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Toxicology of *Kalopanax pictus* Extract and Hematological Effect of the Isolated Anti-Rheumatoidal Kalopanaxsaponin A on the Freunds Complete Adjuvant Reagent-Treated Rat

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We have reported that kalopanaxsaponin A (KPS-A) isolated from *Kalopanax pictus* have anti-rheumatoidal activity in the rat treated with Freunds complete adjuvant (FCA) reagent. In addition, it has been also reported that KPS-A is a potent antioxidant in the rheumatoidal rat. This research was undertaken to examine whether the saponins of KPS-A and -I could adjust the abnormal lipid metabolisms and hematological changes in immunological diseases. KPS-A significantly inhibited the increases in both triglycerides and total proteins in addition to the decrease in total cholesterol induced by FCA reagent treatment. KPS-A treatment decreased the number of leucocytes elevated by FCA reagent treatment. Excess dose of the methanol extract produced no severe toxicity on the body weight, wet organ weights and hepatic functions. Since LD_{50} value of *K. pictus* methanol extract was shown to be 4,033 mg/kg, it could be estimated to be a safe agent for anti-rheumatoidal herbal medicines.

Key words: *Kalopanax pictus*, Kalopanaxsaponin A, Freunds complete adjuvant, toxicity, hematology, lipid metabolism

INTRODUCTION

Kalopanacis Cortex, the stem bark of *Kalopanax pictus* Nakai, has been used, most of all, for the treatment of rheumatoidal arthritis in the folkloric society of Korea. However, this is also applicable for the diseases of adult people such as neurotic pain and *diabetes mellitus* (Moon, 1991). This traditional drug contains several of kalopanaxsaponins as major components and others such as liriodendrin, syringin and coniferylaldehyde (Sano *et al.*, 1991; Shao *et al.*, 1991a and b). These saponins are contained in this crude drug as monodesmosidic and bisdesmosidic forms of hederagenin. Previously, we have reported various biological activities of these saponins including cytotoxicity, antimutagenicity (Lee *et al.*, 2000), anti-diabetes mellitus (Park *et al.*, 1998) and growth inhibition of fungus (Kim *et al.*, 1998).

Recently, we have reported anti-rheumatoidal action of kalopanaxsaponin A (KPS-A) in Freunds complete reagent (FCA)-treated rats along with analgesic activities of this saponin (Choi *et al.*, 2000a). In our continuing studies, we also found that this saponin could be a potent antioxidant due to the actions on the hepatic drug-metabolizing system in FCA reagent-treated rats (Choi *et al.*, 2000b). FCA reagent is dead *Mycobacterium tuberculosis* and surfactant-added fluid paraffine (Ezeamuzie *et al.*, 1992; Saito *et al.*, 1990). FCA reagent, in general, induces chronic inflammation in two weeks in contrast to the induction of acute inflammation by carrageenan administration. In our previous studies, the most potent anti-rheumatoidal principle of *K. pictus* was revealed to be KPS-A, which was partitioned in an ehtylacetate-soluble fraction (EtOAc fraction). The EtOAc fraction was the most active one containing hederagenin monodesmosides than other ones. The anti-rheumatoidal activity of *n*-BuOH fraction was not significant, which contained hederagenin bisdesmosides rather than its monodesmosides (Choi *et al.*, 2000c). Therefore, we studied hematological effects of EtOAc extract fractionated from *K. pictus* methanol extract (MeOH extract) and the saponins, KPS-A and I, isolated

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from the EtOAc extract.

Rheumatoidal arthritis is a disease that the inflammation occurs on joints by self-immunological reaction in the body. It is well known that high levels of reactive oxygen species in patients with rheumatoidal disease provoke lipid peroxidation and result in several of tissue injuries (Simon *et al.*, 1981). Self-immunological diseases are known to result in abnormalities of lipid metabolism (Blanchette-Mackie *et al.*, 1989). In the present study, we found that FCA reagent treatment caused the increases in triglyceride and total protein in contrast to the decrease of total cholesterol. Anti-rheumatoidal saponin, kalopanaxsaponin A, considerably normalized those changes. Based on the capability of Kalopanacis Cortex to treat rheumatoidal arthritis, we conducted toxicological tests together with the hematological investigation on Kalopanacis Cortex and KPS-A and I in FCA reagent-treated rats.

