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Chronic Toxicity of a Combined Preparation of Ticlopidine and *Ginkgo Biloba* Extract (EGb 761) Orally Administered to Rats for 13 Consecutive Weeks

Sang K. Kim, Sung Y. Kim, Mi Y. Yoon, Soo J. Oh, Hye S. Kim, Ja Y. Lee, Sung A. Kang†, Kyung H. Lee† and Young C. Kim*

College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul 151-742 and Research Center, YuYu Industrial Co., Suksoo-Dong, Anyang-Si, Kyonggi-Do, Korea
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ABSTRACT *: Toxicity of a combined preparation of ticlopidine and Ginkgo biloba extract (EGb 761) in a ratio of 10 : 4 was examined in male and female Sprague-Dawley rats. Rats were treated with the test substance intragastrically at a dose of 0 mg/kg, 17 mg/kg, 52 mg/kg or 156 mg/kg for 91 consecutive days. No death or abnormal clinical sign was observed throughout the administration period. There was no difference in body weight gain, food intake or water consumption among different dose groups. Test substance-related differences were not observed in urinalysis. In hematological results mean corpuscular hemoglobin (MCH) of low and high dose male group was increased. Prothrombin time of medium and high dose female group was decreased. A significant increase in serum total cholesterol was observed in both sexes of rats treated with a daily dose of 156 mg/kg, but all the other values obtained in serum chemistry appeared to be within normal ranges. A dose dependent increase in the relative liver and kidney weights was observed in both male and female rats. There were no gross pathological findings at terminal sacrifice. Microscopic histopathological examination did not show any lesion associated with administration of the test substance. The results suggest that under the conditions employed in this study no observable effect level (NOEL) of the test substance be greater than 17 mg/kg/day, but less than 52 mg/kg/day.*

Key Words *: Ticlopidine, Ginkgo biloba extract, Chronic toxicity, Rats*

I. INTRODUCTION

Ticlopidine is a thienopyridine that is currently used for prevention of thrombosis in cerebral vascular and coronary artery disease (Majerus *et al.*, 1996). Ticlopidine inhibits platelet aggregation and clot retraction. Side effects of this drug include bleeding, nausea, diarrhea and severe neutropenia in approximately 1% of patients (Molony, 1993). Induction of neutropenia by this substance has been suspected to be associated with free radicals generated during its biotransformation.

Ginkgo biloba extract (EGb 761) has been prescribed for its vasoregulating activity in various arteriopathies and symptomatology. Much effort has been made to elucidate the mechanism that underlies the positive therapeutic actions of *Ginkgo biloba* extract (Funfgeld, 1988). It has been suggested that the pharmacological actions are associated with the free radical scavenging activity of the flavonoid antioxidants from *Ginkgo biloba* extract (Ferradini *et al.*, 1992).

In an attempt to decrease the potential oxidative damage resulting from metabolite(s) of ticlopidine, a combined preparation of this drug and *Ginkgo biloba* extract mixed in a ratio of 10 : 4 has been being developed. In the present study we report the results of chronic toxicity testing of this preparation orally administered to rats for 91 consecutive days.

II. MATERIALS AND METHODS

1. Animals

Eighty Sprague-Dawley rats, 40 male and 40 female, obtained from Toxicology Research Center of Korea Food and Drug Administration (KFDA) were used in

*To whom correspondence should be addressed

this study. The rats were approximately 5 week old when obtained. They were acclimated in environmentally controlled rooms (temperature: 22±2°C, relative humidity: 55±5%, air circulating frequency: 13~15 times/hr, artificial light: 300 Lux from 7 am to 7 pm, noise: < 50 db) in Animal Center for Pharmaceutical Research in Seoul National University for 10 days before initiation of drug administration. Rats were housed in a polycarbonate cage (26.5 cm×42 cm×14 cm). The number of rats in a cage was four or less. Regular lab chow (Purina Co., Seoul) and tap water were provided *ad libitum*.

