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Subchronic Toxicity of a Combined Preparation of Ticlopidine and *Ginkgo Biloba* Extract Orally Administered to Rats for 30 Days

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***ABSTRACT** : The subchronic toxicity of a combined preparation of ticlopidine and ginkgo biloba extract (EGb 761) mixed in a ratio of 10 : 4 was examined in male and female Sprague-Dawley rats. Rats were treated with the test substance at a dose of 52 mg/kg, 156 mg/kg, or 467 mg/kg intragastrically for 30 consecutive days. Control rats were treated with vehicle only. Each group consisted of 10 rats. No death or abnormal clinical signs were observed throughout the administration period. A transient decrease in body weight gain and food intake was observed in the rats treated with the high dose (467 mg/kg), which was recovered to normal in a week. There were no drug-related differences in urinalysis and hematological results. A significant increase in serum total cholesterol and total protein was observed in both sexes of the rats treated with a dose of 467 mg/kg daily, but all the other values obtained in serum chemistry appeared to be within normal range. A dose dependent increase in liver weight was observed in both male and female rats. Relative kidney weight was also increased in the high dose groups. There was no gross pathological finding at terminal sacrifice. Microscopic histopathological examination did not show any lesion in terms of correlation 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 52 mg/kg/day.*

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

I. INTRODUCTION

Ticlopidine is an antiplatelet drug currently used for prevention of thrombosis in cerebral vascular and coronary artery disease. It has been proposed that ticlopidine is metabolically converted into an active component since this drug is relatively ineffective in inhibiting platelet aggregation when added to platelets *in vitro* directly (Majerus *et al.*, 1996). The most serious side effect of this drug is neutropenia observed in approximately 1% of patients (Molony, 1993). It is suspected that this effect be also associated with free radicals generated from ticlopidine during its biotransformation.

Extracts of *ginkgo biloba* leaves were first introduced into medical practice in 1965 as a remedy for cerebral and peripheral blood flow disturbances. Since then it has been reported by many authors that this agent exerts anti-ischemic, anti-edema, anti-hypoxic, radical-scavenging and metabolic actions, as well as improving rheology in various *in vitro* and *in vivo* models (DeFeudis, 1991). With regard to its mechanism of pharmacological action, the flavonoid anti-oxidants from *ginkgo biloba* extract have been suspected to play an important role in degenerative disease processes implicated with active oxygen species (Ferradini *et al.*, 1992).

In an attempt to increase the clinical efficacy and to decrease the potential oxidative damage resulting from reactive metabolites of ticlopidine a combined preparation of this drug and *ginkgo biloba* extract mixed in a ratio of 10 : 4 has been designed and currently under development. In the present study the subchronic toxicity of a combined pre-

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paration of ticlopidine and *ginkgo biloba* extract administered orally to rats for 30 consecutive days was assessed.

II. MATERIALS AND METHODS

1. Animals

One hundred Sprague-Dawley rats, 50 male and 50 female, were obtained from Toxicology Research Center of Korea Food and Drug Administration (KFDA). The rats were 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 8 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 adjusted to be 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 by 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 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), and "Standard Operating Procedures in Toxicology" (Inveresk Research International, 1979) was referred for detailed experimental procedures.

A total of 40 male and 40 female rats were used in this study. Rats of the same sex were randomly assigned to 4 groups. Thus, each group consisted of 10 rats. The largest dose of the test substance administered to rats was 476 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 156 mg/kg, and 52 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 (476 mg/kg) of the test substance employed was equivalent to 100-fold of an anticipated clinical dose given to a man weighing 60 kg. A single dose of the test substance was administered to a rat intragastrically using a curved blunt-ended metal cannula attached to a disposable syringe between 9 : 30 am and 10 : 30 am. The adminstration was repeated everyday for 30 consecutive days.

Clinical signs were observed at least once daily for the whole period of the test substance administration. Body weight of each rat was measured twice in a week starting from one week prior to initiation of experimentation till terminal sacrifice. Food and water intake by the cage was determined twice in a week.

