

THE PROTECTIVE EFFECT OF MUSHROOMS IN EXPERIMENTALLY INDUCED DIABETES IN MICE

CORNELIA MIRCEA¹, VERONICA BILD², DANIELA ZAVASTIN³,
OANA CIOANCA^{4*}

¹University of Medicine and Pharmacy „Grigore T. Popa” Iași, Faculty of Pharmacy, Department of General and Applied Biochemistry, 16 Universitatii Street, Romania, Iasi, 700115

²University of Medicine and Pharmacy „Grigore T. Popa” Iași, Faculty of Pharmacy, Department of Pharmacodynamics and Clinical Pharmacy, 16 Universitatii Street, Romania, Iasi, 700115

³University of Medicine and Pharmacy „Grigore T. Popa” Iași, Faculty of Pharmacy, Department of Physical Chemistry, 16 Universitatii Street, Romania, Iasi, 700115

⁴University of Medicine and Pharmacy „Grigore T. Popa” Iași, Faculty of Pharmacy, Department of Pharmacognosy, 16 Universitatii Street, Romania, Iasi, 700115

*corresponding author: oana.cioanca@gmail.com

Abstract

Mushrooms represent an important food source and are recommended for diabetics because of their low glycemic index due to content nutrients. The beneficial effect of products from mushrooms was evaluated under experimental diabetes in mice. The study was performed on extracts and powder respectively from three species of fungi, two of which are edible and cultivated (*Pleurotus ostreatus* - powder and extract, *Agaricus bisporus* brown - powder and extract) and one is parasite (*Fomes fomentarius* - extract).

Among the extracts a positive effect on blood glucose was recorded in the group treated with *Fomes fomentarius* extract (31.35% reduction) and from the group treated with *Pleurotus ostreatus* powder (20.24% reduction). Cholesterol reduction level was between 5.23% (*Fomes fomentarius*) and 15.46% (*Agaricus bisporus* brown extract). For *A. bisporus* brown the extract registered better results, while for *P. ostreatus* the powder was more active. Extracts administration did not result in a return to the control group registered values for liver function tests (ALT, AST, alkaline phosphatase), but determined evident beneficial effects compared with diabetic group and could counteract hepatocellular damage in diabetes.

Rezumat

Ciupercile prin conținutul în elemente nutritive sunt alimente importante, iar la diabetici sunt de recomandat datorită indicelui glicemic mic. Efectul benefic al produselor obținute din ciuperci a fost evaluat în condiții de diabet experimental la șoareci. Studiul a fost efectuat pe extracte și respectiv pulbere provenite de la trei specii de ciuperci, dintre care două sunt comestibile și de cultură (*Pleurotus ostreatus* - pulbere și extract, *Agaricus bisporus* brown – pulbere și extract) și una este parazită (*Fomes fomentarius* - extract). Dintre extracte efectul benefic asupra glicemiei a fost înregistrat la lotul tratat cu extract de

Fomes fomentarius (reducere a nivelului glicemiei cu 31,35%), iar dintre pulberi la lotul tratat cu pulbere de *Pleurotus ostreatus* (reducere cu 20,24%). Gradul de reducere a colesterolemiei a fost între 5,23% (extract de *Fomes fomentarius*) și 15,46% (extract de *Agaricus bisporus* brown). Pentru *A. bisporus* brown mai eficient este extractul, în timp ce pentru *P. ostreatus* mai eficientă este pulberea. Extractele nu au determinat revenirea la valorile înregistrate la lotul martor a testelor de evaluare a funcției hepatice (ALT, AST, fosfataza alcalină), dar determină efecte benefice evidente comparativ cu lotul diabetic și pot contracara afectarea hepatocelulară din patologia diabetică.

Keywords: mushrooms, diabetes, glucose, transaminases

Introduction

Mushrooms represent an important food source and are recommended for diabetics because of their low glycemic index due to their content nutrients such as proteins, lipids, carbohydrates, vitamins, minerals and phenolic compounds [1, 2, 4, 10, 16, 17]. Bioactive substances content of mushrooms depends on: species, substrate type and environment, growing conditions, stage of development, storage conditions and type of processing [17]. All this leads to a very high variability of their biological effects.