2. Test substance

Ticlopidine hydrochloride and *Ginkgo biloba* extract (EGb 761) were supplied from YuYu Industrial Co., Seoul. Ticlopidine and *Ginkgo biloba* extract were mixed in a ratio of 10 : 4 in 0.5% carboxymethylcellulose (CMC). This suspension was prepared every week and stored in a refrigerator (≤4°C) until use. The test substance was stirred and resuspended on a hot plate immediately prior to use. The volume of adminstration was adjusted to 5 ml/kg body weight.

3. Study design

Experiments were conducted according to "*Guidelines for Toxicity Testing of Pharmaceuticals*" (KFDA, 1996). For detailed experimental procedure "*Standard Operating Procedures in Toxicology*" (Inveresk Research International, 1979) was referred.

A total of 40 male and 40 female rats were used in this study. Rats of each sex were randomly assigned to 4 groups. The largest dose of the test substance administered to rats was 156 mg/kg. This preparation was used as the high dose followed by sequential dilution with 0.5% CMC in a ratio of 1 : 3, to 52 mg/kg, and 17 mg/kg, for the medium dose, and the low dose, respectively. Control animals were treated with an identical volume of 0.5% CMC only. The high dose (156 mg/kg) of the test substance employed was equivalent to 33-fold of an anticipated clinical dose given to a man weighing 60 kg. The test substance was administered to a rat intragastrically using a curved blunt-ended metal cannula attached to a disposable syringe between 9 : 30 and 10 : 30 am every morning. Rats were treated with the test substance 7 days a week, for 13 consecutive weeks.

Rats were observed for abnormal clinical signs at least once a day for the whole period of the test substance administration. Body weight of each rat, food and water intake by the cage, were measured twice in a week for the first one month and once in a week thereafter.

Urine was collected from each rat once in the final week of administration. The parameters determined in urinalysis included glucose, bilirubin, ketone, specific gravity, occult blood, protein, urobilinogen, pH, nitrite, and white blood cell. After the administration period of 91 days blood was collected from abdominal aorta in rats under light ether anesthesia and used for hematology and serum chemistry measurements. For hematological measurements white blood cell (WBC), red blood cell (RBC), hematocrit, hemoglobin, platelet, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), prothrombin time (PT), partial thromboplastin time (PTT), and differential leucocyte count were determined. The serum chemistry parameters included total protein, total bilirubin, glucose, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), total cholesterol, blood urea nitrogen (BUN), creatinine, and electrolytes such as Na^+, K^+, Cl^-. After sacrifice by exsanguination all major organs and tissues were examined grossly and the weight was measured. Major organs and tissues including brain, liver, heart, kidney (R/L), lung, adrenal gland (R/L), spleen, testis (R/L), ovary (R/L) and bone marrow from femoral bone were fixed in 10% neutral buffered-formalin solution and processed for microscopic examination.

4. Statistical analysis

All results expressed as means±SD were analyzed by oneway ANOVA followed by Dunnett's *t*-test. For nonparametric urinalysis data, Kruskal-Wallis' H test was used and differences between groups were determined by distribution-free multiple comparison test. Fisher's exact test was employed to analyze clinical

signs, necropsy and histopathology findings.

III. RESULTS

1. Mortality and clinical signs

There was no death among the rats used in this study during the observation period of 13 weeks. Throughout the experimental period, there was no abnormal behavior or appearance associated with administration of the test substance among the animals regardless of sex and dose levels employed.

2. Body weight

The body weight of rats was monitored for 13 weeks (Fig. 1 and Fig. 2). There was no difference in mean body weight increase among the different dose groups of each sex throughout the experimental period.

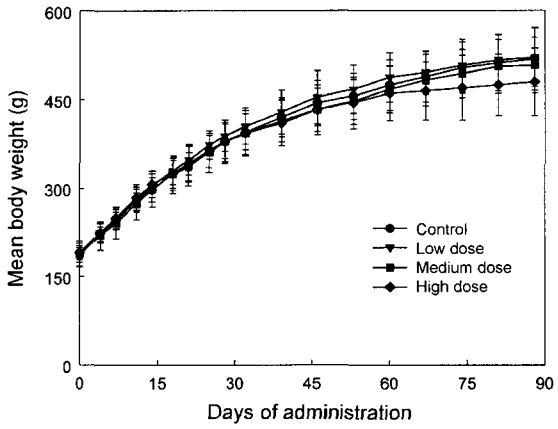

Fig. 1. Body weight increases in male rats treated with the test substance for 13 weeks.

