Study of the main chemical components of Ganoderma lucidum

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[Purpose]

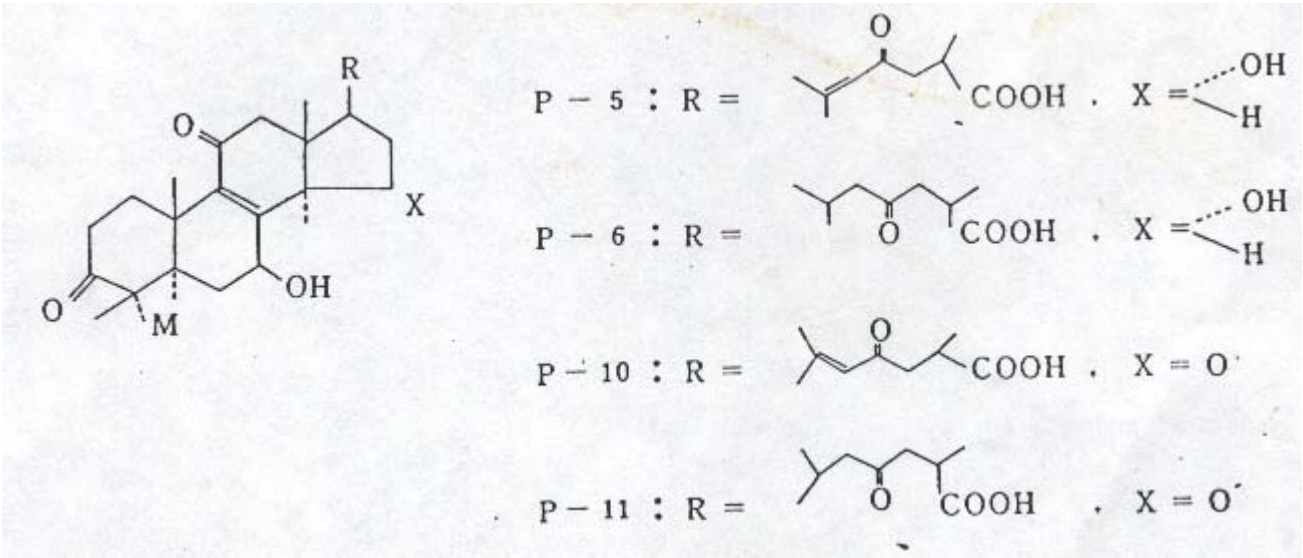
As part of the means for exerting quality control on Ganoderma lucidum 50% ethanol extracts were subjected to HPLC analysis and over a dozen peaks were observed. Four ingredients out of the peaks were isolated to study the structure of each individual component.

[Experiment and results]

The strongly acidic fractions which contain most of the more than one dozen peaks mentioned above, were purified with silica gel columns, LH20 columns, and sampled HPLC to isolate P-5, P-6, P-10, and P-ll.

It was found that the spectrum data of P-6 (C30 H44 O7) were the same as those of ganoderic acid A, which had already been isolated and its structure had been defined (T. Kubota et al. Heir. Cluin. Acta. 65, 611 (1982)].

According to the results of the examination of 'H and 13CNMR spectrum it was also found that P-5 (C30 H42 O7) was the D20, E type derivative of P-6, and that P-10 (C30 H40 O7) and P-ll (C30 H42 O7) had the oxidized forms of quindecahydroxyl of P-5 and P-6, respectively.



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(TRANSLATION)

Toxicological Studies of Ganoderma lucidum Karst

Mamoru Sugiura and Hitoshi Ito (Received September 1, 1977)

MATERIAL AND METHODS

1. Preparation of Test Substance

The preparation method is outlined in Fig. 1. A hot water extraction was initially prepared from Ganoderma lucidum and tested as Fr. A. Fr. A was further precipitated with alcohol and dialyzed to obtain Fr. B, a high molecular weight fraction composed mainly of polysaccharides, which was tested similarly.

