IN VITRO AND IN VIVO ANTIINFLAMMATORY ACTIVITY OF THE METHANOLIC EXTRACT OF CALOCYBE INDICA P.&C.

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ABSTRACT

In vitro and in vivo antiinflammatory activity of Milky mushroom (Calocybe indica) exhibited significant results for its protein denaturation (20.92±0.28 at 1000 µg/ml) and proteinase inhibitory activity (31.55±0.06% at 1000µg/ml) in a dose-dependent manner and in Carrageenan induced acute inflammatory model (48.55% at 200µg/kg body weight). Presence of phytochemicals namely flavonoids, polyphenols, saponins, tannins and terpenes may be responsible for such antiinflammatory activity. These results reveal that, Calocybe indica can be used as a potential antiinflammatory agent.

Keywords: Calocybe indica, antiinflammatory activity, phytochemicals, polyphenols, flavonoids.

INTRODUCTION

Mushrooms are nutritionally functional food as well as a source of physiologically beneficial and nontoxic medicines. Since ancient times, they have been used in folk medicine throughout the world[1]. Milky mushroom, Calocybe indica is a tropical edible mushroom. It has become the third commercially grown mushroom in India, after button and oyster mushrooms[2]. It can grow at temperature range of 25-35°C. Its robust size, sustainable yield, attractive color, delicacy, long shelf-life, moderate protein content and lucrative market value have attracted the attention of both mushroom consumers and prospective growers. At present this variety is being commercially cultivated in South India. Recently its production has started in North
India. The nutritive value of *Calocybe indica* is comparable with other edible mushrooms\(^3\text{-}^5\). Recent literature showed that, edible mushroom *Calocybe indica* possessed profound antioxidant, antibacterial, antitumour, antioxidant, antidiabetic and antiinflammatory activities \(^6\). As there is no reference on the antiinflammatory activity of the *Calocybe indica*, the present investigation has been carried out on its antiinflammatory activity.

**MATERIALS AND METHODS**

The fruiting bodies of *Calocybe indica* P.&C. were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamilnadu, South India. Sample preparation\(^7\), preliminary phytochemical screening\(^8\), *in vitro* antiinflammatory activity namely inhibition of protein denaturation\(^9\) and proteinase inhibitory activity\(^10\) and *in vivo* antiinflammatory activity of the extract were evaluated by Carrageenan induced edema in hind paw rats followed the method reported previously\(^11\).

**Animal ethics**

Animal experiments were carried out according to the guidelines of the committee for the purpose of control of experiments on animals (Reg. No.: CPCSEA/265) and approval of the Institutional Animal Ethics Committee was obtained.

**Statistical analysis**

The results are expressed as mean values and standard deviation. Data were analyzed using one way analysis of variance (ANOVA) followed by Turkey’s multiple comparison post hoc tests using SPSS software 16.0 versions. Values of \(p < 0.05\) were considered as statistically significant.

**RESULTS AND DISCUSSION**

**Preliminary phytochemical screening of the extract of *Calocybe indica***

The qualitative phytochemical screening of the methanolic extract of *Calocybe indica* revealed the presence of flavonoids, polyphenols, saponins, tannins and terpenes but the absence of alkaloids and glycosides (Table 1).
Table 1. Qualitative phytochemical screening of milky mushroom (*Calocybe indica*)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituent</th>
<th><em>Calocybe indica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Terpenes</td>
<td>+</td>
</tr>
</tbody>
</table>

*Key:* “+” denotes present, “-” denotes absent

Di-Cartlo et al. [12] reported that polyphenols have been shown to possess antiinflammatory properties. In the present study also methanolic extract of milky mushroom showed presence of polyphenols and flavonoids which may be responsible for antiinflammatory activities. The high content of saponins in the mushroom is useful in medicinal and pharmaceutical industry due to its foaming ability that produces frothy effect in the food industry [13]. Tannin and terpenes concentration detected in the mushroom have been found to possess astringent properties, which hasten the healing of wounds and inflamed mucous membrane [13].

**In vitro antiinflammatory studies**

**Inhibition of protein denaturation test**

Extract of *Calocybe indica* at different concentrations provided significant protection against denaturation of proteins. The *Calocybe indica* extract showed maximum percentage inhibition of 20.92±0.28 at 1000 µg/ml with IC$_{50}$ value of 1188.60±3.26 µg/ml. Most of the investigators have reported that denaturation of protein is one of the cause of inflammation. Production of autoantigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [14-16]. From the results of present study it can be stated that methanolic extract of *Calocybe indica* is capable of controlling the production of autoantigen and inhibits denaturation of protein in inflammatory disease.
Inhibition of proteinase activity test
Proteinases have been implicated in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors\[^{17}\]. Results of the present study indicated that *Calocybe indica* extract inhibited the proteinase trypsin in dose-dependent manner displaying the most potent inhibitory activity of 31.55±0.06% at 1000µg/ml with IC\(_{50}\) value of 292.63±0.54µg/ml (Table 2).