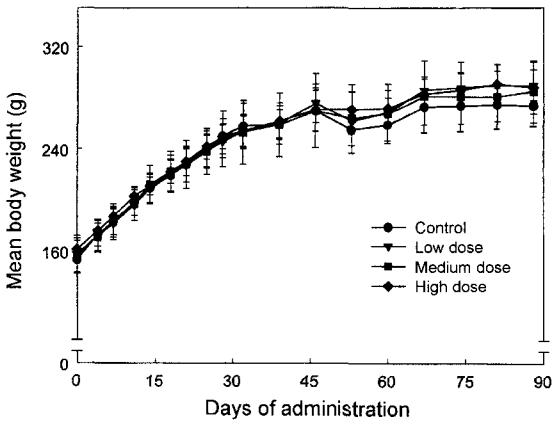

Fig. 2. Body weight increases in female rats treated with the test substance for 13 weeks.

3. Food and water consumption

A transient increase in food intake was observed in the low dose group of male rats immediately following the initiation of experiment (Fig. 3). However, the increase disappeared in the first week of drug administration. There was no difference in water intake in male rats throughout the administration period (Fig. 5). Incidental decreases in food and water consumption observed once in female rats did not appear to reflect the real changes (Figs. 4, 6).

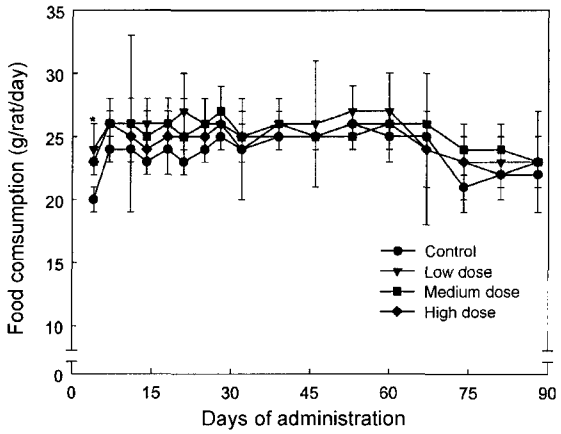

Fig. 3. Food consumption of male rats treated with the test substance for 13 weeks. *Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P < 0.05$).

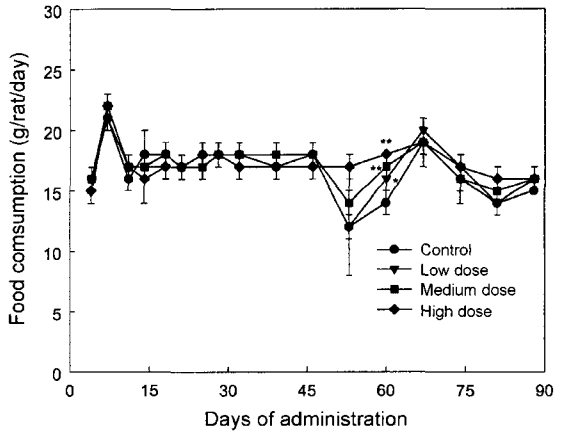

Fig. 4. Food consumption of female rats treated with the test substance for 13 weeks. *,**Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P < 0.05$, $P < 0.01$, respectively).

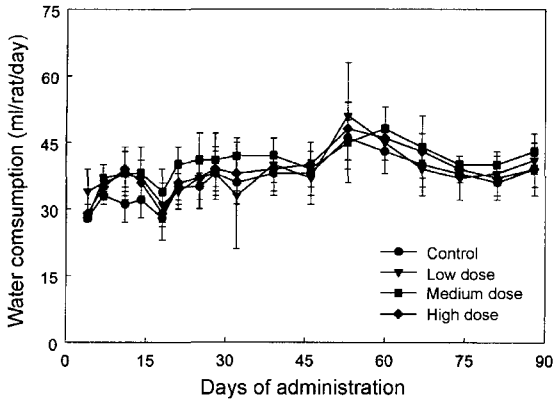

Fig. 5. Water consumption of male rats treated with the test substance for 13 weeks.

