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Three Month Subacute Toxicity Study of *Ginkgo Biloba* Extract(EGb 761) in Rabbits

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ABSTRACT : *Group of 12 male and 12 female rabbits was given daily intravenous injections of different dosage of Ginkgo biloba extract(EGb 761), 7.5 mg/kg/day (low dosage group), 15 mg/kg/day (middle dosage group), or 30 mg/kg/day (high dosage group) for 3 month by ear vein according to Established Regulation of Korean National Institute of Safety Research (1994. 4. 14). Appearance, behavior, mortality, and food consumption of rabbits of treated groups were not affected during the experimental periods. No significant Ginkgo biloba extract(EGb 761)-related changes were found in urinalysis, hematology, serum chemistry, and organ weight. No histopathological lesions were seen in both control and treatment groups. Our results strongly suggest that no toxic changes should be found in rabbit treated intravenously with Ginkgo biloba extract(EGb 761) for 3 month.*

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

I. INTRODUCTION

Ginkgo biloba extract made of leaves of Ginkgo tree (*Ginkgo biloba Linne*) plays a role as antagonist of platelet activating factor and have a function as microvascular permeability and bronchoconstriction (Chung K. F., *et al.*, 1987). In addition, *Ginkgo biloba* extract stimulates blood flow in arteries, capillaries, and veins (Jos K. *et al.*, 1992). Therefore, *Ginkgo biloba* extract pays attention to treatment of circulatory disorders (Jos K. *et al.*, 1992). Recently, *Ginkgo biloba* extract treated with cerebral dysfunctions such as loss of memory, dizziness, and headache in aging people (Semlitsch H. V., *et al.*, 1995). In Germany, *Ginkgo biloba* extract was widely used for cerebral dysfunction from early 1990 (Jos K. *et al.*, 1992). It has been reported that there were no side effect and drug interaction between other dugs in toxicity tests for *Ginkgo biloba* extract (DeFeudis F. G. *et al.*, 1991).

The present study was examined to investigate subacute toxicity, by giving rat injected with *Ginkgo biloba* extract(EGb 761) from YuYu Co., Ltd. for 3 month according to Established Regulation of Korean National Institute of Safety Research (1994. 4. 14).

II. MATERIALS AND METHODS

1. *Ginkgo biloba* Extract(EGb 761)

Ginkgo biloba extract(EGb 761) used in this study was supplied by YuYu Co., Ltd. *Ginkgo biloba* extract (EGb 761) was made of leaves of Ginkgo tree (*Ginkgo biloba Linne*). It was a brown crystalline powder and contained 25.3% Ginkgoflavonglycosides.

2. Experimental Animals

Thirty New Zealand White rabbits, 15 male and 15 female, respectively, were purchased from Samyuk Laboratory Animal Center. The rabbits were acclimated to laboratory conditions for 7 days prior to assignment to the study. 12 male and 12 female rats were selected for experiment. Rabbits

were housed on wood shaving in transparent polycarbonate boxes (45×50×31 cm, MyoungJin Company, Republic of Korea) and maintained at an ambient temperature (23+3°C), a relative humidity of 40-60% (50+10°C) with a minimum of 10 complete changes of 100% conditioned fresh air per hour, and a light/dark cycle of 12 hour light (from 7:00 AM to 7:00 PM) and 12 hour dark with no twilight. The brightness is 150-200 Lux. Feed and water were available ad libitum throughout the experimental period.

3. Experimental Design

Twelve male New Zealand White rabbits were randomly assigned to three treatment groups and one control group. Also, twelve female New Zealand White rabbits were randomly assigned to three treatment groups and one control group. In each treatment group, rabbits were given daily intravenous injections of different dosage of *Ginkgo biloba* extract(EGb 761), 7.5 mg/kg/day (low dosage group), 15 mg/kg/day (middle dosage group), or 30 mg/kg/day (high dosage group) for 3 month by ear vein.

General conditions such as appearance, behavior and toxic signs were observed daily on all the rats as described in Established Regulation of Korean National Institute of Safety Research (1994. 4. 14). Body weight and feed consumption were recorded regularly. Trials to collect urine was performed three times by artificial urination during the whole experiment.

All rats were esanguinated from the abdominal aorta under light anesthesia by ether. Blood samples were collected from jugular vein into ethylenediaminetetraacetic acid (EDTA)-containing tubes for hematology and serum chemistry. At autopsy, gross lesions were recorded. Weights of liver, spleen, kidney, adrenal gland, heart, lung, salivary gland, brain, and testis were measured, fixed with 10% neutral buffered-formalin and embedded in paraffin. Tissues were routinely processed and sectioned (5 μm). Tissues were deparaffinized with xylene, rehydrated through traded alcohols, air dried and stained with hematoxylin and eosin.

