Mushroom Insoluble Non-Starch Polysaccharides (MINSP) Attenuate Ochratoxin A-Oxidative Stress and Hepato-Renal Dysfunctions in Rat

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ABSTRACT

The aim of the present study was to investigate the effect of mushroom insoluble non-starch polysaccharides (MINSP) on Ochratoxin A-induced hepato-renal damage in rat. MINSP (75 and 150 mg/kg) administered daily for 15 days in concomitantly with Ochratoxin A (OA) (1.7 mg/Kg), intraperitoneal (i.p). Normal groups treated with MINSP showed significant decrease in serum levels of γ-glutamyl transferase (GGT), aminotransferases (ALT & AST), creatinine, uric acid, total antioxidant capacity (TAC), tumor necrosis factor alpha (TNF-α) and carcinoembryonic antigen (CEA). In liver and kidney homogenates there were significant decreases in malonaldehyde (MDA), and nitric oxide (NO); while superoxide dismutase (SOD) content increased after treatment with MINSP. OA-treated rats showed significant elevation in serum ALT, AST, TNF-α, CEA, Uric acid, creatinine, MDA and NO and reduction of SOD level in hepatic and renal tissues. The combined treatment with MINSP + OA significantly ameliorated the tested parameters when compared with OA- treated group. It improved the antioxidant activity of liver and kidney in dose-dependent manner. Histopathological examination revealed that MINSP administration protected hepatocytes and renal tissues from the damage induced by OA. Immunohistochemical staining of iNOS expression and DNA damaged area decreased in liver and kidney tissues after administration of MINSP. Conclusion: MINSP are fat replacer in processing low fat diet. MINSP represent a good functional food and have detoxifying effect against ochratoxosis in rat.

Key words: Ochratoxin, Mushroom insoluble non-starch polysaccharides, oxidative stress, rat, TNF-α, IL-6, iNOS, caspase-3, liver, kidney.

Introduction

Ochratoxin A (OA) has been found in improperly stored food and feeds such as barley, oats, rye, wheat, coffee beans, and other plant products, (Bennet and Klich, 2003) and in dry foods such as soybeans, garbanzo beans, nuts and dried fruit, also in grapes and grape products, coffee, and pork (Sage et al, 2004). OA causes a wide array of toxicological effects in animal models, including genotoxicity, nephrotoxicity, nephrocarcinogenicity, teratogenicity, neurotoxicity and immunotoxicity (Mally et al., 2005; EFSA, 2006; Clark and Snedeker, 2006; Abdel-Aziz et al., 2010). OA has been classified as a class 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 1993). OA is one of the most potent renal carcinogens studied to date ( Adler et al., 2009). OA toxicity has been associated with inhibition of protein synthesis, DNA and RNA synthesis, mitochondrial dysfunction, formation of DNA adducts, disruption of calcium homeostasis, and the generation of reactive oxygen species (Marin-Kuan et al., 2006; Rached et al, 2007). OA induced lipid peroxidation (LPO), formation of reactive oxygen species (ROS) and consequent oxidative DNA damage (Meki and Hussein, 2001; and Farag et al., 2010). Schaff et al. (2002) found an increase of ROS levels, depletion of GSH levels and an increase in oxidative DNA damage in rat proximal tubular cells and in LLC-PK1 cells treated by OA.

Mushrooms are considered to be an important source of nutrients and physiologically beneficial and non-toxic medicines (Wasser, 2002). Currently mushroom-derived substances were shown to exhibit antitumour, and immunomodulating properties Borchers et al. (1999), anti-platelet aggregation (Hokama and Hokama, 1981), hypocholesterolaemic (Aletor, 1995), cardioprotective, hypoglycaemic effect (Manzi et al., 2004), hepatoprotective (Nada et al., 2010), antibacterial, antiviral, antifungal and antiparasitic effects (Breene, 1990). The dry matter of mushroom fruit bodies is about 5–15%, having a very low fat content and containing 19–35% proteins (Cheung and Lee, 1998). Cultivated oyster mushrooms are the source of biologically active glucans (Synnysya et al., 2009). Mushroom fruit bodies are plentiful of vitamins, mainly B1, B2, C and D2 (Mattila et al., 2000). The content of carbohydrates, which are mainly present as polysaccharides or

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glycoproteins, ranges 50–90%. Most abundant mushroom polysaccharides are chitin, hemicelluloses, b- and a-glucans, mannans, xylans and galactans (Manzi and Pizzoferrato, 2000). The isolation and purification of these polysaccharides from mushroom mycelia can be carried out with minimal effects (Mizuno et al., 1995). It is of important to emphasize that 15% mushroom products are based on extracts from mycelia (Salama, 2001).