Urine was collected from each rat once in the final week of adminstration. The parameters determined in urinalysis included glucose, bilirubin, ketone, specific gravity, occult blood, protein, urobilinogen, pH, nitrite, and white blood cell. After the adminstration period of 30 day blood was sampled 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. Level of significance was established either at $P<0.05$ or $P<0.01$.

III. RESULTS

1. Mortality and Clinical Signs

Rats were observed daily for any abnormal clinical signs. Throughout the experimental period of 30 days, there was no abnormal behavior or appearance associated with administration of the test substance among the animals regardless of the dose levels employed. Also no death was found in the rats till terminal sacrifice conducted after the drug adminstration for 30 consecutive days.

2. Body Weight

A transient decrease in body weight gain was observed in the high dose group of male rats immediately following the initiation of experiment (Fig. 1). However, the decrease disappeared in the first week of drug administration. There were no differences in body weight increase in the other dose groups of male rats or in female rats treated with the test substance at various dose levels throughout the experimental period (Fig. 2).

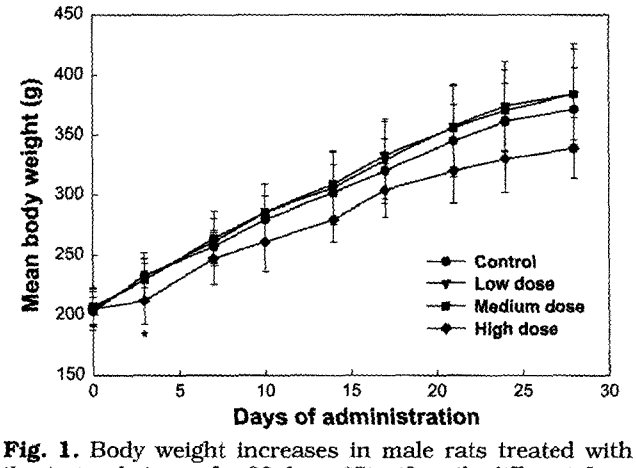

Fig. 1. Body weight increases in male rats treated with the test substance for 30 days. *Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P<0.05$).

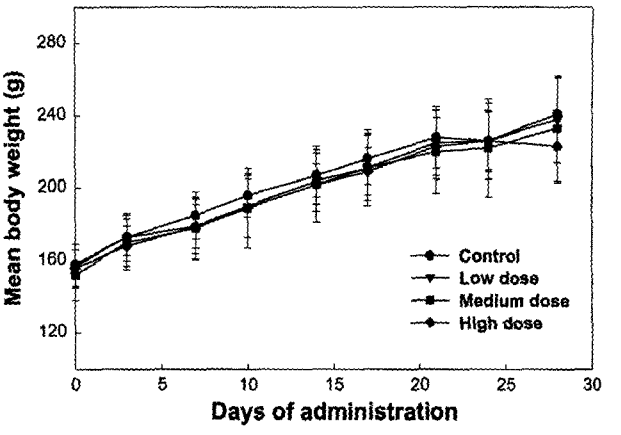

Fig. 2. Body weight increases in female rats treated with the test substance for 30 days.

3. Food and Water Consumption

In both male and female rats treated with the high dose food intake was decreased right after the initiation of experiment, but returned to normal in a week (Figs. 3, 4). There were no differences in this parameter for the rest of administration period. The

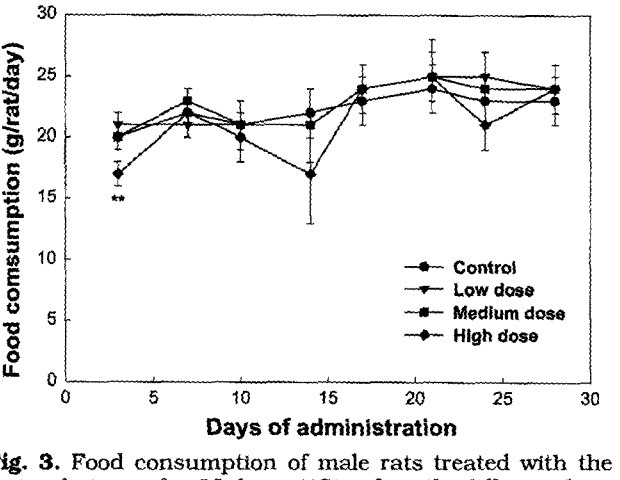

Fig. 3. Food consumption of male rats treated with the test substance for 30 days. **Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P<0.01$).