The preliminary studies indicated an *in vitro* antioxidant potential for the extracts obtained from mushrooms, thus leading us to assessing their protective effect in conditions of experimental diabetes in mice. The study was conducted on three species of mushrooms, two of which come from culture and are edible (*Pleurotus ostreatus*, *Agaricus bisporus* brown) and one is a tree parasite (*Fomes fomentarius*).

Materials and Methods

Experimental procedures were conducted with the approval of the Ethics Committee of the University of Medicine and Pharmacy „Grigore T. Popa” Iași and in accordance to the European Communities Council Directive 86/609/EEC. Also, efforts were made to minimize animal suffering and to reduce the number of animals used. Experimental diabetes was induced by intraperitoneal administration of streptozocin (50 mg/kg bw, in citrate buffer pH 4.5). The control group received only sodium citrate solution pH 4.5. Three days after the streptozocin administration and the onset of diabetes, animals were divided into groups of 10: group *M* (control); group *D* (experimental diabetes), group *AbbE* (experimental diabetes + *Agaricus bisporus* brown extract), group *AbbP* (experimental diabetes + *Agaricus bisporus* brown powder), group *PoE* (experimental diabetes + *Pleurotus ostreatus* extract), group *PoP* (experimental diabetes +

Pleurotus ostreatus powder), group FF (experimental diabetes + *Fomes fomentarius* extract). Both extracts as well as powder administered animals were given 200 mg/kg/day orally for 14 days. Groups M and D received only 0.1% carboxymethyl cellulose solution.

Animals received standard food and water *ad libitum*, and three hours prior to slaughter access to food was stopped. Upon completion of the experiment blood was collected on anticoagulant solution, suitable for biochemical determinations.

Determination of glucose: glucose is oxidized under the action of glucose oxidase with the release of hydrogen peroxide, which causes the oxidative condensation of phenol with 4-aminoantipyrine to form a compound with maximum absorbance at 520 nm [11].

Determination of proteins (Lowry's method): proteins react with copper ions in alkaline medium, reduce Folin's Reagent and form a blue compound with maximum absorbance at 750 nm [21].

Determination of lipids: lipids react with the phospho-vanilic reagent to form a pink compound with maximum absorbance at 530 nm [6].

Determination of cholesterol: cholesterol is oxidized by the action of cholesterol oxidase with the release of hydrogen peroxide which determines oxidative condensation of phenol with 4-aminoantipyrine to form a compound with maximum absorbance at 505 nm [15].

Determination of alkaline phosphatase (ALP): the enzyme determines the hydrolysis of p-nitrophenyl phosphate to release p-nitrophenol with maximum absorbance at 405 nm [20].

Determination of transaminases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) catalyze the transamination reactions with formation of ketoacids which are reduced in the presence of NADH to form compounds with maximum absorbance at 340 nm [5].

Results and Discussion

The results indicated that food (to 12.40%) and water (9.97%) consumption increased for the diabetes group, while weight varied slightly compared to the control group. Treating animals with extracts and powder of mushroom respectively caused a reduction of food consumption compared to the diabetic group (values between 8.75% AbbP, PoE and 27% PoP), water intake reduction (varying between 7.65% PoE and 34.48% PoP) and body weight increase (between 1.97% AbbE and 10.33% PoE) except PoP group which lost weight by 17.29%.

The observed phenomena are similar to those in humans to which the establishment of antidiabetic therapy results in reductions of food intake,

whereas water intake and body weight increases due to the glucose regulatory mechanisms intervention directing excess glucose towards synthesis of lipids.

Plasma glucose concentration for treated groups decreased, varying dependent on the type of extract or powder used (Table I, Figure 1). The registered results are statistically significant ($p < 0.05$).

