**Hepato-renal Protective Effect of Edible Mushroom on Ochratoxin A Toxicity in Sprague Dawley Rats**

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**Abstract:** Ochratoxins are mycotoxins produced by *Penicillium* and *Aspergillus* fungi. The present study was carried out to study the protective effect of mushroom (*Agaricus sp.*) against ochratoxin A in rats. Results indicated that normal groups treated with two doses of mushroom (100 and 200 mg/Kg,P.O.) caused significant increase in ALT and AST values than control group. Ochratoxin A (OA) 1.7 mg/ Kg administration resulted significant elevation in serum ALT and AST than normal control group. The combined treatment with OA and mushroom significantly reduced ALT and AST in lower dose 100 mg/Kg only were (33.27 % and 30.06 %, respectively). OA–treatment significantly elevated kidney functions (serum creatinine and uric acid) compared with normal control; in combined treatment, these values significantly decreased in the lower dose of mushroom (100 mg/Kg); while the higher dose of mushroom had slight decrease in creatinine and uric acid levels. Superoxide dismutase (SOD) activity was increased significantly in the groups of rats treated with mushroom alone or in combination with OA when compared with OA alone treatment group. In conclusion: the lower dose of mushroom (100 mg/kg) was the effective dose that minimized the hepatorenal toxicity induced by OA. Our study needs further continuation in this respect.

**Key words:** Ochratoxin A, hepato – renal toxicity, rat, mushroom.

**INTRODUCTION**

Ochratoxin (OA) is present in a large variety of foods because it is produced by several fungal strains of the *Penicillium* and *Aspergillus* species that have varied physiologies and ecologies. OA is considered to be nephrotoxic, teratogenic, hepatotoxic and immunotoxic, and has been classified by the IARC as a Class 2B carcinogen, probable human carcinogen (Radovanovic et al., 1991). Ochratoxin A is found in wheat, corn, and oats having fungal infection and in cheese and meat products of animals consuming ochratoxin-contaminated grains (Aish et al., 2004). *A. ochraceus* is found on dry foods such as dried and smoked fish, soybeans, garbanzo beans, nuts, and dried fruit. *A. carbonarius* is the major pathogen in grapes and grape product including raisins, wines, and wine vinegars (Garcia et al., 2003). Although reported to occur in foods around the world, the main regions of concern are Europe and Africa (Abarca et al., 2001; Wafa et al., 1998).

Exposure to low concentrations of this toxin causes morphological and functional changes in kidney and liver of several domestic and experimental animals (Hussein and Brasil 2001). The toxin has also been found in human sera from people living in areas where Balkan endemic nephropathy occurs (Pfohl-Leszkowicz et al., 2002 ; Radić et al., 1997), and it is suggested to be a possible determinant of this fatal human disease (Kuiper-Goodman and Scott , 1996). The molecular mechanisms involved in OA-induced nephrotoxicity was described by Krogh (1992) and Nada et al. (1994), carcinogenesis (Pitt 2000), teratogenic effects Abdel- Wahhab et al.(1999), immunosupression and inhibition of mitosis (Abdel-Aziz et al., 2010).The genotoxic effects, inhibition of DNA synthesis and mitosis, as well as histopathological effects on the nuclei of OA-treated cells may be explained by OA-inflicted DNA damage, which include DNA adduct formation and DNA single strand breaks (Farag et al., 2010).

Higher *Basidiomycetes* have become subject of great interest, due to their nutritional value and pharmacological properties. They have been largely consumed to prevent cancer (Reshetnikov et al., 2001) and heart diseases, alleviate the blood stream and reduce cholesterol level. Moreover, these mushrooms are used to combat physical and emotional stress, to stimulate immunity (Borchers et al.,1999), to improve life quality of diabetics, to combat diseases such as osteoporosis and gastric ulcer and to act as an effective antioxidant (Ghaly et al. 2011; Nada et al., 2010 and Lakshmi et al., 2004). Nevertheless, scientific studies about their biological properties are still insufficient,(Gutierrez et al., 2004). Mushrooms have become attractive as a functional food and as a source of drugs development and nutraceuticals (Mahendra et al.,2005). It is well established that many mushroom-extracted compounds are commonly used as immuno-modulators or as biological response modifiers. The basic strategy underlying immuno-modulation is to identify aspects of the host response that can
be enhanced or suppressed in such a way as to augment or complement a desired immune response (Ooi and Liu, 1999). Whether certain compounds enhance or suppress immune responses depends on a number of factors, including dose, route of administration, timing of administration of the compound, mechanism of action, and site of activity (Lorenzen and Anke, 1998). Knowledge of the specific components of cytokine networks and signaling pathways and their role in the regulation of immune responses is important in designing strategies to augment these responses. Immuno-modulators isolated from more than 30 mushroom species have shown anticancer action in animals (Wasser et al., 2000).