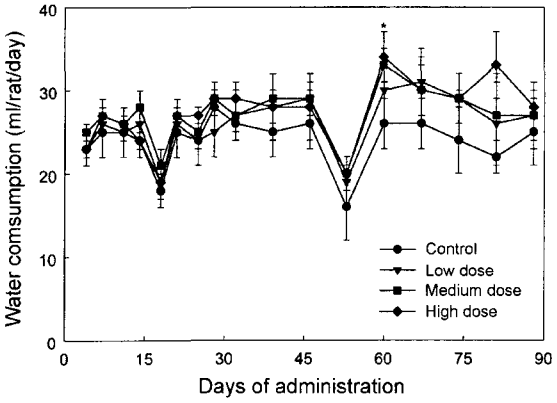

Fig. 6. Water consumption of female rats treated with the test substance for 13 weeks. *Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P < 0.05$).

4. Urinalysis

The parameters determined in urinalysis did not demonstrate statistical differences among the groups except for the decrease in ketone level and specific gravity in the low dose group of male rats (Table 1). However, the changes were not dose-dependent, and not considered to be related to the test substance.

5. Hematology

Hematological readings are shown in Table 2. There were small changes in red blood cells, white blood cells, hematocrit, prothrombin time, mean corpuscular hemoglobin and partial thromboplastin time. Red blood cells and hematocrit were lower in the male low dose group, and partial thromboplastin time was longer in the male medium dose group. Mean corpuscular hemoglobin was increased in the male low dose and high dose group. In female rats white blood cells was lower in the medium dose group and prothrombin time was shorter in the medium and high dose group. However, all the differences observed are small and did not show any dose-dependency. Furthermore, all the values appeared to be in normal range.

There were no significant differences in differential leucocyte count except for the decrease in lymphocyte of the high dose female rats (Table 3).

6. Serum chemistry

Serum biochemical values are summarized in Table 4. In the male rats small differences among the different dose groups included an increase in alkaline phosphatase (ALP) activity in the high dose group and an increase in blood urea nitrogen (BUN) in the medium dose groups. Elevation of glutamic pyruvic transaminase activity in the low and medium dose group and an increase in total protein content in the high dose group were observed in female rats. Also in female rats sodium and potassium levels were changed. However, all the small differences in BUN, GPT, sodium and potassium appeared to be inconsistent in terms of the dose administered or sex except for the increase in total cholesterol in the high dose group of both sexes.

7. Autopsy and organ weight

After the administration period of 91 days all animals were sacrificed, and major organs and tissues were examined grossly. No lesions were observed in the animals regardless of the dose of the test substance administered.

Absolute and relative organ weights are shown in Table 5 and 6. In both male and female rats the most significant difference was the increase in relative liver weight. The kidney weights were also increased slightly in female rats. There were no other changes in the organ weight associated with administration of the test substance among the different dose groups.

8. Histopathology

Major organs and tissues in the control and the

Table 1. Urinalysis of male and female rats treated with the test substance orally for 13 weeks

