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In vitro Antioxidant Activity of *Calocybe indica* on Mercury Chloride Induced Hemolysis in Chronic Renal Failure Patients

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Oxidative processes may be increased in patients with chronic renal failure (CRF), and this is a possible factor contributing to the development of anemia and atherosclerosis, which are characteristic complications of CRF. The objective of the present study was to determine the antioxidant activity of *Calocybe indica* (Edible mushroom) against mercury chloride induced haemolysis in chronic renal failure patients. The analysis includes the estimation of TBARS, G-S-H, superoxide dismutase, catalase, vitamin C and vitamin E. Variation in glucose, lipid profile and haematological parameters such as erythrocyte count and haemoglobin were also assessed before and after haemolysis when compared with the control group. The results of the present study revealed elevated levels of TBARS and G-S-H in CRF patients and mercury chloride treated subjects.The levels of enzymatic, non-enzymatic antioxidants were found to be decreased in chronic renal failure patients and mercury chloride treated subjects. Further more, the level of haematological and lipid parameters were altered in CRF and mercury chloride treated patients. To conclude the addition of *Calocybe indica* extract was found to reverse the lipid peroxidation effect on mercury chloride induced haemolysis in CRF patients. The phytochemical analysis of *Calocybe indica* indicates the presence of phenols, β-carotene and lycopene which contributes to the antioxidant activity of *Calocybe indica*. Therefore, it has been suggested that *Calocybe indica* may serve as an antioxidant supplement for CRF patients.

Key words: Antioxidants; *Calocybe indica*; Haemolysis; Chronic renal failure; Bioactive compounds.

Renal failure is accompanied by oxidative stress (Himmelfard and Hakim 2003; Galle 2003) which consists of damage in biological structures by reactive oxygen species due to their excessive generation and impaired efficiency of antioxidant defence mechanisms. It has long been known that anemia (Eschbach and Adamson 1985) and cardiovascular diseases are characteristic complications of chronic renal failure (CRF) and there is accumulating evidence indicating that oxidative reactions may play a part in these events (Marshall *et al*., 1985 and Santos *et al*., 1980). Recent data suggest that oxidative processes may be increased in patients with CRF. The enzymatic antioxidant system is impaired in erythrocytes from patients with CRF and that the resulting oxidant load may play a role in the development of some complications of CRF (Loughrey *et al*., 1994 and Jackson *et al*., 1995).

Lipid peroxidation inhibition capacity of the edible mushrooms used as models for the lipid peroxidation damage in biomembrances, namely,

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inhibition of β- carotene bleaching in the presence of linoleic acid radicls, inhibition of erythrocytes haemolysis mediated by peroxy radicals, and inhibition of thiobarbituric acid reactive substances (TBARS) formation in brain cells.

MATERIAL AND METHODS

Sample collection

In order to study the antioxidant effect of the mushroom in invitro study, CRF patients and normal patient's blood were collected in Rohini hospital, Thanjavur. Ten healthy male volunteers (40-50years) and ten CRF patients belonging to same age group were selected for blood collection. Five groups were taken for the study.

Group - I : Healthy control
Group - II : CRF blood sample before hemolysis
Group - III : CRF blood sample after hemolysis
Group - IV : 0.6ml *Calocybe indica* extract treated group.
Group - V : 0.8ml *Calocybe indica* extract treated group.

Drug preparation

Calocybe indica was collected from Vishnavi milky mushroom, Thanjavur. They were shade dried and finally powered which was sieved through muslin cloth. Then 5g powder was weighed, mixed with 100ml water. It was allowed to stand for 1 hour. Then different doses (0.6ml, 0.8ml) were used for treatment.

Determination of bioactive components

Bioactive components in the mushrooms extracts were determined by colorimetric assays, based on procedure described by Barros & Ferreira *et al*., (2007).

Hemolysis of blood

Percentage of hemolysis calculated by Tanaka & Nakai (1977).

Estimation of malondialdehyde (LPO) and glutathione

Thiobarbuturic acid substance was estimated by the method of Beuge & Aust (1978). Total reduced glutathione was determined according to the method of Moron *et al.*, (1979).

Estimation of enzymatic antioxidant activity

SOD activity was determined by the modified method of NADH- phenazine methosulphate-nitrobluetetrazolium formazon inhibition reaction spectrophotometerically at 560 nm (Kakkar *et al*., 1984). Catalase was assayed calorimetrically as described by Sinha (1972).

Estimation of non-enzymatic antioxidant activity

The level of ascorbic acid was estimated by the method of Omaye *et al.*, (1979). α - tocopherol was estimated by the method of Baker *et al.*, (1980).

Lipid and biochemical analyses

Total cholesterol level was estimated by the method of Allin (1974). Low density lipoprotein cholesterol (LDL-C) was calculated by Friedwald *et al*., 1972.

Triglycerides was estimated by the method of Jacobe & Denmark (1960), Albumin was estimated by the method of Rodey (1965), Sugar was estimated by Folins Wu method (1920) and Haemoglobin was estimated by Sahli's method. Then no of cells in undiluted blood is calculated by Varley, 1969.

