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Methanol Extract of *Agaricus blazei Murill* Reduces Hepatic Damage Induced by CCl_4 and High Fat and Improves Liver Lipid Profile in Rats*

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The effect of methanol extract from *Agaricus blazei Murill* on the hepatotoxicity was investigated. CCl_4 is one of the oldest and most widely used toxins for the induction of hepatic damages and fibrosis in experimental animals. In this study, male Sprague-Dawley(SD) rats were randomly divided into 6 groups of the control(C), CCl_4(T), CCl_4 and high fat group(TL) with matching sub-groups of *Agaricus blazei Murill* extract-fed groups of CA, TA and TLA. Methanol extracts of *Agaricus blazei Murill* were fed 50 mg/kg B.W daily via drinking water. A 1.2 mL of CCl_4/kg body weight was administered by oral intubation twice a week for total of six times. The levels of total-cholesterol, TG, LDL and LDL-phospholipids were elevated by CCl_4 treatment as compared to the control(C). However, *Agaricus blazei Murill* methanol extract feeding in the group of TA and TLA significantly($p<0.05$) decreased TG by 53.1% and 17.9% compared to the internal control of T and TL, respectively. Triglyceride of TL was increased by 3.33 times($p<0.05$) compared to the control(C) with CCl_4 and high fat administration from 3.78 mg/g to 12.60 mg/g liver. The extract(CA) also reduced kidney weight compared to the control(C). With the administration of high fat and CCl_4(TLA), the extract reduced the organ weight of both liver and kidney and further, significantly reduced TG, total cholesterol and GTP activity. Hepatoprotective effects of *Agaricus blazei Murill* on GOT, GPT, AP and LDH activities were enhanced by the extract feeding. Electronmicrograph showed that CCl_4 deteriorated the structure of cytoplasmic matrix with its uneven distribution. However, the extract reconstituted the damaged cytoplasm and stimulated mitochondriogenesis. The above results suggest that *Agaricus blazei Murill* may have a possible protective effect against chemically induced liver damage and further help to reduce the symptoms of fatty liver.

Key words: *Agaricus Blazei Murill,* CCl_4, liver, Electronmicrograph, Cholesterol

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INTRODUCTION

Mushroom is a fungus classified as ascomycetes belonging to euascomycetidae species which spreads through forming fruit body. Mushroom, a species of higher fungus, contains various nutrients such as carbohydrate, protein, lipid, mineral and vitamins.[1-4] It also carries unique flavor and taste and is used as food stuff as well as medicinal materials.

Studies on the biological effectiveness of *Agaricus blazei Murill* have been focused on its functional aspects of anticancer, stroke, bio-regulation and heart attack.

Agaricus blazei Murill has been known to stimulate immune function particularly after surgical operation of cancer such as gastric, esophagus, duodenum, colon, rectum and liver cancer. It also is known to be effective on irregular menstruation, cervical bleeding, intestinal bleeding, detoxification and intestinal vitality. Although the anticancer functional mechanism of polysaccharide extracted from *Agaricus blazei Murill* is not yet elucidated, it is reported that the anticancer activity is closely related to its immune-boosting action through macrophage or complement system.[5] Though the longevity of human has been increased, rapid economic devolvement, diversification of diet and changes of life style lead to the increase of senile diseases.[6]

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Particularly, high meat and fat intake, fast food consumption and high calorie intake have become major factors of mortality causing obesity, hypertension, atherosclerosis, diabetes and cancer.[7-9] Kirby et al.[10] using oat bran and alfalfa reported low serum triglyceride in experimental animals and Akiba et al.[11] indicated that epigallocatechin gallate and epicatechin gallate reduces serum cholesterol and increases HDL. Lim et al.[12] also reported that mugwort and *Cirsium Maackii Maxim* decrease serum lipid in hyperlipidemic rats. Other efficacy studies include edible mushroom,[13] *Lentinus edodes,*[14] bellflower, *Codonopsis lanceolata*[15] and *Angelica utilis Makino.*[16] Kim et al.[17] reported antimutagenic effects of Korean wild mushroom and previously antimutagenic and cytotoxic effect of *Agaricus blazei* and *Phellinus linteus*[18] were also reported.

