A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Effects of *Ginkgo biloba* Leave Powder and Extract on Plasma and Liver Lipids, Platelet Aggregation and Erythrocyte Na^+ Efflux in Rats Fed Hypercholesterolemic Diet

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DEPARTMENT OF FOOD SCIENCE AND NUTRITION

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

February, 2011

고콜레스테롤혈증 흰쥐에 있어서 은행잎 분말과 추출물이 체내 지질 수준, 혈소판 응집 및 적혈구막 Na^+ 유출에 미치는 영향

指導敎授 姜 晶 淑

金 智 美

이 論文을 理學 碩士學位 論文으로 提出함

2011 年 2 月

金智美의 理學 碩士學位 論文을 認准함

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濟州大學校 大學院

2011 年 2 月

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A thesis submitted in partial fulfillment of the requirement
for the degree of Master of Science

2011. 2.

This thesis has been examined and approved.

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Abstract

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This study was conducted to investigate the effects of *Ginkgo biloba* (Gb) leave powder (10%) and extract (3%) on plasma and liver lipids, platelet aggregation, erythrocyte Na^+ efflux and angiotensin converting enzyme (ACE) in SD rats fed 0.5% cholesterol diet. Average daily gain was significantly lower in rats fed Gb extract than the control ($p<0.05$) with no difference in food intake. Plasma HDL-cholesterol was increased in rats fed Gb powder ($p<0.05$) compared with control. There were no difference in plasma and liver total-cholesterol and triglyceride among groups. TBARS production in liver and PRP was decreased in both Gb groups, but not significantly different. Platelet aggregation in maximum and initial slope were significantly decreased compared with the control (both, $p<0.05$). Intracellular Na^+ was significantly higher in rats of Gb extract than the control ($p<0.05$) without difference in intracellular K^+. Erythrocyte Na-K ATPase and total Na^+ efflux were significantly increased in rats of Gb extract compared with other groups ($p<0.05$).

Plasma glucose was significantly lower in Gb powder than the control. Plasma GOT/GPT and glucose were significantly lower in Gb extract compared with the control (all, $p<0.05$).

Ginkgo biloba which contains bioactive ginkgolide and bilobalide seems to have protective effects from cardiovascular diseases with favorable actions on HDL-cholesterol, platelet aggregation and erythrocyte Na^+ channels.

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Ⅰ. INTRODUCTION

Recently, incident and mortality rate of cardiovascular disease (CVD) is constantly increasing due to the lack of exercise, increase of stress and high lipid intake by the rapid economic growth and westernized life style. According to the WHO statistics in 2007, over 1,700 people are dying of CVD annually in world wide which ranked in the first place on the 30% of mortality rate (World Health Statistic, 2007). According to the 2007 statistics Korea, the primary cause of death is cancer, the secondary cause of death is the cerebral vascular disease, the thirdly cause of death is the CVD, the fifth cause of death is the diabetes mellitus and the ninth cause of death is the hypertension. The mortality rate of CVD related diseases and diabetes mellitus showed higher than the mortality rate of cancer (Statistics Korea, 2007). Major risk factors of CVD is hypertension, hypercholesterolemia, obesity, smoking and lack of exercise and minor ones are highly related to high blood lipid (Martin *et al.*, 1986; Mazur *et al.*, 1990).

Among circulatory diseases, hypertension, called "silence killer" does not have symptoms itself, but is associated with some CVD complications such as cerebral hemorrhage, angina, myocardial infarction and renal insufficiency (Victor R, 2004). High incidence of hypertension in Korea may be partially due to high intake of sodium. According to WHO, standard hypertension defines sytolic/diastolic blood pressure of over 160/90mmHg, borderline hypertension is systolic of 140-160mmHg, diastolic of 90-95mmHg and normal blood pressure is of less than 140/90mmHg (Bishop and Detweiler). There are two categories of hypertensions which are essential hypertension and secondary hypertension (Reid and Rubin, 1989). Essential hypertension comprised

with 90% of all hypertension patients is naturally or genetically occurred without specific causes. Essential hypertension may be involved in abnormal responses to endocrine and neural systems and high sensitivity to sodium intake and stress.

Cholesterol plays roles as the component of cell membrane and the basic materials for synthesis of bile acid, steroid hormones and vitamin D. Cholesterol of 300mg daily is ingested from ordinary diet, and some organs such as liver, adrenal cortex and skin synthesize certain amount of cholesterol for cholesterol balance. Blood cholesterol is transported in forms of lipoprotein. LDL-cholesterol transports cholesterol to extrahepatic organs and cells where cholesterol can be used for synthesis of steroid hormone and vitamin D. Dietary cholesterol is packed into chylomicron in intestine and transported to general circulation, unloading triglyceride to tissues, and chylomicron remnant is taken up by liver where it is resynthesized and released in form of VLDL. LDL-cholesterol, the major cholesterol transporter is formed from VLDL or IDL in blood and is taken up by liver with the recognition site of Apo-B100. LDL-cholesterol is atherogenic lipoprotein and associated with CVD. Body cholesterol is well controlled by two liver enzymes, HMG-CoA reductase and cholesterol 7-hydroxylase. HMG-CoA reductase is the rate limiting enzyme in *de novo* cholesterol synthesis, controlling of the velocity formation of mevalonate from HMG-CoA. Cholesterol 7-hydroxylase is the key enzyme in biosynthesis of bile acids, which is the end product of cholesterol catabolism in body. About 99% bile acid released through jejunum are reabsorbed in ileum, returning to the liver via enterohepatic circulation, therefore only small amount of bile acid is newly synthesized to replace the daily loss of bile acid in feces (Martin *et al.*, 1983). Dietary fiber is effective in excretion of dietary cholesterol and bile acid through feces. Increased small dense LDL particle and phospholipase A_2

(PLA_2) are considered as emerging CVD risk factors in subjects with metabolic syndrome (Filippatos *et al.*, 2007) and the number of LDL particle is a more sensitive indicator of CVD risk than LDL-cholesterol (william *et al.*, 2007). Chapman (2006) reported that low level of HDL-cholesterol in subjects with type 2 diabetes, the metabolic syndrome and dyslipidemia was associated with the elevated risk for premature CVD.

Platelets are small, granulated bodies 2-4μM in diameter and have a 4 days of half life with concentration of 300,000/μl in blood (Ganong, 1983). They are generated from megakaryocyte in bone marrow and normally functions as the first aids to stop bleeding in injured blood vessel. Platelets can adhere the collagen layer of injured vessels, aggregating and releasing various reactive substances such as ADP, serotonin, TXA_2 and platelet-derived growth factor (PDGF) which are involved in CVD complications. Serotonin and TXA_2 from platelets cause blood vessel contract, increasing blood pressure and PDGF is known to induce blood vessel thickening, resulting in athrosclerosis. Aggregating platelets cause thrombosis and stroke. Blood is continually clotting and dissolving depending on the circumstance. Hyperlipidemia, hypertension, high blood sugar and smoking can activate platelet to aggregate (Carvalho *et al.*, 1974; Tomizuka *et al.*, 1990). Free radical and low level of antioxidant nutrients are another factor for platelet aggregation to cause thrombosis (Buczynski *et al.*, 1993; Aviram *et al.*, 1998). Besides platelet thrombosis, atherosclerotic plaque detached from vessel wall can cause embolism which block brain capillary and coronary artery, leading to stroke and myocardial infarction (Dyerberg and Bang, 1978; Gibson, 1982).

Electrolytic Na^+ and K^+ are important in controlling extracellular fluid volume including blood. It has long been recognized that alternation of

Na^+ distribution within the cells can be related to the trigger mechanism of hypertension. If the amount of Na^+ is increased within the cells, there can be change in cell volume followed by increased cell membrane Ca^{2+} and electric pressure. These changes cause an increase in blood vessel resistance and blood pressure (David *et al.*, 1989). There are four Na^+ transport pathways; Na-K ATPase, Na-K cotransport, Na-(Li^+) counter transport and Na-passive transport. Na-K ATPase is known to function on Na^+ reabsorption in collecting duct of kidney and glucose absorption in intestine where cotransported Na^+ with glucose is pumped out by Na-K ATPase. In nerve cells, Na-K ATPase is very active after action potention exerts, creating more Na^+ and K^+ concentration gradients across cell membranes (Sydney 1983). Na-K ATPase pumps out 3 Na^+ in exchange 2 K^+ into cell, transporting these ions against concentration using one ATP. Na-K ATPase is known to be sensitive and inhibited by a cardiac glycoside, ouabain and Na-K cotransport is known to be sensitive and inhibited by furosemide. Both ouabain and furosemide have natriuretic and diuretic effects, thereby being used for hypotensive drugs (Hart *et al.*, 1998). Use of red blood cells for measurement of Na^+ efflux has great advantage, because it can be gotten easily. Na-K ATPase is used for the indicator of the study as the pathological adjunctive factors of diseases such as hypertension, diabetes mellitus, uremia, arthritis and cardiac disorder (Earl *et al.*, 1979; Haddy *et al.*, 1980; Kiziltunc *et al.*, 1997; Masoom-Yasinzai, 1996). Na-K ATPase in blood vessel and heart ventricle was more suppressed in the hypertensive patients than the normotensive entity (Edmondson *et al.*, 1975; Kiziltunc *et al.*, 1997). An ^{23}Na-NMR study showed that erythrocyte Na-K pump was low and intracellular Na^+ was high in uremic patient, implying the presence of an endogenous Na-K pump inhibitor in uremic plasma (Jean *et al.*, 1990).