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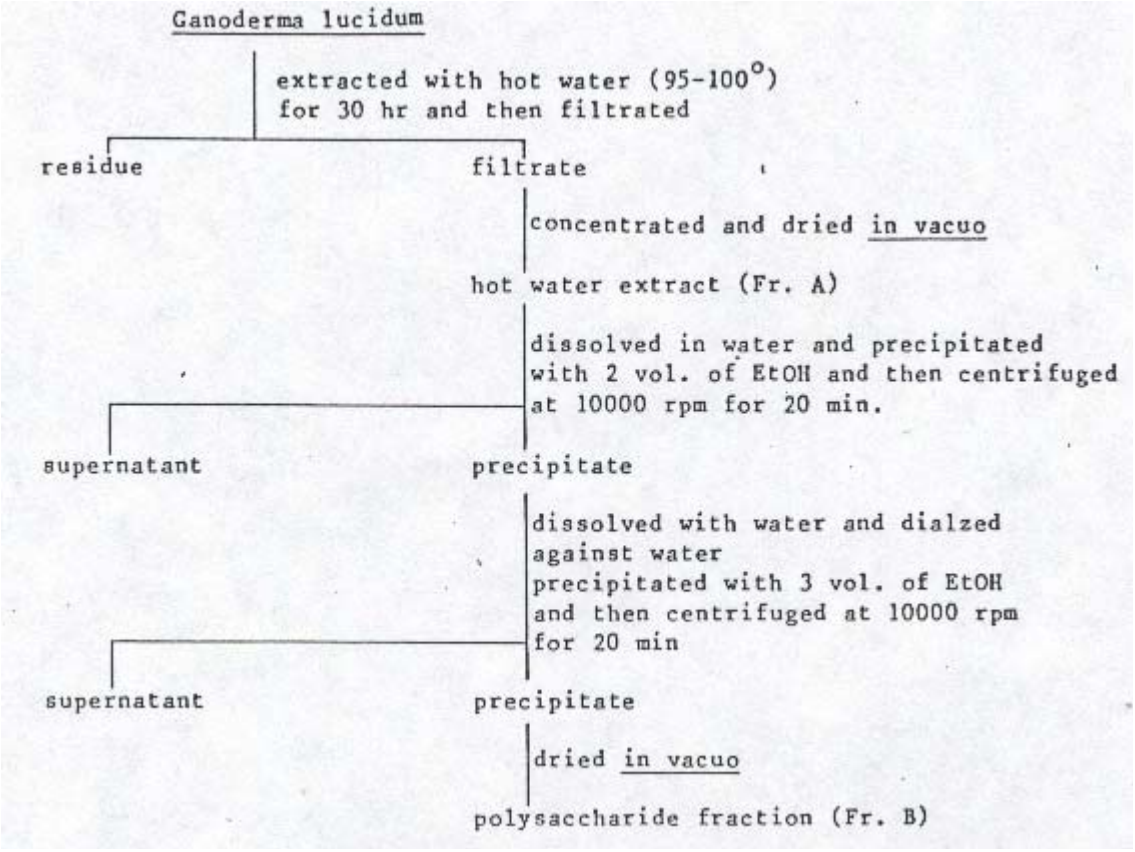

Above: Fig. 1. Procedure for Extraction of Polysaccharide. Fraction from Ganoderma lucidum

2. Chemical Composition of Test Substance

(1) Composition of Fr. A

The test substance is a saccharide composed principally of glucose. A minute quantity of ash was detected. The free amino acid composition is summarized in Table I.

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Below: TABLE 1. Free Amino Acids in Fr. A

Amino acid	mg%	Amino acid	mg%
Aspartic acid	20.0	Methionine	0.2
Threonine	5.8	Leucine	5.7
Serine	8.9	Isoleucine	0.5
Glutamic acid	4.6	Tyrosine	5.0
Proline	3.0	Phenylalanine	3.9
Glycine	4.9	Lysine	5.6
Alanine	17.9	Histidine	0.9
Valine	8.2	Arginine	11.4

(2) Composition of Fr. B

Fr. B is a high molecular weight fraction consisting mainly of polysaccharides. Degradation yields monomers such as glucose, maltose and galactose. As conjugated amino acids, there is a slightly high percentage of acidic amino acids such as aspartic acid and glutamic acid. The composition of Fr. B does not characteristically differ from that of other mushrooms belonging to Ganoderma lucidum.

3. Acute Toxicity

Swiss mice (body weight about 25g), Sprague Dawley -JCL rats (body weight about 150g), and Japanese guinea pigs (body weight about 450g) were used as experimental animals. Healthy animals showing normal body weight gain after a 1-week acclimatization period were employed. The animals were raised at a room temperature of 25 ± 2 C and a relative humidity of 55 ± *5%.* Solid food (Oriental NMF) and tap water were supplied ad libitum.

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Both test substances were suspended in physiological saline. Maximum concentrations capable of being physically administered were used, i.e., a 5% solution for Fr. A and a 10% solution for Fr. B. The maximum dosage volume administered p.o. was 0.25 ml in the mouse, 5 ml in the rat and 8 ml in the guinea pig.

Any changes in the general condition of the animals were observed for 7 days after treatment.