MATERIALS AND METHODS

Chemicals

The reagents of NAD^+ and NADH were supplied by Sigma Co. (USA). Freunds complete adjuvant (FCA) reagent was purchased from Difco Co.. Kit reagents of aminotranferase and γ-glutamyltranspeptidase were purchased from Youngdong Pharm. Co. and those of sorbitol dehydrogenase, lactate dehydrogenase, cholesterol and triglyceride were supplied by Sigma Co..

Plant material and extraction, fractionation and Isolation

Kalopanax pictus Nakai was collected in August, 1998 in Kangwon province, Korea, and the plant was identified by prof. S.Y. Yun (Division of Applied Plant Sciences, Sangji University, Wonju, Korea). A voucher specimen (*#NATCHEM*-19) was deposited in the herbarium of Life Science and Natural Resources, Sangji University, Wonju, Korea. Dried stem bark (4.8 kg) of *Kalopanax pictus* was cut and extracted three times under reflux. The extract was filtered and evaporated on a rotary evaporator under reduced pressure to give a viscous mass (630 g) of MeOH extract. This extract was fractionated and isolated to give EtOAc extract and KPS-A and I as reported previously (Choi *et al.*, 2000c). Physicochemical data of isolated saponins were taken and the values were in good agreement with those of authentic specimens, respectively. Optical rotations were measured at 22°C.

KPS-A (Kalopanaxsaponin A): Colorless needles from MeOH, mp 265-268°C (dec.), $[a]_D$ +18°C (MeOH, *c* 0.3), ^{1}H-NMR and ^{13}C-NMR: literature (Park *et al.*, 1999)

KPS-I (Kalopanaxsaponin I): Colorless needles from MeOH, mp 218-220°C (dec.), $[\alpha]_D$ +11° (MeOH, *c* 0.28), ^{1}H-NMR and ^{13}C-NMR: literature (Park *et al.* 1999)

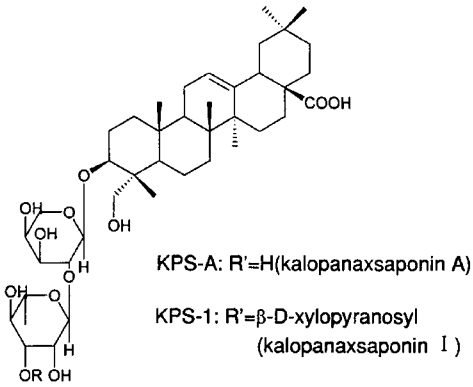

Fig. 1. Structures of kalopanaxsaponin A and -I isolated from the stem bark of *K. pictus*

Animals

Experimental animals were purchased from Korean Experimental Animal Co. and were adapted in a constant condition (temperature: 20 ± 2°C, humidity: 40-60%, light/dark cycle: 12 h) for more than two weeks. For the animal experiment, ICR male mice weighing 20-25 g and Sprague-Dawley male rats weighing 100-120 g were used. From 24 h before the experiments, only water was given to the animals. Considering the variation of enzyme activity during one day, the animals were sacrificed at fixed time (10:00 A.M.-12:00 A.M.).

Induction of rheumatoidal disease and administraion of test samples

For the induction of rheumatoidal disease, each 0.05 ml of FCA reagent was injected to the sole of right feet of rats. After two weeks, the induction of inflammation in rats was confirmed. The test samples, EtOAc fractions and KPS-A and -I were dissolved in dimethylsulfoxide (DMSO) and those solutions with various concentrations were prepared by being diluted with saline. After the preliminary experiment on the dose, 250 mg/kg and 500 mg/kg of EtOAc fraction were administered orally but 5, 10 and 20 mg/kg of the isolated saponins were injected intraperitoneally.

Determination of LDH activity in blood

For determination of lactate dehydrogenase (LDH) activity in FCA reagent-treated rats, the lactate substrate method (Young *et al.*, 1975), which is coloring methods, was used. This experiment produces diformazine by being reduced from nitrotetrazolium blue under 1-methoxy-5-ethylphenazynium methylsulfate by NADH formed in the dehydrogenation process from lactate to pyruvate.