Angiotensin converting enzyme (ACE) found in pulmonary circulation

is involved in water and Na^+ retention by converting angiotensin Ⅰ to the fully active angiotensin Ⅱ which stimulates adrenal cortex to release aldosterone. Aldosterone functions on Na^+ reabsorption in collecting duct of kidney and blood vessel contraction, thereby increasing blood pressure (Ganong, 1983) (**Figure 1**). ACE inhibitor, captopril normalized the suppressed RBC membrane Na-K pump activity and reduced blood pressure in essential hypertensive subjects (Golik *et al.*, 1996). ACE inhibitor increased blood flow by reducing vasoconstriction and improved neurovascular function in streptozotocin-induced diabetic rats and improved myocyte contractility and electrophysiology in pigs (Mary *et al.*, 2001; Francis *et al.*, 1998).

Ginkgo biloba (Gb), known as the Maidenhair tree, is originated in China thousands of years ago and is mentioned in the Chinese *Materia Medica* 5000 years ago (Deng, 1988). Today, Gb is the only one surviving species of the Ginkgoaceae family and Ginkgoales order. Gb is commonly called a "living fossil", because it has changed little for over 100 million years (Nakanishi, 2005). Gb leaves of fan-shaped are very unique among seed plants. Gb leaves contain various flavonoids (e.g kaempferol, quercetin and isohamnetin derivatives) and terpenes (e.g ginkgolides and bilobalide) (Beek *et al.*, 1991; Sticher, 1993). The specific concentrations of these substances in the leaves varies with season, and different Gb extracts also is used in different formulations (Beek *et al.*, 1991; Sticher, 1993; Smith *et al.*, 1996). The extract of Gb leaves is well known popular herbal medications and has been used pharmaceutically. Gb extract extracted from green leaves of Gb was first introduced in Germany in 1965 and one such extract, EGb 761, is standardized extract (Kyung-Su *et al.*, 2005). Gb extract is composed of approximately 24% flavone glycosides (primarily composed of quercetin, kaempferol, and

isorhamnetin), 6% terpene lactones (2.8-3.4% ginkgolides A, B, and C, and 2.6-3.2% bilobalide) (Blumenthal, 1998; Blumenthal, 2000) (**Figure 2**). Ginkgolides have a cage molecule structure with six five-membered rings and a tert-butyl group (Maruyama *et al.*, 1967; Woods *et al.*, 1967; Hiroki *et al.*, 1999). Rong *et al.* (1996) and Pietri *et al.* (1997) showed that EGb 761 is the most commonly prescribed herbal medicine to preventing ischemia-induced oxidation. Antioxidant components of Gb leaves are kaempferol 3-O-(2"-O-β-D-glucopyranosyl)-α-L-rhamnopyranoside, kaem -pferol 3-O-α-(6"'-p-coumaroyl-glucosyl-β-1,4-rhamnoside), and quercer -tin-3-O-α-(6"'-p-coumaroylglucosyl-β-1,4-rhamnoside) and have inhi- bitory effect on linoleic acid autoxidation with scavenging effects on DPPH radical and superoxide radicals (Byeong-soo *et al.,* 2000). EGb 761 was effective in minimizing oxidative stress in cardiovascular surgery, where highly bioavailable terpene played some positive roles (Sylvia *et al.*, 1997). Shen (1995) and Yasuo *et al.* (1996) reported that Gb extract had beneficial effects on myocardial and retinal injuries from reperfusion after ischemia, indicating a potential of Gb extract as an anti-oxidant. Gb extract have protective effect on neurons against oxidative stress induced by hydrogen peroxide (Yasuo *et al.*, 1992; Yasuo *et al.*, 1996). Recent studies have shown that Gb extract is effective on blood flow improving cerebral insufficiency (Jos and Paul, 1992). EGb 761 has been an effective remedy for a variety of disturbances of cerebral function by improving peripheral and cerebral circulations; alleviating multi infarct dementia from a cerebral ischemic damage and minimizing the early cognitive decline in senile dementias (e.g Alzheimer's disease) or cognitive decline from mixed origins (e.g psychoorganic origin) (Clostre, 1999; Koch *et al.*, 2000). Gb extract enhanced spatial memory and motivation with significant changes in the content and metabolism of monoamines in several brain regions (Kamilla *et al.*, 2009). Braquet

(1988) and Jos *et al.* (1992) reported treatment with Gb extract lowered fibrinogen levels and decreased plasma viscosity. Ginkgolides B of Gb extract is known to act as a platelet activating factor antagonist, and flavonoid fraction of Gb extract showed free radical scavenging effect (Smith *et al.*, 1996). In general, Gb has been attracting attention from researchers because of the positive effects on circulation to brain and central nervous system, thereby enhancing memory and brain function.

In present study, we focused on the therapeutic and preventive effects of *Ginkgo biloba* in cardiovascular disease, measuring platelet aggregation, Na^+ efflux and angiotensin converting enzyme, and cholesterol metabolism in rats fed diets containing 10% Gb powder and 3% Gb extract. We also examined the effects of antioxidant, hypoglycemia, liver protective by measuring TBARS, blood glucose and GOT/GPT, seeking a possibility to use Gb as the alternative medicine or the food supplements.

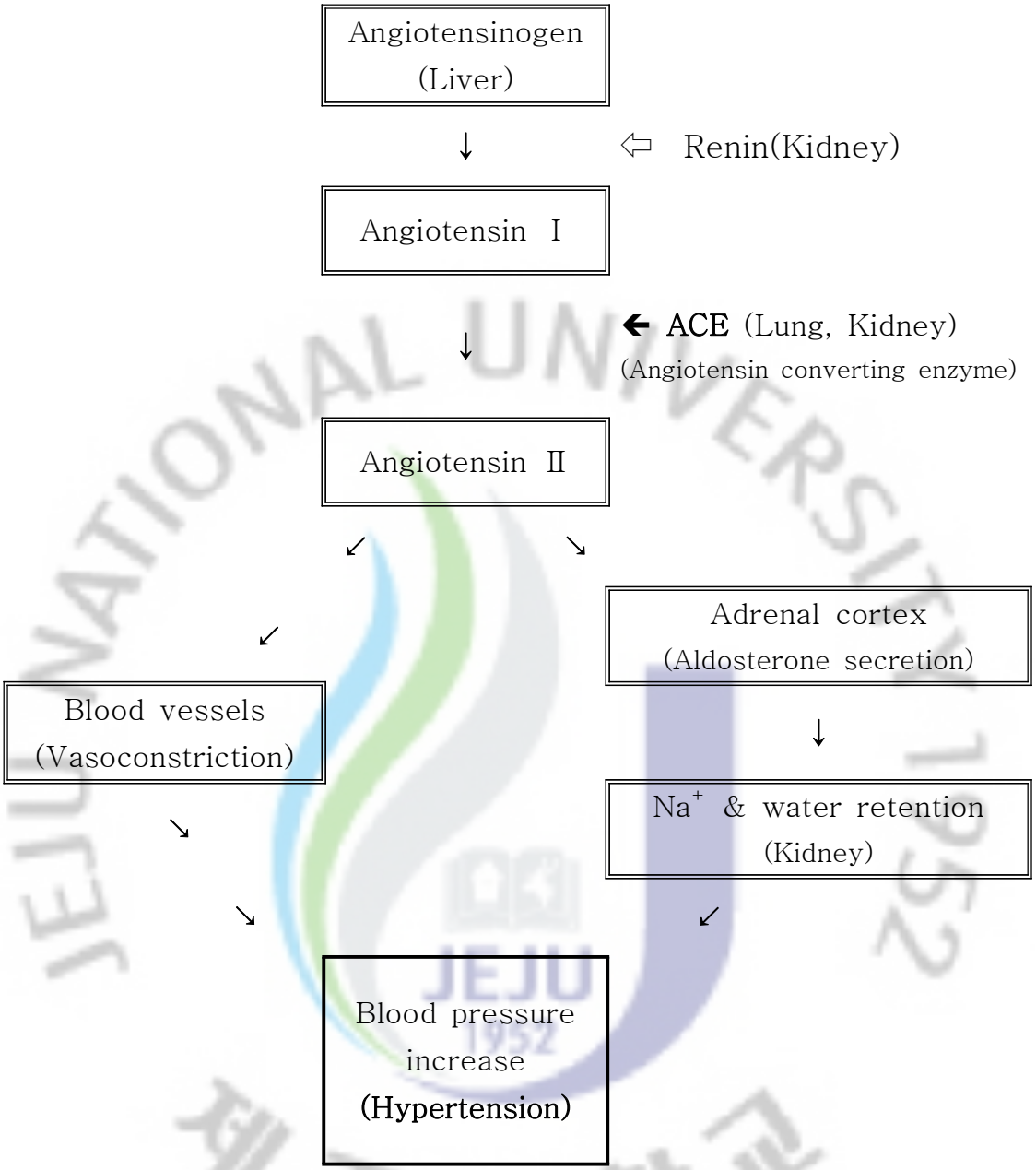
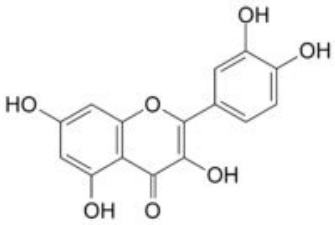
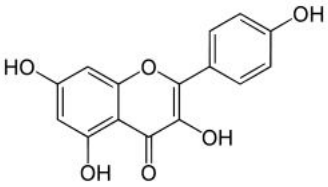


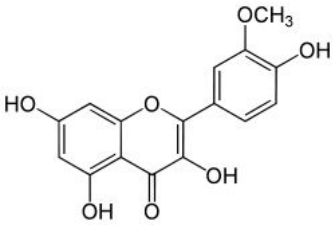
Figure 1. The hypertension mechanism of renin-angiotensin system