4. Subacute Toxicity Studies

Swiss mice and Sprague Dawley - JCL rats were used as experimental animals. Ten mice of each sex and 5 rats of each sex were tested. The animals were raised at a temperature of 22 ± 2°C and a relative humidity of 50 ± 5%. Solid food (Oriental NMF) and tap water were supplied ad libitum.

Fr. A was suspended in physiological saline and administered in doses of 5000 mg/kg and 1000 mg by oral gavage using a stomach tube for 30 days.

After initiating treatment, body weights and food consumption were measured and behavioral changes examined at 3-day intervals. Urinalysis was performed for urine samples obtained on Day 30. On Day 31 after commencing treatment, the animals were anesthetized with ether, a blood sample was taken by venipuncture. The animals were

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then sacrificed by exsanguination and immediately autopsied and examined macroscopically. The major organs were removed, weighed, fixed in 10% formalin, embedded in paraffin, sliced, stained with hematoxylin and eosin, and examined histopathologically. The blood samples obtained by venipuncfcure underwent hematological examination.

1) Urinalysis: pH, protein and glucose were determined using Combistix. Bilirubin was measured by sinotest No. 6.

2) Hematology: Erythrocytes (RBC) and leukocytes (WBC) were determined by a Toa autoanalyzer. Hematocrit (Ht) was measured by the capillary tube method. Hemoglobin (Hb) was determined by the cyanmethemoglobin technique.

RESULTS

1. Acute Toxicity

Fr. A and Fr. B did not cause any deaths at the maximum doses capable of being administered by either route in the mouse, rat and guinea pig (Tables II - IV). The LD50 was thus unable to be calculated.

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As for general condition, after i.p. injection both Fr. A and Fr. B produced sedation, followed by an ataxic gait in which the hind limbs were slightly dragged. Some animals also exhibited a supine position, but all recovered after 24 hr. None of the animals displayed serious signs. There were no deaths.

2. Subacute Toxicity

1) General condition

Although some mice given 5000 mg/kg of Fr. A exhibited transient inhibition of spontaneous motility and atax-ic gait after treatment, all recovered to normal by the next dose.

Rats given Fr. A did not display any noteworthy signs during the treatment period in either sex.

2) Body weight

Mice: As shown in Fig. 2 and 3, the body weight of males given 5000 mg/kg of Fr. A was slightly high, but neither sex showed any significant differences compared with the control during the treatment period.

Rats: As shown in Fig. 4 and 5, rats given Fr. A showed normal body weight gain, similar to the control and mice, throughout the treatment period.

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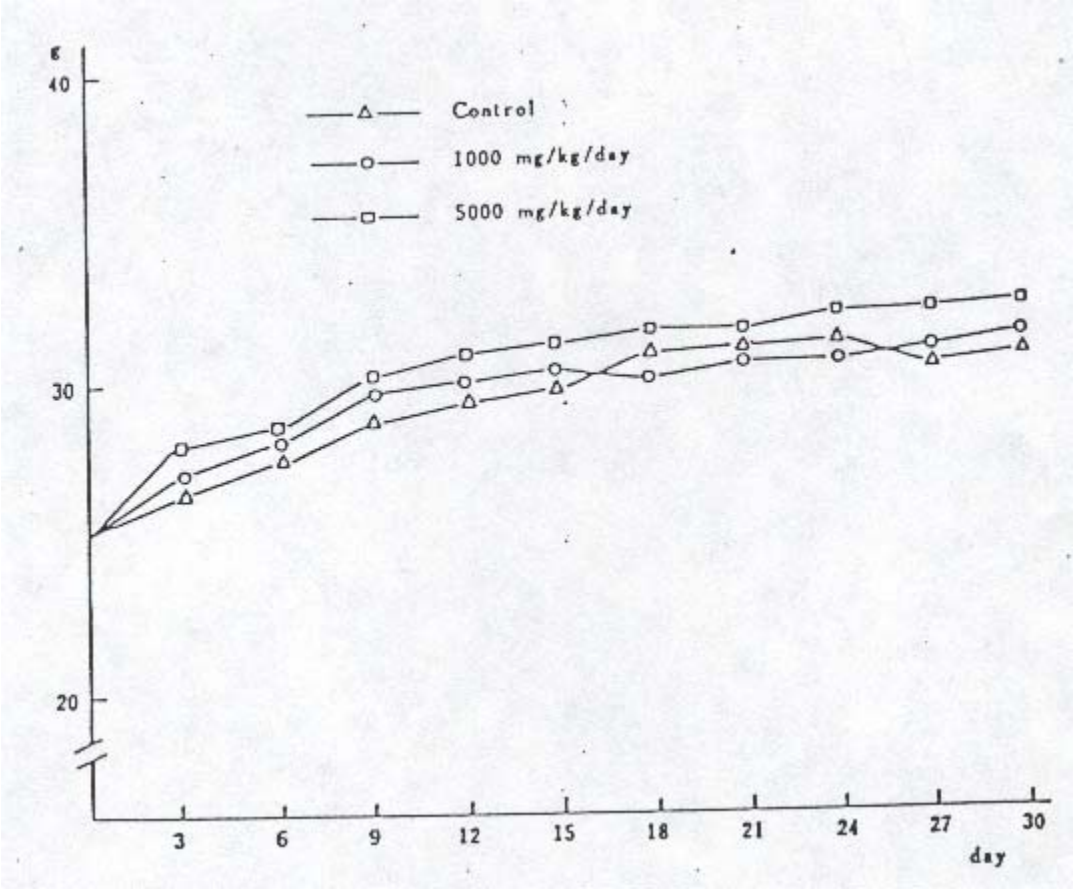
Below: TABLE II. Acute Toxicity of.Fr. A and Fr. B on Mice