Data were analyzed by non-parametric one way Kruskal-Wallis test, Terpstra-Jackheere test and Chi-square test.

III. RESULTS

1. Clinical Signs

The intravenous injection of *Ginkgo bibola* extract(EGb 761) developed no adverse effect on normal behavior of the animals in treatment and control group throughout the whole experiment. No dose-related death of rabbits was observed during the 3 month experimental period.

2. Body Weight

No significant difference changes of body weight between *Ginkgo biloba* extract(EGb 761)-treatment and control group (Figs. 1 and 2). However, there was a higher body weight (2900.00±100.00, 3100.00±346.41) in males of middle and high dosage group compared with control group (2650.00±86.60) at 25 days.

3. Feed and Water Consumption

In the feed and water consumption, there was no significant difference between the treatment and control group (Figs. 3, 4, 5 and 6). However, there was a higher feed and water consumption (260.00±45.82) in males of higher dosage group (30 mg/kg/day)

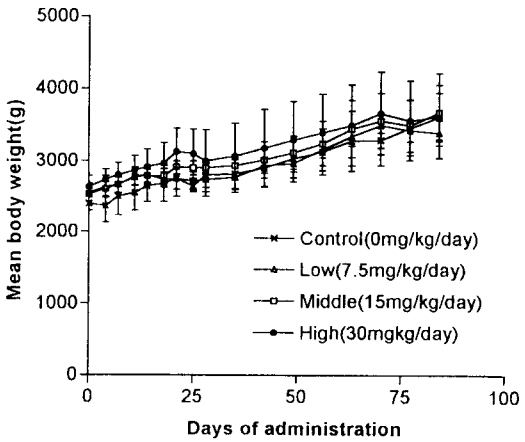

Fig. 1. Mean body weights in male rabbits intravenously injected with *Ginkgo biloba* extract(EGb 761). Each value represented the mean±SD of 3 rabbits.

compared with control group at 21 days. there was a high feed and water consumption (256.67±23.09) in males of middle dosage group (15 mg/kg/day) compared with control group at 77 days. There was a higher feed and water consumption (196.67± 30.55, 213.33±5.77) in females of low and high dosage group compared with control group at 28 days. There was a lower feed and water consumption (290.00±52.91) in females of high dosage group compared with control group at 84 days.

4. Urinalysis

The appearance and pH of the urine in the treatment groups were comparable to those in the control

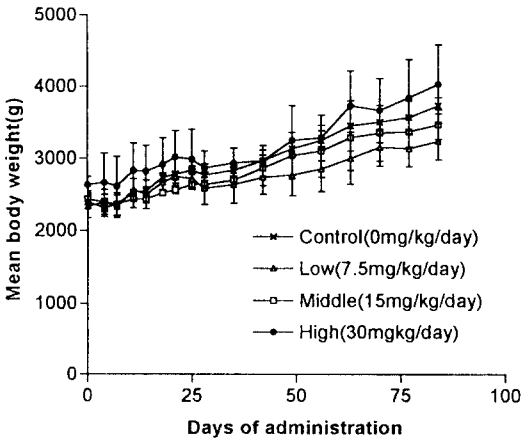

Fig. 2. Mean body weights in female rabbits intravenously injected with *Ginkgo biloba* extract(EGb 761). Each value represented the mean±SD of 3 rabbits.

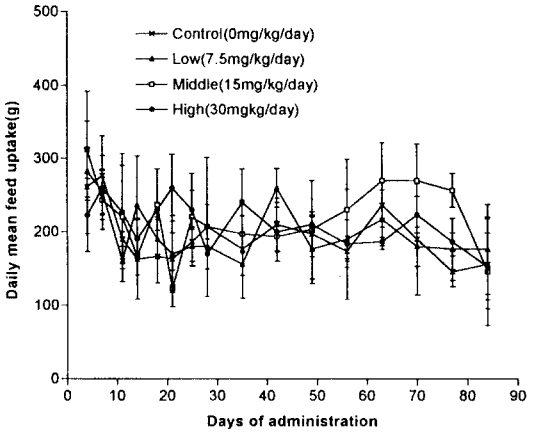

Fig. 3. Daily mean Feed uptake in male rabbits intravenously injected with *Ginkgo biloba* extract (EGb 761). Each value represented the mean±SD of 3 rabbits.