(a) Quercetin

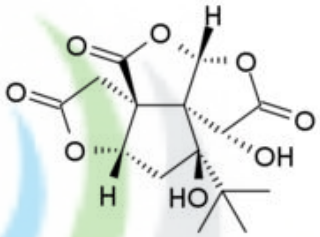


(b) Kaempferol



(c) Isorhamnetin

(1) Flavone glycosides in Gb extract



Bilobalide

Ginkgolide

	Ginkgolides				
	A	B	C	J	M
R1	OH	OH	OH	OH	H
R2	H	OH	OH	H	OH
R3	H	H	OH	OH	OH

(2) Terpene lactones in Gb extract

Figure 2. Compositions of structures in Gb extract

Ⅱ. MATERIALS & METHODS

1. Experimental Materials

1.1 Animals and Diets

Male Sprague Dawley (10 weeks old) rats were purchased from Orient Bio company (Ltd, Gapyung, Korea). Rats weighing approximately 330g were divided into three groups and fed the AIN-76 (American Institute of Nutrition-76) based following experiment diets; 0.5% cholesterol based control diet for the control group, control diet plus 10% *Ginkgo biloba* powder for the *Ginkgo biloba* powder group, control diet plus 3% *Ginkgo biloba* extract for *Ginkgo biloba* extract group (Table 1).

Rats were housed in individual cage in a room maintained at 20-25℃ room temperature, 40-60% humidity with 12 hours dark-light cycle. Rats had free access to their respective diets and water for 4 weeks. Food intake for individual rats was monitored every 2 days and animals were weighted every 2 days during the feeding period.

Gb powder was purchased from a herbal medicine shop in Jeju.

For ethanolic extracts from Gb powder, two kilograms of Gb powder was mixed with 6 liter of 80% ethanol and placed in shaking incubator for 24 hours at room temperature. The mixtures were filtered with whatman filter paper and the residue were reused three times. The filtered liquid was turned through a rotary vacuum evaporator at 40℃ to removed all ethanol. This concentrate was dried by using a freeze dryer and 230g of extract powder was procured.

Table 1. Composition of experimental diets (%)

Ingredient	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
	Cholesterol based diet		
Casein[a)]	20.0	20.0	20.0
L-methionine[a)]	0.3	0.3	0.3
Lard[b)]	9.0	9.0	9.0
Soybean Oil[c)]	1.0	1.0	1.0
Choline chloride[d)]	0.2	0.2	0.2
Vitamin mix[e)]	1.0	1.0	1.0
Mineral mix[f)]	3.5	3.5	3.5
Sucrose[c)]	20.0	20.0	20.0
Corn starch[g)]	39.3	34.3	36.3
Cellulose[h)]	5.0	-	5.0
Cholic acid[h)]	0.2	0.2	0.2
Cholesterol[h)]	0.5	0.5	0.5
Ginkgo biloba powder		10	
Ginkgo biloba extract			3.0
Total (%)	100.0	100.0	100.0

a) Teklad, Harlan Madison WI, USA

b) Samlip Yugi Co.

c) Jeil Jedang Co.

d) Junsei Chemical Co., Ltd.

e) Vitamin mixture(mg/100g) : Thiamine HCl 60.0, Riboflavin 60.0, Pyridoxine HCl 70.0, Nicotinic Acid 300.0, D-Calcium Pantothenate 160.0, Folic Acid 20.0, D-Biotin 2.0, Vit. B_{12} 0.1, Vit. A 80.0, Vit E 2000.0, Vit. D_3 0.25, Vit. K 0.5, Sucrose 99247.15

f) Mineral mixture(g/100g) : $CaHPO_4$ 50.0, NaCl 7.4, $K_3C_6H_5O_7 \cdot H_2O$ 22.0, K_2SO_4 5.2, MgO 2.4, Manganous carbonate(43-48%Mn) 0.35, Ferric citrate(16.7%Fe) 0.6, Zinc carbonate(70%Zn) 0.16, Cupric carbonate(53-55%Cu) 0.03, KIO_3 0.001, $Na_2SeO_3 \cdot 5H_2O$ 0.001, $CrK(SO_4)_2 \cdot 12H_2O$ 0.055, Sucrose 11.804

g) Samyang Genex Co.

h) Sigma Chemical Co., USA

2. Collection of Samples

2.1 Blood Sample

At the end of 4 weeks, rats were anesthetized with ether after fasted for 12 hours, and blood was collected by cardiac puncture into vacuum tubes containing heparin. Plasma was obtained by centrifugation of blood sample at 1000×g for 15 minutes and stored at -20℃ for total cholesterol, HDL-cholesterol and triglyceride analysis. Hematocrit of the whole blood was determined using Hematocrit centrifuge.

2.2 Liver and Lung Sample

The liver for total cholesterol, triglyceride and lung for ACE inhibition activity analysis were quickly removed and weighted and frozen at -20℃ until analyzed.

3. Analysis of samples

3.1 Plasma Total-cholesterol, HDL-cholesterol, HDL-C/Total-C (HTR, %), Triglyderide, and Glucose

Total cholesterol, HDL-cholesterol, Triglyceride, and glucose were measured using commercial enzymatic assay kit (ASAN Pharmaceutical Co., Ltd, Korea). For total cholesterol, triglyceride, and glucose assay, 10 ㎕ each of plasma sample was used in a quantitative respect and added 1.5㎖ color reagent, then incubated at 37℃ water bath for 5 minutes and 10 minutes respectively. For HDL-cholesterol assay, 0.1㎖ each of plasma samples were used and added 0.1㎖ precipitating reagent, then incubated at room temperature for 5 minutes. The plasma samples were centrifuged at 1000×g for 10 minutes to obtain a clear supernatant. Each of samples were added 1.5㎖ color reagent and mixed well, then incubated at 37℃ for 5 minutes. The absorbance for total cholesterol, HDL-cholesterol and glucose was read at 500nm and triglyceride at 550nm using spectrophoto -meter (Uvikon XS, Secomam Co., France).

The HDL-C/Total-C (HTR, %) is calculated following the formula.

HDL-C/Total-C (HTR, %) = [HDL-C] / [Total-C] × 100

3.2 Liver Total-cholesterol and Triglyceride

Liver samples were prepared by modifying the method described in Folch *et al.* (1957) to determine cholesterol and triglyceride. One gram of liver tissue was homogenized in 6㎖ chloroform/methanol mixture (2/1, v/v) and 2㎖ distilled water using a tissue homogenizer for 5 minutes and centrifuged at 1000×g for 10 minutes. The chloroform fraction containing cholesterol and triglyceride is the bottom layer. For liver total cholesterol, five hundreds ㎕ of chloroform fraction in the bottom layer was transferred and leaved to dry for 24 hours. Fifty ㎕ Triton X-100 : chloroform (1:1, v/v) was added and vortexed again. Ten ㎕ was transferred to a new tube and leaved to dry under clean bench, then 1.5 ㎖ color regent (ASAN Pharmaceutical Co., Ltd, Korea) was added and incubated at 37℃ water bath for 5 minutes. The absorbance of the incubation medium was read at 500nm using spectrophotometer.

For liver triglyceride, Ten ㎕ of chloroform fraction in bottom layer was transferred to a new tube and leaved to dry under clean bench, then 50㎕ methanol was added and vortexed. To this solution, 1.5㎖ color regent (ASAN Pharmaceutical Co., Ltd, Korea) was added and incubated at 37℃ water bath for 10 minutes. The absorbance of the incubation medium was read at 500nm using spectrophotometer (**Figure 3**).