Fr. A				Fr. B			
Route	Dose (mg/kg)	Sex	Motality	Route	Dose (mg/kg)	Sex	Motality
p.o.	5000	♂ ♀	0/10 0/10	p.o.	1000	♂ ♀	0/10 0/10
i.p.	5000	♂ ♀	0/10 0/10	i.p.	1000	♂ ♀	0/10 0/10
LD_{50}	(mg/kg)>5000			LD_{50}	(mg/kg)>1000		



Above: Fig. 2. Changes in Body Weight of Male Mice on Oral Administration of Fr. A for 30 Days

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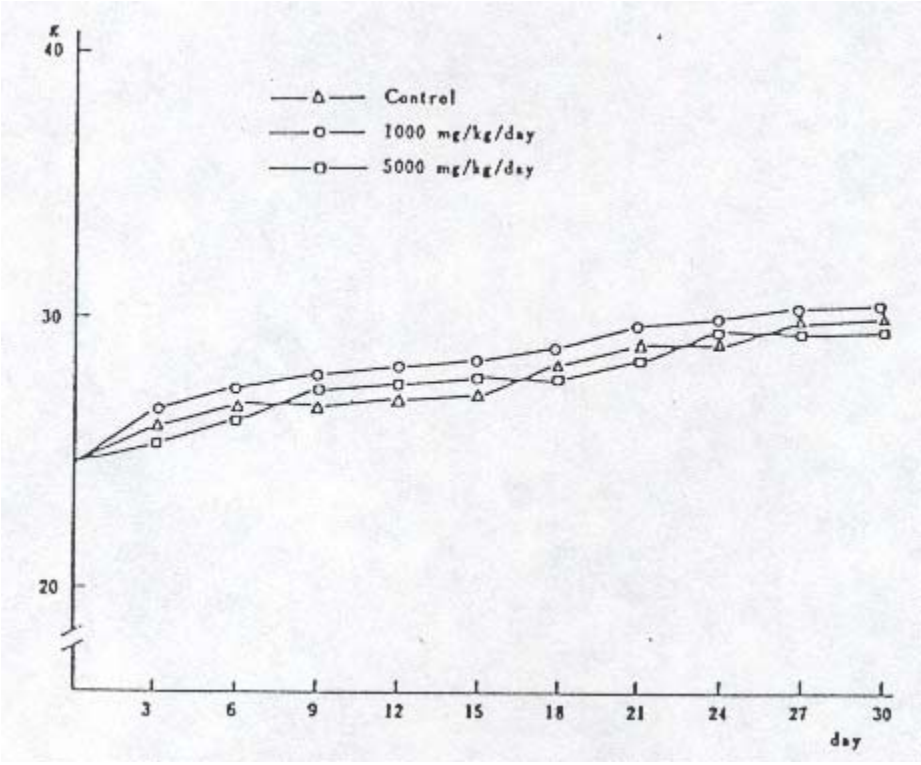
Below: TABLE III. Acute Toxicity of Fr. A and Fr. B on Rats

