Antihyperglycemic, antistress and nootropic activity of roots of
Rubia cordifolia Linn

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Effect of alcoholic extract of roots of Rubia cordifolia was studied on elevated blood glucose level in alloxan treated animals. The extract reduced the blood sugar level raised by alloxan. Effect of alcoholic extract was also investigated on cold restraint induced stress and scopolamine-induced memory impairment. Alcoholic extract enhanced brain γ-amino-n-butyric acid (GABA) levels and decreased brain dopamine and plasma corticosterone levels. Acidity and ulcers caused due to cold restraint stress were inhibited by alcoholic extract. Animals treated with alcoholic extract spent more time in open arm in elevated plus maze model. It also antagonized scopolamine induced learning and memory impairment. Baclofen induced catatonia was potentiated by alcoholic extract.

Keywords: Antihyperglycemic activity, Antistress, Nootropic activity, Root extract, Rubia Cordifolia

Rubia cordifolia Linn (Rubiaceae) is a climber growing in the north-west Himalaya and other hilly areas of India. The methanolic extract of R. cordifolia has anticancer activity.1 Ethanolic extract of the aerial parts of the plant shows hypoglycemic activity in rats.2 Roots are used in folkore medicine to cure ulcers.3 Diabetes patients have shown impairment in learning and memory and mental and motor speed.4 The triterpenes isolated from the petroleum ether extract of R. cordifolia possess anticonvulsant property.5 Tripathi and his associates have shown that the ethanolic extract of R. cordifolia has lipoxgenase inhibitory activity and its ethyl acetate fraction is most potent in this regard.6 Our laboratory has also reported the anti-inflammatory and analgesic activity of R. cordifolia.7 Most of the established nootropic plants like Brahmi, Shatavari, Shankhpushpi, Siris contain high concentration of saponin. Preliminary phytochemical work showed that roots of Rubia cordifolia contain saponins. Therefore, we investigated antihyperglycemic, antistress, and nootropic activity of the alcoholic extract of Rubia cordifolia roots. In addition acute toxicity, neurotoxicity, anxiolytic activity and effect on baclofen-induced catatonia were also assessed to study the probable mode of action.

Materials and Methods

Extraction — The dried roots of Rubia cordifolia were obtained from Aushadhi Bhavan, Ayurved Seva Sangh, Nashik, India. The plant material was identified and authenticated as roots of Rubia cordifolia by Dr. D. R. Mahajan of the Botany Department, KTHM College Nashik. The roots were extracted successively with petroleum ether (60°-80°C) and alcohol (70%v/v) using Soxhlet’s extractor till the material was exhausted as indicated by a blank thin layer chromatography. Extract was concentrated under vacuum. Alcoholic extract of Rubia cordifolia (AERC) was dissolved in distilled water and administered intraperitoneally (ip).

Animals — Albino mice (NIN strain) of either sex (weighing 20-25 g) and albino rats (NIN strain) of either sex (weighing 150-175 g) were housed into groups of 8 animals under standard laboratory conditions. The experiments were performed during 0900-1600 hour in the laboratory, at 23°C±1°C. The protocol of this study was approved by the Institutional Animal Ethics Committee.

Drugs — The drugs used were—Alloxan (Burgoyne Burbidges, India), (±) baclofen (Sun Pharma, India), diazepam (Ranbaxy, India), piracetam (Uni-UCB, India), Scopolamine (German Remedies, India). All drug solutions were prepared in distilled water immediately for use.

Acute toxicity — AERC was administered at the doses of 100, 200, 400, and 1000 mg/kg intraperitoneally (ip) and percentage mortality was observed after 24 hr in mice.
Activity on rotating rod — Mice were previously trained to remain on the rod (2.54 cm diam) rotating at a speed of 20 rev/min for a period of 5 min. On the next day the animals were randomly divided into groups of 5 each. The alcoholic extract of Rubia cordifolia (100, 200, and 400 mg/kg, ip) or diazepam (1 mg/kg, ip) or vehicle (5 ml/kg, ip) was administered 30 min before the test and the time required to fall off the rotating rod was noted for each animal.

Assessment of blood glucose level — Blood glucose level was measured using the One Touch Glucometer. Mice (n=5) were treated with alloxan (200 mg/kg, sc) 24hr before alcoholic extract of Rubia cordifolia (100, 200, or 400 mg/kg, ip) and after 60 min, blood was withdrawn from retro-orbital plexus. One drop of blood was taken on glucometer strip and blood glucose level (mg/dl) was obtained within 40 sec.

