ORIGINAL ARTICLE

Hepatoprotective Efficacy of Oyster Mushroom (*Pleurotus ostreatus*)

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ABSTRACT

Oyster Mushroom (Pleurotus ostreatus) is typically an edible mushroom. It is also a medicinal mushroom that is traditionally used for curing various diseases. The aim of the present study was to perform chemical screening and evaluate the hepatoprotective activity of powder and extracts of Pleurotus ostreatus in CCl_4-induced hepatotoxic rat. The hepatoprotective efficacy of mushroom powder as well as extracts was compared with the reference drug Silymarin (Trade name Silybin, Square Pharmaceutical Ltd, Bangladesh). The level of Bilirubin, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were significantly decreased in Pleurotus ostreatus powder as well as extracts-treated hepatotoxic rats while concentration of total Protein and serum Albumin were significantly increased.

KEYWORDS: Extracts, Hepatotoxicity, Long Evan Rats, Pleurotus ostreatus, Powder.

1. Introduction

Mushrooms are poor in calories but rich in proteins, fibers, carbohydrates, important vitamins such as Vit-B complex, Vit-C and minerals [1–5]. From various studies it has been known that the regular consumption of mushrooms is beneficial to health. They are usually considered functional foods or nutraceutical products [6, 7]. Medicinal mushrooms are used in traditional oriental therapies and their metabolites are increasingly being used to treat a wide range of diseases [8, 9]. Edible mushrooms are not consumed only as food but also in medicinal purpose as some of them have been shown to be rich in bioactive compounds [14]. Mushrooms contain many bioactive compounds such as alkaloids, flavonoids, terpenoids, proteins, polysaccharides, fats, nucleic acids etc. which show various biological activities including antioxidant [10-12], antitumor/anticancer [13], antimicrobial [14], immune-modulatory [15], anti-inflammatory [16, 17], anti-atherogenic [18] and hypoglycemic actions [19]. Additionally, hepatoprotective properties have also been reported for mushroom extracts and mushroom-derived molecules [20, 21]. Oyster mushroom (*Pleurotus ostreatus*) is a popular edible mushroom with high nutritional values [22, 23]. It is also a valuable source of biologically active compounds [24]. For this, *Pleurotus ostreatus* is used as food and also ingredients in pharmaceuticals. In the present study, we observed hepatoprotective potency of *Pleurotus ostreatus* in CCl4-induced hepatotoxic Long Evan rats.

2. Materials And Methods

2.1 Pleurotus ostreatus

Fruiting bodies of mushroom were collected by the corresponding author from Chapainawabganj Horticulture center, Bangladesh and were identified in the Department of Zoology and brought to the Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh to screen their pharmacological efficacy.

2.2 Experimental Animal

Long Evan rats were selected as experimental animal to carry out this study. Rats, weighing 100-180 gm, were collected from the Animal Resource Division of ICDDR'B Mohakhali, Dhaka, Bangladesh.

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2.3 Preparation of powder and extracts of oyster mushroom

Fresh mushrooms were washed and disinfected by treating with $HgCl_2$ and washed again. The mushrooms were dried in shade under room temperature for eight to ten days, powdered with grinding machine and sieved. 100 gm of fine powder was separately dissolved in methanol (200 ml) and ethyl acetate (200 ml) and regularly shaked up to 10 days. Then the extracts were filtered, concentrated and dried in a rotary flash evaporator at 40-45°C for absolute dehydration. The dried extracts were preserved in an air-tight container at temperature below 20°c for further investigation.

