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Hepatoprotective and Antioxidant Activity of Edible Mushroom *Termitomyces Hemii* in Ccl_4-Induced Hepatotoxicity in Albino Rats

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Abstract:

The present study included the assessment of hepatoprotective efficacy of the edible mushroom *Termitomyces hemii* against Carbon Tetrachloride (CCl_4) hepatotoxicity in albino Wistar rats. The aqueous extract of the fruiting bodies showed the presence of several pharmacologically important biochemical constituent compounds including flavonoids, alkaloids, phenolics, tannins, saponins, among others. The in-vitro antioxidant analysis of the extract showed a strong free radical scavenging activity against DPPH (1, 1- diphenyl-2- picryl hydrazyl) radicals using Ascorbic acid as reference standard. At 100 µg/ml concentration, the extract exhibited 47.58% DPPH radical scavenging activity in comparison to the 55.14% DPPH radical scavenging activity of the standard reference Ascorbic acid. The in-vivo studies showed that a dose of 500 mg/Kg Body weight/day of the extract in CCl_4-induced hepatotoxic rats resulted into significant ($p \leq 0.05$) improvement in the blood levels of liver function parameters, such as bilirubin, serum albumin, total protein, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). The marked hepatoprotective efficacy of the present mushroom may be attributed to its rich content of bioactive and potent biochemical constituent compounds having marke antioxidant potentialities.

Key words: Mushroom, Hepatoprotective, Antioxidant, Termitomyces hemi, Carbon tetrachloride

Introduction:

Mushrooms are actually the macrofungi with fruiting bodies large enough to be seen through naked eyes, which can be either epigeous or hypogeous. They belong to two major groups, Ascomycota and Basidiomycota, and possess variable degree of edibility. Studies have reported that there are around 140,000 species of macrofungi found across the globe, out of which only 10% (14,000) species have been described to date (Chang and Miles, 2004). Among these, approximately 2000 species are considered as edible, and about 700 species have been considered to possess medicinal or pharmacological properties (Chang and Miles, 2004; Karaman *et al*., 2012). The edible mushrooms have been reported to contain essential mineral nutrients, fibres, amino acids, fatty acids, vitamins, polysaccharides etc., and are considered to be

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a rich source of proteins and bioactive secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, saponins, among others (Kues and Liu, 2000; Mattila *et al*., 2002; Aidaa *et al*., 2009). Mushrooms are typically regarded as functional foods or nutraceutical products since they are nutritionally beneficial when consumed regularly or when their isolated bioactive constituent compounds are consumed (Lakhanpal and Rana, 2005).

The liver is a complex organ of the body, which is involved in carrying out a number of vital metabolic and physiological functions, such as drug elimination and detoxification, metabolism of food molecules and maintenance of blood glucose levels by glycogenesis and glycogenolysis, synthesis and secretion of bile, synthesis of several plasma proteins including blood clotting factors and many others physiological and metabolic functions (Gowri Shankar *et al*., 2008). Liver damage or liver related disorders, therefore may produce serious health consequences, sometimes may prove fatal. Liver damage is a prevalent and widespread condition that typically entails oxidative stress and is marked by a steady progression from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular cancer (Kodavanti *et al*., 1989). Carbon Tetrachloride (CCl4), a toxic substance to the liver, is commonly employed in scientific research to induce hepatotoxicity in animal models and to assess the efficacy of any hepatoprotective agent or medicines (Seifert, 1994). The induction of hepatotoxicity by CCl_4 includes the binding with Cytochrome P450 and thereby release of highly reactive free radical i.e. Trichloromethyl ($CCl_3\cdot$), which then mediates cascade of reactions leading to enhanced lipid peroxidation and cellular damage, consequently leading to hepatocellular injury and hepatic necrosis (Kadhska *et al*., 2000). Any chronic or acute hepatotoxicity produced by the hepatotoxic agent leads to the abnormal levels of specific biochemical parameters or enzymes in the blood or liver tissues, such as bilirubin, serum albumin, total protein, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) (Giannini *et al*., 2005; Maity and Ahmad, 2012).

Termitomyces hemii is an edible macrofungus belonging to family Lyophyllaceae, and is commonly found in South Asia. This fungus lives in a symbiotic association in or on termite nests. Puttaraju *et al*., (2006) studied the phenolic content and antioxidant efficacy of 23 species of edible mushrooms collected from different locations of India, and found *T. hemi* as the best variety with highest phenolic content and most prominent free radical scavenging activity. The marked antioxidant efficacy of *T. hemi* was attributed to its rich phenolic content. Since mushrooms generally contain a number of potent biochemical components with several pharmacologically important functions, the present work had been undertaken to qualitatively analyze the mycochemical constituent compounds and to determine the antioxidant potentiality and hepatoprotective efficacy of the present edible mushroom *T. hemi*.

