BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 14, MARCH 1985
P. 7-16

Studies on the Protective Effects of *Ganoderma applanadum* Long Term Administrated Rats Against *Escherichia coli* Exotoxin

HUEY-FANG SHANG, CHENG-CHUANG TSENG, HSUEH-CHUANG WU*
LENG-FANG WANG** AND YIH-CHIH TUNG**

ABSTRACT

The rats treated with hot water extract of Ling Chih for a long period had been distinctly improved their physical functions, and also had excellent defensive ability against the challenges of the purified exotoxin of capsular polysaccharide-synthesizing Escherichia coli. Two hours after the challenge of exotoxin (2mg/kg), we found that (1) The difference in arterial blood pressure and heart rate: The systolic pressure of the G. applanadum and G. lucidum administered rats were decreased by 11.0 mmHg and 14.0 mmHg, respectively. The changes of G. applanadum group were milder than the control group (32.0 mmHg). The heart rate of the G. applanadum, G. lucidum and control groups were increased by 38 ± 15.5 beats/min, 22 ± 24.2 beats/min, and 46 ± 14.0 beats/min, respectively. (2) Change in blood cells: Leukopenia and hemoconcentration of the G. applanadum and G. lucidum groups were milder than those of the control group. (3) Change in the kidney function test datas: BUN: G. applanadum, G. lucidum and control groups were increased by 0.6 mg/dl, 1.9 mg/dl and 4.6 mg/dl, respectively. Creatinine: By 0.20 mg/dl, 0.08 mg/dl and 0.3 mg/dl. Uric acid: By 1.47 mg/dl, 0.77 mg/dl and 1.13 mg/dl respectively. (4) The liver function test data: S-ALP: G. applanadum, G. lucidum and control groups were respectively increased by 8.2 unit, 14.2 unit and 33.1 unit. S-GPT: By 7.4 unit, 6.0 unit and 10.1 unit. S-GOT: By 76.1 unit, 50.1 unit and 70.4 unit. (5) Plasma glucose concentration of G. applanadum group had been distinctly increased (110.8 ± 3.4 mg/dl) compared with G. lucidum (87.7 ± 3.1 mg/dl) and control groups (85.3 ± 4.0 mg/dl).

The defensive ability against the E. coli infection, and the survival time after transplantation of Sarcoma-180 cells, of the Ling Chih pretreated mice were showed excellent results.

Departments of Microbiology, Medical Technology* and Biochemistry**, Taipei Medical College.
Received for Publication: April 18, 1984.

Key words: *Ganoderma Applanadum, Exotoxin of Escherichia Coli, Ling Chih.*

INTRODUCTION

"Ling Chih" has been thought to be a magic drug of Chinese herbs[1]. It has been applied to treat hepatitis, nephritis, peptic ulcer, hypertension, arthritis, neurasthsia, bronchitis, asthma and leukopenia etc.[2] Recently it was also applied to the therapy of tumor[3,4]. After administration of the hot water extract of the fruiting body of *G. applanadum*, the mice could resist the infection of *E. coli*[5]. We also found that the physiological effect of rats, such as the function of kidney and liver, the concentration of blood sugar and cholesterol could be improved after long-term oral administration of the extract of *G. applanadum* in normal Wister strain rats[6].

Because the wide usage of antibiotics or the hospital instruments (such as intravenous catheter etc.) and the life elongation of chronic patients, the ratio of bacteremia and septicemia by Gram-negative enteric bacteria infection increased with time[7-9]. In order to understand the defensive effects of Ling Chih against the Gram-negative bacilli, the rats were injected with the purified exotoxin of *E. coli*[10,11] and observed their changes in the hematology, biochemical and serological examinations[12]. We also observed the antibacterial activity against *E. coli* infection and the survival time after the transplantation of Sarcoma-180 cells.