2.4 Maintenance of animals

Animals were maintained under standard laboratory conditions at ambient temperature of 25-28 °C and relative humidity at 60-65 %, with dark light cycle of 12 hrs. They were fed with diet those were made by our research group. Forty-two (42) rats were randomly divided into seven groups (Table 1)

Table1: Grouping of experimental rats

Group	No. of Rats	Average Body weight (gm)	Age (week)	Dose /kg body weight of Long Evan rats.
Gr-I (Normal)	6	110.80	6-7	Normal diet
Gr-II (Normal control)	6	112.20	6-7	Normal diet (50 gm/kg.b.wt)
Gr-III (Hepatotoxic)	6	114.50	6-7	Normal diet
Gr-IV (Experimental powder)	6	107.30	6-7	Mushroom powder (50 gm/ kg.b.wt)
Gr-V (Experimental Extract)	6	113.20	6-7	Methanol extract (200mg/ kg.b.wt)
Gr-VI (Experimental Extract)	6	109.30	6-7	Ethayl acetate extract (200mg/ kg.b.wt)
Gr-VII (Standard)	6	111.60	6-7	Silymarin (100mg/kg.b. wt)

Each rat was numbered with a permanent marker for experimental purpose, weighed and recorded; seven cages (containing 6 rats) were kept in the departmental animal house. The animals were fed standard laboratory diet with fresh water and kept at 250c. Rats were accustomed to the laboratory conditions for one week before experimental works were started.

2.5 Acute toxicity studies

Various doses of powder and extracts (50gm and 200 mg respectively)/kg b.wt were administered orally by oral feeding tube to Long Evan rats. The animals were observed for gross behavior, neural and autonomic toxicity as described on OECD guidelines (OECD 2004) [24]. No rat died over the whole period of experiment.

2.6 Preparation of standard drug solution for oral administration

About 140 mg of Silymarin (Trade name Silybin, containing Silymarin BP 100mg/tablet; Square pharmaceuticals Ltd.) was suspended uniformly in 5ml distilled water and mixed well with a vortex mixture. The drug was not completely dissolved but dispersed in water. This dispersed drug was fed orally daily once with the help of a dropper to the experimental rats at equal dose.

2.7 Induction of hepatic injury

Hepatic injury was induced by a single intraperitoneal injection with a dose of 0.5 ml/kg. b. wt. of Carbon tetrachloride (CCl_4) to the experimental rats of Gr-III, Gr-IV, Gr-V, Gr-VI and Gr-VII.

2.8 Collection of blood

After treatment of 10 consecutive days, CCl_4 (0.5ml/ kg.b.wt.) was intraperitoneally injected on 11^{th} day. At 24 hrs. post–CCl_4 administration experimental rats were anaesthetized by putting each one in a glass jar containing ether-soaked cotton wool for about 5mins. When the heart rhythm was completely stopped, blood sample (5 ml) was then collected from retro orbital sinus using a sterile syringe and allowed to coagulate for 30mins followed by centrifugation at 2500 rpm for 15mins. Separated serum was used for estimating various blood parameters.

2.9 Estimation of blood parameters for assessment of hepatoprotective efficacy

Serum Alanine Transaminases (SALT/SGPT) and Aspartate Amino Transferase (SAST/SGOT) activity was measured following the standard method of Reitman and Frankel [25] and serum Alkaline Phosphatase (SALP) activity was estimated following the standard method of Bessey et al. [26], total Bilirubin was estimated by the standard method of Malloy and Evelyn [27], total Protein and Albumin was measured following the standard method of Kingsley and Frankel [28].

2.10 Statistical analysis

All values were expressed as mean ± standard error of mean (SEM) for six rats in each group. Statistical analysis was carried out using one-way ANOVA followed by the software 'Statistical Package for Social Sciences (SPSS)', where significant values are **p<0.01 and *p<0.05 compared to hepatotoxic (Gr-III) and tp<0.01 compared to normal (Gr-I).

