Advanced Herbal Medicine, Winter and Spring 2021; 7(1): 1-13

herbmed.skums.ac.ir

Hepatoprotective effect of *Fomes fomentarius* extract against carbon tetrachloride-induced acute liver injury in rat

Zahra Chegini Nejad[1], Mahdieh Kurepaz-mahmoodabadi[2], Azadeh Manayi[2*]

[1] School of Pharmacy, International Campus, Tehran University of Medical Sciences, Tehran, Iran

[2] Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Original Article

Received: 9 October 2021 Accepted: 17 October 2021

Abstract:

Background and aims: Medicinal mushrooms are known for their potential for the treatment of various diseases such as cancer and gastrointestinal disorders. *Fomes fomentarius* is a fungus belonging to the Polyporaceae family with anti-inflammatory, antioxidant, antidiabetic, and antitumor effects.

Methods: This study aimed to investigate the hepatoprotective effect of *F. fomentarius* extract in a rat model of carbon tetrachloride (CCl4)-induced toxicity along with its scavenging activity against 2,2-diphenyl-1-picrylhydrazyl.

Results: Better antioxidant activity was obtained for ethanol extract of the fungus (IC_{50}: 206.20 µg/mL) compared with its aqueous extract (IC_{50}: 414.33 µg/mL). Levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase decreased after administration of the ethanol extract especially at 100 mg/kg compared with higher doses (200 and 400 mg/kg).

Conclusion: Treatment with the lower dose of the extract reduced alkaline phosphatase more pronouncedly than silymarin, indicating the beneficial properties of the fungus for biliary tract diseases. Histopathological findings also showed that *F. fomentarius* extract

*Corresponding author: Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98 - 64121229 Email: manayi@tums.ac.ir

at the lower dose could protect against CCl4-induced liver injury, and the mechanism may be related to its antioxidant activity.

Keywords: Alkaline phosphatase, Alanine aminotransferase, Antioxidant, Aspartate aminotransferase, Fungus, Liver enzymes

INTRODUCTION

Liver plays an essential role in the protection of the body against ingested xenobiotics and drugs. Hepatic necrosis and apoptosis mostly result from hepatic injury due to poisons or drugs. These agents cause oxidative damage that plays a pivotal role in hepatic injury (1).

The development of hepatoprotective agents with maximal hepatoprotective activity and minimal toxicity has become a purpose frequently sought after for liver protection. Compounds with antioxidant and free radical-scavenging activity can protect liver against oxidative damage. Some natural products such as silymarin are well known for antioxidant and hepatoprotective effects, which are primarily attributed to reducing lipid peroxidation (2). In this regard, mushrooms with their salience in traditional medicine and long history of application as a nutritional supplement can serve as effective alternatives for the protection of liver.

Fomes fomentarius (L.) Fr. (Polyporaceae) is a woody and perennial fungus with a large body that develops as saprophyte or parasite on deciduous trees. The fungus has been used for the treatment of various diseases including inflammation, gastrointestinal problems, oral ulcers, cirrhosis of the liver, and cancers for many centuries. Antioxidant, antidiabetic, and anti-inflammatory activities of *F. fomentarius* have been observed in recent studies (3-7). Different classes of primary and secondary metabolites including polysaccharides, polysaccharide-protein complexes, triterpenes, fungi sterol linoleate, betulin 28-O-acetate, 7-ergostenol, β-sitosterol, (22E)-ergosta-7,22-dien-3-one, paulownin, daphnetin, and volatile compounds have been isolated from the fungus (8).

Modern science is seeking to explore and establish beneficial properties of traditionally used mushrooms using modern scientific techniques and methods. The subject of few researches has been chemical properties and biological activities of *F. fomentarius*; however, according to traditional resources, the plant can be a valuable source of active ingredients for protecting the liver. Therefore, the

present study was aimed to investigate the antioxidant and hepatoprotective activities of the fungus in acute liver toxicity induced by tetrachloride carbon (CCl4) in rats.

MATERIALS AND METHODS

Extraction:

The fruit bodies of the fungus were collected from Neka forest in Mazandaran province, north of Iran in the summer 2015. The identification of the species was done by an expert at the Herbarium of Research Institute of Forests and Rangelands (Mazandaran Agricultural and Natural Resources Research and Education Center). The mushrooms were then air-dried and grounded into small pieces. The grounded mushrooms (980 g) were extracted using ethanol and then distilled water. The sample was extracted with ethanol using percolation apparatus (3×72 h), and then the residue was boiled with distilled water for 4 h to obtain more polar compounds. Both extracts were separately concentrated by a rotary evaporator (Heidolph, Germany) at 40 ºC and freeze-dried (Alpha 2-4 LDplus, Germany).