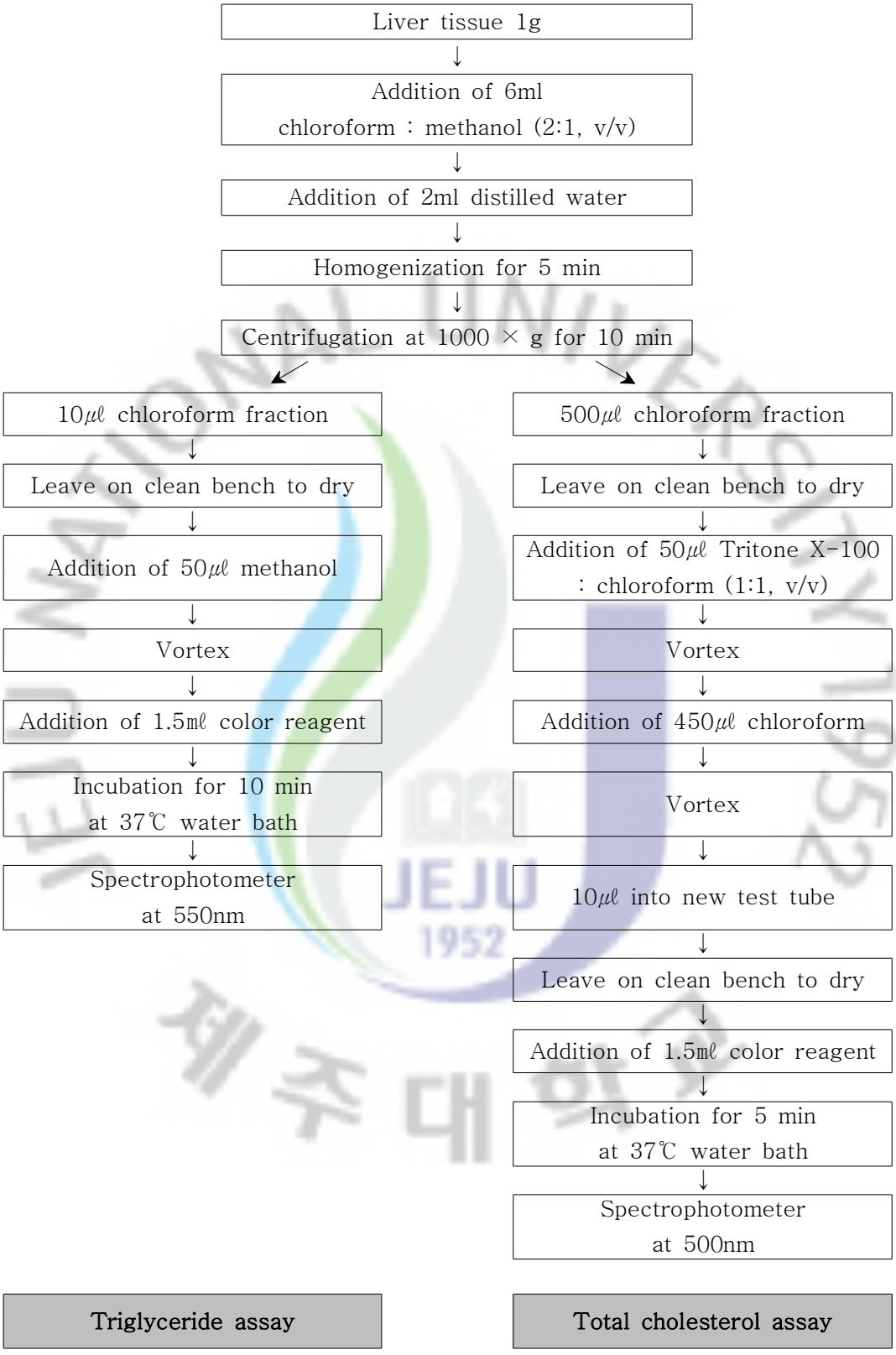


Figure 3. Analytical scheme for extraction of liver lipids

3.3 Platelet Rich Plasma (PRP) TBARS Production

Platelet rich plasma (PRP) were obtained after centrifuging whole blood at 3000×g for 10 minutes. PRP TBARS (Thiobarbaituric Acid Reactive Substance) production was measured with a modified Buege and Aust method (1978). TBARS, malondialdehyde (MDA) is an end product of fatty acid peroxidation.

An half ㎖ PRP in 1.5㎖ PBS (Phosphate buffered saline) was incubated at 100℃ oil bath for 20 minutes after adding 2㎖ TBA solution (15g TBA, 0.139g TCA, 1.81㎖ 12N HCl in 85㎖ D-H_2O). After cooled under tap water, the incubated mixture was centrifuged at 1000×g for 10 minutes. Finally the TBARS in supernatant was measured with the absorbance at 532nm on spectrophotometer using deionized H_2O as blank.

3.4 Liver TBARS Production

Buege and Aust method (1978) was used for the liver TBARS. One gram of minced liver sample in 2㎖ PBS was incubated at 100℃ oil bath for 20 minutes after adding 2㎖ TBA or nonTBA solution (0.139g TCA, 1.81㎖ 12N HCl in 85㎖ D-H_2O). The incubated mixture was cooled down and centrifuged at 1000×g for 10 minutes. The TBARS in supernatant was measured of spectrophotometer at 532nm using deionized H_2O as blank. All samples were performed in duplicate and the values of TBARS was the outcome subtracting the concentration of the nonTBA treated from the TBA treated.

3.5 Protein Analysis

Liver protein was measured with Lowry method (Lowry et al., 1951) using bovine serum albumin (BSA) as standard protein. The absorbance for protein concentration was read at 750nm with spectrophotometer using D-H_2O as blank.

3.6 Plasma GOT and GPT

Plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) was measured with a spectrophotometric diagnostic kit purchased from the Asan pharmaceutical Company (Korea). Briefly, 1 ㎖ GOT/GPT substrate solution was incubated at 37℃ shaking water bath for 5 minutes and 0.2㎖ plasma sample was added. After GOT/GPT incubated at 37℃ for 60 minutes and 30 minutes respectively, 1㎖ of color reagent was added to each of samples and incubated for 20 minutes at room temperature. After incubation, 10㎖ 0.4N NaOH was added mixed well, incubated for 10 minutes at room temperature. The absorbance at 505nm was read, using spectrophotometer and results are expressed as IU/ℓ.

3.7 Whole Blood Platelet Aggregation

Platelet aggregation was measured using Aggrolink attached Chronolog whole blood platelet aggregometer (Chrono-Log 200-CA Havertown, Pennsylvania, USA). The whole blood was diluted with isotonic saline (0.9% NaCl) (1:4) to give a platelet concentration of approximately 400,000/㎕. Two mM adenosine diphosphate (ADP) was added to initiate aggregation, and three readings of impedance (Ω) changes were taken for each rat and the mean value was used. Platelet aggregation causes an increased in impedance across two platinum electrodes in whole blood and the impedance gain was set 20Ω in recorder response. The impedance method using fresh whole blood has the advantage of measuring platelet aggregation under nearly physiologi-cal conditions in the presence of other blood components.

3.8 Erythrocyte Na^+ Efflux

1) Red Cell Preparation

Blood was centrifuged at 1000×g for 10 minutes, and the plasma and buffy coat were removed. Red blood cells were washed 5 times with C.W.S. (choline chloride washing solution) [150mM choline chloride, 10mM Tris-4-morpholinopropane sulfonic acid (MOPS), pH 7.4 at 4℃] and centrifuged at 1000×g for 5 minutes after each wash. The RBC pellet was resuspended in the choline chloride washing to give 40-50% hematocrit, which was also measured for intracellular Na concentration, Na-K ATPase activity, Na-K cotransport, and Na-passive transport.

2) Intracellular Na^+ and K^+ Concentration

For intracellular Na^+ concentration measurement, 50㎕ aliquot of the RBC suspension was added to 5㎖ 0.025% acationox (metal free detergent, Scientific products, McGraw Park, Illinois, USA), and Na^+ concentration was measured using automic absorption spectrophotometer (AA6701F Shimazu Corporation, Japan). For intracellular K^+ RBC suspension in acationox was diluted with deionized water in 1 to 10 and K^+ concentration was measured using AAS.

3) Na-K ATPase activity

Ouabain sensitive Na-K ATPase can be blocked by ouabain, and Na^+ efflux via Na-K ATPase is calculated from the difference between the efflux into $MgCl_2$ medium with and without ouabain.

Three ㎖ each of erythrocyte was added to 30㎖ medium 1 [70mM $MgCl_2$, 10mM KCl, 85mM sucrose, 10mM glucose, 10mM Tris-MOPS, pH 7.4 at 37℃] and medium 2 [medium 1 plus 10mM ouabain] then mixed gently and aliquot 10 tubes. The tubes were transferred in duplicates to

an ice bath after incubation at 37℃ in a shaking water bath for 0, 2, 4, 6, 8 minutes. The tubes were subsequently centrifuged at 3000×g for 10 minutes, the supernatant was removed.

4) Na-K cotransport

Furosemide sensitive Na-K cotransport can be blocked by furosemide, and Na^+ efflux via Na-K cotransport is calculated from the difference between the efflux into choline chloride medium with and without furosemide.

One and half mℓ each of erythrocyte was added to 30mℓ medium 3 [150mM choline chloride, 1.0mM ouabain, 10mM glucose, 10mM Tris-MOPS, pH 7.4 at 37℃] and medium 4 [medium 3 plus 0.3308g/L furosemide] then mixed gently and aliquot 10 tubes. The tubes were transferred in duplicates to an ice bath after incubation at 37℃ in a shaking water bath for 0, 10, 20, 30, 40 minutes. The tubes were subsequently centrifuged at 3000×g for 10 minutes, the supernatant was removed for determining Na^+ concentration.

5) Na-passive transport

Na-passive trasnport is the efflux into choline chloride medium containing ouabain and furosemide under condition of both Na-K ATPase and Na-K cotransport blocked.

For Na-passive transport measurement, erythrocyte was added medium 4 [150mM choline chloride, 1.0mM ouabain, 10mM glucose, 0.3308g/L furosemide, 10mM Tris-MOPS, pH 7.4 at 37℃] .

Na^+ concentration of all Na^+ channel (Na-K ATPase, Na-K cotrans-port, Na-passive transport) were measured of automic absorption spectrophotometer (AA6701F Shimazu Corporation, Japan).

