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Histological Changes in the Fetal Brain of Albino Mouse after Maternal Treatment with *Panax ginseng*

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Abstract.-This study was conducted to evaluate the efficacy and safety of a herb *Panax ginseng*. It was found that maternal treatment with *Panax ginseng* negatively affected the fetal brain and increased the glial cells with a concomitant reduction in number of neurons. Although *in vitro* embryotoxicity may not reflect the true situation in humans, further investigation and monitoring of the adverse effects of the ginseng during pregnancy are warranted. Herbal therapies should not be used indiscriminately unless there is adequate evidence of their safety and efficacy.

Key words: Neurons, neuroglia, *Panax ginseng*, fetal brain, anomalies.

INTRODUCTION

The history of herbal medicine practice is as old as the civilization itself. Amid the various herbal medicines practiced in the world, ginseng is one of the commonly used and highly researched herbs (Kiefer and Pantuso, 2003), as it contains the maximum number of active constituents and has the most extensive pharmacological effects and specific mechanism of actions (Cheng *et al*., 2005). *Panax ginseng* is regarded as a tonic with adaptogenic stimulant and aphrodisiac properties (Attele *et al*., 1999). It enhances phagocytosis, improves physical and mental performance, increases resistance to exogenous stress factors and affects hypoglycemic activity (Kiefer and Pantuso, 2003). The recognized primary active components of ginseng are a group of 30 different triterpene saponins, also referred to 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 (Nah, 1997; Kitts and Hu, 2003). The dammarane saponins are derivatives of either protopanaxadiol or protopanaxatriol; commonly available ginseng extracts usually contain a mixture of ginsenosides, and GRb1, GRc, GRe, and GRg1 are present in high concentrations (Liu *et al*., 2006). Of numerous ginsenosides that have been identified six (Rb1, Rb2, Rc, Rd, Re, and Rg1) have been chosen for reference standards for ginseng products. The concentration of any individual ginsenoside varies based on the source, plant part and time of year harvested (Coleman *et al*., 2003). Recent studies have also identified an acidic polysaccharide referred to as "Ginsen' with noted immuno-stimulatory activity. Other constituents present in the root extract are volatile oils, anti-oxidants, polysaccharides, fatty acids, vitamins, heavy metals and polyacetylenes (Kitts and Hu, 2003).

The mechanism by which the herbal remedy exerts its effects is most likely through hypothalamic-hypophysial-adrenal axis and through immunostimulation (Kwon *et al*., 2003). Ginsenoside produce different effects from one another, with each ginsenoside having the power to initiate multiple actions in the same tissue. These results create an overall complex pharmacological picture (Coleman *et al*., 2003).

It is generally believed that natural herbal medicines are better and safer than conventional medicine. Herbal medicines are in fact associated with serious toxic effects (Ong *et al*., 2005). Up to 64% of women are reported taking herbal supplements including ginseng during their pregnancy. Despite widespread usage of ginseng during pregnancy, information concerning the potential effects of ginseng on the developing fetus *in vivo* are lacking. Various *in vitro* studies prove that ginsenoside exert direct teratogenic effects on rat and mouse embryos and there is a significant

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variability in embryotoxic effects of different ginsenoside (Liu *et al*., 2006; Chan *et al.*, 2003; Chan *et al.*, 2004; Liu *et al.*, 2006; Liu *et al.*, 2005; Ashmaoui *et al.*, 2003). Most published research studies have used a standardized ginseng extract in a dosage of 200 mg per day. Capsule formulas are generally given at a dose of 100-600 mg per day usually in divided doses (Kitts and Hu, 2003)**.** The maximum tolerated dose of ginseng as described by the European Committee for Herbal Medicines is 2-9g/day (EAEMP, 1999).

This study was designed to determine toxic effects of *Panax ginseng,* if any, on the fetal brain of mouse.

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). 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% ginsenoside was obtained from Sigma (USA). The dosage of ginseng was determined by maximum tolerated dose (MTD), and human therapeutic dose (HTD)

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

Following three groups were used in the experiment, Group 1 (control group) which was given 0.1ml of distilled water orally throughout pregnancy, Group 2 (low dose treated group), which was given orally throughout pregnancy the human therapeutic dose of ginseng (780 mg/kg/day) dissolved in 0.1ml of distilled water, and Group 3 (high dose treated group), which was given orally throughout pregnancy the maximum tolerated dose of ginseng (1560 mg/kg/day) dissolved in 0.1ml of distilled water.

