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Research Article

ANTIHEPATOTOXIC EFFICACY OF MUSHROOM, *AGARICUS BLAZEI* MURRILL AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN MALE ALBINO WISTAR RATS

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ABSTRACT

Mushrooms have a long history of use in folk medicine, and higher Basidiomycetes have become matters of great interest, due to their many-fold nutritional, medicinal, and pharmacological properties. Mushroom extracts are widely sold as nutritional supplements and touted as beneficial for health. *Agaricus blazei* is widely used for nonprescript, medicinal purposes, both as an edible mushroom and in the form of extracts. According to tradition, it helps against a variety of diseases, including cancer, diabetes, arteriosclerosis and hepatitis. The present study was undertaken to scientifically prove the traditional use of the mushroom against liver disorders. The therapeutic potential of *A. blazei* on liver damage was evaluated by carbon tetrachloride induced hepatotoxicity in rats. Male albino wistar rats were orally treated with *A. blazei* (250 and 500 mg/kg body weight) or silymarin (25 mg/kg) daily with administration of carbon tetrachloride (1 ml/kg body weight- ip) only for seven days. Carbon tetrachloride induced liver damage and significantly increased the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and cholesterol whereas protein level was decreased as compared with control. Treatment with *A. blazei* or silymarin consecutively for twenty eight days could significantly decrease the elevated activities of AST, ALT, ALP, bilirubin and cholesterol where as enhance the level of protein in serum when compared with carbon tetrachloride alone treated rats.

Key words: *Agaricus blazei*, Carbon tetrachloride, Liver marker enzymes, Cholesterol.

INTRODUCTION

Liver diseases have become one of the major causes of morbidity and mortality all over the globe (Dinakar *et al.*, 2010). Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine (Girish *et al.*, 2009). Several studies were conducted in the field of drug discovery and development but due to the side effects of modern medicine, natural remedies are considered to be effective and safe alternate treatments for hepatotoxicity (Madukiran *et al.*, 2012).

Carbon tetrachloride is one of the most widely used models to study hepatic damage which lead to progressive hepatic fibrosis and finally to cirrhosis (Natarajan *et al.*, 2006). Fibrosis can be considered as an excessive accumulation of connective tissue in parenchymal organs. In the liver, fibrosis represents a very frequent event which follows chronic insult of sufficient intensity to trigger a "wound healing"-like reaction (Poli, 2000). Activated portal fibroblasts, myofibroblasts of bone marrow origin, and particularly hepatic stellate cells (HSCs), have been identified as major collagen-producing cells in the injured liver, playing a role in fibrogenesis (Bataller and Brenner, 2005). These cells are activated by fibrogenic cytokines such as TGF-beta1, angiotensin II, and leptin. After liver injury, HSCs become activated, converting themselves into a myofibroblast-like cells (Moreira, 2007).

A. blazei Murill is a well known edible mushroom and considered as one of the most favorite culinary medicinal mushroom. The fungus has been used as nutritional therapies for many common diseases such

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asatherosclerosis, hepatitis, hyperlipidemia, diabetes, dermatitis, and cancer (Firenzuoli *et al.,* 2008). Previous studies have demonstrated the immune-stimulant and anticancer effects of *A. blazei* fruit body (Ellertsen *et al.*. 2006), and showed its antioxidant function (Izawa and Inoue 2004; Watanabe *et al.* 2008; Soares *et al.* 2009), anti-inflammatory and anti-diabetic activities (Kim *et al.*, 2005; Padilha *et al.*, 2009). Recently, its antitumor activities and the inhibitory mechanisms to the growth of cancer cells were reported (Yu *et al.,* 2009; Ziliotto *et al.,* 2009). However there are no reports regarding the antihepatotoxic efficacy of *A. blazei*. The present study is aimed to evaluate the antihepatotoxic role of *A. blazei* against CCl_4-induced liver injury in rats.

MATERIALS AND METHODS

Procurement and rearing of experimental animals

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (27 ± 2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 160-200 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water *ad libitum* and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment.

Preparation of aqueous extract

The collected *A. blazei* were air dried and powdered. The powdered *Agaricus blazei* were kept in airtight containers in a deep freeze until the time of use. A sample containing 250 g of *Agaricus blazei* was mixed with 1000 mL of distilled water and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extracts was approximately 14.8 g.

The suitable optimum dosage schedules were identified by administering the aqueous extract of *A. blazei* extracts at different dosages (250, 500 and 1000 mg/kg body weight) in a day daily for twenty eight days. The optimum doses were selected as 250 and 500 mg/kg body weight of the animals for twenty eight days respectively.

Experimental design

The animals were divided into 6 groups of 6 rats each.

Group 1 : Control rats given physiological saline solution 10 mL/kg body wt.

Group 2 : Rats given carbon tetrachloride (1 ml/kg body wt./ip) for seven days.

Group 3 : Rats given carbon tetrachloride + *A. blazei (*250 mg/kg body wt.) administered orally using an intragastric tube.

Group 4 : Rats given carbon tetrachloride + *A. blazei* (500 mg/kg body wt.) administered orally using an intragastric tube.

Group 5 : Rats given carbon tetrachloride + silymarin (25 mg/kg body wt.) administered orally using an intragastric tube.

Group 6 : Rats given *A. blazei* (500 mg/kg body wt.) alone administered orally using an intragastric tube.

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. The liver tissues were excised immediately and washed with chilled physiological saline.