Determination of the contents of triglyceride and total cholesterol

The content of total cholesterol was determined by the enzyme method dependent on kit reagent (Stein, 1986). The enzyme solution 3.0 ml was added to 200 ml of the serum and mixed. After it was incubated at 37°C for 5 min, the absorbance of the resultant solution was measured at 500 nm. The triglyceride content was determined using a kit reagent according to the McGowns method (McGown *et al.*, 1983).

Measurement of erythrocyte, leucocyte, hemoglobin, hematocrit and platelet and determination of total protein

Effects on erythrocyte, leucocyte, hemoglobin, hematocrit and platelet were measured using Sysmex K-1000 Cell-Counter according to Fonios method (Kim *et al.*, 1984). The content of total protein was determined using the kit according to Biuret's method (Franco *et al.*, 1971).

Measurement of body weights and organ weights

To search for the effects of test samples on the experimental animal, the body weight was recorded every week (1-4 weeks) after the starting day of the sample administration with various doses (250, 500, 1,000, 1,500 mg/kg). The differences between obseved body weights and that of the initial day were recorded. Four weeks after the initial day, the weight of each organ was measured and calculated to the weight per 100 g body weight.

Determinations of AST, ALT, LDH, SDH, and γ-GT activities in serum

The activities of aminotransferases (AST, ALT) were determined by Reitman and Frankels methods (Reitman *et al.*, 1957). In brief, the substrate solution of 1.0 ml were added to the kit reagent, alanine transaminase, containing 1,780 mg DL-alanine and 29.2 mg α-ketoglutaric acid per 100 ml and preincubated at 37°C for 5 min. After the preincubation, alanine transaminase was reacted for 30 min and aspartate transaminase for 60 min. The reagent, 2,4-dinitrophenylhydrazine (19.8 mg per 100 ml solution), was added to terminate the reaction. This was mixed with 0.4N-NaOH and allowed to stand for 10 min at room temperature. The absorbance of this final one was taken at 505 nm using UV spectrophotometer. By the calibration curve, the activity was calculated and expressed as karmen unit/ml serum. The activity of sorbitol dehydrogenase (SDH) was determined by Weinsers method (Weisner *et al.*, 1965). The reagents of 0.2M triethanolamine HCl buffer (pH 7.4) and 12 mM NADH in 1% $NaHCO_3$ were added to 200 ml of serum and followed by adding 4M fructose immediately. The absorbance of this final one was measured at 340 nm on a UV spectrophotometer. Three minutes after this measurement, the absorbance was again recorded at the same wavelengths. Using the difference of the two absorbances, SDH activity was determined as the unit of mU/ml that represents the oxidized amount from NADH to NAD^+ for 3 min by fructose. For the activity of γ-glutamyltransferase (γ-GT) (Szasz, 1969), 100 ml of the serum was added to the buffer solution (pH 8.2) containing 40 nmole of glycylglycine. The production rate of *p*-nitroaniline was determined by the measurement of the absorbance of this final solution.

Determination of LD_{50}

LD_{50} of the extract in mice was determined in five groups with 30 mice each by Behrens-Karber method (Ju *et al.*, 1993). In brief, variable doses of the MeOH extracts (3,000, 3,500, 4,000, 4,500 and 5,000 mg/kg) were injected intraperitoneally. Twenty four h after the injection, the survival or death was observed and the LD_{50} value was calculated according to the following equation: $LD_{50}=D-\Sigma zd/m$, where D is a dose occurring complete death, and z is number of dead animals by the adjacent dose amount, and d is an amount difference between the adjacent dose amounts, and m is number of animals in a group.

Statistics

The data were shown by means ± S.D.. Statistical analysis was done by Duncan's multiple range test.

RESULTS

Effect on LDH activity in FCA reagent-treated rats

Table I. Effect of EtOAc extract of *K. pictus* and KPS-A and I on the LDH activity in rats by Freunds complete adjuvant reagent

Group	Dose (mg/kg)	Activity Wroblewski unit (U/l)	Inhibition rate (%)
Normal		724.6 ± 46.00^e	100
Control		1111.8 ± 87.95^a	0
EtOAC extract	250	903.7 ± 25.70^c	53.7
	500	879.4 ± 30.00^{c,d}	60.0
KPA-A	5	906.4 ± 28.43^c	53.0
	10	853.2 ± 33.23^{c,d}	66.8
	20	830.1 ± 24.57^d	72.8
KPS-I	5	909.3 ± 23.79^c	52.3
	10	1006.5 ± 32.98^b	27.2
	20	903.9 ± 26.55^c	53.7

Normal: Untreated with FCA reagent; Control: Treated with FCA reagent; Dose: EtOAc extract (*p.o.*), KPS-A and I (*i.p.*); Values represent means ± S.D. (n=10).
Values with the same superscripts are not significantly different from normal ($p<0.05$). ns: not significant.