Antioxidant activity:

The free radical-scavenging activities of the extracts were examined using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) reagent, which is a stable free radical (7). For this purpose, 1 mL of the extracts at different concentrations (100-400 µg/mL of ethanolic extract and 1000-2000 µg/mL of aqueous extract) were added to 2 mL of DPPH (4×10^{-2} mg/mL in methanol). The absorbance of the samples was measured at 517 nm after 30 min. The half maximal inhibitory concentration (IC50) of the free radical was determined using the plot of inhibition percentage against the extracts' concentrations.

Procedure:

In the present study, male Wistar rats with an average weight of 230(10) g were procured from the Animal House of Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS), Tehran, Iran and quarantined for seven days prior to use.

Room temperature was set at 22-24 ˚C with a relative humidity of 50-55% and alternating natural light/dark photoperiod (12/12 h). All animals received standard laboratory food and

fresh water *ad libitum*. The care protocol conformed to the animal welfare regulations approved by the TUMS/Pharmaceutical Sciences Research Center Ethics Committee to reduce trouble, distress, and pain in the animals.

The animals were randomly divided into seven groups of six each, six groups of which were intraperitoneally treated with a single dose of CCl4 (0.5 mg/kg) and the respective treatments were administered for seven consecutive days.

Group 1 served as negative control (intact animals) and only received 0.9% normal saline; group 2 was administered with CCl4 without any other therapeutic intervention; group 3 was administered with silymarin 100 mg/kg; groups 4-6 were treated with 100, 200, and 400 mg/kg of ethanolic extract of *F. fomentarius*, respectively; and group 7 received solvent (10% v/v dimethyl sulfoxide in ethanol). All the groups were administered with similar volume (2 mL) of the respective treatments. The extract doses were selected based on one of our previous studies in which the extract of the fungus extract (50-500 mg/kg) was intraperitoneally administered to the rats and no toxic effects were observed (7).

Animals were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) to collect their blood samples by cardiac puncture (without anticoagulant). Serum was subsequently extracted using a refrigerating centrifuge at 4000 rpm for 15 min to analyze biochemical contents. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkanine phosphatase (ALP), lactate dehydrogenase (LDH) activities and total protein of serum were determined using diagnostic assay kits (Pars Azmoon, Tehran, Iran).

Histopathological investigations:

The livers were removed after an abdominal incision and fixed in 10% buffered formalin. Tissue samples were embedded in paraffin, sectioned in thickness of 5 μm and stained with hematoxylin and eosin. Histological changes were observed in the prepared slides using an optical microscope. Bleeding, sinusoidal dilatation, and lobular changes (according to the number of Kupffer cells) were studied to investigate changes in the liver tissue.

Data analysis:

All the experiments were done in triplicate and results were expressed as mean [standard deviation (SD)] with 95% confidence interval. Significant differences between groups were investigated using one-way ANOVA followed by Tukey's test for multiple comparisons. Significance level (*P*) was considered to be <0.05.

RESULTS

Antioxidant activity of the extracts:

Antioxidant activities of the extracts were examined using DPPH, a stable free radical. Ethanol *F. fomentarius* extract at 400 µg/mL with inhibition percentage of 84.05(0.94)% showed the highest antioxidant activity against DPPH (Table 1). The inhibition percentages of 200 and 100 µg/mL of the ethanol extract were calculated at 50.66(0.49)% and 36.52(1.25)%, respectively. The aqueous extract at 1000, 1500, and 2000 µg/mL scavenged DPPH with inhibition percentages of 71.26(0.58)%, 51.5(1.56)%, and 35.92(1.18)%, respectively. The values of IC50 of the aqueous and ethanol extracts were calculated at 414.33 µg/mL and 206.20 µg/mL, respectively. Therefore, ethanol *F. fomentarius* extract with higher antioxidant activity was administered to investigate the hepatoprotective activity of the fungus in the rat model of CCl4-induced acute liver injury.

