

PROTECTIVE EFFICACY OF THE AQUEOUS EXTRACT OF *VOLVARIELLA VOLVACEA* (BULLIARD EX FRIES) SINGER AGAINST ETHANOL INDUCED HEPATIC DAMAGE

SHWETHA.V.KALAVA*, SUDHA.G.MENON

Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore 641029, Tamilnadu, India.

Email: s.shwetha215@gmail.com

Received: 29 Mar 2012, Revised and Accepted: 03 Jun 2012

ABSTRACT

Alcoholic liver disease is a major cause of liver disease. It arises from the excessive ingestion of alcohol. Aqueous extract *Volvariella volvacea* (500, 1000 mg/kg, p.o) showed significant hepatoprotective activity against ethanol induced hepatotoxicity in rats by normalizing the levels of serum AST, ALT, ALP, LDH, total bilirubin and protein. The extract improved the activity of the antioxidant enzymes, catalase and superoxide dismutase and hepatic glutathione (GSH) content and depleted the lipid peroxidation levels in a dose dependent manner. Silymarin was used as the standard drug. Histopathological analysis of the hepatic tissue further confirmed the protective nature of the extract.

Keywords: Mushrooms, *Volvariella volvacea*, Ethanol, Hepatoprotective.

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences¹⁻³. Many risk factors, including hepatic viruses, alcohol consumption and chemical agents and environmental pollutants, have been considered to be the main factors that cause liver diseases^{4,5}.

Alcohol related disorders are one of the challenging current health problems with far reaching medical, social and economic consequences. Long term use of alcohol potentially results in serious illness, including alcoholic liver disease, hyperglyceridaemia, cirrhosis, cardiovascular disease and inflammation of the pancreas⁶. Alcohol consumption is associated with a number of changes in cell function and the oxidant-antioxidant system; it is known to cause direct and indirect toxic effects by the effect of its byproducts such as acetaldehyde and acetate⁸. Acetaldehyde, a primary metabolic product of alcohol in the liver, appears to be a key generator of free radicals.

Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status⁹.

Mushrooms are macrofungi and they have had a notable place in folk medicine throughout the world since ancient times¹⁰. The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms¹¹. Some of the substances from mushrooms have been demonstrated to possess significant antitumor, cardiovascular, antiviral, antibacterial, and antidiabetic activities¹². Medicinal effects have been demonstrated for many traditionally used mushrooms¹³, like *Agaricus campestris*¹⁴, *Lentinus edodes*¹⁵, *Pleurotus ostreatus*¹⁵, *Tricholoma* sp^{16,17} and *Coriolus versicolor*¹⁸.

Volvariella volvacea, well known as the paddy straw mushroom, is cultured in rice straw in the Philippines and Southeast Asia. *Volvariella volvacea* is a species used extensively in Asian cuisines. It has been reported to produce a hypotensive response in animals including humans. They have been used, as an auxiliary treatment for diabetes patients. It is reported to contain various alkaloids, flavonoids and phenolic acids. *V.volvacea* is also reported to exhibit hypocholesteremic and antioxidative activity.

The present study was undertaken to evaluate the hepatoprotective potential of the aqueous extract of *Volvariella volvacea* (Bulliard ex Fries) Singer against ethanol induced hepatic damage.

MATERIALS AND METHODS

Preparation of the sample

The mushroom was obtained from Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. The fruiting bodies were shade dried and powdered. 10g of the powder was extracted with 100 ml of water at 100°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°C until further use.

Drugs and chemicals

Ethanol was procured from E. Merck chemicals pvt. Ltd, Mumbai, India, silymarin were 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 ± 2°C) 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, Tamilnadu) 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 oral gavage of ethanol¹⁹.

Group I: Rats in this group served as a control.

