Egypt. J. of Appl. Sci., 35 (11) 2020 *127-142*

EFFECT OF MUSHROOM FUNGUS FEEDING ON INDUCED HEPATOTOXICITY RATS

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Key Words: Mushroom *(Agaricus bisporus)*, Extract, Hepatotoxicity, Rats.

ABSTRACT

In last decades, Mushroom is used widely in feeding for its medicinal properties, many studies confirmed its role as antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects; they also protect against tumor development and inflammatory processes. The aim of the present study was to investigate the effect of mushroom *(Agaricus bisporus)* as a part of diet on hepatotoxic rats. Thirty adult male albino rats (Sprague-Dawley strain), weighing about (200±10g) were divided randomly into two main groups as follow: the first group (-ve control= 6 rats) was fed on basal diet. The second group (24 rats) were fed on basal diet and injected with CCl_4, using a five necrogenic dose to induce acute liver damage, then divided into 4 groups from group 2 to group 5. Group 2 (+ve control) fed on basal diet. Group 3 and 4 fed on basal diet supplemented with 20% of dry uncooked mushroom and 20% of dry cooked mushroom, respectively. Finally, group 5 fed on basal diet supplemented with10% of duple (aquatic and ethanolic) extract of mushroom. At the end of the experimental period (4 weeks), rats were scarified and serum was collected for biochemical analyses. Results indicated that serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and malondialdehyide (MDA) level was significantly increased ($P< 0.05$) in the positive control group compared with the negative control and significantly ($P<0.05$) decreased in serum of Reduced Glutathione (GSH), superoxide dismutase (SOD) and catalyzes (CAT). It also indicated that supplemented diet with dry uncooked, dry cooked and its extract reversed these changes that caused by CCl_4 administration. Histological examinations of studied rat's livers sustained biochemical and enzymatic results. It could be recommended that Mushroom is worthy treating on Hepatotoxicity.

INTRODUCTION

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites **(Ozougwu and Eyo, 2014)**. A total loss of liver function could leads to death within minutes, demonstrating the liver's great importance **(Ozougwu, 2014)**.

The liver is a reddish-brown wedge-shaped organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg, and has a width of about 15 cm .It is both the heaviest internal organ and the largest gland in the human body **(Cotran *et al.*, 2005)**.

The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion. It also plays a role in metabolism, regulation of glycogen storage, decomposition of red blood cells and hormone production. The liver is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids **(Tortora *et al.*, 2008)**.

Treatment options for common liver diseases are limited, and therapy with modern medicine may lack in efficacy. So, there is a need for effective therapeutic agents with a low incidence of side effects. The natural antioxidants, more recently, have attracted considerable attention of users and researchers largely on account of adverse toxicological reports on some synthetic antioxidants and growing awareness among consumers **(Ramalakshmi *et al.*, 2007)**.

In fact, mushrooms may have diversity of chemicals ranging from bitter compounds that stimulate digestive system, phenolic compounds for antioxidant and many other pharmacological properties, including antibacterial and antifungal, tannins that work as natural antibiotics, diuretic substances, and alkaloids. The usage of natural drugs for the treatment of liver diseases has increased all over the world. Developing therapeutically effective agents from mushrooms as natural source may reduce the risk of toxicity when the drug is used clinically **(Girish and Pradhan, 2008; Chang and Wasser, 2012 and Finimundy *et al.*, 2013)**.

Mushrooms have been considered as ingredient of gourmet cuisine across the globe; especially for their unique favor and have been valued by humankind as a culinary wonder. More than 2,000 species of mushrooms exist in nature, but around 25 are widely accepted as food and few are commercially cultivated. Mushrooms are considered as a delicacy with high nutritional and functional value, and they are also accepted as nutraceutical foods; they are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance **(Chang and Miles, 2008 and Ergo¨nu¨l *et al.*, 2013)**. However, there is not an easy distinction between edible and medical mushrooms because many of the common

edible species have therapeutic properties and several used for medical purposes are also edible **(Guillamon, 2010)**.