MATERIALS AND METHODS

Ling Chih[5,6]: The fruiting bodies of Ling Chih (including the natural *G. applanadum* and the cultivated *G. lucidum*) were washed with 90% alochol and then were disintegrated. The crushed powders were extracted with 10X-20X volumes of hot water (100°C) for 4 hrs. The hot water extract was filtered, concentrated by evaporation under lower pressure and then lyophilized. The administrating method of the extract was the same as previous method[6]. All the experiemental rats were female Wistar rats (about 50 gm).

Purification of exotoxin of *Escherichia coli*[10,11]: A capsular polysaccharide-synthesizing strain of *E. coli* (E-4) was cultivated in heart-infusion broth (Difco) and 18 hr after the culture supernatant was subjected to 40% saturated ammonium sulfate precipitate and filtrated through sephadex G-150 (Pharmacia, Sweden) column (3 x 70 cm). The fraction obtained at near the void volume was the opalescent fraction, which mainly contained lethal exotoxin (mice i.p. LD_{50}: 0.261 mg/kg, and rats i.v. LD_{50}: 1.6 mg/kg). In this experiment, the dosage for the rat was 2 mg/kg.

The test items and methods[13]: The rat was anesthetized by intraperitoneal injection of sodium pentobarbital. The blood was taken from femoral arteries by polyethylene tube connected to a syringe for hematological and biochemical examinations:

(1) The measure of the arterial blood pressure and heart rate: Record the difference in femoral arterial blood pressures and heart rates by polygraph (Multi-purpose polygraph RM 150,

NIhon Kohden Kogyo Co.)

(2) The examination of blood cells[14]: Count the total numbers of WBC and RBC by the hemocytometer. The hematocrit was calculated by the capillary method.

(3) The biochemical examination of serum: This test included 7 items. Blood sugar: measured by 0-toluidine method[15]. Cholesterol: measured by the principle of Liebermann-Burchard reaction[16]. BUN: measured by the reaction of urea and diacetyl monoxime[15]. Creatinine: quantified by a red product which was formed by creatinine and alkaline picrate[17]. Uric acid: quantified by alkaline phosphatungstate[15]. Alkaline phosphatase: measured by p-nitrophenylphosphate[18].

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT): measured by the method of Reitan and Frankel[18].

RESULTS

The pharmacological effects of the *E. coli* exotoxin on the Ling Chih long-term oral administrated rats:

1. The influence on the blood pressure (Table 1): After 2 mg/kg exotoxin injection, the systolic pressure of the control group was lowered by 32 mmHg ($P < 0.05$), the *G. applanadum* and *G. lucidum* groups were lowered by 11 mmHg ($P > 0.05$) and 14 mmHg ($P < 0.01$). So the *G. applanadum* could clearly decrease the influence of exotoxin on the blood pressure.
2. The effects on the heart rates (Table 1): The heart rates of all experimental rats were increased after the exotoxin injection. The increased rates of the *G. applanadum*, *G. lucidum* and the control groups were 38 beats/min, 22 beats/min and 46 beats/min, respectively. The Ling Chih could also improve the

Table 1. Changes of Arterial Blood Pressure and Heart Rate of *G. applanadum* Long-Term Administered Rats Against *Escherichia coli* Exotoxin

Subject	Group (n)	Exotoxin treatment (2mg/kg; iv)		d ± SEd
		Before	After (2 hr)	
Blood pressure (mmHg)				
Systolic	*G. applanadum* (5)	146 ± 2.2#	135 ± 4.5	-11.0 ± 6.40
	G. lucidum (5)	124 ± 4.6	110 ± 4.0	-14.0 ± 2.45**
	Control (5)	125 ± 8.3	93 ± 2.2	-32.0 ± 7.50*
Diastolic	*G. applanadum* (5)	101 ± 2.2	95 ± 5.3	- 6.0 ± 4.85
	G. lucidum (5)	83 ± 2.7	74 ± 4.3	- 5.6 ± 5.48
	Control (5)	84 ± 8.2	68 ± 4.1	-15.3 ± 5.55
Heart rate (Beat/min)	*G. applanadum* (5)	395 ± 28	433 ± 40	+38 ± 15.5
	G. lucidum (5)	404 ± 12	426 ± 20	+22 ± 24.2
	Control (5)	422 ± 11	468 ± 11	+46 ± 14.0*

Mean ± standard error.
By paired t test: *$P < 0.05$ and **$P < 0.01$.

