INTRODUCTION

The use of herbal medicinal products, usually marketed as dietary supplements is rapidly increasing. According to a survey carried out on 1200 pregnant Nigerian women, 12% reported the use of native herbs and of these 42% were nulliparous. A similar survey conducted in South Africa showed that out of 229 pregnant women, 55% used herbal medicine during pregnancy.1 In the absence of adequate safety data, caution must be exercised in using herbal medicines as health supplements during pregnancy. Among the more popularly used herbal supplements, Ginkgo biloba, also known as maidenhair or gymnosperm tree, is one of the oldest living species in the world and is considered a living fossil.2 The standardized extract (EGb 761) of the leaves contains 5-7% ginkgolides and bilobalide collectively called terpene trilactones, along with 22-24% of flavonoids, such as quercetin, kaempferol, and isohamnetin. Other constituents include proanthocyanadins, glucose, rhamnose, organic acids, D-glucaric acid and ginkgolic acid.3 The flavonoids are collectively purported to be responsible for the free radical scavenging and antioxidant activity of Ginkgo biloba preparations.4 The ginkgolides have been shown to inhibit platelet-activating factor (PAF).5 Ginkgo is said to be effective for a wide range of clinical conditions, although it is popularly used for stroke and dementia in elderly women. It is also prescribed in the childbearing period for memory boosting, asthma, mountain sickness, varicose veins, and premenstrual syndrome (PMS) or sometimes for idiopathic cyclic edema.6 The most common usage among young women is for premenstrual syndrome (PMS). A standardized extract of Ginkgo biloba is effective against the congestive symptoms of PMS, particularly breast symptoms. A study on 165 women showed that neuropsychological symptoms along with congestion and breast tenderness abated during their premenstrual syndrome.7 Cyclic edema, which is a manifestation of pre-menstrual syndrome, has been treated by Ginkgo biloba because flavonoids present in the Ginkgo extract are capable of normalizing capillary permeability.8 More recently, it showed a growing popularity in the treatment of sexual dysfunction which is presumed to be due to selective serotonin reuptake inhibitors.9 Young women with connective tissue disorders also use it and, the World Health Organization has recommended the use of Ginkgo for Raynaud’s disease.10

ABSTRACT

Objective: To determine the gross structural malformations to the mice fetuses of the mothers given Ginkgo biloba during pregnancy.

Study Design: Experimental study.

Place and Duration of Study: The Experimental Research Laboratory, University of Health Sciences, Lahore, from May 2006 to December 2006.

Methodology: The teratogenic effects of Ginkgo biloba extract (78 mg/kg/day and 100 mg/kg/day) dissolved in water were studied on the gross features of mice fetuses. Three groups (A, B and C) of 6 females each were mated with 2 males in two cages with 3:1 ratio of females to males. The first two groups (A and B) served as experimental and the third (C) was used as a control. Pregnancy was confirmed by a vaginal plug and gravid female mice (6) were separated from the males. Group A was treated with human therapeutic dose (78 ppm) while group B was given a high dose (100 ppm). Group C was given water only. Both experimental groups were given the drug orally throughout the gestational period. Result were compared using ANOVA with significance at p < 0.05.

Results: Forty-nine fetuses from B and C groups and 50 fetuses from A group were recovered. There was a significant (p < 0.05) decrease in weight and crown-rump length of fetuses in group B as compared to those from group A and C. Further, fetuses from groups A and C did not show any gross abnormalities, whereas those from group B exhibited a high frequency of malformations including round shaped eye and orbits, syndactyly, malformed pinnae, nostrils, lips and jaws.

Conclusion: The results obtained substantiate the early finding that Ginkgo biloba can be teratogenic when given to pregnant mothers.

Key words: Ginkgo biloba. Aqueous extract. Gross malformations. Teratology.
Despite its being widely used medicinally, teratogenic effects of *Ginkgo biloba* have not been studied extensively *in vivo* or *in vitro*. Studies on effects of *Ginkgo biloba* extract on embryogenesis and on fetal development have been suggested. In view of limited work on the teratogenic effects of *Ginkgo biloba*, the present study was conducted to evaluate the effect of *Ginkgo biloba* extract on developing conceptus, using mice as experimental model. It is assumed that the results obtained will provide necessary information on the safety of this agent during pregnancy.