The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus spp*. and *Flammulina velutipes*. Mushrooms production continuously increases, China being the biggest producer around the world **(Aida *et al.*, 2009 and Patel and Goyal, 2012)**. Mushrooms could be an alternative source of primary and secondary metabolites that necessary for human nutrition as vitamins, peptides and proteins **(Alves *et al.*, 2012)**. Mushrooms have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fibers, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) **(Mattila *et al.*, 2001 and Heleno *et al.*, 2010)**. Thus, they could be an excellent source of many different nutraceuticals and might be used directly in human diet and to promote health for the synergistic effects of all the bioactive compounds present **(Ferreira *et al.*, 2010 and Vaz *et al.*, 2010)**.

MATERIALS AND METHODS

Materials:

Mushroom *(Agaricus bisporus)* was obtained from Ministry of agricultural in giza. Carbon tetrachloride CCl_4 was obtained from Sigma-Aldrich, Germany. The contents of the basal diet; casein, all vitamins, minerals, cellulose, choline and starch were obtained from El-Gomhoria Company, Cairo, Egypt. Kits for biochemical analysis required for estimating parameters used in this study were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt. Experimental animals: Adult male Sprague-Dawley rats (n =30) which weighing (200+10g) were purchased from Farm of experimental animals in Giza, Egypt.

Methods:

1. Preparation of mushroom samples

The fungal materials (15) kg of healthy apparatus fruiting bodies of *Agaricus bisporus* mushroom) were brought to the laboratory in sterile bags and processed within a few hours after. The fungal materials were rinsed gently in running water to remove dust and debris. After proper washing, stems and fruiting bodies samples were cut into small pieces and dried with air. The dry mushroom, then divided to three parts: the first was dry, the second was dry and cooked and the later was extracted with aqueous and ethyl alcohol.

2. Induction of Hepatotoxicity

Hepatotoxicity was induced using the method describe by **Pawa and Ali ,(2004)**, were employed carbon tetrachloride (CCl_4) to induce hepatic toxicity in rats, using a five necrogenic dose (1.5 ml/kg body weight of 80%

CCl_4 in corn oil) which was equivalent to 1/5 of the oral LD_{50} in mice **(Abou Gabal *et al.*, 2007)** for one week After this period, a blood samples were taken from injected rats for measuring serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) , alkaline phosphatase (ALP) and total bilirubin concentration to be sure that all rats have been suffering from hepatotoxicity.

2. Preparation of Basal Diet and Experimental Animal Design:

The basal diet was formulated according to AIN-93M diet **(Reeves *et al.*, 1993)**.

Animals (30 rats) were divided into five groups and housed in well aerated cages under hygienic conditions of humidity, temperature (20-25°C) and light (12-h light: 12-h dark cycle) and fed on basal diet for one week before starting the experiment for acclimatization in animal biological studies Lab of RCMB - Al Azhar University. They were left for seven days as adaptation period and they were allowed to feed standard laboratory food and water.

The first main group (G1, 6 rats) was fed on the basal diet during the experimental period as a negative control group. The rest of the animals (n=24) were induce hepatic toxicity in them and divided into 4 groups (G2 to G5, 6 rats in each) as follows: Group (2): six rats with hepatotoxicity were fed on basal diet only as positive control group, Group (3): six rats with hepatotoxicity were fed on basal diet supplemented with 20% of dry uncooked mushroom, Group (4): six rats with hepatotoxicity were fed on basal diet supplemented with 20% of dry cooked mushroom and Group (5): six rats with hepatotoxicity were fed on basal diet supplemented with 10% of duple (aquatic and ethanolic) extract of mushroom.

At the end of the experimental period (4weeks), rats were sacrificed after overnight fasting. Blood samples were immediately collected in clean and dry tubes from the portal vein and left to clot at room temperature. Blood samples were centrifuged at 3000 rpm for 15 minutes to separate serum. Serum was carefully separated into dry clean tubes and allowed to be frozen at -20 c until the determination of the tested parameters.

