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PROTECTIVE EFFICACY OF THE EXTRACT OF *VOLVARIELLA VOLVACEA* (BULLIARD EX FRIES) SINGER. AGAINST CARBONTETRACHLORIDE INDUCED HEPATIC INJURY

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ABSTRACT

Carbon tetrachloride is a xenobiotic that produces hepatotoxicity. Aqueous extract *Volvariella volvacea* (500, 1000 mg/kg, p.o) showed significant hepatoprotective activity against carbontetrachloride induced hepatotoxicity in rats by normalizing the levels of serum AST, ALT, ALP, LDH and total bilirubin. The extract improved the activity of Catalase (CAT), Superoxide dismutase (SOD), and hepatic glutathione (GSH) content and depleted the lipid peroxidation levels in a dose dependent manner. Silymarin was used as the standard drug.

INTRODUCTION: Liver, the largest organ in the vertebrate body, is the major site of intense metabolic activities. Liver injury induced by chemicals and drugs is a well-recognized toxicological problem. The hepatotoxicity possibly results from toxic intermediates that bind covalently to the hepatocytes, causing a centrilobular hepatic necrosis. Liver diseases are a serious health problem. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [1].

Carbon tetrachloride (CCl_4) is a xenobiotic producing hepatotoxicity in human beings and animals [2]. In fact, it has been shown that the trichloromethyl radical, formed in the metabolism of CCl_4 via the liver microsomal cytochrome P450 system, reacts rapidly with molecular oxygen to produce trichloromethyl peroxy radical. These radicals react with unsaturated fatty acids of phospholipids present in cell membranes, initiating lipid peroxidation (LPO) in liver cells [3]. This leads to the formation of lipid peroxides, and a depression of protein synthesis [4] and elevated levels of serum marker enzymes such as AST, ALT and ALP[5]. The antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced hepatopathy [6].

CCl_4-induced liver injury is therefore considered as a model for the screening of hepatoprotective drugs [7]. A number of investigators have previously demonstrated that antioxidants would prevent CCl_4 toxicity by inhibiting lipid peroxidation [8, 9].

Many natural products of herbal origin are in use for the treatment of liver aliments. Phytochemicals derived from plants are excellent antioxidants. Antioxidants appear to act against diseases by raising the levels of endogenous defense, by up-regulating gene expressions of the antioxidant enzymes [10, 11].

Edible mushrooms are nutritionally endowed fungi. Mushrooms accumulate a variety of qualitatively good protein, crude fiber, minerals and vitamins but are poor sources of lipids and are rich in secondary metabolites [12].

Various mushrooms viz. *Ganoderma lucidum* [13, 14], *P. ostreatus* [15] *Antrodia camphorate* [16], *Phellinus linteus* [17]and *Phellinus rimosus* [18] have found to possess protective effects against liver diseases. *Volvariella volvacea* (Bulliard Ex Fries) Singer. is generally known as rice straw/ paddy straw mushroom, is cultivated throughout East and Southeast Asia and used extensively in Asian cuisines The mushroom is found to possess antibacterial activity [19, 20].

The present study was undertaken to evaluate the hepatoprotective potential of the aqueous extract of *Volvariella volvacea* (Bulliard ex Fries) Singer. against CCl_4-induced liver injury.

MATERIALS AND METHODS:

Preparation of the sample: Mushroom was obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The fruiting bodies were shade dried and powdered. 10g of the powder was extracted with 100 ml of water at 100^{o}C for 4 hours, centrifuged at 5000rpm for 15 minutes and filtered through Whatman No. 1 filter paper. The residue was extracted twice with 100ml portions of water, as described above. The extracts were combined and vacuum evaporated. The extract obtained after vacuum evaporation was freeze dried and stored at 4^{0}C until further use.

Drugs and chemicals: Silymarin was obtained from Himedia, Bangalore, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental Animals: Female Sprague Dawley rats, weighing, 160g-180g were purchased from, Small Animal Breeding Centre, College of Veterinary and Animal Science, Mannuthy, Kerala, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (AVM Cattle Feeds, Coimbatore, Tamil Nadu) and water *ad libitum.* Experimental animals were handled according to the University and Institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental Design: The animals were divided into 5 groups of six animals each. Hepatotoxicity was induced by intraperitoneal injection of CCl_4 in paraffin oil [21].