There is a growing interest towards the utilization of these polysaccharides in the production of fiber gel to be used functionally in place of traditional fats and oils (Hussien et al., 2008). Most of these fiber gels are currently produced from rice bran or barley flour (Inglett et al., 2004) or corn hulls (Inglett and Carrier, 2001).

The present study was designed to investigate the protective effect of mushroom insoluble non-starch polysaccharides (MINS) against ochratoxicosis in rats.

Materials and Methods

Detection and Determination of ochratoxin A:

Extraction:

OA was extracted from YES broth (2% yeast extract, 4% sucrose, 1000ml distillate water; pH 5.8 ± 0.2) according to Tsubouchi et al. (1985).

A Sep-Pak C18 Column prewashed twice with methanol: water: sodium bicarbonate (3%). (v/v/v). OA was eluted with 8 ml ethyl acetate: methanol: acetic acid (95: 5: 0.5 v/v/v). The extract was dried and prepared for quantitative HPLC analyses according to AOAC (2000) method.

Preparation of MINS:

Mycelia of Pleurotus ostreatus were obtained from the mushroom cultivation and production unit at National Research Centre, Dokki, Cairo, Egypt. The MINS in the mushroom was prepared by using the solvent extraction methods as outlined by (Cheung and Lee, 2000). The mixture in each extraction step was filtered through cheese cloth to separate the extracted solvent from the insoluble residue. The final insoluble residue was dialyzed and washed several times with distilled water and freeze dried. The tested doses of MINS 75 and 150 mg/kg were suspended in 2.5% gum acacia.

Chemicals and reagents were purchased from Riedel-de Haen, Germany and Bodiagnostic, Cairo, Egypt. Kits used for biochemical analysis were purchased from Bodiagnostic, Inc., (Egypt). Carcinoembryonic antigen (CEA) and tumor necrosis factor alpha (TNF-a) kit were produced by Diaclone Research Co., France.

Animals:

Sprague Dawley rats of both sexes weighing 120 –130 g were used throughout the experiments. Animals were housed under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues (Zimmermann, 1983).

Experimental design:

The animals were distributed into six equal groups (8 rats each). Rats treated orally for 15 days as follows: group 1: untreated control orally administered the vehicle (10 ml/kg, gum acacia 2.5%); group 2: treated with OA (1.7 mg/kg b.w) this dose according to Nada et al. (1994), groups 3 and 4, treated with 75 mg and 150 mg/kg MINS, respectively. Groups 5 and 6 treated with MINS 75 mg or 150 mg/kg concomitantly with OA (1.7 mg/kg), respectively.

At the end of the treatment period, all animals were fasted for 12 hr. Blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia and left to clot. The sera were separated using cooling centrifugation and stored at –80 °C until analysis.

All animals were sacrificed and the liver and kidney tissues of each animal were dissected. Tissues were divided into two portions, 1st portion was homogenized and prepared for biochemical analysis; the 2nd portion was prepared for histopathological examination.

Serum biochemical analysis:

Blood samples were withdrawn from the retro-orbital venous plexus, under light anesthesia by diethyl ether. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15 min. The obtained serum was serum was analyzed for determination of γ-glutamyl transferase (GGT) according to Rosalki et al. (1970), amino-transferases (ALT and AST) activities by method of Reitman and Frankel (1957), total antioxidant capacity (TAC) (Koracevic et al., 2001) carcinoembryonic antigen (CEA) in serum was carried out by immunoradiometric assay (Begent, 1984), tumor necrosis factor alpha (TNF-a) was done by ELISA method.
(Corti et al., 1992), creatinine was determined by Bartles et al. (1972) and uric acid (Barham and Trinder, 1972).

