D:/Biomedica Vol.25, Jul. – Dec. 2009/Bio-1.Doc P. 88 – 92 (WC)

HISTOLOGICAL CHANGES IN FETAL LIVER AFTER MATERNAL TREATMENT WITH PANAX GINSENG

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ABSTRACT
It is generally believed that 'natural' herbal medicines are better and safer than conventional medicines. Various in vitro studies have proved that Ginsenosides exert direct teratogenic affects on rat and mouse embryos and there is a significant variability in embryotoxic effects of different Ginsenosides. Histological examination revealed signs of hepatocyte degeneration, sinusoidal congestion and erythrocyte infiltration in the sections of liver. The changes encountered in the treated groups were dose dependant; these were marked in the high dose treated group. Ginsenosides present in the commercially available Ginseng products have teratogenic effects in vivo, although results from animal teratogenicity may not reflect the circumstances in humans. Our investigation indicates that Ginseng products have teratogenic effects in vivo and suggest that further investigations and monitoring of effects of Ginsenosides on human embryoes are warranted.

***Key words**: Ginseng, Hepatocytes, teratogenicity.*

INTRODUCTION

Nearly 200 years ago Herbal medicines were the sole source of treatment in the world; with the establishment of pharmacology as a leading branch of therapeutics 'Herbalism' went into a rapid decline. Many developing countries never abandoned herbal practice of medicine, as Ayuvedic medicines in India, Kampo medicine in Japan, and Chinese Herbalism in China. Amid the various herbal medicines practiced in the world, Ginseng is one of the commonly used and highly researched herbs.[1,2]

Ginseng is the common name of two species of 'Panax' of the family Araliaceae. The herb has characteristic branching roots extending from the middle of the main root resembling a human figure. Panax Ginseng is available in a wide range of forms and preparations (e.g. fresh root, alcoholic extracts, capsules, teas, cigarettes) both alone and in combination with a wide range of other ingredients.[3] The active ingredients of Ginseng are triterpene saponins called Ginsenosides[2]; based on the dammarane structure more than forty Ginsenosides have been identified by the chemists, and one Ginsenoside Ro is derived from Olenoic acid.[4] The concentration of any individual Ginsenoside varies with source, plant part and time of year harvested.[5] Ginsenosides produce different effects from one another, with each Ginsenoside having the ability to initiate multiple actions in the same tissue. These results create an overall complex pharmacological picture [6]. The mechanism by which the herb exerted its activity was reported to be through the hypothalamus-pituitary-adrenal axis and through immuno-stimulant effect.[4]

The growing popularity of medicinal herbs may increase the deliberate or inadvertent use of medicinal herbs during pregnancy, raising the possibility of adverse fetal or neonatal effects,[7] when used by pregnant women.[8] In a recent survey, as much as 15% of women were reported to have consumed Ginseng during their pregnancy and the reason given for consumption was that it is good for healthy pregnancy and fetuses.[9]

Ginseng has numerous potential beneficial effects; there is, however, limited information about its adverse effects and much less is known about its effects on embryonic development.[10] Ginseng may contain an endocrine-like active substance, which can affect neonate development.[4]. There is a significant variability in embryotoxic effects of Ginsenosides; various in vitro studies prove that Ginsenoside exerts direct teratogenic effects on rat embryos.[11-15]

Previous studies have explored the teratogenic effects of Ginseng in vitro and none of the published data implies that the product is safe for use during pregnancy; the current study is, therefore, designed to explore the possible effects of Ginseng on fetuses of albino mice.

MATERIALS AND METHODS

Thirty albino mice (twenty-four female and six males) 6-8 weeks old 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. 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).[3]. Pregnant mice were randomly divided into three groups; each group contained eight female and two male mice. Commercially available Panax Ginseng root powder containing 3% Ginsenosides was obtained from Sigma. The dosage of Ginseng was determined by:

1. Maximum tolerated dose (MTD).
2. Human Equivalence therapeutic dose (HTD).

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

Grouping

Group A: Animal receiving distilled water for the whole duration of pregnancy

Group B: Animal receiving HTD (780/mg/kg/day) dissolved in 0.1ml of distilled water for the whole duration of pregnancy – low dose treated group.

Group C: Animal receiving MTD (1560/mg/kg/day) dissolved in 0.1ml of distilled water for the whole duration of pregnancy – high dose treated group.

Microscopic Examination

The pregnant mice were sacrificed on the 18th day of gestation and the fetuses were delivered. The fetuses were dissected to remove the livers, which was fixed in 10% formaline for 48 hours, processed and microscopic slides were prepared. The slides thus prepared (five slides per tissue of each fetus) were stained with haematoxylin and eosin.

Statistical analysis

The statistical analysis was carried out using computer software, Statistical package for social sciences (SPSS) version 15. For numerical values student't' test was used and for nominal values chi-square test was used.