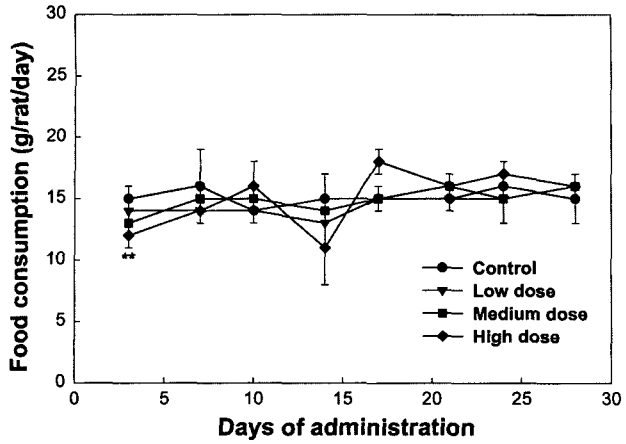

Fig. 4. Food consumption of female rats treated with the test substance for 30 days. **Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P<0.01$).

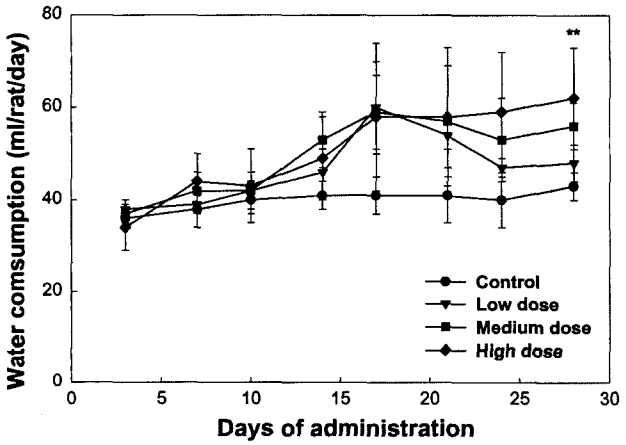

Fig. 5. Water consumption of male rats treated with the test substance for 30 day. **Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P<0.01$).

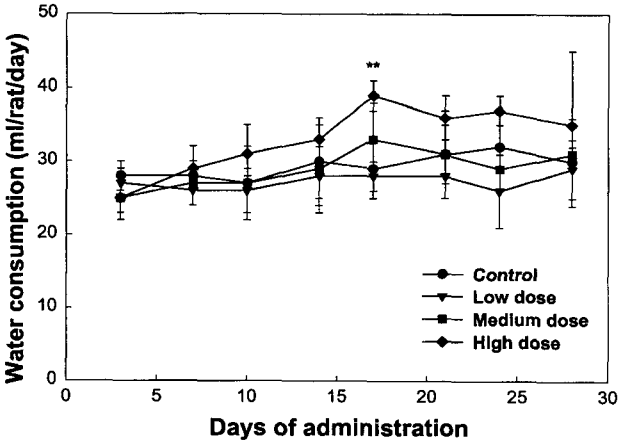

Fig. 6. Water consumption of female rats treated with the test substance for 30 days. **Significantly different from the control group (oneway ANOVA followed by Dunnett's *t*-test, $P<0.01$).

incidental increases in water consumption observed once in male and female rats during the period did not appear to reflect the real changes in water intake (Figs. 5, 6). It was concluded that there were no differences in water intake in both sexes of rats irrespective of the dose of the test substance used for each group.

4. Urinalysis

The parameters determined in urinalysis did not demonstrate significant differences among the groups except for the decreases in protein in the male low dose group and in specific gravity/occult blood in the female medium dose group (Table 1). However, the changes were not dose-dependent, and did not appear to be related to the test substance.

