

Hepatoprotective efficacy of edible macrofungi *Dacryopinax spathularia* (Schwein) and *Schizophyllum commune* (Fries) against Carbon tetrachloride induced hepatotoxicity in albino Wistar rats

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Abstract

In the present study the hepatoprotective efficacy of two edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune* has been assessed against CCl₄-induced hepatotoxicity in albino wistar rats. The administration of CCl₄(1ml/Kg) resulted into significant ($p < 0.05$) rise in the levels of liver function marker enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and bilirubin and decrease in the levels of total protein and albumin in blood. On administration of low dose (250mg/Kg) and high dose (500mg/Kg) of both macrofungal extracts in different hepatotoxic group of rats, the serum levels of AST, ALT, ALP and bilirubin significantly ($p < 0.05$) lowered down and the levels of total protein and albumin significantly ($p < 0.05$) increased in comparison to the hepatotoxic group of rats, reflecting the hepatoprotective impact of both the extracts.

Keywords: Carbon tetrachloride, Hepatotoxicity, Hepatoprotective, Macrofungi

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INTRODUCTION

Liver is a large, complex and vital organ of the body, involved in diversified functions like metabolism of food molecules, detoxification of toxic agents, drugs or waste products of metabolism such as ammonia, recycling of breakdown products of RBCs and bile synthesis, synthesis of plasma proteins including clotting factors, maintenance of blood glucose level through glycogenesis and glycogenolysis and many other biochemical and physiological functions. Therefore any hepatotoxic factor may produce serious health consequences by inducing liver damage, which is indicated by abnormal levels of marker enzymes in blood like Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Serum Albumin, Total Protein and Bilirubin (Das and Sattegiri, 2018; Maity and Ahmad, 2012). Liver damage is a widespread problem across the world and in most of the cases of liver damage oxidative stress is involved, which is characterized by a progressive change from steatosis to chronic hepatitis, fibrosis, cirrhosis and sometimes hepatocellular carcinoma (Kodavanti *et al.*, 1989). Moreover, Oxidative stress has been in-

involved in the pathogenesis of a variety of pathophysiological conditions of liver damage, such as exposure to hepatotoxins, alcoholic liver injury, viral hepatitis, intrahepatic cholestasis, biliary disease, liver ischemia and liver necrosis (Adeyemi, 2014; Stephens, 2003). Carbon Tetrachloride (CCl₄) is toxic to liver and kidney and is used frequently in scientific research works to induce the hepatotoxicity in animal models and to evaluate the hepatoprotective potentiality of the hepatoprotective agents (Seifert *et al.*, 1994)

Macrofungi, also called as Mushrooms, belong to two major groups Ascomycota and Basidiomycota, which include approximately 10000 species and among these about 3000 species are considered as edible, in which 700 species have been found to have pharmacological properties (Chang and Miles, 2004; Karaman *et al.*, 2012; Wasser and Weis, 1999.). Mushrooms are not only prized for their value as a rich nutritional dietary source but also for their pharmacological efficacy (Lindequist *et al.*, 2005). Edible macrofungi or mushrooms contain various potent bioactive chemical constituent compounds like tannins, saponins, alkaloids, flavonoids, phenolics etc., which possess many pharmacological properties like

antioxidant (Peralta *et al.*, 2008; Dandapat and Sinha, 2015), anti-inflammatory (Moro *et al.*, 2012), anti-diabetic (Hu. *et al.*, 2006), antimicrobial (Barros *et al.*, 2007), immunomodulatory and anti-cancerous (Moradali *et al.*, 2007). Moreover, hepatoprotective impact of macrofungi has been reported by many workers (Jayakumar *et al.*, 2006; Andreia *et al.*, 2013).

Dacryopinax spathularia (Schwein) and *Schizophyllum commune* (Fries) are two edible macrofungi belonging to group Basidiomycota and are traditionally used as anti-diabetic, antibacterial, anti-inflammatory, hepatoprotective and nephroprotective food supplement. Kumar *et al.* (2018) have reported the mycochemical composition and antioxidant potentiality of these two edible macrofungi and found that both the macrofungi have significant antioxidant capacity. The present work was aimed to assess the hepatoprotective efficacy of *D. spathularia* and *S. commune* and to evaluate them as hepatoprotective nutraceutical agents using albino rats as animal model.

MATERIALS AND METHODS

Animals: Wistar albino rats (*Rattus norvegicus*) weighing about 175-200 g were used in the study. The rats were maintained under standard laboratory conditions at a temperature of 25±5°C and relative humidity of 50±15%. The Dark-light cycle of 12 hrs. was maintained throughout the experimental period of time. The animals were fed with commercial pellet diet and water *ad libitum*. The rats were kept in Polypropylene cages with paddy husk as bedding material. The experiment was carried out as per the approval of Ethics committee of Ranchi University, Ranchi.