The drug was given throughout the pregnancy and at term the fetusus were checked for any gross abnormalities and fixed in 10% buffered formalin for histological studies. Routine histological technique and procedure were followed and sections were cut at 5-6 µm thickness and stained with haematoxylin and eosin.

Statistical analyses

The statistical analyses were carried out using computer software Statistical Package for Social Sciences (SPSS) version 13. The difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS

The fetal heads were grossly well formed and did not manifest any malformations of brain or the spinal cord. Transverse sections of the whole fetal skull were taken and the area selected for counting neurons and neuroglia was adjacent to the cavity of third ventricle at the level of pinna. The numbers of neurons as well as neuroglia were counted using a 10 x 10 reticule under 40X objective magnification.

In the histological sections of both the treated and control groups the large multi-polar neurons were dispersed among the neuroglial cells and were characterized by numerous processes extending in different directions from the perikaryon or the cell body (Fig. 1).

The average number of neurons present in the transverse section was 27 per mm^2 in the low dose (n=47) treated group (Fig. 2), 20 per mm^2 in the high dose (n=43) treated group (Fig. 3) as compared to 35 per mm^2 (n=52) in the control group (Fig. 1). The difference in the number of neurons seen in the treated groups compared with the control group was statistically significant ($p < 0.05$).

The neuroglia constitute the supporting frame work of the nervous system; and were seen as

deeply staining bodies that were smaller in size than the multipolar neurons. Both the fibrous and protoplasmic astrocytes were markedly increased in number, with the number much more in the high dose treated group. Fibrous astrocytes were seen in the vicinity of the blood capillaries and exhibited a small cell body, large oval nucleus and a dark staining nucleolus with processes extending from the perikaryon. Protoplasmic astrocytes were characterized by their abundance of cell processes and a smaller size of the perikaryon (Fig. 1).

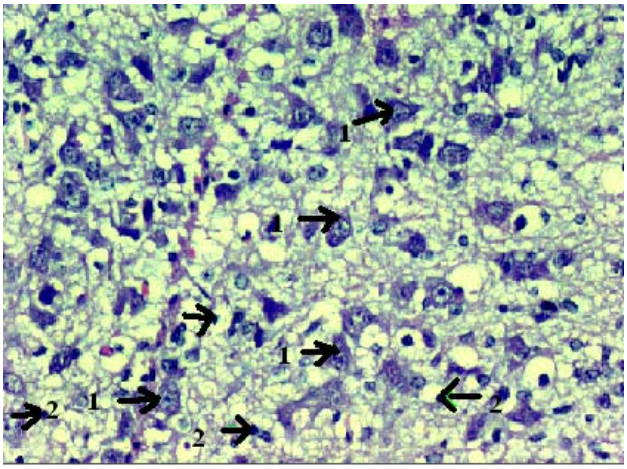

Fig. 1. Histological structure of fetal brain of control group demonstrating neurons (1) and neuroglia (2). 300 X; H & E.

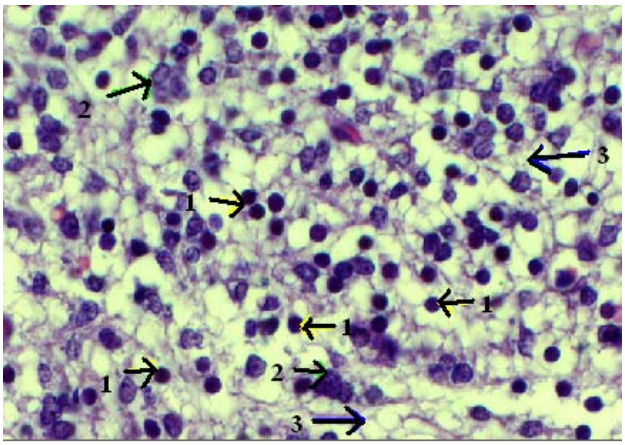

Fig. 2. Histological structure of low dose treated group showing fewer neuroglia (1) amid wealth of neurons (2). Dispersed in between the neurons and neuroglia is white matter (3). 300 X; H & E.

The ventricles were lined by ependymal cells that were high cuboidal to columnar in shape. The nuclei of the ependymal cells were darkly staining and oval to round in architecture. Long axis of the ependymal cells was perpendicular to the long axis of the cavity. The ependymal cells observed in the treated groups were not as well formed as seen in the control group. The nuclei at places were seen to form aggregates and the cilia were less frequently observed (Fig. 4).