**Preparation of liver and kidney homogenate:**

Immediately after blood sampling, animals were sacrificed by cervical dislocation and the liver and kidney tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. A weighed part of each tissue was homogenized in ice-cooled saline (0.9% NaCl) to prepare 20% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. at 4°C. The aliquot was divided into three parts; the 1st and 2nd parts were used for determination of lipid peroxidation (LPO) as malondialdehyde (MDA) according to Ruiz-Larrea et al. (1994) method, and nitric oxide (NO) content (Montgomery and Dymock, 1961). The 3rd part was used to analyze SOD activity by Marklund and Marklund (1974) method.

**Histological examination:**

The 2nd portion of the tissues were instantly fixed in 10% formalin, embedded in paraffin, cut into 5 μm pieces and mounted on slides. The samples were stained with hematoxylin and eosin (H&E) for histopathological examination (Bancroft and Gamble, 2002); other slides were stained with Feulgen reaction to be applied for the histochemical demonstration of DNA

**Quantitative Analysis of DNA by Image analysis:**

Using computer-assisted digital image analysis workstation (LEICA QWin 500 Image Analyzer, LEICA Imaging Systems Ltd, Cambridge, England; image analyzer unit, Pathology Department, National Research Centre, Cairo, Egypt); composed of high precision illuminator, digital camera and computer with specific image analysis software. The degree of reaction was chosen by the color detect menu and the areas of reactivity were masked by a red binary color and area was measured using an objective lens of magnification 40, and eye lens 10 the total magnification was 400. Ten fields were chosen in each specimen and the mean values were calculated.

**Immunohistochemistry of iNOS and caspase-3:**

Immunohistochemical staining of iNOS and caspase-3 was performed with streptavidin- biotin. Sections of 4 μm thick were deparaffinized and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti iNOS antibody as the primer antibody at a 1:100 dilution and caspase-3 (1: 100). The specimens were counter stained with H & E. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

**Statistical Analysis:**

Results were analyzed by ANOVA single factor at P< 0.05, using EXCELL Microsoft office 2007, followed by Least significant difference (LSD) of the means between groups, whereas, the different capital letters above columns are significantly different.

**Results**

MINPS –treated groups (75 and 150 mg/Kg) had no effect on serum liver enzymes (ALT, AST and GGT); while they increased TAC level when compared with control values. MINSP (two doses) had no effect on serum creatinine and uric acid concentration when compared with the control group. Moreover, MINSP treatment had no effect on CEA and TNF-α (Fig 2: a, b).

Ochratoxin A significantly elevated the liver enzymes (ALT, AST and GGT) and kidney function test (creatinine and uric acid) as shown inFig. 1: d, e. TAC showed significant inhibition in OA-treatment alone when compared with any treated groups (Fig. 1: f).

The combined treatment with MINSP and OA significantly decreased the elevated values of ALT, AST, GGT, TAC, creatinine and uric acid and increased TAC level toward the control values in dose response manner as shown in Fig. (1).

CEA value significantly elevated by OA-treatment alone when compared with the control or combined treatment with MINSP (75 - 150 mg/kg). However, the two doses of MINSP significantly inhibited CEA-elevation caused by OA-administration in dose dependant manner; the combined treatment with the higher dose of MINSP and OA normalized CEA value (Fig. 2 a).

Moreover, MINSP –treatment caused significant decrease in serum TNF-α than control value in dose response manner. OA-treatment significantly elevated TNF-α when compared with all treatment groups. The combined treatment with the two doses of MINSP normalized the increased value of TNF-α comparing with the control group (Fig. 2b).
MINSP- treatment groups showed significant decreases in MDA and NO levels in liver and kidney homogenates. SOD value did not changed in the groups treated with MINSP (75 -150 mg/Kg ) comparing with the control values.

Ochratoxin A- administration significantly elevated MDA and NO values and inhibited SOD activity especially in the renal tissue compared with the other treatment groups (Fig. 2: f) However, the combined treatment with MINSP + OA ameliorated the oxidative stress (MDA, NO levels) and increased SOD activity in the hepatorenal tissues homogenate(Fig. 2: c, f).