1. Group I: Rats in this group served as a control.
2. Group II: Hepatotoxicity was induced with CCl_4 in paraffin oil (1:5, v/v) (1.5 ml/kg body weight, i.p) 3-times in a week for 5 weeks (total 15 doses)
3. Group III: Treated with 500mg/kg body weight/ day of aqueous extract of *V. volvacea* (VVAE) orally, to the animals one hour prior to each CCl_4 injection.
4. Group IV: Treated with 1000mg/kg body weight/day of aqueous extract of *V. volvacea* (VVAE) orally, to the animals one hour prior to each CCl_4 injection.
5. Group V: Treated with 25mg/kg body weight of silymarin administered orally, to the animals one hour prior to each CCl_4 injection.

Biochemical Analysis: At the end of the last injection, the animals were subjected to fasting for a period of 12 hours. At the end of 12 hours fasting the animals were sacrificed, blood was collected and the liver, were excised and washed in saline. 10% homogenate of the liver tissues was prepared with 0.1 M Tri-HCl buffer, pH 7.4. Serum was prepared from whole blood. The homogenates were centrifuged at 3000 rpm for 15 min at 4^{o}C for cytosolic separation.

The levels of serum bilirubin were determined based on the method of by the method of Malloy and Evelyn [22]. The activity of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) by the method of Reitman and Frankel [23] Alkaline phosphatase was determined by King and Armstrong [24] and Lactate dehydrogenase by King [25]. The enzymatic activity of hepatic superoxide dismutase (SOD) was assessed according to the method of Das et al [26] and Catalase (CAT) by the method of Sinha [27], Glutathione (GSH) content of hepatic tissues were assessed using Ellman's reagent according to the method described by Ellman [28]. Protein levels were determined as described by Lowry [29]. Rat liver homogenate lipid peroxide (LPO) levels were determined by measuring MDA content according to the method of Niehus and Samuelsson [30].

Histopathological Examination: The hepatic tissue of each animal were dissected out then fixed in buffered formalin for 12 hours and processed for histopathological examination. Four µm thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol.

Statistical Analysis: The data are expressed as mean ± S.D. Statistical comparison was done at significance level, P<0.05 using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

RESULTS: Table 1 represents the levels of bilirubin and the activity of the liver marker enzymes- AST, ALT, ALP and LDH in the serum of the experimental animals. There was observed a significant (p<0.05) raise in the activity of the enzymes and levels of bilirubin in the CCl_4 intoxicated rats. The treatment with VVAE to the animals of group III and IV at a dose of 500mg/kg b wt and 1000 mg/kg b wt, respectively, resulted in a significant (p<0.05) decrease in the activity of the enzymes and serum bilirubin levels in a dose dependent manner. Silymarin administration to the group V animals, also resulted in a marked reduction (p<0.05) in the serum bilirubin levels and the activity of the enzymes.

TABLE 1 EFFECTS OF AQUEOUS EXTRACT OF *VOLVARIELLA VOLVACEA* ON SERUM BILIRUBIN, PROTEIN AND THE ACTIVITIES OF MARKER ENZYMES

Groups	Bilirubin (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)
Control	0.58 ± 0.01^{b}	147.41 ± 4.12^{b}	97.41±5.51^{b}	78.15 ±1.92^{b}	152.21 ±3.24^{b}
CCl_4 (1.5 ml/kg b wt)	3.52 ± 0.09^{a}	215.81 ±3.45^{a}	272.88 ±9.48^{a}	232.89 ±7.01^{a}	214.24±3.59^{a}
VVAE (500mg/kg.bwt) + CCl_4	2.36 ±0.03^{ab}	174.99±3.29^{b}	170.87±4.14^{ab}	154.45±3.93^{ab}	173.58±1.12^{ab}
VVAE (1000mg/kg.bwt) + CCl_4	0.92±0.04^{b}	156.94 ±1.66^{b}	148.08 ±5.90^{b}	105.31±2.41^{b}	156.94 ±1.66^{b}
Silymarin (25 mg/kgb.wt) + CCl_4	0.59±0.01^{b}	147.78 ±1.37^{b}	142.91 ±4.81^{b}	97.07 ±1.76^{b}	147.41±10.12^{b}

Group I- Control; Group II- CCl_4 (1.5 ml/kg b wt); Group III- VVAE (500mg/kg b wt) + CCl_4; Group IV- VVAE (1000mg/kg.b wt) + CCl_4; Group V- Silymarin (25mg/kg b wt) + CCl_4

