IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com *Volume 1 Issue 1. 2014 Pages: 93-104*
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

RESEARCH ARTICLE

STUDIES ON ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF MOREL MUSHROOM MYCELIUM

R.RAVIKUMAR[1] AND A.KALIDOSS[2]

[1]Department of Biotechnology, J.J.College of Arts and Science, Sivapuram, Pudukkottai Tamil Nadu, India.
[2]Department of Microbiology, Mohamad Sathak college ge of Arts and Science, Chennai, Tamil Nadu, India.
Corresponding Author: ravimicrobiotech@gmail.com

Abstract

Species of Morchella are known as morels. Morchella is a brain like fungus, it tastes so good. It is often found under oak, maple, likory and other hard wood especially in May. It is an economically important mushroom. Attempts to grow Morchella species have not been successful. Hence their mycelia are produced commercially by submerged fermentation. Morel mycelia can be used as a good source of protein supplement to vegetarian diets. Proteins from the mycelia are comparable to vegetable protein used by several sections of the population in our country. *Morchella esculenta* is an important morel mushroom belonging to the family Morchellaceae of Ascomycotina Division and is locally known as Guchhi. Morchella grows naturally on the forest floor rich in humus they are difficult to grow commercially. In India this mushrooms is found growing in the forest of Jammu and Kashmir and Himachal Pradesh. In 1982 *Morchella esculenta* was reported to be grown under laboratory condition. *Morchella esculenta* is an expensive product because of its rich nutritional value and unique flavour. It is cooked as food and used in medicinal and health care systems by the traditional societies and also considered as important for clinical use.

Key words: Morchella, Morchellaceae, Ascomycotina, medicinal, health care systems.

Introduction

It is interesting to note that the oxygen, which is most essential for the very existence of higher organism can become a monostrously dangerous molecule when combined with free electron. In the living cell several electrons can be leaked out from the electron transports chain and get attached to the acceptor molecule in tissue. A free radical in any species is capable of independent existence, that contain one or more electrons with in its outer orbital. The unpaired electron alter chemical reactivity of an atom or molecule making it more reactive than the corresponding nonradical atom or molecule.

Reactive oxygen species is a collective name which is used by biologist which includes not only the oxygen radicals (superoxide-O_2 and hydroxyl radicals-OH) but also some derivatives of oxygen that do not contain unpaired electrons such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2) and peroxynitrate (ONOO). Most of the reactive oxygen species come from the endogenous source as a by-product of

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

normal essential reaction such as generation of energy from mitochondria or the detoxification reactions involving the liver cytochrome P-450 system. The exogenous sources include bacterial, viral or fungal infections. Reactive oxygen species (ROS) are considered to be important factors in etiology of several pathological conditions (Hemanani and Prihar,1998).Recent studies on free radical and biochemical research in infectious diseases show involment of free radical generation in several diseased conditions.

Human liver is the main organ that contain complex parenchymal cells that perform multiple and diverse functions essential for life. Hepatocytes have a unique regeneration capacity as well as a marked capacity to respond to increased metabolic demands of the organisms.

Liver directly receives, process, and stores the materials absorbed from the digestive tracts such as aminoacids, carbohydrates, fatty acids and cholesterol as well as vitamins and is capable of releasing metabolites of these compounds on demands. Many plasma proteins including 1,2 and 3 globulin, clotting factors and transport proteins are synthesized by the liver.

The liver is the main organ of detoxification and is the site of metabolic conversion of endogenous and exogenous compounds. Another major functions of liver is to synthesize bile acids from cholesterol and to secrete these compounds from the hepatocytes into the intestine, there by generating bile flow and facilitating dietary fats, encalcification and absorption. Tetracycline, ethanol, carbontetrachloride, bromobenzene, paracetamol sulfonamide, erythromycin etc are the chemicals that induces hepatotoxicity.

Carbon tetrachloride is a pungent volatile fluid used as a drycleaner because of its ability to easily dissolve greasy stains. Its lipid solubility allows it to cross cell membrane rapidly and any tetrachloride methane taken into the body is quickly distributed to all organs. When inhaled or swallowed it may severely damage the heart, liver and kidney, causing cirrhosis and necrosis and it can also affect the optic nerves. Treatment is by administration of the oxygen.