Recent advances in chemical technology have allowed the isolation and purification of some compounds in mushrooms, especially polysaccharides which possess strong immuno-modulation and anti-cancer activities (Nada et al., 2010). They are used as biological response modifiers. The polysaccharides isolated from mushroom fruiting bodies are either water soluble or insoluble glucans and hetero-polysaccharides with different main and side-chains. There is great interest in these molecules because they can act as biological response modifiers. This study aimed to evaluate the edible mushroom as a protective agent against ochratoxicosis in rats.

MATERIALS AND METHODS

Detection and Determination of Ochratoxin A:

1. Extraction

Extraction of OA from YES Media:

OA was extracted from YES media according to Tsubouchi et al. (1985).

2. Clean Up:

A Sep-Pak C18 Column was placed on vacuum manifold ports, column prewashed twice with 2 ml methanol, 2 ml water, and 2 ml sodium bicarbonate (3%). Five ml bicarbonate extract were added to the C18 column, followed by 2 ml phosphoric acid (0.1M) and 2 ml water, and washings were discarded. OA was eluted with 8 ml ethyl acetate: methanol: acetic acid (95:0.5:v/v/v). The elute was collected in vial containing 2 ml water and the elute was shaken with tube shaking machine (vortex genie) to mix the two phases. Piped OA extract (upper phase) to 7 ml screw-capped vial. Rinse remaining upper phase from tube with 2 x 1 ml ethyl acetate and add to OA. Evaporate extract just to dryness on steam bath under nitrogen for subsequent HPLC analyses (AOAC 2000).

3. Determination of OA by HPLC:

The foregoing columns elutes were dissolve in 500 µl mobile phase consists of acetonitrile: water: acetic acid (99:99.2) and filter through 0.45 µm micro filter into 5 ml screw-cap vial for subsequent HPLC analyses. High performance liquid chromatography (HPLC) was used to ochratoxin A Determination. The system equipped with (Waters 600) delivery system. HPLC column a reverse phase analytical column packed with C18 material (Spherisorb 5 µm ODS2, 15cm x 4.6mm). The detection was performed using the fluorescence detector was operated at an excitation wave length of 330 nm and an emission wave length of 460 nm. The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. Data were integrated and recorded using a Millennium Chromatography. Manager Software 2010 (Waters, Milford MA 01757).

4. Quantization:

Calculated from chromatographic peak areas using the standard curve.

5. Mushroom (Agaricus sp.) Preparation:

Mushroom (Agaricus sp.) was obtained from National Research Centre, Egypt. It was dried and Grinded to powder, 100 mg and 200 mg/kg of rats were used and suspended in gum acacia (2.5%, v/v).

6. Animals:

Sixty male Sprague-Dawley rats (purchased from Animal House Colony, Giza, Egypt) weighing 150 ± 6 g were maintained on OA-free standard laboratory diet (protein: 16.04%, fat: 3.6%, fiber: 4.1% and metabolic energy: 2887 kcal/kg) and water ad libitum at the Animal House, National Research Centre, Dokki, Cairo, Egypt. After an acclimation period for one week, animals were divided into four groups (10 rats/group) and housed in filter-top polycarbonate cages housed in a temperature-controlled and artificially illuminated room free from any sources of chemical contamination. All animals received humane care in compliance with the guidelines of the Ethical Committee of National Research Centre, Egypt.
7. Experimental Design:

The animals were distributed into four treatment groups and treated orally for 15 days as follows: group 1: untreated control, group 2: treated with OA (1.7 mg/kg b.w) this dose according to Nada et al. (1994), group 3: treated with extract alone (100 mg/kg b.w) and (4) treated with the extract (200 mg/kg b.w) + OA (1.7 mg/kg b.w). At the end of experimental period and under ether anesthesia, blood samples were collected from the retro-orbital sinus plexus from all animals after being fasted for 12 h for different biochemical analysis. Blood samples were left to clot and centrifuged at 3000 rpm at 4 °C for 15 min to separate the serum.

The obtained serum was used to estimate the activities of AST, ALT, SOD, Creatinine and Uric acid. Determination of AST (Aspartate Amino-Transferase) and ALT (Alanine Amino-Transferase ALT) activities using a test reagent kit according to, (Reitman and Frankel, 1957). Determination of Superoxide Dismutase SOD according to Nishikimi et al. (1972).