Table I
Blood parameters registered in experimental groups

Parameter*	Groups of animals						
	M	D	AbbE	AbbP	PoE	PoP	FF
Glucose (mg/dL)	108.77 ± 5.19	155.56 ± 11.37	113.08 ± 3.87	123.74 ± 3.36	121.05 ± 5.61	124.07 ± 4.28	106.79 ± 11.76
Proteins (g/dL)	4.60 ± 0.32	4.13 ± 0.18	4.44 ± 0.22	4.54 ± 0.22	4.26 ± 0.10	5.33 ± 0.19	4.60 ± 0.26
Lipids (mg/dL)	461.85 ± 20.43	493.14 ± 7.43	480.98 ± 15.18	481.85 ± 13.97	480.52 ± 7.38	478.63 ± 7.97	487.87 ± 9.87
Cholesterol (mg/dL)	143.53 ± 14.12	183.96 ± 6.14	155.51 ± 16.60	172.82 ± 8.91	169.68 ± 3.30	167.12 ± 3.00	174.33 ± 5.67
ALP (U/L)	28.81 ± 8.25	74.82 ± 21.16	39.16 ± 6.26	56.91 ± 10.85	63.13 ± 6.56	68.68 ± 4.25	68.22 ± 7.49
ALT (U/L)	33.16 ± 0.98	73.67 ± 2.82	68.07 ± 2.14	65.68 ± 0.23	61.96 ± 1.79	69.22 ± 4.56	66.64 ± 2.23
AST (U/L)	37.75 ± 0.60	48.05 ± 0.22	42.43 ± 2.13	38.89 ± 1.43	42.54 ± 1.63	48.37 ± 1.32	40.69 ± 1.17

* data represents the mean value ± SD (n = 10 animals)

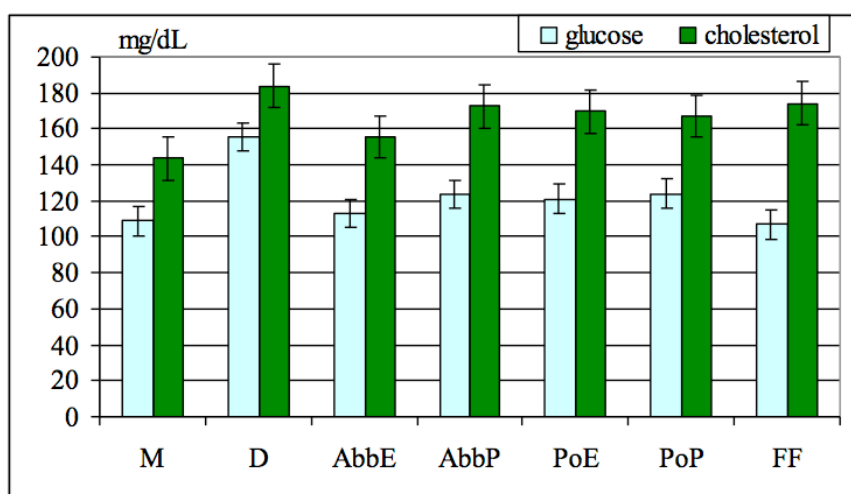


Figure 1

Glucose (mg/dL) and cholesterol (mg/dL) mean values variation in the experimental groups

Among the studied extracts, the most effective in reducing blood glucose levels proved to be FF extract which caused a mean decrease value by 31.35% (below the control group recorded value), and the most effective powder was PoP sample which induced a reduction of 20.24%. Lee *et al.* (2005), using an extract of *F. fomentarius* observed a significant reduction in blood glucose levels which might explain the presence of certain insulin-like substances in the extract or that there are compounds able to increase the rate of glucose utilization in the periphery [13].

The lower effect of the mushroom powder could be caused by the presence in its composition of carbohydrates that can be absorbed in the gut, whereas solvent extraction does not allow these components to become part of the extract.

Similar results have been achieved by Jeong (2010) who has noticed that in experimental conditions extracts from *A. bisporus* improve food intake, blood sugar levels (reduced by 24.70%), however their effect is less significant for body weight and total cholesterol value [9].

The degree of cholesterol reduction is lower than for glucose; the values rank between 5.23% (FF) and 15.46% (Abbe) (Table I, Figure 1). The data obtained do not indicate a higher extracts efficiency over the powder because when we analyse the two types of fungi for which we have studied both forms the results are variable: for *A. bisporus* brown the extract is more efficient, while for *P. ostreatus* the powder is more efficient.

For the *P. ostreatus* sample the higher powder efficiency could be explained by the presence of lovastatin in its composition; once the lovastatin compounds permeate the body they will block the hepatic synthesis of cholesterol. On the other hand the fibers that are present in the powder can reduce the intestinal absorption of cholesterol from food [2, 8, 19]. Compared to mushrooms from the *Agaricus* genus, the *Pleurotus* genus contains a larger amount of lovastatin, even 3 times higher, but a lower content of ergothioneine, a substance with obvious antioxidant action [3, 7, 18]. For *A. bisporus* the presence of unsaturated fatty acids of 18:2 type could explain the cholesterolemia lowering effect; it is a known fact that acids have a positive effect on the lipid metabolism [12, 14].