Tetrachloromethane is metabolized by the p450 system to give the trichloromethyl radical (CCl_3)

$$CCl_4 \longrightarrow CCl_3 + Cl$$

This CCl_3 is brought about by the cytochrome p450 molecule itself. The trichloromethyl radical then to attracts a hydrogen atom from the membrane lipid, setting of the chain reaction lipid peroxidation. Products of peroxidation are known to inhibit protein synthesis and the activity of the certain enzymes.

Cancer is the second largest single cause of death in both men and women worldwide. Cancer arises from the abnormal and uncontrolled division of cells, then invade and destroy the surrounding tissue. Spread of cancer cells may occur via the blood streams or the lymphatic channels, thus setting up secondary tumor. Hepatocellular carcinoma is the one of the most common malignances with limited effective options. Chronic hepatitis B virus (HBV) are the etiological factor's. Chemotherapy, radiotherapy and surgery are more effective treatment of cancer. But these treatments are found to damage the normal cells. .

Mushrooms are widely distributed in nature. They are the largest reproductive structure of edible fungi.Humanities use of mushrooms extends to ancient days. About 10,000 species of mushrooms are known all over the world. Mushrooms are rich source of proteins vitamins and minerals.

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

A large number of mushrooms have been reported to possess medicinal properties. Many of them are found useful to treat number of disease conditions. Some important medicinal properties of mushrooms are antibiotic, immunoregulatory, cardiotonic, hypocholesteremic, antiviral and antitumor. Extract and powders of mushroom's (mycelium, sporocarp) in the form of sugar coated tablets are being marketed for the treatment of diseases such as diabetes, cancer etc. Attempt have been made in many parts of the world to explore the use of mushroom metabolites for the treatment of human sufferings (Janardhanan, 1999)
However, information on the medicinal properties of this valuable mushroom is fragmentary. Since, commercial cultivation of morel mushroom have not been successful, the cultured mycelium of morel are extensively used as food and flavour. Thus it was considered desirable to investigate the medicinal properties of the cultured mycelium. The present investigations where undertaken to evaluate the antioxidant and hepatoprotective activity of the ethanolic extract of the mycelium of Morchella esculenta.

Materials and methods

Experimental materials

Mycelia of *Morchella esculenta*

Animals

Wistar rats purchased from Small Animal Breeding Center, Kerala Agriculture University, Mannuthy, Thrissur and were used. The animals were kept for a week under environmentally controlled conditions with free access to standard food (Lipton, India) and water.

Methods

All the ingredients were dissolved in 1000ml distilled water. The medium was poured into Roux bottles and sterilized by autoclaving at 121°c for 30 minutes. The medium was inoculated with 10 days old culture of *M.esculenta* and incubated at 30°C for 20 days as stationary culture.

Preparation of extract

After 20 days growth the liquid medium was filtered and the mycelium was separated. The mycelium was washed and dried at 40°-50°c for 48 hours. Powdered mycelium was extracted by boiling with 50% ethanol repeatedly for a period of 10 hours. The extracts were pooled and evaporated at low temperature. The alcoholic extracts thus obtained was named as the ethanol extract and was employed in the experiments.

Determination of in vitro antioxidant activity

Superoxide scavenging activity

.

Procedure

Superoxide radicals (O_2^-) are generated by the photoreduction of riboflavin was detected by NBT reduction method of McCord and Fridovich(1968).

The reaction mixture contained EDTA (6mM) 3µg NaCN, riboflavin (2mM), NBT(50mM), KH_2PO_4-Na_2HPO_4 (67mM, ph 7.8) and various concentration of the ethanolic extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15 min. The optical density (OD) at 530 nm was measured before and after illumination. The inhibition of superoxide radicals was determined by comparing the absorbance value of the control with that of the treatments.

Inhibition of lipid peroxidation

Procedure

The reaction mixture contained the rat liver homogenate of 0.1ml (25% W/V) in Tris-Hcl

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

buffer (20mM,pH7.0); KCL (30mM); $FeSO_4$ (NH_4); SO_4 $6H_2O$ (0.16mM); ascorbate (0.06mM) and various concentration of mycelium extract to a final volume of 5.0ml. The reaction mixture was incubated at 37° C for 1 hour. After the incubation period, 4ml was taken and treated with 0.2ml sodium dodecyl sulphate (SDS) 8.1%; 1.5ml thiobarbituric acid (0.8%) and 1.5ml acetic acid (20%, pH 3.5). The total volume was made up to 4ml with distilled water and kept in a water bath at 95° to 100° C for 1 hour.
After cooling, 1.0 ml of the distilled water and 5.0ml of the n-butanol pyridine mixture (15:1) was added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance at 532 nm was read. Inhibition of the lipid peroxidation was determined by comparing the OD of treatment with that of the control.

