EVALUATION OF SKELETAL MUSCLE RELAXANT ACTIVITY OF AQUEOUS EXTRACT OF CINNAMOMUM VERUM BARK ON SWISS ALBINO MOUSE

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ABSTRACT

Objective: To evaluate the muscle relaxant activity of aqueous extract of bark of Cinnamomum verum (synonyms: Cinnamomum zeylanicum, Ceylon cinnamon) [AEBCV] on swiss albino mouse.

Methods: Twenty-two albino mice (25-30 g) were taken and divided into four groups. The first group was kept as a control (Distilled water), second as the standard (Diazepam) and remaining two groups as test 1 and 2 were given different doses of AEBCV (50 and 100 mg/kg). Skeletal muscle relaxant activity on rotarod and actophotometer were performed. Statistical analysis was carried out by using ANOVA, followed by Tukey's multiple comparison tests, using GraphPad Prism software. P-value <0.05 was considered significant.

Results: In rotarod test, AEBCV (50 mg/kg, 100 mg/kg) significantly reduced motor coordination of tested animals when compared to control (P<0.05). Maximum muscle relaxation was observed with 100 mg/kg. In actophotometer, AEBCV showed highly significant reduction (P<0.05) in the locomotor activity when compared to control that is 74% and 81%. AEBCV also showed a significant reduction when compared to standard.

Conclusions: Our data indicates that AEBCV possesses skeletal muscle relaxant activities.

Keywords: Cinnamomum verum, Cinnamomum zeylanicum, Ceylon cinnamon, Rota rod, Actophotometer, Muscle relaxant

INTRODUCTION

A muscle relaxant is a type of medication that helps alleviate muscle spasms, stiffness, and associated pain. These drugs work by either acting directly on the muscles or targeting the central nervous system to reduce muscle contractions. Muscle relaxants are commonly prescribed for conditions such as muscle strains, sprains, spasms, and certain neurological disorders. Neuromuscular blockers exert their effects at the neuromuscular junction, directly targeting muscle fibers to induce transient paralysis, while spasmylics, acting centrally, are employed to alleviate musculoskeletal pain and reduce spasticity across various neurological conditions [1].

The bark of Cinnamomum verum (CV) is commonly added as spices and flavoring agent in food products. CV belongs to the family Lauraceae and is commonly known as Ceylon or True cinnamon. In Assamese, the vernacular name is 'Dalcheni'. In ancient times cinnamon was prized as much for its sweet, sharp and sensuous fragrance as it was for its taste. Cinnamon abundant in antioxidants and beneficial compounds, plays a crucial role in blood sugar level maintenance in diabetes, shields against cancer heart disease and mitigates inflammation and discomfort [2].

As early as 2000 BC, cinnamon was being brought into Egypt through imports [3]. In Egypt, it was being utilized for embalming rituals as well as in religious observances. Throughout medieval Europe, cinnamon was employed both as a spice to enhance flavor and as a medicinal remedy for addressing cold symptoms, including coughs, hoarseness and sore throats. Later, it emerged as the top revenue-generating spice during the Dutch East India Company's trading endeavors [4]. Now-a-days, it is found widely in Sri Lanka but also grows in Malabar, Cochin-China, Sumatra and in Eastern Islands too. Besides India, it is also cultivated in Brazil, Mauritius, Jamaica and in other countries also. Hence, this study is undertaken to elucidate the skeletal muscle relaxant properties of CV.

MATERIALS AND METHODS

This is an experimental study conducted within the period of one month at Jorhat Medical College and Hospital after obtaining approval from the Institutional Animal Ethics Committee (IAEC) filed under the No. IAEC/JMCH/09/2023/006.

Preparation of plant extract

The bark of CV was obtained from a local market and was authenticated by Dr. Iswar Chandra Barua, Principal Scientist, Department of Agronomy, Assam Agricultural University (Weed Herbarium Accession no: AUU-WH-5493). The aqueous extract of powdered bark was prepared using Soxhlet's apparatus in the Department of Pharmacology. The extract was kept at room temperature, which was dined under vacuum and protected from direct sunlight.

The powdered form of cinnamon (50 mg) was dissolved in 200 ml distilled water and then transferred to the thimble, with filter paper, of Soxhlet apparatus. It was subsequently boiled for 3 cycles (each lasting for 6 h), after which the solid residue left in the thimble was discarded. The concentrated remained in the boiling flask was filtered through Whatman filter paper (no.1) and the filtrate is evaporated using spirit lamp [5]. Percentage of extract yielded was 40% (solid remains following evaporation 20 g).

