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Original Article

FREE RADICAL SCAVENGING ACTIVITY OF *PLEUROTUS OSTREATUS* AGAINST CCL_4-INDUCED HEPATIC DAMAGE IN WISTER RATS

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ABSTRACT

Objective: The present study was aimed to evaluate the anti-oxidant effect of *Pleurotus ostreatus* using *in vitro* and *in vivo* methodology.

Methods: Methanolic extract of *Pleurotus ostreatus* (MEPO) was prepared by cold maceration technique. Rats weighing 150-200 g were divided into 6 groups of six each (n=6). Group I served as control (0.9% Nacl), group II was considered as the positive control, administered with CCl_4 dissolved in olive oil (intraperitoneal injection at 0.5 mg/kg body weight), group III was given with CCl_4+standard drug sylimarin (100 mg/kg body weight, p. o.), group IV, V and VI were administered with CCl_4+MEPO at 200, 400 and 800 mg/kg orally for a period of 21 d. The liver injury was induced by the administration of CCl_4 intraperitoneally with a single dose of CCl_4 (0.5 mg/kg body weight) as a 1:1 (v/v) solution in olive oil.

Results: In the Hydroxyl free radical scavenging assay, the IC_{50} values of the test extract was found to be 72.1±1.68 as compared with the standard drug (IC_{50}-66.3±1.02), which was considered as significant (***p<0.001). In the reducing power assay, all the absorbance values were comparable to standard ascorbic acid and showed maximum reducing ability at the concentration of 500 µg/ml. In the *in vivo* anti-oxidant study, administration of MEPO to injury-induced rats at the dose of 400 and 800 mg/kg, significantly increased (**p<0.001) the levels of SOD and CAT, with a significant reduction in the MDA levels.

Conclusion: The current study explored a potential source for anti-oxidants in the methanolic extract of *Pleurotus ostreatus,* thus seems to be protective to liver in CCl_4-induced hepatic injury.

Keywords: *Pleurotus ostreatus*, Anti-oxidant effect, Reactive oxygen species, Hepatic damage

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INTRODUCTION

Oxidative stress comprises an imbalance between the formation of reactive oxygen species (ROS) and the scavenging system by antioxidants [1]. An uncontrolled production of deleterious ROS substantially causes serious damage to the proteins, lipids and DNA. A biological system has ROS as a critical type of free radicals, developed through the electron transport chain present in mitochondria. Free radical generation contributes to human disorders such as aging-related diseases, cardiovascular diseases, cancer and inflammatory diseases [2]. Additionally, free radicals deplete the anti-oxidant system, alter gene expression and may generate the induction of protein synthesis abnormally. More than or equal to 5% of the oxygen (O_2) inspired is converted to reactive oxygen species (ROS) such as O_2^{--}, H_2O_2, and OH radicals. In the majority, the liver controls all the functions such as digestion of food, glycogen reserve, metabolic control, detoxification of drugs and production of hormones [3]. The liver is considered a detoxifying organ, *in vivo* liver systems represent a better experimental approach to generating free radicals and investigating antioxidant agent effects.

In order to neutralize the free radical generation, anti-oxidants are used. They combat free radicals and protect all humans against infestations and certain diseases. Anti-oxidants exist in two forms–Natural and synthetic. Butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters are the synthetic ones that act as chelating agents, thereby effects the participation in the process. In humans, there might be every chance that synthetic anti-oxidants promote negative consequences like mutagenesis and carcinogenesis [4]. As a result, there is a growing tendency to substitute synthetic antioxidants with naturally occurring ones that can prevent diseases caused by free radicals.

Free radicals and activated oxygen species generation are regulated by natural antioxidants, and they can also prevent their reaction with biological structures. Such anti-oxidants include antioxidative enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and small nonenzymatic antioxidant molecules, such as glutathione and vitamins C and E [5]. Apart from these natural ones, many herbs, spices such as sage, basil, pepper, clove, nutmeg and saffron and extracts of plants with their medicinal properties and ethnobotanical uses were found to possess anti-oxidant components.