Acute toxicity studies: The OECD guidelines (2004) have been followed for acute toxicity studies. Different doses of both macrofungal extracts were administered to two different groups of 10 rats, where each group received one macrofungal extract. The extracts were fed orally by oral feeding tube. No mortality was observed up to the doses of 2000 mg/kg body weight (BW) /day within 48 hrs.

Induction of hepatotoxicity and Evaluation of hepatoprotective efficacy: Hepatotoxicity was induced by intra-peritoneal (i.p.) administration of 1 ml/kg BW every 72 hrs. for 14 days of a mixture

containing 30% CCl₄ and liquid paraffin (1:2 V/V). Animals were equally divided into six groups (Group 1, 2, 3, 4, 5, 6) containing 10 animals each and the experiment was carried as follows:

Group 1: Served as Control, received 1 ml of distilled water orally

Group 2: Considered as hepatotoxic, received 1ml/Kg i.p. of CCl₄ every 72 hrs.

Group 3: Hepatotoxic rats treated as above, received 250 mg/Kg BW/day of *D. spathularia* extract (LD); LD= Low Dose

Group 4: Hepatotoxic rats treated as above, received 500 mg/Kg BW/day of *D. spathularia* extract (HD); HD= High Dose

Group 5: Hepatotoxic rats treated as above, received 250 mg/Kg BW/ day of *S. commune* extract (LD)

Group 6: Hepatotoxic rats treated as above, received 500 mg/Kg BW/day of *S. commune* extract (HD)

Sample collection and assessment of biochemical parameters:

The experiment was carried for 14 consecutive days. At the end of the experimental period all the animals were kept fasting overnight and then blood was collected by retro-orbital bleeding under light ether anesthesia. Three blood samples were collected randomly from each group. The blood samples were placed in test tubes and allowed to clott for 30 minutes. Then the blood samples were centrifuged at 2500 rpm for 10 minutes to get the clear serum and biochemical investigations were carried out. Total protein and Albumin were estimated following the method of Kingsley and Frankel (1939), serum ALT (U/L) and AST (U/L) were measured following the method of Reitman and Frankel (1957) and serum AST (U/L) was measured following the method of Bessey *et al.* (1964).

Statistical analysis: Data was taken in triplicate and expressed as mean± standard error of mean. Statistical analysis was done by one way ANOVA followed by student's t-test, statistical significance of values was considered at $p<0.05$.

RESULTS

The hepatoprotective effect of *S. commune* extract on hepatotoxicity-induced rats is shown in Table 1. The concentration of liver function marker enzymes AST, ALT, ALP and bilirubin significantly

Table 1. Hepatoprotective efficacy of *Schizophyllum commune* extract against CCl₄-induced hepatotoxicity in rats (Data expressed as mean±SE, n=3). ^aStatistically significant when compared to control group($p<0.05$); ^bStatistically significant when compared to hepatotoxic group($p<0.05$).

Animal Groups	Total Protein (g/dL)	Serum Albumin(g/dL)	Bilirubin (mg/dL)	AST(U/L)	ALT(U/L)	ALP(U/L)
Group 1(Control)	8.26±0.34	3.48±0.12	0.68±0.09	48.30±2.56	57.46±3.35	94.52±4.35
Group2(CCl ₄ treated/ Hepatotoxic)	4.78±0.26 ^a	2.02±0.52 ^a	2.14±0.22 ^a	124.20±9.68 ^a	168.36±12.25 ^a	134.91±7.15 ^a
Group5(Hepatotoxic+ LD of <i>S. commune</i> extract)	7.92±0.46 ^{ab}	3.34±0.16 ^b	0.86±0.14 ^a	63.62±4.23 ^{ab}	71.83±5.28 ^{ab}	104.12±5.79 ^{ab}
Group 6(Hepatotoxic + HD of <i>S. commune</i> extract)	8.51±0.56 ^b	3.57±0.22 ^b	0.72±0.14 ^b	51.46±6.22 ^b	63.16±5.18 ^{ab}	102.08±6.63 ^{ab}

Table 2. Hepatoprotective efficacy of *Dacryopinax spathularia* extract against CCl₄-induced hepatotoxicity in rats (Data expressed as mean±SE, n=3).