Parameter	\Sex	Male				Female			
	\Group	Control	Low	Medium	High	Control	Low	Medium	High
	\Dose (mg/kg)	0	17	52	156	0	17	52	156
	\No. of animals	10	10	10	10	10	10	10	10
Glucose (g/dl)	-	10	10	10	10	10	10	10	10
	+/- 0.1	0	0	0	0	0	0	0	0
	+ 0.25	0	0	0	0	0	0	0	0
	++ 0.5	0	0	0	0	0	0	0	0
	+++ 1.0	0	0	0	0	0	0	0	0
	++++ 2	0	0	0	0	0	0	0	0
Bilirubin	-	10	10	10	10	9	10	8	9
	1+	0	0	0	0	1	0	1	1
	2+	0	0	0	0	0	0	1	0
	3+	0	0	0	0	0	0	0	0
Ketone (mg/dl)	-	1	8	1	2	9	9	10	9
	+/- 5	2	1	1	7	1	1	0	1
	+ 15	7	1	8	1	0	0	0	0
	++ 40	0	0	0	0	0	0	0	0
	+++ 80	0	0	0	0	0	0	0	0
	++++ 160	0	0	0	0	0	0	0	0
Specific gravity	≤1.000	0	0	0	0	0	1	0	0
	1.005	0	6	3	0	2	2	3	0
	1.010	4	4	4	0	4	0	3	3
	1.015	0	0	1	2	3	2	2	1
	1.020	4	0	1	4	0	1	0	1
	1.025	0	0	0	0	0	1	0	1
	≥1.030	2	0	1	4	1	3	2	3
Occult blood	-	4	5	7	4	4	0	2	4
	+/-	6	5	3	2	3	5	4	3
	1+	0	0	0	2	0	3	2	1
	2+	0	0	0	1	2	0	0	0
	3+	0	0	0	1	1	2	2	2
Protein (mg/dl)	-	0	0	0	0	5	5	8	0
	+/-	1	4	1	2	3	4	2	9
	+ 30	7	5	3	4	2	1	0	1
	++100	2	0	5	4	0	0	0	0
	+++ 300	0	1	1	0	0	0	0	0
	++++ 1000	0	0	0	0	0	0	0	0
Urobilinogen (EU/dl)	0.1	10	10	10	10	10	10	10	10
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
pH	5.0	0	0	0	0	0	0	0	1
	6.0	3	0	0	3	1	2	4	5
	6.5	0	0	1	4	1	1	0	1
	7.0	1	0	0	2	1	0	2	1
	7.5	4	2	3	1	3	3	4	1
	8.0	1	6	2	0	4	3	0	1
	8.5	1	2	4	0	0	0	0	0
Nitrite	-	10	10	9	10	10	9	9	10
	+	0	0	1	0	0	1	1	0
White blood cell	-	10	10	9	7	10	9	8	8
	+/-	0	0	1	1	0	0	0	1
	+	0	0	0	2	0	1	2	1
	++	0	0	0	0	0	0	0	0
	+++	0	0	0	0	0	0	0	0

Ketone level and specific gravity in the low dose group of male rats were statistically lower than control group (Kruskal-Wallis' H test followed by distribution free multiple comparision test, $P < 0.05$).

Table 2. Hematological values of male and female rats treated with the test substance orally for 13 weeks

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	17	52	156	0	17	52	156
\No. of aminals	10	10	10	10	10	10	10	10
WBC (thousand/mm^3)	8.0±2.4	8.8±1.9	6.1±1.3	8.0±1.2	7.8±2.1	6.1±2.3	5.3±1.1**	7.1±1.5
RBC (millions/mm^3)	7.64±0.28	7.00±0.42**	7.29±0.60	7.29±0.27	6.95±0.46	6.82±0.44	6.87±0.28	6.70±0.73
Hb (g/dl)	14.4±0.4	13.9±0.4	14.4±0.9	14.4±0.7	14.2±0.5	13.7±0.7	13.7±0.5	13.5±0.9
Hct (%)	46±1	42±2**	44±2	44±3	42±2	41±3	41±1	40±4
Platelet (thousand/mm^3)	791±75	766±154	833±130	784±122	848±95	797±116	849±132	816±143
MCV (fl)	60±2	61±1	60±2	61±3	61±2	61±1	60±2	60±2
MCH (pg)	18±1	19±1*	19±1	19±1*	20±1	20±1	19±1	20±1
MCHC (%)	31±1	32±1	32±1	32±1	33±1	33±2	33±1	33±2
PT (sec)	14.9±0.4	15.3±0.5	15.6±2.0	14.9±0.5	15.6±0.6	15.4±0.4	14.9±0.6**	14.5±0.5**
PTT (sec)	20±2	26±4	29±11**	23±2	28±5	30±5	25±5	23±3

*,**Significantly different from the control (oneway ANOVA followed by Dunnett's t-test, $P < 0.05$, $P < 0.01$, respectively).