Group II: Hepatotoxicity was induced with ethanol orally at a dose of 5g/kg b wt, once every 12 hours (total of 3 doses)

Group III: Treated with 500mg/kg b wt / day of aqueous extract of *V.volvacea* (VVAE) orally, to the animals for 7 days. Hepatotoxicity was induced on the 7th day with ethanol at a dose of 5g/kg b wt, once every 12 hours (total of 3 doses)

Group IV: Treated with 1000mg/kg b wt / day of aqueous extract of *V.volvacea* (VVAE) orally, to the animals for 7 days. Hepatotoxicity was induced on the 7th day with ethanol at a dose of 5g/kg b wt, once every 12 hours (total of 3 doses)

Group V: Treated with 20 mg/kg b wt of silymarin orally, to the animals for 7 days. Hepatotoxicity was induced on the 7th day with ethanol at a dose of 5g/kg b wt, once every 12 hours (total of 3 doses)

Biochemical Analysis

After the third dose of ethanol, 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°C for cytosolic separation. The levels of serum bilirubin were determined based on the method of by the method of Malloy and Evelyn²⁰. The activity of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) by Reitman and Frankel²¹, Alkaline phosphatase was determined by King and Armstrong²² and Lactate dehydrogenase by King²³. Protein levels were determined²⁴.

The enzymatic activity of hepatic superoxide dismutase (SOD) was assessed according to the method of Das et al²⁵ and Catalase (CAT) by the method of Sinha²⁶ Glutathione (GSH) content of hepatic tissues were assessed using Ellman's reagent according to the method described by Ellman²⁷.

Rat liver homogenate lipid peroxide (LPO) levels were determined by measuring MDA content according to the method of Niehus and Samuelsson²⁸.

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 protocol²⁹.

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

Effects of aqueous extract of *V. volvacea* on serum biochemical parameters

Table 1 depicts the effect of VVAE on the activities of hepatic marker enzymes, total bilirubin and protein levels in serum of experimental animals.

The activities of serum hepatic marker enzymes namely AST, ALT, ALP and LDH and serum total bilirubin level showed a significant (p<0.05) increase in group II animals that were induced with ethanol alone as compared to control group and the total serum protein levels ion was significantly (p<0.05) decreased in ethanol control (group II) animals. Administration of VVAE at 500mg/kg b.wt and 1000 mg/kg b.wt to the animals of group III and group IV respectively was found to significantly reduce the levels of serum AST, ALT, ALP and LDH as compared to the animals treated with ethanol alone and the total serum bilirubin and protein levels were also normalized on treatment with VVAE in a dose dependent manner as compared with ethanol alone group. Treatment with silymarin to the group V normalized the enzyme activities and serum bilirubin and protein levels effectively.

Effects of aqueous extract of *V. volvacea* on hepatic antioxidant levels and MDA

The effect of VVAE on the hepatic MDA levels, GSH content and activities of hepatic SOD and CAT are presented in Table 2.

There was a significant (p<0.05) increase in MDA content and reduction in GSH, SOD and CAT activities of ethanol intoxicated animals. Treatment with silymarin (20mg/kg b wt p.o) and VVAE significantly (p< 0.05) prevented increase in MDA levels and brought them near to normal level, where as significantly (p<0.05) improved the level of hepatic GSH and activities of SOD, CAT levels.

Table 1: Effects of aqueous extract of *Volvariella volvacea* on bilirubin, protein and the activities of marker enzymes in serum of experimental animals

Groups	Bilirubin (mg/dl)	Protein (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)
Control	0.49 ± 0.03 ^b	5.56 ± 0.41 ^b	114.58 ± 8.71 ^b	90.32 ± 7.12 ^b	82.03 ± 2.19 ^b	149.89 ± 4.18 ^b
Ethanol induced (5g/kg b.wt)	2.67 ± 0.21 ^a	2.58 ± 0.12 ^a	230.30 ± 11.12 ^a	251.78 ± 9.13 ^a	288.11 ± 8.13 ^a	229.31 ± 9.32 ^a
VVAE (500mg/kg.bwt)+Ethanol	1.24 ± 0.08 ^{ab}	4.54 ± 0.23 ^{ab}	170.47 ± 4.12 ^b	140.45 ± 3.15 ^{ab}	143.28 ± 5.19 ^b	188.19 ± 4.23 ^b
VVAE (1000mg/kg.bwt)+Ethanol	0.69 ± 0.04 ^b	5.34 ± 0.19 ^b	120.51 ± 3.63 ^b	104.21 ± 5.11 ^b	110.48 ± 3.75 ^b	173.7 ± 8.17 ^b
Silymarin (20mg/kgb.wt)+Ethanol	0.64 ± 0.05 ^b	5.18 ± 0.21 ^b	118.24 ± 7.38 ^b	106.15 ± 6.74 ^{ab}	91.07 ± 1.26 ^b	165.15 ± 5.14 ^b