3. Biological Parameters: feed intake (FI)**,** body weight gain % (BWG%) and feed efficiency ratio (FER) were measured using the following equations as described by **Chapman *et al.*, (1959)**; **BWG %** = (Final body weight – Initial body weight) / Initial body weight X 100

FER = Body weight Gain (g)/ Food consumed (g).

Chemical analysis of serum:

Serum aspartate aminotransferase (AST) was measured using Spectrophotometer at 505 nm according to themethod described by **Young, (2001)**, and Serum alkaline phosphatase (ALP) was determined according to **Roy, (1970)**. Serum total bilirubin concentration was determined according to **Young, (2001)**. Serum total cholesterol was determined according to the

method described by **Allain *et al.*, (1974)**. Serum cholesterol was determined according to the method described by **Fossati and principel, (1982)**. Serum HDL-C was determined according to **Albers *et al.*, (1983)**. Concentration of VLDL-c and LDL-c were estimated according to the method described by **Friedewald *et al.*, (1972)**.

Determination of Oxidative stress and Antioxidant Biomarkers:

The animals were sacrificed at the end of treatment and the liver was perfused and excised. The liver portion was rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation (MDA). A part of homogenate after precipitating proteins with Trichloroacetic acid was used for estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD, and CAT activity.

Reduced Glutathione (GSH) was determined according to **Ellman, (1959)**; Super Oxide dismutase (SOD) was determined according to **Kakkar *et al.*, (1984)**; catalase (CAT) was determined according to **Aebi, (1974)** and malondialdehyde (MDA) determined according to **Mansour *et al.*,(2016)**.

Histopathological examination:-

The liver of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness, stained with heamtoxylin and eosin stain for histopathological examination as described by **Carleton, (1979)**, then observed under a light microscope (Olympus, Japan).

Statistical analysis:

Results of biochemical analysis and biological evaluation of each group were statistically analyzed as mean ± SD using one way ANOVA as described by **SAS, (2006)**.

RESULTS AND DISCUSSION

Among the antioxidant compounds in mushrooms, both polysaccharides and phenolic compounds have attracted much attention. In many studies comparing antioxidant activities in alcoholic extracts from different mushrooms, positive correlations were found with the total phenolic content **(Savoie *et al.*, 2008; Jiang *et al.*, 2010 and Liu and Wang, 2012)**.

The detoxification effects of mushroom and mushroom extract on CCl_4 induced hepatic injury in rats are shown through the following results. The results on **Table (1)** showed the effect of supplemented with dry uncooked mushroom; dry cooked mushroom and duple (aquatic and

ethanolic) extract of mushroom on feed intake (FI), body weight gain % (BWG%) and feed efficiency ratio (FER) of 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.

Table (1): Effect of Mushroom and Mushroom Extract on Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) of Hepatotoxic Rats:

Parameters / Groups	Biological evaluations		
	FI(g/d)	BWG %	FER
G1:-ve control	17.8	12.32±1.64[a]	0.68±0.09[a]
G2:+ve control	14.70	2.08±0.62[d]	0.14±0.04[d]
G3:20%uncooked mushroom	18.9	5.31±0.50[c]	0.27±0.03[c]
G4:20% cooked mushroom	18.6	10.03±1.19[b]	0.054±0.10[b]
G5: 10% of duple extract of mushroom	18.2	13.86±1.39[a]	0.75±0.07[a]

***Mean values are expressed as means ± SD.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

Adding mushroom on rat's diet was bustle in the taste. Thus, its impact on the total weight as a positive effect and these results were striking with previous studies on other materials and extracts **(Nwanjo and Orjiako, 2006; Chen *et al.*, 2008 and Wasser, 2011)**.

As seen in **Table (2)** serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin of hepatotoxic rats were significantly increased (P<0.05) elevated by CCl_4 administration (positive control group) compared with negative. It was observed significant (P<0.05) reduce in serum ALT,AST,ALP and total bilirubin levels for all treated groups with mushroom and the duple (aquatic and ethanolic) extract of mushroom compared to the positive control group. The highest improvement for liver functions was observed at the group that fed on 10% of duple (aquatic and ethanolic) extract of mushroom.