Histopathological findings:
The liver:
The normal liver tissue section showed hepatic cells, sinusoidal with central vein and portal tracts (Fig. 3 A). The group treated with ochratoxin showed extensive areas of pericentral necrosis with loss of hepatic architecture, vacuolar fatty change and inflammatory cell infiltration with pyknotic nuclei and densely stained (Fig.3B). Dilatation and congestion in hepatic arteries, central veins and hepatic sinusoids, bridging necrosis, presence of early fibrosis.
Fig. 2: Effect of MINSP (two dose levels: MINSP1, 75mg/kg and MINSP2, 150 mg/Kg) concomitantly with Ochratoxin A (OA) (1.7 mg/kg, i.p.) on serum tumor necrosis factor alpha (TNF-a)(a), carcinoembryonic antigen CEA (b) in rat serum and on MDA (c, d), SOD (e, f), and NO (g, h) in liver and kidney homogenates after 15 days of daily treatment. (Means ± SE; number= 6). ANOVA—one way, at P< 0.05. The different capital letters above columns are significantly different.

The lower dose of MINSP (75 mg/kg) did not prevent the toxic effect of OA, and necrotic areas were still present (Fig. 3 C). However, the higher dose of MINSP (150 mg/kg) markedly prevented liver necrosis, showing minimal hepatic damage (Fig. 3D).

**The kidney:**

The kidney of the control rats showed normal architecture of renal glomeruli with intact Bowman's capsule. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with brush border and distal convoluted tubules lined by cuboidal cells (Fig. 4 A).
Fig. 3: A. Control liver of rat with central vein (CV) and surrounding hepatocytes (H), sinusoids (S) and nucleus (N). B. Rat treated with OA showing pericentral necrosis (arrow head) with inflammatory cell infiltration around portal tract and bridging necrosis with early fibrosis (long arrow). C. Rat orally given MINSP (75 mg/kg) and OA showing necrotic cells (arrow head), dilatation of hepatic sinusoids (S), the nuclei appear nearly normal (N) and activated kupffer cells (K). D. Rat administered MINSP (150 mg/kg) and OA showing dilatation of hepatic sinusoids (S), the nuclei appear nearly normal (N) and activated kupffer cells (K).

Fig. 4: A. Kidney of control rat showing normal histological structure glomerulus (G) proximal convoluted tubules (P), and distal convoluted tubules (D). B. Kidney of rat treated with OA showing degeneration, hypercellularity of the glomerular with thickening the basement membrane (G) with glomerular and tubulo-interstitial infiltration of lymphocytes (I), renal tubules became vacuolated and lost their brush borders (D & P). C. Rat orally administered MINSP (75 mg/kg) and OA showing less degeneration of the glomerular (G) with less glomerular and tubulo-interstitial infiltration of lymphocytes (I), renal tubules became more or less normal (D & P). D. Rat treated with MINSP (150 mg/kg) and OA showing the glomerular (G) and renal tubules became more or less normal (D & P) with less glomerular and tubulo-interstitial infiltration of lymphocytes (I).

Kidneys of the OA treatment group showed vacuolar degeneration, hypercellularity of the glomerulai with infiltration of lymphocytes and thickening of the capillary basement membrane in the glomeruli. The renal tubules became vacuolated and lost their brush borders. Also in OA-treatment group showed loss of cellular architecture, associated with glomerular and tubulo-interstitial necrosis which was characterized by hydropic degeneration of the glomerular (Fig. 4 B).

However, concomitant treatment with OA and MINSP (75 & 150 mg/kg) ameliorated renal histological lesions in dose dependent effect (Fig.4 C). However, the higher dose of MINSP showed remarkable
improvement in the pathological changes produced by OA - treatment and restored the normal structure of renal tissue (Fig.4 D).

**Quantitative analysis of DNA reaction in liver and kidney sections:**

The optical density of DNA contents of liver and kidney sections significantly decreased in OA-treated group when compared with the control groups as shown (Fig 5, A&B). However, animals administered MINSP and OA significantly increased DNA staining intensity in liver and kidney sections when compared with OA-treated group in dose dependent manner (Fig. 5, A &B). DNA content in the renal was normalized by the higher dose of MINSP, while in the liver did not reached to the normal figures.