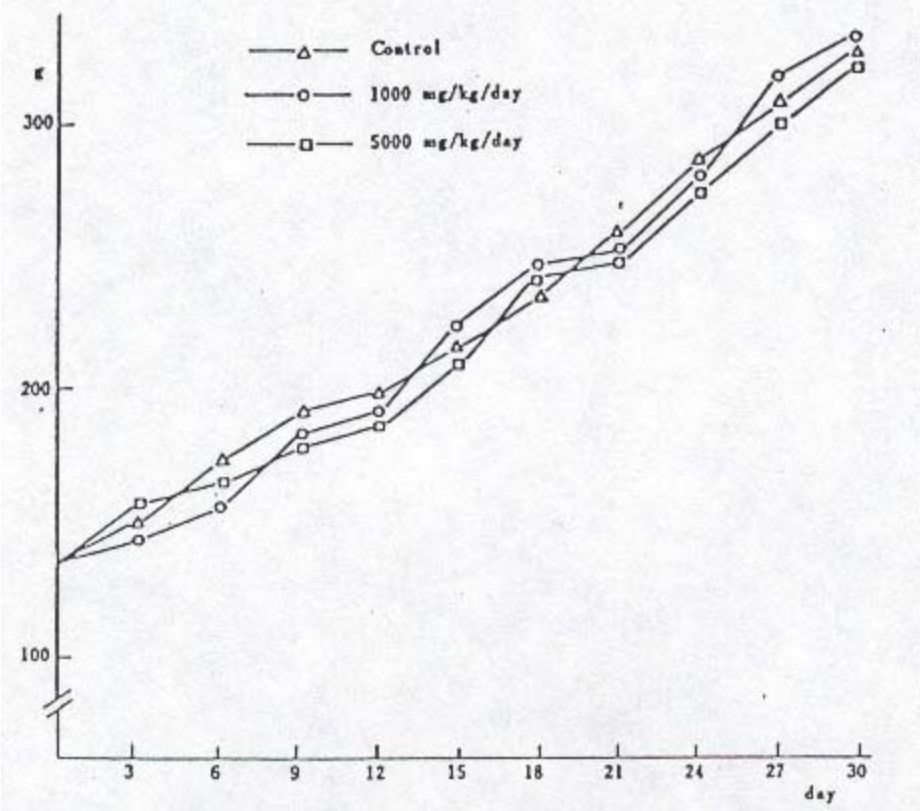
Fr. A				Fr. B			
Route	Dose (mg/kg)	Sex	Motality	Route	Dose (mg/kg)	Sex	Motality
p.o.	16700	♂	0/6	p.o.	3330	♂	0/6
		♀	0/6			♀	0/6
LD_{50}	(mg/kg)>16700			LD_{50}	(mg/kg)>3330		

Below: TABLE IV. Acute Toxicity of Fr. A and Fr. B on Guinea-pigs

Fr. A				Fr. B			
Route	Dose (mg/kg)	Sex	Motality	Route	Dose (mg/kg)	Sex	Motality
p.o.	8890	♂	0/6	p.o.	1780	♂	0/6
		♀	0/6			♀	0/6
LD_{50}	(mg/kg)>8890			LD_{50}	(mg/kg)>1780		



Above: Fig. 3. Changes in Body Weight of Female Mice on Oral Administration of Fr. A for 30 Days



Above: Fig. 4. Changes in Body Weight of Male Rats on Oral Administration **of Fr. A** for 30 Days

Below: TABLE V. Urianalysis of Rats on Oral Administration of Fr. A for 30 days

Sex	Dose mg/kg/day	No. of rats	Glucose		pH					Protein				Bilirubin	
			−	+	5	6	7	8	9	±	+	++	+++	−	+
Male	5000	5	5	0	0	4	0	1	0	0	3	2	0	5	0
	1000	5	5	0	0	3	1	1	0	0	3	2	0	5	0
	Control	5	5	0	0	4	0	1	0	0	2	2	1	5	0
Female	5000	5	5	0	0	4	1	0	0	0	3	2	0	5	0
	1000	5	5	0	0	3	2	0	0	0	2	3	0	5	0
	Control	5	5	0	0	3	2	0	0	0	1	4	0	5	0

3) Food consumption

Mean daily food consumption in rats is graphically depicted in Fig. 6 and 7. Rats given 1000 mg/kg and 5000 mg/kg of Fr. A did not display any differences in food consumption compared with the control in either sex.

