Effect of bio-fractions isolated from *Ficus bengalensis* bark on clonidine induced catalepsy

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**ABSTRACT**

Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining normal posture. Clonidine, a $\alpha_2$-adrenoceptor agonist, induces dose dependent catalepsy in mice, which is inhibited by histamine H$_1$ receptor antagonists but not by H$_2$ receptor antagonist. Hence this screening is useful to evaluate antihistaminic activity. Anti-cataleptic fractions were isolated from aqueous extract of *Ficus bengalensis* bark. Six fractions frc.1 to frc.6 were isolated using column chromatography, Silica gel (60-120#) as stationary phase. The effect of these fractions on clonidine induced catalepsy in mice were studied and it was found that frc.4 inhibited significantly clonidine induced catalepsy as compare to control group at 50 mg/kg, i.p. dose. Phytochemical analysis showed that presence of phenol, flavonoid and terpenoid.

**Keywords:** *Ficus bengalensis*, cataleptic activity, antihistaminic, clonidine

**INTRODUCTION**

*Ficus bengalensis* Linn. (Moraceae) is a very large tree reaching about 30 m. high and sending down many aerial roots from the branches. Bark is tonic, cooling, astringent, diuretic, antidysenteric, antidiabetic and used in inflammation. Bark of plant showed different pharmacological activities, immunomodulatory and antioxidant; prevent liver damage, hypoglysemic, antidiabetic, anti-atherogenic. Bark showed antistress and antiallergic effects in asthma. Mukharjee, (1998) showed anti-diarrhoeal activity of ethanol extract of roots. Aqueous extract of bark significantly inhibited clonidine-induced catalepsy (Taur et al 2007). In our previous studies we evaluated various extracts i.e petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *Ficus bengalensis* bark on clonidine induced catalepsy and found that aqueous extract was most significant. The aim of present research was to isolate anti-cataleptic biofraction from aqueous extract.

**MATERIAL AND METHODS**

**Plant material**

Bark of *F. bengalensis* were collected from Ahmednagar district of Maharashtra and authenticated at Botanical Survey of India, Pune, where a sample specimen (voucher number: TDJ1) has been deposited.

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**Animals**

Male Swiss albino mice weighing 25-28 g were housed under standard laboratory conditions, in groups of five. The animals had free access to food and water. The animal ethical committee of the institute approved all the protocols of the study.

**Drugs and Chemicals**

Clonidine (Unichem, Ltd.); Chlorpheniramine maleate (Alkem, Mumbai); Ethyl acetate A R (Research Lab, Pune); Methanol A R (Research Lab, Pune).

**Extraction**

Dried and coarsely powdered bark of *F. bengalensis* was macerated with chloroform water for 48 hrs. The extract obtained was concentrated and completely dried over a regulated water bath maintained at 40°C. Dried extract produce 5.68%w/w, yield.

**Statistical Analysis**

The results were reported as mean±SEM and analyzed for statistical significance using one-way ANOVA followed by Dunnet’s test. $P<0.01$ and $P<0.05$ was considered significant

**Isolation of fraction from aqueous extract:**

Aqueous extract (5.1 g) of *F. bengalensis* bark was dissolved in small volume of ethanol-water (1:1) and applied to silica gel G (60-120) column (2.9 X 44 cm), which was eluted by using ethyl acetate; yielding Frac.1 (4.0%); ethyl acetate: methanol (1:1) yield Frac.2 (22.0%); ethyl acetate: methanol (1:9) yield Frac.3 (12.0%) and Frac.4 (20.0%); further eluted with methanol yield Frac.5 (8%) and lastly with ethanol: water (1: 1) yielding Frac.6 (18%). These fractions then screened for clonidine induced catalepsy in mice.

**Clonidine-induced catalepsy in mice:**

Bar test was used to study effect of extracts on clonidine-induced catalepsy, to determine indirect antihistaminic activity.
Table no.1 Effect of various fractions isolated from aqueous extract of F. bengalensis bark on clonidine induced catalepsy in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration of catalepsy at Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>40.14±1.08</td>
</tr>
<tr>
<td>II</td>
<td>Frc.150 mg/kg, i.p</td>
<td>42.83±4.82*</td>
</tr>
<tr>
<td>III</td>
<td>Frc.250 mg/kg, i.p</td>
<td>27.21±5.25*</td>
</tr>
<tr>
<td>IV</td>
<td>Frc.350 mg/kg, i.p</td>
<td>24.50±3.94**</td>
</tr>
<tr>
<td>V</td>
<td>Frc.450 mg/kg, i.p</td>
<td>9.54±2.02**</td>
</tr>
<tr>
<td>VI</td>
<td>Frc.550 mg/kg, i.p</td>
<td>23.04±0.69**</td>
</tr>
<tr>
<td>VII</td>
<td>Frc.650 mg/kg, i.p</td>
<td>38.63±1.4*</td>
</tr>
<tr>
<td>VIII</td>
<td>CPM 10mg/kg i.p</td>
<td>9.47±3.48</td>
</tr>
</tbody>
</table>

n= 5, in each group. ** P<0.01, * P< 0.05, one way ANOVA followed by Dunnett’s Test.

Phytochemical study: The active fraction Frc.4 was subjected to phytochemical analysis for identification of constituents using standard procedures.12,13

RESULT AND DISCUSSION

The isolated fractions frc.1 to frc.6 from aqueous extract was screened for anti-histamine activity by using clonidine induced catalepsy in mice. Clonidine induces maximum (140.37 ± 5.8) catalepsy after 90 min in saline (control) treated group as shown in table no. 1. Out of these fractions the frc.4 significantly (P<0.001) protect clonidine induced catalepsy in mice (32.02 ± 1.4) after 60 min. as compared to control group. Standard drug CPM inhibited catalepsy after 90 min. Clonidine, a Gα adrenoceptor agonist induces dose dependent catalepsy in mice, which was inhibited by histamine H1 receptor antagonists but not by H2 receptor antagonist.14 Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions.15 Catalepsy produced by clonidine is mediated by histamine via H1 receptors. Significant inhibition of clonidine induced catalepsy by frc.4 reveals that antihistaminic potential of frc.4. Phytochemical study of frc.4 observed that this antihistaminic property is due to presence of phenol, flavonoids or terpenoids.

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