Materials and Methods:

Collection of sample and preparation of extract:

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Fresh fruiting bodies of *T. hemi* have been collected from East Singhbhum region of Jharkhand, India and brought to the laboratory. The fruiting bodies were thoroughly washed, disinfected with 0.1% $HgCl_2$ followed by repeated washing with deionized water. Now the fruiting bodoes were dried under shade for 7-8 days, and upon complete drying, and then powdered using an electric grinder. The powder was then sieved and stored for further use. 20 gm of the powdered sample was subjected to solvent extraction using water (500 ml) as solvent with the help of Soxlet apparatus. The extract was then filtered and stored for further use.

Qualitative biochemical analysis of T. hemi extract:

The preliminary qualitative analysis for the presence of different secondary metabolites was done according to previously established standard tests (Bhaskar and Kumar, 2012).

Assessment of Antioxidant activity:

The antioxidant activity of the extract was determined by DPPH (1, 1- diphenyl-2- picryl hydrazyl) radical scavenging assay following the previously established standard methods (Moon and Terao, 1998; Kumar et al., 2008). Different quantities of samples (10µg, 50µg, and 100µg) were taken in Dimethyl Sulfoxide (DMSO) and methanol was added to bring the volume up to 500µl. These tubes were then filled with 5 ml of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH; Sigma-Aldrich, Bangalore), followed by vigorous shaking. A control solution was kept with the equal amount of methanol but without the test component. The tubes were let to stand at room temperature for 20 minutes. At 517 nm, the samples' absorbance was measured. The reference standard used was butylated hydroxy anisole (BHA).

Free Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{(\text{control OD - sample OD})}{\text{control OD}} \times 100.$$

Animals and acute toxicity studies:

Adult albino wistar rats (*Rattus norvegicus*) weighing between 180-220 gm were maintained at a temperature of 25±5ºC and a relative humidity of 50±15% over a paddy husk bed under standard laboratory conditions. The rats were fed with commercial pellet diet and water ad libitum. The experiment was undertaken with prior approval from "Institutional Ethical Committee" of Ranchi University, Jharkhand, India. The staircase method was used for acute toxicity studies following the OECD guidelines (2004). The rats were divided into five groups of ten rats each and fed with increasing concentration of the extract. No mortality was observed upto the concentration of 2000mg/kg Body Weight per day within a period of 48 hrs.

Animal groups and Research design:

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A mixture of CCl4 (30%) and liquid paraffin (1:2 V/V) was injected intra-peritoneally (i.p.) every 72 hrs. for 14 days. The animals were divided into three groups with ten rats in each group and the experiment was carried out as follows:

Group A (Control): received 1 ml of normal saline orally

Group B (CCl_4 treated): received 1ml/Kg i.p. of CCl_4 every 72 hrs

Group C (CCl_4 treated + Extract): hepatotoxic rats, received 500 mg/BW/day of *T. hemii* extract

The experiment was continued for 14 days. At the end of 14th day, all experimental animals were kept fasting overnight, and then the blood samples were collected randomly from each group in triplicates. The blood samples were centrifuged at 3000 rpm for 10 minutes and the clear serum was collected for further estimation of biochemical parameters and enzymes. Serum ALT (U/L) and AST (U/L) were estimated using the Reitman and Frankel (1957) method, total protein and albumin were assessed using the Kingsley and Frankel (1939) method, and serum AST (U/L) was quantified using the method of Bessey *et al*. (1964).

Results:

Qualitative biochemical analysis of *T. hemi* extract:

The result of qualitative analysis of secondary metabolite content of the aqueous extract of fruiting bodies of T. hemi has been shown in Table 1.

Table 1: Showing qualitative analysis of aqueous extract of *T. hemii* extract

Biochemical constituents	Presence/Absence (+/-)
Reducing sugars (Carbohydrates)	+
Proteins	+
Amino acids	+
Alkaloids	+
Flavonoids	+
Phenolics	+
Saponins	+
Tannins	+
Sterols and steroids	+
Quinons	+

Antioxidant activity of *T. hemi* extract:

Table 2 shows the DPPH radical scavenging activity of the *T. hemi* extract using Ascorbic acid as the standard reference.

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Table 2: Showing % DPPH radical scavenging activity at various concentrations of *T. hemi extract*

Concentration (µg/ml)	% Free radical scavenging activity of Pt-AgNPs	% Free radical scavenging activity of Ascorbic acid
25	24.57	31.63
50	37.91	44.21
100	47.58	55.14

Hepatoprotective activity of *T. hemi* extract:

The results of analysis of hepatoprotective activity of *T. hemi* extract have been shown in Table 3.