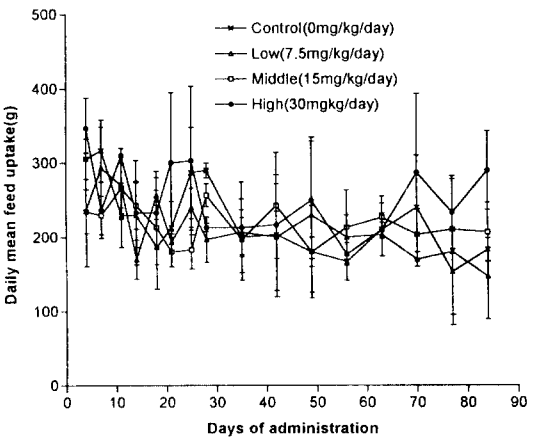

Fig. 4. Daily mean Feed uptake in female rabbits intravenously injected with *Ginkgo biloba* extract (EGb 761). Each value represented the mean±SD of 3 rabbits.

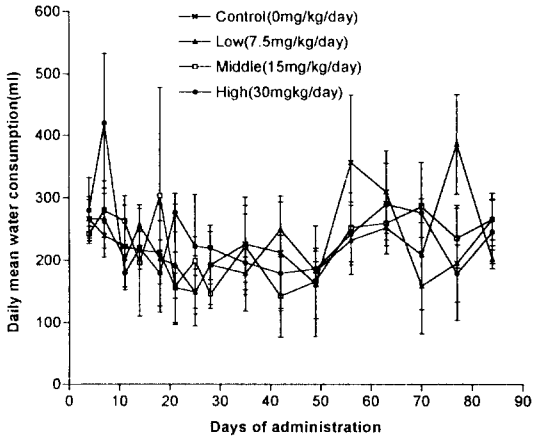

Fig. 5. Daily mean water consumption in male rabbits intravenously injected with *Ginkgo biloba* extract(EGb 761). Each value represented the mean±SD of 3 rabbits.

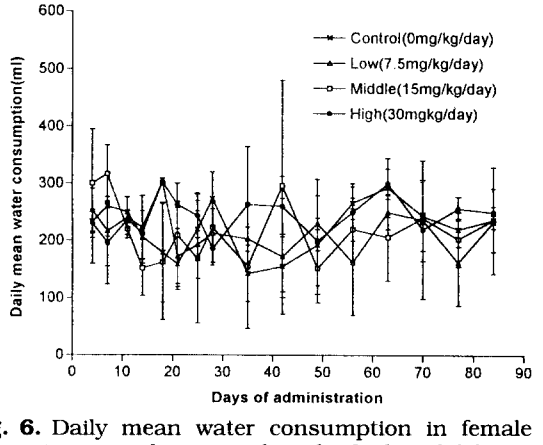

Fig. 6. Daily mean water consumption in female rabbits intravenously injected with *Ginkgo biloba* extract (EGb 761). Each value represented the mean±SD of 3 rabbits.

group and were within normal range of variation.

5. Serum Biochemistry

Serum biochemical data are summarized in Tables 1 and 2. There was a lower chloride and cholesterol in males of low dosage group (7.5 mg/kg/day). There was a lower chloride, total protein, potassium, and albumin in males of middle dosage group (15 mg/kg/day). There was a lower total bilirubin, chloride, blood urea nitrogen, and albumin in male middle dosage

Table 1. Serum biochemical values of rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 6 weeks (Dose unit: mg/kg/day)