Hydroxyl radical scavenging activity

Procedure

The reaction mixture contained deoxyribose (2.8mM); Fecl3 (0.1mM); KH_2PO_4-KOH buffer (20mM, pH 7.4): EDTA (0.1mM): H_2O_2 (1.0mM); ascorbic acid (0.1mM) and various concentrations of the extract to a final volume of 1ml. The reaction mixture was incubated at 37°C for 1hr. The TBARS formed was estimated by the above method of Ohkawa and Oshishi (1979). The activity was determined by comparing the absorbance of the control with that of the treatment.

Nitric oxide scavenging activity

Procedure

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method of Marcocci et al (1994). Immediately before the experiment, 10mM stock solution of sodium nitroprusside was prepared in PBS (pH 7.4), Various concentrations of the extract and sodium nitriprusside (1mm) in PBS to a final volume of 3ml were incubated at 25° C for 150 minutes. After incubation, 0.5ml of the solution was taken and diluted with 0.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% naphthylethylene diamene). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with the naphthylethylene diamenedihydrochloride was read immediately at 546nm.

Statistical analysis

All the data are expressed as mean ± SD.

Determination of the hepatoprotective activity

Hepatoprotective activity was determined using Carbon tetrachloride (CCl_4) induced acute hepatotoxicity in rats.

Experimental Procedure

Hepatoprotective activity was determined by the method of Lin *et al*: (1995) with some modifications (Ajith and Janardhanan, 2002).

Animals were divided into four groups of six animals each. Group 1 was administrated with normal saline and kept as normal. Group 2 was given CCl_4 paraffin oil (1:IV/V, 1.5ml/kg), intraperitonially and kept as control. Group 3 and 4 were administrated orally with the ethanolic extract (500, 250mg/kg body weight). The administration of the drug continued for three consecutive days. One hour before the administration of last dose of the extract, the CCl_4 was given intrapertonially to group 2, 3, 4. There after the experimental animal were sacrificed after 24 hours.

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

Collection of Liver and Serum

Blood was collected from the animals by puncturing of the heart. Serum from the control and the experimental rats were separated by centrifugation of the blood. The liver was washed, dried with a filter paper, weighed and processed immediately for biochemical analysis.

Preparation of liver homogenate

The liver was homogenized in 50mM phosphate buffer (pH 7.0) to give a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 10 minute in a cold centrifuge at 4° C and the supernatant were used for enzyme assays and protein determination.

Serum sample was used for the estimation of Glutamate oxaloaletate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) by the method of Reitman and Frankel and Alkaline phosphatase (ALP) by the method of Kind and King. Tissue homogenate was used for the assay of superoxide dismutase (SOD) by the method McCord and Fridovich; Malondialdehyde (MDA) by the method of Ohkawa et al using 1,1,3,3-tetramethoxy propane as the standard. Protein was estimated by the method of Lowry *et al.,*(1951) using Bovine serum albumin as standard and catalase by the method of Aebi *et al.,*(1974).

Assay of enzymes

Alkaline phosphatase (ALP)

Procedure

Alkaline phosphatase was assayed by the method of Kind and King,(1980).
500µl of the working buffered substrate (Phenyl Phosphate) was made up to 2ml with purified water. It was incubated for 3 minutes at 37°C. To this 50µl of the serum was added and again incubated for 15 minute at 37°C. To this 1ml of the chromogen reagent was added. The optical density was read at 510nm. 10mg% phenol was used as a standard.

Assay of serum glutamate pyruvate transaminase (GPT)

Procedure

GPT activity was determined according to the method of Reitman and Frankel (1957).
0.25ml of the buffered substrate (2-ketoglutarate and L-Alanine, P^H 7.4) was incubated for 5 minutes at 37°C. 50µl of the serum was added, mixed well and incubated at 37°C for 30 minute. 0.25ml DNPH colour reagent was added and allowed to stand at room temperature for 20 minute . 2.5ml of 4N sodium hydroxide was added to this and allowed to stand at room temperature for 10 minute. The optical density was read against purified water at 505nm. The enzyme activity was calculated from the standard (Sodium pyruvate, 2mM) calibration curve.

