RS2-2

Characterization of *Fomes fomentarius* on their Basic Pharmacological Activities with Bromobenzene-induced Hepatotoxicity and STZ-induced Hyperglycemic Rats

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Fomes fomentarius has been reported in B.C. on birch, alder, balsam poplar, and cottonwood. Elsewhere in North America it has also been found on maple, Douglas-fir (rarely), oak, apple, willow, and *Prunus* spp. *Fomes fomentarius* is a fungus of the polyporaceae family, parasitic on broadleaf trees. Tea of large and white clubs of *F. fomentarius* is a popular drink said to have an anticancer effect and to be good for the health care in Japan.

In this present study, we characterized the pharmacological basic studies of methanol extract of *Fomes fomentarius*(FFM) on the hepatic antioxidant activity and PGE2, NO production, COX-2, iNOS expression.

Material and Methods

1. Aminotransferase (AST, ALT) activity-Reitman and Frankels method
2. Erythrocyte, Leucocyte, Hemoglobin and Hematocrit-Fonios method
3. Phospholipid level-Chen's method
4. LD_{50}- Behrens-Karber method
5. Urinalysis parameter- using Visual/Urine analyzer Urine strips (MDSAVERnet Co.)
6. Blood glucose Concentration -using Lifescan One Touch Test Strip (Lifescan, USA)
7. Triglyceride level-McGowan's method
8. Total cholesterol level-Richmond's method
9. Aminopyrine N-demethylase activity-Nash method
10. Aniline hydroxylase activity-Bidlock's method
11. Xanthine oxidase activity-Stirpe and Della method
12. Lipid peroxide content-Ohkawa's method
13. Glutathion S-transferase activity- Habig's method
14. Epoxide hydroxylase activity-Hammock's method

Results and Discussion

FFM extracts is a relatively stable, water-soluble and their fruit body widely used in neutraceutical foods.

Studies were designed to determine the acute toxicity of FFM when administered orally to both sexes of rats for 24, 48, 72hrs. FFM was not effectively in acute (5,000mg/kg LD_{50}) and subacute toxicity (Table 1).

Streptozotocin (STZ) -induced diabetic effects were analyzed for glucose and triglyceride level. FFM were significantly decreased of blood glucose conc. and lipid levels on STZ-induced hyperglycemic rats. (Table 2,3,4). The activation of hepatic enzymes is reduced during STZ-induced diabetes that might play a role in controlling glucose homeostasis in diabetic animals. Also, FFM were significantly regenerated of lipid peroxide, microsomal enzyme system and epoxide hydrolase on bromobenzene-induced hepatotoxicity rats (Table 5,6,7,8,9).

LPS induced antiimfalmatory effect of RAW 264-7 cell were characterized for PGE2, NO production and an expression of COX-2, iNOS.

Depending on FFM concentrations, which were significantly decreased of nitrate accumulation, PGE2 and TNF-alpha.

From these results, we expected the characterization of *F. fomentarius* on the potentiality of the diabetes effect and antiimflamatory effects.

Table 1. Acute toxicity (LD_{50}) of methanol extract of *F. fomentarius*

Time (hr)	2,000	3,000	4,000	5,000(mg/kg)
	Dead * / treated animal			
24	0/30	0/30	0/30	0/30
48	0/30	0/30	0/30	0/30
72	0/30	0/30	0/30	0/30

*The number of dead mice for 24, 48, 72 hours after intraperitoneally injectio and orally administered of sample

Table 2. Urinalysis parameter in male SD rats orally administered with for methanol extract of *F. fomentarius* one month

Treatment parameter	Glucose					Bilirubin					Ketone				
degree	-	+/-	1+	2+	3+	-	+/-	1+	2+	3+	-	+/-	1+	2+	3+
Normal	5	0	0	0	0	2	3	0	0	0	1	4	0	0	0
10%	5	0	0	0	0	5	0	0	0	0	3	2	0	0	0
20%	5	0	0	0	0	4	1	0	0	0	1	4	0	0	0
30%	5	0	0	0	0	5	0	0	0	0	2	3	0	0	0

Treatment Parameter	Urobilinogen				Occult Blood					pH			
Degree	0.1	1.0	2.0	4.0	-	+/-	1+	2+	3+	7.0	7.5	8.0	8.5
Normal	4	1	0	0	5	0	0	0	0	0	1	4	0
10%	5	0	0	0	5	0	0	0	0	0	2	2	1
20%	4	1	0	0	5	0	0	0	0	0	1	4	0
30%	5	0	0	0	5	0	0	0	0	0	1	3	1

The assay procedure was described in the experimental methods. Values represent means ± S.D. (n=10).