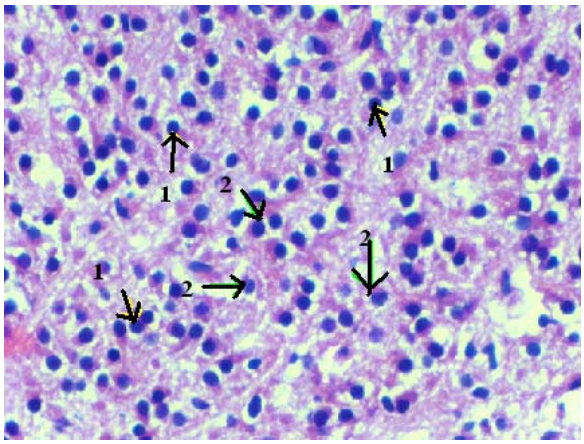

Fig. 3. Histological structure of brain of high dose treated group showing abundance of neuroglia (1) characterized by their smaller size than the neurons (2). The number of neuroglia was markedly increases in the treated groups. 300 X; H & E.

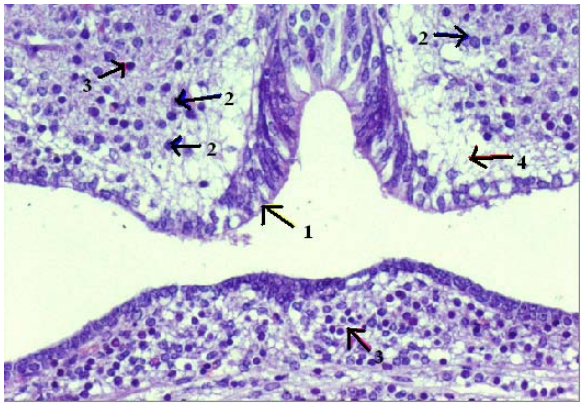

Fig. 4. Histological structure of High dose treated group showing ependymal cells lining the third ventricle (1), neurons (2) and neuroglia (3). Also evident in the photomicrograph is dispersed white matter and un-differentiated connective tissue (4), 300 X; H & E.

The neuroglial cells of ectodermal origin, *i.e.*, astrocytes, oligodandrocytes and ependymal cells were in abundance in the treated groups as compared to the control group (Figs. 2, 3). The number of the neuroglia (astrocytes and oligodandrocytes) was 57per mm^2 in the low dose (n=47) treated group, 95 per mm^2 in the high dose (n=43) treated as compared to 43 per mm^2 in the control (n=52) group. The abundance in the number of neuroglia seen in the treated groups compared with the control group was statistically significant ($p < 0.05$).

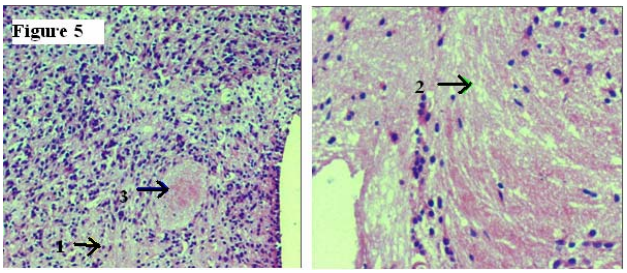

Fig. 5. Histological structure of fetal brain of low dose treated group and control group, showing a decrease in the content of white matter in treated group (1) as compared to the control group (2). A fiber bundle (3) is also evident in the photomicrograph of the treated group. 300 X; H & E.

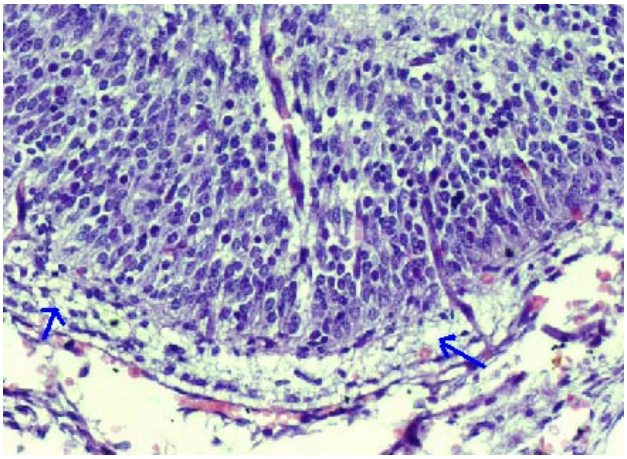

Fig. 6. Histological structure of fetal brain of high dose treated group showing un-differentiated connective tissue (blue arrow). 300 X; H & E.