OBSERVATIONS AND RESULTS

The histological sections of liver demonstrated numerous sinusoids and blood cells in various stages of formation. The unique organization of the liver had its origin in its mode of development, the liver developed from a diverticulum from the foregut which forms a mass of proliferating endodermal epithelial cells invading the mesenchyme of the embryonic septum transversum. In histological sections of the control group, the hepatic cords appeared to be one to three cells thick, radiating from central vein. The hepatic cells that made up the hepatic cords were polyhedral in form and they had sharply defined boundaries. Each cell had a central nucleus with a distinct nuclear envelope and one or two prominent nucleoli. Scattered throughout the cytoplasm of the hepatic cells were small clear areas, representing areas of glycogen (Fig. 1).

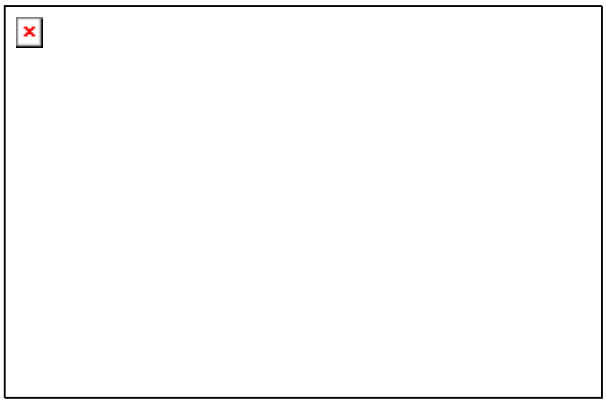


Fig. 1: *Photomicrograph of fetal liver of control group, showing Glisson's capsule (Yellow arrow), Lymphocytes (green arrow), hepatocytes (red arrows), eosinophils (blue arrow), monocytes (lilac arrow), and neutorphil (magenta arrow). X 1500, H & E stain.*

In the treated groups the histological sections of the fetal livers showed signs of degeneration, apparent in the sections were pale homogenous areas with dispersed nuclei. The heaptocytes did not exhibit cellular boundaries and the nuclei were seen to be dispersed (Fig. 2).

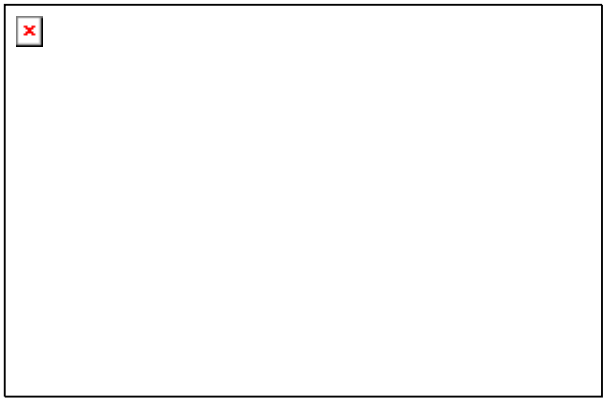


Fig. 2: *Photomicrograph of fetal liver of MTD group exhibiting hepatocyte degeneration (1) and erythrocyte infiltration (2). X 200, H & E stain.*

The degeneration of hepatocytes was hallmarked by nuclear condensation and nuclear fragmentation (Fig. 3).

The malformation in the appearance of the heapatocytes was observed commonly in the high dose treated group as compared to the low dose treated group. The malformations seen in the heapato-

cytes of the treated groups were statistically significant $p < 0.05$ (Table 1).

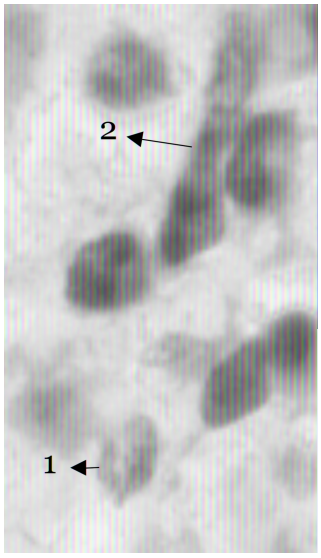
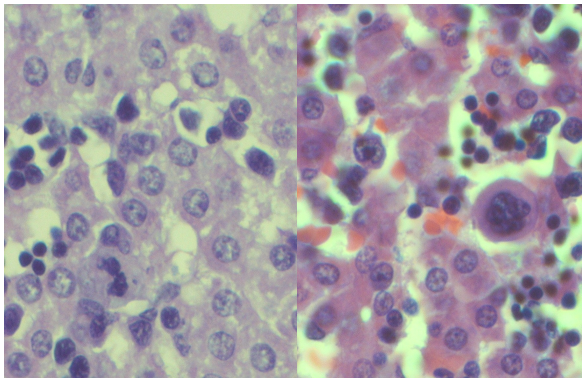


Fig. 3: *photomicrograph of fetal liver of MTD group showing nuclear fragmentation (1) and nuclear condensation (2). X 1500, H & E stain.*

Table 1: *Comparison of hepatocytes malformation of fetuses of control, low dose and high dose treated groups; values were compared to chi-square test.*

G	Degeneration	No degeneration	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).