Mushrooms have been prized for their distinctive flavour and delicate aroma in gourmet cuisine all over the world since ancient times [6]. Many species of mushrooms possessed and generated numerous of inexplicable biological properties [7]. The nutritive value of Mushrooms was found to have much importance in clinically due to the presence of these bioactive compounds. They stood as a remedy for plenty of diseases and also for nourishment [8]. Though mushrooms have a past history of their use in medicine, in the present time few contemporary studies have promoted them in the maintenance of good health and vivacity. There are over 2,70,000 plants on the planet., out of which a tiny part has been explored phytochemically. Amongst the medicinal plants, mushrooms were found to possess approximately high amounts of carbohydrates, proteins, fibre and low levels of fat; rich in vitamins. They also contain bioactive phenolic compounds, carotenoids, and unsaturated fatty acids all of which might help to combat medical conditions via a few properties like anti-oxidant, anti-inflammatory anti-fungal, anti-bacterial, anti-hypertensive, hepatoprotective, anti-allergic, anti-diabetic, and anti-cancer. The fungal species *Pleurotus ostreatus*, known as the oyster mushroom, was noted for being an edible variant. During World War I, it was initially grown as a survival strategy in Germany [9]. Although it may be grown on straw and other materials, one of the more popular wild mushrooms is the oyster mushroom. It smells like benzaldehyde, just the same as bitter almonds [10].

Pleurotus species grow on sawdust, wood and wet areas, require a temperature of 10–32 °C for their growth, and possess medicinal benefits in the traditional system of medicine. Among the different varieties of mushrooms available, considering the medicinal

potential of *Pleurotus ostreatus*, researchers focused and began to investigate the therapeutic efficacy. Globally, the most grown and edible species among the mushrooms is *Pleurotus ostreatus*, (Oyster mushroom).

The natural anti-oxidants present in the human body may fall in deficiency to neutralize the generated free radicals. It follows that adding antioxidants to our diet will help us prevent hazardous illnesses. It becomes easy to consume medicine as a part of our regular diet. Therefore, there is every need to develop Natural Anti-oxidants from natural sources such as plant materials. The fundamental function to promote metabolic equilibrium in the body was taken up by the liver [11]. Whenever a toxicant enters the liver, disrupts or obstructs the liver's production and transportation activities, which may result in malfunction without significantly harming cells. The severity of the damage, the number of cells impacted, and whether the effect is acute or chronic have a significant impact on how sensitive the liver is to chemical assaults. In this context, carbon tetrachloride (CCl_4) was the most commonly used hepatotoxin to induce oxidative stress caused by liver injury. The toxic effects of carbon tetrachloride (CCl_4) were associated with the high content of cytochrome P-450. Anti-oxidants act by antagonizing the harmful effects of free radicals (or) ROS, thus serving as protection to hepatocytes against damage [11]. In the current research, Silymarin was used as a standard drug for *in vivo* anti-oxidant activity. Globally Silymarin was used for all types of liver-related diseases due to its hepatoprotective property. From the research studies, the efficacy of silymarin as an anti-oxidant was emphasized, acts by inhibiting the liver damage along with the maintenance of integrity of the plasma membrane. It also suppresses the release of liver enzymes into the circulation, and halts the apoptosis process in hepatocytes [12].

In the present study, in the evaluation of test drug by *in vitro* method, Ascorbic acid was used as a standard anti-oxidant agent, it acts by participating in oxidation. Ascorbate gets oxidized into monodehydroascorbate and then dehydroascorbate in the presence of ROS, thereby ROS gets reduced to water, while the oxidized form remains stable without causing damage to cells.

This current study involved the investigation of the methanolic extract of *Pleurotus ostreatus* on the oxidative status of carbon tetrachloride (CCl_4) induced oxidative stress in experimental animals.

MATERIALS AND METHODS

Materials

The materials were procured from different companies/sources. Sylimarin was a procured from the Rajiv Enterprices, India. CCl_4 was ordered from Sigma Chemicals Co., St. Louis USA. Other chemicals like HCl, KCl, EDTA, H_2O_2, potassium ferricyanide, trichloro acetic acid and ethanol were bought from SRL Pvt Ltd. Ascorbic acid was bought from SRL Pvt Ltd, while Mannitol was procured from Ralington Pharma LLP, Gujarat, India.

Plant collection and authentication

Pleurotus ostreatus were obtained from the local places of Tirupati, AP. *Pleurotus ostreatus* was authenticated by Dr. K. Madhava Chetty, M. Sc., M. Ed., M. Phil., Ph. D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh with a specimen number PS/SV/201/23.