Liver function assays:

Administration of CCl4 resulted in a two- to three-fold increase in the studied enzymes' levels compared with the control group. As shown in Table 2, the concentrations of ALT, AST, ALP, and LDH significantly increased after CCl4 administration by, respectively, 64.9(4.0), 181.6(33.3), 807.8(18.8), and 857.3(39.2) U/L, compared with those in the control group [38.2 (2.7), 84.6 (16.4), 640.4 (159.7), and 280.4 (56.8) U/L, respectively].

Table 1: Antioxidant activities of *F. fomentarius* extracts against DPPH. The results reported as mean (SD)

Aqueous extract (µg/mL)	Inhibition%	**Ethanolic extract** (µg/mL)	Inhibition%
2000	71.26 (0.58)	400	84.05 (0.04)
1500	51.59 (1.56)	200	50.66 (0.49)
1000	35.92 (1.18)	100	36.52 (1.25)
IC_{50}: 414.33		IC_{50}: 206.20	

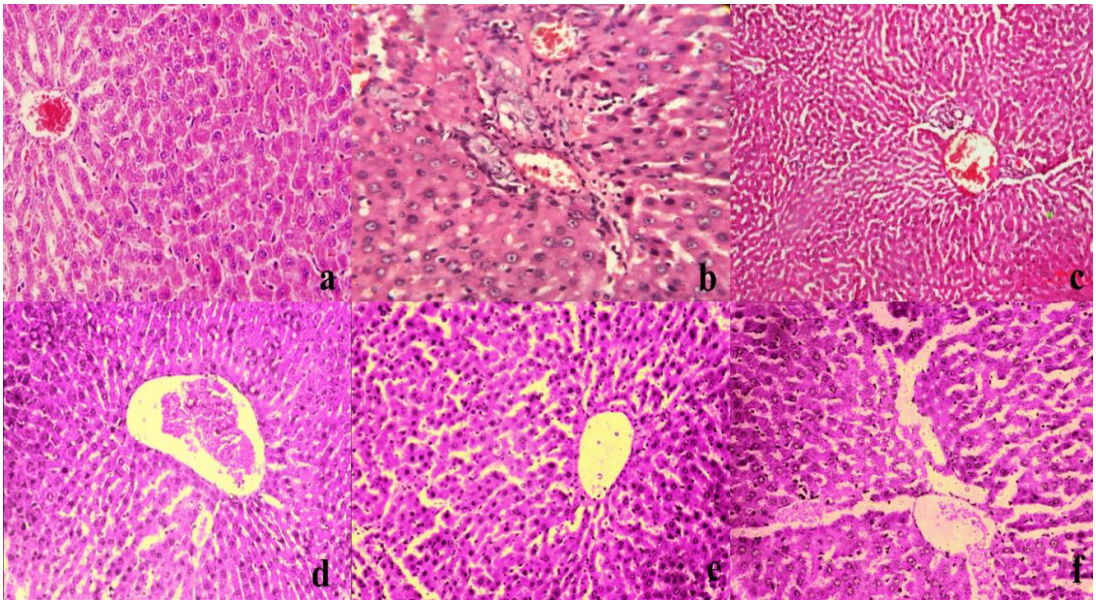

Figure 1. Histopathological changes induced by CCl4 and treatment with silymarin and ethanolic extract of *F. fomentarius*.

a: control (intact animals); b: 0.5 mg/kg CCl4 without any treatment, exhibited severe hepatocyte degeneration and necrosis; c: 100 mg/kg silymarin, showed mild sinusoidal dilation and normal amount of Kupffer cells; d: 100 mg/kg ethanolic extract of the fungus, hepatocytes are normal but mild bleeding can be seen with normal portal spaces and Kupffer density is almost normal; e: 200 mg/kg ethanolic extract of the fungus, accumulation of blood and Kupffer cells were higher than control group; f: 400 mg/kg ethanolic extract of the fungus; cellular damage and bleeding is high. Kupffer cells accumulation in dilated sinusoids is higher than control

Table 2. Biological parameters in rats treated with *F. fomentarius*.
Group 1 served as negative control (intact animals) and only received 0.9% normal saline; group 2 was administered carbon tetrachloride without any other therapeutic intervention; group 3 was administered silymarin 100 mg/kg; groups 4-6 were treated with 100, 200, and 400 mg/kg of ethanol extract of *Fomes fomentarius*, respectively; group 7 was given solvent (10% v/v dimethyl sulfoxide and ethanol); * significant difference between groups and control group ($p<0.05$); # significant difference between groups and carbon tetrachloride-treated group ($p<0.05$).

