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The Protective Role of *Agaricusbisporus*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 Agariusbiporus protective effects in experimental mice of liver injury.Forty adult female albino mice were divided to five groups (Ten mice for eachgroup), the first group give normal saline as control, the second one treated with 2ml/kg body weight of peroxide(H_2O_2) withdrinking water,the third one treated with water aqueous extract (25) mg/ml ofA.bisporusorallywith H_2O_2 in drinking water and finally the fourth one treated withaqueous 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. bisporustreated group with peroxide inducednon-apoptotic. Histopathological examination of liver cells exposed to H_2O_2 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/kgbody weight of A.bisporus and H_2O_2 with drinking water showedslightly congested blood vesseland aggregation of mononuclear cells in liver parenchyma. The liver tissue in mice treated with (2) ml/kgbody weightof A.bisporus appeared approximately like the normal tissue.We conclude that the aqueous extract of* Agaricusbisporus*can protect the liver against peroxide induced oxidative damage in mice and is an efficient antioxidant agent and hepatoprotectiveagainst peroxide induced liver injury.*

Keywords: *Agaricusbisporus, Liver, Peroxide, and Mice.*

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I. Introduction

Hydrogen peroxide (H_2O_2) 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 (H_2O_2) 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 H_2O_2 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 H_2O_2 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].*Agaricusbisporus* is one of the many known medicinal mushrooms, with high nutritive value [4]. It considered as neutraceutical due to its low calories with a nutritional value,carbohydrate and sodium content as will as a high content of,potassium, several vitamins and some trace element [5]. *Agaricus .bisporus*hassignificant medicinal properties such as antioxidant, anti-inflammatory, antibacterial antifungal,antiviral, hepatoprotective, antitumor activities and antithrombotic [6]. Because of commonly consumption of mushrooms by most people inthe world,especially Iraqi people , this study was carried out and aims to evaluate the protective action of an aqueous extract of *A bisporus* in detoxificationof peroxide byan experimental model of female albino mice

II. Materials And Methods

2.1PREPARATION OF AQUEOUS EXTRACT OF *AGARICUS BISPORUS*

Agaricusbisporus 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) °C, 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].

2.2 EXPERIMENTAL ANIMALS

Female albino mice of strain *Musmusculus* weighting (32-35 g) were divided into fourgroups,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 H_2O_2 with drinking water.
Group 4: Animals received 2ml/kg body weight for H_2O_2 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*is given orally by intragastric tubeand 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. Byusing 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 H_2O_2 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 re suspended 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 res suspended in IX Binding Buffer and adjust cell density to ($2\text{-}5x10^5$)cells/ml.

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

4- At room temperaturein 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:

$$\text{Apoptotic Rat (\%)} = \frac{\text{Number of positive staining cells}}{\text{Number of total cells}} \text{ X } 100$$

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 *Agaricusbisporus*. 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]. H_2O_2 is an effective apoptosis inducer, but the dose range and the exposure time differ by cell type to avoid cell death primarily by necroptosis or necrosis [13]. *A bisporus*have a high amount of anti-oxidant and phenolic compounds which have pro oxidative properties therefore its able 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].

Table (1-1): Apoptosis for treated groups

Groups	Apoptosis
Control (aqueous extract)	0.0451 ±0.02
Peroxide	2.0112±0.432*****
Peroxide + aqueous extract	0.065±0.0050

***** 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 H_2O_2. Since H_2O_2 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 theincidence of cancer. Recent studies showed benefit with selenium in reducing cancer, specifically in the liver, lung, colon, and prostrate[21]. In the present study *Abisporus* mushrooms showed protective effect against liver damage by H_2O_2-induced toxicity.

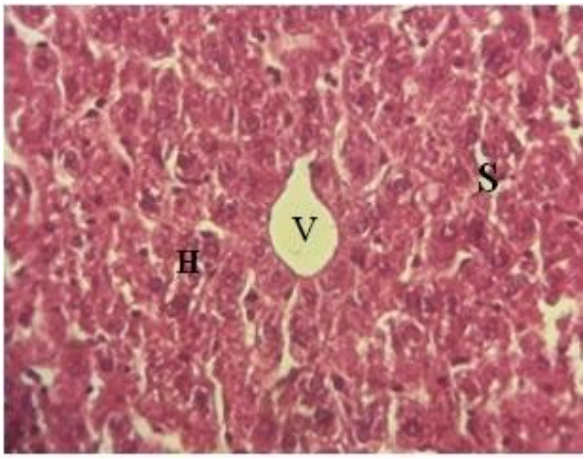


Fig (1): Histopathological section of control group shows central vein (V), hepatocytes(H) and sinusoide (S) (H&E stain 40X)