Methods

Samples preparation and extraction

The *Pleurotus ostreatus* fungus was thoroughly cleaned with water, evacuated and then thinly sliced. Then dried at room temperature and strained through Sieve Mesh 60, again dried in the oven at 450 °C. A cold maceration technique was used to extract 150 g with 500 ml of methanol for 72 h along with agitation. By means of a muslin cloth, the solution was filtered, then filtered through Whatman No. 1 filter paper and obtained a stock solution of plant extract. The extract was concentrated in a rotary evaporator that was held at a temperature of no more than 600 °C. About 20 ml of the extract was collected and dried in an oven at 450 °C, leaving behind a semi-liquid. The extract thus obtained was utilised for further testing and analysis [13].

Phytochemical screening

The presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates was carried out by standard tests in methanolic extract of *Pleurotus ostreatus* (MEPO) [13,14].

Evaluation of oxidative stress by *in vitro* and *in vivo* methods

Hydroxyl radical scavenging assay

An assay described by Elizabeth and Rao was followed with slight modifications. This particular assay competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (The Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in Thiobarbituric acid reactive substances (TBARS) formation. The reaction mixture contained deoxyribose (2.8 mmol), $Fecl_3$ (0.1 mmol), H_2O_2 (1 mm), ascorbate (0.1 mmol), KH_2PO_4-KOH buffer (20 mmol, pH 7.4), EDTA (100 µM); H_2O_2 (1.0 mmol); Ascorbic acid (100 µM) and various concentrations of 50–200 µg/ml) of the test sample or reference compound. This was incubated t 37 °C for 1 h, then added 1 ml 2.8% Trichloro acetic acid (TCA), then 1 ml 1% aqueous Thiobarbituric acid (TBA), then the whole mixture was incubated for 15 min at 90 °C for the development of color. The mixture was cooled and absorbance was measured at 532 nm, readings were recorded in triplets. Mannitol, a classical OH. Scavenger was used as a positive control [15].

The percentage inhibition was calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \text{ X } 100$$

$IC_{50,}$ which is the concentration of sample required to scavenge 50% of the free radicals, was calculated.

Reducing power assay

A method developed by Oyaizu, 1986 was used to determine the reducing power assay. Different concentrations of extracts were prepared and mixed with 2.5 ml of phosphate buffer (0.2M) (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was kept in a water bath at 50 °C for 20 min. After cooling, 2.5 ml of 10% Trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. 2.5 ml of the upper layer of solution was mixed with 2.5 ml of distilled water and freshly prepared 0.5 ml (0.1%) of $FeCl_3$. The absorbance was measured at 700 nm. Control was prepared similarly, excluding the samples [16].

Acute toxicity tests

Toxicity studies were carried out per the OECD guidelines no 423**.** The extract was orally given to 8 groups of 6 rats each (n=6), with an initial dose of 50 to 2000 mg/kg body weight. The test drug-treated animals were kept under observation for toxicity signs, readings were noted after 48 h of keen observation to check the expected behavior and examine if there were any issues with nervous problems and any lethality [17].

Experimental animals

Wistar rats of weight 150-200 g were brought from the lab bearing a valid registration number, Hyderabad, India. Animals were housed in cages with water and food *ad libitum*, and the animal room temperature was kept at a constant temperature of 20±1 °C on a 12-hour light/12-hour dark cycle. The protocol was prepared and presented in the IAEC (Institutional Animal Ethics Committee) as per CCSEA and it was accepted with no: 1447/PO/Re/S/11/CCSEA-65/A.

Induction of hepatic injury by CCl_4

The liver injury was induced by the administration of CCl_4 (Sigma Chemicals Co., St. Louis USA). Before the administration of the extracts rats were injected intraperitoneally with a single dose of CCl_4 (0.5 mg/kg body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 h. CCl_4 was administered once a week for three weeks [18].

Grouping of animals

Wistar Albino rats were divided into 6 groups of six each (n=6). Group I served as control (0.9% Nacl), group II was considered as a positive control, administered with CCl_4 dissolved in olive oil (intraperitoneal injection at 0.5 mg/kg body weight), group III was given with CCl_4+standard drug silymarin (100 mg/kg body weight, p. o.), group IV, V and VI were administered with CCl_4+MEPO at 200, 400 and 800 mg/kg orally for a period of 21 d. On the 22nd d at the end of the experimental period, all the rats were sacrificed and the liver homogenate was prepared for anti-oxidative enzyme estimation.

Group I: Normal control received distilled water

Group II: CCl_4 in olive oil (intraperitoneal injection at 0.5 mg/kg body weight)

Group III: CCl_4 in olive oil+Silymarin (100 mg/kg, p. o.)

