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GINKGO BILOBA EXTRACT: EFFECTS ON FETAL MORPHOLOGY IN ALBINO RATS

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ABSTRACT:

Objective: To identify the effects of Ginkgo Biloba extract (EGb 761) on gross appearance of fetuses in Albino rats. EGb 761 is popular in women of reproductive age as an antioxidant and anti-stress tonic. In spite extensive use of EGb 761 containing dietary supplement during pregnancy, there is very fewer scientific evidence stating its effects on fetal health. This experimental study was completed in 2016 at the Anatomy department of Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore. **Study Design:** In this study effects on gross appearance was observed after administration of 14mg/kg daily oral dose of EGb761 to mothers. 39 female and 13 male Wistar Albino rats were kept together in cages with 3:1 female to male ratio for breeding purpose. After confirmation of conception by vaginal plug, 30 gravid females were selected by lottery method and divided into 5 groups A, B, C, D & E having equal number of animals. 21 days of gestation was divided into three trimesters (7 days each). Group A was taken as control group (no drug was given). Drug was administrated to group B, C, & D in 1st, 2nd & 3rd trimester respectively. Group E received drug in whole pregnancy. At term, all animals were euthanized and 4 fetuses / animal were taken and observed for any gross anomaly. These qualitative variables were described by frequency and percentages. Data were analyzed statistically by Chi square test while the difference was considered statistically significant when the p-value is greater than 0.05. **Results:** 24 fetuses / group were examined for the gross anomalies. Only group C showed a noticeable anomaly in which abdominal wall was absent in 12.5% fetuses. The finding was statistically significant ($p<0.05$) when compared to control group A. No other anomaly was found in any group. **Conclusion:** The results of current study demonstrated that Ginkgo biloba extract when it is used in second trimester, can cause fetal malformation in Wistar rats.

Key words: Ginkgo Biloba, fetal morphology, Albino rats

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INTRODUCTION

Use of Ginkgo Biloba in Chinese medicinal history is apparent since centuries back.[1] Now a days there is growing trend of the herbal medicinal use as food and nutritional supplements and standard extract of Ginkgo Biloba (EGb 761) is among highest selling herbal medicine for this purpose.[2]

EGb 761 is renowned anti-oxidant tonic,[3] also useful in the treatment of memory impairment, asthma, varicose veins, or sometimes for idiopathic cyclic edema in women of reproductive age.[4] It is found to be advantageous in various inflammatory, respiratory and cardiovascular disorders.[1]

Leaves of ginkgo tree are the source of EGb 761.[5] Final phytomedicinal product is standardization of 5–7% terpene trilactones and 22–24% of flavonol glycosides.[3, 6-7] 2.8-3.4% ginkgolides and 2.6-3.2% bilobalide are the primary terpene lactones in EGb 761.[3-5, 7] It also contains organic acids, ginkgolic acid and related alkylphenols (e.g., ginkgol, bilobol).[7-8]

Flavonoids can reach fetal tissues by crossing the placenta, where they obtain greater concentration than in maternal tissues.[9] In vitro reproductive toxicity by EGb761 was reported in a study on Wistar rats, treated with 7 and 14mg/kg/day of EGb761. According to this study EGb761 given to rats in the periods of organogenesis and fetogenesis might be a reason of fetal intra-uterine growth retardation (IUGR) but doesn't show any noxious effects on mothers.[10] Very limited studies have been performed to evaluate effects of EGb761 on developing fetal organs. Despite its wide medicinal use, tetratogenic effects of ginkgo biloba had so far not been established conclusively. This study is planned to assess the possibilities of morphological malformations caused by EGb761 in Wistar Albino rats.

MATERIALS AND METHODS:

This experimental study was completed in 2016 at the department of Anatomy Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore.

Dosage of Ginkgo biloba: The extract of Ginkgo biloba 120mg/5ml (24mg/ml) was purchased from Medisave Pharmaceuticals in liquid form containing 24% flavone glycosides & 6% terpene lactones. The concentration used in this experimental study was 14mg/kg/day. To identify the human reproductive risk, four times greater dose (14 mg/kg/day) was commonly administrated in animals than the highest dose in humans.[8] Route of administration was oral.