Therefore, this study has intended to identify the biological function of *Agaricus blazei Murill* and the effect on cholesterol and lipid metabolism of the liver in rats.

MATERIALS AND METHODS

1. Extraction of Specimen

Specimen of *Agaricus blazei Murill* was obtained locally in Kangwon province, and methanol(70%)-extracted with 10 times the sample(w/w) for 24 hours and concentrated with rotary evaporator(Eyela, Japan). The specimen reaching 25% of yield was frozen for 24 hours and then lyophilized for 48 hours.

2. Experimental Animals and Design

Weanling male Sprague Dawley(SD) rats weighing approximately 70-80 g were maintained in a 12:12 dark and light cycled room. Before the start of the experiment, animals were fed chow diet for a week and then divided randomly into six groups of high fat and CCl_4 administration. Body weight and food consumption were measured three times a week.

3. Experimental Diet Composition and Administration of CCl4

Animals were fed *ad libitum* with powered mixed diet based on AIN-76 formula. Control diet was consisted of 17% of casein, 4% of corn oil, 3.5 % of AIN-76 mineral mixture, 1% of AIN-76 vitamin mixture, 0.18% of DL-methionine, 5.0% of alpha-cellulose, 20% of corn starch and 52.32% of sucrose. High fat diet contained 10% of lard and 1% of cholesterol. A sample of 50 mg/kg body weight of *Agaricus blazei Murill* dissolved in H_2O was fed daily through the drinking water bottle. CCl_4, a potent hepatic toxin, dissolved in olive oil, was administered at 1.2 mL/kg body weight twice a week for 3 weeks, total of 6 times through the stomach tube. Internal control was fed vehicle only.

Table 1. Experimental design and animal groups

Group[1]	Experimental diet
C	Basal diet[2]
CA	Basal diet+Extract[3]
T	Basal diet+CCl_4[4]
TA	Basal diet+CCl_4+Extract
TL	Basal diet+CCl_4+High fat[5]
TLA	Basal diet+CCl_4+High fat+Extract

[1] C; basal diet only fed, CA; basal diet and extract fed, T; basal diet and CCl_4 administered group, TA; basal diet and CCl_4 and extract fed group, TL; Basal diet, CCl_4 and high fat fed group, TLA; Basal diet, CCl_4, high fat and extract fed group
[2] AIN-76 diet composition formula
[3] *Agaricus blazei Murill* methanol extract of 50 mg/Kg body weight was fed via drinking bottle daily
[4] CCl_4 of 1.2 mL/Kg body weight was administered through stomach tube twice a week for 3 weeks from a week after the start of extract feeding
[5] High fat contained 10% of lard and 1% of cholesterol in the diet

4. Preparation of Tissue Specimen from Experimental Animal

Animals were fasted for 12 hours and weighed before sacrificed by ether anesthesia and cervical dislocation. Blood was taken by cardiac puncture, and organs including liver and kidney were taken, weighed and rinsed with physiological saline. Liver sample of 1 g was homogenized with 0.25 M sucrose buffer and centrifuged at 600 g (Microspin 24S, Sorvall instruments, USA) for 15 minutes, and pellets were discarded and the supernatants were obtained and kept frozen at -70 ℃ for enzyme assay.

5. Analysis of Liver Lipids

Analysis of liver lipids was performed by the modified method of Folch et al.[19]

In order to obtain total liver lipids, 1 g of rat liver was homogenized in a solution of chloroform : methanol (2:1, v/v), centrifuged at 3,000 rpm for 10 minutes, and the chloroform layer was removed, and then it was centrifuged again with 3 mL of chloroform, and after vacuum drying, total liver lipids were measured. Liver triglyceride(TG), total cholesterol, LDL, LDL-phospholipids were measured at 505 nm using Beckman spectrophotometer(DU series-70) and kit(Wako Co., Japan).