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	TP (mg/mL)
	Mean (SD) CI 95%				
Control	38.2 (2.7) (34.8, 41.5)	84.6 (16.4) (64.4, 105.1)	640.4 (159.7) (442.0, 838.7)	280.4 (56.8) (209.8, 350.9)	6.14 (0.27) (5.8, 6.5)
CCl4	64.9 (4.0) * (53.8, 76.0)	181.6 (33.3) * (89.0, 274.1)	807.8 (18.8) (755.5, 860.1)	857.3 (39.2) * (748.4, 966.1)	6 (0.07) (5.8, 6.2)
Silymarin	35 (2.5) # (27.9, 42.0)	103 (4.1) (91.4, 114.5)	586.2 (31.7) # (497.9, 674.4)	334.4 (66.8) (148.7, 520.0)	5.76 (0.2) (5.0, 6.5)
100 mg/kg	35.0 (2.2) # (28.8, 41.1)	109 (8.0) (86.5, 131.4)	411.6 (20.2) *# (355.3, 467.8)	480.4 (100.9) (200.0, 760.7)	6.0 (0.1) (5.7, 6.4)
200 mg/kg	45.2 (3.7) # (34.7, 55.7)	133.4 (21.3) (74.2, 192.6)	579.6 (48.9) # (443.8, 715.3)	571 (67.4) * (383.7, 758.2)	6.1 (0.2) (5.6, 6.6)
400 mg/kg	54.2 (2.9) * (46.1, 62.2)	177.4 (7.1) * (157.7, 197.0)	617.6 (70.7) (421.3, 813.8)	656.6 (70.2) * (461.5, 851.6)	6.1 (0.2) (5.4, 6.9)
Vehicle	58.6 (3.9) * (47.6, 69.5)	174 (16.7) * (127.5, 220.4)	686 (51.7) (542.2, 829.7)	777.1 (11.0) * (746.4, 807.8)	6.3 (0.1) (6.1, 6.6)

Ethanol *F. fomentarius* extract at all doses (100-400 mg/kg) prevented CCl4-induced elevation of the enzymes. The fungus extract at 100 mg/kg more pronouncedly reduced the levels of ALT [35.0(2.2)], AST [109.0(8.0)], ALP [411.6(20.2)], LDH [480.4(100.9)] U/L compared with higher concentrations of the fungus extract. Although the levels of all studied enzymes decreased after administration of 200 and 400 mg/kg of the fungus extract, the extract at 100 mg/kg reduced the enzymes' levels more pronouncedly. Silymarin, a hepatoprotective compound, at 100 mg/kg significantly decreased the levels of the studied enzymes [ALT 35.0(2.5), AST 103.0(4.1), ALP 586.2(31.7), LDH 334.4(66.8) U/L] compared with the group that only received CCl4. The amounts of total proteins in all groups remained relatively unchanged (5.7-6.14 mg/mL).

Histopathological examination of liver tissue:

Microscopic studies of the liver samples indicated completely normal and healthy liver tissue structures in the control group, while administration of CCl4 led to substantial pathological liver injuries including bleeding, cell damage, Kupffer cell aggregation, sinusoidal dilatation, and necrosis. Treatment with 100 mg/kg of the ethanol extract and silymarin significantly attenuated the CCl4-induced damage and restored histological condition (Fig. 1). Mild degenerative changes were observed in rats treated with 200 mg/kg of the extract. Although tissue damages in rats receiving the extract at 400 mg/kg were lower compared to the intoxicated rats, sinus dilatation, aggregation of Kupffer cells, and bleeding were obvious in the liver tissue.

Discussion

The mean level of SOD activity increased in different experimental groups on the 14th day as compared to the control group and this increase was statistically significant in experimental groups 1 and 2, so that it caused a significant difference in SOD activity in these groups compared to that in the control group.

Despite the increase in the mean activity of SOD in the three experimental groups compared with the

control group on the 28th day, this increase was statistically significant only in experimental group 3 so that a significant difference in the mean level of the enzyme was observed compared to that in the control group. It can be argued that hydroalcoholic extract of safflower at low and medium doses significantly increases the mean level of SOD in the short term, and the body will adjust to the low and medium doses in the long term and only high doses of the extract lead to increased mean SOD level on the 28th day.

Therefore, despite the increasing effect of safflower extract on the mean level of SOD, the extract cannot be used in the long term as a stimulant for the production of SOD because of the acquired adaptation to consumption of this extract. It can be used as an additive of SOD from safflower extract in the short term.

