Original Article

Hepatoprotective And Antioxidant Activities Of The Edible Mushroom, (*Agaricus Bisporus*) On Carbon Tetrachloride-Induced Chronic Hepatitis In Rats

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DOI: 10.47750/pnr.2023.14.S01.36

Abstract

In this study the hepatoprotective and antioxidant activities of the edible mushroom (white button mushroom, *Agaricus bisporus*), as a part of diet, were investigated in Wistar rats. Various biometric and biochemical parameters were examined. Chronic hepatitis model was induced by intraperitoneal (ip) administration of CCl4. Twenty-eight adult male albino rats were utilized in this experiment arranged randomly in four groups, seven rats each, as follows: The first main group (n= 7) rats were fed on basal diet and served as a negative control group. The second main group (n=21) rats were intraperitoneally injected by CCL4 dissolved in olive oil (in a dose of 0.5 mg/Kg) twice weekly for four weeks. These rats were distributed into three subgroups as follows: The first subgroup: fed on basal diet and served as a positive control group. The other subgroups fed on basal diet supplemented with 7.5% and 15% dried mushroom, respectively. At the end of the experimental period (6 weeks), rats were sacrificed and blood sampled were withdrawn then centrifuged to obtain then clear serum which used for biochemical analysis. Results indicated that diet supplementation with mushroom significantly *(P<0.05)* increased superoxide dismutase (SOD) enzyme and decreased malondialdehyde (MDA) levels. Diet supplementation with mushroom significantly *(P<0.05)* improved the activity of AST, ALT, ALP and total bilirubin and increased the concentrations of albumin and total protein, as well as improvement daily feed intake (FI), body weight gain percentage (BWG %) and feed efficiency ratio (FER). The obtained results from this study revealed that mushroom is a good antioxidant and hepatoprotective agent against carbon tetrachloride-induced chronic hepatitis in rats, which would be exploited as a potentially natural dietetic agent for hepatic protection, and it can be recommended for food and health applications aiming at reduction prospect oxidative stress.

Keywords: *Agaricus bisporus*; Mushroom; Diet supplementation; CCl4; chronic hepatitis; Hepatoprotective

INTRODUCTION

Agaricus bisporus is known as the white button mushroom and is commonly referred to as a “mushroom”. It is one of the most economically important and widely cultivated mushrooms in Egypt [1].

A. bisporus is not only a valuable source of food but also exhibits medicinal values. The bioactive compounds with nutritional value in A. bisporus contribute to human health [2]. As known edible mushrooms have been consumed as food, functional foods or dietary supplements in many parts of the world, it could be viewed as source of useful bioactive metabolites for disease prevention and human health promotion [3]. Recently some researchers reported the protective action of A. bisporus mushroom on experimentally induced liver injuries in rats [4], [5] and [6]. Several studies exhibited the bioactivity of mushrooms extract; for example genus of Agaricus and its beneficial activities in many fields rather than nutrition as efficient biocontrol agents [7].

Nowadays the modern research refers to the efficiency of edible mushrooms in many clinical trials; they have been natural therapeutic bioactive components may are responsible for antioxidant activities in mushroom extracts [8]

and [9]. Some other researchers have also, confirmed the presence of antioxidants in A.bisporus and it exhibits comparatively higher antioxidant potential compared with other important edible mushrooms [10], [11] and [12]**.** Numerous health-promoting substances were isolated from fruiting bodies of this species as well as from its mycelium [13] and [14]. Major toxicological problems associated with several diseases have been centered around the effects on the liver [15]. Usually, liver cells are affected by hepatotoxic agents through the induction of oxidative damage [16]. The use of natural drugs for the treatment of liver diseases has increased all over the world. Developing therapeutically effective agents from mushrooms as a natural source may reduce the risk of toxicity when the drug is used clinically [17], [18] and [19].

The aim of the present study was to evaluate the antioxidant and hepatoprotective properties of the edible mushroom. White button mushroom, Agaricus bisporus that is cultivated in Egypt, as a part of rat's diet using model of CCl4-induced chronic hepatitis in Wistar adult male rats.