Assay of serum glutamate oxaloacetate transaminase (GOT)

Procedure

GOT activity was determined according to the method of Reitman & Frankal (1957).
0.25ml buffered substrate (Aspartate and α-Ketoglutarate pH 7.4) was incubated for 5 minutes at 37°C. 50 µl of serum was added to this and again incubated at 37°C for 60 minute. 0.25ml of DNPH colour reagent was added, mixed well and allowed to stand at room temperature for 20 minutes. 2.5ml of 4N Sodium hydroxide was added and allowed to stand at room temperature for 10 minutes. The optical density was read against purified water at 505nm. The enzyme activity was calculated from the

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

standard (Sodium pyruvate 2mM) calibration curve.

Assay of superoxide dismutase (SOD)

Superoxide dismutase activity was determined by NBT reduction method of McCord and Fridovich (1969). The reaction mixture contained, EDTA (6mM) containing 3 µg, NaCN ; riboflavin (2mM); NBT (50µM); KH_2PO_4-Na_2HP buffer (67mM, pH 7.8) and tissue homogenate to a final volume of 3.0 ml, the tubes were illuminated under incandescent lamp for 15 minutes. the optical density (O.D) at 530nm was measured before and after illumination. The superoxide dismutase (SOD) was determined by comparing the absorbance values of the control.

Inhibition of tissue lipid peroxidation

The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. 0.4 ml of tissue homogenate was treated with 0.2 ml SDS (8.1%); 1.5 ml TBA (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95°-100°C for 1 hour. After cooling, 1.0 ml distilled water, 5.0 ml n-butanol and pyridine mixture (15:1) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was removed and its absorbance at 532 nm was measured. Inhibition of lipid peroxidation was determined by comparing the O.D of the treatment with that of control.

Inhibition of serum lipid peroxidation

The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. 0.4 ml of serum was treated with 0.2 ml SDS (8.1%); 1.5 ml TBA (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95°-100°C for 1 hour. After cooling 1.0 ml distilled water and 5.0 ml n-butanol and pyridine mixture (15:1) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was removed and its absorbance at 532 nm was measured. Inhibition of lipid peroxidation was determined by comparing the O.D of the treatment with that of control.

Catalase activity

catalase activity in mice liver homogenate was determined by the method of Aebi *et al*. The reaction mixture contained liver homogenate 0.1 ml in 1.9 ml phosphate buffer (0.2 mM : pH 7.0) mixed H_2O_2 instantly with the reaction mixture and measured hydrogen absorbance at 240 nm for 1 minutes interval at 4 times. Catalase activity was determined by comparing the optical density of treatment with that of control.

Assay of protein estimation

Procedure described by Lowry *et al.,*(1951) was used.

Procedure

10 µl of tissue homogenate (10%) was made up to 1.0 ml with distilled water. Mixed with 5.0ml of alkaline copper reagent, incubated at room temperature for 10 minutes in a final volume of 6.0 ml. After the incubation, 0.5ml of Follins reagent (1:1 with distilled water) was added and kept in room temperature for 30 minutes. The optical density of the coloured solution was read at 660 nm using the reagent blank. A standard curve was prepared similarly using various concentration of BSA (10-200 µg/ml).

Statiscal analysis

All experimental data were as mean ± SD.

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

Results

Antioxidant activity

Super oxide radical scavenging activity

Ethanol extract of the mycelim of *Morchella esculenta* was found to be a scavenger of superoxide generated by photoreduction of riboflavin. (Table 1). The extract showed superoxide inhibiting activity. The extract inhibited 47.13% superoxide generated at a concentration of 1000 µg/ml.

Inhibition of lipid peroxidation

Ethanol extract of the mycelim of *Morchella esculenta* was effective in inhibiting the lipid peroxidation induced by $Fe^{2\pm}$ ascorbate system in rat liver homogerate (Table-1). The generation of malondialdehyde (MDA) and related substance that react with thiobarbituric acid (TBARS) was found to be inhibited by the extract. This indicated the significant lipid peroxidation inhibition activity of the extract. The extract inhibited 56.2% lipid peroxidation at a concentration of 1000µg/ml.

Hydroxyl radical scavenging activity

The degradation of deoxyribose TBARS by hydroxy radical generated from $Fe^{2\pm}$ – ascorbate – EDTA – H_2O_2 system was markedly decreased by ethanolic extract of the mycelim of *Morchella esculenta* (Table-1). The extract inhibited 64.98% of hydroxyl radical at a concentration of 1000µg/ml. This indicated the significant hydroxyl radical scavenging activity of the extract.