Table 2. Hematological Changes of the *G. applanadum*–Administered Rats after *Escherichia coli* Exotoxin Challenge

Subject	Treated with (n)	Exotoxin challenge		d ± SEd
		Before	After (2 hr)	
WBC (mm^3)	Control (5)	4,790 ± 264#	1,480 ± 168	-3,310 ± 233**
	G. lucidum (5)	5,170 ± 560	2,160 ± 351	-3,010 ± 858*
	G. applanadum (5)	5,260 ± 296	2,380 ± 454	-2,880 ± 632*
RBC (x 10^7)	Control (5)	560 ± 20	529 ± 8	+23 ± 14
	G. lucidum (5)	553 ± 8	418 ± 93	-35 ± 16
	G. applanadum (5)	584 ± 19	731 ± 35	+147 ± 43*
Hematocrit (%)	Control (5)	36.2 ± 0.97	41.6 ± 1.17	+5.4 ± 1.72*
	G. lucidum (5)	38.8 ± 0.80	39.0 ± 2.17	+0.2 ± 2.58
	G. applanadum (5)	41.0 ± 7.07	41.6 ± 1.33	+0.6 ± 1.87

2 mg/kg of exotoxin of *Escherichia coli* was intravenously injected, 2 hr after injection the treated rats was bleeded.
Mean ± standard error.
By paired t test: *P < 0.05 and **P < 0.001.

influence of exotoxin on the heart rates.

3. The effects on the blood cells (Table 2): After the exotoxin injection, the RBC number of the *G. applanadum* group was increased by 22.8% (vs 4.6% of the control group $P < 0.001$). But the *G. lucidum* group was decreased by 6.3%. The hematocrit of the three groups were increased by 17.1%, 0.9% and 0.5%. The total WBC number of the three groups were decreased by 69.1%, 54.9% and 58.2% ($P < 0.001$, $P < 0.005$ and $P < 0.005$). Although after the injection of exotoxin, the leukopenia in the Ling Chih groups was milder than that of the control group, the difference was nonsignificant ($P > 0.05$).
4. Effects on the serum total protein (Table 3): After the exotoxin injection, the serum total protein of the Ling Chih treated groups was clearly decreased, such as the protein content of *G. applanadum* group was decreased from 7.68 ± 0.17 gm% to 5.80 ± 0.17 gm% (24.5%, $P < 0.001$) and the *G. lucidum* group was decreased by 21.1% ($P < 0.001$), but the control group was decreased only by 3.0%.
5. Effects on the liver and kidney function (Table 4): From the datas of BUN, Creatinine and Uric acid, we found that the kidney function of the Ling Chih group still influenced by the exotoxin. But comparing to the control group, the influence was milder. (vs

Table 3. Effect of *G. applanadum* on Serum Protein Change of Rats after *Escherichia coli* Exotoxin Challenge

Administered with (n)	Serum total protein (gm%)		
	Before	After (2 hr)	t
G. applanadum (5)	7.68±0.17#	5.80±0.17	6.99*
G. lucidum (5)	7.40±0.36	5.83±0.13	9.19*
Control (5)	6.70±0.25	6.50±0.07	1.76

* Administration of *G. applanadum* see this text.
2 mg/kg of exotoxin of *Escherichia coli* was intravenously injected, 2 hr after injection the treated rats was bleeded.
Mean ± Standard error.
t: By student t test. *P < 0.001.