Materials and Methods

Dried mushroom preparation

Fresh mushroom (*Agaricus bisporus)* was obtained from the Agriculture Research Center, Giza, Egypt. The fungal materials of healthy apparatus fruiting bodies of *Agaricus bisporus* mushroom were brought to the laboratory in sterile bags. Samples were cut into small pieces and dried by solar energy, at the National Research Center, Giza, Egypt, then grinded to powder in a mortar and stored at 4-8 °C in a refrigerator.

Animals and biological experimental design

The basal diet was formulated according to AIN-93M diet [20]. Twenty-eight adult male rats were housed in well conditions in biological studies lab of Faculty of Home Economics, Helwan University. They were kept in aerated cages under hygienic conditions at room temperature (25 ± 3 °C) with a 12 h dark/light cycle and fed on basal diet for one week for adaptation. After week, rats were divided into 4 groups, seven rats each, as follows: The first main group (n= 7) rats were fed on basal diet and served as a negative control group (G1: Control -ve). The second main group (n=21) rats were subjected to intraperitoneal (IP) injection of CCl4 dissolved in olive oil (in a dose of 0.5 mg/Kg) twice weekly for two weeks. These rats were divided into three subgroups as follows: The first subgroup: fed on basal diet and served as a positive control group (G2: Control +ve). The second subgroup: fed on basal diet supplemented with 7.5% *A.bisporus* dried mushroom (G3: 7.5% *A.bisporus*).The third subgroup: fed on basal diet supplemented with 15% *A.bisporus* dried mushroom (G4*:*15% *A.bisporus*).

Biological Evaluation

Biological evaluations were carried out by determination of daily feed intake (FI) which was recorded every day throughout the experimental period. Body weight gain percent (BWG %) and feed efficiency ratio (FER) were determined according to [21].

Biochemical parameters estimation

At the end of the experimental period (6 weeks), the rats were fasted overnight before sacrificing; blood samples were collected from the hepatic portal vein then centrifuged 3000 r.p.m for 15 min. to obtain serum then stored at -20^{o}c until biochemical analysis.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Young [22], alkaline phosphatase (ALP) was determined according to. [23]. Serum total bilirubin concentration was determined [24]. Albumin and total protein and were determined according [25] and [26], respectively**.** Superoxide dismutase (SOD) and malondialdehyde (MDA) were determined according to the methods described by [27] and [28].

Statistical analysis

The results were expressed as mean ± standard error (SE). The statistical analysis was carried out by using SPSS, PC statistical software (Version 18.0 SPSS Inc., Chicago, USA) using the Dunk 'test multiple range post-hoc test. Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at $P < 0.05$ [29].

Results

Biometric parameters

The effect of diet supplementation with *A.bisporus* on daily feed intake (FI), body weight gain (BWG%) and feed efficiency ratio (FER) in rats with induced chronic hepatitis are shown in **Table (1).**The mean value of FI of negative control group was 26.00 (g/day/rat) while in positive control group decreased to reach 20.50 (g/day/rat). Rat groups suffering from hepatitis that fed diet supplemented with dried mushroom showed increase in FI values,

as compared to the positive control group. Tested groups which supplemented with 7.5 and 15% *A.bisporus* had mean values 22.00 and 24.50 g/day/rat, respectively.

The mean value of BWG% in the negative control group reached 50.14 %, whereas it decreased in the positive control group to reach 14.99%. Rat group suffering hepatitis that fed diet supplemented with 7.5% *A.bisporus* and rat group suffering hepatitis that fed diet supplemented with 15% *A. bisporus* showed significant higher (P<0.05) BWG% values than that of the positive control group reached 26.88 and 42.98 %, respectively.

