Original Article

Side Effect of Ginsenosides

# Renal Mal-Development – A Devastating Side Effect of Ginsenosides

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## ABSTRACT

**Objectives:** To determine the side effects of ginseng product on human.

**Study Design:** Experimental study.

**Place and Duration of Study:** This study was conducted at the Department of Anatomy, University of Health Sciences, Lahore from Jan 2005 to December 2007.

**Materials and Methods:** Thirty adult albino mice were split into three groups of 10 mice (eight pregnant females and two males) in each group. Group A received distilled water for full term of gestation. Group B received HTD (780 mg/kg/day) mixed in 0.1ml of distilled water and Group C received MTD (1560/mg/kg/day) mixed in 0.1ml of distilled water for full term of gestation. Embryos were taken by doing C-section on gestational day 18. The fetuses were prospected and kidneys were removed. The kidneys were fixed; processed and microscopic slides were prepared.

**Results:** Histological examination showed signs of renal tubular malformation as well as varying degrees of un-differentiated mesenchymal connective tissue along with congestion and erythrocyte infiltration in the tissue preparations. These alterations were dose dependent in experimental groups. These changes were remarkable in the group C as compared with the group A or group B. Our study shows that Ginseng has embryotoxic consequences and indicates that more researches and close observation of embryotoxic outcomes of Ginsenosides on pregnancy are required.

**Conclusion:** Our investigation indicates that Ginseng products have teratogenic effects in vivo and suggest that further investigations and monitoring of embryonic effects of Ginsenosides on human pregnancy are warranted.

**Key Words:** Kidney, Renal, Malformation, Congenital, Ginseng

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## INTRODUCTION

In modern world, herbal medicine practice virtually vanished from the therapeutic map, however, many developing countries never abandoned herbal medicine practice, among all alternative therapies practiced worldwide Panax Ginseng is the most abundantly used herbal drug[1,2], as it contains the highest number of functional elements and has the wide range of pharma-codynamics and distinct mechanism of actions[3]. As the Ginseng root symbolizes the human body, the plant is assumed as a mean of healing for all ailments of the body[4]. It is used for augmenting fertility and sexual activity, and for increasing the strength the body, mood elevation and health building[5, 6].

Triterpene saponins called Ginsenosides are the main functional constituents of Ginseng[2]; of various Ginseng saponins that have been discovered, six (Rb1, Re, Rc, Rd, Rb2 and Rg1) have been selected as judgement tool for other Ginseng commodities. Ginsenosides act in different ways as it produces different actions in the same tissue so Ginsenosides have complex pharmacokinectics.[5] Ginsenosides produce its effects by acting on hypothalamus-pitutary-adrenal axis and by stimulating immune system[5,7,8].

The Maximum Tolerated Dose of Ginseng is 2-9gm/ day as designated by the European Committee for Herbal medicines[9]. It was reported in many studies that 15% of women use Ginseng in pregnancy as it is thought to be beneficial for fetuses [10].

It is documented fact in many studies that ginsenosides affect rat embryos directly[11,12,13,14]. There may be an endocrine like active substance in Ginseng which effect the development of embryo[15]. Placental membrane is freely crossed by the unconjugated steroid hormones[16]. There is a significant variability in teratogenicity of different Ginseng saponins[17].

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## MATERIALS AND METHODS

Thirty albino mice (twenty-four female and six males) of 6-8 weeks age were procured for use in this study. The experimental animals were kept in experimental animal room of UHS Lahore. Rodent chow and water was provided ad libitum. Female mice were left for mating and the day on which vaginal plug was detected was taken as day 0 of gestation[3]. Experimental animals were arbitrarily split into three groups. There were eight female and two male mice in each group. Commercially

pocurable Panax Ginseng root powder was purchased from Sigma containing 3 % Ginsenosides.

**Grouping**

**Group A**: Animals received distilled water for the full term of gestation.

**Group B**: Animals received HTD (780mg/kg/day) mixed in 0.1ml of distilled water for full term of gestation - low dose treated group

**Group C**: Animals received MTD (1560/mg/kg/day) dissolved in 0.1ml of distilled water for full term of gestation – high dose treated group

**Microscopic Examination:** The gravid mice were killed on the 18th day of pregnancy and the fetuses were taken out. The fetuses were resected and kidneys were excised. The kidneys were preserved in neutral buffer 10% formaline for two days. After processing (paraffin embedding) sections were prepared and stained with hematoxylin and eosin and PAS staining for microscopic examination.

# RESULTS

The data was analyzed by using computer software Statistical package for social sciences (SPSS) version 15. For quantitative data student 't' test was used and for qualitative data chi-square test was used.

**Morphological and histological features of the fetal kidney:** In histological sections, the cortical region composed of straight tubules alternating with regions containing glomeruli and convoluted tubules.(Figure 1).