Table 4. Biochemical Examination of Sera of *G. applanadum* Administered Rats Against *Escherichia coli* Exotoxin

Tests	Exotoxin treatment (2mg/kg; iv)	Control (n=5)	*G. applanadum* (n=5)	t (vs control)	*G. lucidum* (n=5)	t (vs control)
Glucose (mg/dl)	Before	102.3 ± 7.7#	81.6 ± 2.6	5.69***	87.4 ± 5.0	3.63***
	After (2 hr)	85.3 ± 4.0	110.8 ± 3.4	10.87***	87.7 ± 3.1	1.06
	(t:vs before)	(4.38)**	(6.69)***		(0.11)	
Cholesterol (mg/dl)	Before	50.9 ± 5.5	47.3 ± 2.7	1.31	46.5 ± 2.0	1.68
	After (2 hr)	60.4 ± 11.3	47.4 ± 2.2	2.52*	45.1 ± 2.9	2.93*
	(t:vs before)	(1.69)	(0.06)		(0.89)	
BUN (mg/dl)	Before	21.1 ± 0.9	16.7 ± 0.4	10.08***	18.2 ± 0.4	6.64***
	After (2 hr)	26.4 ± 1.1	17.3 ± 0.6	16.35***	20.1 ± 0.8	10.38***
	(t:vs before)	(8.38)***	(1.66)		(4.77)**	
Creatinine (mg/dl)	Before	0.76 ± 0.03	0.65 ± 0.02	5.80***	0.76 ± 0.04	0.00
	After (2 hr)	1.06 ± 0.14	0.85 ± 0.04	3.32*	0.84 ± 0.12	2.68*
	(t:vs before)	(4.24)**	(9.43)***		(1.41)	
Uric acid (mg/dl)	Before	1.60 ± 0.31	0.88 ± 0.09	4.95**	1.63 ± 0.17	0.19
	After (2 hr)	2.73 ± 0.31	2.35 ± 0.19	2.31*	2.40 ± 0.29	1.74
	(t:vs before)	(5.76)***	(13.86)***		(5.07)***	
S-ALP (u)	Before	3.4 ± 0.4	2.2 ± 0.9	2.75*	2.9 ± 0.3	2.47*
	After (2 hr)	36.5 ± 5.2	10.4 ± 3.4	9.38**	17.1 ± 3.9	6.67***
	(t:vs before)	(14.18)***	(4.66)**		(8.13)***	
S-GPT (u)	Before	27.9 ± 3.5	11.3 ± 0.9	10.09***	19.1 ± 2.1	4.80**
	After (2 hr)	38.0 ± 8.6	18.7 ± 2.5	4.84**	25.1 ± 8.4	2.40*
	(t:vs before)	(2.46)*	(5.57)***		(1.55)	
S-GOT (u)	Before	259.6 ± 16.2	119.0 ± 5.9	18.24***	157.2 ± 17.1	9.72***
	After (2 hr)	320.0 ± 56.6	195.1 ± 11.5	4.84**	207.3 ± 42.1	3.57**
	(t:vs before)	(2.30)*	(11.77)***		(2.47)*	

Mean ± standard error.
By student t test: *P < 0.05, **P < 0.01 and ***P < 0.001.

the control group: *G. applanadum* group $P < 0.05$ - $P < 0.001$, *G. lucidum* group $P < 0.05$ - $P < 0.001$ except Uric acid). The datas of S-alkaline phosphatase, SGPT and SGOT also showed that the liver function of the Ling Chih treated groups was influenced by the exotoxin, but the influence was still milder. (vs the control group: both of the Ling Chih treated groups $P < 0.05$ - $P < 0.001$). The blood sugar content was also influenced by the exotoxin. The sugar contents of the *G. applanadum*, the *G. lucidum* groups increased by 35.8% ($P < 0.001$), 0.3% ($P > 0.05$), but that of the control group was decreased by 16.6% ($P < 0.01$). So the injection of the *E. coli* exotoxin would increase the blood sugar on the Ling Chih groups.

