The Protective Role of Agaricus bisporus in Experimental Liver Injury in Mice

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Abstract: Hepatic disorders can develop by many causative agents like Reactive oxygen species (ROS). Various antioxidants have been ensured to protect against hepatic damage. The aim of this study is to ensure Agaricus bisporus protective effects in experimental mice of liver injury. Forty adult female albino mice were divided into five groups (Ten mice for each group), the first group given normal saline as control, the second one treated with 2ml/kg body weight of peroxide(H2O2) with drinking water, the third one treated with water aqueous extract (25) mg/ml of A. bisporus orally with H2O2 in drinking water and finally the fourth one treated with aqueous extract (25) mg/ml of A. bisporus orally by use of intragastric tube. Results for apoptosis test showed peroxide treated group induced apoptosis while aqueous extract of A. bisporus treated group with peroxide induced non-apoptotic. Histopathological examination of liver cells exposed to H2O2 showed necrosis and degenerated liver cells by the evidence of tendency to forming granuloma. In sinusoids showed dilation with severe congestion of blood vessel while the tissue treated with (2) ml/kg body weight of A. bisporus and H2O2 with drinking water showed slightly congested blood vessel and aggregation of mononuclear cells in liver parenchyma. The liver tissue in mice treated with (2) ml/kg body weight of A. bisporus appeared approximately like the normal tissue. We conclude that the aqueous extract of Agaricus bisporus can protect the liver against peroxide induced oxidative damage in mice and is an efficient antioxidant agent and hepatoprotective against peroxide induced liver injury.

Keywords: Agaricus bisporus, Liver, Peroxide, and Mice.

I. Introduction

Hydrogen peroxide (H2O2) is a reactive oxygen species (ROS). It is produced as a short-lived product in biochemical processes of human and animals during metabolism processes like respiration. Peroxide (H2O2) plays an active role in the regulation of various physiological processes. In spite of that, the access amount of it results in oxidative stress that can lead to extensive cellular damage. Furthermore, high levels of H2O2 have been implicated in many pathological conditions including diabetes, neuronal cell death, cardiovascular diseases, and cancer[1]. In a dilute solution, Hydrogen peroxide (H2O2) is a very pale blue liquid which appears colorless. It is a weak acid slightly more viscous than water. It has strong oxidizing properties, but has also found use as a disinfectant and as an oxidizer and apparently able to cross cell membranes [2]. Recently high levels of H2O2 are cytotoxic to many animal, plants and bacterial cells. The toxicity is due to oxidation of membrane lipids, DNA and proteins, by the peroxide ions. [3] Agaricus bisporus is one of the many known medicinal mushrooms, with high nutritive value [4]. It considered as neuaceutical due to its low calories with a nutritional value, carbohydrate and sodium content as well as a high content of potassium, several vitamins and some trace element [5]. Agaricus bisporus has significant medicinal properties such as antioxidant, anti-inflammatory, antibacterial antifungal, antiviral, hepatoprotective, antitumor activities and anti-thrombotic [6]. Because of commonly consumption of mushrooms by most people in the world, especially Iraqi people, this study was carried out and aims to evaluate the protective action of an aqueous extract of A. bisporus in the detoxification of peroxide by an experimental model of female albino mice.

II. Materials And Methods

2. Preparation of Aqueous Extract of Agaricus Bisporus

Agaricus bisporus was obtained from the Mushrooms Production Unit in the College of Agriculture, University of Tikrit. Mushroom was dried by oven, at a ratio of (1:10) the obtained powder was sinked in D.W. (w/v) and for (30) minutes boiled in (60) oC, then left covered for (30) minutes. The precipitant were then removed by filtration through gauze and for (30) min, at 4°C further centrifuged at (10,000) rpm. Supernatants were filtered after collection through filter paper (Whatman (No.1)). Then by using freeze dryer, we obtained freeze dried extract powders and stored at (4± 2°C)[7].

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2.2 EXPERIMENTAL ANIMALS

Female albino mice of strain Mus musculus weighting (32-35 g) were divided into four groups, each group contains (10) mice as follows:

**Group 1:** Animals treated with normal saline as a control.

**Group 2:** Animals only received (2) ml/kg body weight of H₂O₂ with drinking water.

**Group 4:** Animals received 2ml/kg body weight for H₂O₂ with drinking water and treated with water aqueous extract (25) mg/ml of A. bisporus daily.

**Group 4:** Animals were received with water aqueous extract (25) mg/ml of A. bisporus daily.

The aqueous extract of A. bisporus given orally by intragastric tube and peroxide given with drinking water. Animals treated for 30 days. After (30) days of the treatment, blood were collected by heart puncture method and putted in eppendorftube for apoptosis test.

All mice were sacrificed by cervical dislocation. Liver was removed and dissected out. It was washed with normal saline and fixed in formalin (10%). After that samples were dehydrated in ascending grades of ethanol and then embedded in paraffin wax. By using a rotary microtome then it cut into section of (4–6) μm thickness. Staining made with Haematoxylin and Eosin (H&E) according to [8]. The examination was made by using a light microscope and photographs were taken with digital camera.

2.3 APOPTOSIS

Apoptosis was induced by using 2ml/kg body weight of H₂O₂ with drinking water for 30 days and we measured it according to [9].

1- By using centrifuge, the cells were harvested. The supernatant was discarded and the pellet was resuspended in cold phosphate buffer saline (PBS) and by gentle shaking the cell was washed or by mixing in pipette tip up side down. The supernatant was discarded after washing again by centrifuge.