Creatinine was determined according to Bartles et al., (1972). Uric acid was determined according to Barham and Trinder (1972).

Statistical Analysis:

In the present study, all results were expressed as mean ± standard error of the mean. Data were analyzed by one-way analysis of variance (ANOVA) at P<0.5. When the variation among groups was proved significant, the least significant difference (LSD) test was performed to compare the significance between groups.

Results:

Liver Function: Activities Of AST And ALT Enzymes:

Data in Table (1) showed ALT activity was increased significantly by 32.85% and 66.44%; AST by 16.00 % and 40.22 %, respectively in mushroom treatment (100 and 200 mg/Kg) when compared with normal in dose response manner. Moreover, AST activities also elevated in the serum of normal groups administered 100 and 200 mg/Kg mushroom doses when compared with control group. Meanwhile, ochratoxin A–treatment group resulted significant elevation in serum ALT and AST than normal control groups. Combined treatment with OA and mushroom (100 mg/Kg) significantly reduced ALT and AST to 33.27 % and 30.06 %, respectively; the higher dose of mushroom (200 mg/Kg) exhibited slight decrease in the activities of serum ALT and AST levels compared with OA treated groups to be 4.40 % and 6.34 %, respectively.

Table 1: Effect of mushroom on liver function (serum ALT and AST) of normal and/or in concomitant with OA.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Ochratoxin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>SE</td>
<td>1.08</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>% of effect</strong></td>
<td>0.0</td>
<td>32.85</td>
</tr>
<tr>
<td>AST (IU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>75.00</td>
<td>87.00</td>
</tr>
<tr>
<td>SE</td>
<td>2.08</td>
<td>2.93</td>
</tr>
<tr>
<td><strong>% of effect</strong></td>
<td>0.0</td>
<td>16.00</td>
</tr>
</tbody>
</table>

* Percentage of effect relative to its control value: normal and Ochratoxin A-treated groups.
ANOVA – one way, different capital letters are significantly different at P < 0.05 between groups.

Mush-1: 100 mg/Kg body weight.
Mush-2: 200 mg/Kg body weight.
OA: Ochratoxin A (1.7 mg/Kg)

Data in table (2) revealed that, mushroom impaired renal function in dose dependent manner. Creatinine concentration increased significantly in mushroom two dose treatments (100 mg and 200 mg/Kg) to be higher than normal values (55.55 % and 92.59%, respectively). Moreover, Uric acid concentration also elevated in the serum of normal groups administered mushroom (100 and 200 mg/Kg) when compared with control group; the percentage of increase was (32.83% and 82.08%, respectively) relative to the normal control values. Meanwhile, no significant changes were seen in kidney functions (creatinine and uric acid) in the group treated with OA and mushroom (200 mg/Kg) when compared with OA treatment alone. While, the co-administration with the lower dose of mushroom and with OA (1.7 mg/Kg) ameliorated OA effect on kidney function in both serum creatinine and uric acid (-27.63 % and -29.86 %, respectively), but still above normal control groups as illustrated in Table (2).
Table 2: Effect of mushroom on kidney function (Creatinine and Uric acid serum) of normal and/or in concomitant with OA (Ochratoxin A 1.7 mg/Kg).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Mush-1</th>
<th>Mush-2</th>
<th>OA</th>
<th>OA+ Mush-1</th>
<th>OA+ Mush-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine mg/dl</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>X</td>
<td>0.27 ± 0.55</td>
<td>0.42 ± 0.52</td>
<td>0.76 ± 0.55</td>
<td>0.73 ± 0.52</td>
<td>X</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>SE</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>*% of effect</td>
<td>0.0</td>
<td>55.55</td>
<td>92.59</td>
<td>0.0</td>
<td>- 27.63</td>
<td>- 3.94</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>X</td>
<td>0.67 ± 1.01</td>
<td>0.89 ± 1.22</td>
<td>1.44 ± 1.01</td>
<td>1.38 ± 1.01</td>
<td>X</td>
<td>0.019 ± 0.022</td>
</tr>
<tr>
<td>SE</td>
<td>0.015</td>
<td>0.015</td>
<td>0.017</td>
<td>0.015</td>
<td>0.031</td>
<td>X</td>
</tr>
<tr>
<td>% of effect</td>
<td>0.0</td>
<td>32.83</td>
<td>82.08</td>
<td>0.0</td>
<td>- 29.86</td>
<td>- 4.16</td>
</tr>
</tbody>
</table>

* Percentage of effect relative to its control value: normal and OA groups.
ANOVA – one way, different capital letters are significantly different at P < 0.05 between groups.
Mush-1: 100 mg/Kg body weight.
Mush-2: 200 mg/Kg body weight.
OA: Ochratoxin A (1.7 mg/Kg)