· Calculations ;

- Cotransport ;

$$\frac{[\text{Na } \mu g/\mu \ell]}{[\text{min}]} \times \frac{[60\text{min}]}{[\text{hr}]} \times \frac{[\mu\text{mole}]}{[23\mu g]} \times \frac{[44-(4\times\text{HCT})]}{[4\times\text{HCT}]} = \text{Na mmole}/\ell \text{ rbc} \cdot \text{hr}^{-1}$$

- Intracelluar Na^+ ;

$$\frac{[\text{Na } \mu g]}{[\text{m}\ell]} \times \frac{[\mu\text{mole}]}{[23\mu g]} \times \frac{[101]}{[\text{HCT}]} = \text{Na mmole}/\ell \text{ rbc}$$

- Intracelluar K^+ ;

$$\frac{[\text{K } \mu g]}{[\text{m}\ell]} \times \frac{[\mu\text{mole}]}{[39\mu g]} \times \frac{[101]}{[\text{HCT}]} = \text{K mmole}/\ell \text{ rbc}$$

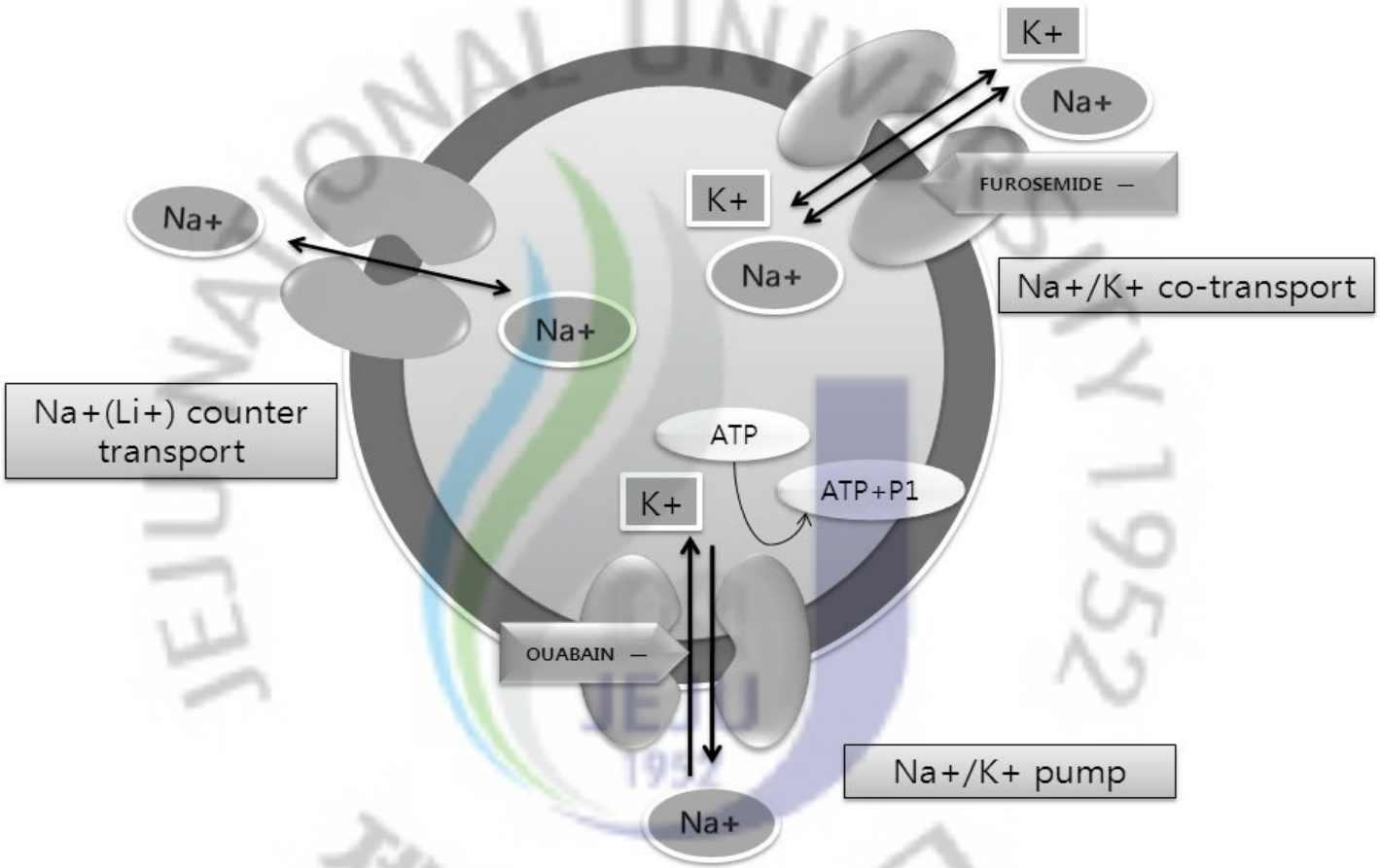


Figure 4. Model of erythrocyte Na^+ efflux channel

3.9 Angiotensin converting enzyme (ACE) Inhibitor Activity

Angiotensin converting enzyme (ACE) inhibitory was determined with modified methods of Cushman and Cheung (1971) and Dominik *et al.* (1997) measuring HA (Hippuric acid) released from HHL (Hippuryl-L-His-tidyl-L-Leucine) in incubation medium (**Figure 5**). Two hundreds ㎎ of lung was homogenized with 2㎖ 20mM potassium phoshpate buffer (1M K_2HPO_4 plus 1M KH_2PO_4, pH 8.3) containing 0.01% triton X-100 and centrifuged at 600×g for 10 minutes. Supernatant containing membrane bound ACE was transferred to a clean tube. Total 250㎕ medium including 10㎕ supernatant, 100㎕ 5mM HHL, 190㎕ 100mM potassium phosphte buffer with 30mM NaCl in effendorf tube was incubated at 37℃ for 30 minutes, then reaction was stopped by 500㎕ 1N HCl and medium was centrifuged at 1000×g for 10 minutes. Supernatant of 500㎕ was transferred to a glass tube containing 1.5㎖ ethyl acetate, vortexed for 15 seconds and centrifuged at 1000×g for 5 minutes. One ㎖ of upper ethyl acetate layer was transferred to a glass tube and dried for 30 minutes on 120℃ heating block. Dried HA was dissolved in deionized water and read the absorbance at 228nm on UV spectrophotometer using each blank prepared with the same procedure without HHL in incubation medium. Standard HA was prepared in the same procedure as tissue samples.

ACE inhibitory effect of Gb powder and extract was calculated with the following formula.

$$\text{ACE inhibition rate (\%)} = [\ 1 - (\ Sa - Sb) / (\ Sb\ )\ ] \times 100$$

· Sa : Absorbance of sample with substrate (HHL)

· Sb : Absorbance of sample without substrate (HHL)

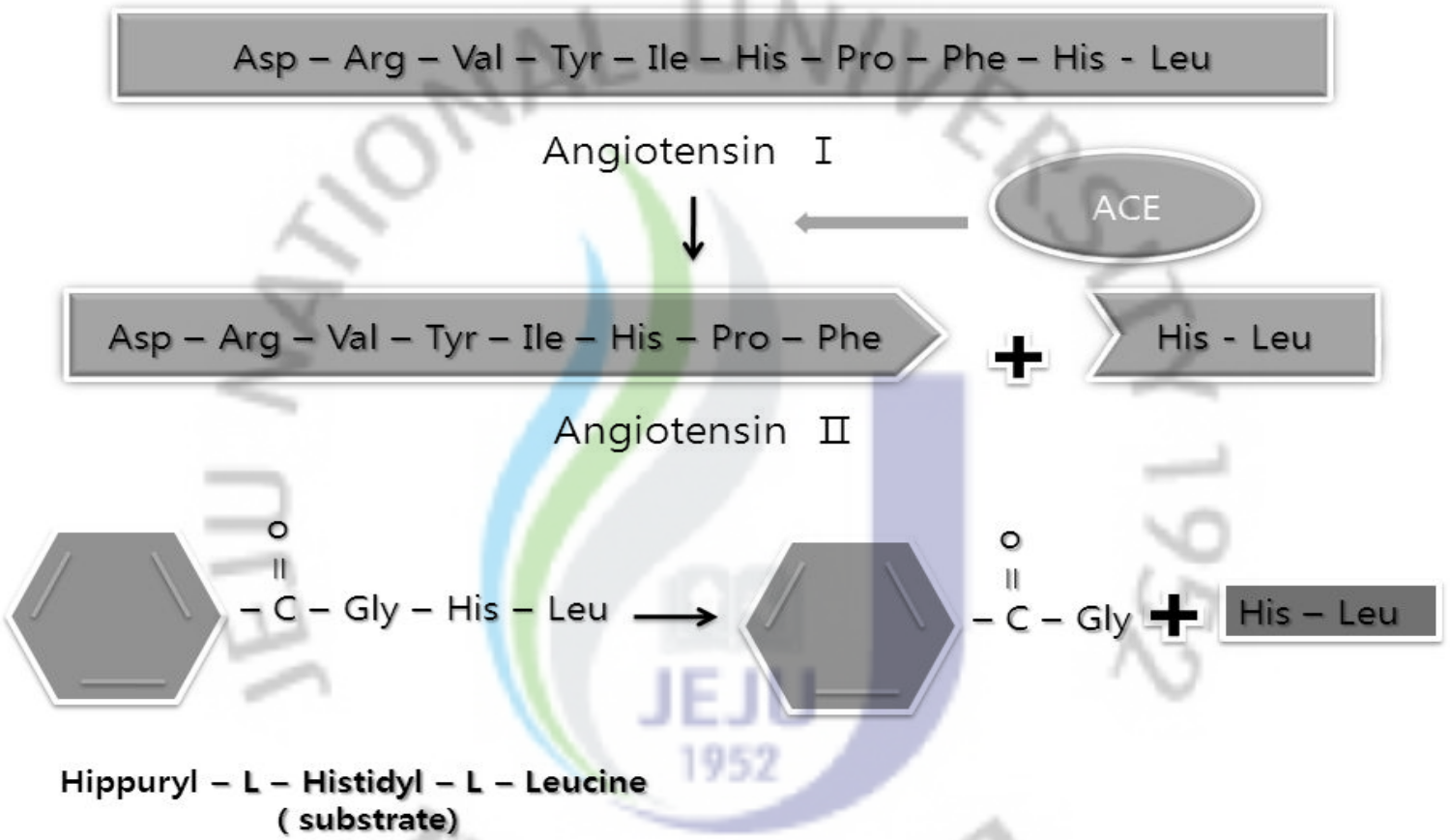


Figure 5. Diagram of the action of Angiotensin Converting Enzyme (ACE)

4. Statistical Analysis

Values were analyzed using the SAS package (SAS, 1994). Analysis of variance were conducted in a completely randomized block design. Duncan's multiple test was applied to compared individual means when F-value was significant ($p<0.05$).