Regarding FER, the mean value of FER in the positive control group showed significant decrease (P<0.05), as compared to all other experimental rat groups. The mean value of FER in the negative control group reached 0.087, whereas it decreased in the positive control group to reach 0.033. The FER values of rat group suffering hepatitis that fed diet supplemented with 7.5% *A.bisporus* and rat group suffering hepatitis that fed diet supplemented with 15% *A.bisporus* reached 0.055 and 0.079, respectively , showed significant higher (P<0.05) FER values than that of the positive control group. It was noticed that, there is no statistically significant difference (P>0.05) in the mean value of FER, for the rat group suffering hepatitis that fed diet supplemented with 15% *A.bisporus* , as compared to the negative control group.

Table (1): The effect of *A.bisporus* supplementation on FI, BWG% and FER in rats with induced chronic hepatitis.

Parameters / Groups	FI (g/day/rat)	BWG%	FER
G1: Control (-ve)	26.00	50.14±2.50a	0.087±0.037a
G2: Control (+ve)	20.50	14.99±0.68d	0.033±0.015c
G3: 7.5% *A.bisporus*	22.00	26.88±1.93c	0.055±0.038b
G4:15% A.*bisporus*	24.50	42.98±1.32b	0.079±0.024a

Values are expressed as means ± SE; Values at the same column with different letters are significantly different at P<0.05.

Biochemical parameters

Effect of supplementation with dried A. bisporus mushroom on blood serum enzymes and total bilirubin levels

The effect of supplementation with dried *A. bisporus* mushroom on blood serum enzymes is presented in Table (2) the serum levels of AST, ALT and ALP in the positive control group was significantly (P<0.05) increased compared to the negative control group. The levels of AST, ALT and ALP in the positive control group were recorded 158.27, 70.30 and 321.35 µ/L, respectively. Whereas these values decreased to reach 76.96, 55.17and 189 µ/L, respectively in the negative control group. In comparison with positive control groups, supplemented rats with dried *A. bisporus* mushroom exhibited a significant (P<0.05) reduction in AST, ALT and ALP levels which reached 121.25, 37.50 and 242.50 µ/L in rat group suffering hepatitis that fed supplemented with 7.5 % *A. bisporus.* The corresponding values in rat group suffering hepatitis that fed supplemented with 15 % *A. bisporus* were 95.62, 27.50 and 225.10 µ/L.

As regard total bilirubin level, results in Table (2) showed that, the level of total bilirubin in the negative control group reached 3.52 mg/dl, whereas it increased in the positive control group to reach 8.99 mg/dl. The total bilirubin in rats was significantly (P<0.05) decreased by the supplementation with dried *A. bisporus* mushroom, as compared to the positive control group. Rat group suffering hepatitis that fed supplemented with 15 % *A. bisporus* had a lower total bilirubin (4.26 mg/dl) than those supplemented with 7.5 % *A. bisporus* (6.01 mg/dl).

Table (2): The effect of *A. bisporus* supplementation on blood serum enzymes and total bilirubin levels in rats with induced chronic hepatitis.

Parameters / Groups	AST	ALT	ALP	T. Bilirubin
	(µ/L)			(mg/dl)
G1: Control (-ve)	76.96±2.22d	55.17±1.83b	189.60±2.44d	3.52±0.16d
G2: Control (+ve)	158.27±1.60a	70.30±1.44a	321.35±2.48a	8.99±0.10a
G3: 7.5% *A.bisporus*	121.25±4.00b	37.50±1.91c	242.50±2.24b	6.01±0.17b
G4:15% *A.bisporus*	95.62±2.30c	27.50±2.55d	225.10±1.97c	4.26±0.24c

Values are expressed as means ± SE; Values at the same column with different letters are significantly different at P<0.05.

Effect of supplementation with dried A. bisporus mushroom on albumin and total protein levels

The comparison between four experimental rat groups in albumin and total protein levels in blood serum is shown in Table (3). The values of albumin and total protein in positive control group were significantly (P<0.05) decreased, as compared to the negative control group.

Table (3): The effect of *A.bisporus* supplementation on total protein and albumin levels in rats with induced chronic hepatitis.