6. Measurement of Liver Function Index Enzymes

Glutamic oxaloacetic transaminase(GOT) and glutamic pyruvic transaminase(GPT) exist abundantly in the liver and heart and are used to detect hepatitis and liver cirrhosis. As hepatitis, cirrhosis or apoptosis develops, the activity of these enzymes sensitively reacts and increases. Alkaline phosphatase(AP) and lactate dehydrogenase(LDH) were measured with a kit using Johnson and Johnson Ektachem(USA) analyzer. GOT, GPT, AP and LDH were expressed as unit/L of serum.

7. Ultrastructural Observation of Liver Tissue

Liver tissue in a size of approximately 4-6 mm from each experimental group was prefixed for 2 hours in a solution of 1% paraformaldehyde and 1% glutalaldehyde, rinsed in a buffer solution and fixed in 2% osmium tetroxide. Fixed slide was dehydrated and sliced by Reicurt Yung ultramicrotome after embedding with Lowicryl HM20. Slide attached with grid was double stained with lard citrate and uranyl acetate and was observed by Zeiss(Swiss) EM 109 Transmission Electron Microscope.

8. Statistical Analysis

Data analysis was carried out using SAS(statistical analysis system) version 8.0(SAS Institute, Cary, NC, USA) program. Data from all experiments were expressed as the means±standard error of means(SEM). The level of significance was set at $p<0.05$. General lineal model(GLM) with Duncan's multiple range test was used to assess the differences in mean levels of continuous variables among the groups. Student's *t*-test was used to compare the means of several variables between the experimental group and each paired internal control.

RESULTS AND DISCUSSION

1. Body, Hepatic and Other Weight Changes by the Extract

Changes of body weight, diet consumption and food efficiency of experimental animals during the four weeks of experimental diet feeding are shown in Table 2. Body weight and diet consumption were decreased in *Agaricus blazei Murill* extract fed groups comparing to the control with no statistical significance. The result was similar to the observation in *Pueraria lobata*,[20] green tea,[21] red tea[22] and mugwort[23] that the extract feeding resulted in body weight decrease. Kim et al.[24] also observed similar results in *Ganoderma lucidum, Lentinus edodes Sing, Auricularia auricula.*

Table 2. Body weight, food intake and food efficiency of the rats fed *Agaricus blazei Murill* methanol extract and treated CCl_4

Group	Body weight gain (g/day)	Food consumption (g/day)	Food efficiency ratio
C[1]	6.029 ± 0.572[ns,3),4)]	15.854 ± 2.173[ns]	0.363[2)]
CA	5.824 ± 0.447	15.314 ± 3.456	0.350
T	5.033 ± 0.614	12.704 ± 1.254	0.396
TA	5.654 ± 0.815	13.959 ± 3.172	0.405
TL	5.925 ± 0.925	14.679 ± 2.863	0.404
TLA	4.403 ± 0.713	15.447 ± 3.245	0.290

1) Refer to Table 1 for group legends
2) Food efficiency ratio=body weight gain/food consumption
3) ns: not significant
4) Mean±S.E.M

Table 3. Weight of liver, kidney and spleen of rat fed *Agaricus blazei Murill* methanol extract

(unit: g)

Group	Liver	Kidney	Spleen
C[1]	8.796 ± 0.273[c,2,3)]	2.063 ± 0.067[a]	0.657 ± 0.025[a]
CA	8.773 ± 0.267[c]	1.943 ± 0.038[b]	0.656 ± 0.006[a]
T	8.830 ± 0.248[c]	1.707 ± 0.040[b]	0.616 ± 0.018[a]
TA	8.221 ± 0.445[c]	1.706 ± 0.062[b]	0.687 ± 0.024[a]
TL	12.901 ± 0.389[a]	1.950 ± 0.041[b]	0.721 ± 0.032[a]
TLA	11.074 ± 0.172[b]	1.444 ± 0.102[c]	0.691 ± 0.052[a]

1) Refer to Table 1 for group legends
2) Mean±S.E.M
3) Values with different superscript within the same column are significantly different at $p<0.05$ using general linear model with Duncan's multiple range test to assess the differences in the mean levels among the groups.