Ⅲ. RESULTS & DISCUSSIONS

1. Weight gain and Food efficiency

Weight gain and food efficiency are shown in Table 2. The final body weight was tended to decrease in all the *Ginkgo biloba* (Gb) groups compared with initial body weight but not significantly different. The weight increase of the control group was 31.5% of the initial body weight, while the weight increase of Gb powder and extract were 28.9% and 21.0% respectively. The average daily gain (ADG) and feed efficiency ratio (FER) were decreased in the Gb powder and extract compared with the control and there are significant differences between the control and the Gb extract in FER and ADG ($p<0.05$) without difference in average feed intake among groups. L.W/B.W was significantly increased in the Gb of powder and extract compared with the control and significantly different between the Gb powder and the control ($p<0.01$).

Yukari *et al.* (2008) reported that the Gb extract diet group did not significantly affect body weight, but significantly increased the liver to body weight ratio by approximately 1.4-fold compared with the control group in rats. Kazumasa *et al.* (2002) observed that the Gb extract diet did not change the body weight of rats, but significantly increased liver weight by approximately 1.4-fold. In rats fed Gb extract, the weight and size of the liver were markedly increased. Huang *et al.* (2005) showed that liver weight increased with age but was proportionally less than body weight, the ratio of liver weight to body weight declined. The ratio increased after treatment with Gb extract. It is generally recognized that the deposition of glycogen and cholesterol into the liver or inflammation of the liver results in hypertrophy.

In present study, rats fed Gb powder and extract had higher liver to body weight ratio with low liver triglyceride and plasma GOT/GPT, increased liver size in Gb groups does not necessarily mean that Gb aggravates fatty liver in cholesterol fed rats.

Table 2. Effects of *Ginkgo biloba* powder and extract on growth rate and feed intake in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Initial B.W[1] (g)	337.33 ± 46.54	338.71 ± 33.29	338.54 ± 33.00
Final B.W (g)	443.46 ± 39.19	436.54 ± 32.83	409.54 ± 41.16
ADG[2] (g/d) *	3.76 ± 1.13[a)]	3.48 ± 0.44[a)]	2.53 ± 0.77[b)]
ADFI[3] (g/d)	26.45 ± 3.81	25.49 ± 2.15	25.11 ± 2.55
F.E.R[4] *	0.141 ± 0.03[a)]	0.137 ± 0.02[a)]	0.101 ± 0.03[b)]
L.W/B.W[5] (%) **	5.33 ± 0.50[b)]	6.21 ± 0.33[a)]	5.66 ± 0.44[b)]

1) Body weight after exercise adaptation
2) ADG : Average daily gain
3) ADFI : Average daily feed intake
4) F.E.R : Feed Efficiency Ratio
5) L.W/B.W : Liver weight / Body weight ratio
Values are means ± SD of 10 rats
* Values in the same row not sharing the same superscript differ ($p<0.05$)
** Values in the same row not sharing the same superscript differ ($p<0.01$)

2. Plasma Total-cholesterol, HDL-cholesterol, Triglyceride and Glucose

Plasma total-cholesterol, HDL-cholesterol, triglyceride and glucose are shown in Table 3. Plasma total-cholesterol was tended to increase in rats fed Gb extract compared with the control and Gb powder. HDL-cholesterol was increased in Gb powder and extract, and there was significant difference between Gb powder and the control ($p<0.05$). The HTR % (HDL-C/Total-C) was significantly increased in rats fed the Gb powder compared with the control and Gb extract ($p<0.05$). Plasma triglyceride was tended to decrease in both Gb groups, but not statistically different compared with the control. Plasma glucose was decreased in both Gb groups and there was significant difference between Gb extract and the control ($p<0.05$).

Yang-Hee *et al.* (2007) observed that plasma total-cholesterol was increased in the statin treated group compared with the control, and CoQ10 or green tea further increased these levels, but was not statistical difference among groups. HDL-cholesterol was increased in groups with CoQ10 and green tea. Conquer *et al.* (1997) reported that quercetin supplementation did not modify serum total, LDL or HDL-cholesterol or triglyceride levels. Kim *et al.* (2006) reported that supplementation with flavonoid naringin resulted in a significant decrease in the plasma cholesterol and triglyceride concentrations without changing HDL-cholesterol in the 6-week trial, therefore significant higher in HTR.

Rai *et al.* (2003) indicated that pretreatment with Gb extract to accute stress (AS)-induced hyperglycemia decreased blood glucose compared with untreated AS group. Qian *et al.* (2007) reported that low or high dense of Gb extract markedly reduced blood glucose. Lei *et al.* (2010) indicated that Gb extract inhibited glucose consumption and improved glucose tolerance.

In present study, Gb powder and extract have anti hyperglycemic effect by lowering fasting glucose level. *Ginkgo biloba* is rich in various flavonoids such as quercetin, kaempferol, isorhamnetin, proanthocyanidins, and catechins, and it also contains high terpene lactones in forms of bilobalide and ginkgolides.

Bioactive Gb powder and extract have protective effects on several pharmacological targets in hypercholesterolemia and diabetes with unknown mechanisms which should be further studied.

Table 3. Effects of *Ginkgo biloba* powder and extract on plasma Total-cholesterol, HDL-cholesterol, Triglyceride, and Glucose content in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Plasma (㎎/dℓ)			
Total cholesterol	149.26 ± 54.47	142.79 ± 34.98	167.47 ± 60.57
HDL-cholesterol *	28.23 ± 4.88[b)]	35.35 ± 6.81[a)]	32.52 ± 5.18[ab)]
HTR[1)] (%) *	20.12 ± 3.74[b)]	25.69 ± 4.46[a)]	20.09 ± 4.38[b)]
Triglyceride	75.47 ± 9.37	69.72 ± 5.50	70.20 ± 4.59
Glucose *	135.92 ± 12.72[a)]	123.01 ± 13.82[ab)]	120.19 ± 13.98[b)]

1) HTR (%) : [HDL-cholesterol] / [Total cholesterol] × 100

Values are means ± SD of 10 rats

* Values in the same row not sharing the same superscript differ ($p<0.05$)

3. Liver Total-cholesterol and Triglyceride

Liver total-cholesterol and triglyceride are shown in Table 4. The Liver total-cholesterol showed a tendency to decrease in both Gb groups compared with control. The Gb groups are also tended to decrease in liver triglyceride, but not statistically different between any two groups.

Yang-Hee *et al.* (2007) reported that liver total cholesterol was significantly decreased in the group with green tea compared with other groups. Liver triglyceride was decreased in groups of statin and green tea compared with the control, and the difference between groups of the control and green tea was significant. Song-Hae *et al.* (1999) showed that tangerine peel extract or a mixture of citrus bioflavonoids substantially reduced the excretion of fecal neutral sterols compared to the control. It appeared to compensate for the decreased cholesterol biosynthesis in the liver. Lin *et al.* (2009) suggested that the flavonoid-enriched *Nelumbo nucifera* leaf extract supplement significantly decreases blood lipid and liver lipid. Gnoni *et al.* (2009) observed that quercetin inhibits triglyceride synthesis and very-low-density lipoproteins (VLDL) formation by depressing *de novo* fatty acid synthesis and diacylglycerol acyltransferase activity in liver cells from normal rats.

In present study, flavonoid rich *Ginkgo biloba* was effective in decreasing absorption of dietary cholesterol and hepatic cholesterol synthesis.

Table 4. Effects of *Ginkgo biloba* powder and extract on the liver Total-cholesterol and Triglyceride content in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Liver (㎎/g)			
Total-cholesterol	12.13 ± 4.45	11.72 ± 3.92	9.42 ± 3.39
Triglyceride	16.90 ± 4.46	14.16 ± 4.40	13.74 ± 4.98

Values are means ± SD of 10 rats

4. Platelet Rich Plasma (PRP) and Liver Hepatic Thiobarbituric Acid Reactive Substances (TBARS) Production

Platelet rich plasma (PRP) and liver hepatic thiobarbituric acid reactive substances (TBARS) production are shown in Table 5. The PRP TBARS was not statistically different among groups. The liver TBARS was tended to decrease in both Gb groups, but there was not statistical difference compared with the control.

Yang-Hee *et al.* (2007) observed that TBARS production in PRP was significantly decreased in the groups with CoQ10 and green tea compared with the control and statin groups. Punithavathi and Prince (2009) reported that quercetin and α-tocopherol showed significant decrease in plasma lipid peroxidation products. Bok *et al.* (2002) showed that the overall potential for antioxidant protection was significantly enhanced by the quercetin dihydrate supplements through lowering the plasma and TBARS levels. The present data indicate that the increase in erythrocyte malondialdehyde (MDA) levels might be related to the oxidative damage. Gb extract 761 will strengthen the antioxidant defense system of an organism. Huang *et al.* (2005) showed that the level of MDA in Gb extract liver tissue was significantly decreased. Gb extract is used as a standardized recipe preparation and contains two groups of major substances (e.g flavonoid glycosides and terpenoids). And Kose *et al.* (1997) observed that the inhibitory effect of Gb extract 761 on MDA production in the control and Behcet's disease (BD) patient groups in the erythrocytes was increased. There was a significantly increase in MDA formation in the patient group with no Gb extract 761 as compared to the control. Kose *et al.* (1995) indicated that Gb extract 761 has been found to be more effective than ascorbic acid, glutathione and uric acid as effective as α-tocopherol and retinol acetate.