Parameters Groups	**Albumin(g/dl)**	**Total protein (g/dl)**
G1: Control (-ve)	7.46±0.26a	10.10±0.40a
G2: Control (+ve)	3.72±0.19d	4.72±0.21d
G3:7.5% *A.bisporus*	4.78±0.07c	6.44±0.25c
G4:15% *A.bisporus*	6.06±0.19b	7.88±0.31b

Values are expressed as means ± SE; Values at the same column with different letters are significantly different at P<0.05.

The levels of total protein and albumin in the negative control group reached 7.46 and 10.10 g/dL, respectively. The corresponding values in the positive control group were decreased to reach 3.72 and 4.72 g/dL.

Albumin content in rat group suffering hepatitis that fed supplemented with 7.5 % *A. bisporus* and rat group suffering hepatitis that fed supplemented with 15% *A. bisporus* increased to reach 4.78 and 6.06 g/dL, respectively. The corresponding values in total protein contents increased to reach 6.44 and 7.88 g/dL, as compared to the values of the positive control groups.

Effect of supplementation with dried A. bisporus mushroom on SOD and MDA

The data in Table (4) represent the changes in levels of SOD and MDA as oxidative stress parameters The negative control group showed a significant higher (P<0.05) SOD level reached 81.50 U/mg, whereas it increased in the positive control group to reach 48.65 U/mg. Rat groups suffering hepatitis that fed diet supplemented with *A.bisporus* dried mushroom showed a significant increase (P<0.05) in SOD level, as compared to the positive control group. Rat group suffering hepatitis that fed diet supplemented with 15% *A.bisporus* had a significant higher (P<0.05) SOD level (75.02 U/mg) than rat group suffering hepatitis that fed diet supplemented with 7.5% *A.bisporus* (62.10 U/mg).

Table (4): The effect of A.bisporus supplementation on superoxide dismutase (SOD) and malondialdehyde (MDA) levels in rats with induced chronic hepatitis.

Parameters Groups	**SOD (U/mg)**	**MDA (µmol/dL)**
G1: Control (-ve)	81.50±1.74a	40.97±1.59d
G2: Control (+ve)	48.65±1.40d	86.75±1.03a
G3:7.5% *A.bisporus*	62.10±1.46c	63.07±1.67b
G4:15% *A.bisporus*	75.02±1.93b	49.07±1.80c

Values are expressed as means ± SE; Values at the same column with different letters are significantly different at P<0.05.

With respect to the MDA formation, the MDA level in the positive control group was significantly increase (P<0.05) to reach 86.75 µmol/dL, as compared to the negative control group which reached 40.97 µmol/dL. Rat groups suffering hepatitis that fed diet supplemented with dried *A. bisporus* mushroom showed significant lower (P<0.05) levels of MDA than that of the positive control group. Rat group suffering hepatitis that fed diet supplemented with 15% *A.bisporus* had a significant lower (P<0.05) MDA level (49.07 µmol/dL) than rat group suffering hepatitis that fed diet supplemented with 7.5% *A.bisporus* (63.07 µmol/dL).

Discussion

In the current study the antioxidant and hepatoprotective activities of the dried mushroom, *Agaricus bisporus*, as a part of diet, were investigated in Wistar rats. The chronic hepatitis model was induced by intraperitoneal (IP) injection of CCl4. These effects were examined along with biometric and biochemical parameters.