![Fig 5: Effect of MINSP (MINSP 1, 75 mg/Kg; MINSP 2 , 150 mg/kg) on DNA –optical density of liver (A) and renal tissues (B) after 15 days of Ochratoxin A(OA) oral administration. (Means ± SE ; number= 8) ANOVA –one way, at P< 0.05. The different capital letters above columns are significantly different.]

**Immunohistochemistry of iNOS, caspase-3 expression for liver and kidney:**

Weakly iNOS immunopositive was shown in the liver (Fig 6A) and kidney (Fig.7 A) of control group. Strong iNOS immunopositive reaction was observed in the group treated with OA-alone in liver and kidney sections comparing with control groups as shown in (Fig. 6 & 7 A). Mild iNOS immunopositivity was found in liver and kidney (Fig.6 C & 7C) treated with MINSP at low dose (75 mg/kg), however at high dose (150 mg/kg) iNOS was found to be nearly similar to that of control (Fig 6 D & 7D).

![Fig. 6: A. liver section from control rat stained with iNOS Immunohistochemistry, showing weak iNOS expression. B. Rat administered OA showing strong iNOS expression. C. Rat administered MINSP (75 mg/kg) and OA showing mild iNOS expression. D. Rat administered MINSP (150 mg/kg) and OA showing iNOS expression nearly normal]

Immunohistochemical analysis of caspase-3 expression in rat livers and kidney revealed strong caspase-3 immunopositive reaction in the livers of OA-treated rat (Fig.8 B & 9 B), whereas weak caspase-3 immunopositive stain was obtained in livers and kidneys of control rat (Fig.8 A & 9 A). Treatment with MINSP significantly reduced caspase-3 expression in dose dependent effect (Fig. 8 & 9: C&D) when compared with OA treatment alone.

As shown in results, MINSP completely abrogated OA-, mediated caspase-3 activation at low dose of
MINSP whereas at the high dose prevented caspase-3 activation.

**Fig. 7:** A. Kidney section from control rat stained with iNOS Immunohistochemistry, showing weak iNOS expression. B. Rat administered OA showing strong iNOS expression. C. Rat administered MINSP (75 mg/kg) and OA showing mild iNOS expression. D. Rat administered MINSP (150 mg/kg) and OA showing iNOS expression nearly normal.

**Fig. 8:** A. Weak caspase-3 expression was found in liver of the control rat. B. Strong caspase-3 expression was found in liver of OA-treated rat. C. Mild caspase-3 expression in liver rat orally given MINSP (75 mg/kg) and OA. D. nearly normal caspase-3 expression in rat liver administered MINSP (150 mg/kg) and OA.

**Fig. 9:** A. Weak caspase-3 expression was found in kidney of the control rat. B. Strong caspase-3 expression was found in kidney of OA-treated rat. C. Mild caspase-3 expression in kidney rat orally given MINSP (75 mg/kg) and OA. D. nearly normal caspase-3 expression in rat kidney administered MINSP (150 mg/kg) and OA.
Discussion:

Ochratoxin A (OA) is a mycotoxin produced by several species of the fungal genera *Aspergillus* and *Penicillium* and is found in cereal and grain products OA is hepatonephrotoxic (Nada et al., 1994), immunosuppressive (Singh et al., 1990), teratogenic (Abdel-Wahhab et al., 1999), apoptotic (Seege et al., 1994), genotoxic (Pfohl-Leszczewicz and Manderville, 2012) agent and it has oxidative stress property (Nada et al., 1994). It cause cytotoxicity and increased ROS production in rat and monkey kidney cell lines (Schaaf et al., 2002). Beside, OA was suggested to cause an imbalance between oxidant/antioxidant parameters in rat kidney and liver tissues (Palabiyik et al., 2013).

Several antioxidants have been applied to provide protection against OA-induced hepato-renal toxicity (Yenilmez et al., 2010).

In the present study, OA-treated rats showed significant increase in the serum levels of ALT, AST, GGT, creatinine, uric acid, TNF-α and CEA comparing to the control values. OA treatment caused elevation in MDA and NO and Inhibition in SOD activity in both liver and kidney homogenates. However, OA exerts its toxic effects by accumulation in the target organs (kidney and liver) and potentially imposing serious damage to these organs. Petzinger and Ziegler (2000) suggested that OA enhanced lipid peroxidation, inhibited protein synthesis, interfered the metabolic systems involving phenyl alanine, disrupted calcium homeostasis, inhibited mitochondrial respiration, and cause DNA damage (Cavin et al., 2009, Pfohl-Leszczewicz and Manderville, 2012).