Table 3. Posttreatment of methanol extract of *F. fomentarius* on the body weight changes in STZ-induced rats

Treatment	Dose (mg/kg)	Body weight Change (g)
Normal		25.8 ± 1.92a
STZ	50	-32.4 ± 5.59c
FFM	50	-28.0 ± 5.70b, c
	100	-23.7 ± 4.15b

Sample were administrated orally from 2 weeks after STZ injection. The rats were sacrificed 24 hours for last treated materials. The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments. 2) Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 4. Posttreatment of methanol extract of *F. fomentarius* on the level of glucose in STZ-induced rats

Treatment	Dose (mg/kg)	Concentration (mg/dl)
Normal		95.2 ± 9.98c
STZ	50	341.0 ± 31.6a
FFM	50	330.1 ± 19.5a
	100	270.6 ± 18.9b

Sample were administrated orally from 2 weeks after STZ injection. The rats were sacrificed 24 hours for last treated materials. The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments. 2) Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 5. Effect of methanol extract of *F. fomentarius* on hepatic lipid peroxide content in bromobenzene-treated male rat

Group	Dose (mg/kg)	Content MDA n moles/g of tissue
Normal		18.0 ± 1.18d
BB	460	56.4 ± 1.77a
FFM	50	46.2 ± 3.12b
	100	38.7 ± 2.98c

Sample were administrated orally from 2 weeks and rats were sacrificed 24 hours for last treated materials. Bromobenzene(BB, 460mg/kg) was intraperitoneally injected twice a day. The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments, 2) Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 6. Effect of methanol extract of *Fomes fomentarius* on hepatic aminopyrine N-demethylase and aniline hydroxylase activities in bromobenzene-treated male rats

Group	Dose(mg/kg)	AD	AH
Normal		4.17 ± 0.24c	0.64 ± 0.090c
BB	460	9.34 ± 0.37a	1.26 ± 0.087a
FFM	50	9.02 ± 0.22a	1.17 ± 0.073a
	100	8.18 ± 0.25b	0.91 ± 0.061b

The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments, 2) Values sharing the same superscript letter are not significantly different each other($p<0.05$) by Duncan's multiple range test.
AD: aminopyrine N-demethylase: formaldehyde nmole/mg protein/min
AH: aniline hydroxylase: p-aminophenol nmole/mg protein/min

Table 7. Effect of methanol extract of *F. fomentarius* on hepatic xanthine oxidase activity in bromobenzene-treated male rats

Group	Dose(mg/kg)	Activity*
Normal		2.18 ± 0.11b
BB	460	3.37 ± 0.18a
FFM	50	3.45 ± 0.20a
	100	3.50 ± 0.17a

The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments, 2) Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.
*uric acid nmole/mg protein/min

Table 8. Effect of methanol extract of *F. fomentarius* on hepatic glutathione S-transferase activity activity in bromobenzene-treated male rats

Group	Dose(mg/kg)	Activity*
Normal		263.4 ± 17.7a
BB	460	243.8 ± 7.68a,b
FFM	50	239.6 ± 10.4b
	100	259.6 ± 9.25a,b

The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments, 2) Values sharing the same superscript letter are not significantly different each other($p<0.05$) by Duncan's multiple range test.
*1,2-dinitro-4-nitrobenzene nmole/mg protein/min

Table 9. Effect of methanol extract of *F. fomentarius* on hepatic epoxide hydrolase activity activity in bromobenzene-treated male rats

Group	Dose(mg/kg)	Activity*
Normal		14.80 ± 0.60a
BB	460	4.16 ± 0.13d
FFM	50	5.36 ± 0.41c
	100	9.37 ± 0.22b

The assay procedure was described in the experimental methods. 1) Values are expressed mean ± S.D. for groups of 6 experiments, 2) Values sharing the same superscript letter are not significantly different each other($p<0.05$) by Duncan's multiple range test.
*TSO nmle/mg protein/min