Table 3. Differential leucocyte count of male and female rats treated with the test substance orally for 13 weeks

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	17	52	156	0	17	52	156
\No. of aminals	10	10	10	10	10	10	10	10
Neutrophil Seg (%)	12±6	14±8	21±10	17±5	17±5	18±9	15±6	21±5
Neutrophil Stab (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Lymphocyte (%)	87±6	85±8	79±10	83±5	83±5	82±9	84±6	78±5*
Monocyte (%)	0±0	0±1	0±0	0±0	0±0	0±0	0±0	0±0
Eosinophil (%)	1±1	0±0	0±0	0±0	0±0	0±0	1±1	1±1
Basophil (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

**Significantly different from the control (oneway ANOVA followed by Dunnett's t-test, $P < 0.01$).

Table 4. Serum biochemical values of male and female rats treated with the test substance orally for 13 weeks

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	17	52	156	0	17	52	156
\No. of aminals	10	10	10	10	10	10	10	10
T-Protein (g/dl)	6.3±0.2	6.3±0.2	6.3±0.4	6.7±0.4	6.9±0.3	6.8±0.4	7.1±0.4	7.4±0.4*
T-Bilirubin (mg/dl)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Glucose (mg/dl)	137±7	149±15	147±14	137±10	140±11	146±15	143±7	130±17
GOT (IU/l)	183±36	208±61	157±44	146±19	149±29	176±33	173±27	170±27
GPT (IU/l)	62±19	85±43	52±12	44±6	38±6	55±11**	53±18*	39±8
ALP (IU/l)	268±69	294±45	257±43	213±32*	122±33	133±29	142±36	93±22
T-Cholesterol (mg/dl)	64±11	74±22	74±17	86±15*	69±13	72±8	75±17	121±14**
BUN (mg/dl)	17.6±2.0	19.3±1.6	20.4±2.3**	17.0±1.4	17.6±5.0	19.0±2.9	18.3±2.7	22.1±6.3
Creatinine (mg/dl)	0.4±0.0	0.4±0.0	0.5±0.1	0.4±0.0	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Sodium (mEq/l)	137±1	139±1	137±1	137±2	136±2	134±2*	134±1*	134±1*
Potassium (mEq/l)	5.9±0.2	6.2±0.4	6.0±0.3	5.6±0.3	5.5±0.2	6.0±0.4*	6.1±0.2**	5.1±0.5*
Chloride (mEq/l)	103±1	104±1	103±1	102±2	103±1	104±2	104±2	102±1

*,**Significantly different from the control (oneway ANOVA followed by Dunnett's t-test, $P < 0.05$, $P < 0.01$, respectively).

high dose groups were processed for microscopic histopathological examination (Table 7). Small numbers of incidence of microgranuloma and cell infiltration of Glisson's sheath were observed in livers of both male and female rats. Vacuolation of renal tubules in cortex was observed both in the control and in the high dose groups. The incidence of renal cortex vacuolation appeared to be increased in the high dose group of both

Table 5. Absolute organ weights of male and female rats treated with the test substance orally for 13 weeks