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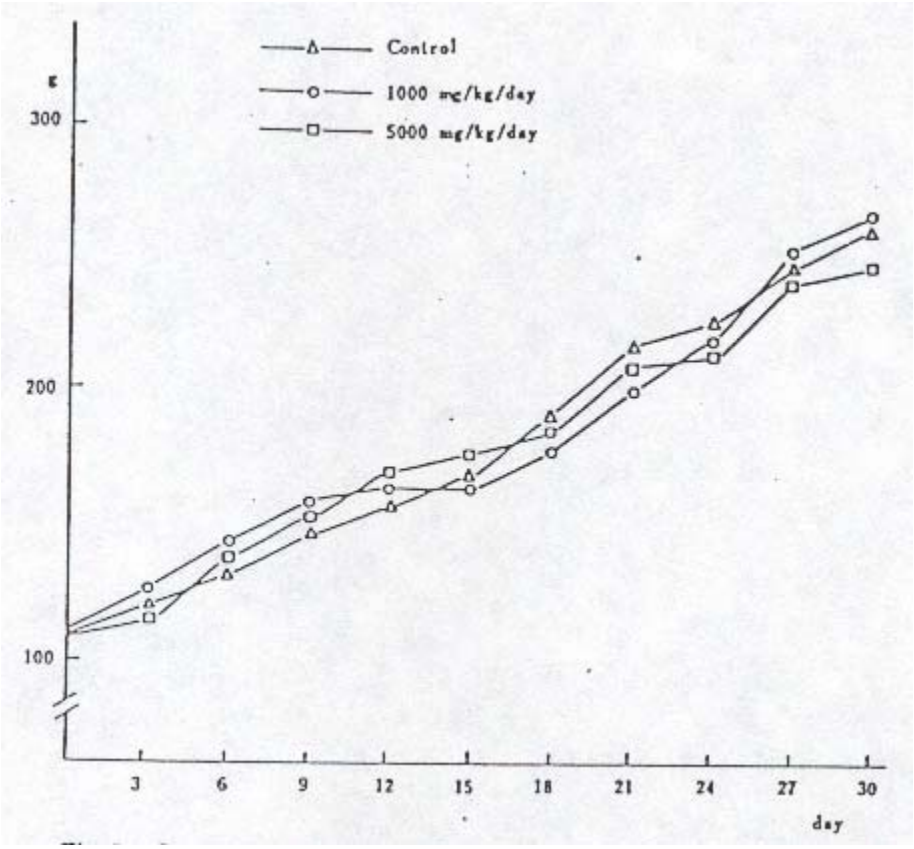
4) Urinalysis

The results of urinalysis in the rat are summarized in Table V. Urinary protein was

detected in + (trace) to. +++ (300 mg/100 ml) concentrations in some rats in both the control and treated groups regardless of dosage. Abnormalities in glucose and bilirubin were not seen in any animals. The pH range was 6.0 - 8.0 in all groups including the control. Effects attributable to Fr. A were not noted.



Above: Fig. 5. Changes in Body Weight of Female Rats on Oral Administration of Fr. A for 30 Days

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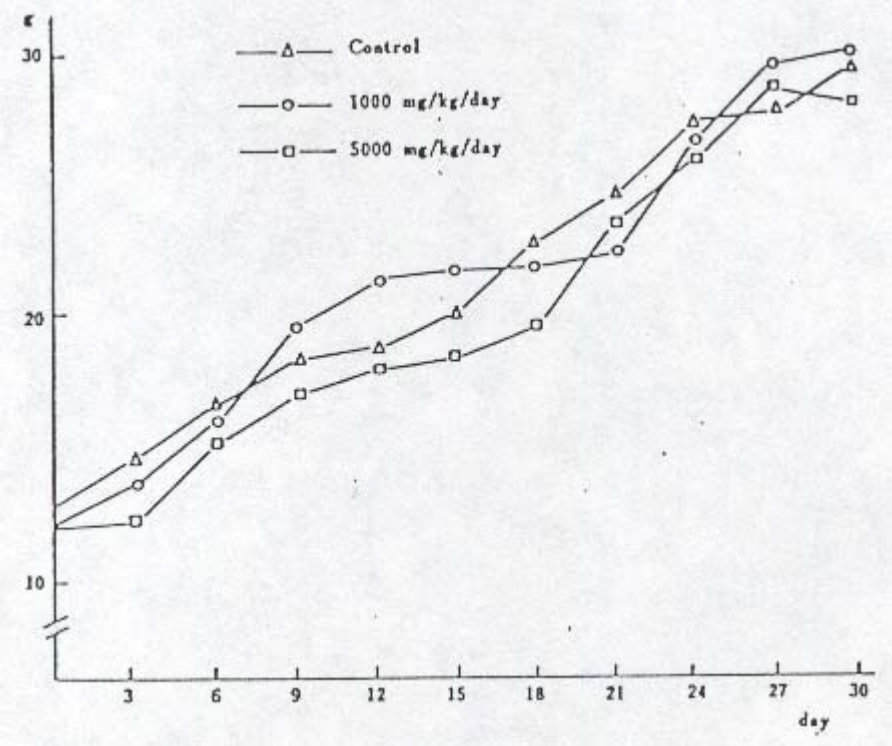
5) Hematological examination

Mice: As shown in Table VI, significant fluctuations in RBC, WBC, Hb and Ht compared with the control were not seen in any group. Rats: As summarized in Table VII, similar to mice, there were no significant fluctuations compared with the control in any group.