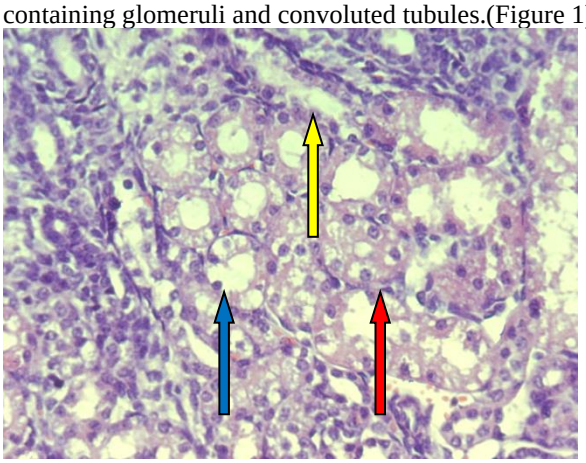

Figure No.1: Photograph of fetal renal tissue from control group demonstrating completely differentiated renal tubules. Evident in the section are well formed PCT (red arrow), DCT (blue arrow), and collecting tubule (yellow arrow) are evident in the section. X 200, H & E stain.

The cortical nephrons ranged from 1-6 per $mm^2$, 1-4 per $mm^2$ in group B and group C respectively as compared to 3-6 per $mm^2$ in group A. The difference in the number of glomeruli in experimental groups compared to the group A was statistically significant $p< 0.05$. (Table 1).

**Table 1: Comparison of number of renal glomeruli in experimental and control groups. Values were analyzed according to student 't' test.**

| Group | 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*** |

Figure in parenthesis demonstrates the number of fetuses in each group. (* Group A Vs Group B; ** Group A Vs Group C; *** Group B Vs Group C).

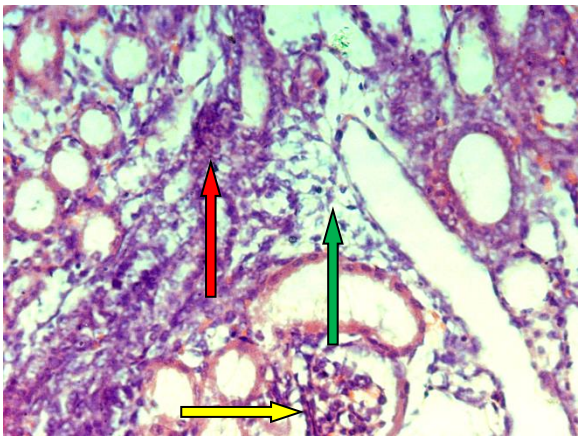

Figure No.2: Photograph of fetal kidney of high dose treated group, showing areas of tubular degeneration (red arrow), hemorrhages in the Bowman's capsule (yellow arrow) and mesenchymal tissue (green arrow). X 150, H & E Stain.

**Table 2: Association of malformations of renal glomeruli of fetuses of group A, B and C; data was analyzed by using chi-square test.**

| Group | Fetuses with malformations | Fetuses with no malfor-mations | 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 | | | |

Values in parenthesis show total number of fetuses in each group. (* Group A Vs Group B; ** Group A Vs Group C; *** Group B Vs Group C).

The Bowman's capsule was composed of visceral and parietal layers, between the two layers of renal corpuscle was the Bowman's space. An evident malformation of architecture of Bowman's capsule was observed in the treated groups (Figure 2, Figure 3) as compared to the Group A. The malformation observed in groups B and C as compared to the group A was statistically significant ($p < 0.05$: Table 2).

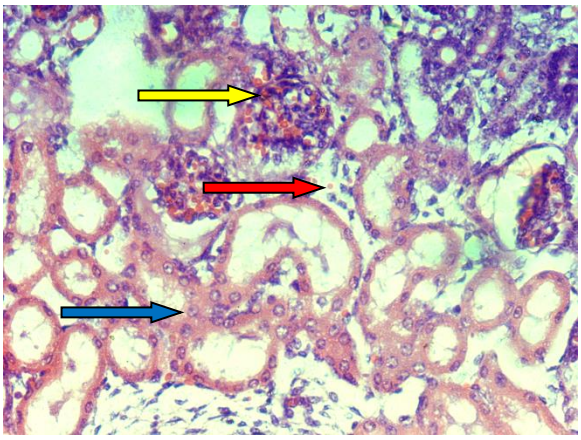

Figure No.3:.Photograph of fetal renal tissue of Group B, depicting mesenchymal tissue (red arrow) and tubular degeneration (blue arrow). Also evident in the section are hemorrhagic areas (yellow arrow), and PCT (pink arrow).X 200, H & E stain.

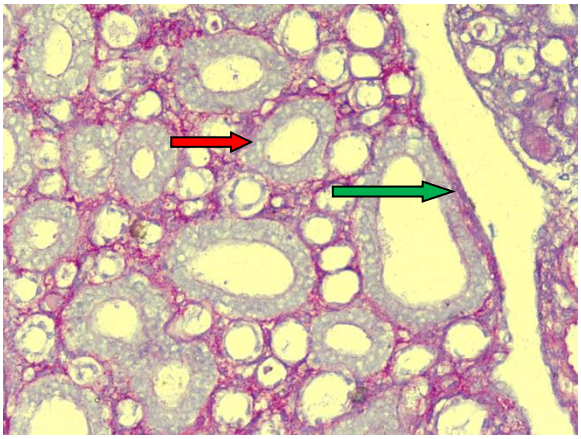

Figure 4: Photograph of renal embryonic tissue in group B showing renal tubules (red arrow) and collecting tubules (green arrow) are also visible. X 200, P.A.S. Stain.