\Sex	Male				Female			
Parameter \Group	Control	Low	Middlie	High	Control	Low	Middlie	High
\Dose	0	7.5	15	30	0	7.5	15	30
\No. of animal	3	3	3	3	3	3	3	3
ALT (u/l)	56.50± 4.95[a]	60.33±17.16	77.50± 56.57	61.00±16.70	51.00± 2.83	53.50± 2.12	35.00± 56.57	36.00±10.54
AST (u/l)	23.50± 3.54	26.00±10.53	85.00±100.41	31.00±11.31	12.00±14.14	23.50± 9.19	14.00±100.41	32.33±28.04
CHOL (mg/dl)	38.50± 4.95	32.67± 4.72*	47.00± 4.24	59.67±17.67	60.00±18.38	97.50±53.03	65.00± 4.24	76.00± 5.56
GLU (mg/dl)	135.00± 5.66	142.00±74.51	158.00± 53.74	114.33± 5.13	111.50±12.02	100.50± 0.70	108.00± 53.74	109.66±20.79
TB (mg/dl)	0.30± 0.00	0.37± 0.06	0.40± 0.00	0.27± 0.06*	0.25± 0.07	0.30± 0.14	0.40± 0.00	0.36± 0.05
TP (g/dl)	6.43± 0.22	6.65± 0.06	5.43± 0.23	6.12± 0.46	6.18± 0.04	6.12± 0.96	6.11± 0.23	6.79± 0.64
TG (mg/dl)	77.50±23.33	120.00±70.93	187.00±108.89	107.00±75.94	83.50±21.92	71.50±14.85	49.00±108.89	68.33±14.57
ALP (u/l)	126.00±76.37	118.00±15.87	97.00± 22.63	118.00±19.70	116.50± 2.127	88.50± 7.78	103.00± 22.63	60.00±26.05
CL (meq/l)	11.50± 2.12	104.33± 3.79*	102.00± 1.41*	102.00± 3.46*	99.00± 5.66	105.00± 0.00	104.00± 1.41	105.66± 2.88
CREAT (mg/dl)	1.60± 0.14	1.40± 0.20	1.30± 0.00	1.43± 0.15	1.35± 0.07	1.55± 0.35	1.00± 0.00	1.33± 0.37
BUN (mg/dl)	42.50± 7.78	28.33± 5.13	30.50± 6.36	25.00± 6.56*	28.00± 1.41	27.50± 3.54	34.00± 6.36	27.66± 8.32
K (meq/l)	8.60± 0.28	8.83± 0.40	7.55± 0.21*	8.00± 0.72	7.65± 0.21	8.30± 0.57	8.30± 0.21	8.66± 1.41
Albumin (g/dl)	4.40± 0.14	4.80± 0.46	3.80± 0.00*	4.10± 0.10*	4.20± 0.14	4.15± 0.35	4.00± 0.00	3.86± 0.51

[a] Values were expressed mean±S.D.

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

ALT, alanine transaminase; AST, aspartate transaminase; CHOL, cholesterol; GLU, glucose; TB, total bilirubin; TP, total protein; TG, triglyceride; ALP, alkaline phosphatase; CL, chloride; CREAT, creatinine; BUN, blood urea nitrogen.

Table 2. Serum biochemical values of rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 6 weeks (Dose unit: mg/kg/day)

\Sex	Male				Female			
Parameter \Group	Control	Low	Middlie	High	Control	Low	Middlie	High
\Dose	0	7.5	15	30	0	7.5	15	30
\No. of animal	3	3	3	3	3	3	3	3
ALT (u/l)	45.50± 3.53[a]	72.50± 23.33	40.00±29.46	67.00± 0.00	23.50± 7.77	21.50± 4.94	19.50±12.02	30.66±10.26
AST (u/l)	43.00±11.31	49.00± 32.14	26.33±27.15	29.00±13.45	20.50±14.84	24.50± 6.36	27.00±32.52	26.50± 4.94
CHOL (mg/dl)	39.50±14.84	31.33± 3.05	32.00± 7.81	39.00±15.62	33.50±26.26	110.00± 9.89*	39.00±16.97	59.33± 6.50
GLU (mg/dl)	219.00±48.08	248.66±103.55	119.66±52.69	175.33±21.82	125.50±54.44	87.00±32.52	95.50±20.50	145.00±10.14
TB (mg/dl)	0.45± 0.07	0.46± 0.25	0.30± 0.14	0.43± 0.11	0.30± 0.00	0.35± 0.07	0.40± 0.14	0.33± 0.20
TP (g/dl)	6.75± 0.01	7.68± 0.60	4.64± 1.63*	6.87± 0.05	4.96± 3.33	6.17± 0.42	4.60± 2.30	7.06± 0.83
TG (mg/dl)	186.00±31.33	153.33± 93.64	108.66±76.53	86.33±17.00	111.00±69.29	251.50±67.17	97.00±98.99	119.00±78.46
ALP (u/l)	96.50±60.10	92.33± 14.22	71.00±32.35	122.00± 7.54	48.00± 2.82	101.50± 7.77	6.05± 4.17	46.66±37.81
CL (meq/l)	107.50± 2.12	110.66± 1.52	79.66±57.76	113.00± 4.35	97.50±21.92	102.00± 1.41	91.50±16.26	106.33± 3.51
CREAT (mg/dl)	2.05± 0.63	1.76± 0.05	1.20± 0.60	1.73± 0.40	1.10± 0.70	1.35± 0.07	0.90± 0.14	1.50± 0.36
BUN (mg/dl)	43.50±16.26	29.33± 3.51	22.33±13.31	25.00± 3.46	30.00± 5.65	17.50± 0.70	19.50± 0.70	32.33±10.40
K (meq/l)	8.95± 0.21	8.40± 0.50	6.46± 4.53	8.80± 0.26	7.70± 3.11	7.70± 1.27	5.45± 2.19	8.70± 0.20
Albumin (g/dl)	4.70± 0.00	5.30± 0.50	3.00± 1.77*	4.46± 0.32	4.30± 0.98	3.00± 0.28	3.45± 0.35	4.23± 0.40

[a] Values were expressed mean±S.D.