A b

Fig. 4: *Photomicrograph of fetal liver of control group compared to HTD group, showing sinusoidal congestion and erythrocyte infiltration. X200, H & E stain.*

The hepatic sinusoids made up the intra-lobular system of blood capillaries which coursed centripetally through the lobule. The sinusoids were lined by discontinuous endothelial cells, and seen to be anastomosing, irregularly separating the hepatic cords from one another. In the treated groups the sinusoids were narrower. The congestion of the sinusoids was more pronounced in the high dose treated than the low dose treated group (Fig. 4a).

Erythrocytes were accumulating in the lumen of the central vein as well as in the sinusoidal spaces of the treated groups; these changes were more frequent in the high dose treated group (Fig. 4b).

The sinusoidal congestion and erythrocyte infiltration in treated groups as compared to the control group was statistically significant ($p < 0.05$: Table 2).

Table 2: *Comparison of malformation of central vein (liver) of fetuses of control, low dose treated and high dose treated groups; values were compared using chi-square test.*

G	Sinusoid malformation	No malformation	X^2
A (52)	00	52	25.7*
B (47)	15	32	32.2**
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).

DISCUSSION

The use of herbal medicine is rapidly increasing in both the developed as well as the developing countries. In recent years assessment of the safety and efficacy of these alternative therapies is an important issue for the health professions.[10]

The Herbal remedies have a greater consumption during pregnancy as they are considered safe and are also reported to have beneficial effects in the treatment of intra uterine growth retardation as other nutritional products[16]; these products, however, have adverse effects on the developmental process. Steroidal saponins like Ginseng,[17] which share structural features with steroid hormones, possess numerous physiological activities, partly due to the nature of the steroid structure. Ginseng with its structural similarities with the steroids may contain an endocrine-like active substance which can affect neonate development.[18]

The effects of Panax Ginseng are controversial; it is documented to repair the damaged tissues of kidney, liver and brain.[19,20] On the other hand con-

stituents of herbal medicines, including Ginseng, are considered toxic, resulting in tissue damage.[21,22]

Panax Ginseng showed toxic effects on the developing liver in our study, damaging the hepatocytes (Table 1); the damage was more pronounced in the high dose treated group as compared with the low dose treated or the control group.

Hepatocytes from the control group showed prominent nuclei and well demarcated cell boundaries (Figure 1) as compared to malformed hepatocytes with ill-defined margins, nuclear fragmentation and condensation in the treated groups (Figs. 2, 3); the changes in the hepatocyte probably resulted from the cellular damage produced by Ginsenosides during the process of development. The degenerative changes seen in the histological sections of liver were probably the result of apoptosis or cell degeneration triggered by Ginseng saponins. Several Ginsenosides have shown to posses cytotoxic and growth inhibitory effects against tumuor cells, others had been shown to induce differentiation and inhibit metastasis[1]; Ginsenoside Rh_2 inhibited growth and arrested cell cycle at the G_1 stage.[23] The Ginsenosides with structural similarities with steroids can traverse cell membranes freely,[24] 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.

In Apoptosis, frequently referred to as "programmed cell death", initially there is a change in shape and size of cell due to loss of intracellular water, the cells become elongated and shrunken. This is followed by condensation of nuclear chromatin, degeneration of nuclear envelope and ultimately nuclear fragmentation; these signs were seen in the histological sections of fetal liver (figs. 2, 3) in our study. Any toxic insult to proliferating and differentiating cells during development shall lead to their degeneration and death. It has been speculated that Compound K, the major protopanaxadiol saponin absorbed by the intestine,[25] readily penetrates the mitochondria and activates caspase 9.[23] Caspases are a family of proteins that are activated in the early stages of apoptosis, these proteins breakdown or cleave key cellular substrates that are required for normal cellular function.

Nitric oxide is a reactive oxygen and nitrogen species, which carry a reactive lone pair of electrons that undergoes an oxidative process to form an active intermediate which is capable of toxic nitrosylation. Prolonged exposure to a large amount of NO inhibits the activity of several enzymes, such as aconitase, cytochrome C oxidase and ribonucleotide reductase; as a result NO could become cytotoxic or cytostatic which is the basis for pathological functions of NO.[26] Ginsenosides increase the level of NO end products NO_2 and NO_3[27,] prolonged exposure to ginsenosides, as during pregnancy, increases the NO concentration and its at high concentrations can cause circulatory shock and cell death.[26]

It is **concluded** that 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.

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