Group I- Control; Group II- Ethanol induced (5g/kgb.wt); Group III- VVAE (500mg/kg.bwt) +Ethanol

Group IV- VVAE (1000mg/kg.bwt) + Ethanol; Group V- Silymarin(20mg/kgb.wt)+Ethanol

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

Table 2: Effects of aqueous extract of *Volvariella volvacea* on the activity of antioxidant enzymes, GSH and MDA levels in liver of the experimental animals.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/mg protein)	MDA (nmoles /min/mg protein)
Control	5.69 ± 0.16 ^b	25.55 ± 0.87 ^b	15.17 ± 0.96 ^b	1.02 ± 0.07 ^b
Ethanol induced (5g/kg b.wt)	1.86 ± 0.10 ^a	9.72 ± 0.07 ^a	7.17 ± 1.83 ^a	5.98 ± 0.08 ^a
VVAE (500mg/kg.bwt)+Ethanol	4.21 ± 0.25 ^b	18.42 ± 1.10 ^b	12.86 ± 1.02 ^b	3.80 ± 0.20 ^{ab}
VVAE (1000mg/kg.bwt)+Ethanol	5.10 ± 0.31 ^b	22.29 ± 0.5 ^b	14.92 ± 0.91 ^b	2.91 ± 0.19 ^{ab}
Silymarin (20mg/kgb.wt)+Ethanol	5.83 ± 0.12 ^b	25.34 ± 2.02 ^b	14.83 ± 0.63 ^b	1.17 ± 0.04 ^b

Group I- Control; Group II- Ethanol induced (5g/kgb.wt) Group III- VVAE (500mg/kg.bwt)+Ethanol

Group IV- VVAE (1000mg/kg.bwt) +Ethanol; Group V- Silymarin(20mg/kgb.wt)+Ethanol

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

Figure 1 Effect of aqueous extract of *V. volvacea* on histopathology of the hepatic tissue

Figure 1(a-e) shows effect of aqueous extract of *V. volvacea* and silymarin on ethanol induced hepatotoxicity in rats.

Figure 1a reveals the tissue section of the normal control group I animals showed normal lobular architecture with normal appearance of the hepatocytes and central vein and radiating hepatic cords.

Figure 1b reveals the tissue sectioning of the ethanol control animals. Severe necrosis and hemorrhage was observed. Infiltration of the inflammatory cells was seen.

Figure 1c presents the section of liver tissue of the group III animals that were treated with VVAE at 500mg/kg b wt dose 7 days prior to ethanol. The section shows mild necrosis and milder infiltration of inflammatory cells.

Figure 1d presents the section of liver tissue of the group IV animals that were treated with VVAE at 1000mg/kg b wt dose 7 days prior to ethanol. The section shows absence of necrosis and considerable reversal to normal architecture.

Figure 1e presents the section of liver tissue sectioning of the group V animals that were treated with silymarin at 20 mg/kg b wt dose 7 days prior to ethanol induction. The section shows normal architecture of hepatocytes.