Study Design: 39 mature female and 13 mature male rats of Wistar strain were purchased from Veterinary Research Institute, Lahore. Weight of female and male rats was 200-250 and 250-300 grams respectively. After acclimatization of 15 days, for mating purpose, three nulliparous females with one male were housed in a cage for seven days. Female rats were observed every morning for vaginal plug and day of its confirmation was taken as day zero of gestation. After confirmation of conception 30 females were selected randomly and kept in separate cages with proper labeling.

The gravid female rats were randomly divided into five groups, A, B, C, D, and E. Each group contained 6 pregnant rats. Gestational period in rats is of 21 days and divided in to three equal trimesters of 7 days each.

Group A was the control group, no drug was administrated to this group. Drug was administrated to group B from day 1 to day 7 (first trimester), group C from day 8 to day 14 (second trimester), group D from day 15 to day 21 (third trimester) and group E from day 1 to day 21 (all trimesters).

After approval of research ethical committee of Institutional Review Board (IRB), all animals were euthanized on the day 21 by injecting calculated dose of pentobarbital intraperitoneally; the conventional agent for anesthesia acts swiftly and gently kills the rodents. The intraperitoneal dose of injection pentobarbital is 200mg/kg.[11] The animals were sacrificed immediately after euthanasia. In supine position, layers of abdomen were opened one by one after midline incision. In the lower abdomen, the pregnant uterus, with two horns was identified by its "beads on a string" appearance. Position of fetuses and placentae was identified. Average litter size was taken 4/animal so four fetuses per animal were selected randomly by lottery method. The total number of fetuses was 120 (Sample size was 24/group). The fetuses with their membranes were taken out and examined for presence of congenital anomalies. Fetuses were sacrificed by euthanasia and preserved in phosphate buffer. Fetuses were observed for any gross abnormality in all five groups.

Statistical Analysis: All qualitative variables were described by frequency and percentages. The qualitative data were analyzed statistically by Chi square test. The difference was considered statistically significant when the p-value is greater than 0.05.

RESULTS:

The fetuses of control group-A appeared normal, orbits; eyelids; external auditory meatus; pinnae were well formed. Similarly other facial features like nostrils; jaws; lips were also well formed. Both fore and hind limbs were distinctly divided into three segments, with claws having five digits in fetuses of all five groups. Tails were directed cranially and were nearly of equal length in all fetuses (Approximately 0.8-1 cm).

The fetuses of experimental groups appeared normal like control group. However 3 out of 24 (12.5%) fetuses of group C showed absent abdominal wall (Fig.1). Chi-Square value among all groups was 9.973 and p-value was 0.041, which was statistically significant (Table-1). Absence of abdominal wall was statistically significant among group A vs C, B vs C, C vs D and C vs E, when compared by Chi-Square test (Table-2)

DISCUSSION:

The purpose of present study was to know the effects of Ginkgo biloba extract (EGb761) on detailed morphology of fetuses in Albino rats. It always remained point of discussion regarding the safety of ginkgo during pregnancy.[8] It is suggested that components of EGb761 may cross the placenta and altered the process of organogenesis. [9]

A previous study on effects of EGb761 on embryo-fetal development showed no significant findings regarding morphology of fetuses.[12] But in current study, statistically significant finding observed while using EGb761 in second trimester was absent abdominal wall. It is showing that high doses of EGb 761 administrated in 2nd trimester had altered the external fetal morphologies. According to a previous study structural changes in DNA, could lead to external malformations among rat fetuses. In addition, it had been suggested that ginkgolide B, a constituent of EGb, significantly retarded mouse embryonic development, implantation and embryogenesis by reduction of inner cell mass.[13]

In another study to evaluate the adverse properties of alkylphenols (constituent of EGb 761), different fractions were evaluated for their embryotoxic effects in the hen's egg test. These results confirmed the high toxic potential of ginkgolic acids.[8]

It is therefore, suggested that the reduction in the inner cell mass lineage may reduce embryonic viability and development.

