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Teratological Studies of *Ginkgo biloba* Extract(EGb 761) in Rabbits

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ABSTRACT : *A teratological study was performed using New Zealand White rabbits to examine the teratological potential of Ginkgo biloba extract(EGb 761), which is a known strong platelet activating factor antagonist. Ginkgo biloba extract(EGb 761) was administered per intravenously during the organogenesis period (day 6th to 18th of gestation) of rabbits at dose levels of 7.5, 15, and 30 mg/kg/day. All pregnant females were sacrificed on day 29 of gestation and teratological abnormalities of their fetuses was examined. No statistically significant difference of body weight change between control and treated groups during experimental periods was noted. There was no statistically signifiant difference of numbers of corpus lutes and implantations, fetal death ratio, fetal sex ratio, and placental weight between control and rabbits exposed to three different concentration ranges of Ginkgo biloba extract (EGb 761). No marked external, visceral and skeletal abnormalities related to Ginkgo biloba extract(EGb 761) were observed in the fetuses. In conclusion Ginkgo biloba extract(EGb 761) does not show any effect on implantation or embryonic development.*

Key Words : *Ginkgo biloba extract(EGb 761), Teratological study, Rabbit*

I. INTRODUCTION

Ginkgo biloba extract has a strong pharmacological effect on vascular physiology. As a strong antagonist of platelet activating factor, *Ginkgo biloba* extract can increase microvascular permeability, induce bronchoconstriction, and has vasoregulatory activity of blood vessels. Therefore *Ginkgo biloba* extract might be a good model compound to develop as a new therapeutic agent for circulatory disturbance (Jos, K. *et al.*, 1992). Recently, *Gingko biloba* extract has also been used for treating peoples with several neural dysfunctions such as headache, dizziness, and memory loss (Jos, K. *et al.*, 1992, Schneider B., 1992, and Semlitsch, H.V. *et al.*, 1995).

Ginkgo biloba extract is known to have no general toxicity and can be coadministered with other drugs without compromisng the effect of other drug (DeFeudis, F. G. *et al.*, 1991).

This study was performed to examine the teratologenicity of *Ginkgo biloba* extract(EGb 761) according to the Good Laboratory Procedure guideline for Reproduction Experiments to Evaluate the Safety of Drugs issued by Korean National Institute for Safety Research center in 1994.

II. MATERIALS AND METHODS

1. Chemical

Ginkgo biloba extract(EGb 761) was extracted and provided by Yu-Yu, Inc. *Ginkgo biloba* extract (EGb 761) was dissolved in citrate buffer solution before use.

2. Methods

Fifty five, 5-month-old, virgin, female rabbits were purchased from Samyuk Laboratory Animal

Co. Ltd.. After mating with rabbits of the same strain, the rabbits were allowed to acclimate for one week and 48 pregnant rabbits were selected and used throughout the experiment. Animals had a free access to a commercial laboratory food and water and were maintained on a 12-hr light:dark cycle with a temperature range of 24±3°C and humidity range of 55±10%.

The rabbits were randomly divided into 4 groups. Each group was injected per intravenously with 30mg/kg (group 1), 15mg/kg (group 2), and 7.5 mg/kg (group 3) of test chemical or vehicle only (group 4) for 12 days consecutively between day 6 and day 18 of post implantation.

Body weight, food and water consumption of each rabbit were monitored every 3 days. The rabbits were euthanized day 29 (one day before delivery) after the treatment was initiated and pregnancy index (number of corpus leuteum, implantation, absorption, and fetal body weight and sex) was examined. Teratological examination was performed by Gleich and Frohberg and Kimmel and Trammel's methods. The results were statistically analyzed by dunnett's t-test, Wilcoxon-Mann-Whitney rank-sum test and Nemenyl-Kruskal-Wallis multiple comparison.

III. RESULTS

1. Effect on Body Weight and Food and Water Consumption (Tables 1, 2, and 3)

No significant body weight change of treated groups was noted compared to that of control group. Food consumption of group 1 was significantly decreased at day 0 and water consumption of group 1 was significantly decreased at days 9, 12, 15 post initiation compared to those of control group.