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

ALT, alanine transaminase; AST, aspartate transaminase; CHOL, cholesterol; GLU, glucose; TB, total bilirubin; TP, total protein; TG, triglyceride; ALP, alkaline phosphatase; CL, chloride; CREAT, creatinine; BUN, blood urea nitrogen.

group (15 mg/kg/day). There was a lower total bilirubin, chloride, blood urea nitrogen and albumin in males of high dosage group (30 mg/kg/day) compared with control group. There was a lower cholesterol in females of lower dosage group.

6. Hematology

Hematological data are summarized in Tables 3 and 4. There was a higher red blood cell (RBC), hemoglobin (HGB), and hematocrit in males of low

Table 3. Serum biochemical values of rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 6 weeks
(Dose unit: mg/kg/day)

\Sex	Male				Female			
Parameter \Group	Control	Low	Middlie	High	Control	Low	Middlie	High
\Dose	0	7.5	15	30	0	7.5	15	30
\No. of animal	3	3	3	3	3	3	3	3
Neutrophil ($\times 10^3/\mu l$)	0.74± 0.96[a]	0.17± 0.06	0.14± 0.05	0.27± 0.16	0.77± 0.41	0.27± 0.07	3.92±3.64	0.29± 0.12
Lymphocyte ($\times 10^3/\mu l$)	5.87± 3.64	9.01± 1.37	5.32± 2.35	8.72± 1.64	6.49± 1.69	7.96± 1.54	6.09±5.09	5.88± 2.70
Monocyte ($\times 10^3/\mu l$)	0.02± 0.00	0.03± 0.02	0.02± 0.01	0.03± 0.01	0.08± 0.04	0.03± 0.00	0.22±0.08*	0.03± 0.01
Eosinophil ($\times 10^3/\mu l$)	0.04± 0.01	0.03± 0.01	0.02± 0.01	0.04± 0.02	0.11± 0.10	0.05± 0.00	0.10±0.09	0.04± 0.01
Basophil ($\times 10^3/\mu l$)	0.07± 0.02	0.04± 0.02	0.04± 0.02	0.05± 0.01	0.12± 0.04	0.06± 0.02	0.21±0.15	0.05± 0.01
Leucocyte ($\times 10^3/\mu l$)	0.15± 0.11	0.15± 0.03	0.06± 0.01	0.02± 0.01	0.32± 0.12	0.17± 0.02	1.94±1.59	0.16± 0.01
WBC ($\times 10^3/\mu l$)	6.87± 2.56	9.43± 1.44	5.58± 2.26	9.27± 1.83	7.84± 0.89	8.49± 1.65	9.45±4.15	6.43± 2.53
RBC ($\times 10^3/\mu l$)	5.96± 0.10	6.86± 0.63*	6.32± 0.14	6.63± 0.41	6.93± 0.60	6.54± 0.13	6.31±0.53	5.86± 0.52
HGB (g/dl)	12.80± 0.65	14.30± 0.81*	13.36± 0.25	13.50± 0.60	13.60± 0.42	13.05± 0.49	12.40±0.14	12.00± 0.95
HCT (%)	38.86± 1.77	42.93± 3.10*	38.60± 0.62	39.63± 1.25	40.80± 2.82	38.30± 0.56	37.80±0.84	35.46± 1.84
MCV (fL)	65.13± 2.33	62.66± 1.10	61.10± 1.22*	59.80± 1.99*	58.85± 0.91	58.50± 0.42	60.20±6.50	60.73± 5.75
MCH (pg)	21.50± 0.91	20.86± 0.73	21.13± 0.32	20.33± 0.46	19.70± 1.13	20.00± 1.13	19.75±1.48	20.60± 2.48
MCHC (g/dl)	33.00± 0.26	33.33± 0.66	34.56± 0.20*	34.00± 0.45*	33.40± 1.41	34.20± 1.69	32.95±1.06	33.83± 1.00
PLT ($\times 10^3/\mu l$)	296.00±91.92	348.66±102.06	139.00±178.06	501.66±90.23	295.50±335.87	556.50±95.45	433.00±5.65	458.33±164.93

[a] Values were expressed mean ± S.D.
* significantly different from control group ($p < 0.05$).
WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corposcular hemoglobin; MCHC, mean corposcular hemoglobin concentration; PLT, platelet.