Nitric oxide radical scavenging activity

Incubation of solution of sodium nitroperoxide in phosphate buffered saline at 25°C for 150 min. resulted in generation of nitric oxide. The ethanolic extract of the mycelim of *Morchella esculenta* effectively reduced the generation of nitric oxide radicals. The extract inhibited 54.07% nitric oxide radical generation at a concentration of 1000µg/ml. This showed marked nitric oxide scavenging activity of the extract (Table-1).

Hepatoprotective activity

In living system livers is considered to be highly sensitive to toxic agents. Hepatic dysfunction due to ingestion (or) inhalation of hepatotoxins are increasing world wide. Carbontetrachloride (CCl_4) is a well known dry cleaning agent, that produces acute hepatictoxicity.

In modern medicine, there is hardly any drug that stimulates liver function, offer protection to liver from damage and help regeneration of hepatic cells.

In the present study, a dose of CCl_4 was used to induce liver damage in the rats CCl_4 caused significant changes in the liver function enzymes. A significant elevation of serum marker enzymes namely GOT, GPT and ALP was observed.

Serum ALP levels registered drastic increase in CCl_4 treated rat as compared to normal. Administration of ethanolic extract of the mycelim of *Morchella esculenta* (250 and 500 mg/kg body wt.) also significantly reduced the increase in serum ALP levels (Table 2) Administration of (500 mg/kg body weight) extract reduced the increase in serum ALP levels to almost normal level.

Serum GOT and GPT levels registered a significant increase in CCl_4 treated rat as compared to normal. Administration of ethanolic extract of *Morchella esculenta* (250 and 500 mg/kg. body weight) significantly reduced the increase in serum GOT and GPT levels (Table 2).

Super oxide dismutase activities in the liver significantly decreased in CCl_4 treated rat as compared to the normal. Administration of ethanol extract of the mycelium of

IJCRCPS

International Journal of Current Research in ry

and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

Table .1 Invitro antioxidant activity of ethanol extract of mycelium of *morchella esculenta* (% inhibition)

Activities	Ethanol Extract		
	1000 µg/ml	500 µg/ml	250 µg/ml
Super oxide scavenging activity	47.13	41	26.78
Hydroxyl radical scavenging activity	64.98	55.53	45.07
Lipid Peroxidation inhibiting activity	56.25	53.12	40.62
Nitric oxide scavenging activity	54.07	50.61	40.62

Table.2 effect of ethanol extract of *Morchella esculenta* mycelium on hepatic enzymes consequent to the challenge with CCl_4 activities of GPT, GOT, ALP levels in serum (values are mean ± SD, for animals)

GROUP	TREATMENT (mg/kg)	SGPT	SGOT	ALP (KA/dl)
Normal		73±11.08	128±5.53	139.5±11.05
Control	CCl_4+paraffin oil(1:1)	428±60.3	281±15.9	373±62.14
CCl_4 + ME	500	181.33±24.7	140.3±8.9	181±7.52
CCl_4 + ME	250	284.33±22.7	190±12.05	252.5±25

ME = Mycelium Extract

Table.3 effect of ethanol extract of *Morchella esculenta* mycelium on hepatic antioxidant enzyme activities and lipid peroxide (MDA) levels consequent to CCl_4 challenge (values are mean± SD, for 6 animals)

GROUP	Treatment mg/kg	SOD (µg/mg protein)	CAT (µg/mg protein)	MDA	
				Tissue (nmole/mg protein)	Serum nmole/ml
Normal	--	18.25±1.57	63.47±5.7	1.30±.32	1.53±0.16
Control	CCl_4+paraffin oil(1:1)	1.39±.31	16.47±3.41	2.78±.44	2.66±0.52
CCl_4 + ME	500	10.91±4.2	58.80±7.2	1.38±.35	1.64±0.45
CCl_4 + ME	250	8.4±1.79	24.20±5	2.07±.13	2.24±0.17

ME = Mycelium Extract

IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 93-104
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

Morchella esculenta to CCl_4 treated animals significantly increased the SOD activities as compared to control group (Table 3). The catalase activity was significantly decreased in CCl_4 treated group as compared to normal. Administration of the extract of caused significant increase in catalase activity(Table3).The concentration of malondialdehyde in liver and serum significantly increased in CCl_4 treated group as compared to normal. Ethanolic extract of *Morchella esculenta* treatment significantly decreased malondiadehyde levels as compared to control group (Table 3) indicating the in vivo inhibition of lipid peroxidation.