Below: TABLE VI. Hematolotical Examination of Mice on Oral Administration of Fr. A for 30 Days

Sex	Dose mg/kg/day	No. of mice	RBC x 10^4/mm^3	Hb g/dl	Ht %	WBC x 10^2/mm^3
Male	5000	10	757 ± 29	15.2 ± 0.9	47.0 ± 2.5	59.2 ± 8.9
	1000	10	723 ± 21	13.4 ± 0.6	45.2 ± 1.3	52.6 ± 4.9
	Control	10	725 ± 34	14.6 ± 0.7	44.6 ± 1.4	60.2 ± 6.4
Female	5000	10	789 ± 29	14.0 ± 0.4	49.0 ± 0.9	53.2 ± 3.9
	1000	10	769 ± 30	14.9 ± 1.4	46.0 ± 0.7	57.2 ± 8.1
	Control	10	756 ± 31	14.9 ± 1.7	49.2 ± 1.2	50.4 ± 6.3

mean ± SD



Above: Fig. 6. Dietary Consumption of Male Rats on Oral Administration of Fr. A for 30 Days

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TABLE VII. Hematological Examination of Rats on Oral Administration **of** Fr. A for 30 Days

Sex	Dose mg/kg/day	No. of rats	RBC x 10^4/mm^3	Hb g/dl	Ht %	WBC x 10^2/mm^3
Male	5000	5	567 ± 21	14.0 ± 1.4	46.8 ± 0.8	100 ± 2.2
	1000	5	557 ± 27	14.9 ± 0.9	42.3 ± 0.5	109 ± 2.8
	Control	5	600 ± 34	14.7 ± 0.5	44.3 ± 0.9	131 ± 4.6
Female	5000	5	552 ± 34	14.9 ± 0.9	41.2 ± 1.7	89 ± 4.2
	1000	5	549 ± 61	15.0 ± 0.5	41.0 ± 4.0	99 ± 4.0
	Control	5	541 ± 39	14.6 ± 0.4	40.9 ± 4.2	93 ± 6.9

mean ± SD

6) Autopsy findings and organ weights

Neither mice nor rats exhibited any noteworthy findings in major organs such as the heart, lungs, liver, kidneys, spleen, gastrointestinal tract, adrenals and thymus.

The wet organ weights of the heart, liver, kidneys and spleen in the mouse, summarized in Table VIII, did not

differ significantly from the cont'rol. The relative organ weights (g/100 g) of the heart, liver, kidneys and spleen, summarized in Table IX, did not show any significant differences with the control.

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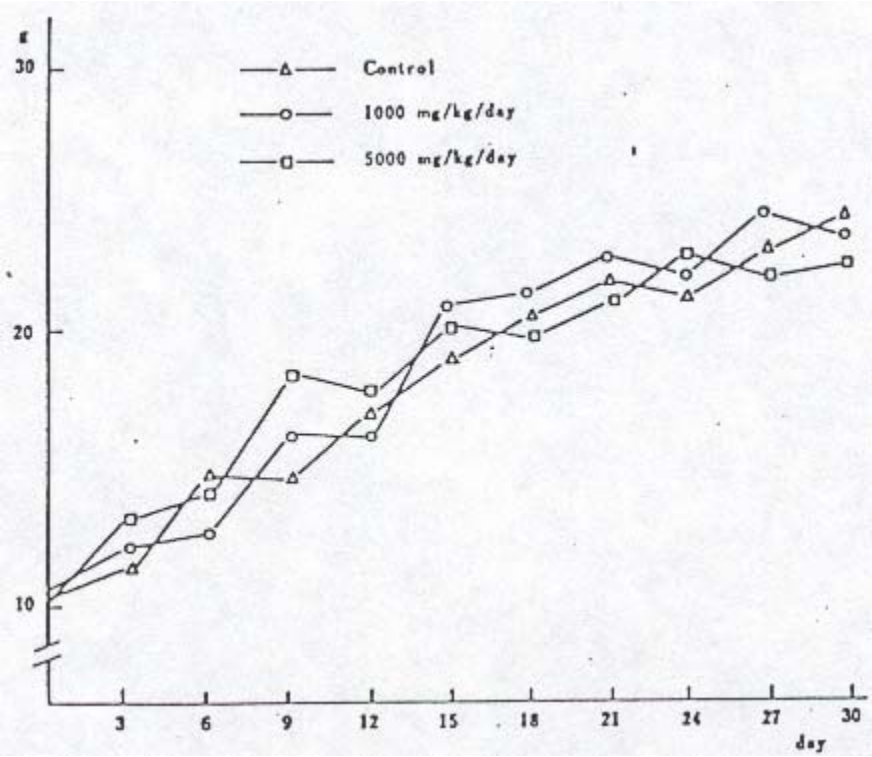