6. The influence of the serum cholesterol concentration was almost the same on

the three groups.

The antibacterial activity of Ling Chih treated mice against *E. coli* (Table 5): The mice were continuously injected intraperitoneal with 200 μg/mouse/day of the extract of *G. applanadum* or *G. lucidum* for 4 days. The *G. applanadum* group had the better antibacterial activity against the culture broth of *E. coli*. The mortality of the *G. applanadum*, *G. lucidum* and the control groups were 42.9%, 87.5% and 100%, respectively.

Table 5. Protection of *G. applanadum*-Pretreated-mice Against *Escherichia coli*

Pretreatment (μg/mouse)	Mortality (0.5 ml/mouse)
G. applanadum (800)	3/7 (42.9)#
G. lucidum (800)	7/8 (87.5)
Control (0)	5/5 (100.0)

Mice were injected intraperitoneally with 200μg/mouse/day of *G. applanadum* and *G. lucidum* for 4 times, respectively. 24 hrs after final immunogens pretreatment the pretreated-mice were challenged intra-peritoneally with 0.5 ml of 24 hr heart-infusion broth culture of *Escherichia coli*. The results were observed for one week.

No. of death/No. of test; figures in parenthesis show the percentage.

The survival time of Ling Chih treated mice after Sarcoma-180 transplantation (Table 6): The mice were treated with the extract of *G. applanadum* and *G. lucidum* as above, then they were i.p. injected with Sarcoma 180 cells at the dose of $1x10^6$ cells/mouse. After transplantation of the Sarcoma 180, the mice were continuously injected with the same amount of Ling Chih until the tenth day. The *G. applanadum* group had a better resistance against the tumor cells, the survival time was 28.3 day. It was distinctly different from that of the *G. lucidum* group (10.8 day) and the control group (11.9 days). But if we injected the extract of Ling Chih for 10 days, then we transplanted the Sarcoma cells, the antitumor activity of the rat decreased distinctly. Although the difference of the survival time between the test groups and the control groups was limited, we could still discovered that the *G. applanadum* treated group had the better resistance against the tumor cells. From the above results,

Table 6. Effect of *G. applanadum* on the Survival Time of the ICR Mice Challenged by Sarcoma-180 Cells

Method of administration (200 μg/mouse/day)	Time of survivors (day)		
	G. applanadum	*G. lucidum*	Control
S-180 (10^6 cells; ip) ↓ ——— (day) ↑ stop	28.3 (n=15)	10.8 (n=15)	11.9 (n=12)
S-180 (10^6 cells; ip) ↓ ——— (day) ↑ stop	16.1 (n=15)	14.9 (n=15)	15.6 (n=10)

we concluded that the antitumor activity of Ling Chih might be a cytostatic activity[19].

DISCUSSION

After long-term oral administration of the hot water extract of the fruiting bodies of *G. applanadum*, the physiological effects of the rat were clearly improved, except the induction of hypertenion[6]. This improvement will help the hosts to get a stronger protection against the bacterial infections. In this experiment, the *G. applanadum* treated rats were injected by the exotoxin of *E. coli*. The exotoxin could still cause the hypotention, the increase of the heart rates, the decrease of the WBC count, and the interference on the function of liver and kidney. But comparing to the control group, the influence to the treated rats was much milder.

Escherichia coli is the main normal flora in the human intestinal tract. They are responsible for maintaining the balance of microbial population. The pathogenic substances of *E. coli* include enterotoxins and lethal exotoxin[11,20-23]. The exotoxin of *E. coli* may be one of the main substances which might cause a secondary shock whenever the bacteremia of *E. coli* happened[12]. Recently the secondary infection and nosocomial infection are the most considered in bacteria infections. Their prevention and treatment are studied by many experts, especially about the non-specific cross protection[24-29]. In this respect, the *G. applanadum* may be a available material.