TABLE-1

Effect of ginkgo biloba extract on gross appearance of fetal abdominal wall in control and experimental groups based on Chi-Square test

Group	Abdominal wall		Total
	Normal	Abdominal wall absent	
A	24	0	24
B	24	0	24
C	21	3	24
D	24	0	24
E	24	0	24
Total	**117**	**3**	**120**

Chi-Square Value = 9.973 **p = 0.041***

A Control Group
B Experimental Group (treated with ginkgo biloba in 1st trimester)
C Experimental Group (treated with ginkgo biloba in 2nd trimester)
D Experimental Group (treated with ginkgo biloba in 3rd trimester)
E Experimental Group (treated with ginkgo biloba in all trimesters)
* Statistically significant difference ($p < 0.05$)

TABLE-2

Comparison of Chi-Square values of absent abdominal wall among the control and experimental groups (Mothers treated by ginkgo biloba extract)

Contrast Tests

Comparison among groups	Chi-Square values	Sig.
A vs B	0	1.000
A vs C	4.359	0.037*
A vs D	0	1.000
A vs E	0	1.000
B vs C	4.359	0.037*
B vs D	0	1.000
B vs E	0	1.000
C vs D	4.359	0.037*
C vs E	4.359	0.037*
D vs E	0	1.000

A Control Group
B Experimental Group (Mothers treated with ginkgo biloba in 1st trimester)
C Experimental Group (Mothers treated with ginkgo biloba in 2nd trimester)
D Experimental Group (Mothers treated with ginkgo biloba in 3rd trimester)
E Experimental Group (Mothers treated with ginkgo biloba in all trimesters)
Sig. Statistical Significance
* Statistically Significant Chi-Square value among the groups
Based on Chi-Square test

CONCLUSION

The results of present study demonstrated that ginkgo biloba extract when it is used in second trimester, can cause the birth defects in Wistar rats, a mammal which has a close functional semblance to human beings.

RECOMMENDATIONS

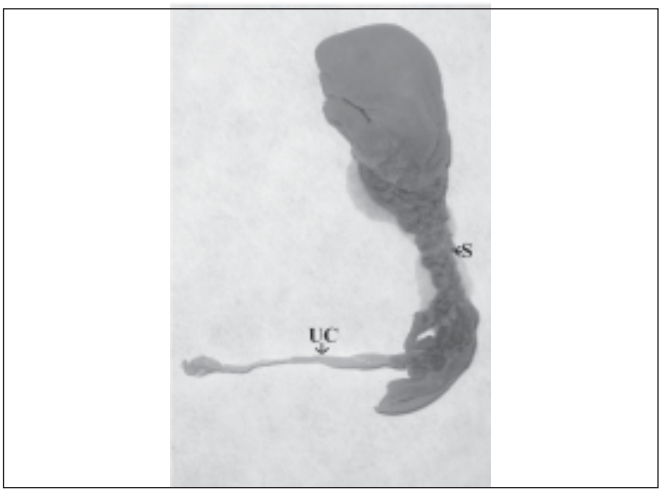
Current work indicates the adverse effects of EGb761 on fetal development. The problem of using EGb761 during pregnancy can be addressed by providing proper knowledge of EGb761 related adverse effects by medical community.

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FIGURE-1

Post-fixed photograph showing absent abdominal wall in fetus of group C (Mothers treated with ginkgo biloba extract in 2nd trimester)



UC Umblical cord
S Spine

of ginkgo (Ginkgo biloba) during pregnancy and lactation. Can J Clin Pharmacol 2006; 13(3): 277-84.
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Authors contribution:
* Whole Process ** Data Collection Write-up *** Data Collection, Write-up.

Conflict of interest: This study is planned to assess the possibilities of morphological malformations caused by EGb761 in wistar Albino rats.