Assessment of antistress activity — Stress ulcers were induced in mice (n = 5) by subjecting them to restraint stress for 2 hr at 4°C as described earlier. Alcoholic extract of Rubia cordifolia (25, 50 and 100mg/kg, ip) or diazepam (1mg/kg, ip) was administered 30 min before stress. Whole brain contents dopamine, γ-amino-n-butyric acid, plasma 11-hydroxycorticosteroids, total acidity and the severity of ulcers were measured.

Assessment of anxiolytic activity — Elevated plus maze was used as described earlier. In brief, mice (n = 5) were placed individually in the center of elevated plus maze (25 × 5 cm, open arm and 25 × 5 × 20 cm, closed arm, elevated to the height of 70 cm) facing an enclosed arm. The time spent by mice in open arm was noted. The mice were treated with vehicle, diazepam (0.5 or 1.0 mg/kg, ip), or AERC (25, 50, and 100 mg/kg, ip) 30 min before the test.

Effect on learning and memory — Mice were individually placed at the end of one open arm facing away from the central platform and the time it took to move from open arm to either of the enclose arm (Transfer Latency, TL) was recorded. On the first day mice were allowed to explore the plus-maze after measurement of TL. Mice were sent back to their home cages after the first trial. After 24 hr, mice were treated with vehicle, alcoholic extract of Rubia cordifolia (25, 50, and 100 mg/kg, ip), piracetam (100 mg/kg, ip) or scopolamine (0.3 mg/kg, ip) and placed again on the elevated plus maze individually as before and TL was noted again.

Table 1 — Effect of alcoholic extract of R. cordifolia on fall-off time in mice using Rotarod test

<table>
<thead>
<tr>
<th>Treatment (conc., mg/kg)</th>
<th>Falling time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle AERC</td>
<td>247.25 ± 35.58</td>
</tr>
<tr>
<td>100</td>
<td>206.6 ± 57.63</td>
</tr>
<tr>
<td>200</td>
<td>114.5 ± 30.27*</td>
</tr>
<tr>
<td>400</td>
<td>224.8 ± 33.92</td>
</tr>
<tr>
<td>Diazepam (1)</td>
<td>99.2 ± 9.11*</td>
</tr>
</tbody>
</table>

*P<0.05, Student’s t test.
decreased the blood glucose level significantly to 84.8 ± 3.58, 77.75 ± 3.30, and 108.8 ± 7.83 mg/dl, respectively when measured after 1 hr of AERC treatment. The observations are given in Table 2.

Assessment of antistress activity — In vehicle treated mice, cold restraint stress (CRS) significantly increased ulcer index from 0.4 ± 0.06 to 1.34 ± 0.06. Alcoholic extract of Rubia cordifolia (25, 50, and 100 mg/kg, ip) significantly decreased ulcer index in a dose-dependent manner in animals under CRS. Diazepam (1 mg/kg, ip) significantly decreased ulcer index in animals under CRS as compared to stress control animals.

In vehicle treated animals, CRS significantly increased acidity, plasma corticosterone level. Alcoholic extract of Rubia cordifolia (100 mg/kg, ip) per se insignificantly increased acidity, but significantly increased plasma corticosterone level. Alcoholic extract of Rubia cordifolia (25, 50, and 100 mg/kg, ip) significantly decreased acidity and plasma corticosterone level in a dose-dependent manner in animals under CRS. Diazepam (1 mg/kg, ip) significantly decreased acidity and plasma corticosterone level as compared to stress control animals. Brain content of dopamine increased significantly in animals treated with vehicle under CRS. Alcoholic extract of Rubia cordifolia (100 mg/kg, ip) per se significantly decreased brain content of dopamine. Alcoholic extract of Rubia cordifolia (25, 50 and 100 mg/kg, ip) significantly decreased brain content dopamine, in dose-dependent manner in animals under CRS. Diazepam (1 mg/kg, ip) significantly decreased brain content, dopamine, in animals under CRS.

In vehicle treated animals, CRS significantly decreased γ-amino-n-butyric acid in brain. Alcoholic extract of Rubia cordifolia (100 mg/kg, ip) per se significantly increased brain γ-amino-n-butyric acid. Alcoholic extract of Rubia cordifolia (50 and 100 mg/kg) significantly increased γ-amino-n-butyric acid in dose-dependent manner in animals under CRS. Alcoholic extract of Rubia cordifolia (25 mg/kg, ip) caused insignificant increase in brain content of γ-amino-n-butyric acid in animals under CRS. The observations are given in Table 3.