The hepatoprotective effects of mushroom (*Macrocybe gigantea*) ethanolic extract on CCl_4 induced hepatic injury in mice are recorded by **Acharya *et al.*, (2012)**. The CCl_4 receiving group as expected, revealed significantly higher increase in liver function indices such as SGPT, SGOT and ALP (P<0.05) compared to the normal group. Treatment with ethanolic extract significantly (P<0.05) lowered the activities of serum marker enzymes, bilirubin, comparable to standard drug silymarin and towards normalization. Serum transaminases (SGPT and SGOT) were inhibited by 55.02% and 62.59% respectively compared with the control group animals whereas the extract showed inhibition of 39.26% in ALP level with respect to the control set. The increased activity of serum enzymes may explain cell membrane break down and death **(Kaplowitz *et al.*, 1986)**. CCl_4 intoxication

even produced a significant (P<0.05) rise in serum bilirubin thereby indicating hepatic damage **(Plaa and Hewitt, 1982)**.

Table (2): Effect of Mushroom and its Extract on serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin of hepatotoxic rats.

Parameters / Groups	Liver enzymes			
	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (Mg/dl)
G1:-ve control	81.16±2.85^{e}	34.17±2.31^{b}	80.84±0.34^{d}	0.19±0.04^{c}
G2:+ve control	450.83±8.6^{a}	145.17±7.75^{a}	140.66±0.21^{a}	0.53±0.08^{a}
G3:20%uncooked mushroom	220.66±5.89^{b}	38.50±1.87^{b}	111.29±0.47^{b}	0.31±0.10^{b}
G4:20% cooked mushroom	173.6±8.91^{c}	33.67±2.06^{b}	107.97±0.50^{bc}	0.22±0.05^{c}
G5: 10% of duple extract of mushroom	161.33 ±5.60^{d}	19.42±2.24^{c}	104.35±0.25^{c}	0.19±0.05^{c}

***Mean values are expressed as means ± SD.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

The enhancement of results on liver functions may be due to that mushroom contains Phenol, flavonoid, ascorbic acid and carotenoids concentrations contained in the five *Agaricus sp.* mushroom extracts. Phenols were the major antioxidant components found in the Agaricus extracts **(Andrera *et al*., 2009)**. Ergosterol, a phenolic compound extracted from white button mushroom *(Agaricus bisporus*) showed inhibitory effect on cancer cell line *in vitr*o by aromatase inhibition without side effect **(Lillian *et al*., 2002)**.

The hepatoprotctive property of mushroom extract may also be because it other properties like anti-inflammatory. **(Chang and Schiano, 2007; Barros *et al*., 2008 and Maares and Haase, 2016)** reported that phenolic and antioxidant properties of *(Agaricus bisporus*) caused the substantial attenuation of cell inflammation.

Data in **Table (3)** also revealed that; TC, TG, LDL-C and VLDL-C were significantly (P< 0.05) increased in the positive control group compared with the negative control group. Results showed that all groups that were treated with mushroom significantly decreased (P< 0.05) in serum TC, TG and VLDL-C compared to the positive control group.

Serum LDL-C level, results showed that all groups treated with mushroom significantly decreased (P< 0.05) compared to the positive control group. Rats were fed on 10% of duple (aquatic and ethanolic) extract of Mushroom had the best result for reducing serum LDL-C level. Regarding serum HDL-C level, results demonstrated a significant (P< 0.05) decrease in serum HDL-C level of the positive control group compared to the negative control group. Also, it was observed that all treated groups with

mushroom significantly increased (P< 0.05) compared to the positive control group. Rats with fed on Rats were fed on 10% of duple (aquatic and ethanolic) extract of mushroom considered the best result for increasing serum HDL-C level. The highest improvement for lipid profile was observed at the group that fed on 10 % of duple (aquatic and ethanolic) extract of mushroom. Previous studies highlights on the Flavonoids and polyphenols as active metabolites in mushroom and playing important role as antioxidant and antilipidemic agents **(Shieh *et al.*, 2001; Wang *et al.*, 2002; Lakshmi *et al .*, 2006 Hu *et al.*, 2006; Du *et al.*, 2015 and Alshammari *et al.*, 2017)**.