Discussion

Reactive oxygen species (ROS) and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Sreejayan and Rao, 1997). A potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids, degradation of DNA and oligonucleosomal fragments (Hemanani and Prihar, 1998). Nitric oxide or reactive nitrogen species, formed during its reaction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4 and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage (Lin *et al.,* 1995). Mycelial extract of *Morchella esculenta* (50% ethanol) shows potent scavenging activity of free radical such superoxide, hydroxyl and nitric oxide and inhibition of lipid peroxidation activity. The significant antioxidant activity of the extract, thus suggests the possible therapeutic potentials of this mushroom.

Studies on the hepatoprotective activity in experimental model indicate that CCl_4 is first metabolized by cytochrome P_{450} in the liver endoplasmic reticulum to the highly reactive CCl_3 radical. The main cause of CCl_4 induced liver injury is lipid peroxidation by free radical derivaties of CCl_4 (Recknagel, 1967).

The results of the present study reveal that the crude ethanolic extract of *M.esculenta* mycelium (250,500 mg/kg) have preventive action of CCL_4 induced hepatotoxicity in a dose dependent manner. The amelioration of liver toxicity by the extract is evident from its significant effect on the serum transaminases (SGOT and SGPT) and Alkaline Phosphatase (ALP) levels. The protective effect of the extract of *M.esculenta* on CCl_4 induced hepatoxicity was evident from the increase in the generation of antioxidant enzymes like superoxide dismutase and catalase. The lipid peroxidation was also lowered by the administration of the extract. This indicates that antioxidant activity of the extract plays a significant role in the amelioration of hepatoxicity induced by CCl_4. The marked *in vitro* antioxidant activity of the extract also support this conclusion. The experiment results reveal that ethonolic extract of the mycelium of *Morchella esculenta* possess profound antioxidant and hepatoprotective effects. The findings thus suggest the potential therapeutic use of moral mushroom mycelium.

References

Abei.,E, Gergmeyered, H.U.1974. Catalase estimation, Method of enzymatic analysis, Verlag chem., Weinheim, 2, 673-684.

Ajith.T.A., Janardhanan, K.K. 2002. Antioxidant, Antihepatoxic activity of wood inhibiting macrofungus P*hellinus rimosus*, J.Ethanopharmacol., 81, 387-391.

Bastis, M., Ashwood.S 1994. Protective effect of carrot (Daccus carota) against lindane induced hepatotoxicity in rats. Journal of Elhanopharmacol, 12, 434-436.

Babior, B.M. and Woodman, R.C. 1990. Chronic Granulomatous disease, 27, 247-259.

Beal, M.F. 1997. Oxidative damage in neurodegenerative diseases, Neuroscientist, 3, 21-27.

Bishyee, S. and Balasubramanian, A.S. 1979. Assay of lipid peroxidation, J.Neuro.Chem., 16, 909-913.

Byun, S.H. and Kim, D.H. 1987. Studies on the concurrent administration of drugs VII, Effects of concurrent administration of *Ganoderma Lucidium* on the liver damaged induced by carbon tetrachloride in rats, 31, 133-139.

Chang, R. 1996. Functional properties of edible mushrooms, Nurt Rev., 45(11), 91-93.

Chang,S.T., Arora,K.K., Mukerji, K.G., Marth, E.H., Marcel Deckkes. 1994. Hand Book of applied Mycology, New York, Based Hong Knog, 3, 221-240.

Chatterjee, S.1995. Effect of Optiliv on alcohol plus CCl_4 induced hepatotoxocoty in albino rats, Ind.J.Indg. Med., 7, 27-31.

Cornelius, C E. 1963. Relation of body weight to hepaticglutamic pyruvic transaminase activity,Nature 2002, 580-581.

Duncan,C.J., Pugh.N., Pasco,D.S., Ross, S. A. 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*, J. Agric. Food Chem., 50(20), 5683-85.

Eisenhur, R and Fritz, D.1991. Medically effective and health promoting compounds of edible mushrooms. Gartenbrauwis-seneschaft, 56(6) 266-270.

Fridovich, I. Beal M.F. 1989. Superoxide dismutase, an adaptation to a paramagnetion gas., J. Biol. chem., 264, 7761-7764.