Group IV: CCl_4 in olive oil+MEPO (200 mg/kg, p. o.)

Group V: CCl_4 in olive oil+MEPO (400 mg/kg, p. o.)

Group VI: CCl_4 in olive oil+MEPO (800 mg/kg, p. o.)

Determination of Anti-oxidant enzymes by *in vivo* method

Superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidase (LPO) were endogenous enzymes that counteract the free radicals generated during liver damage. In the present study, levels of these antioxidant enzymes were estimated in the liver homogenate of the animals using standard procedures. The enzyme levels were expressed in terms of units/mg of protein.

Dissection and preparation of liver homogenate

The animals were euthanized under light anesthesia with ether and dissected to remove the liver.

Assay of superoxide dismutase (SOD)

The activity of superoxide dismutase was assayed spectrophotometrically by the method of Kono, 1978. Estimation of SOD is based on the detection of O_2^- by oxidation of hydroxylamine hydrochloride yielding nitrite, which is measured colorimetrically. O_2^- can also be generated during the autooxidation of hydroxylamine. Accompanying the autoxidation of hydroxylamine at pH 10.2 NBT is reduced and nitrite is produced in the presence of EDTA, which can be detected colorimetrically. 100 µl of 5 % of liver homogenate in 0.2 M sucrose in phosphate buffer (pH 7.4) was taken in the test tube; to this, a mixture containing 1 ml of sodium carbonate, 0.4 ml of NBT and 0.2 ml of EDTA was added and zero-minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 ml of 1 mmol Hydroxylamine hydrochloride to the test tube. The reaction mixture was incubated at 25 °C for 5 min, and the reduction of NBT was measured at 560 nm. A parallel control without liver homogenate was also treated in a similarly manner as the test [19].

Assay of catalase (CAT)

Catalase activity was measured by the method of Aebi, 1983. Catalase activity was done by determining the decomposition of H_2O_2 at 240 nm in an assay mixture containing phosphate buffer. Dissolve 100 µl of 5 % liver homogenate in 0.15 M KCl buffer and 1.9 ml of phosphate buffer pH 7 and absorbance was measured at 240 nm. To this reaction mixture,1 ml of 30 mmol hydrogen peroxide solution was added and the absorbance was measured after allowing it to stand for 1 min at 240 nm using phosphate buffer as a blank solution [20].

Estimation of lipid peroxidase (LPO)

The level of lipid peroxidation in liver homogenate was determined by the method of Okhawa 1979. The assay was based on the reaction of malondialdehyde (MDA,one of the products of lipid peroxidation) with TBA (Thiobarbituric acid) to form TBARs (Thiobarbituric acid reactive substances), which have a pink colour with absorption maxima at 540 nm [21].

Preparation of standard

A 10 m mol/l stock standard of MDA was prepared by dissolving 123.5 µl of 1,1,3,3–tetraethoxy propane in 50 ml ethanol (40% ethanol by volume). MDA was used as an external standard and different dilutions of 12.5,25,50 and 100 µmol/l were prepared with 1,1,3,3–tetraethoxy propane.

In the procedure, 5% liver homogenates were prepared by taking 1 g of liver in 20 ml of cold 0.15 M Kcl and centrifuging at 800Xg for 10 min. The supernatant 1 ml was collected and added to 1 ml TCA, 1 ml TBA and 1 ml HCl in test tubes. All the tubes were kept in a boiling water bath for 20 m at 80 °C, and then the tubes were kept in an ice bath and centrifuged at 2500X g for 10 m. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm allied with reagent blank without tissue homogenate. The amount of TBARs was expressed as nmoles/mg of protein.

Statistical analysis

The data values were compiled and represented in mean±SEM, analysed by one-way ANOVA followed by Dunnett's test in Graph Pad Prism 5. The groups were compared to that of control. Values were considered significant at p values: ***p<0.001, **p<0.01, *p<0.05, NS-Non significance.

RESULTS

Preliminary screening of phytochemical constituents

The preliminary phytochemical screening showed the presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEPO.

Acute toxicity studies

Acute toxicity tests revealed that there was no toxicity observed, and the MEPO was found to be safe for the study to be carried out because of no signs of death. The maximum tolerated dose of MEPO was 2000 mg/kg; the test doses were selected in a geometric pattern. Hence, in the current study, rats were administered lower, moderate and higher doses of methanolic extract of *Pleurotus ostreatus* (200, 400 and 800 mg/kg body weight, p. o).

