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Medicinal Efficacies of *Ganoderma lucidum* (XV) Anti-HIV Activities of *Ganoderma lucidum*

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ABSTRACT: To examine components of *Ganoderma lucidum* for anti-HIV activity, the aqueous and methanol extracts of its basidiocarps were respectively separated into the two and eight fractions. These fractions were used in XTT antiviral assay which showed cytophathic effects of HIV-1 on human T lymphoblastiod cells. Virus multiplications were also assayed by measuring its reverse transcriptase (RT) activity cells in the supernatant of Jurkat T lymphocytes that were infected with HIV-1 in the presence of each fractions. The results showed that the low-molecular-weight fraction of the aqueous extract strongly inhibited virus multiplication. The total methanol extract (A) showed a strong antiviral activity. Its hexane (B), ethyl acetate (C), neutral (E), and basic (G) fractions showed excellent antiviral activities. The results of the RT assay showed that the C and G fractions had significant antiviral activity. These results were in agreement with those of the XTT assay. These data indicate that the basidiocarps of *G. lucidum* had several components responsible for the inhibition of HIV multiplication.

1 INTRODUCTION

The fruit body of *Ganoderma lucidum* (Fr.) Karst. (Polyporaceae) is a well known traditional crude durg in the Orient to treat hepatopathy, chronic hepatitis, nephritis, gastric ulcer, hypertension, arthritis, neurasthenia, insomnia and bronchitis.

Recently, biological activities of the components of this mushroom have been elucidated due to the successful growing of this mushroom (reviewed by Jong and Birmingham 1992). The water extract of *G. lucidum* showed various biological activities such as adrenaline-induced lipolysis (Kubo *et al.* 1980), inhibition of platelet aggregation (Shimizu *et*

al. 1985), secretion of insulin (Kimura *et al.* 1983) and growth-promotion of mouse hair (Miyamoto *et al.* 1985).

The first structure-elucidated compounds from *G. lucidum* were ganoderic acids A and B that have bitter tastes (Kubota *et al.* 1982). The bitter tastes were also found in ganoderenic acids and lucidenic acids (Hirotani *et al.* 1985); Nishitoba *et al.* 1985). The fact that *G. lucidum* was traditionally used to treat bronchitis was confirmed scientifically by the inhibition of histamine release from mast cells by ganoderic acids (Kohda *et al.* 1985). Ganoderic acids also inhibited angiotensin converting enzyme that is responsible for the hypertension (Komoda *et al.* 1985). Some ganoderic acids inhibited growth of liver cancer cells (Toth *et al.* 1983).

On the other hand, polysaccharides from *G. lucidum* have various biological activities such as anticancer activity (Kim *et al.* 1980, Miyazaki *et al.* 1981), antihypertension (Morinaka Milk Industry Co., 1981, Park *et al.* 1987) and decrease of blood glucose (Hikino *et al.* 1985). From this fungus, immunomodulatory protein, Ling Zhi-8, was also isolated (Kino *et al.* 1989). Its complete amino acids were sequenced (Tanaka *et al.* 1989), cDNA for Ling Zhi-8 was cloned (Murasugi *et al.* 1991) and biological activities such as prevention of insulitis (Kino *et al.* 1990) and inhibition of anitbody production by ling Zhi-8, were reported. Moreover the extracts of *G. lucidum* did not show any conceivable toxicities (Sugiura *et al.* 1977, Kim *et al.* 1986), thus it is considered as a very safe mushroom.

As the lignin-protein complex of *Lentinula edodes* showed anti-HIV activity, we investigated whether extracts of *G. lucidum* have antiviral activities against human immunodeficiency virus. Here we report the results that some fractions of this mushroom showed anti-HIV activities.

2 MATERIALS AND METHODS

2.1 *Fractionation of G. lucidum*

The basidiocarp (1.7 kg) of G. *lucidum* was minced and extracted with hot water for 5 hours. The water extract was filtered, concentrated to 1/10 of the original volume and added with 3 volumes of ice-cold ethanol to precipitate high molecular weight components. The resulting precipitate was dissolved in small volume of distilled water and dialyzed for 7 days at 4 °C against streaming distilled water to remove contaminated low molecular weight components. The dialysate was freeze-dried to obtain 10.0 g and named GL-HMW fraction. On the other hand, the solution left after removing the precipitate was evaporated and freeze-dried to obtain 50.3 g and named GL-LMW.