Values are expressed as mean ± SD for six animals. Group comparison and statistical significance at p<0.05: ^{a}: Group I vs. II, III, IV, V ^{b}: Group II vs. I, III, IV, V

The effect of VVAE on the activity of the antioxidant enzymes (SOD and CAT), hepatic GSH content and lipid peroxidation levels are presented in **Table 2**. A significant reduction in the activity of SOD, CAT and GSH were observed in the group II animals that served as CCl_4 control animals. Lipid peroxidation levels as MDA content was observed to be markedly (p<0.05) elevated in the group II animals. Treatment with VVAE at 500mg/kg b wt and 1000 mg/kg b wt to the animals of group III and IV respectively resulted in a marked improvement in the activity of SOD and CAT and a significant (p<0.05) raise in the levels of GSH and decline in MDA content. Group V animals that were supplemented with the standard, silymarin effectively normalized (p<0.05) the activity of the enzymes- SOD and CAT, increased the hepatic GSH content and depressed the MDA levels.

TABLE 2: EFFECTS OF AQUEOUS EXTRACT OF *VOLVARIELLA VOLVACEA* ON THE ACTIVITY OF SOD, CAT AND THE LEVELS OF MDA AND GSH IN LIVER

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/mg protein)	MDA (nmoles /min/mg protein)
Control	6.69±0.16^{b}	4.28±0.02^{b}	13.45 ± 0.59^{b}	1.02 ± 0.07^{b}
CCl_4 (1.5 ml/kg b wt)	3.98±0.10^{a}	2.22±0.15^{a}	7.29 ± 0.49^{a}	2.98 ± 0.06^{a}
VVAE(500mg/kg.bwt) + CCl_4	6.05 ±0.56^{b}	3.36 ± 0.26^{ab}	8.58 ± 0.16^{ab}	1.33 ± 0.02^{b}
VVAE(1000mg/kg.bwt) + CCl_4	6.85 ±0.42^{b}	4.28 ±0.12^{b}	12.22 ± 0.75^{b}	0.93 ± 0.04^{b}
Silymarin(25mg/kgb.wt) + CCl_4	6.83 ± 0.07^{b}	3.74 ± 0.27^{b}	12.58 ± 0.98^{b}	1.13 ± 0.05^{b}

Group I- Control; Group II- CCl_4 (1.5 ml/kgb.w); Group III- VVAE (500mg/kg. b. wt) + CCl_4; Group IV- VVAE (1000mg/kgb.wt) + CCl_4; Group V- Silymarin (25 mg/kg b.wt) + CCl_4

Values are expressed as mean ± SD for six animals. Group comparison and statistical significance at p<0.05: ^{a}: Group I vs. II, III, IV, V ^{b}: Group II vs. I, III, IV, V

The results of the histopathological analysis of the liver tissue of the experimental animals are presented in **figure 1 (a-e)**. Figure 1a represents the liver sectioning of the group I animals. Tissue presents normal architecture of the hepatocytes and the central vein. Figure 1b presents the hepatic tissue of the group II CCl_4 control animals. The sectioning depicts severe hemorrhage and necrosis of the hepatocytes. Figure 1c- The hepatic sectioning of the group III animals treated with 500mg/kg b.wt VVAE.

The tissue presents mild inflammation. Figure 1d- The slide represents the liver section of the animals treated with 1000mg/kg b.wt VVAE. The sectioning reveals negligible inflammation and near normal architecture. Figure 1e- The hepatic sectioning of the standard, silymarin treated group V animals. The section presents near normal architecture of the hepatocytes.

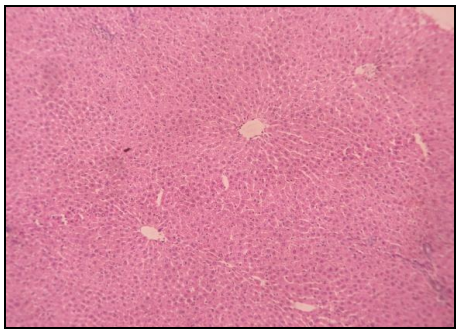

FIGURE 1A: GROUP I (CONTROL)