Parameters	\Sex	Male				Female			
	\Group	Control	Low	Medium	High	Control	Low	Medium	High
	\Dose (mg/kg)	0	17	52	156	0	17	52	156
	\No. of aminals	10	10	10	10	10	10	10	10
Body weight (g)		513±54	522±50	503±45	475±59	262±10	283±11*	282±22*	275±20
Liver (g)		14.4±2.5	15.2±2.3	15.3±2.5	17.0±3.1	7.5±0.3	8.4±0.8*	9.2±0.8**	9.9±1.0**
Spleen (g)		0.80±0.14	0.86±0.07	0.76±0.09	0.77±0.08	0.52±0.05	0.52±0.09	0.55±0.09	0.51±0.08
Heart (g)		1.41±0.16	1.59±0.19*	1.43±0.16	1.39±0.10	0.86±0.06	0.90±0.06	0.95±0.06*	0.91±0.07
Lung (g)		2.52±0.36	2.44±0.29	2.11±0.18*	2.35±0.36	1.63±0.16	1.62±0.09	1.66±0.23	1.74±0.29
Brain (g)		2.05±0.11	2.06±0.10	2.10±0.05	2.05±0.11	1.89±0.05	1.94±0.09	1.95±0.06	1.93±0.07
Testis (g)/	Right	1.69±0.22	1.80±0.48	1.62±0.12	1.68±0.10	73.8±9.8	79.0±13.3	80.6±9.8	77.5±8.3
Ovary (mg)	Left	1.76±0.13	1.68±0.15	1.62±0.12	1.68±0.10	65.8±7.3	80.1±17.8*	79.2±9.9*	71.1±5.1
Kidney (g)	Right	1.63±0.16	1.68±0.19	1.58±0.15	1.68±0.24	0.86±0.05	0.96±0.09*	1.01±0.08**	1.03±0.06**
	Left	1.62±0.17	1.67±0.20	1.61±0.15	1.64±0.22	0.84±0.06	0.94±0.07**	0.99±0.07**	1.03±0.07**
Adernal grand	Right	36.9±6.4	35.6±6.5	40.3±6.9	29.1±4.8*	29.9±5.2	35.7±7.9	36.0±3.5*	30.1±4.0
(mg)	Left	35.9±4.1	36.9±7.7	39.2±6.9	31.8±6.8	31.5±3.8	34.0±3.7	36.3±1.8**	33.5±3.5

*,**Significantly different from the control (oneway ANOVA followed by Dunnett's *t*-test, $P < 0.05$, $P < 0.01$, respectively).

sexes. Also there were several cases of thickening of alveolar wall, accumulation of hemosiderin in red pulp of spleen. But incidence of these lesions was identical both in the control and in the high dose groups, thus, did not appear to be related to administration of the test substance. No other lesions were found in either male or female rats.

IV. DISCUSSION AND CONCLUSION

In this study we examined the chronic toxicity of a combined preparation of ticlopidine and *Ginkgo biloba* extract (EGb 761) mixed in a ratio of 10 : 4 in male and female SD rats. The test substance was administered intragastrically to rats at a daily dose of 156 mg/kg (high dose), 52 mg/kg (medium dose), or 17 mg/kg (low dose) for 91 consecutive days. Control rats received an identical volume of vehicle only.

Throughout the administration period rats were observed for any abnormal behavior or appearance. There was no abnormal behavior, appearance or death among animal till the terminal sacrifice. Transient changes in food and water intake were observed, but appeared to be incidental.

There were small changes in the parameters determined in urinalysis or in hematological examinations. But the changes were not either dose-dependent or consistent. Furthermore, all the values appeared to be in the normal range. In serum chemistry total cholesterol concentrations were increased in the high dose group of both sexes. The other small changes in BUN, GPT, sodium or potassium did not show any dose-dependency, thus, appeared not to be related to the test substance administration.

There were no significant lesions observed grossly in major organs and tissues at the end of administration period. But the relative liver weight was increased as the dose of test substance was multiplied in both sexes. In female groups treated with the test substance an increase in kidney weight was also noted. Microscopic evaluation of major organs and tissues did not demonstrate any significant abnormal finding associated with the test substance administration.

In conclusion it is suggested that the only significant change associated with administration of the test substance in rats is the increase in relative liver weight and serum total cholesterol concentration. This result is in good agreement with the conclusion obtained in the previous study (Kim *et al.*, 1998). These results indicate that the target organ of the test substance would be the liver. However, differences in other hepatic parameters among different dose groups were not significant or consistent. The increases in liver and kidney weights, serum cholesterol level were also observed by other investigators (Castaigne, 1974; Daiichi Seiyaku, 1975) who examined the toxicity of ticlopidine in rats. The other significant effect of the test substance observed in this study, an increase in serum cholesterol level, is a well established side effect of ticlopidine administered to patients clinically (Molony, 1993). Therefore, it is suggested in this study that the

Table 6. Relative organ weights of male and female rats treated with the test substance orally for 13 weeks