To isolate low molecular weight components from this mushroom, the basidiocarp (3.5 kg) was extracted three times by sonication at room temperature for each 5 hrs. The extracts were combined and concentrated to obtain 219.2 g and named GLA as a total methanol extract. The GLA was further fractionated into GLB, GLC, GLD, GLE, GLF, GLG and GLH with n-hexane, ethylacetate and chloroform. All the fractions were completely dired and added to the culture assay system by dissolving in dimethylsulfoxide.

2.2 *Maintenance of human immunodificiency virus*

The virus, human immunodeficiency virus-1 lllb varient, used in this investigation was maintained in H9 cell line. The H9 cell was cultured in RPMI 1640 containing 10% fetal calf serum and 50 μg/ml gentamicin. H9 cell was replaced by new batch every two months to maintain consistent condition. The medium of HIV-1 infected H9 cell was replaced once a week. The infected H9 cell was adjusted to a concentration of 0.1×10^6 cells/ml in 200 μl. The culture supernatant was centrifuged after 6 days to obtain virus stock solution, which was frozen in liquid nitrogen tank. Virus titer assay was performed by syncytium assay (Nara *et al.* 1988). Its assay result was expressed as syncytium-forming unit (SFU)/ml. The average titer of the frozen virus was about 3×10^4 SFU/ml.

2.3 *Anti-HIV activity assay*

Target cell for anti-HIV activity was CEM-IW T cell line which grows well in the absence of interleukin-2. The viability of the target cell was assessed by XTT.

[2,3-bis(2-methoxy 4 nitro-5-sulfophenyl)-5-(phenylamino)carbonyl)-2H-tetrazolium hyroxide] assay in which yellow XTT formazan produced by the viable cell was measured by optical density at 450nm (Weislow et al., 1989). For anti-HIV assay, 100 μl of HIV-1 infected CEM-IW cells (1,000 cells) in 96-well cultured plate were mixed with 100 μl of serial diluted fractions of *G. lucidum* were cultured for 7 days in 5% CO_2 and 37 °C incubator. At day 8, each 50 μl of XTT tetrazolium solution was added to the culture wells and incubated further for 4 hrs. Then the homogenized culture solutions were measured for optical density at 450 nm.

3 RESULTS

3.1 *Fractionation of G. lucidum*

Hot water extraction method of the minced basidiocarp of *G. lucidum* was employed to obtain high- and low-molecular weight fractions. From 1.7 kg of the basidiocarp, 10.0 g (0.58%) and 50.3 g (2.95%) were obtained as high- and low-molecular weight fractions, respectively (Table 1).

Table 1. Extraction yield of *G. lucidum* fractions with hot water.

Fraction	Weight (g)	Yield (%)
Fruit bodies	1,700.0	—
GL-HMW	10.0	0.58
GL-LMW	50.3	2.95

In case of methanol extraction, total extraction was 219.2 g (6.26%) from 3.5 kg of the basidiocarp. Major fractions were GLC, GLD and GLE yielding 105.2 g (3.01%), 100.7 g (2.87%) and 56.4 g (1.61%), respectively (Table 2).

Table 2. Extraction yield of *G. lucidum* fractions with methanol.

Fraction	Weight (g)	Yield (%)
Fruit bodies	3,500.0	—
GLA	219.2	6.26
GLB	13.2	0.38
GLC	105.2	3.01
GLD	100.7	2.87
GLE	56.4	1.61
GLF	27.2	0.78
GLG	0.5	0.02
GLH	21.1	0.60

3.2 *Anti-HIV activities of fractions of G. lucidum*

HIV-1 infected CEM IW cells (1,000 cells/well) were cultured for 7 days in the absence or presence of serial diluted fractions of *G. lucidum*. When the high-molecular weight fraction was added to the cells in the absence of the virus, the viability of the cells were above 84% at 125.0 μg/ml which was the highest concentration used. No dead cells were observed at 62.5 μg/ml concentration, indicating that the high-molecular weight fraction of *G. lucidum* did not have any toxicity to the target cells. When we

assayed the viability of the target cells in the presence of HIV-1 virus and various concentrations of the high-molecular weight fraction, the viable cells were below 10% in almost every concentration (Table 3). This suggests that the high-molecular weight fraction did not have any anti-viral activity.

Table 3. Anti-HIV effect of GL-HMW that was extracted with water from the fruit bodies of *Ganoderma lucidium*.