2. Effect on Body Weight Gain and Relative Organ Weight (Table 4)

Compared to control group, no statistically significant difference was noted in all three treated groups.

3. Effect on Pregnancy Index (Table 5)

Pregnancy index between control and treated groups was not statistically different.

4. Teratological Effect on Fetuses (Table 6)

Table 1. Body weight of dams intravenously injected with *Ginkgo biloba* extract(EGb 761) on day 6-18 of gestation

(unit: g)

Group	Control	Low	Middle	High
Dose (mg/kg/day)	0	7.5	15	30
No. of dams Gestation day	12	12	12	12
0	3833.33[a)]±478.79	4116.67±453.44	4108.33±444.07	4041.67±527.36
3	4125.00 ±492.90	4458.33±436.32	4408.00±501.29	4312.50±546.94
6	4083.33 ±485.86	4454.17±479.80	4404.17±479.80	4354.17±449.98
7	4216.67 ±487.73	4441.67±517.35	4408.33±481.87	4408.33±450.67
8	4283.33 ±487.26	4550.00±434.85	4450.00±470.01	4416.67±438.66
9	4275.00 ±485.47	4512.50±542.77	4437.50±509.96	4325.00±524.62
10	4279.17 ±517.19	4508.33±435.28	4388.33±559.76	4375.00±517.20
11	4362.50 ±487.63	4533.33±467.75	4375.00±606.59	4329.17±440.17
12	4383.33 ±493.75	4645.83±458.98	4379.17±592.55	4362.50±462.27
13	4370.83 ±481.69	4587.50±393.19	4345.83±606.95	4362.50±497.78
14	4400.00 ±462.70	4625.00±407.60	4358.33±593.08	4379.17±495.64
15	4433.33 ±514.33	4650.00±482.89	4458.33±636.34	4458.33±502.19
16	4487.83 ±481.69	4620.83±456.00	4454.17±620.29	4516.67±501.06
17	4504.17 ±549.57	4720.83±457.99	4479.17±580.15	4525.17±493.81
18	4491.67 ±543.91	4700.00±430.64	4470.83±591.02	4533.33±480.69
21	4516.67 ±501.97	4662.50±422.18	4516.67±613.98	4562.50±493.19
24	4545.83 ±531.92	4700.00±440.56	4487.17±663.14	4579.17±479.80
27	4537.50 ±491.81	4691.67±454.69	4529.17±663.14	4658.33±450.17
29	4600.00 ±493.13	4716.67±442.79	4566.67±639.01	4700.00±445.18

[a)] presented mean±S.D.

Table 2. Feed consumption (unit: g)

Group Dose (mg/kg/day) No. of dams Gestation day	Control 0 12	Low 7.5 12	Middle 15 12	High 30 12
0	272.50[a)]±40.03	218.33± 59.37	236.67± 68.40	200.83*± 74.52
3	268.33 ±82.66	291.67± 62.21	282.50± 74.24	266.67 ±109.49
6	231.67 ±39.50	254.17±101.31	244.17± 81.40	195.83 ± 68.82
9	236.00 ±31.55	245.00± 44.82	199.17± 85.97	215.83 ± 93.46
12	212.50 ±43.56	211.25± 61.87	197.50± 64.65	199.17 ± 57.28
15	205.42 ±67.47	189.58± 70.53	227.08±114.23	204.17 ± 56.48
18	224.83 ±73.52	222.50± 77.59	254.58± 99.30	272.92 ± 63.33
21	218.33 ±71.71	196.50± 71.19	226.25± 99.30	170.83 ± 71.15
24	184.42 ±80.44	231.25± 69.48	196.67± 78.32	215.83 ± 69.99
27	176.67 ±89.68	235.00± 72.55	231.67± 83.86	209.58 ± 61.07
29	175.00 ±88.57	235.00± 72.67	230.00± 82.13	213.75 ± 66.71

[a)] presented mean±S.D.

* significantly different from control group ($p < 0.05$).