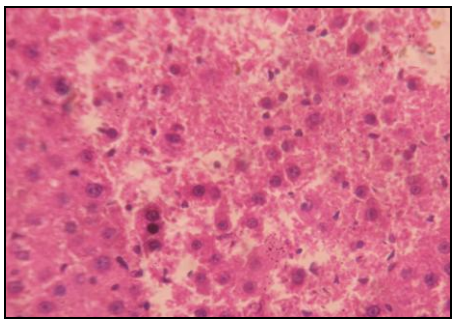

FIGURE 1B: GROUP II (CCl_4)

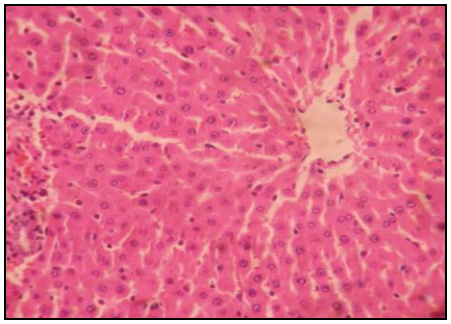

FIGURE 1C: GROUP III
(500mg/kg b. wt VVAE+ CCl_4)

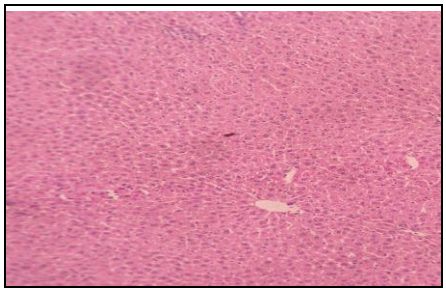

FIGURE 1D: GROUP IV
(1000mg/ kg b.wt VVAE+ CCl_4)

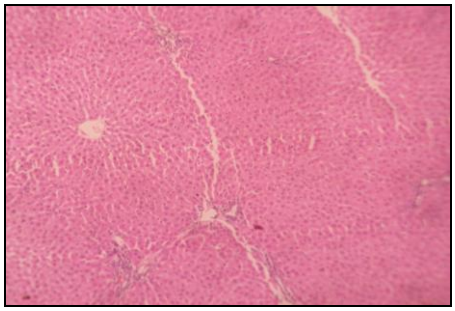

FIGURE 1E: GROUP IV
(Silymarin 25mg/kg b.wt + CCl_4)

DISCUSSION: Serum bilirubin levels are indicators of hepatic damage. It is well known that necrotizing agents like CCl_4 produce sufficient injury to hepatic parenchyma to cause large increases in bilirubin content.

In the liver it has been shown that toxicity of CCl_4 is mediated by the Cyt P450-dependent mixed oxidase-mediated biotransformation product, trichloromethyl free radical (CCl_3) and subsequent derivative Cl_3COO [31]. These free radical combines with the cellular lipid and proteins to produce lipid peroxidation, measured through its catabolite, malondyaldehyde (MDA), resulting in structural changes of endoplasmic reticulum and other biomembranes and loss of metabolic activity leading to liver damage [32].

A high concentration of bilirubin in serum is an indication for increased erythrocyte degeneration rate [33]. Due to the liver injury caused by the hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum [34]. The raised levels of bilirubin in the serum of the group II animals that served as CCl_4 control group, suggest the hepatocellular injury caused by CCl_4. Prior treatment with VVAE was observed to prevent severity of liver damage caused by CCl_4 as evidenced by the low level of bilirubin in the serum in a dose dependent manner.

Silymarin also reduced the serum bilirubin levels, thereby protecting the hepatocytes.

The results obtained were found to be in coordination with Shanmugasundaram and Venkataraman [35]. *Hippophae rhanmoides* L was found to decrease the raised bilirubin levels after induction with CCl_4 [36] and *Aerva lanata* was exploited for its hepatoprotective activity also reduces the serum bilirubin levels upon CCl_4 induction [37].

Liver injury induced by is the best-characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening the hepatoprotective activity of drugs [31, 38, 39]. Serum AST, ALT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage, because these are cytoplasmic in location and are released into the circulation after cellular damage [40].

In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum, and soluble enzymes like AST will also be similarly released. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membranes in liver [3, 41].

Thus, the increase in the serum levels of AST, ALT, ALP and LDH suggest the liver injury due to toxic insult of CCl_4. The treatment with VVAE was found reduce the serum activities of the enzymes in a dose dependent manner. The results suggest the capacity of the extract in counteracting against the damage caused by CCl_4.