The white matter dispersed in between the neurons and neuroglia appeared to be well formed but less in amount as seen in the treated groups when compared to the control group (Fig. 5).

There was also evidence of un-differentiated connective tissue, present in the vicinity of the ventricles, in the treated groups (Fig. 6).

DISCUSSION

In recent years assessment of the safety and efficacy of these alternate therapies is an important issue for the health professions. Literature review has elicited a controversy regarding beneficial role of *Panax ginseng. Panax ginseng* is documented to repair the damaged tissues of kidney, liver, brain and endothelial cells (Kitts and Hu, 2003). On the other hand, constitutes of herbal medicines are considered toxic resulting in liver, kidney or other tissue damage (Fogden and Neuberger, 2001; Va'zquez and Aguera-Oritz, 2002).

Histological examinations of fetal brain *in situ* demonstrated an untoward increase in the number of neuroglial cells. This increase was dose dependant with the highest number seen in the high dose treated group. The altered ratio between the neurons and neuroglia might be a result of an increased production of neuroglial cells or it might be a result of degeneration or apoptosis of the neurons. Stem cells are capable of proliferation, self renewal and production of differentiated functional progeny that are characteristic of the organ from which they were derived (Hall and Watt, 1989). The proliferation and differentiation of neural stem cells are under the control of various intracellular and extracellular signaling molecules and growth factors (Weissmann, 2000; Chan *et al*., 2002). The exogenous small molecules that can mimic the activity of the growth factors could, in principle, induce neurogenesis or gliogenesis *in situ*, in the brain and the endogenous neural stem cells (Dai and Holland, 2003; Connor and Dragunow, 1998; Kruger and Morrison, 2002). In a recent study it was shown that Ginsenoside Rd promoted the production of astrocytes from neurospheres in treated groups with regard to control, and the relative production of neurons was decreased as compared to control group. It was concluded that Rd could play a role on neuron/glia differentiation pathway resulting in differentiation of neual stem cells into astrocytes

(Shi *et al.*, 2005; Jeong and Nah, 2005). These are in accordance with our findings suggesting that ginsenoside may influence the neural progenitor cells during the developmental process resulting in an increased number of glial cells.

Also evident in the sections of brain were loci of un-differentiated connective tissue. These loci were statistically significant ($p<0.05$) in treated groups as compared with the control group. Ginseng possesses cytotoxic activity that is also the basis for its anticancer activity (Shibata, 2001; Chang *et al.*, 2003). The cytotoxic activity of ginseng probably arrested the growth of mesenchymal cells into their mature form.

Herbal remedies and alternative medicines are used throughout the world and in the past herbs were often the original sources of most drugs. Complementary and alternate medicines (CAM) are widely used in affluent countries such as USA, Canada, UK, Germany and Australia and are taken not only for curative but also for preventive purposes (Wilson *et al.*, 2006). It seems that less than half of people using CAM therapies do, in fact, have disorders for which they seek cure (Matsuda *et al.*, 2003). The usage of herbal remedies is reported to be more frequent in women than in men, more frequent amongst middle-aged than elderly people, and more frequent amongst well-educated than low-educated individuals. In a recent survey it was seen that 9.1% pregnant women consumed herbal remedies including ginseng during their pregnancy. This percentage was raised to 10 in Asian countries (Grieve, 2005).

The unguarded rise in use of herbal medicines raises a question as to how safe are these preparations for the unborn fetus. The dilemma facing most regulatory authorities is that the public considers these products as either traditional medicines or natural products that are safe for consumption and require no regulation what so ever. Most countries have laws concerning foods, drugs, and cosmetics. It is our ill-fate that such laws are not established in Pakistan. Our society has no hindrances in procuring these remedies as these are considered safer and better than most of the conventional or allopathic medicines. If we adopt a causal attitude to the potential embryotoxic affects of ginsenoside, it might induce some kind of severe consequence in humans, such as low birth weight infant or even abnormality of spirit and behavior etc (Weissmann, 2000).

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