Body weight gain of T group with CCl_4 administration was 5.03 g/day, a decrease of 16.6% compared to the control(C). Diet consumption and food efficiency showed no difference from the control. The extract feeding in combination with CCl_4 and high fat(TLA) stimulated the decrease of body weight compared to the corresponding control group of TL, decreasing 25.7% of body weight daily.

The effect of the extract on the organ weight of rats is shown in Table 3. Extract feeding(CA) tended to reduce the organ weight of liver, kidney and spleen. This corresponds to the reduction of weight and diet consumption in the extract fed group compared to the control(C).

Takeda et al.[25] reported that the liver accumulation of lipids corresponded to the increase of liver weight. Liver weight was reported to be reduced by the extract feeding of *Ixeris sonchifolia* Hance.[26] The liver weight of TL was increased by 46.7%($p<0.05$) compared to that of the control. However, the extract feeding significantly($p<0.05$) reduced the liver and kidney weight in the group of CCl_4 and high fat. Spleen weight was not affected by the extract.

Table 4. Effects of *Agaricus blazei Murill* methanol extract on the concentration of the lipids in the liver of rat treated with CCl_4

(mg/g liver)

Group	TG	Total -cholesterol	LDL -cholesterol	LDL -phospholipid
C[1)]	3.78 ± 0.183[cd,2),3)]	9.12 ± 1.231[c]	1.43 ± 0.219[b]	3.15 ± 0.249[b]
CA	2.71 ± 0.426[d]	7.88 ± 0.474[c]	1.29 ± 0.047[b]	2.86 ± 0.287[b]
T	4.73 ± 0.555[c]	12.84 ± 1.289[c]	1.70 ± 0.104[b]	3.26 ± 0.236[b]
TA	2.22 ± 0.392[d]	9.074 ± 0.704[c]	1.33 ± 0.182[b]	3.09 ± 0.531[b]
TL	12.60 ± 0.982[a]	35.43 ± 2.587[a]	9.08 ± 1.251[a]	8.11 ± 0.981[a]
TLA	10.34 ± 1.303[b]	25.23 ± 2.217[b]	9.87 ± 1.583[a]	10.07 ± 2.374[a]

1) Refer to Table 1 for group legends
2) Mean±S.E.M
3) Values with different superscript within the same column are significantly different at $p<0.05$ using general linear model with Duncan's multiple range test to assess the differences in the mean levels among the groups.

Our results are similar to the observation of Menson et al.[27] that CCl_4 can damage hepatic cell membrane and thus increase permeability and eventually can cause edema and lipid accumulation in the liver. It is considered that the extract can reduce the CCl_4 induced hepatic and renal toxicity.

2. Effect of the Extract, CCl4 and High Fat on the Liver Lipid Metabolism

The extract feeding in the group of TA and TLA significantly($p<0.05$) decreased TG by 53.1% and 17.9% compared to the internal control of T and TL, respectively. Triglyceride of TL was increased by 3.33 times ($p<0.05$) compared to the control(C) with CCl_4 and high fat administration form 3.78 mg/g to 12.60 mg/g liver. These results are similar to those of Kim et al.[28] with various mushroom extracts. Total cholesterol of TLA was reduced by 28.2% ($p<0.05$) by the extract in the CCl_4 and high fat group. LDL cholesterol and LDL-phospholipids were not affected by the extract. CCl_4, an environmental contaminant, is produced in the industry of manufacturing glass, rubber and solvent and can cause cell damage and lipid peroxidation converted into CCl_3 in the human body. The results indicate that the extract of *Agaricus blazei Murill* may be involved in the detoxification process in the liver that is caused by CCl_4.