Table 1: Percentage inhibition of MEPO in hydroxyl radical scavenging assay with IC_{50} Values

Concentration of test and standard drugs (µg/ml)	% Inhibition of hydroxyl radical	IC_{50} Values
MEPO (µg/ml)		
MEPO (100)	34.5±0.52	72.1±1.68
MEPO (200)	58.6±1.28	
MEPO (300)	75.41±1.52	
MEPO (400)	84.45±6.52	
MEPO (500)	87.45±1.52	
Standard drug (µg/ml)		
Mannitol (100)	44.1±0.26	66.3±1.02
Mannitol (200)	61.2±1.39	
Mannitol (300)	78.67±1.09	
Mannitol (400)	81.45±1.43	
Mannitol (500)	95.89±1.72	

Number of experiments (n=3); mean±SEM

Hydroxyl radical scavenging assay

In the present study, when compared with the control, the MEPO considerably scavenged the hydroxyl radicals produced by the EDTA/H_2O_2 system at all tested doses of 100, 200, 300, 400 and 500 µg/ml, respectively. The amount of OH^-radicals that MEPO was able to scavenge increased in a dose-dependent manner. As compared to Mannitol at a higher concentration of 500 µg/ml (95.89±1.72 as % of inhibition), the test extract produced 87.45±1.52 as % of inhibition. The IC_{50} values of test extract were found to be 72.1±1.68 as compared with the standard drug (IC_{50}-66.3±1.02), which was considered as significant (***p<0.001) (table 1 and fig. 1)

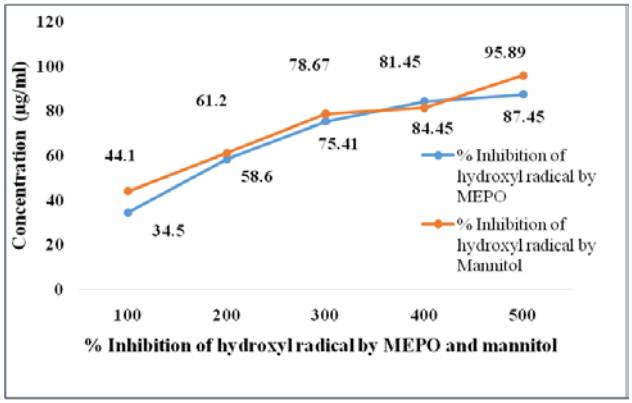

Fig. 1: Percentage (%) inhibition of MEPO and standard drug mannitol in hydroxyl radical scavenging assay, number of experiments (n=3); mean±

Reducing power assay

In the reducing power assay method, the reducing power of the test extract was determined and compared to the standard used; absorbance with increased concentration significantly increased the reducing power. The MEPO showed an absorbance of 1.98 at 500 µg/ml (table 2). All the extracts exhibited a significant (**p<0.001) reducing ability. All the absorbance values showed maximum reducing ability at 500 µg/ml comparable to standard ascorbic acid. The standard graph for both the Hydroxyl radical scavenging assay and the Reducing power assay was displayed in fig. 2.

***In vivo* anti-oxidant study**

The intensified free radical production causes damage to proteins and DNA, which finally.

Table 2: Absorbance of MEPO and standard Ascorbic acid at various concentrations in reducing power assay

Concentration (µg/ml)	Methanolic extract of *Pleurotus ostreatus*	Ascorbic acid
50	0.20±0.005	0.35±0.003
100	0.22±0.005	0.50±0.003
200	0.78±0.005	1.02±0.005
300	1.01±0.005	1.59±0.005
400	1.20±0.005	1.90±0.005
500	1.98±0.005	2.50±0.001

Number of experiments (n=3); mean±SEM

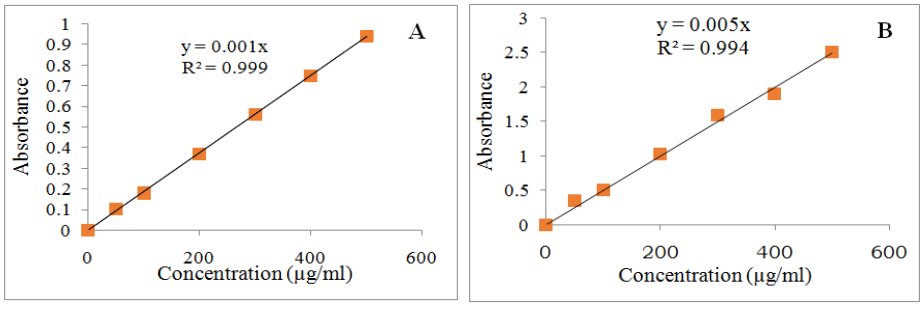

Fig. 2: Standard graph of Ascorbic acid in A) Hydroxyl scavenging assay and B) Reducing power assay