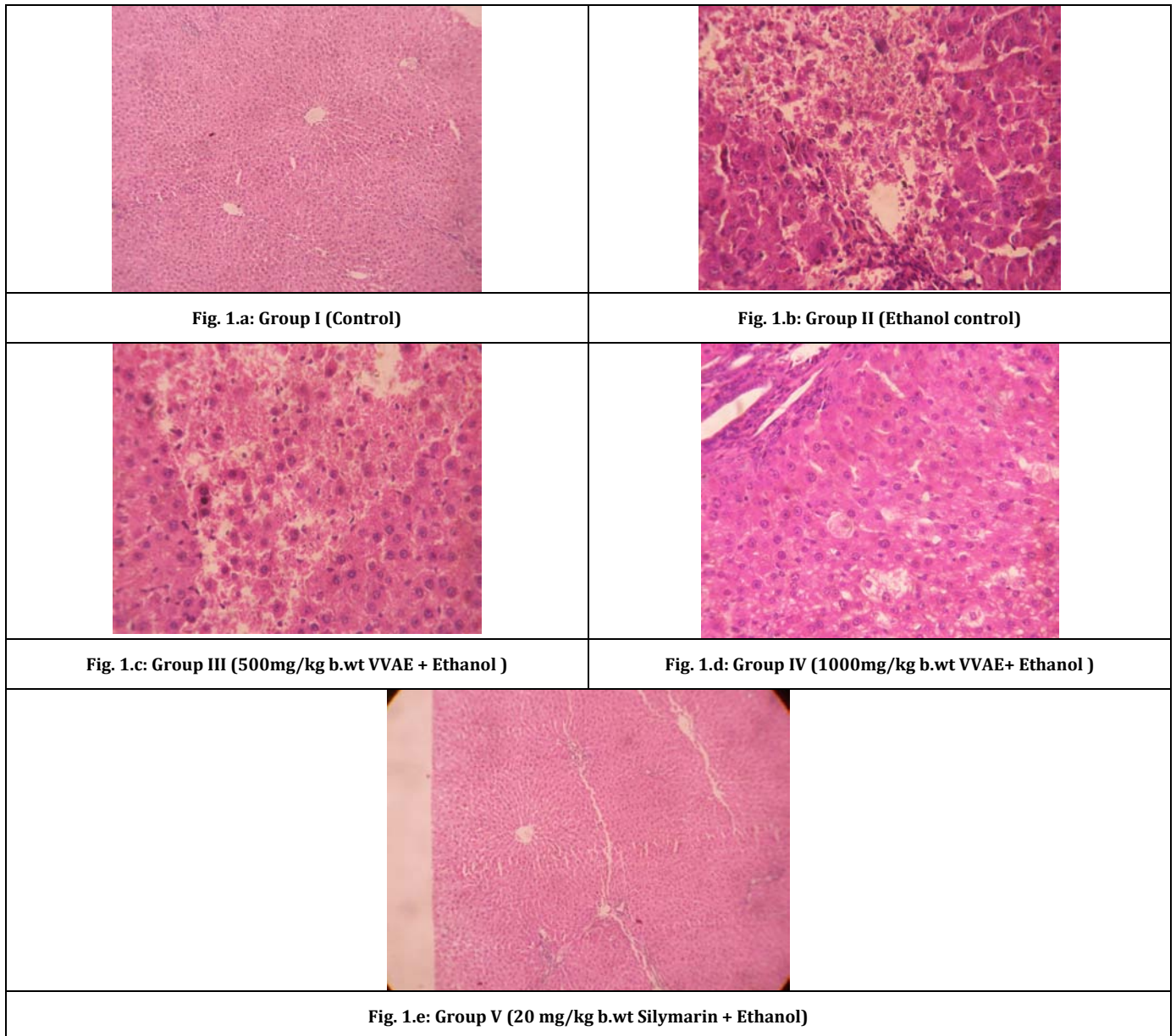


Fig. 1: Effect of aqueous extract of *V. volvacea* on histopathology of the hepatic tissue

DISCUSSION

Alcoholic liver disease is the major cause of liver disease. It arises from the excessive ingestion of alcohol. Chronic heavy drinkers develop liver damage. High alcohol consumption results in critical problems in the body including alcoholic liver disease (ALD)⁷. At least 80% of heavy drinkers have been reported to develop

steatosis, 10–35% alcoholic hepatitis, and approximately 10% liver cirrhosis. One of the factors that play a central role in many pathways of alcohol induced damage is oxidative stress. During metabolism of alcohol via microsomal enzyme oxidizing system (MEOS) pathway, ethanol can increase liver concentration of cytochrome P450 2E1 (CYP2E1) up to ten folds. This induction is responsible for oxidative damage in hepatocytes³⁰.

Liver injury induced by ethanol is the best-characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening of the hepatoprotective activity of drugs³¹. 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 damage³².

A high concentration of bilirubin in serum is an indication for increased erythrocyte degeneration rate³³. It is well known that necrotizing agents like ethanol produce sufficient injury to hepatic parenchyma to cause large increases in bilirubin content. VVAE prevented severity of liver damage caused by ethanol as evidenced by the low level of bilirubin in the serum. 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 of bilirubin in serum³⁴.