Fig. 7. Dietary Consumption of Female Rats on Oral Administration of Fr. A for 30 D»yS

TABLE VIII. Organ Weight in Mice on Oral Administration of Fr. A for 30 Days

Sex	Dose mg/kg/day	No. of mice	Heart g	Liver g	Kidneys g	Spleen g
Male	5000	10	0.87 ± 0.04	5.37 ± 0.25	2.40 ± 0.05	0.54 ± 0.03
	1000	10	0.79 ± 0.24	6.71 ± 0.69	2.44 ± 0.05	0.57 ± 0.03
	Control	10	0.85 ± 0.03	5.45 ± 0.43	2.42 ± 0.02	0.56 ± 0.04
Female	5000	10	0.51 ± 0.04	5.41 ± 0.12	1.69 ± 0.06	0.49 ± 0.04
	1000	10	0.49 ± 0.03	5.12 ± 0.29	1.77 ± 0.08	0.48 ± 0.04
	Control	10	0.52 ± 0.04	5.40 ± 0.34	1.99 ± 0.23	0.47 ± 0.05

mean ± SD

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TABLE IX. Relative Organ Weight in Rats on Oral Administration of Fr. A for 30 Days

Sex	Dose mg/kg/day	No. of rats	Heart mg	Liver mg	Kidneys mg	Spleen mg
Male	5000	5	367 ± 36	4157 ± 249	809 ± 74	251 ± 28
	1000	5	370 ± 29	4344 ± 281	794 ± 80	249 ± 79
	Control	5	395 ± 32	4069 ± 233	820 ± 65	229 ± 46
Female	5000	5	383 ± 31	3994 ± 240	840 ± 49	277 ± 29
	1000	5	376 ± 29	4014 ± 249	893 ± 63	279 ± 52
	Control	5	385 ± 29	4079 ± 199	872 ± 59	260 ± 39

mean ± SD

7) Histopathological examination

Microphotographs of the stomach, small intestine, heart, lung, liver, kidney, spleen and pancreas are presented in Fig. 8 and 9. There were no appreciable differences between the control and treated groups or noteworthy findings, there were likewise no changes in the pancreas, thymus, testis, ovary, adrenal and bone marrow.

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DISCUSSION

The present study was designed to investigate the toxicity of Fr. A (hot water extract) and Fr. B derived from Ganoderma lucidum Karst, a mushroom of the Ganoderma lucidum family.

After a single dose of Fr. A and Fr. B, the toxic signs of slight sedation and ataxic gait were seen in animals treated by i.p. injection. There were no serious toxic manifestations or deaths even after the maximal dose capable of being administered. Although the LD50 could not be calculated from the dosage of the present study, the oral LD50 estimated based on the maximum dose levels used was greater than 5000 mg/kg in the mouse, 16,700 mg/kg in the rat and 8890 mg/kg in the guinea pig for Fr. A and greater than 1000 mg/kg in the mouse, 3330 mg/kg in the rat and 1780 mg/kg in the guinea pig for Fr. B.

In subacute toxicity studies by continuous administration, the mouse and rat were given 1000 mg/kg of Fr. A and 5000 mg/kg of Fr. B p.o. for 30 consecutive days.

No noteworthy changes in general condition were noted in any group during the treatment period. None .of the animals died during any part of the study. There were no differences in body weight gain and food consumption compared with the control. Likewise, Urinalysis and hematological examinations carried out at the completion of the experiment did not reveal any abnormalities. Autopsy and organ weight analysis similarly did not indicate any effects of the test substance.

Furthermore, abnormalities specific to the treated groups were not detected at histopathological examination; characteristic toxic manifestations ascribed to the test substance were not detected. Therefore, the safe level of Fr. A after 30 days of p.o. administration in the mouse and rat can be regarded as 5000 mg/kg, although the minimum toxic level and certain toxic level could not be determined based on the results of the present study.

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