Robak *et al.* (1988) reported that flavonoids are considered important dietary antioxidants. Rice *et al.* (1998) indicated that Quercetin, one of the ingredients, was reported to inhibit hypoxanthine-xanthine oxidase activity and scavenge super oxide, hydroxyl radicals, and peroxynitrite in vitro. The antioxidant properties of flavonoids involve mainly scaveging of free radical reactions.

In present study, diets with Gb powder and extract decreased liver TBARS production. With results from previous research, we assumed that bioactive compounds of *Ginkgo biloba* flavonoids and terpene lactones have an action to reduce oxidative stress.

Table 5. Effects of *Ginkgo biloba* powder and extract on the platelet rich plasma (PRP) and liver TBARS productions in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
TBARS			
PRP[1)] (*nmol/㎖ plasma*)	7.13 ± 0.90	7.99 ± 0.61	7.17 ± 1.40
Liver (*μmol/㎎ protein*)	0.30 ± 0.15	0.22 ± 0.12	0.17 ± 0.07

1) PRP : Platelet rich plasma (PRP) were obtained after centrifuging whole blood at 3000×g for 10 min.

Values are means ± SD of 10 rats

5. Plasma GOT and GPT

Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT) are shown in Table 6. The GOT (AST) and GPT (ALT) were decreased in Gb groups, and both enzymes were significantly different between Gb extract and the control ($p<0.05$).

GPT is found in the liver, kidney, heart, and skeletal muscle, and GOT is also found the liver, kidney, heart, skeletal muscle, brain and red blood cell. GOT and GPT are important enzymes associated with liver parenchymal cells and leak out to blood as liver cells are damaged. Significantly increased level of GOT and GPT often suggest the evidence of clinical problems such as viral hepatitis, heart failure, liver damage.

Kazumasa *et al.* (2002) observed that serum transaminase, GOT and GPT, were not changed by the Gb extract. Marcos *et al.* (2008) showed that serum GOT and GPT levels were lower in the Gb extract diethylnitrosamine (DEM) treated group than in the respective control group. Shenoy *et al.* (2001) reported that stimultaneous treatment with Gb protects the liver against CCl_4 induced hepatotoxicity. It is posssible that the mechanism of hepatoprotection of Gb is due to its antioxidant effect.

However, Yukari *et al.* (2008) reported that serum GOT level of aged SHR was not changed, but serum GPT level significantly increased to greater than 1.2-fold.

In present study, supplementation of cholesterol cause lipid peroxication in fatty liver and probably elevated in the levels of GOT and GPT. But rat fed flavonoids (quercetin, kaempferol, isorhamnetin), terpene lactones (bilobalide, ginkgolides) rich Gb extract showed a decreased the levels of GOT and GPT compared with control. Therefore, we can suggest that Gb extract is effective of inhibiting inflammation and preventing harm of liver.

Table 6. Effects of *Ginkgo biloba* powder and extract on the plasma GOT and GPT in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Plasma (Unit/l)			
GOT[1) *]	77.82 ± 13.35[a)]	64.86 ± 17.57[ab)]	56.99 ± 19.42[b)]
GPT[2) *]	10.96 ± 6.67[a)]	7.86 ± 4.33[ab)]	4.90 ± 2.26[b)]

1) GOT (AST) : Glutamic oxaloacetic transaminase

2) GPT (ALT) : Glutamic pyruvic transaminase

Values are means ± SD of 10 rats

* Values in the same row not sharing the same superscript differ ($p<0.05$)

6. Hematocrit and Whole Blood Platelet Aggregation

Hematocrit and whole blood platelet aggregation are shown in Table 7.

Hematocrit was not statistically different among groups. The maximum and initial slope of whole blood platelet aggregation were significantly decreased in both Gb groups compared with the control and showed a significant difference between the control and the Gb extract ($p<0.05$).

Blood platelets are required for hemostatic plug formation when normal vessels are injured. The platelet aggregation is stimulated by ADP, thromboxane (TXA_2), and α-2 receptor activation. Platelet activation initiates the arachidonic acid pathway to produce TXA_2. Schwartz *et al.* (1990) and Cho *et al.* (2007) reported that interactions between platelets and collagen can cause circulatory disorders, such as thrombosis, and myocardial infraction.

Carvalho *et al.* (1974) reported that an increased platelet function is related to hyperlipoproteinmia. Stuart *et al.* (1980) showed that cholesterol-rich human platelet had hypersensitivity to TXA_2 which activates platelet aggregation. According to recently studies, the two factors, platelet aggregation and plasma cholesterol level, are related each other. Sugatani *et al.* (2004) reported that platelet aggregation factor (PAF) actyltransferase in PAF biosynthesis was increased by LDL-cholesterol and decreased by tea catechin. Akiba *et al.* (1998) reported that ginkgolides A, B and C inhibited platelet-activating factor-induced aggregation, but not oxidant-induced aggregation. The suppressive effect of the extract is specific on platelet aggregation stimulated by oxidative stress, and that this effect is involved in the mechanism related to its protective effect upon cerebral or myocardial injuries. Cho *et al.* (2007) showed that ginkgolide C significantly inhibited the collagen-stimulated platelet aggregation. Koch (2005) showed that the

PAF antagonistic effect of ginkgolides could be responsible for hemorrhage in patients taking Gb extract 761. Kudolo *et al.* (2002) observed that Gb extract has been shown to significantly reduce platelet aggregation in healthy subjects and in subjects suffering from type 2 diabetes mellitus.

In present study, platelet of rat fed ginkgolide rich Gb extract showed a decreased platelet aggregation and suggested that ginkgolides may have antiplatelet actions preventing thrombosis and stroke.

Table 7. Effects of *Ginkgo biloba* powder and extract on hematocrit and platelet aggregation in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Hematocrit (%)	41.54 ± 2.64	41.67 ± 1.89	41.67 ± 2.16
Platelet Aggregation			
Maximum[1)] (Ω) *	11.58 ± 1.19[a)]	10.50 ± 1.05[ab)]	9.98 ± 1.11[b)]
Initial Slope[2)] (Ω/min) *	12.69 ± 2.18[a)]	11.07 ± 1.61[ab)]	10.67 ± 1.04[b)]

1) Maximum aggregation is ohm at the point where aggregate dissociated

2) Initial slope ohm change for the first one minute of aggregation

Values are means ± SD of 10 rats

* Values in the same row not sharing the same superscript differ ($p<0.05$)

7. Erythrocyte Na^+ Efflux

Erythrocyte Na^+ efflux is shown in Table 8. Intracellular Na^+ of groups with Gb were higher than the control, and there is significantly different between the control and the Gb extract ($p<0.05$). Intracellular K^+ was also increased in all the Gb groups compared with the control, but not statistically different. Intracellular K^+ is 100 times higher than intracellular Na^+.

Na^+ efflux through plasma membranes is composed of Na-K ATPase (Na-K pump), Na-K cotransport, Na-passive transport. Na-K ATPase was increased in both Gb groups compared with the control, showing a significant difference between the control and the Gb extract ($p<0.05$). Na-K cotransport was increased in all Gb groups, but not statistically different between each groups. Na-passive transport was decreased in the Gb powder compared with the control, but was increased in the Gb extract compared with the control. On the whole, total Na^+ efflux was increased in all Gb groups compared with the control and showing a significant difference between the control and the Gb extract ($p<0.05$).

The Na-K ATPase is a membrane-bound enzyme that plays a crucial role in cellular ion homeostasis. Na-K ATPase pumps out 3 Na^+ in exchange 2 K^+ into cell, transporting these ions against concentration using one ATP. Na-K ATPase is known to play roles in Na^+ reabsorption in collecting duct of kidney and glucose absorption in intestine.

Ferrandi *et al.* (1996) observed that both young and adult milan hypertensive rats (MHS) are characterized by an increased enzymatic activity of the outer medulla Na-K ATPase. This increase is already present before the development of hypertension and is due to a higher number of functionally active pump sites on the cell membrane surface.

On the other hand, Hanaa *et al.* (2009) reported that significant increase in striatum Na-K ATPase activity of Gb extract group was occurred at all experimental days as compared to control. The protective effect of the present medicinal plants could be due to the antioxidant properties of Gb extract, Nicolson *et al.* (2007) and Hanaa *et al.* (2009) demonstrated that administration of anti-oxidants can prevent excess oxidative membrane damage, restore mitochondrial and other cellular membrane functions. Also Pierre *et al.* (1999; 2008) indicated that the observations of cellular adhesion and Na-K ATPase suggest that Gb extract 761 can prevent the changes in endothelial function elicited by exposure to oxidized LDL-cholesterol. Moreover Gb extract 761 prevents lipoperoxidation and the impairment of active ion transport that accompanies cerebral ischemia.

In the present study, Na efflux (Na-K ATPase, Na-K cotransport, and Na-passive transport) from rats of Gb extract showed the highest in all of them and suggested flavonoids (quercetin, kaempferol, isorhamnetin), terpene lactones (bilobalide, ginkgolides), proanthocyanidins, and catechins of *Ginkgo biloba* may have anti-oxidation action of cell membrane and prevent plenty of Na^+ efflux.

However, it is still difficult to draw definitive conclusions regarding the specific function of Na-channel and disease.

8. Angiotensin Converting Enzyme (ACE) Inhibitor Activity

Angiotensin converting enzyme (ACE) inhibitor activity is shown Table 8. ACE inhibitor activity was tended to increase in all the Gb groups but was not statistically different between groups.