5. Hematology

Results from hematological measurements are summarized in Table 2. There were no significant changes except for the small differences in hemoglobin concentration and in blood clotting time. Hemoglobin concentration was lower in the male low dose group, and prothrombin time was shorter in the male high dose group whereas partial thromboplastin time was prolonged in the medium dose group. In female rats prothrombin time was shorter in the high dose group. However, all the differences observed are small, did not show any dose-dependency, and furthermore, the values appeared to be in normal range.

There were no significant differences in differential leucocyte count in both sexes of rats irrespective of the dose administered (Table 3).

6. Serum Chemistry

Among the parameters measured in the serum biochemical analysis, total plasma protein and cholesterol level were increased in both sexes of rats treated with the high dose of the test substance (Table 4). Other differences among the different dose groups included an increase in glucose in the male high dose group and an increase in blood urea nitrogen (BUN) in the female low and medium

Table 1. Urinalysis of male and female rats treated with the substance orally for 30 days

Parameter	\Sex	Male				Female			
	\Group	Control	Low	Medium	High	Control	Low	Medium	High
	\Dose (mg/kg)	0	52	156	467	0	52	156	467
	\No. of aminals	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	-	4	7	5	7	10	9	10	10
	1+	6	3	3	3	0	1	0	0
	2+	0	0	0	0	0	0	0	0
	3+	0	0	2	0	0	0	0	0
Ketone (mg/dl)	-	1	5	5	1	9	10	10	10
	+/-5	3	4	4	6	1	0	0	0
	+15	6	1	1	3	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	0	0	0
	1.005	0	2	1	0	0	3	2	0
	1.010	3	4	5	3	2	4	6	3
	1.015	2	2	2	3	1	1	1	1
	1.020	2	0	0	1	3	0	1	2
	1.025	1	1	1	0	0	0	0	1
	≥1.030	2	1	1	3	4	2	0	3
Occult blood	-	4	2	5	7	2	2	8	6
	+/-	5	2	3	2	2	5	2	3
	1+	0	2	0	1	1	1	0	0
	2+	1	1	2	0	2	0	0	1
	3+	0	3	0	0	3	2	0	0
Protein (mg/dl)	-	0	2	1	0	0	4	1	0
	+/-	1	5	5	2	1	2	3	4
	+30	4	2	3	5	8	4	6	6
	+100	4	1	0	3	1	0	0	0
	+300	1	0	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	1	0	1
	6.0	0	0	1	2	5	0	0	5
	6.5	1	1	1	2	1	1	0	1
	7.0	2	0	1	1	0	2	0	0
	7.5	4	5	6	3	2	3	5	3
	8.0	3	1	1	2	2	3	4	0
	8.5	0	3	0	0	0	0	1	0
Nitrite	-	9	9	10	10	10	10	10	10
	+	1	1	0	0	0	0	0	0
White blood cell	-	0	2	0	0	6	8	4	7
	+/-	4	3	0	2	1	1	3	1
	+	5	5	9	7	3	1	3	2
	++	1	0	1	1	0	0	0	0
	+++	0	0	0	0	0	0	0	0

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

dose groups. The small differences in BUN or glucose are inconsistent, lacking dose-dependency, thus, considered to be incidental.

7. Autopsy and Organ Weight

At terminal sacrifice all the animals were autopsied,

Table 2. Hematological values of male and female rats treated with the test substance orally for 30 days