**METHODOLOGY**

Twenty four albino mice (18 females and 6 males), 6-8 weeks old, weighing 20-35 grams were obtained from the National Institute of Health, Islamabad. The animals were acclimatized for 4-5 days in the Experimental Research Laboratory, University of Health Sciences, Lahore. Food and water were allowed *ad libitum* and they were maintained on a 12-hour light/dark cycle, 20-25°C and 45-65% humidity. Three females and one male mouse were housed in a single cage for mating. The females were examined every morning for the presence of vaginal plug and were separated from the male and housed in separate cages after the plug had been observed; noon of the day was considered as gestational day 0.5.

The pregnant females were divided into three groups (A, B and C), of 6 mice each. Group C served as control and the other two, A and B, were used as experimental groups. A standardized extract of *Ginkgo biloba* was purchased from GNC (General Nutrition Centre) laboratories in the form of capsules. The *Ginkgo biloba* extract, equivalent to a human therapeutic dose (78 mg/kg/day) and high dose of 100 mg/kg/day were selected on the basis of the maximum tolerated human dose which was 1.6 gm/kg/day.11

Group A and B received the normal human therapeutic and high dose, respectively, dissolved in distilled water throughout the gestational period. Group C was given an equal amount of distilled water throughout the period. The animals were sacrificed on the 18th day of gestation after being anaesthetized with ether. The fetuses were dissected free of the uterine wall under the dissecting microscope and placed in a phosphate buffer and were examined for gross abnormalities. The body weight of the fetuses in grams through a weighing scale and crown-rump length in centimeters through a measuring scale were also recorded.

The statistical analysis was carried out using SPSS version 13.0. The difference between the means of the three groups was computed using ANOVA. The difference was regarded statistically significant if the ‘p’ value was < 0.05.

**RESULTS**

The “Litter size” in all groups, control and experimental, was nearly equal. The average weight of the fetuses in Group C (control) was 1.90±0.13 g, Group A 1.83± 0.10 g and in Group B was 0.78±0.11 g. ANOVA showed that there was significant dose related decrease in the weight of fetuses of experimental groups A and B. A gradual decrease in the mean body weight was evident with an increasing dose of the drug. The ANOVA and Tukey test showed that the difference in the mean weight of the A, B and C groups was statistically significant (Table I a, b).

### Table Ia: F-value between groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=50</td>
<td>n=49</td>
<td>n=49</td>
<td></td>
</tr>
<tr>
<td>Mean weight (g) ± SD</td>
<td>1.830±0.10</td>
<td>0.786±0.11</td>
<td>1.90±0.13</td>
</tr>
<tr>
<td>Comparisons and</td>
<td>Vs. B = 0.000</td>
<td>Vs. C=0.000</td>
<td>-</td>
</tr>
<tr>
<td>p-value</td>
<td>Vs. C=0.007</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

*Sample size.

Average crown rump length (CRL) recorded for the control group fetuses was 2.82±0.18 cm. The experimental groups A and B had CRL 2.83±0.22 cm and 1.71±0.27 cm respectively. There was a significant decrease in the crown-rump length (CRL) of fetal mice of B group as compared to the other two groups A and C (Table II a,b). The difference in the mean crown rump length of groups A and C was statistically insignificant (p=0.986). The results suggest that only a high dose of *Ginkgo biloba* was effective in producing effects on the CRL.

### Table IIa: F-value between groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=50</td>
<td>n=49</td>
<td>n=49</td>
<td></td>
</tr>
<tr>
<td>Mean CRL (cm) ± SD</td>
<td>2.83±0.12</td>
<td>1.71±0.27</td>
<td>2.82±0.18</td>
</tr>
<tr>
<td>Comparisons and</td>
<td>Vs. B = 0.000</td>
<td>Vs. C=0.000</td>
<td>-</td>
</tr>
<tr>
<td>p-value</td>
<td>Vs. C=0.986</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Sample size.

For gross morphological examination, all fetuses were examined under a Wolfe stereo dissecting microscope. The control (C) and the treated groups (A and B), showed indistinct fontanellae; hindbrain, forebrain, liver and heart bulges were also inconspicuous. The orbits were elliptical and the eyelids were fused in all fetuses of the C and A groups (Figure 1a).
In group B (100 mg/kg/day), 24 out of 49 fetuses (48%) had rounded orbits and the eyelids were normal (Figure 1b). External auditory meatuses were well formed in fetuses of all groups and were completely concealed by the well-formed pinnae in the C and A groups only (Figure 1a). Malformed pinnae were found in 22 out of 49 (44.1%) fetuses in group B (Figure 1c).