Antioxidant Activity (SOD Activity):

Results in Table (3) showed that, the level of SOD was significantly increased in rats treated with mushroom (200 mg/Kg) when compared with group treated with mushroom (100 mg/Kg). While, the lower dose of mushroom (100 mg/Kg) had no effect on SOD level; it is non-significantly changed than normal control value. On the other side OA-treated groups, their SOD value severely depleted more than any treated groups. Surprisingly, SOD activity was increased significantly in the groups of rats treated with OA and the lower dose of mushroom (100 mg/Kg) than that higher dose (200 mg/Kg mushroom); the recorded % of increase were (704.78 % and 582.04%) in OA + 100 mg mushroom and OA + 200 mg mushroom, respectively, versus OA values. These figures did not reach to the normal group value.

Table 3: Effect of mushroom on antioxidant activity in serum SOD in normal and/or in concomitant with OA treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Mush-1</th>
<th>Mush-2</th>
<th>OA</th>
<th>OA+ mush-1</th>
<th>OA+ mush-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (IU/ml)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>X</td>
<td>226.50 ± 226.71</td>
<td>251.00 ± 22.17</td>
<td>178.42 ± 151.21</td>
<td>X</td>
<td>1.89</td>
<td>4.62</td>
</tr>
<tr>
<td>SE</td>
<td>2.43</td>
<td>4.62</td>
<td>1.77</td>
<td>0.015</td>
<td>4.62</td>
<td>2.43</td>
</tr>
<tr>
<td>*% of effect</td>
<td>0.0</td>
<td>10.81</td>
<td>82.08</td>
<td>0.0</td>
<td>704.78</td>
<td>582.04</td>
</tr>
</tbody>
</table>

* Percentage of effect relative to its control value: normal and OA groups.
ANOVA – one way, different capital letters are significantly different at P < 0.05 between groups.
Mush-1: 100 mg/Kg body weight.
Mush-2: 200 mg/Kg body weight.
OA: Ochratoxin A (1.7 mg/Kg)

Discussion:

OTA poses a critical hazard to human and animal health because of its nephrotoxic, teratogenic, and carcinogenic activity (Kuiper-Goodman and Scott, 1996; Pitt, 2000; Wei and Sulik, 1993). The ability of OA to generate free radicals which may lead to DNA breakage, inhibition of protein biosynthesis and gluconeogenesis, lipid peroxidation, disruption of oxidative phosphorylation in mitochondria, inhibition of blood clotting and apoptosis, and its interference with signal transduction in some cell types causing different organ pathogenesis (Abdel-Aziz et al., 2010; Pitt, 2000).

In our study we present the elevated values of serum ALT, AST, creatinine and uric acid and inhibition of SOD activities by OA-administration; these results were in agreement with most researches in worldwide like that taken by Amra et al. (1996) ; Abdel-Wahhab et al (1999) ; Ikekawa (2001); Molitoris (2002); Boa (2004); Coletto (2005) ; Nakajima et al. (2007); Vetter (2003); and Sang-Woo et al. (2007).

However, the combined administration of Mushroom and OA significantly reduced the increased values of the studied parameters (ALT, AST, Creatinine and uric acid) and significantly reduced SOD value by OA-treatment alone , the effect of the lower dose of mushroom was more effective than the higher dose as previously mentioned in the results section.

This suggests that mushroom may be able to protect against the oxidation of hepatic cellular membrane damage via a free-radical scavenging property (Nada et al.,2010). Jayakumar et al. (2006) demonstrated that the ethanolic aqueous extract of the P. ostreatus is able to significantly alleviate the hepatotoxicity induced by CCl4 in the rat. The protective mechanism of Mushroom may also involve enhanced immunity and down-regulation of key cytokines (Vetvicka and Yvin, 2004). Moreover, Mushroom fruit bodies are rich with vitamins (B1, B2, C and D2), polysaccharides and glycoproteins (such as chitin, hemicelluloses, b- and a-glucans, mannans, xylans and galactans); all these contents have strong antioxidant, radical scavenging and other beneficial biological activities (Manzi et al., 1999; Mattila et al., 2000; Synytsya et al., 2009).
From animal experiment we can conclude that, the lower dose of mushroom 100 mg with ochratoxin A was the best treatment to minimize hepato-renal toxicity in rats induced by OA during 15 day of treatment. It is clearly demonstrated that, mushroom was a good functional food due to it is a source of many nutraceutical compounds and has a powerful antioxidant activity which should be regulated according to its usage and duration.

REFERENCES