The antioxidant effects of safflower are due to a powerful antioxidant compound of its oil named p-n control or serotonin (19). It is known that the antioxidant causes the fibroblast's progressive activity of mice and humans in the environment and prevents proinflammatory cytokine production in human monocytes. The antioxidant activity and preventing role in the production of the proinflammatory cytokine from human monocytes is another effect of this plant (20,21).

SOD is the first enzyme of the antioxidant defense system and it seems that the extract of safflower flower has the greatest effect on this antioxidant. SOD is the first natural antioxidant acting against the toxicity of free radicals. This enzyme is a powerful antioxidant that protects the body from damage caused by superoxide, which is the toxic radical generated in the mitochondria. Free radicals are highly reactive molecules that are produced by cells during normal metabolism. Free radicals can accumulate and cause damage to mitochondrial DNA, nuclei and proteins inside the cell.

One of the iron-sulfur containing hydrates in metabolic pathways is disabled by superoxide.

Regarding the antioxidant properties of medicinal plants, Meena et al. (2012) reported that the plant protects brain cells against free radicals and treats the neurological problems caused by free radicals by producing

antioxidant effects (22). Arjmandi et al (2018) observed no significant differences between activities of catalase and GPX in control group in comparision to experimental groups on the 14th and 28th days of study ($p>0.05$) (23).

Khoshvaghti et al. (2013) reported that after 14 days of administering hydroalcoholic safflower flower extract, the activities of glutathione peroxidase and SOD increased in a dose-dependent situation (24).

In this regard, Khorasani et al. (2012) observed walnut's effect in increasing serum glutathione peroxidase level, and that walnut and lemon juice significantly reduced MDA level in blood plasma (25).

Regarding the medicinal effects of the plant, Asgary et al. (2012) reported that the hydroalcoholic extract of safflower flower was effective in the treatment of diabetes (26). Dehkordi et al. (2014) investigated the effect of aqueous extract of safflower on the reproductive system in female rats and observed that the extract was more effective on ovarian activity compared with wounds and had a positive impact on the fertility of female rats (27). Khoshvaghti et al. reported that the continuous short-term use of the safflower could decrease the level of thyroid hormones temporarily, while in the long term, it did not show the same effect (28).

Ellis et al. (2000) found that concomitant use of safflower oil and a high-fat diet caused insulin resistance and increased acyl coenzyme-A (29). The other advantage of safflower is lack of producing any harmful effect on blood pressure, heart rate, and contraction of the pulmonary artery (22,30). Rahimi et al (2014) reported the effect of safflower seed oil in the prevention of diabetes-related complications (31).

CONCLUSION

Taken together, *F. fomentarius* at low doses can inhibit liver injury, at least in part, by suppression of oxidative stress and it would be therefore interesting to investigate whether the fungus extract or its ingredients can ameliorate oxidative stress or play role as pro-oxidants in higher concentrations. The more potent effect of *F. fomentarius* extract for reduction

of ALP than that of silymarin, highlights the potential of the fungus for the treatment of biliary tract diseases. However, additional studies are needed to confirm the therapeutic effect of the fungus on biliary system problems.

Acknowledgement

The project was part of a Pharm.D thesis and supported by Tehran University of Medical Sciences, grant number of 93-04-169-28163.

References

1. Taub R. Hepatoprotection via the IL-6/Stat3 pathway. J Clin Invest. 2003;112(7):978-80.

2. Chen IS, Chen YC, Chou CH, Chuang RF, Sheen LY, Chiu CH. Hepatoprotection of silymarin against thioacetamide-induced chronic liver fibrosis. J Sci Food Agric. 2012;92(7):1441-7.

3. Kolundžić M, Grozdanić ND, Dodevska M, Milenković M, Sisto F, Miani A, et al. Antibacterial and cytotoxic activities of wild mushroom *Fomes fomentarius* (L.) Fr., Polyporaceae. Ind Crops Prod. 2016;79:110-5.

4. Chen W, Zhao Z, Chen S-F, Li Y-Q. Optimization for the production of exopolysaccharide from *Fomes fomentarius* in submerged culture and its antitumor effect in vitro. Bioresour Technol. 2008;99(8):3187-94.