Table 4. Hematological values of rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 12 weeks
(Dose unit: mg/kg/day)

\Sex	Male				Female			
Parameter \Group	Control	Low	Middlie	High	Control	Low	Middlie	High
\Dose	0	7.5	15	30	0	7.5	15	30
\No. of animal	3	3	3	3	3	3	3	3
Neutrophil ($\times 10^3/\mu l$)	0.86± 0.14[a]	1.21± 0.45	1.06± 0.33	1.21± 0.21	1.35± 0.42	1.14± 0.36	2.35± 0.94	0.99± 0.05*
Lymphocyte ($\times 10^3/\mu l$)	3.69± 2.15	5.09± 2.62	4.02± 1.97	8.47± 0.07	8.02± 1.08	7.86± 7.65	4.77± 1.92	6.79± 0.74
Monocyte ($\times 10^3/\mu l$)	0.14± 0.01	0.18± 0.04	0.13± 0.05	0.13± 0.00	0.18± 0.01	0.14± 0.00	0.17± 0.06	0.16± 0.05
Eosinophil ($\times 10^3/\mu l$)	0.16± 0.03	0.18± 0.01	0.22± 0.04	0.21± 0.04	0.18± 0.02	0.20± 0.12	1.98± 2.55	0.19± 0.02
Basophil ($\times 10^3/\mu l$)	0.11± 0.05	0.14± 0.10	0.15± 0.08	0.17± 0.01	0.24± 0.08	0.07± 0.01*	0.15± 0.07	0.12± 0.01
Leucocyte ($\times 10^3/\mu l$)	0.27± 0.01	0.50± 0.20	0.38± 0.14	0.43± 0.12	0.55± 0.15	0.47± 0.30	0.49± 0.43	0.42± 0.05
WBC ($\times 10^3/\mu l$)	5.16± 1.41	7.22± 2.94	5.86± 1.83	10.17± 0.57	10.60± 0.98	5.41± 1.83*	9.03± 2.41	8.64± 0.72
RBC ($\times 10^3/\mu l$)	6.01± 0.04	6.28± 0.27	6.31± 0.24	6.52± 0.61	6.85± 0.07	6.20± 0.19	7.07± 0.72	5.96± 0.49
HGB (g/dl)	12.86± 0.40	13.70± 1.04	13.73± 0.24	13.43± 0.90	13.65± 0.49	13.65± 0.77	14.35± 0.63	12.60± 0.78
HCT (%)	37.03± 1.44	39.63± 2.77	39.66± 1.05	39.06± 2.50	40.80± 1.69	38.55± 1.06	42.75± 3.46	37.53± 2.00
MCV (fL)	61.46± 2.21	63.06± 1.89	62.80± 0.81	59.93± 1.85	59.55± 3.04	62.15± 0.35	60.50± 1.41	63.33± 7.39
MCH (pg)	21.40± 0.70	21.73± 0.86	21.73± 0.45	20.63± 0.55	19.90± 0.84	21.95± 0.49	20.40± 1.27	21.26± 2.54
MCHC (g/dl)	34.76± 0.55	34.46± 0.47	34.60± 0.79	34.40± 0.17	33.40± 0.14	35.40± 0.98	33.70± 1.27	33.56± 0.25
PLT ($\times 10^3/\mu l$)	100.33±88.12	267.33±219.41	298.00±338.22	380.33±136.84	331.50±20.50	121.50±156.20	514.00±189.50	383.33±59.55

[a] Values were expressed mean ± S.D.
WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corposcular hemoglobin; MCHC, mean corposcular hemoglobin concentration; PLT, platelet.

dosage group (7.5 mg/kg/day). There was a higher mean corpuscular volume (MCV) and platelet in males of middle dosage group (15 mg/kg/day), while there was a lower mean corpuscular hemoglobin concentration (MCHC) compared with control group. There was a higher MCV in males of high dosage group (30 mg/kg/day), while there was a lower MCHC compared with control group. There was a higher MCHC in females of low dosage group (7.5 mg/kg/day), while there was a lower basophil and white blood cell count compared with control group.

7. Coagulation

Coagulative data are summarized in Tables 5 and 6. There was a lower partial thromboplastin time (PTT) in males of low and high dosage group. There was a higher prothrombin time (PT) in females of low, middle and high dosage group. There was a lower PTT on females of low and middle dosage group.

8. Relative Organ Weights

Relative organ data are summarized in Table 7. There was no significant different relative organ weights between treatment and control group.