Biometric parameters

The present study results showed statistically significant improvement (P<0.05) in all biometric parameters tested in the groups of rat suffering hepatitis that fed diet supplemented with *A.bisporus,* as compared to the positive control group. Supplementation with *A.bisporus* dried mushroom in diet for feeding rat suffering hepatitis has an enhancement of BWG % and FER , that may be a result of increasing FI , by rising the supplementation with *A.bisporus* to 15% in diet for feeding rats suffering hepatitis, that may be increase the bioavailability of the diet. Adding mushroom on rat's diet was bustle in the taste. Thus, its impact on the total weight as a positive effect. In agreement with these results recently, Shehtata *et al.*[30] who studied the effect of supplemented with dry uncooked; dry cooked mushroom and duple (aquatic and ethanolic) extract of *A.bisporus* mushroom on FI, BWG% and FER of CCL4- hepatotoxic rats. Feed intake was increased in the negative control group, compared to the positive control group. While treated groups were close to negative control group. Also, in this way, El-kholy *et al.*[31] who found that, feed intake and body weight gain were significantly increased in rats feed on ration mixed with dried mushroom (shiitake "*Lentinus-edodes*"), while the group injected CCL4 showed significantly decreased. The groups feed on dried mushroom with injection of CCl4 improved the body weight gain and feed intake and showed significantly increased when compared with group subcutaneous injection of CCl4. Handayani *et al.* [32] compared the dose response effects of oat and mushroom (Shiitake mushroom) enrichment diets on the amount of FI, BWG % and fat deposition of rats fed a high-fat diet (HFD). FI value did not differ among the groups of dietary intervention, while the rats fed with the mushroom enrichment diet had lower BWG % and total fat mass compared with the oat enrichment diet. On the other hand, data of the present study may be inconsistent with those of Sumy *et al.* [33] who found that no body mass variations were evidenced in Wistar albino rats supplemented with oyster mushroom (*Pleurotus florida*) extract, orally administered for 30 days, after paracetamol induced liver damage.

Biochemical parameters

In the present study, the experimental model of chronic hepatitis was induced in Wistar albino rats. Direct evidence of this chronic hepatitis was noted in the occurrence of alterations in various hepatic parameters. CCl4-induced chronic hepatitis was manifested by significant increase (P< 0.05) in the activities of marker enzymes (AST, ALT, and ALP) and total bilirubin, and by significant (P< 0.05) decrease in the albumin and total protein levels in serum, as well as by significant (P< 0.05) increase MDA levels and significant decreases in the activity of SOD, as the obtained results in the positive as compared to the negative control group. The hepatoprotective effects of *A. bisporus* were observed for the rat suffering hepatitis that fed diet supplemented with dried *A. bisporus* mushroom, as preventing the increased activities of marker enzymes (AST, ALT, and ALP) and total bilirubin, and as preventing the decreased albumin and total protein levels in serum, as well as reduced the formation of MDA and enhanced the activity of SOD.

As regard liver function biomarkers, our findings are in agree with those of several researchers, Huang *et al.* [34] reported that two kinds of polysaccharides (ABP-1 and ABP2) isolated from *A. bisporus* have been showed to significantly decrease the concentrations of the ALT and AST in serum in a dose dependent manner and reduce the hepatocellular degeneration and necrosis, as well as inflammatory infiltration. Liu *et al.*[35] reported that aqueous extract of *A.bisporus* at dose of 0.5 g/kg body weight daily , decreased the liver index , serum GGT, ALT , ALP and AST activities in the CCl4-treated by necrogenic dose (1.5 ml/kg body weight of 80% CCl4 in corn oil) in rats model. Ali *et al.*[36] concluded that the aqueous extract of *A. 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. Shehata *et al.* [30] reported that *A. bisporus* mushroom can play an important role as hepatoprotective agent using rats. Serum concentrations of AST, ALT, ALP and total bilirubin, were significantly increased in the positive control group compared with the negative control. Supplemented diet with dry uncooked, dry cooked and its extract reversed these changes that caused by CCl4 administration [6] suggested that *A. bisporus* is a good antioxidant and hepatoprotective agent against carbon tetrachloride-induced liver injury in rats, which would be exploited as a potentially natural nutraceutical for hepatic protection. *A. bisporus* exhibited dose dependent hepatoprotection indicated by almost normalized biomarkers, including enzymatic liver function parameters (AST, ALT, GGT and ALP) and nonenzymatic parameters, namely, total protein, albumin, globulins, total bilirubin, conjugated bilirubin and unconjugated bilirubin.