Assessment of anxiolytic activity — Alcoholic extract of Rubia cordifolia (100 mg/kg) and diazepam (1 mg/kg, i. p.) significantly increased time spent in open arm. Alcoholic extract of Rubia cordifolia (25 and 50 mg/kg) and diazepam (0.5mg/kg) insignificantly altered time spent in open arm (Table 4).

Effect on learning and memory — In vehicle treated group, TL on day 1 was 32.33 ± 1.85 sec. In the same group (vehicle treated group) TL significantly decreased on day 2 and day 9. Alcoholic extract of Rubia cordifolia (25 mg/kg) decreased TL

<table>
<thead>
<tr>
<th>Treatment(conc, mg/kg)</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>65.4 ± 9.53</td>
</tr>
<tr>
<td>Alloxan (200, sc) (after 24 hr)</td>
<td>147.2 ± 8.21@</td>
</tr>
<tr>
<td>Alloxan (200, s.c.) +AERC (100) (after 1 hr)</td>
<td>84.8 ± 3.58**</td>
</tr>
<tr>
<td>Alloxan (200, sc) +AERC (200) (after 1 hr)</td>
<td>77.75 ± 3.30**</td>
</tr>
<tr>
<td>Alloxan (200, sc) +AERC (400) (after 1 hr)</td>
<td>108.8 ± 7.83*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, vs. alloxan treated group (Student’s t test). @P<0.01, vs. vehicle (Student’s t test).

Table 3 — Antistress activity of alcoholic extract of R. cordifolia in mice

<table>
<thead>
<tr>
<th>Treatment (conc, mg/kg)</th>
<th>Ulcer index (mm)</th>
<th>Acidity (meq/l/100g)</th>
<th>Corticosterone (mg/cg/100ml blood)</th>
<th>Dopamine (ng/g)</th>
<th>GABA (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without stress</td>
<td>0.4 ± 0.06</td>
<td>4.2 ± 0.56</td>
<td>1.35 ± 0.29</td>
<td>415.4 ± 22.3</td>
<td>474.74 ± 45.4</td>
</tr>
<tr>
<td>Stress control</td>
<td>1.34 ± 0.06</td>
<td>26.81 ± 1.01</td>
<td>9.6 ± 0.12</td>
<td>588.38 ± 37.19@</td>
<td>243.18 ± 12.81@</td>
</tr>
<tr>
<td>AERC(25)+stress</td>
<td>0.57 ± 0.08</td>
<td>5.72 ± 1.06</td>
<td>3.18 ± 0.42</td>
<td>173.02 ± 14.6£</td>
<td>646.58 ± 5.17£</td>
</tr>
<tr>
<td>AERC(50)+stress</td>
<td>0.94 ± 0.01</td>
<td>12.54 ± 0.74</td>
<td>4.7 ± 0.43</td>
<td>363.63 ± 16.37**</td>
<td>257.75 ± 17.49</td>
</tr>
<tr>
<td>AERC(100)+stress</td>
<td>0.81 ± 0.05</td>
<td>8.36 ± 1.28</td>
<td>2.46 ± 0.18</td>
<td>264.23 ± 10.1**</td>
<td>445.45 ± 30.9**</td>
</tr>
<tr>
<td>Diazepam(1)+stress</td>
<td>0.67 ± 0.05</td>
<td>6.32 ± 0.93</td>
<td>2.32 ± 0.16</td>
<td>125.94 ± 1.71**</td>
<td>603.02 ± 12.1**</td>
</tr>
</tbody>
</table>

Wt. of brain = 0.33 ± 0.01 g

* P<0.05, ** P<0.01, vs. alloxan treated group (Student’s t test).

Table 2 — Effect of alcoholic extract of R. cordifolia on blood glucose level in mice treated with alloxan

[Values are mean ± SE of 5 animals in each group]
by 2.78 per cent on day 2, while it increased by 52.77 per cent on day 9. Alcoholic extract of *Rubia cordifolia* (50 and 100 mg/kg) and piracetam (100 mg/kg) decreased TL on day 2 and 9. Inflexion ratio significantly increased with alcoholic extract of *Rubia cordifolia* (100 mg/kg) and piracetam (100 mg/kg) on day 9 as compared to vehicle.