9. Histopathology

There was a hepatic coccidiosis in association with infiltration of heterophils in portal areas in rabbits of control group. There was a hepatic coccidiosis in association with infiltration of heterophils in portal areas in rabbits of low dosage group.

In rabbits of middle dosage group, glycogen accumulation and increased granularity was seen in the hepatocytes around central vein. There was a slightly increased megakaryocytes in the spleen. Peribronchiolar lymphoid hyperplasia was seen. Lipofuscins were infiltrated into uriniferous tubules. Vaculated degeneration was seen in renal tubular epithelium. Fibrinoid necrosis was seen in the coronary artery. Encephalitozoon was observed

Table 5. Prothrombin time and thromboplastin time of male and female rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 6 week (unit: second)

Sex		\Group \Dose (mg/kg/day) \No. of animal	Control 0 3	Low 7.5 3	Middle 15 3	High 30 3
Male	PT	Mean	15.67	18.33	18.00	18.67
		S.D.	0.58	1.53	1.73	3.21
	PTT	Mean	30.67	24.33*	28.00	23.33*
		S.D.	4.16	2.08	2.00	1.53
Female	PT	Mean	15.00	21.00	16.67	20.00
		S.D.	3.00	1.00	3.79	1.00
	PT	Mean	28.67	25.00	27.33	23.67
		S.D.	1.53	1.00	2.08	3.21

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

Table 6. Prothrombin time and thromboplastin time of male and female rabbits intravenously treated with *Ginkgo biloba* extract(EGb 761) after 12 week (unit: second)

Sex		\Group \Dose (mg/kg/day) \No. of animal	Control 0 3	Low 7.5 3	Middle 15 3	High 30 3
Male	PT	Mean	12.00	12.67	8.33	11.00
		S.D.	1.73	1.53	0.58	3.00
	PTT	Mean	23.67	22.67	23.67	29.67
		S.D.	7.23	2.52	2.31	3.21
Female	PT	Mean	12.33	14.33*	10.33*	10.00*
		S.D.	0.58	0.58	0.58	1.00
	PT	Mean	32.33	25.33*	24.00*	28.00
		S.D.	6.66	0.58	1.73	2.65

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

Table 7. Relative organ weights of rabbits intravenously treates with *Ginkgo biloba* extract(EGb 761) after 12 weeks (unit: %)

\Sex	Male				Female			
Parameter \Group	Control	Low	Middlie	High	Control	Low	Middlie	High
\Dose[a]	0	7.5	15	30	0	7.5	15	30
\No. of animal	3	3	3	3	3	3	3	3
Liver	2.42±0.08[b]	2.30±0.20	2.15±0.33	2.03±0.11	1.94±0.35	1.85±0.01	2.03±0.27	2.16±0.08
Spleen	0.09±0.06	0.04±0.00	0.04±0.01	0.05±0.01	0.06±0.00	0.06±0.00	0.06±0.00	0.07±0.01
Kidney left	0.28±0.00	0.26±0.03	0.24±0.00	0.24±0.02	0.25±0.01	0.26±0.03	0.26±0.02	0.27±0.09
Kidney right	0.28±0.00	0.27±0.02	0.24±0.00	0.25±0.00	0.25±0.02	0.25±0.01	0.26±0.02	0.27±0.09
Adrenal [c]gl. left	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Adrenal gl. right	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Heart	0.25±0.00	0.25±0.03	0.28±0.06	0.26±0.06	0.19±0.02	0.01±0.00	0.23±0.02	0.26±0.07
Lung	0.37±0.01	0.37±0.02	0.44±0.07	0.42±0.13	0.37±0.10	0.21±0.01	0.43±0.00	0.49±0.08
Thymus	0.19±0.01	0.12±0.08	0.11±0.06	0.12±0.02	0.19±0.15	0.45±0.04	0.15±0.09	0.17±0.07
Brain	0.26±0.01	0.29±0.01	0.29±0.05	0.30±0.04	0.27±0.00	0.08±0.00	0.27±0.00	0.26±0.06
Testis left	0.09±0.02	0.11±0.03	0.12±0.02	0.12±0.02	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Testis right	0.09±0.02	0.10±0.02	0.11±0.02	0.11±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00

[a]Dose unit was mg/kg/day; [b]Values were expressed as mean±S.D. [c]gl., gland; *significantly different from control group ($p < 0.05$).

in brain.