2- For preparing a sufficient cell suspension (100) μl per assay, the pellet was resuspended in IX Binding Buffer and adjust cell density to (2.5x10⁵) cells/ml.

3- Add 5 μl of PI and 5 μl of annexin V-FITC. Then mixed gently.

4- At room temperature in dark, the stained cells incubated for 15 min.

5- The cells were centrifuged after incubation period and resuspended pellet in (100) μl of binding buffer.

6- By using flow cytometry, the stained cell was analyzed, as soon as possible.

We calculate Apoptotic Rat (%) by the following equation:

\[
\text{Apoptotic Rat} \% = \frac{\text{Number of positive staining cells}}{\text{Number of total cells}} \times 100
\]

III. Statical Analysis

Analysis of variance was used to analyze data. The significant level was at (P<0.05). Variables were expressed as mean± SE. Data was analyzed using SPSS software [10].

IV. Result And Discussion

Hydrogen peroxideH₂O₂ is used in experimental models to induce oxidative stress in mice. Hydrogen peroxide can induce liver damage through the formation of reactive intermediates such as ROS [11]. Table (1-1) showed that peroxide treated group induced apoptosis however aqueous extract treated group with peroxide failed to induce apoptosis, due to the significant increase in the number of cells and a decrease when treated with aqueous extract of Agaricus bisporus. These results indicated that fungus extract results in non-apoptotic condition. In adult tissue and during fetal development, programmed cell death (Apoptosis) plays critical role in a wide variety of physiological processes. As a result of necrosis, defect in apoptotic cell death regulation and many diseases physiological cell death occurs by apoptosis [12]. H₂O₂ is an effective apoptosis inducer, but the dose range and the exposure time differ by cell type to avoid cell death primarily by necrosis or necrosis [13]. A bisporus have a high amount of anti-oxidant and phenolic compounds which have pro oxidative properties therefore its ability to activate the cellular formation of ROS, the major role of ROS might be signal transmission and regulation of processes including cell proliferation, phagocyte activation and apoptosis [14]. In normal cells, damages caused by ROS induce cells to repair the abnormal damages or activated the affected cells towards the cell death program, that is, to either apoptosis or autophage cell death and tumor cell which appear to be sensitive to ROS induced apoptosis [15].
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Table 1: Apoptosis for treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Apoptosis</th>
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<tr>
<td>Control (aqueous extract)</td>
<td>0.0451 ±0.02</td>
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<tr>
<td>Peroxide</td>
<td>2.0112±0.432*</td>
</tr>
<tr>
<td>Peroxide + aqueous extract</td>
<td>0.065±0.0050</td>
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* mean significant at (P<0.05) compare to control group.

The liver is a vital organ found in animals. It has many functions such as amino acid metabolism, drug metabolism and glycolysis. Liver is able to detoxify toxic material. Hepatotoxic agents like hydrogen peroxide H2O2 can cause very serious damages [16]. Liver of control animals showed normal texture as central vein, hepatocyte and sinusoids (Figure 1). Liver from mice exposed to H2O2 being toxic caused severe necrosis in liver parenchyma and dilated sinusoid with congested blood vessel, aggregation of mononuclear inflammatory cells and tendency to form granuloma (Fig 2,3). The result of this study suggested that an extract of Agaricusbisporus is able to provide protection against acute hepatotoxicity induced by H2O2. Since H2O2 induce hepatotoxicity, it clear that mice receiving mushroom extract with peroxide revealed mononuclear cells aggregation (neutrophils and monocytes) Fig (4,5).While liver of mice treated with A bisporus returned like control Fig(6).Agaricusbisporus considered to have therapeutic and protective properties. Studies demonstrated that Agaricusbisporus extract have anti-inflammatory and anti-tumor activities [17], in other study it used in combination with green tea to reduce the risk of breast cancer [18]. It has potent antitumor effect by enhancing immune response. Selenium is an essential trace element for humans and animals. Since Agaricusbisporus contains high level of selenium and a total of 174 metabolic products were detected. There are many medicinal activities for these metabolites including anti-cancer, anti-hypercholesterol, anti-cardiovascular diseases, hepatoprotective and immune enhancer [19]. The work of [20] involving the role of selenium in cancer chemoprevention. Selenium may have role in prevention of cancer through protection of antioxidant and increased immune function.In human studies, there is evidence which suggest that, when selenium taken in high doses, may reduce the incidence of cancer. Recent studies showed benefit with selenium in reducing cancer, specifically in the liver, lung, colon, and prostate[21]. In the present study Abisporus mushrooms showed protective effect against liver damage by H2O2-induced toxicity.

Fig (1): Histopathological section of control group shows central vein (V), hepatocytes(H) and sinusoid (S) (H&E stain 40X)
**Fig (2):** Section in liver of treated group with peroxide shows severe congestion in blood vessel (C), dilated sinusoids (D), degenerated liver cells (DE) and necrosis (N).

**Fig (3):** Section in liver of treated group with peroxide shows: Tendency to forming granuloma (G), severe congestion in blood vessel (C).

**Fig (4):** Section in liver of treated group with peroxide and *Agaricusbisporus* showed: Slightly congested blood vessel (SC). Aggregation of mononuclear inflammatory cells (M) on the margin of slightly congested blood vessel (C).
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Fig(5): Section in liver of treated group with peroxide and Agaricus bisporus Infiltration of inflammatory cells (I) with Aggregation of mononuclear cells in liver parenchyma (A)

Fig (6): Section in liver of Treated group with agaricus bisporus showed central vein(V), hepatocytes(H) and sinusoids(S)

References

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