Table 2. *In vitro* antiinflammatory activities of *Calocybe indica*, inhibition of protein denaturation and proteinase inhibitory activity.

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>Protein denaturation inhibition (%)</th>
<th>IC(_{50}) value (µg/ml)</th>
<th>Proteinase inhibitory activity (%)</th>
<th>IC(_{50}) value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1.62 ± 0.11</td>
<td>4.98 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>8.46 ± 0.26</td>
<td>10.17 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>12.53 ± 0.12</td>
<td>1188.60 ± 3.26</td>
<td>18.11 ± 0.14</td>
<td>292.63 ± 0.54</td>
</tr>
<tr>
<td>800</td>
<td>17.98 ± 0.17</td>
<td>27.02 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>20.92 ± 0.28</td>
<td>31.55 ± 0.06</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means of three independent analyses of the extract ± standard deviation (n = 3) (p<0.05)

*In vivo* antiinflammatory activity
*Carrageenan induced hind paw edema model in rats*
The methanolic extract of *Calocybe indica* showed significant inhibitory effect against induced inflammation in the experimental models. The *Carrageenan* induced acute inflammations were significantly inhibited by the mushroom extract (48.55%). The effect was evident from the inhibition of the paw edema (Table 3).

Table 3. *In vivo* antiinflammatory activities of *Calocybe indica*, Carrageenan induced hind paw edema

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Paw thickness at 0 h (mm)</th>
<th>Paw thickness at 3 h (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control   (Group I)</td>
<td>0.567 ± 0.08</td>
<td>1.967 ± 0.29</td>
<td>-</td>
</tr>
<tr>
<td><em>Calocybe indica</em> (Group II) (200mg/kg b.w)</td>
<td>0.630 ± 0.08</td>
<td>1.012 ± 0.12</td>
<td>48.55</td>
</tr>
<tr>
<td>Indomethacin (Group III) (10mg/kg b.w)</td>
<td>0.65 ± 0.15</td>
<td>0.58 ± 0.13</td>
<td>70.51</td>
</tr>
</tbody>
</table>

Each values expressed as ± standard deviation of five observations (n = 5) (p < 0.001)
The maximum of edema inhibition 1.012±0.12 mm was obtained after 3 h of treatment with *Calocybe indica* (200 mg/kg b.w.), while standard, a powerful synthetic antiinflammatory agent showed 0.58±0.13 mm. These results clearly indicated that the methanolic extract of *Calocybe indica* has potentials in controlling antiinflammatory activity (*p* < 0.001). Jose et al. [18, 19] reported that methanolic extracts of *Pleurotus pulmonarius* and *Pleurotus florida* cause decrease of induced paw edema and ameliorated acute inflammation in mice. Ajith and Janardhanan [20] found that the methanolic extract of *Phellinus rimosus* (Berk.) Pilat. (Cracked Cap mushroom) inhibited significant antiinflammatory activity in acute and chronic inflammations in mice.

Selvi et al. [21, 22] studied that the aqueous and ethanolic extracts of milky mushroom (*Calocybe indica*) for its antilipidperoxidative activity through *in vitro* model of goat liver homogenate and RBC ghosts. These *in vitro* studies exhibited to a good extend by the mushroom extracts and the extend of significant inhibition in both the model.

The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophil and macrophages. It is believed that currently used drugs such as Opioids and Nonsteroidal antiinflammatory drugs (NSAID) are not useful in all cases of inflammatory disorders, because of their side effects (most important being the gastrointestinal irritation); economy and potency. For chronic diseases such as osteoarthritis and rheumatoid arthritis, lifelong dependency on drugs is necessary as a result, a search for an ideal antiinflammatory drug which is safe and effective is still continuing [23]. At this juncture milky mushroom may serve as a promising plant based antiinflammatory drug.

**CONCLUSION**

The methanolic extract of *Calocybe indica* with its significant antiinflammatory activity in rats, suggests its therapeutic potential for the prevention and the control of inflammation. Earlier researchers concentrated only on the *in vitro* antiinflammatory studies of the milky mushroom. The outcome of the present study fulfills the gap of *in vivo* antiinflammatory studies of milky mushroom and brings out a novel mushroom therapeutics. This work gives scope for further research on the mechanism of action.
Conflict of interest statement: We declare that we have no conflict of interest.

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REFERENCES


10. Oyedepo OO, Femurewa AJ. Antiprotease and membrane stabilizing activities of extracts


