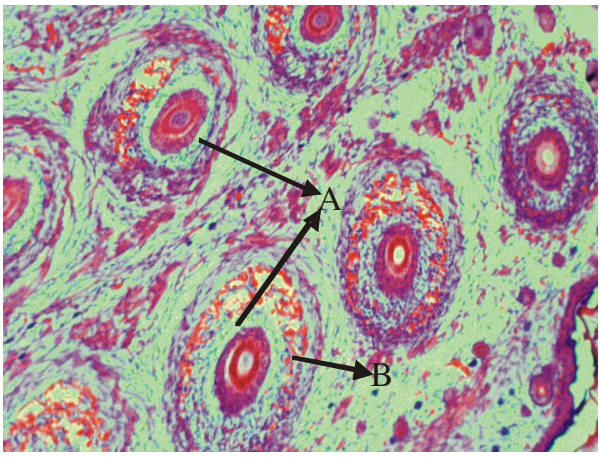

Fig. 2: *Photomicrograph of fetal hair follicles of high dose treated group showing RBC infiltration (A), and areas of degeneration (B). X 400 & E stain.*

Table 3: *Comparison of RBC infiltration in hair follicles of fetuses of control, low dose and high dose treated groups; values were compared by chi-square test.*

G	*Degeneration*	*No degeneration*	*X^2*	*P value*
A (52)	00	52	15.10	< 0.05*
B (47)	10	37	32.22	< 0.05**
C (43)	18	21	-	-

Figure in parenthesis indicate total number of fetuses in each group. (*Control Vs low dose treated group;** Control Vs High dose treated group)

trol group (Table 3, Figure 2, 3).

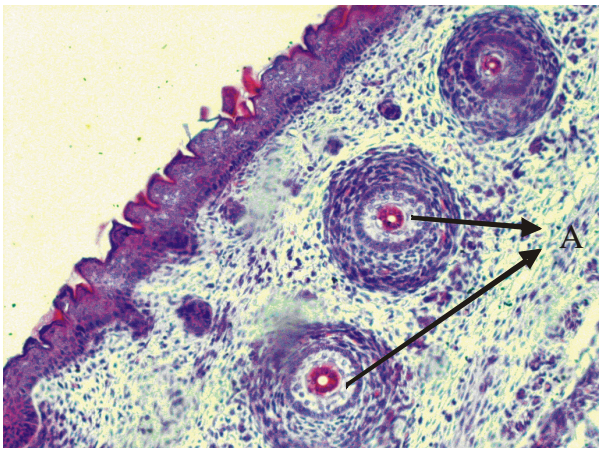

Fig. 3: *Photomicrograph of fetal hair follicles of low dose treated group showing areas of degeneration (A). X 400 H & E stain.*

DISCUSSION

In recent years assessment of the safety and efficacy of the alternate therapies for treatment of various conditions is an important issue for the health profession. Adverse effects of Panax ginseng had been reported in the literature.[15-17] Histological examinations of fetal hair follicles in our investigations demonstrated an increase in the number of cells, which is in accordance with the findings of Matsuda et al, (year ?) who also reported an increase in the number of follicules by ginseng saponins. It was also reported that number of the major constituents of P. ginseng, ginsenoside – Rb_1 (G-Rb_1) exhibited activity, but ginsenoside – Rg_1 (G-Rg_1) and – Ro (G-Ro) were ineffective. Additionally, (20 (S) – ginsenoside–Rg_3) (20 (S)-G-Rg_3) formed by processing red ginseng from the crude root of P. ginseng also showed hair growth promoting activity,[18] the increase was dose dependant and statistically significant as in our study (Table 1).

Stem cells are capable of proliferation, self renewal and production of functionally differentiated progeny that are characteristic of the organ from which they were derived.[19] The proliferation and differentiation of stem cells are under the control of various intracellular and extra cellular signaling molecules and growth factors.[20] The exogenous small molecules that can mimic the activity of growth factors could, in principle, induce mitosis from the endogenous stem cells causing an increase in the number.[21,22] The number of hair follicles was increased due to stimulation of an endogenous growth factor.

The degeneration evident in the follicles probably resulted from arrested growth inflicted by ginsenosides.

Several Ginsenosides have shown to posses cy-

totoxic and growth inhibitory effects against tumor cells, others have been shown to induce differentiation and inhibit metastasis,[1,23] Ginsenoside Rh_2 inhibited growth and arrested cell cycle at the G_1 stage.[24] The Ginsenosides with structural similarities with steroids can traverse cell membranes freely,[25] and inflict cellular damage. It has been postulated that steroid hormones bind with nuclear receptors and are believed to affect primarily the transcription of mRNA and subsequent protein synthesis leading to cell death.[25]

In addition to the degenerative changes of hair follicles encountered in our investigations, there was an abundance of blood cells observed in the histological sections. Different types of Ginsenosides have different effects on endothelial cell, some promote endothelial cell proliferation while other cause cell death. Ginsenosides Rg_1 from Panax ginseng stimulates migration, proliferation and tube formation of Human umbilical vein endothelial cells (HUVEC), and also promotes angiogenesis in a mouse sponge implant model.[26,27] Tube formation was significantly enhanced by HUVEC migration in the presence of Ginsenosides; additionally it was reported that the effects of Ginsenosides Re on HUVEC proliferation, migration and tube formation were dose dependent. The density of neocapillaries and the tissue hemoglobin content in the extra-cellular matrix were significantly enhanced by Ginsenosides Re.[28] The increase in erythrocyte number encountered in the histological sections was probably due to angiogenic promotion by Ginsenosides.

In *conclusion* 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 also. Although results from animal study may not reflect the circumstances in humans, findings in our study suggest that further investigations and monitoring of teratogenic effects of Ginsenosides on developing human are warranted.

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