The above results are similar to the earlier study reported on the effect of *Ganoderma lucidum* in CCl_4 induced hepatotoxic rats [14] and the effect of *Aerva lanata* on CCl_4 induction [37].

Our findings also correlate with the study of Ajith and Janarthanan [43] in *Phellinus rimosus* (Berk) and with the investigations of Zhou *et al*., [44] BJ-JN, a Chinese formulation on CCl_4 induction. The results are in agreement with those obtained by Jadon *et al*., [45] who showed that gallic acid, at 50 mg/kg body weight, could decrease plasma AST and ALT activities elevated by acute hepatic damage.

This evidenced that the administration of VVAE and silymarin showed hepatoprotective effect under CCl_4-induced oxidative stress.

Samudram *et al.,* [46] investigated the effect of Bi-herbal formulation on CCl_4 induced hepatic damage in rats. The formulation decreased the elevated serum levels of the marker enzymes ALP, ACP, AST, ALT, LDH, 5'NT and γ-GT in the toxic control group animals.

Living tissues are endowed with innate antioxidant defence mechanisms, such as the presence of the enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx). A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes [47, 48].

Administration of CCl_4 leads to generation of peroxy radical, O^{2-}; which is associated with inactivation of CAT and SOD enzymes. This probably explains the significantly reduced activities of CAT, SOD and Gpx observed in rats challenged with CCl_4 (group II). In rats receiving CCl_4 and VVAE (group III and IV) the activities of CAT and SOD were significantly higher than in Group II rats, and very similar to the values noted in normal (group I rats). This suggests a hepatoprotective effect by VVAE extract, which is a very encouraging finding. The extract possibly confers this protective effect by dampening the generation of free radicals that is induced by CCl_4.

Ohta *et al*., [49] suggested that the reduced activities of these enzymes might reflect a feed-back inhibition or oxidative inactivation of protein caused by excess generation of ROS. So also, in the present study, significantly lower activities of these enzymes were noted in rats that had received CCl_4, when compared to the levels in normal rats.

GSH is the major non-enzymatic antioxidant and regulator of intracellular redox homeostasis, ubiquitously present in all cell types [50]. Studies with a number of models show that the hepatotoxicity of xenobiotics often is produced by GSH depletion [51, 52]. Significant increase in the hepatic GSH content suggests the protective role of VVAE.

Treatment *of Z. mauritiana* extract was found to modulate and increase the levels of vitamin E and glutathione in the CCl_4 induced rats [53].

Grape seed extract was found to normalize the enzyme activities [54]. Yang *et al*., [55] reported that *Ganoderma lucidum* when administered at a dose of 500mg exhibited hepatoprotective activity in rats with CCl_4 induced toxicity which is in agreement with our study. A significant restoration of antioxidant enzyme activities by treatment with *Phellinus rimosus* (Berk) Pilat [43], *A. camphorate* [16] and *P. Ostreatus* [14] in CCl_4 intoxicated animals also supports our study. Hsu et al. [57] reported that *D.salina* increased the activity of the antioxidant enzymes in CCl_4- induced hepatic damge in rats.

Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. It has been hypothesized that one of the principal causes of CCl_4- induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCl_4 [3, 58].

The observation of elevated levels of hepatic MDA in Group II rats (administered CCl_4 alone) in the present study is consistent with this hypothesis. Thus, the maintenance of near normal levels of hepatic MDA in Group III and IV rats (administered with mushroom extracts) is of great interest since it provides additional evidence to suggest a hepatoprotective role for *Volvariella volvacea* extract.

The results of our investigations are in accordance with that of Hu *et al*., [42] who have reported the effect of *Ganoderma lucidum* against CCl_4 induced hepatotoxicity and Hwang *et al*., [59] who have reported the activity of *A.continentalis* against CCl_4 induced hepatic damage.

Histoarchitectural improvement on treatment with VVAE suggests the protective effects of the extract in a dose dependent way against CCl_4 induced hepatic injury. Reduction in serum bilirubin and marker enzymes (AST, ALT, ALP, and LDH), and augmentation of endogenous antioxidants and suppression of MDA content supports the hepatoprotective and antioxidant activity.

This protective efficiency of VVAE may be due to its potent antioxidant activity/or by scavenging free radicals.

CONCLUSION: The results observed thus suggest the mushroom extract at both doses (500mg/kg b wt and 1000mg/kg b.wt) effectively ameliorated the toxic effect of CCl_4 in a dose dependent manner.

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