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	52	156	467	0	52	156	467
\No. of aminals	10	10	10	10	10	10	10	10
WBC (thousand/mm^3)	4.1±1.4	3.2±0.7	4.3±1.4	4.2±1.3	3.1±1.0	2.8±1.2	2.9±0.4	3.4±1.1
RBC (millions/mm^3)	6.51±0.32	6.08±0.38	6.22±0.40	6.28±0.23	6.56±0.25	6.39±0.27	6.38±0.36	6.32±0.41
Hb (g/dl)	14.0±0.6	13.3±0.4*	13.9±0.6	13.7±0.5	13.6±0.4	13.5±0.5	13.3±0.6	13.0±0.5
Hct (%)	43±10	43±2	43±2	44±2	44±3	43±3	42±3	42±3
Platelet (thousand/mm^3)	620±80	581±55	660±43	665±54	630±90	625±64	597±79	619±90
MCV (fL)	72±3	72±4	71±4	70±3	67±2	68±2	66±1	66±1
MCH (pg)	21±2	22±1	22±1	21±1	20±1	21±1	21±1	20±1
MCHC (%)	30±2	30±1	31±1	31±1	30±1	31±1	31±1	31±1
PT (sec)	13.4±0.2	13.7±0.2	13.7±0.5	12.9±0.2**	14.4±0.5	14.2±0.6	13.9±0.3	13.7±0.5**
PTT (sec)	21±1	21±3	26±6**	23±3	17.8±2.0	19.2±2.5	17.7±0.7	18.7±1.4

*,**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 30 days

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	52	156	467	0	52	156	467
\No. of aminals	10	10	10	10	10	10	10	10
Neutrophil Seg (%)	25±5	28±7	29±6	27±7	25±5	24±4	21±3	23±4
Neutrophil Stab (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Lymphocyte (%)	75±5	72±7	70±6	73±6	76±4	75±4	78±4	76±3
Monocyte (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Eosinophil (%)	0±1	0±1	0±1	0±0	0±1	1±1	1±1	1±1
Basophil (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

Table 4. Serum biochemical values of male and female rats treated with the test substance orally for 30 days

Parameters \Sex	Male				Female			
\Group	Control	Low	Medium	High	Control	Low	Medium	High
\Dose (mg/kg)	0	52	156	467	0	52	156	467
\No. of aminals	10	10	10	10	10	10	10	10
T-Protein (g/dl)	5.9±0.2	5.7±0.3	5.9±0.2	6.5±0.3**	6.2±0.4	6.4±0.3	6.4±0.4	7.0±0.3**
T-Bilirubin (mg/dl)	0.1±0.0	0.0±0.1	0.0±0.1	0.1±0.1	0.1±0.1	0.0±0.1	0.0±0.0**	0.0±0.1
Glucose (mg/dl)	147±13	150±15	150±11	132±9*				
GOT (IU/L)	192±34	191±41	188±24	197±20	199±43	188±38	171±32	171±33
GPT (IU/L)	43±6	56±19	51±12	55±9	57±32	49±10	44±6	46±10
ALP (IU/L)	559±71	541±81	571±113	530±79	281±99	304±65	301±56	252±61
T-cholesterol (mg/dl)	67±10	74±12	79±11	107±24**	70±10	80±10	91±5**	122±14**
BUN (mg/dl)	15.6±2.7	18.2±2.8	18.0±3.0	16.9±2.0	14.8±2.1	18.9±2.1**	17.5±2.1*	16.8±2.9
Creatinine (mg/dl)	0.5±0.1	0.6±0.1	0.5±0.1	0.5±0.1	0.5±0.0	0.5±0.1	0.5±0.0	0.5±0.1
Sodium (mEq/L)	139±1	138±2	139±1	138±1	138±2	139±2	139±1	139±2
Potassium (mEq/L)	5.2±0.5	5.4±0.6	5.5±0.2	5.2±0.3	4.7±0.3	4.7±0.5	4.7±0.3	4.7±0.2
Chloride (mEq/L)	102±2	102±2	103±1	101±1	101±1	102±2	102±1	100±2

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

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 liver weight. The kidney and lung weights were also increased slightly in the high dose groups. There were no other changes in the organ weight among the different dose groups.