In FCA reagent-treated rats, effects of EtOAc (250 and 500 mg/kg, *p.o.*) extract and KPS-A (5, 10, 20 mg/kg, *i.p.*) and I (5, 10, 20 mg/kg, *i.p.*) on LDH activity in blood were shown in Table I. The only FCA reagent-treated group significantly elevated this enzyme activity from that of a normal group. Administration of three test samples decreased the LDH activity elevated in the control group. Among tested groups, KPS-A treatment group with 20 mg/kg showed the most potent activity by 72.8% reduction.

Effect on triglyceride, total cholesterol total protein in FCA reagent-treated rats

Effects of EtOAc extract, KPS-A and -I on the serum triglyceride were shown in Table II. FCA treatment considerably elevated that of a normal group. Oral administration of EtOAc extract and intraperitoneal administration of KPS-A decreased these values to a nearly normal value. However, KPS-I showed a dose-independent action on triglyceride content. Effects of three test samples on total cholesterol were shown in Table II. FCA reagent treatment significantly reduced serum total cholesterol in rats but KPS-A treatment increased this cholesterol value to a normal range. Since KPS-I did not show this effect, one of active principles was suggested to be KPS-A. These activities was observed dose-dependetly. As shown in Table II, FCA reagent treatment significantly elevated total protein. Oral administration of EtOAc extract and intraperitoneal administration of KPS-A decreased the serum total protein elevated. However, KPS-I treatment showed no significant decrease of total protein of a control group.

Hematological effect in FCA reagent-treated rats

Table III shows the effect of the EtOAc extract and its saponin constituents on erythrocyte, leucocyte, hemo-

Table II. Effect of EtOAc extract of *K. pictus* and KPS-A and -I on the the concentrations of triglyceride, total cholesterol and total protein in rats by Freunds complete adjuvant reagent

Group	Dose (mg/kg)	Triglyceride	Total cholesterol	Total protein
		mg/dl	mg/dl	g/dl
Normal		83.8 ± 4.08^{d} (100)	78.6 ± 3.28^{a} (100)	$7.71 \pm 0.25^{a,b,c}$ (100)
Control		117.6 ± 3.64^{a} (0)	$69.8 \pm 5.00^{b,c,d}$ (0)	8.29 ± 0.36^{a} (0)
EtOAc extract	250	97.4 ± 2.37^{c} (59.8)	$68.3 \pm 5.94^{b,c,d}$ (12.7)	$8.10 \pm 0.33^{a,b}$ (32.7)
	500	85.9 ± 3.01^{d} (93.8)	$70.5 \pm 3.92^{b,c,d}$ (31.4)	$7.84 \pm 0.46^{a,b,c}$ (77.6)
KPS-A	5	95.7 ± 2.90^{c} (64.8)	$69.0 \pm 3.21^{b,c,d}$ (18.6)	$7.65 \pm 0.16^{b,c}$ (110.3)
	10	88.2 ± 1.57^{d} (87.0)	$73.3 \pm 2.45^{a,b,c}$ (55.1)	7.26 ± 0.19^{c} (177.6)
	20	82.8 ± 3.11^{d} (103.0)	$75.3 \pm 5.70^{a,b}$ (72.0)	7.33 ± 0.41^{c} (165.6)
KPS-I	5	106.9 ± 5.23^{b} (31.7)	65.4 ± 3.94^{d} (-11.8)	$8.19 \pm 0.62^{a,b}$ (29.7)
	10	98.8 ± 4.13^{c} (55.6)	$67.9 \pm 3.84^{c,d}$ (9.3)	$8.06 \pm 0.15^{a,b}$ (39.7)
	20	119.4 ± 2.64^{a} (-5.3)	$66.3 \pm 2.11^{c,d}$ (-4.2)	$8.00 \pm 0.23^{a,b}$ (50.0)

Dose: EtOAc extract (*p.o.*), KPS-A and -I (*i.p.*); Values represent means ± S.D. (n=10). Values with the same superscript are not significantly different from normal ($p<0.05$). ns: not significant. Values in the parenthses represent recovery rate (%).