Table (3): Effect of mushroom and its extract on serum concentrations of total cholesterol (TC), triglyceride (TG), high density Lipoprotein cholesterol (HDL-C), low density Lipoprotein cholesterol (LDL-C) and Very Low density Lipoprotein cholesterol (VLDL-C) of Hepatotoxic Rats.

Parameters / Groups	Lipid Profile				
	TC	TG	HDL-C	LDL-C	VLDL-C
	mg/dl				
G1: -ve control	85.40±1.89^{a}	62.05±3.41^{a}	60.00±2.55^{a}	20.07±1.40^{b}	10.83±1.26^{b}
G2: +ve control	92.67±2.61^{b}	97.95±2.94^{d}	43.60±3.72^{c}	23.62±3.21^{a}	21.66±1.26^{b}
G3:20%uncooked mushroom	78.33±3.47^{d}	90.00±3.47^{b}	49.33±1.40^{d}	10.65±0.89^{d}	17.86±1.56^{a}
G4:20% cooked mushroom	81.80±2.28^{c}	92.97±2.59^{a}	48.78±1.98^{d}	17.52±2.004^{c}	18.72±1.56 ^{a}
G5: 10% of duple extract of mushroom	73.55±1.69^{d}	70.53±3.63^{c}	56.55±2.09^{b}	8.58±1.10^{d}	12.10±1.58^{b}

***Mean values are expressed as means ± SD.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

Results **Table (4)** also, showed that serum GSH, SOD, and CAT was significantly decreased (P< 0.05) in the positive control group compared with the negative control group. It was clear that, there was significant (P< 0.05) increase in serum GSH, SOD and CAT for all treated groups with mushroom compared to the positive control group. Rats were fed on 10% of duple (aquatic and ethanolic) extract of mushroom considered the best group for increasing serum GSH, SOD and CAT. Results also, showed that serum MDA level was significantly increased (P< 0.05) in the positive control group compared with the negative control group. Moreover, all treated groups with mushroom were significantly (P< 0.05) decreased in serum MDA level compared with the positive control group. The best group that reduced serum MDA level was the group that treated with 10% of duple (aquatic and ethanolic) extract of mushroom.

Table (4): Effect of Mushroom and its Extract on Reduced Glutathione (GSH), superoxide dismutase (SOD), catalyzes (CAT) and malondialdehyide (MDA) of Hepatotoxic Rats:

Parameters / Groups	Oxidative enzymes			
	Catalase	SOD	GSH	MDA
	(U/mg)			(µmol/dL)
G1:-ve control	6.07±0.29[a]	15.75±0.7[a]	38.32±1.20[a]	10.49±0.23[d]
G2:+ve control	3.09±0.68[c]	8.66±0.62[c]	17.01±0.79[d]	22.18±0.44[a]
G3:20%uncooked mushroom	4.67±0.27[b]	13.64±0.8[b]	33.78±1.1[c]	19.41±0.19[b]
G4:20% cooked mushroom	4.38±0.44[b]	15.56±0.32[a]	34.4 ±1.42[c]	17.02±0.31[c]
G5: 10% of duple extract of mushroom	4.80±0.31[b]	15.94±0.61[a]	35.90 ±0.71[b]	19.52±0.24[b]