RESULTS AND DISCUSSION

The aim of the present work was to prove the phytotherapeutical significance of an official and popular mushroom extract and its main ingredient, on the basis of their antioxidant activity due to their influence on pathological free radical reactions. Experimental reactions were planned and developed in order to measure the antioxidant, free radical scavenging and membrane protecting activities and to monitor the capacity of these natural compounds to reduce the extend of lipid peroxidation.

Commonly researched antioxidants are vitamin E, vitamin C, carotenoids and more recently phenolic compounds (Katz, 2003). Polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers. Some authors have already reported a direct correlation betweem mushrooms antioxidant activity and total phenolic content, although the antioxidant action is raised by other substances such as tocopherols and

Table 1. Bioactive compounds in *Calocybe indica*

Total phenols (mg/g)	β-Carotene (mg/g)	Lycopene (mg/g)
1.09 ± 0.05	0.46 ± 0.06	0.25 ± 0.10

β- carotene (Cheng *et al.*, 2003). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipooxygenase and scavenge free radicals (Decker, 1997).

Phytochemicals compounds in *Calocybe indica* might be responsible for antioxidant properties in $HgCl_2$ induced hemolysis patients (Table 1).

One of the effects of mercurials on erythrocytes is disturbance such as decrease of GSH and (G_6 PD) activity which finally results in haemolysis .The other effect is a action on cell membrane that results in haemolysis. The present results demonstrated that 0.05mM concentration of $Hgcl_2$ cause destabilization of RBC membrane, leading to influx of water and direct action on RBC membrane to an increase in lipidperoxidation and oxidative damage. Concurrent addition of $HgCl_2$ and the mushroom *Calocybe indica* extract to RBC suspension caused significant reduction in the rate

Table 2. Effect of *Calocybe indica* on $Hgcl_2$ induced invitro hemolysis in chronic renal failure samples

Hemolysis (%)	5% of 0.6ml mushroom treated CRF sample (% Reduction)	5% of 0.8ml mushroom treated CRF sample (% Reduction)
59.9 ± 0.4	40.8 ± 0.9*	60.0 ± 0.9**

Values were expressed as mean ± SD for ten CRF patients in each group.
P*< 0.01 significantly different from group III
P**< 0.01 significantly different from group III

Table 3. Biochemical characteriation of *Calocybe indica* in $HgCl_2$ induced invitro hemolysis in chronic renal failure samples

Parameters	Healthy control	CRF patients-Before hemolysis	CRF patients-After hemolysis	Treatment	
				5% of 0.6ml mushroom treated CRF sample	5% of 0.8ml mushroom treated CRF sample
LPO (nmole/ml)	0.09 ± 0.01	2.25 ± 0.16*	3.85 ± 0.11**	1.43 ± 0.11#	1.88 ± 0.10#'
GSH (µg/ml)	25.3 ± 0.62	12.1 ± 0.87*	7.65 ± 0.88**	15.0 ± 1.5#	23.0 ± 0.40#'
SOD (IU/g of protein)	6.46 ± 0.01	4.76 ± 0.08*	3.85 ± 0.08**	4.70 ± 0.01#	4.96 ± 0.01#'
Catalase (IU/g of protein)	4.68 ± 0.12	3.71 ± 0.22*	3.31 ± 0.13**	3.43 ± 0.14#	3.46 ± 0.15#'
Vitamin-C (mg/dl)	43.0 ± 14.6	28.3 ± 1.24*	27.3 ± 0.55**	29.0 ± 0.82#	36.8 ± 0.10#'
Vitamin-C (mg/dl)	32.0 ± 12.0	10.8 ± 0.28*	7.4 ± 0.91**	16.7 ± 0.04#	17.5 ± 0.66#'
Cholesterol (mg/dl)	171 ± 2.6	271 ± 2.0*	249 ± 4.1**	229 ± 1.9#	222 ± 2.2#'
TG (mg/dl)	133 ± 1.6	194 ± 2.2*	184 ± 1.9**	179 ± 1.3#	174 ± 1.6#'
HDL (mg/dl)	234 ± 1.6	214 ± 2.4*	164 ± 1.8**	160 ± 1.2#	158 ± 2.1#'
LDL (mg/dl)	23.8 ± 2.4	26.5 ± 1.7*	22.1 ± 1.8**	20.1 ± 1.9#	20.3 ± 1.8#'
VLDL (mg/dl)	27.4 ± 3.07	38.3 ± 1.08*	35.2 ± 1.78**	33.5 ± 1.33#	32.0 ± 1.97#'
Sugar (mg/100ml)	99.8 ± 12.2	102 ± 3.2*	51 ± 1.8**	61.7 ± 2.2#	67 ± 1.4#'
Hb (g/100ml)	14 ± 1.29	7.1 ± 0.73*	5.4 ± 0.6**	5.2 ± 0.51#	5.37 ± 0.6#'
RBC (million cubic/cells)	4.7 ± 0.36	2.8 ± 0.7*	1.8 ± 0.7**	2.5 ± 0.7#	2.8 ± 0.8#'
protein (g/dl)	5.4 ± 0.94	10.2 ±0.92*	9.6 ± 0.92**	6.5 ± 0.86#	6.9 ± 1.04#'

Values were expressed as mean ± SD for ten CRF patients in each group
P*< 0.01 significantly different from group I
P**< 0.01 significantly different from group I
P#< 0.01 significantly different from group III
P#'< 0.01 significantly different from group III

of hemolysis, which may be due to the presence of its phytochemicals (Table 2).

