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# Assessment of the Antifibrotic Activity of Polysaccharides Extracted from Oyster Mushroom (*Pleurotus ostreatus*) Mycelium in the Rats Liver

**Thesis Submitted for the Award of Ph. D in Biochemistry**

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جامعة عين شمس
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# تقييم النشاط المضاد للتليف لعديدى التسكر المستخلص من مايسيليوم عيش الغراب المحارى (*Pleurotus ostreatus*) فى كبد الجرذان

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# *List of Contents*

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*Al Shaimaa Mohamed Taha Ali Ahmed*


## Abstract

The present study aims at evaluating the hepato-therapeutic activity of polysaccharopeptides (PSP) extracted from *Pleurotus ostreatus* mycelia on thioacetamide (TAA) - induced liver fibrosis in rats. Experimental hepatic fibrosis was induced in rats by intraperitoneal administration of 200 mg TAA/ Kg b.w./ twice weekly for 8 weeks. Two treatment modalities were implemented in this study; Colchicine (reference group) and PSP (therapeutic group). The results of this study revealed that PSP treatment (25 mg/ Kg b.w./ 3 times weekly for 5 weeks) of fibrotic rats restores hepatic matrix metalloproteinase (pro-MMP-2& -9) activities, the concentrations of MDA and GSH, as well as the relative protein expression of Fas receptor back to normal levels. However, more pronounced increases in hepatic TNF-α and NO concentrations were recorded, compared to untreated fibrotic rats. In addition, PSP treatment increased hepatic relative BCl-2 protein expression and produced lower elevations in the hepatic concentrations of TGF-β1, TIMP-1 and hydroxyproline, as well as hepatic caspase-3 activity, compared to TAA group. The histological examination of the liver tissue had demonstrated that PSP treatment reverses TAA-induced alterations, especially fibrosis (F0), apoptosis (G0) and necrosis (G0), compared to the normal control. In conclusion, experimental liver fibrosis can be treated by PSP, due to its immuno-stimulatory, antioxidant, anti-fibrotic and anti-necrotic activities.


***Key words:*** *Pleurotus ostreatus* mycelium – Polysaccharopeptides – Thioacetamide – Colchicine – TGF-β1 – Relative caspase-3 activity – Relative Fas receptor protein expression – Relative BCl-2 protein expression – Histological studies - Rats.

# *List of Abbreviations*

| | |
|---|---|
| **A1R** | Adenosine 1 receptor |
| **AKt** | serine/ threonine-specific protein kinase |
| **ALP** | Alkaline phosphatase |
| **ALT** | Alanine aminotransaminase |
| **Apaf1** | Apoptosis-activating factor1 |
| **APO-1** | Apoptosis receptor |
| **ASGP** | Asialoglycoprotein |
| **A-SMase** | Acidic sphingomyelinase |
| **AST** | Aspartate aminotransaminase |
| **ATP** | Adenosine triphosphate |
| **Bak** | BCl-2-antagonist/killer |
| **Bax** | BCl-2-associated X protein |
| **BCIP** | Bromo-4-chloro-3-indolylphosphate |
| **BCl-2** | B-cell lymphoma-2 |
| **BH3** | Bcl-2 homology region |
| **Bid** | BH3-interacting domain agonist |
| **Bim** | BCl-2-interacting mediator |
| **BMP** | Bone morphogenic proteins |
| **BSA** | Bovine serum albumin |
| **cAMP** | Cyclic adenosine monophosphate |
| **CARD9** | Caspase-recruitment domain 9 |
| **Caspases** | Cysteinyl aspartate specific proteases |
| **CAT** | Catalase |
| **CBP** | cAMP-response element-binding protein |
| **$CCl_4$** | Carbon tetrachloride |
| **CCN2** | Cysteine-rich 61/connective tissue growth factor |
| **cGMP** | Cyclic guanosine mono phosphate |
| **CINC** | Cytokine-induced neutrophil chemo attractant |
| **Col 1A1** | Collagen 1A1 gene |
| **CR3** | Complement receptor 3 |
| **CRD** | Cysteune-rich domain |
| **CSF** | Colony stimulating factor |
| **CTGF** | Connective tissue growth factor |

| | |
|---|---|
| **Cyt. C** | Cytochrome C |
| **DC** | Dendritic cell |
| **DD** | Death domains |
| **DDR** | Discoidin domain receptor |
| **DED** | Death effector domains |
| **DEVD-pNA** | Asp-Glu-Val-Asp-p-nitroaniline |
| **DISC** | Death inducing signaling complexes |
| **DTNB** | 5, 5'-dithiobis-2-nitrobenzoic acid |
| **DTT** | Dithiothreitol |
| **EC** | Endothelial cell |
| **ECE** | Endothelin converting enzyme |
| **ECM** | Extracellular matrix |
| **EMT** | Epithelial-mesenchymal transition |
| **eNOS** | Endothelial nitric oxide synthase |
| **ER** | Endoplasmic reticulum |
| **ET-1** | Endothelin-1 |
| **FADD** | Fas associated protein with death domain |
| **Fas** | F ragment aoptosis stimulating |
| **FasL** | Fas ligand |
| **FGF** | Fibroblast growth factor |
| **GCS** | Gamma-glutamylcysteine synthetase |
| **GPI** | Glycosyl phosphatidyl inositol |
| **GPx** | Glutathione peroxidase |
| **GR** | Glutathione reductase |
| **GSH** | Reduced glutathione |
| **H&E** | Hematoxylin and Eosin |
| **$H_2O_2$** | Hydrogen peroxide |
| **HBV** | Hepatitis C virus |
| **HCV** | Hepatitis B virus |
| **HGF** | Hepatocyte growth factor |
| **HRP** | Horseradish peroxidase |
| **HSC** | Hepatic stellate cell |
| **HSP 70** | Heat shock protein 70 |
| **iC3b** | Inactivated complement 3b |