These results disagree with the finding o*f* [37] who found that aqueous extract of *A. bisporus* (9 g/kg BW) significantly increased plasma bilirubin concentrations compared to normal control**.** Also [38], found that *A. bisporus* aqueous extract (500 mg/kg) significantly, decreased serum total bilirubin which had been greatly increased by Zearalenon (ZEA; 2.4 mg/kg BW) which had hepatotoxic effect compared to control mice. While

the mean differences of serum total bilirubin between control group and animals treated with combination of ZEA and mushroom extract were non-significant.

On the other hand, the findings may be inconsistent with those of Chang *et al.* [39] who found that *Agaricus blazei* at high dose 2000 mg/kg produced slightly elevated serum AST and ALT levels in mice model treated by 40% CCl4/olive oil (1 ml/kg body weight per day, I.P. twice per week for 8 weeks) in comparison with the negative control group. Also [40] found that *Agaricus blazei* extract (2.5 g/kg BW) given for 21 days to albino mice has no significant differences in the levels of serum concentrations of total protein, albumin, and inhibited the increase of gamma-globulin with Ehrlich tumor that is associated with marked decrease in albumin (thus, is considered to be a protein of the negative acute phase) and other protein fractions. This discrepancy could be explained on the basis of methodological and environmental differences.

A. bisporus mushrooms, especially portabellas (brown *A. bisporus*), had higher antioxidant capacity relative to *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Grifola frondosa* [41], [42], [43], [44] determined the main phenolic compounds in ethanolic extract of *A. bisporus* like gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin and suggested that the ethanolic extract of this mushroom had potent antioxidant effect and could be explored as a novel natural antioxidant. Also, many edible mushrooms were reported to have in vitro and in vivo antioxidant properties due to the presence of various putative bioactive compounds [45]. [46] and [47] studied the main components of *Pleurotus ostreatus* mushroom extracts with hepatoprotective potential. They finally proposed that β-D-(1→3)-glucans and other carbohydrates, as well as phenolic compounds and bioactive peptides may exert liver protection in carbon tetrachloride-induced toxicity.

With respect to the oxidative stress parameters, MDA is the end product of the lipid peroxidation, while SOD and other antioxidants are involved in the elimination of free radicals. They are assumed to represent pro-oxidant and antioxidant factors, respectively in the cellular free radical metabolism. The balance of these two factors decides the net result of cellular and/or tissue oxidation/peroxidation state [48], [49], [50]. In the present study, activities of antioxidant enzyme (SOD) were significantly decreased, while the oxidative stress marker (MDA) level was found significantly higher in the rat group suffering chronic hepatitis. Supplementation with *Agaricus bisporus*, significantly ($P<0.05$) improved SOD level and decreasing MDA level. Our findings agree with those of [35] who found that *Agaricus bisporus* polysaccharide at dose (0.5 g/kg body weight daily) significantly decreased MDA contents in liver injured by CCl4 (1.5 ml/kg body weight of 80% CCl4) in mice model, while increased antioxidant capacities of hepatic GSH and SOD. Also, Shehata *et al.* [30] and Rizk *et al.* [6] reported that, the mechanism of the obtained hepatoprotection of *Agaricus bisporus* in carbon tetrachloride-induced hepatotoxicity in rats may be based on impeding the oxidative stress mediated, indicated by reduced MDA, and restored SOD, Catalase and GPx levels. Data of the present study go in the same trend with results of Al-Dbass *et al.*[51] who found that the production of MDA was increased in CCl4-treated rats (1.5 ml/kg body weight of 80% CCl4) unlike what was found in rats treated with *Agaricus blazei* Muril at dose of 0.5 g/kg body weight daily.

Conclusion

The obtained results from this study revealed that mushroom is a good antioxidant and hepatoprotective dietary agent against carbon tetrachloride-induced chronic hepatitis in Wister rats, which would be exploited as a potentially natural agent for hepatic protection. *Agaricus bisporus* mushroom can be recommended for food and health applications aiming at reduction of oxidative stress. Therefore, intake diet supplanted with mushroom may be beneficial for patients who suffer from chronic hepatitis

Financial support and sponsorship
No financial support

Conflicts of interest
There are no conflicts of interest.

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