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Other facial features including nostrils, jaws and lips were well formed in the C and A groups, (Figure 2a). However, 21 out of 49 (42.8%) fetuses showed malformed nostrils, jaws and lips in group B (Figure 2b). Both fore and hind limbs were distinctly divided into three segments, with claws having five digits in fetuses of all three groups (Figure 3). Syndactyly was present in 40.8% fetuses of group B while in the other two groups (A and C), claws were webbed proximally and separated distally (Figure 2b).

**DISCUSSION**

It had been suggested that ginkgolide B, significantly retarded mouse embryonic development, implantation and embryogenesis by reduction of inner cell mass. In an earlier study, reduction of cells in the inner cell mass of a blastocyst by 30% or more produced a high risk of fetal loss or developmental injuries, even in cases where the implantation rate and trophoectodermal cell number were normal. Apoptosis is usually not noticed prior to the blastocyst stage in a normally developing mouse; however, abnormal apoptosis had been observed in response to many teratogens. A five to eight-fold increase in cellular apoptosis in the mouse blastocyst after treatment with ginkgolide in a dose-dependent manner had also been reported; the results indicate that ginkgolides conduce apoptosis in the mouse blastocyst and the induced cell loss occurred more commonly in the inner cell mass rather than the trophoectoderm. The number of cells in the “inner cell mass” is considered to be essential for proper implantation of the embryo; therefore, the reduction in the inner cell mass may reduce embryonic viability and development. The abnormalities seen in the current study can be attributed to the presence of ginkgolides; one of the major components of the agent causing the abnormal apoptosis.

Chan observed that the weight was statistically lower in all fetuses of the ginkgolide B-pretreated group of the control group. The ginkgolide B-pre-treated group had
23% fewer fetuses weighing over 600 mg and was due to the reduced proliferation of cells under the influence of the drug. A similar finding was observed in the present study but there was dose related response in the reduction of weight among fetuses of both treated groups. The study hitherto reported reduced crown-rump length of the fetuses after treatment with Ginkgo biloba. In the present study a reduced crown-rump length in the high dose group was noted as compared to the controls. This observation also points to the restricted proliferation of cells presumably due to the ginkgolide B in the aqueous extract given to the animals.

In the present study, human therapeutic dose, given to the mice throughout the gestational period, did not produce any noticeable external deformity. This finding is consistent with the earlier reports on the effects of ginkgolide B on in vivo development of fetuses. However, a high dose of Ginkgo biloba produced gross external defects including low birth weight and reduced crown-rump length. There are several articles reporting on the use of various herbal supplements during pregnancy, with different patterns of use. It is pertinent to mention that half of the supplement users were taking those supplements on their own without informing their healthcare providers, assuming that if a supplement is “natural”, it is safe. Keeping in mind this trend of herbal medication among the population the effects of using a high dose of Ginkgo biloba were considered and reported. In the current study, facial malformations in the high dose group could be implied due to a failure in the formation, proliferation, migration and differentiation of neural crest cells because migration of the neural crest cells to the cranial region is reported to contribute to the formation of the craniofacial skeleton. According to Chan's observations, ginkgolides reduce cell proliferation, increase the apoptosis in the mouse blastocyst and trigger a developmental delay in post-implantation mouse embryos thus inducing a malformation of the craniofacial skeleton. Identification of colchicine in the placental serum of expecting mothers using Ginkgo biloba, as antagonists at recombinant α1β2γ12 GABAA receptors. European J Pharmacol 2004; 494: 31-8.

Our results indicate that Ginkgo biloba extract is deleterious to the developing fetuses in vivo. There was no evidence from the previous studies suggesting its teratogenicity. The present work indicates the adverse effects of Ginkgo biloba extract on developing conceptus. Although the exact mechanism by which Ginkgo biloba induces gross malformations is still elusive, cellular or molecular changes at the level of neural crest cells may be involved, as suggested by the pattern of anomalies. Further detailed studies are needed in order to explain the changes seen in the present research.

CONCLUSION

Our results indicate that Ginkgo biloba extract is deleterious to the developing fetuses in vivo. There was no evidence from the previous studies suggesting its teratogenicity. The present work indicates the adverse effects of Ginkgo biloba extract on developing conceptus. Although the exact mechanism by which Ginkgo biloba induces gross malformations is still elusive, cellular or molecular changes at the level of neural crest cells may be involved, as suggested by the pattern of anomalies. Further detailed studies are needed in order to explain the changes seen in the present research.