Fridovich, I. 1985. The Biology of Oxygen radicals., Science, 120., 875-881.

Frie, B.1994. Reactive oxygen species and antioxidant vitamins, Mechanism of action, AM.J.Med., 97, S5-S8.

Goetz, M.E., Kunig, G., Riederer, P and Youdin. 1994. Oxidative stress, Free radical production in neurnal degeneration, Pharmacol Therapy, 63, 122-123.

Guillouzo, A. 1998. Liver all models in *invitro* toxicology. Environmental Health perspective, 106(2), 511-532.

Gunde and Cinermann, N.1999. Medicinal value of Genus Pleurotus P.Krast (Agaricutes S.I, Basidomycetes), Internat.J.Med.Mushrooms, 1, 69-80.

Guzman, G.1994. The fungi in the traditional medicine in Meso Americans and Mexico ,Revis Iberoamericana Micol., 11(3), 81-85.

Hansford, R.G., Hogue, B. A. 1997. H_2O_2 formation rat hat mitochondria on substrate availability and donor age, Bioenergy Biomem., 29, 89.

Halliwell B., Gutteridge, J.M.C. 1986. Free radicals in Biology and Medicine, Oxford University press, New York, 186, 139-189.

Halliwell, B. John H and Gutteridge, J.M.C. 1989. Production of hydroxyl radicals in living systems, Free Radicals Biology and Medicine, Clarendon Press, Oxford, 121, 31.

Halliwell, B., Gulteridge, J.M.C.1984, Lipid peroxidation oxygen radicals, Cell damage and antioxidant therapy, Lancet, 1396-1397.

Halliwell, B., Gulteridge, J.M.C.1985, Free radicals in biology and medicine, New York, 1st edt. 279-313.

Hemanani, T. and Prihar, M.S. 1998. Radioactive oxygen species and oxidative DNA change, Indian J. Physiology Pharmacology, 42, 44-45.

Irshad, M and Chaudhary.2002. Oxidant antioxidant system, Role and significance in human body, Indian Journal of Experimental Biology, 40, 1233-1239.

Janardhanan, K.K. 1999. Medicinal Mushroom, Amala Research Bulletin, 19, 23-29.

John Alexopoulous. 1979, Introductory mycology 2nd Edition, Wiley Eastern Pvt. Ltd., New Delhi.

Kamagai, Y. Asimoto, T., Yoshikawa, T. and Sagai, M. 1997. Generation of reactive oxygen species during intraction of diesel exhaust- particle components with NADPH- cytochrome reductase and involvement of the bioactivation in the DNA damage, Free radical Biol. Med., 22, 479-487.

Kensler, T.W. 1997 Chemoprevention by inducer's of carcinogen detoxification enzymes, Environmental Health perspectives. 7, 125-130.

Kind, Prn, King, F.J. 1980. Method of Kind and King. In:Varely, H., Gowenlock, A.H., Bell,M., Method of Practical Clinical Biochemistry,1, 899-900.

Koppenol, W.H., Moreno, J.J., Pryor, W.A. et al., 1992. peroxinitrate, A cloaked oxidant formed by nitric oxide and super oxide, Chem. Res. Toxicol., 5, 834-835.

Lieber, C. S. 2003. Alcoholic liver disease New insight in pathogenesis lead to new treatments, J.Hepatology, 32, 113.

Lin, Z. B., Sun, M. G., Chai, B. 1982. Effect of Tremella-polysaccharide and the activity of macrophages homogenic function of bone marrow and synthesis of proteins, J.Trade.Chin.Med, 23, 389.

Lin, J.M., Chen,C.C., Ujiie, T., Takada, A. 1995. Radicals scavenging and antihepatotoxic activity of *Ganoderma lucidium*, *Ganoderma neojapanicum*,J. Ehano. Pharmacol, 47, 33-41.

Lin, J.M., Lin, C.C., Chiu, H.F., Yang, J.J. 1993. Evaluation of anti-inflammatory and liver protective effect of *Ganoderma lucidium* in rats, Am. J.Chin. Med., 21, 59-69.

Liu, G.T., Bao T.T., Nia, X.U.L. and song, Z.Y.C. 1980. Some pharmacologic actions of the mycelium of *Ganoderma lucidium* and the mycelium and the mycelium of *Ganoderma capense* Teng cultivated by submerged fermentation. Chin.Med.Jer. 92, 496-500.