5. Senyuk OF, Gorovoj LF, Beketova GV, Savichuk NO, Rytik PG, Kucherov II, et al. Anti-infective properties of the melanin-glucan complex obtained from medicinal tinder bracket mushroom, *Fomes fomentarius* (L.: Fr.) Fr.(Aphyllophoromycetideae). Int J Med Mushrooms. 2011;13(1).

6. Park Y-M, Kim I-T, Park H-J, Choi J-W, Park K-Y, Lee J-D, et al. Anti-inflammatory and anti-nociceptive effects of the methanol extract of *Fomes fomentarius*. Biol Pharm Bull. 2004;27(10):1588-93.

7. Vazirian M, Dianat S, Manayi A, Ziari R, Mousazadeh A, Habibi E, et al. Anti-inflammatory effect, total polysaccharide, total phenolics content and antioxidant activity of the aqueous extract of three basidiomycetes. RJP. 2014;1(1):15-21.

8. Grienke U, Zöll M, Peintner U, Rollinger JM. European medicinal polypores–A modern view on

traditional uses. J Ethnopharmacol. 2014;154(3):564-83.

9. Nowacka N, Nowak R, Drozd M, Olech M, Los R, Malm A. Antibacterial, antiradical potential and phenolic compounds of thirty-one polish mushrooms. PLoS One. 2015;10(10):e0140355.

10. Khalaf AAA, Mekawy MEM, Moawad MS, Ahmed AM. Comparative study on the protective effect of some antioxidants against CCl4 hepatotoxicity in rats. Egypt J Nat Toxins. 2009;6(1):59-82.

11. Zeinvand-Lorestani H, Nili-Ahmadabadi A, Balak F, Hasanzadeh G, Sabzevari O. Protective role of thymoquinone against paraquat-induced hepatotoxicity in mice. Pestic Biochem Phys. 2018;148:16-21.

12. Rusu M, Tamas M, Puica C, Roman I, Sabadas M. The hepatoprotective action of ten herbal extracts in CCl4 intoxicated liver. Phytother Res. 2005;19(9):744-9.

13. Shahjahan M, Sabitha K, Jainu M, Devi CS. Effect of *Solanum trilobatum* against carbon tetra chloride induced hepatic damage in albino rats. Indian J Med Res. 2004;120:194-8.

14. Tetangco EP, Shah N, Arshad HM, Raddawi H. Markedly elevated liver enzymes in choledocholithiasis in the absence of hepatocellular disease: Case series and literature review. J Investig Med High Impact Case Rep. 2016;4(2):2324709616651092.

15. Chen W, He Y, Jiang D-w, Hou D-y, Mo L-f, Shen X-r. Protective effect of *Fomes fomentarius* extracts on CCl4-induced acute liver injury in mice. Shizhen Guo Yi Guo Yao. 2013;3.

16. Choe J-H, Yi Y-J, Lee M-S, Seo D-W, Yun B-S, Lee S-M. Methyl 9-Oxo-(10 E, 12 E)-octadecadienoate isolated from *Fomes fomentarius* attenuates lipopolysaccharide-induced inflammatory response by blocking phosphorylation of STAT3 in murine macrophages. Mycobiology. 2015;43(3):319-26.

17. Seo D-W, Yi Y-J, Lee M-S, Yun B-S, Lee S-M. Differential modulation of lipopolysaccharide-induced inflammatory cytokine production by and antioxidant activity of fomentariol in RAW264. 7 cells. Mycobiology. 2015;43(4):450-7.

18. Maljurić N, Golubović J, Ravnikar M, Žigon D, Štrukelj B, Otašević B. Isolation and determination of

fomentariol: Novel potential antidiabetic drug from fungal material. J Anal Methods Chem. 2018;2018:2434691.

19. Galati G, O'brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radic Biol Med. 2004;37(3):287-303.

20. Gupta Y, Sharma M, Chaudhary G. Pyrogallol-induced hepatotoxicity in rats: a model to evaluate antioxidant hepatoprotective agents. Methods Find Exp Clin Pharmacol. 2002;24(8):497-500.

21. Neuman MG, Koren G, Tiribelli C. In vitro assessment of the ethanol-induced hepatotoxicity on HepG2 cell line. Biochemical and biophysical research communications. 1993;197(2):932-41.

22. Mani V, Arivalagan S, Siddique AI, Namasivayam N. Antioxidant and anti-inflammatory role of zingerone in ethanol-induced hepatotoxicity. Molecular and cellular biochemistry. 2016;421(1):169-81.