Table 3. Water consumption (unit: ml)

Group Dose (mg/kg/day) No. of dams Gestation day	Control 0 12	Low 7.5 12	Middle 15 12	High 30 12
0	409.17[a)]± 83.28	370.42±103.49	340.83± 73.91	384.17± 87.64
3	382.92 ±105.06	387.50± 93.53	317.50± 82.37	302.50±110.30
6	408.33 ±112.96	360.83±124.57	305.00± 95.68	333.33±113.80
9	394.17 ±103.15	320.83±104.40	272.50± 89.86*	262.50±109.06*
12	438.33 ± 86.11	345.83±103.08	300.42± 79.04*	333.33±116.41*
15	401.67 ±126.12	303.33±112.76	258.00± 90.70*	267.50± 69.43*
18	388.33 ±113.68	406.25± 98.42	341.25±130.80	345.00±131.04
21	306.67 ±147.18	305.00±120.11	249.17± 98.95	253.33± 93.26
24	331.67 ±134.56	315.83±120.11	303.33±124.27.	244.17±105.53
27	407.50 ±110.63	315.00± 86.50	305.00±106.81	340.00± 88.73
29	401.67 ±118.92	309.17± 85.64	300.00± 83.44	337.50± 89.55

[a)] presented mean±S.D.

* significantly different from control group ($p < 0.05$).

Table 4. Effect of *Ginkgo biloba* extract(EGb 761) on organ weight and relative organ weight of rabbits injected intravenously from day 6 to day 18 og gestation day (wet wt.; g, %)

Group Dose (mg/kg/day) No. of dams	Control 0 12	Low 7.5 12	Middle 15 12	High 30 12
Body weight gains (g) (0-29 day)	716.61±220.88[a)]	600.00±543.44	475.00±543.35	654.17±426.11
Absolute organ weight (g)				
Liver	113.90± 14.47	101.06± 22.34	106.40± 18.17	97.91± 11.21
Spleen	2.37± 0.75	2.55± 0.83	2.38± 0.58	2.31± 0.89
Kidney Left	10.86± 1.28	10.74± 1.86	11.47± 1.95	9.72± 1.58
Kidney Right	10.65± 1.49	10.55± 2.05	11.48± 1.97	10.11± 1.09
Lung	17.68± 1.95	19.26± 3.62	17.67± 3.63	16.08± 2.01
Heart	9.85± 1.52	11.45± 2.26	10.87± 2.19	9.49± 1.31
Relative organ weight (9%)				
Liver	2.56± 0.69	2.20± 0.51	2.22± 0.41	2.21± 0.31
Spleen	0.05± 0.02	0.05± 0.01	0.04± 0.01	0.05± 0.01
Kidney Left	0.24± 0.06	0.23± 0.04	0.23± 0.03	0.21± 0.03
Kidney Right	0.23± 0.06	0.23± 0.04	0.23± 0.03	0.22± 0.02
Lung	0.39± 0.08	0.42± 0.08	0.36± 0.07	0.36± 0.05
Heart	0.21± 0.05	0.25± 0.06	0.22± 0.03	0.21± 0.03

[a)] presented mean±S.D.

Table 5. Effect of *Ginkgo biloba* extract(EGb 761) on aspect of fetus at caesarean section of rabbit dams injected intravenously on day 6-18 gestation

Group	Control	Low	Middle	High
Dose (mg/kg/day)	0	7.5	15	30
No. of dams	12	12	12	12
No. of corpus luteum (mean±S.D.)	11.41± 4.23	9.16± 2.72	9.33± 2.93	10.50±2.84
No. of implants (mean±S.D.)	7.75± 3.44	6.75± 2.86	7.08± 2.35	8.08±2.60
Implantation ratio (%)[a]	70.90±22.14	69.91±10.56	79.91±11.11	78.58±9.73
Dead fetus ratio (%)[b]	7.53	2.47	1.18	5.15
Resorption ratio (%)[c]	1.08	0	0	1.03
Early	1	0	0	1
Late	0	0	0	0
Total	0	0	0	0
Sex ratio (M/F)[d]	1.00± 0.75	1.04± 0.64	1.43± 1.20	0.69±0.56
Litter weight	77	65	77	84
Body weight (mean±S.D.)				
Male	50.14±10.26	55.79± 9.17	58.16± 5.36	58.95±8.87
Female	41.97± 7.32	54.33± 8.03	54.67± 5.73	50.14±9.51
Placental weight (g)	6.67± 1.70	6.31± 1.20	5.96± 0.60	6.56±1.20
Gestational ratio (%)[e]	82.79	80.25	90.58	86.59

[a] (No. of implants/No. of corpus luteum)×100.
[b] (No. of dead fetuses/No. of implants)×100.
[c] (No. of resorption/No. of implants)×100.
[d] (No. of male fetuses/No. of female fetuses)×100.
[e] (No. of fetuses/No. of implants)×100.