The oral administration of VVAE at 500 and 1000mg/kg b.wt, effectively reduced the serum total bilirubin levels. The results obtained were found to be in coordination with Shanmugasundaram and Venkataraman³⁵. Lu et al³⁶ reported that the mycelia of *Antrodia camphorata* and *Armillariella tabescens* reduced the serum bilirubin levels in ethanol-induced hepatic toxicity in rats.

Owing to hepatocellular damage process that is usually responsible for the change in the levels of proteins. The decline in protein value is used as a useful marker in the severity of cellular dysfunction in liver cells³⁷.

In the present study, there was a significant decrease in serum total proteins. These results were in agreement with Ahmed et al³⁸ who reported a decrease in serum total proteins in ethanol-administered rats and he suggested that was due to the decrease in the functional ability of liver in ethanol-administered rats. The treatment of VVAE at both the doses well restored the proteins synthesis.

Ethanol induction significantly increased the mean values of liver enzymes, AST, ALT, LDH and ALP in the serum. These results were in agreement with Rajakrishnan and Menon³⁹ who indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Also, Das et al⁴⁰ reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzymes into blood.

The results observed were similar to the earlier study on *Aerva lanata* on ethanol induction. The extract of *Aerva lanata*, normalized the levels of marker enzymes in serum⁴¹. Leakage of large quantities of enzymes into the blood stream is often associated with massive necrosis of the liver⁴². The elevated level of these entire marker enzymes observed in the group II ethanol treated rats in this present study corresponded to the extensive liver damage induced by toxin.

The administration of VVAE (500mg/kg b.wt and 1000mg/kg b wt) and silymarin to group III,IV and group V animals respectively showed hepatoprotective effect under ethanol induced oxidative stress that is evidenced by the decrease in the level of the marker enzymes in the serum. These results agree with the hypothesis that oxidative damage is neutralized when antioxidants such vitamin E, quercetin, ferulic acid and N-acetyl cysteine are administered before or after the induction of oxidative stress with ethanol⁴³⁻⁴⁵. Thus the protective effect of the mushroom extract could be attributes to the antioxidant capacity.

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^{46,47}.

The decreased levels of antioxidant enzymes viz., SOD and CAT in the ethanol alone (group II) animals may be due, in part, to an overwhelming oxidative modification of the enzymatic proteins by

excessive ROS generation. More so, reduction in the activities of these enzymes may stem from decrease in their rate of synthesis.

The over-production of superoxide radicals due to EtOH intoxication implies low activity of SOD under ethanol induced oxidative stress in the hepatic tissue. Administration of VVAE was observed to improve the activities of SOD and CAT thus protecting the liver from ethanol induced toxication. Similar reduction in lipid peroxidation increased antioxidant enzyme activity levels during plant extract supplementation were recorded^{48,49}.

GSH acts as a non-enzymatic antioxidant that reduces H₂O₂, hydroperoxides (ROOH) and xenobiotic toxicity⁵⁰. The higher level of ethyl alcohol intake develops cirrhosis and liver damage by enhancing lipid peroxidation in the liver.

The observed significant decline in the levels of hepatic GSH content in the group II animals could be due to the toxic insult of ethanol. Acetaldehyde the toxic metabolite of ethyl alcohol depresses the liver glutathione level by conjugating with the sulphhydryl groups of glutathione^{51,52}.

Treatment with VVAE significantly elevated GSH levels. Silymarin has been reported to maintain the GSH homeostasis in the system⁵³ and this might be the reason for elevated GSH levels observed during silymarin treatment.

Similar results were reported by Maruthappan et al⁵⁴. The action of the extract exhibited a dose dependant protection of the hepatocyte against ethanol induced hepatotoxicity.

It was found that the inflammatory reactions and oxidative stress play a major role in alcohol hepatotoxicity⁵⁵. In this investigation, there was a significant increase in serum MDA concentration in ethanol treated rats; these results were in agreement with Saravanan et al⁵⁶ and Das and Vasudevan⁴⁰ reported a significant increase in MDA concentration in ethanol-treated rats and the results suggests that reactive oxygen intermediates, generated during the metabolism of ethanol, these free radicals attack the polyunsaturated fatty acids in membranes and organelles to produce lipid peroxides leading to decrease in the membrane permeability, and ultimately cellular necrosis and death.