3. Effects of the Extract on the Liver Function Index Enzymes of GOT, GPT, AP and LDH

Effects of the extract on the liver function index enzymes are shown in Table 5. Overall, the extract was prone to decrease GOT and GPT activities. GPT, in particular, was significantly($p<0.05$) decreased by 21.7% from 72.33 unit to 56.60 unit. CCl_4 and high fat increased GOT activity by 49.4% compared to the control of

Table 5. Effects of *Agaricus blazei Murill* methanol extract on the activity of serum glutamic oxaloacetic transaminase, glutamic pyruvictransaminase, alkaline phosphatase and lactate dehydrogenase in rat treated with CCl_4

(unit/L)

Group	GOT	GPT	AP
C[1)]	128.17 ± 9.904[b,2),3)]	57.40 ± 3.280[b]	204.83 ± 22.644[b]
CA	122.50 ± 13.978[b]	57.50 ± 9.014[b]	206.40 ± 9.9059[b]
T	177.60 ± 11.192[ab]	56.60 ± 4.082[b]	208.60 ± 4.707[b]
TA	172.75 ± 37.845[bc]	43.75 ± 3.945[b]	214.33 ± 2.728[b]
TL	191.50 ± 14.074[a]	72.33 ± 5.220[a]	267.80 ± 21.411[a]
TLA	158.40 ± 8.165[ab]	56.60 ± 4.082[b]	249.71 ± 10.765[ab]

1) Refer to Table 1 for group legends
2) Mean±S.E.M
3) Values with different superscript within the same column are significantly different at $p<0.05$ using general linear model with Duncan's multiple range test to assess the differences in the mean levels among the groups.

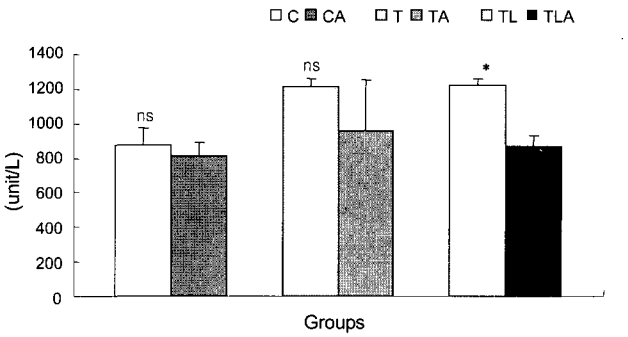

Fig. 1. Lactate dehydrogenase activity of the *Agaricus blazei Murill* extract fed groups.

High fat group with CCl_4, in particular, showed significant($p<0.05$, marked with*) difference when the extract was fed. Overall LDH activity tended to be inhibited by the extract.
1) Refer to Table 1 for group legends.
2) group with different superscript within the paired groups are significantly different at $p<0.05$(marked with*) using Student's *t*-test to compare the means of variables between the experimental and each paired internal control.

128.17 unit/L. Although there was no statistical significance, GOT and AP activities overall tended to decrease by the extract. However, GPT activity was significantly decreased ($p<0.05$) by 17.9% from 72.33 unit to 59.4 unit. LDH activity was also reduced by the extract by 28.9%($p<0.05$) in the CCl_4 and high fat group(Fig 1). The results indicate that the extract might help to restore liver function damaged by CCl_4 and prolonged high fat diet and further, to stimulate liver lipid metabolism.

4. Electron Microscopic Observation of Liver

Control liver maintained an even distribution of mitochondria and endoplasmic reticulum and normal structure of cytosol(Fig 2-1). CCl_4 administration caused cytosolic damages and irregular and uneven distribution of mitochondria and ER(Fig 2-2).

