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.
inhibition of β-carotene bleaching in the presence of linoleic acid radicals, 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 vito study, CRF patients and normal patient’s blood were collected in Rohini hospital, Thanjavur. Ten healthy male volunteers (40-50 years) 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**: 0.6 ml *Calocybe indica* extract treated group.
- **Group - V**: 0.8 ml *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 5 g powder was weighed, mixed with 100 ml water. It was allowed to stand for 1 hour. Then different doses (0.6 ml, 0.8 ml) 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**


**Estimation of malondialdehyde (LPO) and glutathione**

Thiobarbituric 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 formazan inhibition reaction spectrophotometrically 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 Folinus 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 between mushrooms antioxidant activity and total phenolic content, although the antioxidant action is raised by other substances such as tocopherols and Table 1.

<table>
<thead>
<tr>
<th>Bioactive compounds in <em>Calocybe indica</em></th>
<th>Total phenols (mg/g)</th>
<th>β-Carotene (mg/g)</th>
<th>Lycopene (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.09 ± 0.05</td>
<td>0.46 ± 0.06</td>
<td>0.25 ± 0.10</td>
</tr>
</tbody>
</table>

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 HgCl₂ induced hemolysis patients (Table 1).

One of the effects of mercurials on erythrocytes is disturbance such as decrease of GSH and (G₆PD) 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 HgCl₂ 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 HgCl₂ and the mushroom *Calocybe indica* extract to RBC suspension caused significant reduction in the rate

### Table 2. Effect of *Calocybe indica* on HgCl₂ induced invitro hemolysis in chronic renal failure samples

<table>
<thead>
<tr>
<th>Hemolysis (%)</th>
<th>5% of 0.6ml mushroom treated CRF sample (% Reduction)</th>
<th>5% of 0.8ml mushroom treated CRF sample (% Reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.9 ± 0.4</td>
<td>40.8 ± 0.9*</td>
<td>60.0 ± 0.9**</td>
</tr>
</tbody>
</table>

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 characterization of *Calocybe indica* in HgCl₂ induced invitro hemolysis in chronic renal failure samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy control</th>
<th>CRF patients- Before hemolysis</th>
<th>CRF patients- After hemolysis</th>
<th>Treatment 5% of 0.6ml mushroom treated CRF sample</th>
<th>Treatment 5% of 0.8ml mushroom treated CRF sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmole/ml)</td>
<td>0.09 ± 0.01</td>
<td>2.25 ± 0.16*</td>
<td>3.85 ± 0.11**</td>
<td>1.43 ± 0.11*</td>
<td>1.88 ± 0.10*</td>
</tr>
<tr>
<td>GSH (µg/ml)</td>
<td>25.3 ± 0.62</td>
<td>12.1 ± 0.87*</td>
<td>7.65 ± 0.88**</td>
<td>15.0 ± 1.5*</td>
<td>23.0 ± 0.40*</td>
</tr>
<tr>
<td>SOD (IU/g of protein)</td>
<td>6.46 ± 0.01</td>
<td>4.76 ± 0.08*</td>
<td>3.85 ± 0.08**</td>
<td>4.70 ± 0.01#</td>
<td>4.96 ± 0.01#</td>
</tr>
<tr>
<td>Catalase (IU/g of protein)</td>
<td>4.68 ± 0.12</td>
<td>3.71 ± 0.22*</td>
<td>3.31 ± 0.13**</td>
<td>3.43 ± 0.14#</td>
<td>3.46 ± 0.15#</td>
</tr>
<tr>
<td>Vitamin-C (mg/dl)</td>
<td>43.0 ± 14.6</td>
<td>28.3 ± 1.24*</td>
<td>27.3 ± 0.55**</td>
<td>29.0 ± 0.82#</td>
<td>36.8 ± 0.10#</td>
</tr>
<tr>
<td>Vitamin-C (mg/dl)</td>
<td>32.0 ± 12.0</td>
<td>10.8 ± 0.28*</td>
<td>7.4 ± 0.91**</td>
<td>16.7 ± 0.04#</td>
<td>17.5 ± 0.66#</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>171 ± 2.6</td>
<td>271 ± 2.0*</td>
<td>249 ± 4.1**</td>
<td>229 ± 1.9#</td>
<td>222 ± 2.2#</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>133 ± 1.6</td>
<td>194 ± 2.2*</td>
<td>184 ± 1.9**</td>
<td>179 ± 1.3#</td>
<td>174 ± 1.6#</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>234 ± 1.6</td>
<td>214 ± 2.4*</td>
<td>164 ± 1.8**</td>
<td>160 ± 1.2#</td>
<td>158 ± 2.1#</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>23.8 ± 2.4</td>
<td>26.5 ± 1.7*</td>
<td>22.1 ± 1.8**</td>
<td>20.1 ± 1.9#</td>
<td>20.3 ± 1.8#</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>27.4 ± 3.07</td>
<td>38.3 ± 1.08*</td>
<td>35.2 ± 1.78**</td>
<td>33.5 ± 1.3#</td>
<td>32.0 ± 1.97#</td>
</tr>
<tr>
<td>Sugar (mg/100ml)</td>
<td>99.8 ± 12.2</td>
<td>102 ± 3.2*</td>
<td>51 ± 1.88**</td>
<td>61.7 ± 2.2#</td>
<td>67 ± 1.4#</td>
</tr>
<tr>
<td>Hb (g/100ml)</td>
<td>14 ± 1.29</td>
<td>7.1 ± 0.73*</td>
<td>5.4 ± 0.66**</td>
<td>5.2 ± 0.51#</td>
<td>5.37 ± 0.67#</td>
</tr>
<tr>
<td>RBC (million cubic/cells)</td>
<td>4.7 ± 0.36</td>
<td>2.8 ± 0.7*</td>
<td>1.8 ± 0.7**</td>
<td>2.5 ± 0.7#</td>
<td>2.8 ± 0.8#</td>
</tr>
<tr>
<td>protein (g/dl)</td>
<td>5.4 ± 0.94</td>
<td>10.2 ± 0.92*</td>
<td>9.6 ± 0.92**</td>
<td>6.5 ± 0.86#</td>
<td>6.9 ± 1.04#</td>
</tr>
</tbody>
</table>

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).

Lipid peroxidation 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. Altered levels of total C and E were decreased in CRF and hemolysis induced CRF patients. Antioxidant enzymes such as SOD, CAT were decreased in CRF and mercury chloride treated subjects.

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