Previously, many investigators found that administration of OA to rats resulted in enhanced LPO in liver and kidney and promoted hydroxyl radical production. In this respect, membrane lipid peroxidation is an important part of oxidative tissue injury (Rahimtula et al., 1988 and Abdel-Aziz et al., 2010). OA induced LPO by chelating Fe³⁺ and the resulting OA- Fe³⁺ chelate was more readily reducible by the flavoprotein NADPH-cytochrome P450 reductase to an OA-Fe²⁺ complex, which, in the presence of oxygen, provide the active species that initiated LPO. These marked changes in LPO could be contribute to the effect of OA on the antioxidant defense system (Omar et al., 1990). Our data showed that OA- treatment exerts its specific detrimental effects on antioxidant enzyme (SOD) and oxidative parameters, NO and MDA in liver and kidney tissues homogenates. SOD is a copper- and zinc-containing enzyme responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals (O²⁻) to H₂O₂ (Fridovich, 1983). In the present study, SOD showed significant inhibition in liver and kidney homogenates this may be due to OA interact with the copper or zinc molecules in SOD structure consequently depletion in SOD- gene expression occurs (Abdel-Raheim and Hussein, 2001). Moreover, OA reduced protein synthesis by competing phenylalanine in the acylation reaction of phenylalanine- tRNA (Creppy et al., 1983).

The present study, OA administration induced cellular infiltration, congestion, central vein dilatation, pericentral necrosis, vacuolar fatty change within hepatorenal architectures as discussed by Aydin et al. (2003). The observed hepatic congestion may be due to OA – treatment caused disruption in coagulation factors pathway and/or platelets function (Galtier et al., 1975).

Renal tissue is the target organ affected by OA-toxicity, Milicevic et al., (2009) found that OA induced degenerative changes in the renal tubular epithelial cells including: swelling, vacuolar and lipophilic degeneration. It was reported that OA bio-transformed to genotoxic metabolites interacted with DNA caused genetic damage covalently binds to DNA (Mally, et al., 2005).

The present results showed that OA-treatment caused significant inhibition in DNA – contents in liver and kidney tissues. The combined treatment of MINSP and OA significantly normalized DNA in renal contents, while the two dose of MINSP had similar effect to increase DNA content in liver tissue. These effects may be due to the antioxidant nature of MINSP (Nada et al., 2010).

Several studies have provided evidence that the toxicity and genotoxicity of OA could be associated with its biontransformation via peroxidase pathways (Rahimtula et al., 1988).

In the current study, OA was administrated with MINSP to prevent OA toxicity. To our knowledge, there is no report indicating that the use of MINSP counteracts OA toxicity. MINSP is a direct free radical scavenger and indirect antioxidant (Nada et al., 2010).

The much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. In these circumstances, activated macrophages greatly increase their simultaneous production of both nitric oxide and superoxide anions. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite resulting irreversible damage to the cell membrane (Cavin et al., 2009).

OA enhances the release of TNF-α, consequently it activates caspase-3 in kidney and liver sections Sauvant et al., (2005). Co-administration of OA+ MINSP caused significant inhibition caspase-3 activation in dose dependent manner.

MINSP-administration reduced the pathological features presented by OA-administration. The protective mechanism of MINSP may be due to enhanced immunity and down regulation of cytokines (Vetvicka and
Yvin, 2004). Moreover, MINSP fruit bodies are rich with vitamins (B1, B2, C and D2), polysaccharides and glycoproteins (such as: chitin, hemicelluloses, β- and α-glucans, mannans, xylans and galactans); all these contents have strong antioxidant, radical scavenging and other beneficial biological activities (Mattila et al., 2000; Nada et al., 2010; Synytsya et al., 2009).

In conclusion:
OA-toxicity caused biochemical disturbance and oxidant-antioxidant status disruption. MINSP-coadministration with OA greatly diminished the hepatorenal damage induced by OA toxicity. The highly purified MINSP possess an excellent food supplement could to protect hepatorenal tissues from damage induced by OA-toxicity.

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