| | |
|---|---|
| **ICAM** | Intracellular adhesion molecules |
| **IFN-α** | Interferon alpha |
| **IGF** | Insulin-like growth factor |
| **IKK** | Inhibitor of kappa B kinase |
| **IL** | Interleukin |
| **iNOS** | Inducible nitric oxide synthase |
| **kDa** | Kilo Dalton |
| **LacCer** | Lactosylceramide |
| **LAP** | Latency-associated peptide |
| **L-TGFβ** | Latent-transforming growth factor beta |
| **MAPK** | Mitogen-activated protein kinase |
| **MCM** | Mushroom complete medium |
| **MCP-1** | Monocyte chemotactic peptide-1 |
| **MDA** | Malondialdehyde |
| **MIP2** | Macrophage inflammatory protein 2 |
| **MMP** | Matrix metalloproteinase |
| **MPT** | Mitochondrial Permeability Transition |
| **MT1-MMP** | Membrane type 1- Matrix metalloproteinase |
| **MyD88** | Myeloid differentiation factor 88 |
| **$NADP^+$** | Nicotinamide adenine dinucleotide phosphate |
| **NADPH** | Reduced nicotinamide adenine dinucleotide phosphate |
| **NAPQI** | N-acetyl-*p*-benzoquinone imine |
| **NASH** | Hepatitis and nonalcoholic steatohepatitis |
| **NBT** | Niroblue tetrazolium chloride |
| **NEDD** | N-1-naphthylethylenediamine |
| **NEMO/IKKγ** | NF-*k*B Essential Modulator/ |
| **NFAT** | Nuclear factor of activated T cell |
| **NF-*k*B** | Nuclear factor kappa B |
| **NK** | Natural killer cells |
| **NKT** | Natural killer T cells |
| **NO** | Nitric oxide |
| **N-SMase** | Neural sphingomyelinase |
| **PAF** | Platelet activating factor |
| **PAGE** | Polyacrylamide gel electrophoresis |

| | |
|---|---|
| **PBS** | Phosphate-buffered saline |
| **PBST** | Phosphate-buffered saline-Tween 20 |
| **PDGF** | Platelet derived growth factor |
| **PI3K** | Pro-survival kinases phosphoinositol 3 kinase |
| **PKC** | Protein kinase C |
| **PMSF** | Phenylmethylsulfonyl fluoride |
| **pNA** | p-nitroaniline |
| **PSP** | Polysaccharopeptides |
| **PTP** | Permeability transition pore |
| **PUMA** | p53-up-regulated modulator of apoptosis |
| **PVDH** | Polyvinilidene difluoride |
| **RIP** | Receptor-interacting protein |
| **RNS** | Reactive nitrogen species |
| **ROS** | Reactive oxygen species |
| **R-SMAD** | Receptor-associated SMAD |
| **RTK** | Receptor tyrosine kinase |
| **SARA** | SMDA anchor for receptor activation |
| **SDS** | Sodium dodecyl sulphate |
| **SIGNR1** | specific ICAM-3-grabbing non-integrin homolog-related1 |
| **SMAD** | Sma and mothers against decapentaplegic (Mad) homologues |
| **Smurf** | SMAD ubiquitin regulatory factors |
| **SOD** | Super oxide dismutase |
| **SP** | Streptavidin-peroxidase |
| **STAT** | Signal transducer activator of transcription 3 |
| **SYK** | Tyrosine kinase |
| **TAA** | Thioacetamide |
| **TBA** | Thiobarbituric acid |
| **t-Bid** | Truncated Bid |
| **TCA** | Trichloro acetic acid |
| **TEMED** | N, N, Ń, Ń Tetramethyl Ethylene Diamine |
| **TGF-α** | Transforming growth factor- alpha |
| **Th** | T helper cells |

| | |
|---|---|
| **TIMP** | Tissue inhibitor matrix metalloproteinase |
| **TLRs** | Toll like receptors |
| **TMB** | Tetramethyl-benzidine |
| **TNF-R1** | Tumor necrosis factor-receptor1 |
| **TNFRSF6** | Tumor necrosis factor receptor superfamily 6 |
| **TNF-α** | Tumor necrosis factor-alpha |
| **TRADD** | TNF-R1 associated death domain protein |
| **TRAF** | TNF-associated factor |
| **TRAIL** | TNF-α-related apoptosis-inducing ligand |
| **TRAP2** | Type 1 receptor-associated protein 2 |
| **TβR** | Transforming growth factor receptor |
| **UTP** | Uridine triphosphate |
| **$VCl_3$** | Vanadium chloride |
| **VDAC** | Voltage-dependent anion channel |
| **VEGF** | Vascular endothelial cell growth factor |
| **α-SMA** | Alpha-smooth muscle actin |