Table III. Effect of EtOAc extract of *K. pictus* and KPS-A and I on the hematological values in rats by Freunds complete adjuvant reagent

Group	Dose	Erythrocyte	Leucocyte	Hemoglobin	Hematocrit	Platelet
	kg/ml	$10^6/\mu l$	$10^6/\mu l$	g/dl	%	$10^3/\mu l$
Normal		7.4 ± 1.16^{ns}	14.4 ± 2.51^{d}	23.5 ± 3.33^{ns}	35.7 ± 3.65^{ns}	197.8 ± 12.80^{ns}
Control		8.1 ± 1.02	28.7 ± 5.02^{a}	24.3 ± 3.83	37.6 ± 2.91	200.6 ± 12.59
EtOAc extract	250	7.8 ± 0.70	$21.5 \pm 4.22^{b,c}$	23.8 ± 3.08	39.9 ± 2.12	193.7 ± 18.32
	500	8.1 ± 0.97	$24.7 \pm 2.07^{a,b,c}$	24.8 ± 3.26	38.6 ± 1.76	193.3 ± 14.71
KPS-A	5	7.7 ± 1.20	$24.3 \pm 2.53^{a,b,c}$	24.5 ± 3.38	36.7 ± 3.57	205.1 ± 8.68
	10	8.3 ± 1.06	20.1 ± 4.21^{c}	25.7 ± 3.45	37.4 ± 2.81	201.6 ± 12.51
	20	8.0 ± 0.71	$21.6 \pm 3.20^{b,c}$	23.9 ± 3.18	39.8 ± 2.43	198.7 ± 19.20
KPS-I	5	8.8 ± 0.56	$26.5 \pm 2.17^{a,b}$	26.8 ± 4.26	35.6 ± 2.76	200.3 ± 15.32
	10	7.9 ± 1.32	$24.2 \pm 3.22^{a,b,c}$	28.3 ± 5.18	40.0 ± 3.40	199.6 ± 17.28
	20	8.4 ± 1.33	$23.5 \pm 2.17^{a,b,c}$	27.1 ± 4.78	38.3 ± 3.76	203.5 ± 16.42

Dose: EtOAc extract (*p.o.*), KPS-A and -I (*i.p.*); Values represent means ± S.D. (n=10). Values with the same superscripts are not significantly different from normal ($p<0.05$). ns: not significant.

globin, hematocrit and platelet in FCA reagent-treated rats. FCA reagent-treatment exhibited no significant effects on the numbers of etythrocytes, hemoglobins, hematocrits and platelets. Further, KPS-A and I treatment also exhibited no significant effects on those of the control groups. Test samples of EtOAc extract and KPS-A and -I decreased the leucocyte number elevated by FCA reagent treatment. In this test, KPS-A significantly decreased the numbers of leucocyte elevated.

Toxicological Effect of the *K. pictus* MeOH extract on normal rats

As one of the tests on the toxicity of *K. pictus* MeOH extract, the increase of body weight gaining was examined according to the administration of the MeOH extract. As shown in Table IV, high doses more than 250 mg/kg showed significant increases but it seems that the effect was not serious. The prolongation of administration showed the body weight increased to some extent. Four weeks administration with dose amounts of 250, 500, 1,000 and 1,500 mg/kg exhibited no significant changes of the organ weights when compared with those of the normal rat. Table V shows each transformed value indi-cating the organ weight per 100 g body weight. The bio-chemical parameters of the activities of AST, ALT, SDH and γ-GT were measured according to variation of doses (250, 500, 1,000 and 1,500 mg/kg) for 4 weeks. This ex-periment was undertaken to examine the effects of *K. effect* on the liver function of the normal rat. Since the MeOH extract exhibited no significant changes of these parameters, it is not likely that this extract occur negative effect on the hepatic function on normal rats (Table VII). With the dose variation of 3,000-5,000 mg/kg of the MeOH extract, the samples were administered to the five groups (each 30 mice) and observed the death or survival. Table IV shows