3. Results

The effects of mushroom powder and extracts (methanol and ethyl acetate) on blood parameters were studied and their results are given in (Table-2). CCl_4 is mostly used to induce hepatotoxicity on various animals. Administration of CCl_4 to rats produced hepatotoxicity and showed increased serum level of SGPT, SGOT, SALP, S.Bilirubin, by 163.10%, 108.77%, 98.90% and 371% respectively and decreased serum level of total protein as well as serum albumin by 38.36% and 32.03% respectively in comparison to normal group (Gr-I).Oral administration of mushroom powder, methanol extract and ethyl acetate extract reduced SGPT level by 13.83% 26.05% and 30.29% ; SGOT level by 12.85%, 20.69% and 27.11% ; SALP level by 8.88%, 15.835 and 21.33% ; S.Bilirubin level by 18.18%, 30.30% and 33.33% respectively and elevated total protein level by 11.42%,26.53% and 34.69%; serum albumin level by 10.71%, 16.41% and 18.57% respectively with compared to CCl_4 treated hepatotoxic group (Gr-III). Hepatoprotective standard drug silymarin (100mg/Kg.b.wt) retained the serum enzymes near normal level compared to CCl_4-induced hepatotoxic rats.

Table-2: Effect of oyster mushroom *(Pleurotus ostreatus)* powder and extracts (methanol, ethyl acetate) on SGPT, SGOT, SALP, S. Bilirubin, Total Protein and Serum Albumin level in Carbon Tetrachloride (CCl_4) induced hepatotoxic long evan rats.

Grps.	Type of Groups and Doses	SGPT (U/L)	SGOT (U/L)	SALP(U/L)	S.Biliru-bin (mg/dl)	Total Pro.(g/dl)	Serum Albu.(g/dl)
Gr-I	(Normal)	46.1±1.72	67.12±2.21	150.15±3.22	0.35±0.08	7.95±0.17	4.12±0.13
Gr-II	Normal Control (50gm/kg.b.wt)	46.93±2.41	67.21±2.12	149.17±2.18	0.34±0.07	7.97±0.12	4.08±0.15
Gr-III	CCl_4-Treated-(0.5mL/kg.b.wt)	122.12±2.12t	140.13±2.41t	298.66±2.49t	1.65±0.09t	4.90±0.08 t	2.80±0.52 t
Gr-IV	Mush-room powder (50gm/kg.b.wt)	105.2±2.81*	122.12±2.61*	272.11±3.61*	1.35±0.08*	5.46±0.21*	3.15±0.23*
Gr-V	Methanol extract (200mg/kg.b.wt)	90.31±2.14**	111.13±2.12**	252.12±1.12**	1.15±0.07**	6.20±0.10**	3.35±0.41**
Gr-VI	E.Acetate extract (200mg/kg.b.wt)	85.12±2.11**	102.14±2.21**	233.15±3.21**	1.10±0.06**	6.60±0.10**	3.42±0.41**
GrVII	Standard (Sily-marin 100 mg/kg.b.wt)	60.12±2.31**	83.21±2.72**	177.23±2.12**	0.68±0.04**	7.04±0.10**	3.93±0.41**

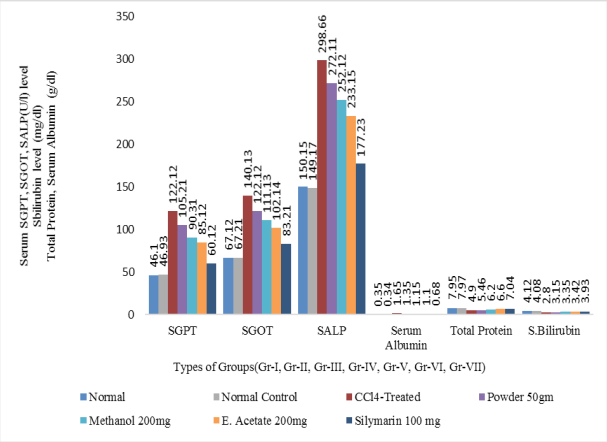

Figure: Effect of mushroom powder and extracts (methanol and ethyl acetate) on serum SGPT, SGOT, SALP, S. Bilirubin, total Protein and S. Albumin in CCl_4-induced hepatotoxic rats.