The blood sugar content of the rat could clearly influenced by the *E. coli* exotoxin. After the injection of exotoxin, the blood sugar content of the control group would clearly decreased from 102.3 ± 7.7 mg/dl to 85.3 ± 4.0 mg/dl ($P < 0.01$). But the *G. applanadum* group got the completely opposite results, the blood sugar content was clearly increased from 81.6 ± 2.6 mg/dl to 110.8 ± 3.4 mg/dl ($P < 0.001$). The shock patients caused by the bacteremia or endotoxemia of G(-) bacilli often had a clear phenomenon of low blood sugar[30]. The endotoxin lowered the activity of liver's Glucose-6-phosphotase, Fructose-1,6-diphosphotase (FDPase), and Phosphoenol pyruvate (PEP) decreased. So the endotoxin could decrease the ability of liver to release the blood sugar. Dr. Berry used the LD_{50} dose of endotoxin to i.p. inject into the mice and found that the blood sugar content, the glycogen amount of liver and muscle also clearly decreased in the treated mice[31]. The endotoxin stimulating hyperdynamic state of anaerobic metabolism would strengthen the sugar catabolism of the peripheral tissue, and the increase of the phagocytosis ability of WBC and insulin-like activity.

In this experiment, the exotoxin of *E. coli* could increase the blood sugar content in the *G. applanadum* administrated rats. Whether the mechanism was related to those described as above, or it was due to the glycogenolysis in liver, or it was due to the other reasons, we still need to discuss it further.

The hot water extract of the fruiting bodies of *G. lucidum* were recommended to treat hyperlipemia, hepatitis, nephritis, gastric ulcer, arthritis, nervous exhaustion,

bronchitis, asthma, leukopenia and cancer etc.[2,32,33]. We have reported that the hot water extract of the fruiting bodies of the cultivated *G. lucidum* could also improve the physiological effects of the rat after long-term oral administration, but the results are not so good as those of the natural *G. applanadum*[6]. In this experiment, although the *G. applanadum* administered group was still influenced by the injection of exotoxin, the influence on the *G. applanadum* group was less than that of the *G. lucidum* group, such as the lowering of blood pressure, the decrease of WBC number, the functions of serological enzymes.

The antibacterial activity of *G. applanadum* pretreated mice was better than that of the *G. lucidum* group. The anticancer activity against the transplantation of Sarcoma-180 also showed the same result.

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Ganoderma applanadum 長期投與大白鼠對大腸桿菌外毒素的保護作用

商惠芳　曾金章　吳雪娟*

汪稜芳**　董一致**

靈芝長期投與大白鼠其生理功能有明顯的改善，且對大腸桿菌莢膜多醣類菌株外毒素之攻擊，具很好的防禦作用。以外毒素（2 mg／kg）注射*G. applanadum* 及*G. lucidum* 長期投與大白鼠 2 小時後發現：

1. *G. applanadum* 及*G. lucidum* 二組之心縮壓分別下降11.0mm Hg，14.0mm Hg。均較對照組緩和。心跳速率的增加亦較對照組少。
2. 白血球的減少及血濃縮現象較對照組輕微。
3. 腎臟功能的檢查：*G. applanadum*，*G. lucidum* 及對照組三組的BUN分別爲0.6mg／dl，1.9mg／dl，和4.6mg／dl. Creatinine：分別爲0.20mg／dl，0.08mg／dl，和0.3mg／dl Uric acid：1.47mg／dl，0.77mg／dl，和1.13mg／dl. 故靈芝組之增加較對照組少。
4. 肝臟功能檢查：除了S-GOT例外，靈芝組之S-ALP，S-GPT之增加較對照組少。

靈芝長期投與大白鼠雖然仍受大腸桿菌外毒素之影響，但其防禦作用較對照組好。此現象亦可發現於靈芝預處理小白鼠對大腸桿菌感染之防禦能力，以及對Sarcoma-180 移植之活存時間。

台北醫學院微生物學科、醫事技術學系*　及生化學科**

民國七十三年四月十八日受理