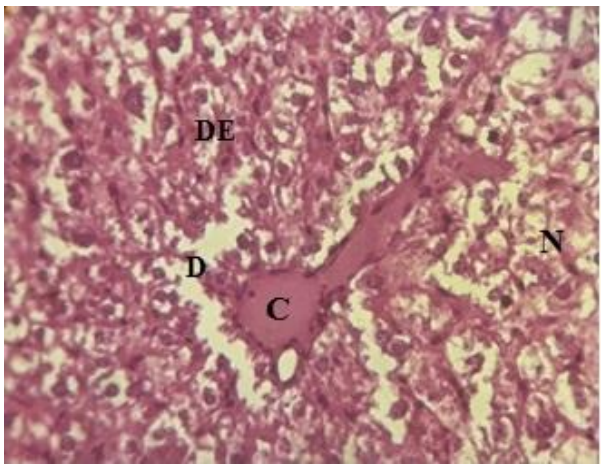


Fig (2): Section in liver of treated group with peroxide shows severe congestion in blood vessel (C), dilated sinusoids (D), degenerated liver cells (DE) and necrosis (N)

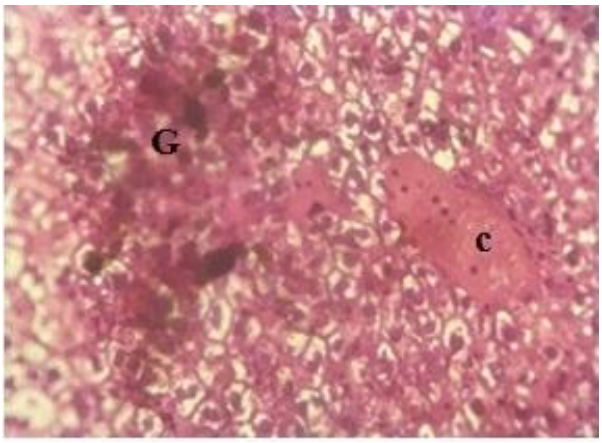
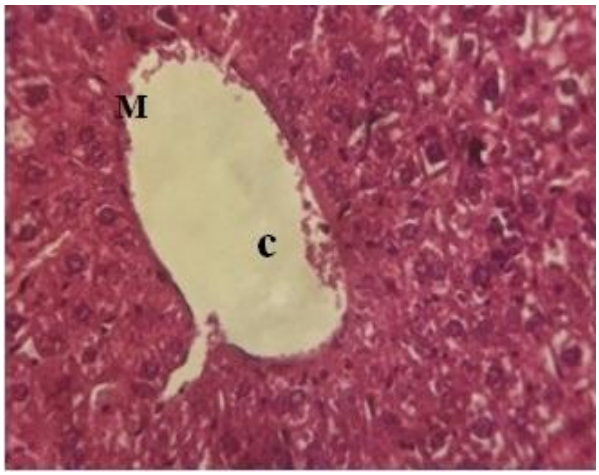
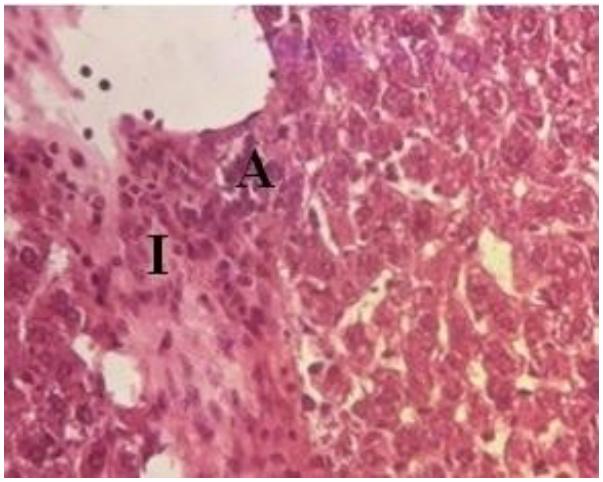


Fig (3): Section in liver of treated group with peroxide shows: Tendency to forming granuloma(G), severe congestion in blood vessel(C)



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)



Fig(5): Section in liver of treated group with peroxide and *Agaricusbisporus* Infiltration of inflammatory cells (I) with Aggregation of mononuclear cells in liver parenchyma(A)

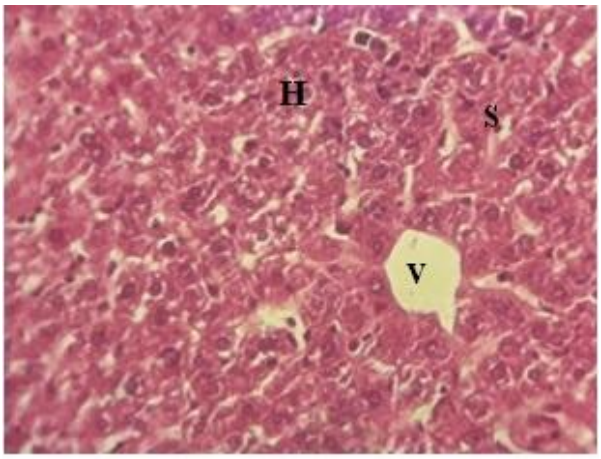


Fig (6): Section in liver of Treated group with agaricusbisporus showed central vein(V),hepatocytes(H) and sinusoids(S)