Table 3: Effect of methanolic extract of *Pleurotus ostreatus* (MEPO) on anti-oxidant enzymes in liver

Groups	Treatment and dose (mg/kg)	SOD (U/mg of protein)	CAT (U/mg of protein)	MDA (µmoles/mg)
I	Normal	56.5±0.85	20.25±1.83	14.34±1.37
II	CCl_4-induced hepatic injury (CI) (0.5 mg/kg, i. p.)	17.67±0.88[a]	5.17±1.16[a]	46.33±1.69[a]
III	CI+Sylimarin (100, p. o.)	48±0.98	11.67±0.95	18±1.48
IV	CI+MEPO (200, p. o.)	15.5±0.76[a]	6.91±0.54[a]	45.17±1.14[a]
V	CI+MEPO (400, p. o.)	20.6±1.22[a]	9±1.06[a]	27.75±2.52[a]
VI	CI+MEPO (800, p. o.)	28.7±1.07[a]	11.08±0.73[a]	25.92±2.70[a]

Number of experiments (n=3); mean±SEM [a]p<0.001; compared to positive control

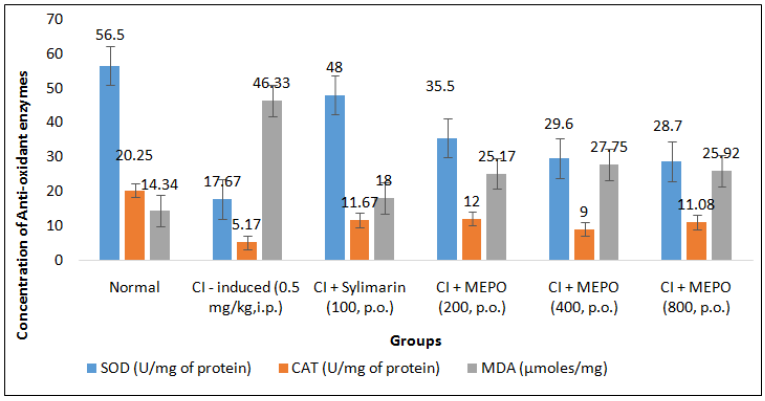

Fig. 3: Effect of methanolic extract of *Pleurotus ostreatus* (MEPO) on anti-oxidant enzymes in liver, number of experiments (n=3); mean±

The results of the *in vivo* antioxidant activity in experimental animals were evaluated and were shown in table 3. The present exploration in table 3 and fig. 3 showed that there is a significant increase (**p<0.001) in liver MDA levels, with a significant decrease (**p<0.001) in SOD and CAT in CCl_4 treated animals in comparison with normal rats. Administration of MEPO to injury-induced rats daily for a period of 21 d at the dose of 400 and 800 mg/kg, significantly increased (**p<0.001) the levels of SOD and CAT. Also there was a significant reduction in the MDA levels.

DISCUSSION

Free radicals are produced continuously in living systems and can significantly harm tissues and biomolecules, resulting in a variety of diseases, including degenerative disorders and significant lysis. About the different treatment options, there are several synthetic drugs available to combat oxidative damage but present adverse effects [22]. Hence, to resolve this problem, it is always better to consume anti-oxidants naturally from the regular diet and might act as a medicine obtained from a plant source. Many natural antioxidants have recently been recognized from various herbs. In this context, Oyster mushroom (*Pleurotus ostreatus*) is a culinary mushroom with a potential source of a plethora of essential nutrients and also the presence of bioactive compounds with their therapeutic effects. The bioactive compounds include peptides, polysaccharides, liposaccharides, glycoprotein, lectin, triterpenoid, fatty acids, essential amino acids and nucleosides [7]. Globally, as mushrooms can be cultivated commercially, it is quite easy for mankind to include them in their regular diet; they can be preferred as food and medicine. It also contains proteins, carbohydrates, vitamins, amino acids, lipids and fibres. *Pleurotus ostreatus* is produced commercially for its culinary, nutritive, and therapeutic benefits. Its ethnobotanical uses emphasize the presence of nutrients such as vitamins B1 (Thiamin) B2 (Riboflavin), B3 (niacin), B9 (folic acid), and ascorbic acid, also it contains internal polysaccharides and exopolysaccharides were found to possess anti-oxidant properties. *Pleurotus ostreatus* has a huge potential to generate unique, value-added products that promote health [8].