***Mean values are expressed as means ± SD.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

The primary function of antioxidant compounds in fungi is to prevent cell damage induced by ROS. Under normal conditions, ROS are cleared from the cell by action of superoxide dismutase (SOD). SOD catalyzes the conversion of O_2 to H_2O_2 which is then decomposed in the presence of catalase (CAT) into water and oxygen. In addition, glutathione (GSH) and glutathione-related enzymes also play an important role against ROS, which are reduced by GSH in the presence of glutathione peroxidase (GPx) and GSH is regenerated by glutathione reductase (GR). The oxidative stress can be removed by the induction of these antioxidant enzymes. In a few *in vivo* studies, generally an aqueous extract of *A. subrufescens* was administered orally to rats or mice. During aging of rats, **de Sa-Nakanski *et al.*, (2014)** showed that *A. subrufescens* was protective mainly to the brain against the oxidative stress by increasing activity of antioxidant enzymes such as SOD and CAT. An improvement in the functionality of mitochondria from brain as evidenced by an increase in the activity of respiratory chain enzymes was also observed. Polysaccharides appear to be involved in this activity of mushrooms related to the antioxidant enzymes, as has been shown for some fungi such as *L. edodes, Ganoderma sp, Auricularia sp, Grifola frondosa, Hericium erinaceus* and *Pleurotus abalones* **(Huang and Nie, 2015)**. Many other edible mushrooms were reported to have *in vitro* and *in vivo* antioxidant properties due to the presence of various putative bioactive compounds such as polysaccharides, vitamins, carotenoids, micronutrients, and polyphenols **(Kozarski *et al.*, 2015)**. SOD, CAT, and GSH-dependent and recycling enzymes are also present in mushroom cells and they contribute to their antioxidant and detoxicant defences.

The histopathological studies was performed to provide direct evidence of the possibility of the mushroom being able to minimize disruption of structure of hepatocytes and accelerates hepatic regeneration thus decreasing the leakage of SGPT, SGOT and ALP into the circulation.

Histopathological examination:-

Histopathological analysis of Rats liver sections using H&E staining (×200) are showed in **photo (1);** A: Section from a liver of negative control rat (fed on basal diet) revealed that no marked histopathological changes.,B: The liver section of positive control rats (fed on basal die and injected with CCl_4) showed that dilatation of hepatic sinusoids and proliferation of oval cells are considered indication of hepatic carcinoma. ,C: Liver Section of rats fed on 20% of dry uncooked mushroom showed a moderate improvement in histological structure of liver and few vacuolar degeneration of hepatocyte ,D: Liver Section of rats fed on 20% of dry cooked mushroom indicated a moderate improvement in histological structure of liver with mild dilatation sinusoids of capillaries and mild proliferation fiber connective tissue in portal area and E: Liver tissue sections prepared from rats fed on 10% of duple (aquatic and ethanolic) extract of mushroom marked improvement in histological structure of liver and nearly disappear of oval cells administration groups exhibited less cavitation and necrosis compared to these shown in B. group.

Results of histopathological examinations of liver were supported by **Wu *et al.*, (2011)** who indicated that CCl_4-treated rats caused a severe hepatocellular degeneration, necrosis, and congestion of the sinusoids, along with periportal mononuclear cell infiltration due to toxicity.

In the other hand, **Standish *et al.*, (2006)**, reported that mice in the negative control group exhibited normal, well-defined histological structures, without any signs of vascular or inflammatory changes; no cavitations, necrosis or fibrosis were found in normal control sections. The histopathological analysis of the liver revealed signs of toxicity after administration of CCl_4. This toxicity was significant in comparison with the negative group and included cavitations, fibrosis in broad areas, mild vascular congestion and moderate inflammatory changes with congested sinusoids, nuclear changes, and centrilobular necrosis. The broad cavitations and fibrosis in livers were somewhat attenuated in mice treated with low or high doses of *Agaricus blazei Murrill (ABM*) during the experimental periods. *ABM* administration for mice did result in fewer cavitations and less fibrosis in the liver. ABM treatment also elevated the survival rate of mice after liver injury induced by CCl_4. *ABM* reduced apparent liver injury caused by CCl_4 for mice by histopathological assessment in a dose-dependent manner.