Scopolamine (0.3 mg/kg) increased TL on day 2 and 9. Alcoholic extract of *Rubia cordifolia* (100, 200 and 400 mg/kg) when given in combination with scopolamine (0.3 mg/kg) significantly decreased TL on day 2 and 9. It significantly increased inflexion ratio on day 2 and 9 (Table 5).

**Effect on baclofen induced catatonia** — In baclofen (5 mg/kg) treated group, the peak catatonia reached after 30 min and maintained peak catatonia till 180 min. Alcoholic extract of *Rubia cordifolia* (200 mg/kg) preponed the peak effect and at a dose of 400 mg/kg potentiated catatonia and peak catatonia was observed after 15 min (Table 6).

**Discussion**

Stress is known to affect the CNS to various extents. It has been shown that stress induces physical and mental disorders. All the systems that raise plasma glucose concentration are activated during stress, which explains why stress exacerbates the symptoms of diabetes. Alcoholic extract of *Rubia cordifolia* (100, 200, 400 mg/kg) significantly decreased the blood glucose level elevated by alloxan.

The U-shaped curve may be because of increased concentration of glucocorticoids as alcoholic extract of *Rubia cordifolia* increases plasma corticosteroid level which results in increased blood glucose level. The extract lowered blood sugar levels in alloxanized mice indicating that the extract has extrapancreatic effects. The exact biological active constituent(s) has neither been known nor the exact mode of action of antihyperglycemic effect determined. Nonetheless,

<table>
<thead>
<tr>
<th>Treatment (conc. mg/kg)</th>
<th>Time spent in open arm (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>77.00 ± 6.02</td>
</tr>
<tr>
<td>AERC (25)</td>
<td>53.75 ± 2.78</td>
</tr>
<tr>
<td>AERC (50)</td>
<td>94.00 ± 7.70</td>
</tr>
<tr>
<td>AER (100)</td>
<td>102.0 ± 2.01*</td>
</tr>
<tr>
<td>Diazepam (0.5)</td>
<td>87.40 ± 7.53</td>
</tr>
<tr>
<td>Diazepam (1)</td>
<td>112.2 ± 6.38*</td>
</tr>
</tbody>
</table>

* P<0.05, Student’s t test

**Table 6** — Effect of alcoholic extract of *R. cordifolia* on (+) baclofen (5 mg/kg, ip) induced catatonia in mice

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Baclofen</th>
<th>AERC (100)+ Baclofen</th>
<th>AERC (200)+ Baclofen</th>
<th>AERC (400)+ Baclofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.40 ± 0.02</td>
<td>0.60 ± 0.19</td>
<td>1.11 ± 0.04</td>
<td>3.96 ± 0.17*</td>
</tr>
<tr>
<td>15</td>
<td>2.17 ± 0.35</td>
<td>3.01 ± 0.13</td>
<td>3.91 ± 0.66**</td>
<td>5.00 ± 0.0**</td>
</tr>
<tr>
<td>30</td>
<td>5.00 ± 0.0</td>
<td>4.41 ± 0.39</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
<tr>
<td>60</td>
<td>5.00 ± 0.0</td>
<td>4.48 ± 0.06</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
<tr>
<td>90</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
<tr>
<td>120</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
<tr>
<td>150</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
<tr>
<td>180</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
<td>5.00 ± 0.0</td>
</tr>
</tbody>
</table>

* P<0.05, ** <0.05, vs. baclofen (ANOVA followed by Dunnett’s test)