Concentration (μg/ml)	Experiment 1		Experiment 2	
	OD	Viability (%)	OD	Viability (%)
Virus uninfected [a]				
Target cell alone	1.40	—	1.69	—
0.97	1.37	98.5	1.66	98.2
1.95	1.40	100.6	1.78	105.2
3.90	1.59	113.9	1.75	103.7
7.81	1.38	99.0	1.57	92.9
15.60	1.35	96.8	1.77	104.7
31.20	1.56	111.6	1.72	101.8
62.50	1.41	101.1	1.27	75.2
125.00	1.18	84.2	1.48	87.7
Virus infected [b]				
Target cell alone	1.40 ± 0.12	—	1.69 ± 0.05	—
0.00	0.11 ± 0.02	7.6	0.15 ± 0.04	8.7
1.95	0.11 ± 0.04	7.7	0.11 ± 0.02	6.2
3.90	0.07 ± 0.01	5.3	0.09 ± 0.01	5.2
7.81	0.06 ± 0.02	3.9	0.12 ± 0.02	6.8
15.60	0.11 ± 0.01	7.8	0.09 ± 0.01	5.3
31.20	0.07 ± 0.03	5.0	0.99 ± 0.01	5.2
62.50	0.06 ± 0.01	4.4	0.18 ± 0.10	10.4
125.00	0.13 ± 0.05	9.3	0.13 ± 0.01	7.8

[a] Done in one well, [b] Done in duplicates

The low-molecular weight fraction of the water extract also did not show any toxicity to the target cells at all the concentrations. However, when the concentration of the low-molecular weight fraction was increased, viabilities of the HIV-infected cells increased reaching maximum viable (71.7%) at 15.6 μg/ml may be due to the presence of unknown interfering components (Table 4).

Table 4. Anti-HIV effect of GL-HMW that was extracted with water from the fruit bodies of *Ganoderma lucidium*.

Concentration (μg/ml)	Experiment 1		Experiment 2	
	OD	Viability (%)	OD	Viability (%)
Virus uninfected [a]				
Target cell alone	1.51	—	1.52	—
0.97	1.59	105.2	1.75	114.7
1.95	1.64	108.3	1.70	111.5
3.90	1.42	93.8	1.75	114.9
7.81	1.69	111.5	1.77	111.5
15.60	1.56	103.1	1.70	104.7
31.20	1.53	101.4	1.53	100.7
62.50	1.60	106.0	1.74	114.3
125.00	1.56	102.9	1.46	96.2
Virus infected [b]				
Target cell alone	1.51 ± 0.10	—	1.52 ± 0.20	—
0.00	0.11 ± 0.02	7.6	0.15 ± 0.04	8.7
0.97	0.17 ± 0.00	11.0	0.20 ± 0.02	12.9
1.95	0.17 ± 0.01	11.4	0.16 ± 0.00	10.3
3.90	0.17 ± 0.04	11.2	0.35 ± 0.12	23.2
7.81	0.56 ± 0.13	37.2	0.37 ± 0.22	24.3
15.60	0.09 ± 0.00	71.7	1.25 ± 0.44	82.5
31.20	0.65 ± 0.01	43.1	0.90 ± 0.00	58.8
62.50	0.83 ± 0.11	54.7	1.13 ± 0.16	74.0
125.00	0.83 ± 0.02	54.6	0.87 ± 0.00	57.0

[a] Done in one well, [b] Done in duplicates

For the methanol extracts, a similar investigation was performed. The concentrations that exhibited the highest viable cells by GLA, GLB, GLC, GLD, GLE, GLF, GLG amd GLH were 31.2 μg/ml (51.9% viable), 15.6 μg/ml (52.4% viable), 31.2 μg/ml (33.2% viable), 125.0 μg/ml (32.1% viable), 15.6 μg/ml (34.9% viable), 31.2 μg/ml (18.5% viable), 15.6 μg/ml (48.5% viable) and 125 μg/ml (45.3% viable), respectively.

4 CONCLUSIONS

The high- and low-molecular-weight fractions of the aqueous extractive of *G. lucidum* had no cytotoxicity on human T lymphocytes. The low-molecular-weight fraction showed anti-HIV activity, but the high-molecular-weight fraction did not. MTT assay showed that the four fractions of hexane, non-hexane, neutral and basic had strong antiviral activities. The acidic fraction did not show any anitviral activity.

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