The cells of the PCT did not show discrete limits when seen under the light microscopy, and the lumen was narrower than that of DCT. However, in treated groups apart from normal looking tubules there were some tubules showing processes of degeneration. Multiple pale-staining homogenous areas were seen in the microscopic preparations of the kidneys of the experimental groups; these were more marked in the group C than in the group B. The areas were extensively distributed throughout the kidney, commonly seen in the sub-cortical or the juxta-medullary region. To rule out the presence of amyloidal deposition, the sections were stained with Congo red and viewed under polarized light. The sections did not take any red color of the stain and there was no bi-fringance under polarized light. The sections were stained by P.A.S. technique; the stain appeared not only to stain the basement membranes but the lumen of the suspected tubules (Figure 4) in comparison to the group A.

The tubular degeneration was pronounced in the group C as compared to group B, and it was statistically significant ($p < 0.05$) when compared with the control group (Table 3).

**Table 3: Comparison of tubular degeneration of fetuses of group A, group B and group C; data was analyzed by using chi-square test.**

| Group | 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 | | | |

Figure in parenthesis indicate total number of fetuses in each group. (*Group A Vs Group B; ** Group A Vs Group C)

# DISCUSSION

About 65-80% of people uses herbal medicines as main health care as guessed by WHO. These herbal medicines should be assessed about their safety and efficacy[8].

All Ginsenosides (except Ro) contain steroids called saponins. Ginseng, one of the steroidal saponins[18], possess endocrine hormone like activities due to structural similarity with steroidal hormones. Ginesenosides have the same biochemical architecture as steroid hormones, it is high probability that they can cross the placental barrier and affect the developmental process[19]. The properties of Ginsenosides are debated; it is reported to have reparative ability of damaged tissue like brain and endothelial cells[6]. On the other hand constituents of herbal medicines, including Ginseng, are thought to be harmful, resulting in tissue damage[20].

The kidneys showed degenerated pale areas in the sub-cortical region; the nuclei were scattered and showed signs of decay; the cells did not exhibit any distinct cell boundaries; the degenerative alterations seen in the microscopic preparations of kidney were may be due to apoptosis (Figure.2) or cell death caused by Ginseng saponins.

Ginsenosides inhibit cell proliferation in tumor cell, others have been shown to cause differentiation and prevent metastasis; Ginsenoside Rh2 interfered with growth and halted cell cycle at the G1 stage[22]. As Ginsenosides, share structural features with steroids, can penetrate the cell membrane freely and can inflict cell injury. Steroid hormones bind with nuclear receptors and alter protein synthesizing capacity of the cell by altering the transcription of mRNA leading to cell death [21].

In programmed cell death, there is a chain of molecular and biochemical events which lead to cell death characterized by sequence of changes. Cells in

apoptosis are shrunk and elongated due to intracellular water loss. It is followed by nuclear condensation, degeneration of nuclear envelope and finally breakdown of nucleus. Apoptic bodies are formed which are nothing but nuclear fragments along with cytoplasmic constituents enveloped by cell membrane. Apoptotic bodies are ultimately shed from the dying cell [22,23]. Chemical agents affecting the differentiation and proliferation of cells can cause cell death as witnessed in renal tubules; Ginseng saponin is known to act on the distal convoluted tubules via Hypothalamus-pituitary-adrenal axis producing steroid like effect, accounting for degeneration of DCT. Compound K is produced as a result of action of intestinal flora after oral intake Ginseng in mammals[24]. It has been assumed that mitochondrial membrane is penetrated by compound K, the major protopanaxadiol saponin which leads to activation of caspase 9 [20]. In early stages of programmed cell death, caspases get activated causing lysis of proteins as well as agents required for normal cellular functions. In addition the caspases also result in degeneration of DNA in the nucleus by stimulating enzymes[25].

The levels of NO and NO2 increases after administration of Ginsenosides[26]. Glomerular endothelial and mesangial cells possess an inducible cytokinase-stimulated NO synthase activity. NO is also produced in the renal epithelial cells of convoluted tubules and macula densa[27]. Nitric oxide produced from enzymes known as nitric oxide synthases (NOS) also activate cellular degeneration. It is implied, therefore, that the signs of cellular degeneration, evident in the renal tubules, probably resulted on account of NO production triggered by Ginsenosides

# CONCLUSION

Ginsenosides found in readily available over the counter preparation can produce embryotoxic effects after oral ingestion. However the findings in animal studies may vary and further investigation and experimentation is therefore suggested.

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

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