Animal groups	Total Protein(g/dL)	Serum Albumin(g/dL)	Bilirubin (mg/dL)	AST(U/L)	ALT(U/L)	ALP(U/L)
Group 1(Control)	8.26±0.34	3.48±0.12	0.68±0.09	48.30±2.56	57.46±3.35	94.52±4.35
Group2(CCl ₄ treated/ Hepatotoxic)	4.78±0.26 ^a	2.02±0.52 ^a	2.14±0.22 ^a	124.20±9.68 ^a	168.36±12.25 ^a	134.91±7.15 ^a
Group3(Hepatotoxic+ LD of <i>D. spathularia</i> extract)	7.86±0.21 ^{ab}	2.97±0.34 ^{ab}	0.89±0.16 ^{ab}	61.70±2.68 ^{ab}	74.32±3.36 ^{ab}	102.51±4.36 ^{ab}
Group4(Hepatotoxic+ HD of <i>D. spathularia</i> extract)	8.12±0.26 ^b	3.35±0.23 ^b	0.74±0.16 ^b	52.38±3.27 ^{ab}	63.76±4.68 ^{ab}	92.45±3.02 ^b

($p=0.01$) increased and the concentration of total protein and albumin significantly ($p=0.01$) decreased in toxicity-induced rats (group2), in comparison to the normal control group. On administration of low dose (250 mg/Kg BW) and high dose (500mg/Kg BW) of *S. commune* extract to the hepatotoxic rats, the concentration of AST, ALT, ALP and bilirubin significantly ($p<0.05$) decreased and concentration of total protein and serum albumin significantly ($p<0.05$) increased, in comparison to the hepatotoxic group of rats.

The results of hepatoprotective effect of *D. spathularia* extract has been shown in Table 2. The administration of LD and HD of *D. spathularia* extract showed significant ($p<0.05$) decrease in the concentration of AST, ALT, ALP and bilirubin and significant ($p<0.05$) elevation in the concentration of total protein and albumin in the blood, in comparison to the CCl₄-treated hepatotoxic rats.

DISCUSSION

The CCl₄ induces hepatotoxicity by transforming into free radicals like trichloromethyl radical (CCl₃·) and trichloromethylperoxy radical (CCl₃OO·), which induces enhanced lipid peroxidation, activation of cytochrome 450 and release of pro-inflammatory mediators like TNF- α which results in necrosis and induces oxidative stress-mediated liver damage (Edwards *et al.*, 1993; Huo *et al.*, 2011). The enhanced oxidative stress and thereby the increased lipid peroxidation can lead to damage in hepatocellular membranes (De Groot *et al.*, 1988). The concentration of liver function marker enzymes like AST, ALT, ALP and Bilirubin in blood increases due to liver biliary obstruction and degradation of hepatic cell membranes (Huo *et al.*, 2011) whereas, the concentration of albumin and protein in blood decreases as a result of damage of intracellular structures like mitochondria, endoplasmic reticulum, DNA etc. (Uru *et al.*, 2013).

Chatterjee *et al.*, (2011) has reported that wild edible mushroom *Calocybe indica* contains bioactive compounds like flavonoids, phenolics etc. and its extract shows hepatoprotective impact by stabilization of hepatocyte membrane and healing of hepatic parenchyma through antioxidant defense mechanism. The two experimental macrofungi

taken for the present study have significant antioxidant activity due to the potent mycochemical constituent compounds present in them like tannins, saponins, alkaloids, flavonoids, phenolics etc. (Kumar *et al.*, 2018). In the present study the two experimental macrofungal extracts have been administered to CCl₄-induced hepatotoxic rats at two respective doses i.e. low dose and high dose to different hepatotoxic groups of rats. The results clearly showed that the administration of extracts of both macrofungi significantly ($p<0.05$) lowered the concentration of AST, ALT, ALP and bilirubin in blood whereas total protein and albumin levels in blood significantly ($p<0.05$) increased (Table 1 and 2). The results of the present work showed that comparatively both the experimental macrofungal extracts have more or less equal hepatoprotective impact on the CCl₄-induced hepatotoxic rats.

It has been reported by many workers that the lowering of AST, ALT, ALP and bilirubin concentration and enhancement in concentration of total protein and albumin in blood back to their respective normal levels are the signs of repair of hepatic toxicity and regeneration of hepatocytes (Thawbrew *et al.*, 1987; Hussain *et al.*, 2017). In the present study the enhancement in AST, ALT, ALP, bilirubin levels and decrease in total protein and albumin in blood following the administration of CCl₄ shows the hepatic toxicity induced by the chemical. Further, the consequential lowering of AST, ALT, ALP and bilirubin levels and enhancement in total protein and albumin levels in blood following the administration of both the macrofungal extracts showed the hepatoprotective activity of both macrofungi studied in this animal model.

Conclusion

Based on the results of present study it can be concluded that the edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune* possess more or less equal hepatoprotective efficacy in the present animal model. Therefore these two macrofungi can be used as hepatoprotective nutraceutical dietary sources and in the development of new potent hepatoprotective agents.

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