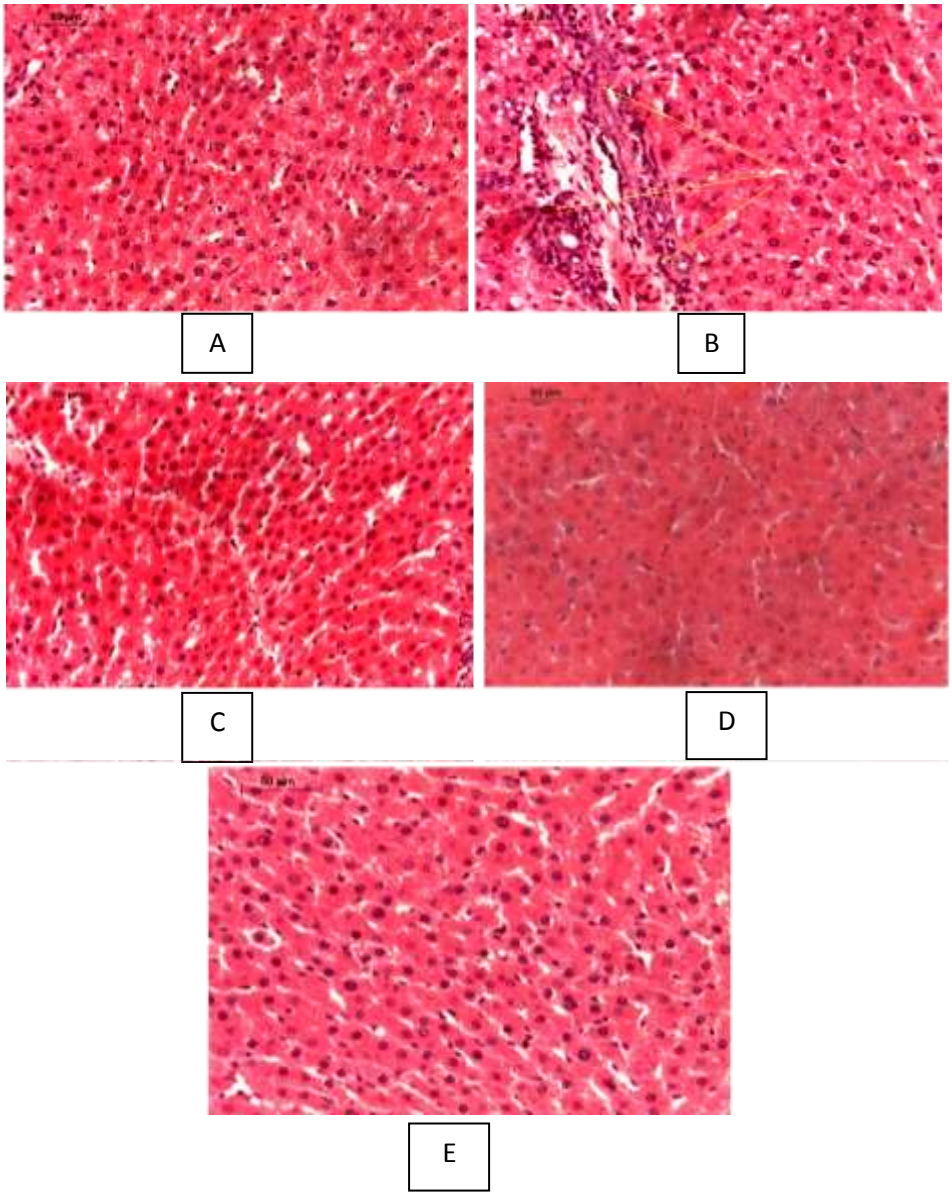

Photo (1): A,B,C,D and E *Histopathological analysis of Rats liver sections using H&E staining (×200). A: Negative control rat (fed on basal diet).,B: Positive control rats (fed on basal die and injected with CCl_4) ,C: Uncooked mushroom ,D: Cooked mushroom and E: Duple (aquatic and ethanolic) extract of mushroom.*

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تأثير التغذية بفطر عيش الغراب علي الفئران المصابة بالتسمم الكبدي