8. Histopathology

The results from microscopic histopathological examination of major organs and tissues are summarized in Table 7. Small numbers of incidence of microgranuloma and focal hemorrhagic necrosis were observed in livers of both male and female rats. Also there were several cases of thickening of alveolar wall. But incidences of these lesions were identical both in the control and in the high dose groups, thus, did not appear to be related to

Table 5. Absolute organ weights of male and female rats treated with the test substance orally for 30 days

Parameters \Sex \Group \Dose (mg/kg) \No. of aminals		Male				Female			
		Control 0 10	Low 52 10	Medium 156 10	High 467 10	Control 0 10	Low 52 10	Medium 156 10	High 467 10
Body Weight (g)		379±35	403±41	392±45	352±32	243±19	239±21	233±30	233±16
Liver (g)		13.4±2.1	15.3±1.9	17.5±2.6**	19.4±2.5**	8.3±1.1	9.2±1.0	10.2±1.7*	13.2±1.7**
Spleen (g)		0.71±0.06	0.75±0.11	0.76±0.12	0.63±0.12	0.53±0.08	0.52±0.07	0.50±0.08	0.50±0.07
Heart (g)		1.28±0.16	1.35±0.11	1.30±0.17	1.24±0.14	0.89±0.11	0.85±0.09	0.84±0.13	0.92±0.08
Lung (g)		1.45±0.13	1.78±0.16**	1.61±0.17	1.52±0.10	1.23±0.10	1.14±0.12	1.08±0.14	1.17±0.09
Brain (g)		1.92±0.08	1.98±0.09	2.02±0.12	1.91±0.11	1.80±0.07	1.86±0.08	1.77±0.07	1.81±0.10
Testis (g)/	Right	1.60±0.05	1.65±0.14	1.65±0.13	1.60±0.11	79.9±8.6	89.9±20.5	85.4±20.5	85.5±11.0
Ovary (mg)	Left	1.60±0.05	1.63±0.12	1.63±0.13	1.60±0.12	77.7±13.6	93.7±18.9	78.3±23.3	84.0±16.8
Kidney (g)	Right	1.34±0.17	1.46±0.19	1.42±0.21	1.44±0.14	0.85±0.08	0.88±0.09	0.84±0.09	0.96±0.12*
	Left	1.31±0.16	1.44±0.19	1.41±0.21	1.43±0.16	0.84±0.10	0.84±0.07	0.83±0.11	0.95±0.13
Adernal Grand	Rgith	31.4±4.1	44.4±10.2**	29.6±10.6	29.3±4.7	34.4±3.8	33.7±9.6	32.1±4.7	29.5±8.6
(mg)	Left	33.9±6.1	40.4±8.2	30.9±9.5	29.5±5.0	36.1±5.1	36.2±7.9	33.5±6.2	34.5±6.1

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

Table 6. Relative organ weights of male and female rats treated with the test substance orally for 30 days

Parameters \Sex \Group \Dose (mg/kg) \No. of aminals		Male				Female			
		Control 0 10	Low 52 10	Medium 156 10	High 467 10	Control 0 10	Low 52 10	Medium 156 10	High 467 10
Liver (%)		3.52±0.29	3.81±0.18	4.44±0.32**	5.51±0.28**	3.42±0.23	3.86±0.20*	4.38±0.33**	5.65±0.48**
Spleen (%)		0.19±0.01	0.19±0.02	0.19±0.02	0.18±0.02	0.22±0.03	0.22±0.02	0.22±0.02	0.22±0.03
Heart (%)		0.34±0.03	0.34±0.02	0.33±0.03	0.35±0.03	0.37±0.04	0.36±0.03	0.36±0.03	0.40±0.03
Lung (%)		0.39±0.04	0.44±0.04**	0.42±0.02	0.43±0.02**	0.51±0.04	0.48±0.05	0.47±0.03	0.50±0.02
Brain (%)		0.51±0.03	0.49±0.03	0.52±0.06	0.55±0.03	0.75±0.06	0.78±0.06	0.77±0.08	0.78±0.05
Testis/	Right	0.43±0.03	0.41±0.04	0.42±0.05	0.46±0.04	0.033±0.004	0.037±0.008	0.037±0.008	0.037±0.003
Ovary (%)	Left	0.43±0.04	0.41±0.3	0.42±0.04	0.46±0.03	0.032±0.006	0.039±0.006	0.034±0.009	0.036±0.007
Kidney (%)	Right	0.36±0.03	0.36±0.02	0.36±0.03	0.41±0.02**	0.35±0.03	0.37±0.02	0.36±0.02	0.41±0.04**
	Left	0.35±0.02	0.36±0.02	0.36±0.02	0.41±0.03**	0.35±0.04	0.35±0.03	0.36±0.02	0.41±0.04**
Adernal Grand	Rgith	0.008±0.001	0.011±0.003*	0.008±0.003	0.008±0.001	0.014±0.001	0.014±0.003	0.014±0.001	0.013±0.003
(%)	Left	0.009±0.002	0.010±0.002	0.008±0.003	0.008±0.001	0.015±0.002	0.015±0.003	0.014±0.002	0.015±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 30 days