**Table 5** — Effect of alcoholic extract of *R. cordifolia* on learning and memory in mice using elevated plus maze

<table>
<thead>
<tr>
<th>Treatment (conc. mg/kg)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 9</th>
<th>Day 2</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>32.33 ± 1.85</td>
<td>21.66 ± 2.18*</td>
<td>14.66 ± 0.88**</td>
<td>0.50 ± 0.06</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>AERC (25)</td>
<td>18.0 ± 0.76</td>
<td>17.5 ± 0.53</td>
<td>27.25 ± 0.87</td>
<td>0.77 ± 0.14</td>
<td>0.33 ± 0.21</td>
</tr>
<tr>
<td>AERC (50)</td>
<td>25.50 ± 0.71</td>
<td>22.5 ± 0.70</td>
<td>17.66 ± 0.68</td>
<td>0.15 ± 0.11</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>AERC (100)</td>
<td>37.00 ± 1.05</td>
<td>28.0 ± 0.53</td>
<td>13.66 ± 0.97</td>
<td>0.35 ± 0.09</td>
<td>2.18 ± 0.29*</td>
</tr>
<tr>
<td>Piracetam (100)</td>
<td>20.33 ± 1.05</td>
<td>12.16 ± 0.47**</td>
<td>6.60 ± 0.50**</td>
<td>0.71 ± 0.08</td>
<td>2.19 ± 0.28*</td>
</tr>
<tr>
<td>Scopolamine (0.3)</td>
<td>83.6 ± 4.55</td>
<td>122.8 ± 13.55</td>
<td>70.25 ± 2.68</td>
<td>-0.29 ± 0.05a</td>
<td>0.24 ± 0.05a</td>
</tr>
<tr>
<td>AERC (100) + Scop. (0.3)</td>
<td>66.4 ± 6.32</td>
<td>24.2 ± 2.45**</td>
<td>13.2 ± 1.15**</td>
<td>1.81 ± 0.28c</td>
<td>4.46 ± 0.94b</td>
</tr>
<tr>
<td>AERC (200) + Scop. (0.3)</td>
<td>59.0 ± 8.31</td>
<td>14.2 ± 2.15**</td>
<td>8.75 ± 0.85**</td>
<td>3.57 ± 0.82c</td>
<td>5.72 ± 1.26b</td>
</tr>
<tr>
<td>AERC (400) + Scop. (0.3)</td>
<td>50.2 ± 5.17</td>
<td>23.5 ± 3.79*</td>
<td>9.5 ± 1.02*</td>
<td>1.20 ± 0.15c</td>
<td>4.72 ± 1.36b</td>
</tr>
</tbody>
</table>

P<0.05, ** <0.01, vs. Day 1 (Student’s t test)

* P<0.05, vs. Vehicle (Student’s t test)

__ ve sign indicates impairment of memory
this observation is consistent with the use of R. cordifolia in folklore diabetes management.

Acute experimental stresses in rats have been shown to increase the synthesis and release in the brain of norepinephrine, dopamine, and 5-hydroxytryptamine (5-HT)23. Also plasma concentration of free dopamine increases in association with events that increase sympathetic tone although to a much lesser degree than seen for norepinephrine, or epinephrine. Thus, a variety of emotional and physical stresses may be associated with increases in plasma free dopamine24.

All the stressors significantly increase levels of norepinephrine, plasma adrenocorticotropic hormone, (ACTH), dihydroxy phenyl acetic acid (DOPAC), and corticosterone. The stressors, such as immobilization, norepinephrine synthesis, reflected by DOPAC changes, are strongly and positively correlated with activity of the hypothalamo-pituitary axis (HPA axis)25. Central GABA-ergic mechanism plays an important role in stress-induced gastric ulcers26. Plant extract increasing γ-amino-n-butyric acid level in brain protected the rats against CRU26. Thus, the results emanated in the present study indicated that alcoholic extract of Rubia cordifolia possessed significant antistress activity. Alcoholic extract of Rubia cordifolia enhanced brain γ-amino-n-butyric acid levels of mice and decreased brain dopamine levels and plasma corticosterone levels. Depression may result from an inability to cope with stresses of diabetes, but may also result from direct effects of diabetes on central nervous system or a combination of both28. Alcoholic extract of Rubia cordifolia (50 and 100 mg/kg) showed increase in time spent in open arm. These observations are in consistency with those described by Pellow15.

Scopolamine induced amnesia is a popular animal model of cognitive dysfunction. Alcoholic extract of Rubia cordifolia (100, 200, 400 mg/kg) prevented the scopolamine induced amnesia in elevated plus maze model. The exact mechanisms involved in cognitive improvement are not known. γ-Amino-n-butyric acid mediated behavior was studied using baclofen (GABA-A agonist) induced catatonia. This was congruent with the observation that alcoholic extract of Rubia cordifolia increased the brain content of γ-amino-n-butyric acid compared to the vehicle treated group. Potentiation of baclofen induced catatonia by alcoholic extract of Rubia cordifolia indicated that weak nootropic activity observed in the present study was unrelated to the effect on GABA-ergic transmission. Alcoholic extract of Rubia cordifolia showed no mortality till 1000mg/kg in mice and it exhibited antihyperglycemic, antistress, scopolamine antagonistic activities. Rubia cordifolia is used in several Ayurvedic medicines. The observations of this study suggest that efforts should be made to obtain novel molecule(s) for the therapeutic use for antihyperglycemic, antistress, and nootropic activity.

References
8 Dunham N M & Miya T S, A Note on simple apparatus for detecting neurological deficit in rat and mice, J Am Pharm Ass, 46 (1957) 208.