References

[1]. Reuter, S.; Gupta, S. C.; Chaturvedi, M. M.; Aggarwal, B. B.(2010) Free Radical,Biol. Med., 49, 1603.
[2]. Halliwell, B., and Gutteridge, J. M. C. (1999) Free Radicals in Biology and Medicine. Third edition, Clarendon Press, Oxford, UK.
[3]. Löffler G. and Petrides, P. E. PhysiologischeChemie. 4 ed., p. 288, Springer, Berlin 1988, ISBN 3-540-18163-6 (in German).
[4]. Chen, S.; Oh, S.R.; Phung, S.; Hur, G.; Ye, J.J.; Kwok, S.L.; Shrode, G.E. and Belury, M. (2006). Anti-aromatase activity of phytochemicals in white button mushrooms (Agaricusbisporus). Cancer Res., 66(24): 12026–34.
[5]. Muna G. A., John,M., Benson, M. and OgoYi, D.(2015) Anti-oxidant properties of cultivated edible mashroom in Kenya, American Journal of Biochemistry, 14(16): 1401-1408.
[6]. Largeteau, M.L. and Savoie, J.M.(2010) Microbially induced diseases of*Agaricusbisporus*:Biochemical mechanisms and impact oncommercial mushroom production.J. *Microbiol. Biotechnol.* 86(1): 63-73.
[7]. Chen and Schluesener,H.J. (2008)Nanosilver: a nanoproduct in medical application. ToxicolLett .176:1–12.
[8]. Luna,L.G. (1968).Manual of histologic staining methodsof the armed forcesintitute of pathology 3rd, New York,McGraw-Hill Boo Companypp:38-76 and 222-223.
[9]. Collins,A.R.;Oscoz,A.A. ; Brunborg,G.;Gaivao,I.; Giovannelli,L.; Kruszewski,M.Smith,C.C. and Stetinaa,R.(2008). The comet assay:topical issues.Mutag.17:1-9.
[10]. Basher, S. Z. (2003). Your guide to the statistical analysis SPSS, version 10. Arab Institute for Statistical and Training Research, Baghdad.
[11]. Ren, Z. ; Guo, Z. ; Meydani,S. N.; and Wu,D (2008)White Button Mushroom Enhances Maturation of Bone Marrow-Derived Dendritic Cells andTheir Antigen Presenting Function in Mice1,2 The Journal of Nutrition Nutritional Immunology J Nutr. 138(3):544-50.
[12]. Wallac.V. ;E.E;Malinin, N.L.; Goltsev,Y.V.; Kovalenko,A.V. and Boldin,M.P.(1999).Tumer necrosis factor receptor fas signaling mechanisms .Annu.Rev.Immunol.17:331-367.
[13]. Xiang, J.;wan, C;guo, R.andguo,D.(2016). Is hydrogen peroxide a suitable apoptosis inducer for all cell types,BioMed Research International
Volume 2016 (2016): 6.
[14]. Barro, L. ; FalcãS. ;Baptist,P ;Freir, C MiguelVilas-BoaIsabel and Ferreir,C.F.R. (2008).Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays Food Chemistry,111 (1): 61-66.

[15]. Swindle E.J., Metcalfe D.D.(2007). The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. Immunol Rev.;217:186-205.
[16]. Subramoniam, A. ; Pushpangadan, P. ; Rajasekharan, S., Evans, D.A;., Latha, P.G., and Valsaraj, R..(1999). Development of phytomedicines for liver diseases. Indian J. Pharmacol. 31: 166–175. Subramoniam, A., Pushpangadan, P., 1996. 33: 283–286.
[17]. Nitha, B.; Meera, C.R. and Janardhanan, K.K. (2007). Anti-inflammatory and anti-tumoractivities of cultured mycelium of morel mushroom. Morchellaesculenta. CurrentScie . 92 (2): 235-239.
[18]. Zhang, M.; Huang, J.; Xie, X. and Holman, C.D. (2009). Dietary intakes ofmushrooms and green tea combine to reduce the risk of breast cancer in Chinesewomen. International Journal of Cancer. 124 (6): 1404-1408.
[19]. Mostafa,E. and Braz,M. (2012). Chemical profile, agaritine and selenium content of *Agaricusbisporus*Arch. Biol. Technol.55:6.
[20]. Clark LC, Combs G F, Turnbull B W, et al., Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin, A randomized controlled trial, Nutritional Prevention of Cancer Study Group, JAMA, 1996; 276:1957-1963.
[21]. Spolara M R, Schafferb E M, Beelmana R B &Milnerb J A, Selenium-enriched Agaricusbisporus mushrooms suppress 7,12-dimethlybenz[a]anthracenebioactivation in mammary tissue, Journal of Chromatography, 2006; 1101: 94–102.

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