Table IV. Effect of *K. pictus* MeOH extract on body weight gain of rats

Group	Dose	Body weight gain (g)			
	(mg/kg)	1 week	2 week	3 week	4 week
Normal		26.4 ± 2.50^{a}	37.8 ± 2.95^{b}	44.3 ± 3.68^{b}	52.5 ± 2.84^{a}
MeOH extract	250	25.6 ± 2.87^{a}	42.7 ± 3.35^{a}	$47.6 \pm 1.75^{a,b}$	$55.2 \pm 3.46^{b,c}$
	500	23.6 ± 1.98^{a}	46.4 ± 2.74^{a}	52.4 ± 3.03^{b}	$60.3 \pm 2.39^{a,b}$
	1,000	25.3 ± 2.49^{a}	44.8 ± 3.40^{a}	51.9 ± 3.53^{a}	64.6 ± 2.31^{a}
	1,500	25.1 ± 2.64^{a}	45.3 ± 3.08^{a}	52.7 ± 2.90^{a}	63.3 ± 3.45^{a}

Rats were orally administered with *K. pictus* MeOH extract (250, 500, 1,000, 1,500 mg/kg) daily for several weeks. Values represent means ± S.D. (n=10). Values with the same superscripts are not significantly different from normal ($p<0.05$).

Table V. Effect of *K. pictus* MeOH extract on wet weight of organs in rats

Group	Dose	g/100 g body weight			
	(mg/kg)	Liver	Kidney	Heart	Spleen
Normal		3.20 ± 0.18^{ns}	0.86 ± 0.07^{ns}	0.35 ± 0.04^{ns}	0.27 ± 0.05^{ns}
MeOH extract	250	3.32 ± 0.21	0.87 ± 0.03	0.32 ± 0.06	0.22 ± 0.03
	500	3.35 ± 0.26	0.85 ± 0.02	0.36 ± 0.03	0.25 ± 0.06
	1,000	3.33 ± 0.34	0.84 ± 0.04	0.33 ± 0.04	0.28 ± 0.07
	1,500	3.41 ± 0.18	0.83 ± 0.06	0.32 ± 0.05	0.23 ± 0.04

Rats were orally administered with *K. pictus* MeOH extract (250, 500, 1,000, 1,500 mg/kg) daily for several weeks. Values represent means ± S.D. (n=10). ns: not significant.

Table VI. Effect of *K. pictus* MeOH extract on the biochemical parameters of liver function in rats

Group	Dose	AST	ALT	SDH	γ-GT
	(mg/kg)	Karmen unit/ml		mU/ml	
Normal		65.7 ± 3.40^{ns}	35.4 ± 4.28^{ns}	19.1 ± 1.82^{ns}	26.0 ± 3.70^{ns}
MeOH extract	250	63.5 ± 4.15	33.2 ± 2.69	18.6 ± 1.59	23.9 ± 3.58
	500	65.3 ± 3.27	35.1 ± 3.75	20.8 ± 1.95	25.3 ± 2.17
	1,000	64.0 ± 2.38	34.2 ± 3.16	17.8 ± 1.37	24.2 ± 4.14
	1,500	68.4 ± 3.45	36.4 ± 4.35	19.5 ± 1.96	27.2 ± 3.00

Rats were orally administered with *K. pictus* MeOH extract (250, 500, 1,000, 1,500 mg/kg) daily for several weeks. Values represent means ± S.D. (n=10). ns: not significant.

Table VII. Toxicity test of *K. pictus* MeOH extract in mice for 50% lethal dose

Dose (mg/kg)	3,000	3,500	4,000	4,500	5,000
	Dead*/Treated				
MeOH extract	0/30	6/30	15/30	22/30	30/30

*: The number of dead mice for 24 h after intraperitoneal injection of sample

that the more dose amount results in the more death ratio. When the dose of 5,000 mg/kg was administered, any of mouse was survived. By the calculation of LD_{50}, the value was shown to be 4,033 mg/kg.