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في العقود الأخيرة، تم استخدام فطر عيش الغراب علي نطاق واسع في التغذية نظرا لخصائصه الطبية الواسعة. أكدت العديد من الدراسات الخواص المميزة له كمضاد للإلتهابات ، خافض لسكر الدم ، مضاد للبكتيريا ومضاد لنشاط الأورام. تمحورت الدراسة فى هذا البحث حول معرفة تأثير فطر عيش الغراب من نوع *الأجاركس بيسبورس* علي مرضى التسمم الكبدى في الفئران. أجريت الدراسة علي ثلاثون فأرا من نوع الألبينو، تتراوح أوزانهم من (200 ± 10 جم) حيث تم تقسيمهم إلي مجموعتين أساسيتين : المجموعة الأولي (6 فئران G1) تم تغذيتهم علي الغذاء الأساسي طوال فترة التجربة وتمثل المجموعة الضابطة السالبة . المجموعة الثانية (24فأرا) تم تغذيتهم علي الغذاء الأساسي و حقنهم تحت الجلد بمادة رابع كلوريد الكربون (CCl_4) فى صورة خمس جرعات متساوية (1.5 مل / كغم من وزن الجسم من 80٪ من (CCl_4) في زيت الذرة) وهو ما يعادل واحد من خمسة أجزاء من الجرعة النصف مهلكة (LD_{50}) للفئران وهى الجرعة الكافية لإحداث سمية الكبد بهم. ثم تقسم المجموعة الثانية إلي أربع مجموعات متساوية فرعية متساوية (G2– G5) شملت كل منها 6 فئران مصابة بالتسمم الكبدى كالتالى : المجموعة الفرعية (2)، تم تغذيتهم علي الغذاء الأساسي فقط وتعتبر مجموعة ضابطة موجبة. المجموعة الفرعية (3)، تم تغذيتهم علي الغذاء الأساسي المحتوي علي 20٪ من فطر عيش الغراب الجاف غير المطبوخ. المجموعة الفرعية (4)، تم تغذيتهم علي الغذاء الأساسي المحتوي علي 20٪ من الفطر المطبوخ الجاف لكل كجم من الغذاء الأساسي. المجموعة الفرعية (5)، تم تغذيتهم علي الغذاء الأساسي المحتوي علي 10٪ من مستخلص ثنائي (ماء ـ كحول) من الفطر. استمرت التجربة لمدة أربعة أسابيع، وفي نهاية فترة التجربة، تم تشريح الفئران والحصول علي دماءها لإجراء التحاليل البيوكيميائية وإجراء الفحوص التشريحية. ونظرا لما يحتويه فطر المشروم من مركبات لها نشاط مضاد للأكسدة، فقد لعبت هذه المواد دورا هاما كمضادات للتسمم الكبدى فى الفئران المريضة التى اشتملت تغذيتها على الفطر فى صوره المختلفة، حيث أظهرت نتائج تحليل مستويات انزيمات الكبد والصفراء الكلية فى دماء الفئران وكذلك الإنزيم المؤكسد من نوع MDA فى النسيج الكبدى للفئران كانت مرتفعة بشكل ملحوظ في المجموعة الضابطة الموجبة مقارنة بالمجموعة الضابطة السالبة، علاوة علي ذلك حدوث انخفاض ملحوظ في مستوي الإنزيمات المؤكسدة من نوع SOD – GSH وكذلك CAT. كما أظهرت النتائج بقوة أن إضافة فطر عيش الغراب ومستخلصه إلى غذاء الفئران المريضة عكس هذه التغييرات التي أحدثها رابع كلوريد الكربون. كما أن التغذية بفطر عيش الغراب خففت من هذه الآثار الضارة لرابع كلوريد الكربون على الأنسجة الكبدية للفئران المصابة فى الدراسات التشريحية. وبالتالي يمكن التوصية بأن فطر عيش الغراب اكتسب أهمية تغذوية وعلاجية واضحة ويمكن اضافته بصور متنوعة لغذاء المرضى المصابون بالتسمم والتليف الكبدى.

الكلمات المفتاحية : فطر عيش الغراب *Agaricus bisporus* ، التسمم الكبدي ، الفئران.