\Sex \Group \Dose (mg/kg) \No. of aminals		Male				Female			
		Control 0 10		High 467 10		Control 0 10		High 467 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	1	10	2	10	2	10	4
Microgranuloma			1		1		2		3
Focal hemorrhagic necrosis			0		1*		0		1
Vacuolation			0		1*		0		0
Spleen		10	0	10	0	10	0	10	0
Heart		10	0	10	0	10	0	10	1
Basophilic materials									1
Lung		10	4	10	3	10	2	10	4
Thickening of alveolar wall			4		3		2		4
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	10	0	10	0	10	0	10	0
	Left	10	0	10	0	10	0	10	0
Adernal Grand	Right	10	0	10	0	10	0	10	0
	Left	10	0	10	0	10	0	10	0

*Observed from the same rat.

administration of the test substance. No other lesions were found in either male or female rats.

IV. DISCUSSION AND CONCLUSION

In the present study subchronic toxicity of a combined preparation of ticlopidine and *ginkgo biloba* extract (EGb 761) mixed in a ratio of 10 : 4 was examined in male and female rats. The test substance was administered intragastrically to rats at a dose of 467 mg/kg (high dose), 156 mg/kg (medium dose), or 52 mg/kg (low dose) for 30 consecutive days. Control rats received an identical volume of vehicle only.

There was no abnormal behavior or appearance among the animals throughout the adminstration period. Also no mortality in animals was observed till terminal sacrifice. In the high dose groups of both male and female rats a transient decrease in food intake was noted immediately following the initiation of administration, but food intake returned to normal in a week. This change was reflected in the slight, transient reduction in body weight gain in the high dose group of male rats in the first week. However, there were no differences in water consumption regardless of the dose level during the period.

There were no consistent or test substance-related changes in the parameters determined either in urinalysis or in hematological examinations, and all the values appeared to be in normal range. In serum chemistry total plasma protein and cholesterol were increased in the high dose group of both sexes. The other small changes in BUN or glucose did not show any dose-dependency, and appeared not to be related to the test substance administration.

At autopsy no significant lesions were found grossly in all major organs and tissues. But the liver weight was increased dose-dependently in both sexes. In the high dose groups an increase in kidney and lung weight was also noted. Microscopic evaluation of major organs and tissues did not demonstrate any abnormal finding associated with the test substance administration. There were incidences of thickening of alveolar wall, but these were observed equally both in the high dose and control groups, and accordingly, attributed to the repeated oral administration of foreign materials by gavage.

In conclusion the significant changes in both sexes of rats associated with administration of the test substance in this study may be summarized as increases in liver weight, total plasma protein, and total cholesterol. These results suggest that the target organ of the test substance would be the liver of the rats. However, other hepatic parameters measured in this study did not show any differences from normalcy. The changes mentioned above (increases in liver weight, plasma protein, cholesterol) and small increases in kidney weight are all in good agreement with the results observed by other investigators (Castaigne, 1974; Daiichi Seiyaku, 1975) who examined the toxicity of ticlopidine in rats. Furthermore, the increase in serum cholesterol level is an established side effect in ticlopidine-treated human patients (Molony, 1993). Thus, the present study suggests that the toxicity resulting from repeated administration of the combined preparation of ticlopidine and *ginkgo biloba* extract is equivalent to that of ticlopidine administration only. Under the conditions employed in this study no observable effect level (NOEL) of the test substance in rats is considered to be 52 mg/kg.

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