DISCUSSION

A control group treated with FCA reagent showed the changes in LDH activity, triglyceride, total cholesterol and total protein in the serum. The LDH activity was increased by FCA reagent treatment. This activity could be increased in the progressive muscular dystrophy, multiple myositis, myeloid leukemia, hemolytic anemia and lymphomia (Kim, 1980). Oral administration of EtOAc extract and intraperitoneal administration of these components, KPS-A and -I, decreased the LDH activity enhanced by FCA reagent treatment. Since the test samples decreased this activity of a control group, it was demonstrated indirectly that KPS-A was effective to rheumatoidal arthritis. To investigate the effects of KPS-A and -I and EtOAc extract on lipid metabolism in FCA reagent-treated rats, the contents of serum triglyceride and cholesterol were measured. The high level of triglyceride is found in patients with diseases of atherosclerosis, hyperlipemia and gout (Kim, 1980). Triglyceride level of KPS-A treatment group (20 mg/kg) was lowered to that of a normal group. In FCA-treated rats, the lowering effect of triglyceride by KPS-A was very strong. This is, to some extent, in agreement with the reports indicating the high viscosities of both total blood and serum in patients with rheumatoidal arthritis. Lower level of cholesterol than a normal range is found in patients with hypertyroidism, malignant tumors, inflammatory diseases, diffuse collagen disease and anemia (Kim, 1980). Dose increase of KPS-A recovered the reduced total cholesterol in a control group. Total serum protein is high in patients with chronic hepatitis, allergic hypersensitivity, purulent arthritis and self-immunological diseases, compared with a normal range (Grever *et al.*, 1980). KPS-A and -I decreased serum total protein level of a control group. From these observations, it seems likely that the administration of KPS-A adjusts abnormal lipid metabolism resulting from immunological reactions. It is also expected that the abnormal lipid metabolism of patients with rheumatoidal diseases could be adjusted by administration of *K. pictus* extract. Leucocytes are increased in infective diseases, inflammatory responses, tissue injuries, malignant tumors and leukemia (Kim, 1980). KPS-A and -I and EtOAc extract exhibited significant reductions of leucocyte numbers of a control group. Between all the treatment groups and a control group, each value of erythrocyte, hemoglobin, hematocrit and leucocyte was not different, respectively.

In order to demonstrate a safety of *K. pictus* extract for clinical applications, detailed toxicological tests were also conducted. The administrations of the MeOH extract with variations of both dose amounts and dose durations exhibited no changes in organ weights and hepatic enzyme activities of aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase. However, the admnistration of MeOH extract for more than 2 weeks and with more than 500 mg/kg dose produced slight increases of body weights. Nevertheless, the organ weights were not significantly changed. Further, LD_{50} value was found to be 4,033 mg/kg. This value was evaluated to belong to a slightly toxic class by the toxicological classification. It was known that many saponins had hemolytic action. In the present study, it was obviously found that oral administration of saponin did not produce hemolytic effect or other serious adverse effects. The toxicological studies of kalopanaxsaponins by intraperitoneal and intravenous administration are under investigation. This research will give informations on a wide application of natural saponins. In conclusion, the anti-rheumatoidal saponin of *K. pictus*, KPS-A, was suggested to be able to adjust abnormal lipid metabolism caused by immunological injuries and to avoid severe hematological changes besides the platelet number. Clinical application of *K. pictus* extract would be suitable for rheumatoidal diseases because it was belong to a slight toxic class by toxicological classification.

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REFERENCES

Blanchette-Mackie, E. J., Dwyer, N. K. and Amende, L. A., Cytochemical studies of lipid metabolism: immunogold probes for lipidprotein lipase and cholesterol. *Am. J. Amat.*, 185, 255-263 (1989).

Choi, J., Chun, H. W., Kim, S. H., Park, H. J. and Han, Y. N., Analgesic effect of *Kalopanax pictus* extract and its saponin components, and their inhibitory effect on Freunds complete adjuvant reagent-induced rheumatoidal arthritis in experimental animals. *The 49th Annual Convention of the Pharmaceutical Society of Korea & International Symposium*, 2000, 236 (2000a).

Choi J., Chun, H. W., Kim, S. H., Lee, K. T., Han, Y. N., Park, H. J., Kalopanaxsaponin A from *Kalopanax pictus*, a potent antioxidant in the rheumatoidal rat induced by Freunds complete adjuvant reagent. *The 49th Annual Convention of the Pharmaceutical Society of Korea & International Symposium*, 2000, 235-236 (2000b).

Choi, J. W., Han, Y. N., Lee, K. T., Kwak, T. S., Kwon, S. H. and Park, H. J., Anti-lipid peroxidative principles of the stem bark of *Kalopanax pictus Nakai*. The 49th Ann. Conv. Pharmaceut. Soc. Korea & International Sym. 2000, 188 (2000c).