In the current investigation, the preliminary phytochemical screening *Pleurotus ostreatus* revealed the presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEPO. The test extract was subjected to an anti-oxidant effect by *in vitro* methods. Maximum biological damage and lipid peroxidation occur because of hydroxyl radicals. In the present study, these are produced by incubation of ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4; when *Pleurotus ostreatus* extract was added to the reaction mixture, the hydroxyl radicals were removed, thereby prevent the reaction. The IC_{50} value indicated that the plant extract is a better hydroxyl radical scavenger as comparable with the standard Mannitol. Similarly, in reducing power assay, the MEPO showed good radical quenching ability as evidenced from the results [23]. The test extract showed maximum and significant *in vitro* anti-oxidant activity. These results indicated the potential anti-oxidant effect of the extracts, which may be due to the presence of phenolics, which are known anti-oxidants. Phenolic compounds are metabolites of plants responsible for colour, nutritional benefits and anti-oxidant properties. It has been reported that there is a strong relationship between phenolic content and anti-oxidant effect.

In the evaluation of anti-oxidant activity, the hepatic injury was induced by CCl_4, a toxicant for the liver, which undergoes metabolism by cytochromes, might be CYP3A, which substantially results in the formation of Trichloromethyl radical, [CCl_3]. [CCl_3]– acts by binding to biological molecules like proteins, lipids and nucleic acids. Hence, it alters the physiological processes, also combined with the oxygen, that triggers lipid peroxidation by [CCl_3 OO]-attacks and destroys polyunsaturated fatty acids [24]. Eventually, the cystolic calcium disrupts, leading to cell injury. The *in vivo* antioxidant study revealed that there was a reduction in anti-oxidative enzymes like SOD and CAT level, and increased levels of MDA concentration which determined the level of LPO in the CCl_4-induced liver tissue [25]. With the treatment of test extract, there was increased activity superoxide dismutase (SOD) and catalase in the liver tissue, with a subsequent decline in the MDA levels. Catalase acts by causing the decomposition of hydrogen peroxide, a ROS, SOD acts by catalysing dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the chances of reaction of superoxide anion with nitric oxide to form reactive peroxynitrite. This increased levels of CAT and SOD explored the *in vivo* anti-oxidant effect, thus mitigating the effect of ROS and damage [26].

In the treatment of many liver diseases, Silymarin has a protective effect for liver damage. Previous studies demonstrated a robust anti-oxidant activity of the drug and exhibited promising protectiveness against toxicity of the liver, by impeding the lipid peroxidation [27]. The presence of phenols in Silymarin is attributed to the anti-oxidant property which in turn is linked to hepatoprotective effect [28, 29]. In the present investigation, the anti-oxidant effect of methanolic extract of *Pleurotus ostreatus* was confined to the presence of flavonoids, which belong to the polyphenolic substances. Some studies reported that flavonoids in the plants exhibited anti-oxidant properties and possess the capability of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals respectively. These in turn stand responsible for their respective pharmacological actions.

CONCLUSION

In conclusion, our study illustrated that the methanolic extract of *Pleurotus ostreatus* had a remarkable effect on oxidative stress in CCl_4 challenged animals. Keeping given the above considerations, it is explored that *P. ostreatus* possess hepatoprotective property with therapeutic effect in a clinical point of view and can be used for the treatment of human liver-associated diseases. Additionally, as the test drug has nutritive value, serves as a functional food and medicine significantly. As it is broadly used for nourishment and has medicinal value, it can be incorporated into the diet and would always have a probability to combat the generation of oxidative stress and related diseases or disorders. For a decade, the *Pleurotus*

ostreatus has been used as a source of therapy and added as a food supplement. Therefore, regarding the proper utilization in the future, an awareness can be created amongst the consumers.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors contributed for the study. Darna Bhikshapathi., designed and involved in readiness of the manuscript. Lakkaraju Chandana., conducted the experiment, analysed and compiled the results and prepared the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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