Table 3: Showing hepatoprotective effect of *T. hemii* extract on serum levels of liver function parameters in CCl_4-induced hepatotoxic rats

Animal Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (g/dL)	Albumin (g/dL)	Total Bilirubin (mg/dL)
Group 1	63.34±7.16	56.32±6.71	127.49±9.53	21.97±3.29	7.08±0.64	1.06±0.18
Group 2	183.29±9.87[a]	189.73±12.39[a]	214.37±13.89[a]	6.58±0.72[a]	3.04±0.52[a]	3.18±0.49[a]
Group 3	71.59±8.33[b]	72.19±8.37[ab]	131.79±10.21[b]	17.39±3.11[ab]	6.22±0.58[b]	1.51±0.23[ab]
Group 4	69.11±8.59[b]	69.27±7.13[ab]	125.63±11.59[b]	20.46±2.58[b]	7.37±0.79[b]	1.26±0.36[b]

*a (significantly different from Group 1); b (significantly different from Group 2) at $p \leq 0.05$

Discussion:

The administration of CCl4 results into abnormal rise in oxidative stress within the hepatic cells or tissues, which leads to enhanced lipid peroxidation and thereby damage to the hepatocellular membranes (De Groot et al., 1988; Acharya *et al*., 2012). The degeneration of hepatocellular membranes and the obstructions in the billiary system of the liver results into an abnormal increase in the blood levels of AST, ALT, ALP and bilirubin (Huo *et al*., 2011). The oxidative stress can also produce damage in the intracellular structures like endoplasmic reticulum, mitochondria along with DNA within the hepatic cells, and consequently the reduced protein synthesis, which results into abnormal decrease in the blood levels of proteins and albumin (Rajendran *et al*., 2009). In the present work the administration of *T. hemi* extract in hepatotoxic group of rats had resulted into significant ($p \leq 0.05$) decrement in the blood levels of AST, ALT, ALP, and bilirubin, and a concurrent and significant ($p \leq 0.05$) rise in the blood levels of albumin and proteins, back towards their normal values, indicating the protective effect of *T. hemii* extract against the CCl4-induced hepatotoxicity. Depletion in the enhanced levels of bilirubin along with decrease in the blood levels of ALP indicates the efficacy of T. hemi extract

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to stabilize billiary disfunctions. Further, the depletion in the blood levels of AST, ALT and bilirubin suggests that the *T. hemii* extract has the ability to minimize the structural damages in the hepatocytes, as well as to accelerate the regeneration in hepatic cells and tissues.

Several previous works have reported that the macrofungi or mushrooms are rich in their potent and bioactive secondary metabolite content, which have marked pharmacological properties (Nada *et al*., 2010). Previous studies reported that the mushrooms extracts or specific biochemical constituents isolated from mushroom extract showed significant hepatoprotective properties. The edible mushrooms like *Lentinus edodes, Grifola frondosa*, *Tricholoma lobayense*, *Ramaria botrytis*, *Calocybe indica* and *Astraeus hygrometricus* are reported to possess marked Hepatoprotective efficacies (Ooi Vec, 1996; Kim *et al*., 2003; Chatterjee *et al*., 2011). Ajith *et al*., (2006) had reported the Hepatoprotective efficacy of the edible mushroom *Phellinus rimosus* against CCl_4-mediated hepatotoxicity in rats. Jayakumar *et al*., (2006) had reported that the extract of edible mushroom *Pleurotus ostreatus* shows marked improvement in the hepatotoxicity caused by CCl_4. Acharya *et al*., (2012) had reported the Hepatoprotective efficacy of the edible mushroom *Macrocybe gigantean* against CCl_4-induced hepatotoxicity in rats. Other works have also reported that edible mushrooms like *Agaricus blazei* (Al-Dbass *et al*., 2012), *Russula albonigra* (Chaterjee et al., 2012), and *Pleurotus cornucopiae* (El-Bohi *et al*., 2009), possess the ability to improve the liver damage from CCl_4-induced hepatotoxicity in rats.

The studies have reported that the marked hepatoprotective efficacy of mushroom extracts is primarily due to their strong antioxidant potentiality, which can be attributed to their bioactive chemical constituent compounds such as flavonoids, phenolics, alkaloids, among others (Di Carlo *et al*., 1999; Al-Dbass *et al*., 2012; Acharya *et al*., 2012). The qualitative analysis of the present mushroom *Termitomyces hemii* had shown the presence of potent antioxidant compounds like flavonoids, alkaloids, phenolics etc. in the extract. Further, the results of the present study also showed the strong antioxidant activity of *T. hemii* extract. Hence, the marked hepatoprotective efficacy of the present edible mushroom, i.e., *T. hemii*, can be attributed to its bioactive chemical constituents or the secondary metabolite content.

Conclusion:

From the results of the present work, it can be concluded that the aqueous extract of *T. hemii* fruiting bodies has marked hepatoprotective activity against CCl_4-induced hepatotoxicity in the present mammalian animal model, i.e., albino Wistar rats, which might be due to its strong antioxidant properties. Hence, the present edible mushroom, i.e., *T. hemii,* can be a potent nutraceutical dietary source, which can further be studied and explored to isolate and develop new drugs and medicinal agents from this mushroom.

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