Ezeamuzie, I. C. and Njoku, A. C., The role of neutrophils in acute and chronic inflammation in rats. *Afr. J. Med. Med. Sci.*, 21, 23-28 (1992).

Franco, J. A. and Savory, J., Evaluation of a "Kit" method for the measurement of total serum globulins. *Am. J. Clin. Pathol.*, 56, 538-542 (1971).

Grever, M. R., Thompson, V. N., Balcerzak, S. D. and Sagone, A. L., The effect of oxidant stress on human lymphocyte cytotoxicity. *Blood*, 56, 284-288 (1980).

Ju, W. K., Laboratory of pharmacology and toxicology, Shin-il Publishing Co., Seoul, p 53 (1993).

Kim, K. H., Clinical application of inspection results, Seoul, Komun-Sa, pp 66-107, 144-159, 164-170 (1980).

Kim, D. W., Bang, K. H., Rhee, Y. H., Lee, K. T. and Park, H. J., Growth inhibitory activities of *Kalopanax pictus* against Human Pathogenic Fungi. *Arch. Pharm. Res.*, 21, 688-691 (1998).

Kim, J. C. and Kim, J. K., Summary of clinical test methods, Seoul, Komun-Sa, p 242, 298, 303, 1112, 1149 (1984).

Lee, K. T., Sohn, I. C, Park, H. J., Kim, D. W., Jung, C. O. and Park, K. Y., Essential Moiety for Antimutagenic and Cytotoxic Activity of Hederagenin Monodesmosides and Bisdesmosides Isolated from the Stem Bark of *Kalopanax pictus*. *Planta Medica*, 66, 329-332 (2000).

McGowan, W., Artiss, J. D., Strandbergh, D. R.: A peroxidase coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.*, 29, 538-542 (1983).

Moon, K. S., The Components and Utilization of Medicinal Plants, Ilweolseogak, Pyongyang, p.419 (1991).

Park, H. J., Kim D. H., Choi, J. W., Park, J. H. and Han, Y. N., A potent anti-diabetic agent from *Kalopanax pitus*. *Arch. Pharm. Res.*, 21, 24-29 (1998).

Reitman, S., and Frankel, S., A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, 28, 56-60 (1957).

Saito, B. Ohashi, T. Togashi, M. and Koyanagi, T., The study of BDN induced bladder cancer in mice, Influence of Freund complete adjuvant and immunological reactions in mice. *Nippon Hinyokika Gakkai Zasshi*, 81, 993-996 (1990).

Sano, K., Sanada S., Ida, Y. and Shoji, J., Studies on the constituents of the stem bark of *Kalopanax pictus*. *Chem. Pharm. Bull.*, 39, 865-870 (1991).

Shao, C. J., Kasai, R., Xu, J. D. and Tanaka, O., Saponins from roots of *Kalopanax septemlobus* (Thunb.) Koidz., Ciqiu: Structures of kalopanax-saponins C, D, E and F. *Chem. Pharm. Bull.*, 37, 311-314 (1989a).

Shao, C. J., Kasai, R., Xu, J. D. and Tanaka, O., Saponins from leaves of *Kalopanax septemlobus* (Thunb.) Koidz: Structures of kalopanax-saponins La, Lb and Lc. *Chem. Pharm. Bull.*, 37, 3251-3252 (1989b).

Simon, R. H., Scoggin, C. M. and Patterson, D., Hydrogen peroxide causes the fetal injury to human fibroblasts exposed to oxygen radicals. *J. Biol. Chem.*, 256, 7181-7186 (1991).

Stein, E. A., Lipids, lipoproteins and apolipoproteins. In: Textbook of clinical chemistry, W. B. Saunders Co., Piladelphia, p 884 (1986).

Szasz, F., Kinetic photometric method for serum γ-glutamyltranspeptidase. *Clin. Chem.*, 15, 124-136 (1969).

Weisner, I. S., Rawnsley, H. M., Brooks, F. P. and Senior, J. R., Sorbitol dehydrogenase in the diagnosis of liver disease. *Am. J. Dig. Dis.*, 10, 147-154 (1965).

Young, D. S., Pestaner, L. E. and Gibberman, V., Effect of drugs on clinical laboratory tests. *Clin. Chem.*, 21, 1D-432D (1975).