Administration of the extract and powder samples to mice resulted in a minor change in the concentration of total lipids compared with the value determined in the diabetic group, with decreases of 1.06% (FF) and 2.94% (PoP) (Table I). Determination of total lipid level is just a test to evaluate disorders of lipid metabolism in diabetics, since it is the sum of the most important lipid compounds present in the blood. The positive effect of P.

ostreatus powder on both lipids and cholesterol emphasizes the effectiveness of its use as cholesterol or lipid-lowering agent [19].

ALT, AST and alkaline phosphatase (Table I, Figure 2) values increased in the diabetic group and indicate an acute liver damage (due to a significant increase of ALT over AST) in terms of experimental data. The increase of alkaline phosphatase indicates hepatocellular damage consequent to lipid metabolism disorders; the liver protective effect is correlated with the decrease of total cholesterol for AbbE group. The extracts did not cause the return to the previously recorded values in the control group, but caused obvious benefits in comparison to the diabetic group and can counteract the hepatocellular damage in diabetes.

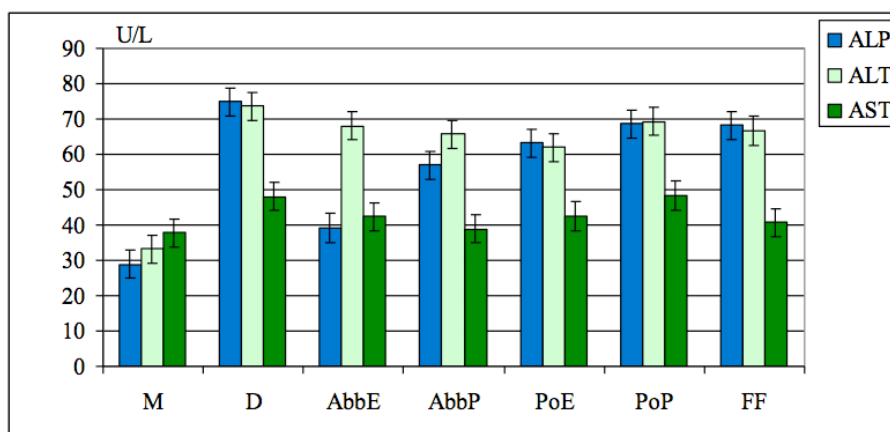


Figure 2

Variation of mean values of alkaline phosphatase and transaminases activities for the experimental groups

The protective effect of extracts and powders is also caused by the presence therein of polyphenols and flavonoids, substances with a known antioxidant effect.

The positive results observed in diabetic mice that received preparations of mushrooms underline the possibility of their use in combination with conventional therapy, obviously only after a full assessment of the side effects risk.

Conclusions

The results indicate a variability of the mechanism of action by which extracts and powder manifest their positive effect on the body, extracts are more effective to regulate blood glucose, whereas for cholesterol and lipids the effect depends on the type of mushroom. Such variables indicate the

different ways in which the bioactive compounds from mushrooms are involved in the regulatory and countervailing mechanisms the body developed during diabetes.

Hepatoprotective and hypoglycaemic effects of fungi can be overlapped with those obtained in other experimental studies, provided that the full analysis of the powder and mushroom extract is performed and products standardized in bioactive compounds are obtained, thus enabling an easy comparison of the biological actions.

The obtained data are encouraging and emphasize that, like medicinal plants, mushrooms can be a source of active substances that should not be ignored, especially if we consider that *A. bisporus* brown and *P. ostreatus* are edible mushrooms. *F. fomentarius* although it is a parasite fungus commonly growing on trees, is rich in antioxidants and extracts with beneficial effects such as those made from *Ganoderma sp.* can be easily obtained.

Acknowledgements

This research was conducted within the internal UMF Grant no. 28215 funded by the University of Medicine and Pharmacy „Grigore T. Popa” Iași.