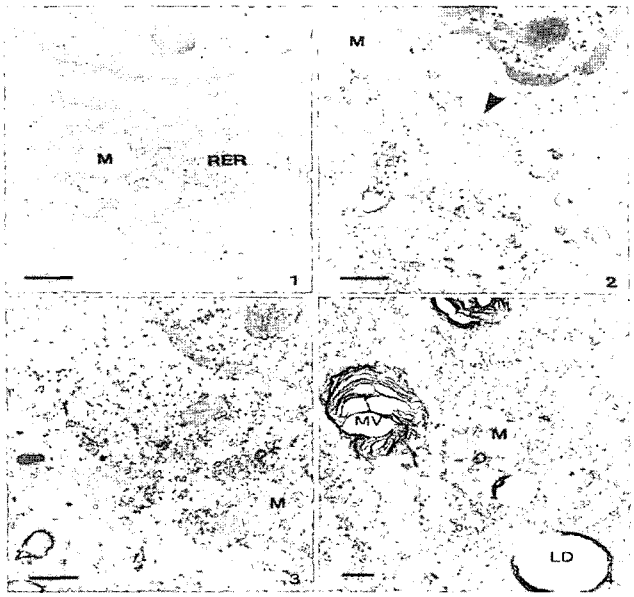

Fig. 2. Electron micrograph of hepatocytes of experimental rat fed *Agaricus blazei Murill* methanol extract. Bar equals 1μm (×12,600)

1) Control rat liver. Regular distribution of rough endoplasmic reticulum(RER) and mitochondria(M) in the cytoplasm were observed.
2) CCl_4 treated rat liver. Mitochondria was partly localized in the cytoplasm. Arrow indicates cytoplasmic damage.
3,4) CCl_4 and *Agaricus blazei Murill* methanol extract fed rat liver. Mitochondria and multivesicle(MV) were seen in the process of repairing. lipid droplet (LD) was observed with the increase of the number of mitochondria.

In addition, a distinctive deterioration of cytosolic components was observed and the accumulation of fibrous filaments as well. Extract of *Agaricus blazei Murill* seemed to revitalize CCl_4-damaged cytosol and to help forming mutivesicles(Fig. 2-3, Fig. 2-4). The increases of mitochondrial number and even distribution of ER were identified with newly synthesized lipid droplets. The above histological and biochemical changes by the extract support the observation in the study that the enhancement of lipid metabolism and decrease of LDL and triglycerides in the liver. The extract was considered to contribute to the increase of mitochondria and cytosolic components. Similar results were observed by Park[29] and Rhee[30] in the studies of onion and ginseng on the reduction of liver cholesterol and lipids.

SUMMARY AND CONCLUSION

Agaricus blazei Murill methanol extract feeding in the group of TA and TLA significantly($p<0.05$) decreased TG by 53.1% and 17.9% compared to the internal control of T and TL, respectively. Triglyceride of TL was increased by 3.33 times($p<0.05$) compared to the control (C) with CCl_4 and high fat administration form 3.78 mg/g to 12.60 mg/g liver. The extract(CA) also reduced kidney weight compared to the control(C). However, with the administration of high fat and CCL_4(TLA), the extract reduced the organ weight of both the liver and kidney and further, significantly reduced TG, total cholesterol and GTP activity. The extract seems to have a role in protecting liver and kidney from damages induced by CCl_4 administration and high fat diet. Although statistically not significant, the extract overall tended to reduce GOT, GPT and LDH activities which were increased by the administration of potent liver toxin of CCl_4. Electron micrography observed that damaged mitochondria and cytosolic components were revitalized and mutivesicles were newly synthesized by the extract. The results in this study suggest that *Agaricus blazei Murill* may have a possible protective effect against chemically induced liver damage and further may help to reduce the symptoms of fatty liver.

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