Lockitch, G. 1989. Clinical significance and analytical concepts, Crt. Rev. Clin.lab. Sci., 27, 483.

Lowry, H.D, Rosenberg, N.J., Farr, A.L. and Randa R.J. 1951. Protien measurement with Folin phenol reagent, J.Biol.chem., 193, 265-275.

Marcocci, L., Maguire, J.J., Droy-lefaix, M.T., Packer, L. 1994. Nitric oxide scavenging properties of *Gilkgo bibolo extract Egb* 761, Bioch. And Biophy. Res. Comouni., 201, 748-755.

Markland, S.L., Holme, E., Hellner, L. 1982. Superoxide dismutase in extracellular fluids, Cli. Chin. Act., 126, 41.

McCord,J.M. and Fridovich.J. 1969. Superoxide dismutase an enzymatic function of erythrocuperin, J.Bio.Chem., 244, 649-650.

Nautyal, S., Maikhuri, R. K., Rao, K. S. 2001. Journal Herb. Spices Med. Plants, 8, 47-64.

Ohkawo, H., Oshishi, W., Yagi, K. 1979, 1995. Assay for peroxidase in animal tissue by thiobarbituric acid reaction, Biochemistry, 95, 351-358.

Oshino, N. 1973. The role of H_2O_2 generation in perfused rate liver and the reaction of catalyses compound and hydrogen donor, Arch. Biochem. Biofis., 157.

Parihar, M.S., Manjula, Y., Bano,S., Hennan,T., Javeri.T., Prakash. 1997. P.Nicotinamide and α-tocophorl combination particularly protects tetrabutylhydroperoxide neurotoxicity . implication for neurodegenerative, Curr.sci., 73(3), 290-293.

Prakash, P. Parihas, M.S., Dubey, A.K. 1996 changes in lipid peroxidation, Superoxide dismutase activity, ascorbic acid and phospholipid contents in liver of fresh water catfish Heperopheustes fossils exposed to elevated temperature. J.Therm.Biol,. 21(5), 323-330.

Prasad, P, Chauhan-Kusam, Kandari, L.S., Maikhuri,R.K., Purohit Aditya, Bhatt, R.P. And Rao, K.S. 2002. *Morchella*

esculenta, Current Science, 9, 1098-1099.

Ray, G., Batra, M. Levy, A. 2000. Lipid peroxidation Free radical production and antioxidant status in breast cancer, Breast cancer Res. Treat,. 54, 163.

Reckngel, R. O. 1967. Carbon tetrachloride hepatotoxicity, Pharmacology review, 19, 145-208.

Retiman, S., Frankel, S. 1957. A calorimetric method for the determination of serum glutamicoxaloacetate and glutamate pyruvic transaminase, Am.J.Clinical Path. 28, 56-63.

Sies, H. 1997. Oxidative stress, oxidant and antioxidants, Experimental Physiology, 82, 291.

Sreejayan, Rao, M. N. A. 1997. Nitricoxide scavenging by curcuminoids, Journal of Pharmacy and Pharmacology, 49, 105-107.

Stobs,S.J., Bagehi, D. 1995. Oxidative mechanism in the toxicity of metal ions, Free Radical Biol.Med., 18, 321-336.

Subrahmanian,C.V. 1995. Mushrooms Beauty Diversity relivance, Curr.Sci., 69, 998-1112.

Uppu, R.M., Squadrito, G.C., Pryor, W.A., 1996. Acceleration of peroxynitrite oxidation by CO_2, Arch. Biochem., 19, 335-343.

Wasser, S.P. and Weiss, A.L., 1999. The edible and medicinal Mushroom Industry in Australia, Int.J.Med.Mushrooms,2, 1.

Wasser,S.P. and Weis, A.L(1993).medicinal properties of mushroom occur in the higher Basidomycetes mushrooms, current perspective, Int.J.Med. Mushroom, 31.89-96.

Wei,Y.Zhang, .T.Y. 2001. Counter current chromatographic separation , J.Chromatgr., 917(1-2), 347-351.

Yoshikawa,T., Tanaka.H., Yoshida,N., Seto,O, and Kondo,M., 1983, Adjuvant arthritis and lipid peroxide protection by superoxide dismutase, Lipid peroxide Res., 7, 1083.

Yoshikawa, T. Tanaka H, Yoshida,N. Seto,O. and Kondo,M. 1993. Adjuvant arthritis and lipid peroxide protection by superoxide dismutase lipid peroxide, 7, 108-110.