References

1. Alam N., Yoon K.N., Lee J.S., Cho H.J., Shim M.J., Lee T.S., Dietary effect of *Pleurotus eryngii* on biochemical function and histology in hypercholesterolemic rats, *Saudi Journal of Biological Sciences*, 2011, 18 (4): 403-409.
2. Barros L., Baptista P., Correia D.M., Casa S., Oliveira B., Ferreira I.C.F.R., Fatty acid and sugar compositions, and nutritional value of wild edible mushrooms from Northeast Portugal, *Food Chemistry*, 2007, 105: 140-145.
3. Virgolici B., Mohora M., Radoi V., Lixandru D., Stoian I., Gaman L., Coman A., Greabu M., Manuel-Y-Keenoy B., Correlations between dysglycemia, markers of oxidative stress and inflammation in diabetic foot patients, *Farmacia*, 2011, 59(2), 216-227
4. Diez A.A., Alvarez A., Compositional and nutritional studies on two wild edible mushrooms from Northwest Spain, *Food Chemistry*, 2001, 75: 417-422.
5. Dufour D.R., Lott J.A. Nolte F.S., Gretch D.R. et al., Diagnosis and monitoring of hepatic injury. Recommendation for use of laboratory tests in screening, diagnosis and monitoring, *Clin Chem*, 2000, 46: 2050-2068.
6. Frings C.S., Fendley T.W., Dunn R.T., Queen Cecelia, Improved Determination of Total Serum Lipids by the Sulfo-Phospho-Vanillin Reaction, *Clinical Chemistry*, 1972, 18 (7): 673-674.
7. Jayakumar T., Thomas P.A., Geraldine P., Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats, *Experimental Gerontology*, 2007, 42: 183-191.
8. Jayakumar T., Thomas P.A., Sheu J.R., Geraldine P., In vitro and in vivo antioxidant effects of the oyster mushroom *Pleurotus ostreatus*, *Food Research International*, 2011, 44 (4): 851-861.

9. Jeong S.C., Jeong Y.T., Yang B.K., Islam R. et al., White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats, *Nutr Res*, 2010, 30: 49-56.
10. Kalač P., Chemical composition and nutritional value of European species of wild growing mushrooms: A review, *Food Chemistry*, 2009, 113:9-16.
11. Kaplan L.A. Glucose. Kaplan A et al., Clin Chem The C.V. Mosby Co. St Louis, Toronto, Princeton, 1984, 1032-1036.
12. Kozarski M., Klaus A., Niksic M., Jakovljevic D. et al., Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidum* and *Phellinus linteus*, *Food Chemistry*, 2011, 129 (4): 1667-1675.
13. Lee J., Effects of *Fomes fomentarius* supplementation on antioxidant enzyme activities, blood glucose, and lipid profile in streptozotocin-induced diabetic rats, *Nutrition Research*, 2005, 25 (2): 187-195.
14. Băcanu E.V., Lixandru D., Stoian I., Virgolici B, Mohora M, Arsene A.L., Ionescu-Tîrgoviște C., Correlations between obesity antropometric markers, adipocytokines and monocytes oxidative stress status in type 2 diabetic patients, *Farmacia*, 2012, 60(2), 194-205
15. Naito H.K., Kaplan A. et al., Cholesterol. Clinical Chemistry, The C.V. Mosby Co. St Louis, Toronto, Princeton, 1984, 1194-1206.
16. Palacios I., Lozano M., Moro C., Arrigo M.D. et al., Antioxidant properties of phenolic compounds occurring in edible mushrooms, *Food Chemistry*, 2011, 128 (3): 674-678.
17. Reis F.S., Martins A., Barros L., Ferreira I.C.F.R., Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples, *Food and Chemical Toxicology*, 2012; 50 (5): 1201-1207.
18. Roupas P., Keogh J., Noakes M., Margetts C., Taylor P., The role of edible mushrooms in health: Evaluation of the evidence, *Journal of Functional Foods*, 2012, 4: 687-709.
19. Schneider I., Kressel G., Meyer A., Krings U. et al., Lipid lowering effects of oyster mushroom (*Pleurotus ostreatus*) in humans, *Journal of Functional Foods*, 2011, 3: 17-24.
20. Tietz N.W., Burtis C.A., Duncan P., et al, A reference method for measurement of alkaline phosphatase activity in human serum, *Clin Chem*, 1983, 29 (5):751-6.
21. Walker J.M., The Lowry Method for Protein Quantitation, The Protein Protocols Handbook, Second Edition, Humana Press, New York, 2002, 7-9.

Manuscript received: November 13th 2012