Lipidperoxidation level was increased in CRF and mercury chloride treated subjects. Antioxidant enzymes such as SOD, CAT were decreased in CRF and hemolysis induced CRF patients. The level of GSH and antioxidant vitamins C and E were decreased in CRF and hemolysis induced CRF patients. Altered levels of total cholesterol, TG, HDL, VLDL, and LDL were observed in CRF and hemolysis induced CRF patients. Diminished level of sugar, albumin, Hb and RBC were observed in CRF and hemolysis induced CRF patients (Table 3).

Thus the mercury induced hemolysis in CRF blood sample was prevented by the mushroom *Calocybe indica* extract. This effect was due to its phytochemicals constituents of *Calocybe indica*. To conclude, the addition of *Calocybe indica* extract was found to reverse the lipid peroxidation effect on mercury chloride induced hemolysis in CRF patients. Further research is needed to identify the active principles responsible for the antioxidant property of *Calocybe indica.*

REFERENCES

1. Eschbach J.W, Adamson J.W: Anemia of end-stage renal disease (ESRD). *Kidney Int*, 1985; **28:** 1-5.
2. Beuge J.A and Aust S.D, The thiobarbituric acid assay, *Methods in Enzymology*, 1978; **52**: 306-307.
3. Friedward W.T, Lery R.T, and Fredrickson D.S, Estimation of low density lipoprotein cholesterol in plama without use of the preparative centrifuge, *Clin Chem*, 1972; **18**: 499.
4. Folin O, and Wu H, *J. boil. Chem*, 1920; **41**: 367. Halliwell B, Gutteridge J.M.C, Free radical in biology and medicine, Oxford, UK, (2003).
5. Jacobe N.J and Van Denmark P.J, Arch Bio Chem, *Bio phy*, 1960; **88**: 250.
6. Moron M.S, Dsepierre J.W, and Manerwik K.B, Level of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, 1979; **582**: 67-68.
7. Omaye S.T, Tumball J.D, Sauberlich H.E, Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids, *Methods in Enzymology*, 1979; **62**: 1-11.
8. Reiko Tanaka, and Kengo Nakai, Haemolysis and morphological changes in rat erythrocytes with mercurials, *Japan. J.Pharmacol*, 1977; **27**: 413-419.
9. Rodey F.L, Direct spectrophotometric determination of albumin in human serum, *Clinical Chemistry*, 1965; **11**: 478-479.
10. Sinha Ka, Colorimetric assay catalase, *Ann.Biochem.* 1972; **47**: 389-394.
11. Decker E.A, Phenolics: prooxidants or antioxidants, *Nutrition Reviews*, 1997; **55:** 396-407.
12. Galle J, Seibold S, Wanner C. Inflammation in uremic patients: what is the link? Kidney *Blood Press Res*, 2003; **26**: 65-75.
13. Cheung L.M., & Cheung P.C.K, Ooi V.E.C, Antioxidant activity and total phenolics of edible mushroom exracts, *Food Chemistry*, 2003; **81**, 249-255.
14. Katz N.B, Antioxidants: focus on free radicals, adding and vitamin X. *Institute for Natural Resources*, 2003; 1-18.
15. Loughrey CM, Young IS, Lightbody JH, McMadster D, McNamee PT, Trimble ER.Oxidative stress inhaemodialysis.*QJM*. 1994; **87**(11): 679-683.
16. Marshall P, Warso MA, Lands WE: Selective micro determination of lipid hydroperoxidase. *Anal Biochem*, 1985; **145:** 192-199.
17. Jackson P, Loughrey CM, Lightbody JH, McNamee PT, Young IS: Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic renal failure. *Clin Chem* 1995; **41**: 1135–1138.
18. Ong-awyooth L, Ong-ajyooth S, Tiensong K, Nilwarangkur S, Reduced free radical scavengers and chronic renal failure, *J Med AssocThai*, 1997; **80**: 101–108.
19. Santos MT, Valles J, Aznar J, Vilches J: Determination of plasma malondialdehyde-like material and its clinical application in stroke patients. *J Clin Pathol*, 1980; **33**: 973-976.
20. Allin C.C, *Clin Chem*, 1974; **20**: 470.
21. Baker H, Frank O, De Angeles B and Feinglod S, Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol, *Nutrition reparts international*, 1980; **21**: 531.
22. Himmelfarb J, Hakim RM. Oxidative stress in uremia. *Curr Opin Nephrol Hypertens*,; 2003; **12**: 593-8.
23. Kakkar P, Das B, Viswananthan P.N, A modified spectroscopic assay of SOD, *Indian Biochem.Biophys*, 1984; **2**: 130-132.