4. Discussion

The aim of the study was to explore the hepatoprotective efficacy of powder and extracts (methanol and ethyl acetate) of *Pleurotus ostreatus* in CCl_4-induced hepatotoxic rats. Activity of liver markers such as concentration of SGPT, SGOT, SALP and Bilirubin was increased in the blood due to liver biliary obstruction and degradation of hepatic cell membrane [29] while serum albumin and serum total protein level was decreased due to damage of intracellular structures such as endoplasmic reticulum, mitochondria, DNA etc. [30, 31]. CCl_4 causes hepatotoxicity by changing to the form of trichloromethyl radical (CCl_3-) and trichloromethyl peroxy radical (CCl_3OO-), which initiates activation of cytochrome P_{450} 2E1, lipid peroxidation, release of pro-inflammatory mediators such as TNF-α results in necrosis and apoptosis of hepatocytes and enhances oxidative stress-mediated hepatic damage by release of reactive oxygen species and reactive nitrogen species [32, 33]. In the present study, the hepatic biomarkers SGPT, SGOT, SALP, and bilirubin level increased and albumin as well as total protein level decreased significantly in hepatotoxic rats due to hepatic damage mediated by oxidative stress. In the present study, *Pleurotus ostreatus* powder and extracts showed phytochemical properties possibly due to the presence of bioactive compounds like alkaloids, flavonoids, terpenoids and phenolics etc. Alkaloids cause anti oxidizing effects by reduction of nitrate generation, which involves protein synthesis [34, 35]. Terpenoids inhibit free radical mediated lipid peroxidation by blocking the propagation of free radicals and inhibiting 3-hydroxy-3-methyl CoA reductase [36-38]. In the present study, chemicals of the powder and extracts contain bioactive compounds possessing antioxidant activity and preventing hepatic damage from free radicals. Chatterjee studied the hepatoprotective efficacy of wild edible mushroom (*Calocybe indica)* in CCl_4-induced hepatotoxic mice and reported that elevated level of SGPT, SGOT, SALP and total bilirubin of hepatotoxic rats significantly decreased due to the antioxidant defense mechanism of bioactive compounds such as alkaloids, flavonoids, terpenoids, proteins, polysaccharides, fats, nucleic acids etc. of *Calocybe indica* extracts [39]. It was found that root extract of *Glycyrrhiza glapra* significantly decreased SGPT, SGOT, SALP level and significantly increased total protein and serum albumin level in CCl_4-treated hepatotoxic rat groups when treated with *G.glapra* extract[29]. In present study, *Pleurotus ostreatus* powder and extracts lowered SGPT, SGOT, SALP activity, while total bilirubin level significantly decreased but total protein and serum albumin level significantly increased in CCl_4-induced hepatotoxic rats. Similar effects have been reported for licorice extract [29] and extract of *Calocybe indica* [39]. It has been reported that for animals, treated with the plant or fungal extract, lowered serum SGPT, SGOT, SALP activity indicates stabilization of plasma membrane as well as repair of hepatic injury and regeneration of hepatocytes with healing of hepatic parenchyma [40, 41].

5. Conclusion

In our study we observed that oyster mushroom *(Pleurotus ostreatus)* exhibited hepatoprotective efficacy against CCl_4-induced hepatotoxic rats comparable to that of standard hepatoprotective drug Silymarin. This finding indicates that *Pleurotus ostreatus* must contain such bioactive compounds that protect hepatotoxicity. After Purifying these bioactive compounds, further studies including human and clinical trials should be carried out for full appreciation to evaluate their safety and clinical effectiveness. With respect to getting safe and good potency, these compounds might be used to manufacture innovative drugs in the treatment of liver injury.

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Refferences

1. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. Nutrients in edible mushrooms: An inter-species comparative study. *Food Chem.* 1999;65: p. 477–482.

2. Kues U, Liu Y. Fruiting body production in basidiomycete. *Appl. Microbiol. Biotechnol.* 2000;54: p.141–152.

3. Mattila P, Salo-Vaananen P, Konko K, Aro H, Jalava T. Basic composition and amino acid contents of mushrooms cultivated in Finland. *J. Agric. Food Chem.* 2002;50: p.6419–6422.

4. Firenzuoli F Gori L, Lombardo G. The medicinal mushrooms *Agaricus blazei* Murrill: Review of literature and pharmacotoxicological problems. *Evid. Based Complement. Alternat. Med.* 2008;5: p.3–15.