ACE inhibitors obstruct the conversion of angiotensin Ⅰ to angiotensin Ⅱ. Angiotensin Ⅱ causes the muscles surrounding blood vessels to contract, narrowing the vessels thereby increasing the blood pressure. It also stimulates adrenal cortisol to release aldosterone which plays Na^{+} reabsorption in renal tubule.

Kwon *et al.* (2006) tested several purified compounds in relation to the ACE inhibition and they observed that among these quercetin had no inhibitory activity. However, Persson *et al.* (2008) showed that Gb extract inhibits ACE activity. A flavonol (quercetin and/or homologs) is the main component responsible for the inhibitory effect of ACE activity. The mechanism of the allopathic ACE inhibitors is their ability to bind Zn^{2+} at the active site of ACE. Flavonoids are known to be able to chelate metal ions such as Zn^{2+} and Fe^{2+}. Satoh and Nishida (2004) reported that terpene-lactones is no effects on ACE activity so the ginkolides A, B, C and bilobalide are not responsible for the beneficial effects on ACE activity due to treatment with the whole extract. On the other hand, Liebgott *et al.* (2000) and Persson *et al.* (2008) reported that synergistic effects of the ginkgolides and bilobalide present in Gb extract can not be excluded. Pinto *et al.* (2009) indicated that no correlation was found between total phenolics and antioxidant activity. In relation to the ACE inhibition, only ethanolic extracts had inhibitory activity. Kubota *et al.* (2006) reported that reduced salt-related elevation of blood pressure in hypertensive rats fed with the Gb extract 761 and suggested that the flavonoids present in the extract may be responsible for such an effect.

But Yukari *et al.* (2008) evaluated the long-term feeding of Gb extract in hypertensive rats and observed no significant decrease on the blood pressure.

In present study, lung tissues from rats fed Gb powder and extract showed a increased ACE inhibitor activity, suggesting flavonoids and terpene lactones of *Ginkgo biloba* might have ACE inhibitor activity, consequently lowering blood pressure by relaxation of blood vessels and reducing renal Na^+ reabsorption. These results could, at least partially, explain the beneficial effects on the CVD including hypertension and stroke.

Table 8. Effects of *Ginkgo biloba* powder and extract on erythrocyte Na^+ efflux and Angiotensin Converting Enzyme (ACE) inhibitor activity in rats

	Control	*Ginkgo biloba* Powder	*Ginkgo biloba* Extract
Intracellular[1] (mmol/l RBC)			
Na^+ *	1.58 ± 0.76[b]	1.87 ± 0.71[ab]	2.45 ± 0.66[a]
K^+	109.05 ± 34.62	115.68 ± 19.59	122.82 ± 14.13
Na^+ Efflux (mmol/l RBC/hr)			
Na-K ATPase[2] *	0.75 ± 0.64[b]	1.39 ± 0.98[ab]	2.19 ± 1.08[a]
Na-K cotransport[3]	0.57 ± 0.32	0.62 ± 0.25	0.67 ± 0.36
Na-passive transport[4]	0.33 ± 0.18	0.25 ± 0.13	0.47 ± 0.37
Total Na^+ Efflux (mmol/l RBC/hr) *	1.65 ± 0.84[b]	2.26 ± 0.94[b]	3.32 ± 1.12[a]
ACE Inhibition activity rate[5] (%)	61.11 ± 10.46	69.16 ± 13.81	65.78 ± 6.27

1) Intracellular Na^+ & K^+ ; upper values are for intact red blood cells

2) Na-K ATPase is ouabain sensitive Na efflux through Na-pump

3) Na-K cotransport is furosemide sensitive Na efflux through Na-pump

4) Na-passive transport is Na efflux under the blockage of Na-K ATPase & Na-K cotransport

5) ACE inhibition activity rate (%) = [1 - (Sa - Sb) / (Sb)] × 100

· Sa : Absorbance of sample with substrate (HHL)

· Sb : Absorbance of sample without substrate (HHL)

Values are means ± SD of 10 rats

* Values in the same row not sharing the same superscript differ ($p<0.05$)

Ⅳ. CONCLUSIONS

This study was carried out to compare the effect of 10% *Ginkgo biloba* (Gb) powder, 3% Gb extract on plasma and liver lipids, RBC Na^+ efflux and ACE, platelet aggregation and GOT/GPT using rats fed diets containing 0.5% of cholesterol diets.

1. The final body weight was lower in both *Ginkgo biloba* (Gb) groups than the control group; Gb extract had lower average daily gain (ADG) with the same feed intake ($p<0.05$), therefore the lower feed efficiency ratio (FER) implying the antiobese effect.

2. Plasma total-cholesterol and triglyceride were not different among groups. HDL-cholesterol was increased in both Gb groups and there was significant difference between Gb powder and the control ($p<0.05$).

Blood glucose was tended to decreased in both Gb groups, showing the significant difference between Gb extract and the control ($p<0.05$).

3. The liver total-cholesterol and triglyceride were decreased in both Gb groups, but not statistically different.

4. Hepatic thiobarbituric acid reactive substances (TBARS) production in both liver and platelet rich plasma (PRP) was the least in Gb extract among groups, but not statistically different.

5. GOT and GPT were decreased in both Gb groups with significant differences between Gb extract and the control ($p<0.05$), implying Gb may have liver protective effects in hypercholesterolemic rats.

6. Gb did not affect hematocrit.

Platelet aggregation in the maximum and the initial slope were decreased in both Gb groups with the statistical significance between Gb extract and the control ($p<0.05$), showing the antiplatelet effect of Gb.

7. Intracellular Na^+ and K^+ were tended to increase in Gb groups, and intracellular Na^+ was significant difference between Gb extract and the control ($p<0.05$). Na-K ATPase was tended to increase in both Gb groups with significant difference between Gb extract and the control ($p<0.05$).

Total Na^+ efflux was increased in both Gb groups with the statistical significance between Gb extract and the contol ($p<0.05$).

ACE inhibitory effect was higher in both Gb groups than the control, implying that Gb depresses ACE, consequently decreasing angiotensin Ⅱ and blood pressure.

Ginkgo biloba which contains bioactive ginkgolide and bilobalide seems to have protective effects from cardiovascular diseases with favorable actions on HDL-cholesterol, platelet aggregation and erythrocyte Na^+ channels.

Ⅴ. REFERENCES

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Ⅵ. 초 록

현대사회는 급속한 경제 성장과 생활양식의 서구화로 고지방 섭취의 증가, 스트레스 증가, 운동부족 등에 의해 순심혈관계질환의 발병률 및 사망률이 증가하고 있다. 은행잎에서 추출되는 물질(flavone glycosides, terpene lactones)들은 혈액순환에 효과적인 것으로 알려져 있으며, 현재 시중에서 혈액순환 개선제로 사용되고 있다.

본 연구에서는 은행잎 분말과 추출물의 식이가 고콜레스테롤혈증 흰쥐의 혈장과 간의 지질 수준, 혈소판 응집 및 적혈구 세포막의 Na^+ 유출에 미치는 영향에 대해서 연구하였다.

4주간 0.5%의 콜레스테롤을 함유한 식이에 은행잎 분말 10%와 추출물 3%을 각각 첨가하여 공급하였다. 최종 체중은 대조군 보다 은행잎 첨가군에서 낮은 경향을 보였다. 평균 일일 증체량에서 은행잎 추출물군이 유의적으로 감소하였고 ($p<0.05$), 식이섭취 비율 또한 낮은 경향을 보였다. 혈장 총콜레스테롤과 중성지방에서는 유의적인 차이가 없었다. HDL-콜레스테롤에서 은행잎 첨가군 모두 증가하는 경향을 보였고, 은행잎 분말군이 통계적 유의차를 보였다 ($p<0.05$). 혈당은 은행잎 첨가군에서 모두 감소하는 경향을 보였고, 은행잎 추출물군이 유의적으로 감소하였다 ($p<0.05$). 간의 총콜레스테롤과 중성지방은 은행잎 첨가군에서 모두 감소하였으나, 유의적인 차이는 없었다. 간과 혈장(PRP)의 TBARS 생성에 있어서 은행잎 추출물군이 가장 낮게 나타났으나 유의적인 차이는 없었다. GOT/GPT에서 은행잎 첨가군 모두 감소하는 경향을 보였고, 특히 은행잎 추출물군에서 유의적인 차이를 보였다 ($p<0.05$). 혈소판 초기응집곡선과 최대 응집치에 있어서 은행잎 첨가군 모두 감소하는 경향을 보였고, 은행잎 추출물군은 유의적으로 감소하였다 ($p<0.05$). 세포내 Na^+와 K^+는 은행잎 첨가군 모두 증가하는 경향을 보였고, 특히 세포내 Na^+는 은행잎 추출물군에서 유의적인 차이를 보였다 ($p<0.05$). Na-K ATPase에서 은행잎 첨가군 모두 증가하는 경향을 보였고, 특히 은행잎 추출물군은 유의적으로 증가하였다 ($p<0.05$). 총 Na^+ efflux에서도

은행잎 추출물군은 통계적 유의차를 보였다 ($p<0.05$). ACE 억제 효과에서 은행잎 첨가군이 높은 경향을 보였으나 유의적인 차이는 없었다.

본 연구의 결과를 종합해 볼 때, 은행잎에 함유된 생리활성물질인 ginkoglide와 bilobalide가 HDL-콜레스테롤, 혈소판 응집 및 적혈구막 Na^+ 유출에 유의적인 차이를 보였고 이것은 심순혈관계질환에 긍정적인 영향을 미칠것으로 사료된다.