Table 6. Effect of *Ginkgo biloba* extract(EGb 761) on external, visceral, skeletal anomalities in rabbit fetuses (F1)

Group	Control	Low	Middle	High
Dose (mg/kg/day)	0	7.5	15	30
No. of dams	12	12	12	12
external anomalities				
No. of examined fetuses	77	65	77	84
No. of abnormal fetuses	0	0	0	0
Fused placenta	0	0	0	0
Hematoma	0	0	0	0
Hemorrhage in the yolk-sac	0	0	0	0
Exencephaly	0	0	0	0
Exophthalmia	0	0	0	0
Annurysm	0	0	0	0
Dislocation of ear	0	0	0	0
abnormal face	0	0	0	0
Hind limb ab.	0	0	0	0
Short tail	0	0	0	0
External malformation (%)	0/77(0%)	0/65(0%)	0/77(0%)	0/84(0%)
visceral anomalities				
No. of examined fetuses	38	32	38	42
No. of abnormal fetuses	0	0	0	0
Aberrant right subclavian artery	0	0	0	0
Esophagus tracheal constriction	0	0	0	0
Ventricular septal defect	0	0	0	0
Dilatation of renal pelvis	0	0	0	0
Renal displacement	0	0	0	0
Dilatation of subarachnoid space	0	0	0	0
Visceral malformation (%)	0/38(0%)	0/32(0%)	0/38(0%)	0/42(0%)

Table 6. Continued

Group Dose (mg/kg/day) No. of dams	Control 0 12	Low 7.5 12	Middle 15 12	High 30 12
skeletal anomalities				
No. of examined fetuses	39	33	39	42
No. of abnormal fetuses	0	0	0	0
Mandibular hypoplasia	0	0	0	0
Parietal hypoplasia	0	0	0	0
Forelimb, Ectrodactyly	0	0	0	0
Fission of thoracic vertebral center	0	0	0	0
Synostosis of sternebrae	0	0	0	0
Shortening of rib	0	0	0	0
Skeletal malformation (%)	0/39(0%)	0/33(0%)	0/39(0%)	0/42(0%)

Treated chemical of three different concentration ranges did not show any marked external, visceral, and skeletal teratological abnormalities.

IV. DISCUSSION AND CONCLUSION

This study was performed to examine the teratological effect of *Ginkgo biloba* extract(EGb 761) when it was exposed to pregnant New Zealand rabbits. Forty, eight rabbits were randomly divided into three treatment groups and one control group. Thirty, 15, and 7.5 mg/kg body weight of *Ginkgo biloba* extract(EGb 761) was administered to the rabbits per intravenously throughout the organogenesis stage (day 6th to 18th of gestation) of rabbits and teratological examination of the fetuses was performed one day before delivery.

No statistically significant difference of body weight change between control and treated groups during experimental periods was noted. However, food consumption of high concentration group at day 0 and water consumption of high and middle concentration group at days 9, 12, and 15 were significantly lower than those of control group, respectively. There was no statistically signifiant difference of numbers of corpus lutes and implantations, fetal death ratio, fetal sex ratio, and placental weight between control and rabbits exposed to three different concentration ranges of *Ginkgo biloba* extract(EGb 761). No marked external, visceral and skeletal abnormalities were found from fetuses.

In conclusion, when *Ginkgo biloba* extract(EGb 761) was administered per intra venously up to concentration of 30 mg/kg/day to pregnant rabbits in organogenesis stage, no significant toxic as well as teratological effects were found on pregnant rabbits and their fetuses.

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