5. Mishra S, Singh RB. Effect of mushroom on the lipid profile, lipid peroxidation and liver functions of aging *Swiss* albino rats. *Open Nutrac. J.* 2010;3: p.248–253.

6. Lakhan pal TN, Rana M. Medicinal and nutraceutical genetic resources of mushrooms. *Plant Gen. Res.* 2005;3: p.288–303.

7. Preeti A, Pushpa S, Sakshi S, Jyoti A. Antioxidant mushrooms: A review. *Int. Res. J.Pharm.* 2012;3: p. 65–70.

8. Linde quist U, Timo HJ, Julich WD. The pharmacological potential of mushrooms. *Evid. Based Complement. Alternat. Med.* 2005;2: p. 285–299.

9. Guillamón E, García-Lafuente A, Lozano M, D'Arrigo M, Rostagno MA, Villares A, *et al*. Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia.* 2010;8: p.715–723.

10. Peralta RM, Oliveira AL, Eler GJ, Soares AA, Bracht, A. Functional properties of edible and medicinal mushrooms. *Curr. Trends Microbiol.* 2008;4: p.45–60.

11. Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SMN, Somasundaram R. Antioxidant activity of indigenous mushrooms. *J. Agric. Food Chem.* 2006;54: p.9764–9772.

12. Ferreira, IC, Barros L, Abreu RM. Antioxidants in wild mushrooms. *Curr. Med. Chem.* 2009;16: p.1543–1560.

13. Moradali MF, Mostafavi H, Ghods S, Hedjaroude GA. Immuno modulating and anticancer in the realm of macro mycetes fungi (macro fungi). *Intern. Immuno pharmacol.* 2007;7: p.701–724.

14. Barros L, Baptista P, Estevinho LM, Ferreira IC. Effect of fruiting body maturity stageon chemical composition and antimicrobial activity of *Lactarius* sp. Mushrooms. *J. Agric.Food Chem.* 2007;55: p.8766–8771.

15. Borchers A, Keen CL, Gershwin M.E. Mushrooms, Tumors, and Immunity: An update.*Soc. Exp. Biol. Med.* 2004;229: p.93–406.

16. Padilha MM, Avila AA, Sousa PJ, Cardoso LG, Perazzo FF, Carvalho J.C. Anti-inflammatory activity of aqueous and alkaline extracts from mushrooms (*Agaricus blazei* Murrill). *J. Med. Food.* 2009;12: p.359–364.

17. Moro C, Palacios I, Lozano M, DÁrrigo M, Guillamón E, Villares A, *et al*. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. *Food Chem.* 2012;130: p.350–355.

18. Mori K, Kobayashi C, Tomita T, Inatomi S, Ikeda M. Anti atherosclerotic of the edible mushrooms *Pleurotus eryngi* (Eringi), *Grifola frondosa* (Maitake) and *Hypsizygus marmoreus* (Bunashimeji) in Apo lipoprotein E-deficient mice. *Nutr. Res.* 2008;28: p335–342.

19. Hu SH, Wang JC, Lien JL, Liaw ET, Lee MY. Anti hyperglycemic effect of polysaccharide from fermented broth of *Pleurotus citrinopileatus*. *Appl. Microbiol. Biotechnol.* 2006;70: p.107–113.

20. Ooi, VE. Hepatoprotective effect of some edible mushrooms. *Phytotherapy Res.* 1996;10: p.536–538.

21. Jayakumar T, Ramesh E, Geraldine P. Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCL_4-induced liver injury in rats. *Food Chem. Toxicol.* 2006;44: p.1989–1996.

22. Chag ST. Mushroom research and development-Equality and Mutual Benefit. In: Royse DJ, editor.

Proceed. II International conf. Mushroom Biology and Mushroom Products. The Pennsylvania State University. *1996: p.1-10.*

23. Wasser SP. Weis A. Medicinal properties of substances occurring in higher Basidiomycetes Mushrooms: current perspectives (review). *Int. J. Medic. Mushrooms.* 1999;1: 31–62.