Parameters	\Sex	Male				Female			
	\Group	Control	Low	Medium	High	Control	Low	Medium	High
	\Dose (mg/kg)	0	17	52	156	0	17	52	156
	\No. of aminals	10	10	10	10	10	10	10	10
Liver (%)		2.80±0.32	2.91±0.21	3.02±0.34	3.57±0.29**	2.87±0.14	2.97±0.30	3.27±0.14**	3.60±0.28**
Spleen (%)		0.16±0.01	0.17±0.02	0.15±0.01	0.16±0.03	0.20±0.02	0.19±0.03	0.20±0.03	0.18±0.03
Heart (%)		0.28±0.02	0.31±0.01*	0.29±0.03	0.30±0.02	0.33±0.02	0.32±0.02	0.34±0.02	0.33±0.02
Lung (%)		0.49±0.05	0.47±0.07	0.42±0.03*	0.50±0.09	0.62±0.05	0.57±0.05	0.59±0.08	0.63±0.10
Brain (%)		0.40±0.04	0.40±0.04	0.42±0.04	0.44±0.05	0.72±0.04	0.69±0.05	0.70±0.06	0.71±0.06
Testis/	Right	0.33±0.05	0.34±0.06	0.33±0.04	0.36±0.04	0.028±0.004	0.028±0.004	0.029±0.004	0.028±0.003
Ovary (%)	Left	0.35±0.04	0.32±0.03	0.32±0.04	0.36±0.05	0.025±0.003	0.028±0.006	0.028±0.004	0.026±0.003
Kidney (%)	Right	0.32±0.02	0.32±0.02	0.32±0.03	0.35±0.03*	0.33±0.02	0.34±0.03	0.36±0.02	0.38±0.03**
	Left	0.32±0.03	0.32±0.02	0.32±0.03	0.35±0.03	0.32±0.03	0.33±0.03	0.35±0.02*	0.38±0.02**
Adernal	Right	0.007±0.001	0.007±0.001	0.008±0.001	0.006±0.001	0.012±0.001	0.013±0.003	0.013±0.001	0.011±0.002
Grand (%)	Left	0.007±0.001	0.007±0.001	0.008±0.002	0.007±0.002	0.012±0.001	0.012±0.001	0.013±0.001	0.012±0.002

*,**Significantly different from the control (oneway ANOVA followed by Dunnett's t-test, $P < 0.05$, $P < 0.01$, respectively).

Table 7. Histopathological findings of male and female rats treated with the test substance orally for 13 weeks

	\Sex	Male				Female			
	\Group	Control		High		Control		High	
	\Dose (mg/kg)	0		156		0		156	
	\No. of aminals	10		10		10		10	
Organ		No. of rats examined	No. of rats with lesions	No. of rats examined	No. of rats with lesions	No. of rats examined	No. of rats with lesions	No. of rats examined	No. of rats with lesions
Liver		10	5	10	4	10	5	10	3
Microgranuloma			2*		2*		4*		3*
Cell infiltration of Glisson's sheath			4*		3*		2*		1*
							0		0
Spleen		10	10	10	10	10	10	10	10
Accumulation of hemosiderin in red pulp			10		10		10		10
Heart		10	0	10	0	10	0	10	0
Lung		10	3	10	4	10	2	10	3
Thickening of alveolar wall			3		4		2		3
Brain		10	0	10	0	10	0	10	0
Bone Marrow		10	0	10	0	10	0	10	0
Testis/	Right	10	0	10	0	10	0	10	0
Ovary	Left	10	0	10	0	10	0	10	0
Kidney	Right : Vacuolation of renal tubules in cortex	10	3	10	6	10	2	10	5
			3		6		2		5
	Left : Vacuolation of renal tubules in cortex	10	3	10	6	10	2	10	5
			3		6		2		5
Adernal	Right	10	0	10	0	10	0	10	0
Grand	Left	10	0	10	0	10	0	10	0

*One animals of each group had lesions of both microgranuloma and cell infiltration of Gisson's sheath.

chro- nic toxicity of a combined preparation of ticlopidine and *Ginkgo biloba* extract administered to rats for consecutive 91 days is not greater than that resulting from sole ticlopidine administration. It is concluded that under the conditions employed in this study no observable effect level (NOEL) of the test substance in rats is considered to be greater than 17 mg/kg, but less than 52 mg/kg daily.

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