The present results showed significant decrease in the levels of MDA upon treatment with VVAE and silymarin, suggesting the hepatoprotective effects reported increased lipid peroxidation with ethanol in their dose dependant studies.

This decrease in the levels of lipidperoxides is due to the ability of the extract to scavenge free radicals that damage the membrane. In addition, the antioxidant activity and/or the inhibition of free radical generation are important in terms of protecting the liver from toxic insults³¹.

Administration of *Plumbago zeylanica* decreased the level of bilirubin and the activities of marker enzymes in the serum of hepatotoxicity induced rats suggesting that it offered protection⁵⁷. *Cassia occidentalis* extract was found to decrease the activity of serum marker enzymes in hepatotoxic rats, thus protecting the hepatocytes against damage⁵⁸.

Histoarchitectural improvement further supported by biochemical changes in liver, reduction in serum bilirubin and marker enzymes (AST, ALT, ALP, and LDH), and augmentation of serum total protein and 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 bwt) effectively ameliorated the toxic effect of ethanol in a dose dependent manner.

ACKNOWLEDGEMENT

The authors are thankful to the Management of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

REFERENCES

- Shahani S. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. *Indian Drugs* 1999; 36: 628-31.
- Subramoniam A, Pushpangadan P. Development of phytomedicine for liver diseases. *Ind J Pharmacol* 1999; 31: 166-75.
- Achliya GS, Wadodkar SG, Dorle AK. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. *J Ethnopharmacol* 2004; 90:229-32.
- Day CP, Yeaman SJ. The biochemistry of alcohol-induced fatty liver. *Biochimica et Biophysica Acta* 1994; 1215: 33-48.
- Szabo G. Pathogenic interactions between alcohol and hepatitis C. *Cur Gastroenterol Rep* 2003; 5: 86-92.
- Ponnappa BC, Rubin E. Modeling alcohol's effect on organs in animal models. *Alcohol. Res. Health* 2000; 24:93-104.
- Sivaraj A, Vinothkumar P, Sathiyaraj K, Devi K, Senthilkumar B. Hepatoprotective and antioxidant properties of *Coccinia grandis* aqueous leaf extract on ethanol induced liver toxicity in albino rats. *J.Pharmacy.Res* 2010; 3(3): 533-36.
- Mallikarjuna K, Chetan K, Reddy KS, Rajendra W. Ethanol toxicity: Rehabilitation of hepatic antioxidant defense system with dietary ginger. *Fitoterapia* 2008; 79: 174-78.
- Vinothkumar P, Sivaraj A, Devi K, Senthilkumar B. Hepatoprotective and antioxidant properties of aqueous rhizome extracts of *Picrorhiza kurroa* on CCl₄ induced liver toxicity in albino rats. *J.Pharmacy.Res* 2010; 3(6): 1280-82.
- Jong SC, Birmingham JM. Edible mushrooms in biotechnology. *Proc Asian Mycol Symp. Seoul, Korea* 1992; 18-35.
- Asfors KE, Ley, K. Sulfated polysaccharides in inflammation. *J. Lab. Clin. Med* 1993; 121: 201-2.
- Begell W, Wasser SP. The first international journal of medicinal mushrooms. *Int. J. Med. Mushrooms* 2001; 3:115.
- Ooi VE, Liu F. A review of pharmacological activities of mushroom polysaccharides. *Int J Med Mushrooms* 1999; 1: 196 - 206.
- Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). *J Endocrinol* 1998; 157: 59-66.
- Kim BK, Park EK. Studies on the constituents of the higher fungi of Korea 12. Anti-neoplastic components of *Coriolus versicolor* Fr, *Pleurotus ostreatus* (Fr) Kummer and *Lentinus edodes* (BERK) Singer. *J Nat Prod* 1979;42:684.
- Wang HX, Liu, WK, Ng TB, Ooi VEC, Chang ST. Immunomodulatory and antitumor activities of a polysaccharide - peptide complex from a mycelial culture of *Tricholoma* sp., a local edible mushroom. *Life Sci* 1995; 57: 269- 81.
- Wang HX, Ng TB, Ooi VEC, Liu WK, Chang ST. A polysaccharide-peptide complex from cultured mycelia of the mushroom *Tricholoma mongolicum* with immunoenhancing and antitumor activities. *Biochem Cell Biol* 1996; 74: 95-100.
- Chu KKW, Ho SSS, Chow AHL. *Coriolus versicolor*: a medicinal mushroom with promising immunotherapeutic values. *J Clin Pharmacol* 2002; 42: 976- 84.
- Nadro MS, Dahiru D, Arungbemi RM. Evaluation of *Moringa oleifera* Leaf Extract on Alcohol-induced Hepatotoxicity. *Tropical J Pharma Res* 2006; 5 (1): 539- 44.
- Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem* 1937; 119: 481-90.
- Reitman S, Frankel AS. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol* 1957; 28: 53-56.
- King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. *Canadian Med. Ass. J* 1934; 31: 376- 9.
- King J. The hydrolases-acid and alkaline phosphatases. In: Van, D. (Ed.), *Pract. Clin. Enzymol.* Norstand Co. Ltd, London 1965; 191-208.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's phenol reagent. *J. Biol. Chem* 1957; 193: 265-75.
- Das S, Vasight S, Snehlata R, Das N, Srivastava LM. Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. *Curr. Sci* 2000; 78: 486-7.
- Sinha AK. Colorimetric assay of catalase. *Anal. Biochem* 1972. 47, 389-94
- Ellman GL. Tissue sulphhydryl groups. *Arch Biochem Biophys* 1959; 82: 70- 7.
- Niehius WG, Samuelsson D. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem* 1968; 6: 126-130.
- Allen CT. *Laboratory Methods in Histochemistry*, 1st ed., American Registry of Pathology, Washington 1992; 53-56.
- Saxena AK, Singh B, Anand K. Hepatoprotective effects of *Eclipta alba* on sub cellular levels in rats. *J Ethnopharmacol* 1993; 40: 155-161.
- Manibusan, M.K., Odin, M. and Eastmond, D.A. Postulated carbon tetrachloride mode of action: a review. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev* 2007; 25: 185-209.
- Sallie R, Tredger JM, Willam R. *Drugs and the liver.* *Biopharm. Drug Dispos* 1991; 12: 251-9.
- Singh B, Saxena AK, Chandan BK, Anand KK, Suri OP, SuriSatti KA, SuriSatti NK. Hepatoprotective activity of verbenaalin on experimental liver damage in rodents. *Fitoterapia* 1998; 69: 134-40.
- Rao RR. Mechanism of drug induced hepatotoxicity. *Indian J Pharmacol* 1973; 5: 313-8.
- Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. *J. Ethnopharmacol* 2006; 104:124-8.
- Lu ZM, Tao WY, Zoub XL, Fub HZ, Ao ZH. Protective effects of mycelia of *Antrodia camphorata* and *Armillariella tabescens* in submerged culture against ethanol-induced hepatic toxicity in rats. *J Ethnopharmacol* 2007; 110: 160-4.
- Samudram P, Rajeshwari H, Vasuki R. Geetha A, Sathiyamoorthi P. Hepatoprotective activity of Bi - herbal ethanolic extract on CCl₄ induced hepatic damage in rats. *Afr. J. Biochem.Res* 2008; 2 (2): 61-5.
- Ahmed B, Alam T, Varshney M, Khan AS. Hepato- protective activity of two plants belonging to the A piaceae and the Euphorbiaceae family. *J.Ethnopharmacol* 2002;79 (3): 313-16.
- Rajakrishnan V, Menon VP. Potential role of antioxidants during ethanol induced changes in the fatty acid composition and arachidonic acid metabolites in male Wistar rats. *Cell Biol. Toxicol* 2001; 17:11-22.
- Das D, Mukherjee MM, Das AS, Mitra C. Aqueous extract of black tea (*Camellia sinensis*) prevents chronic ethanol toxicity. *Curr. Sci* 2005; 88 (6): 952-61.
- Nevin KG, Vijayammal PL. Effect of *Aerva lanata* against hepatotoxicity of carbon tetrachloride in rats. *Environ Toxicol Phar* 2005; 20: 471-7.
- Shyamal S, Latha PG, Shine VJ, Suja SR, Rajasekharan S, Ganga Devi T. Hepatoprotective effects of *Pitosporum neelgherrense*, a popular Indian ethnomedicine. *J. Ethnopharm* 2006; 107: 151-5.
- Molina MF, Sanchez-Reus I, Iglesias I, Benedi J. Quercetin, a flavonoid antioxidant prevents and protects against ethanol-induced oxidative stress in mouse liver. *Biol. Pharm. Bull* 2003; 26(10):1398- 402.
- Ozaras R, Tahan V, Aydin S, Uzun, H, Kaya S, Senturk H. N-Acetylcysteine attenuates alcohol-induced oxidative stress in animals. *World J. Gastroenterol* 2003; 9 (4): 791- 4.
- Rukkumani R, Aruna, K, Suresh VP. Menon, VP. Influence of ferulic acid on circulatory prooxidant-antioxidant status during alcohol and PUFA induced toxicity. *J. Physiol. Pharmacol* 2004; 55 (3): 551-61.
- Krishnakantha TP, Lokesh BR. Scavenging of super oxide anions by spice principles. *Ind. J. Exp. Biol* 1993; 30: 133-4.
- Sheela CG, Angusti, K. Antiperoxide effects of S-allyl cysteine sulphoxide isolated from *Allium sativum* Linn and guggulipid in chleosterol diet fed rats. *Ind. J. Exp. Biol* 1995; 33: 337-41.
- Adamska T, Mlynarczyk W, Jodynis-Liebert J, Bylka W, Matlawska I. Hepatoprotective effect of the extract and