24. Organisation for Economic Cooperation and Development (OECD). OECD guidelines for the testing of chemicals/section 4: Health Effects Test No. 423; Acute Oral Toxicity Acute Toxic Class Method.Pandit A. Sachdeva T. Bafna P. 2012. Drug-induced hepatotoxicity: A review. *J. Appl.Pharm. Sci.* 2004;2: p.233–243.

25. Reitman, Frankel S. Determination of SGOT and SGPT in Serum. *Amer.J.Cli.Path.* 1957;28: p.56.

26. Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *Journal of biological chemistry.*1946;164: p.321-329.

27. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *Journal of biological Chemistry*. 1937;119: p.481-490.

28. Kingsley GR. The determination of serum total protein, albumin and globulin by the biuret method Reaction. *Journal of biological Chemistry.*1939;131: p.197-200.

29. Huo HZ, Wang B, Liang YK, Bao YY, Gu Y. Hepatoprotective and antioxidant effects of licorice extract against CCl_4-induced oxidative damage in rats. *Int. J. Mol. Sci.*2011;12: p.6529–6543.

30. Huang WY, Zhang HC, Liu WX, Li CY. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *J. Zhejiang Univ. Sci. B.* 2012;13: p. 94–102.

31. Uru OM, Emeka IE, Lotanna AD, Ogechukwu UB. Hepatoprotective and anti-hepatotoxic active activities of aqueous leaf extract of Tacazzeab arteria against carbon tetrachlotide induced hepatotoxicity in Albino rat. *Int. Res. J. Pharm.* 2013;4: p.60–65.

32. Edwards MJ, Keller BJ, Kauffman FC, Thurman RG. The involvement of Kupffer cells in carbon tetrachloride toxicity. *Toxicol. Appl. Pharmacol.* 1993;119: p.275–279.

33. Sanghai DB, Kumar VS, Srinivasan KK, Shreedhara CS. Effect of Malvastrumcorom and elianum (L) Gracke leaf extract on CCl4 induced hepatotoxicity in rats. *J. Pharm. Phytother.* 2013;2: p.38–42.

34. Isaac OO, Chinwe JA. The phytochemical analysis and antibacterial screening of extract of Tetrecarpidum conophorum. *J.Chem. Soc. Nig.* 2001;26: p.53–55.

35. Dandapat S, Kumar M, Sinha MP. Therapeutic efficacy of Cinnamomumtamala (Buch.-Ham.) and Aeglemarmelos (L.) leaf. *Balneo Res. J.* 2014;5: p.113–121.

36. Auger C, Caporiccio B, Landrault N, Teissedre PL, Laurent C, Cross G, *et al.* Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolemic golden Syrian hamster (Mesocricetus auratus). *J. Nutr.* 2002;132: p.1207–1213.

37. Chang JJ, Chen TH, Chan P, Chen YJ, Shu FL, Lo MY, *et al*. The in-vitro inhibitory effect of tannin derivatives on 3-hydroxy-3methylglutaryl-coenzyme A reductase on Vero cells. *Pharmacology.* 2001;62: p:224–228.

38. Dandapat S, Sinha MP. Antioxidant and anti-inflammatory activity of Pleurotus tuber-regium (Rumph. ex Fr.) Singer. *Adv.Biol. Res.* 2015;9: p140–145.

39. Chatterjee S, Dey A, Dutta R, Dey S, Acharya K. Hepatoprotective Effect of the ethanolic extract of Calocybe indica on mice with CCl_4 hepatic intoxication. *Int. J. Pharm. Tech. Res.* 2011;3: p2162–2168.

40. Thawbrew MI, Joice PM, Rajatissa WA. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Me.* 1987;53: p 239–241.

41. Acharya K, Chatterjee S, Biswas G, Chatterjee A, Saha GK. Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in mice. *Int. J. Pharm. Pharmaceut. Sci.* 2012;4: p.285–288.