In rabbits of high dosage group, there was a hepatic coccidiosis in association with infiltration of heterophils and lymphocytes in portal areas. Increased granularity was seen in the hepatocytes. Occasionally, hepatocytes were focally necrotic. Heterophils were infiltrated into sinus in the spleen. There was a increased megakaryocyte, hemosiderosis in the spleen. Interstitial nephritis was seen and eosinophilic materials were seen in the Bowman's cavity. Increase granularity was seen in cardiac myofiber with calcification. Calcification was seem in ovary.

IV. DISCUSSION

Ginkgo biloba extract plays a important role as a strong vasoregulatory factor and a treatment of brain dysfunction and peripheral arterial disease such as difficulties of memory, dizziness, tinnitus, headache and emotional instability with anxiety (Semlitsch H. V., *et al.*, 1995).

The present reports describe findings in rabbit injecting intravenously *Ginkgo biloba* extract(EGb 761) with various dosages such as 7.5 mg/kg/day (low dosage group), 15 mg/kg/day (middle dosage group), or 30 mg/kg/day (high dosage group) for 3 month according to Established Regulation of Korean National Institute of Safety Research (1994. 4. 14). No laboratory animals in treatment and control group were dead. *Ginkgo biloba* extract(EGb 761) may alter the body weight at 25 days post-experiment in males of middle and high dosage group. *Ginkgo biloba* extract(EGb 761) may increase feed and water consumption significantly at 21 days post-experiment in males of high dosage group and at 77 days post-experiment in males of middle dosage group. *Ginkgo biloba* extract(EGb 761) may decrease feed and water consumption significantly at 28 days post-experiment of low and high dosage group but increase feed and water consumption significantly at 84 days post-experiment of high dosage group. These alterations of body weight, and feed and water consumption is not dosage-dependent and may be due to experimental error.

In hematological data, *Ginkgo biloba* extract(EGb 761) may increase RBC, hemoglobin, hematocrit significantly in males of low dosage group. *Ginkgo biloba* extract(EGb 761) may decrease MCV and platelet count significantly but increase MCHC in males of middle dosage group. *Ginkgo biloba* extract(EGb 761) may decrease MCV significant but increase MCHC in males of high dosage group. *Ginkgo biloba* extract(EGb 761) may decrease basophil and WBC counts significantly but increase

MCHC significantly in females of low dosage group. *Ginkgo bilba* extract(EGb 761) may increase neutrophils counts significantly in females of high dosage group. The alterations of hematological value are not dosage-dependent and may not be due to *Ginkgo biloba* extract (EGb 761).

In serum biochemical data, *Ginkgo biloba* extract (EGb 761) may decrease chloride and cholesterol in males of low dosage group, chloride total protein potassium and albumin in males of middle dosage group, total bilirubin, chloride, blood urea nitrogen, albumin in males of high dosage group, respectively. The alterations of serum biochemical value are not dosage-dependent and may not be due to *Ginkgo biloba* extract(EGb 761).

In coagulative data, *Ginkgo biloba* extract(EGb 761) may decrease PTT significantly in males of low and high dosage group but there is no correlation between dose of *Ginkgo biloba* extract (EGb 761) and alteration of PTT value. *Ginkgo biloba* extract(EGb 761) may increase PT significantly in females of low, middle and high dosage group. *Ginkgo biloba* extract(EGb 761) may decrease PTT significantly in females of low and middle dosage group. Since males and females in other treatment group is not alter significantly, the alterations of coagulative value may not be due to *Ginkgo biloba* extract(EGb 761).

Histopathologically, lymphocytes infiltration, increased granularity, focal necrosis, diffuse glycogen accumulation seen in the liver may be due to coccidiosis. In the spleen, increased megakaryocytes were seen in males and females of middle and high dosage group. Heterophil infiltration, mild hemosiderosis were also seen in the spleen. Peribronchiolar lymphoid hyperplasia, interstitial nephritis, lipofuscinosis of uriniferous tubule, tubular epithelial vacuolation were also seen.

Since these histopathological lesions were seen in most of treatment and control groups, these pathological alterations have not beed due to *Ginkgo biloba* extract(EGb 761). DeFeudis *et al.* also reported that *Ginkgo biloba* extract tested into patients clinically and found a mild adverse effects such as gastrointestinal compliant, headache, and skin allergin skin reaction (DeFeudis F. G. *et al.*, 1991). Our data and other study (DeFeudis F. G. *et al.*, 1991) suggested that *Ginkgo biloba* extract (EGb 761) could be untoxic.

In conclustion, the intravenous administration of *Ginkgo biloba* extract(EGb 761) in rabbits for 3 month showed no clinical signs and histopathological lesions. Therefore, *Ginkgo biloba* extract(EGb 761) could be nontoxic for subacute toxicity study.

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