Biochemical analysis

Blood samples were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Serum biochemical parameter such as Transaminases (AST and ALT), ALP, Bilirubin, cholesterol and protein levels were estimated according to standard methods (Reitman and Frankel, 1957; King and Armstrong, 1980; Malloy and Evelyn 1937; Zlatkis *et al.*, 1953; Lowry *et al.*, 1951).

Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at $p \leq 0.05$ (Duncan, 1957).

RESULTS

Hepatic serum marker enzymes

The levels of serum AST, ALT and ALP were estimated in normal and experimental rats. Significant elevation in serum AST, ALT and ALP in rats treated with carbon tetrachloride when compared with the corresponding control rats. Oral administration of aqueous extract of *A. blazei (*250 and 500 mg/kg body wt.) *a*nd silymarin to carbon tetrachloride induced hepatic damage rats caused a marked reduction in the activities of these enzymes. Extract alone administered rats did not shows any significant change (Table 1).

Cholesterol, Bilirubin and Protein

The levels of cholesterol, bilirubin and protein were analyzed in normal and experimental rats. There was a significant decrease in protein levels whereas increase the level of cholesterol and bilirubin in rats treated with carbon tetrachloride when compared with the corresponding

control rats. Oral administration of aqueous extract of *A. blazei (*250 and 500 mg/kg body wt.) and silymarin to carbon tetrachloride induced hepatic damage rats caused a marked increase in the levels of protein and decreased the levels of cholesterol and bilirubin when compared with carbon tetrachloride alone treated rats. Extract alone administered rats did not shows any significant change (Table 2).

Table 1. Serum hepatic marker enzyme activities in control and experimental groups.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	96.71 ± 7.65^{a}	49.48 ± 4.36^{a}	167.15 ± 11.34^{a}
Carbon tetrachloride	212.02 ± 10.12^{e}	168.07 ± 11.24^{e}	601.73 ± 15.28^{e}
Carbon tetrachloride + *Agaricus blazci* (250 mg/kg)	123.87 ± 9.44^{d}	65.15 ± 5.02^{d}	224.12 ± 18.44^{d}
Carbon tetrachloride + *Agaricus blazci* (500 mg/kg)	109.42 ± 8.25^{b}	55.20 ± 4.68^{b}	195.42 ± 10.65^{b}
Carbon tetrachloride + Silymarin (25 mg/kg)	117.10 ± 7.18^{c}	62.22 ± 3.74^{c}	214.18 ± 8.72^{c}
Agaricus blazci (500 mg/kg) alone	94.32 ± 6.82^{a}	50.07 ± 4.82^{a}	164.33 ± 10.82^{a}

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level ($P < 0.05$).

Duncan Multiple Range Test (DMRT).

Table 2. Serum bilirubin, cholesterol and protein levels in control and experimental groups.

Groups	Bilirubin (mg/dL)	Cholesterol (mg/dL)	Protein (mg/dL)
Control	0.91 ± 0.02^{a}	91.67 ± 5.24^{a}	7.91 ± 0.18^{d}
Carbon tetrachloride	3.67 ± 0.20^{d}	166.65 ± 9.12^{e}	4.86 ± 0.23^{a}
Carbon tetrachloride + *Agaricus blazci* (250 mg/kg)	1.39 ± 0.01^{c}	117.15 ± 7.42^{d}	6.70 ± 0.19^{b}
Carbon tetrachloride + *Agaricus blazci* (500 mg/kg)	1.29 ± 0.02^{bc}	101.80 ± 6.74^{b}	7.33 ± 0.21^{c}
Carbon tetrachloride + Silymarin (25 mg/kg)	1.28 ± 0.01^{b}	109.81 ± 4.66^{c}	7.13 ± 0.52^{c}
Agaricus blazci (500 mg/kg) alone	0.92 ± 0.02^{a}	94.14 ± 5.18^{a}	8.13 ± 0.39^{d}

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level ($P < 0.05$).

Duncan Multiple Range Test (DMRT).

DISCUSSION

Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India (Dash *et al.*, 2007).

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells. There are however, a number of drugs employed in traditional system of medicine for liver affections. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

The increased levels of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lowhorn, 1978). In view of this, the extract mediated reduction in levels of AST and ALT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by carbon

tetrachloride. This effect is in agreement with the commonly accepted view that serum levels of transminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew *et al.,* 1987). Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow (Ploa and Hewitt, 1989). The use of ALP in chemical induced liver dysfunction has been investigated in this investigation. Carbon tetrachloride induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The aqueous extract *A. blazei* mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction in rat liver during hepatic injury with carbon tetrachloride. ALP was a membrane bound enzyme, which was released unequally depending on the pathological phenomenon. An elevation in the levels of the serum marker enzymes in generally regarded as one of the most sensitive index of the hepatic damage (Kapil *et al.,* 1995).

In the present study administration of carbon tetrachloride treated rats showed an increase the activities of serum AST, ALT, ALP, bilirubin and cholesterol where as decrease the level of protein in rats treated with carbon tetrachloride when compared with the corresponding control rats. Oral administration of aqueous extract of *Agaricus blazei (*250 and 500 mg/kg body wt.) and silymarin to carbon tetrachloride induced hepatic damage rats caused a marked reduction in the activities of these enzymes, bilirubin and cholesterol where as protein level was increased.

CONCLUSION

It is concluded that treatment with aqueous extract of *A. blazei* was effective in bringing about functional improvement of hepatocytes. The enhancement of the level of protein and suppress the elevated activities of liver marker enzymes were confirmed that, aqueous extract of *A. blazei* have a potential hepatotherapeutic properties.

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