- isocytiside from *Aquilegia vulgaris*. *Phytotherapy Res* 2003; 17(6):691-6.
49. Izabela D, Anna S, Zbigniew S, Figaszewski W. Green tea modulation of the biochemical and electrolyte properties of rat liver cells that were affected by ethanol and aging. *Cell Mol Biol Lett* 2004; 9:709-21.
 50. Kadiska MB, Gladen BC, Baird DD, Dikalov AE, Sohal RS, Hatch GB, Jones DP, Mason RP, Barret, JC. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl4 poisoning? *J. Free Rad. Biol. Med* 2000; 28: 838- 45.
 51. Comporti M, Benedetti, A, Chieli E. Studies on invitro peroxidation of lipids in ethanol treated rats. *Lipids* 1973; 8(9): 498-502.
 52. Mahendran P, Shyamla Devi CS. The modulating effect of *Garcinia cambogia* extract on ethanol induced peroxidative damage in rats. *Indian J Pharm* 2001; 33:87-91.
 53. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. *Clin. Drug Investig* 2002 ; 22: 51-65.
 54. Maruthappan V, Sakthi Shree K, Raja MK, Pravin Kumar RJ. Hepatoprotective effect of *Azadirachta indica* (Neem) leaves against alcohol induced liver injury in albino rats. *J Pharmacy Res* 2009; 2(4): 655- 9.
 55. Albano E. Free radicals and alcohol-induced liver injury. In "Ethanol and the Liver". Sherman CDIN, Preedy VR and Watson RR (eds.). London: Taylor and Francis 2002; 153-90.
 56. Saravanan R, Viswanathan P, Pugalendi KVC. Protective effect of urosolic acid on ethanol-mediated experimental liver damage in rats. *Life Sci* 2006; 78: 713- 8.
 57. Kanchana N and Mohamed Sadiq A. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. *Int J Pharm Pharm Sci* 2011; 3(1):151- 154.
 58. Sheeba Ranl M, Emmanuel S, Raja sreekanth M , Ignacimuthu S. Evaluation of *in vivo* antioxidant and hepatoprotective activity of *Cassia occidentalis* Linn. Against paracetamol induced liver toxicity in rats. *Int J Pharm Pharm Sci* 2010; 2 (3): 67-70.
 59. Articles published in IJPPS similar to the present study:
 60. Kanchana N and Mohamed Sadiq A. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. *Int J Pharm Pharm Sci* 2011; 3(1):151- 154.
 61. Sheeba Ranl M, Emmanuel S, Raja sreekanth M , Ignacimuthu S. Evaluation of *in vivo* antioxidant and hepatoprotective activity of *Cassia occidentalis* Linn. Against paracetamol induced liver toxicity in rats. *Int J Pharm Pharm Sci* 2010; 2 (3): 67-70.