Animals

The study was carried out on twenty-two Swiss albino mice of either sex weighing about 20±2 g. Animals were procured from Chakraborty Enterprise, Kolkata, India (Regd. No. 1443/F0/b/11/CPCSEA). Animals were quarantined for 14 d and then housed in the central animal house, Jorhat Medical College and Hospital and were allowed to accustom to the environment for one week. Animals were maintained under standard husbandry conditions. The animals were fed chow diet with water ad libitum, and were maintained under well ventilation with regulation of temperature with proper spacing and isolation. All the animals were taken care of to prevent coprophagy, under ethical consideration. The study was performed in accordance with the CESSE guidelines [6].

Drugs and chemicals

Diazepam, 10 mg/kg was administered in a volume of 10 ml/kg. The extracts were suspended in distilled water and subjected to muscle
relaxant activity using the rotarod apparatus and actophotometer. The extracts were administered orally in a volume of 10 ml/kg of body weight in doses of 50 mg/kg and 100 mg/kg.

Toxicity study
Acute toxicity test was done for the extract of CV following OECD 425 guidelines. The AEBCV was found to be non-toxic up to a dose 2000 mg/kg. Hence, 50 mg/kg and 100 mg/kg were selected for the study [7].

Experimental design
Group 1 (Control-C) = Distilled water (10 ml/kg)
Group 2 (Standard drug-S) = Diazepam (10 mg/kg)
Group 3 (Test drug-T1) = AEBCV (50 mg/kg)
Group 4 (Test drug-T2) = AEBCV (100 mg/kg)

Skeletal muscle relaxant activity
The mice were divided into four groups. Group 1 included four mice and the other remaining groups included six mice each. Group 1 served as the control, which received distilled water 10 ml/kg. Group 2 served as the standard and received diazepam 10 mg/kg, per orally; Group 3 and 4 received the aqueous AEBCV orally at a dose of 50 and 100 mg/kg respectively. The animals remained on rotarod (25 rpm) for five minutes. After administering the control, standard and test drug, the time taken to fall off the rotating rod was recorded 30 min later. The variance in fall-off time between the control and the treated mice was utilized as an indicator of muscle relaxation. Scoring was assigned to each fallen mouse within different time range [8].

Locomotor activity
The assessment of spontaneous locomotor activity was aided by employing a photoactometer [9]. Each animal was monitored for a duration of five minutes with a square enclosed field arena (50 x 30 cm) containing six photocells embedded in the outer wall. The interruption of photocell beams was recorded using a six-digit counter. The baseline activity score for all the animals was recorded. After administering the control, standard and test drugs, the activity score for five minutes was observed after one hour. The difference in activity levels before and after drug administration was recorded. The percentage decrease in motor activity was determined.

Statistical analysis
The results of the study were expressed as mean±standard deviation (mean±SD). Results were analyzed by ANOVA, followed by Tukey's multiple comparison test [10]. The significant difference was established when probability value (p-value) was less than 0.05. The results were calculated with the use of GraphPad Prism software version 5.0.

RESULTS
Rotarod test
The AEBCV in doses 50 and 100 mg/kg demonstrated a significant decrease in the duration animals spent on the rotating rod compared to the control group (P<0.05) (table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>1-19 sec</th>
<th>20-39 sec</th>
<th>40-59 sec</th>
<th>60-79 sec</th>
<th>80-99 sec</th>
<th>100-120 sec</th>
<th>mean score</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>12</td>
<td>5.25±0.95</td>
</tr>
<tr>
<td>S</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td>1.66±0.81*</td>
</tr>
<tr>
<td>T1</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td>3.16±1.16*</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.16±1.16*</td>
</tr>
</tbody>
</table>

C = Control (Distilled water- 10 ml/kg), S = Standard (Diazepam-10 mg/kg), T1 = AEBCV (50 mg/kg), T2 = AEBCV (100 mg/kg), mean scores are expressed as mean±SD, *P<0.05 when compared to control.

Scoring system
Score time (in sec)
1 = 1-19 sec (score 1 assign to each mouse fallen within this time range)
2 = 20-39 sec (score 2 assign to each mouse fallen within this time range)
3 = 40-59 sec (score 3 assign to each mouse fallen within this time range)
4 = 60-79 sec (score 4 assign to each mouse fallen within this time range)
5 = 80-99 sec (score 5 assign to each mouse fallen within this time range)
6 = 100-120 sec (score 6 assign to each mouse fallen within this time range)

Actophotometer test
The AEBCV in doses 50 and 100 mg/kg exhibited a highly significant reduction compared to control and standard groups (table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Actophotometer score</th>
<th>Score activity after administration of the drug</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>178.8±14.22</td>
<td>178.8±14.22</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>192.3±11.78</td>
<td>12.8±4.71*</td>
<td>93%</td>
</tr>
<tr>
<td>T1</td>
<td>180.3±9.39</td>
<td>47±9.89**</td>
<td>74%</td>
</tr>
<tr>
<td>T2</td>
<td>189.5±13.37</td>
<td>36±11.19**</td>
<td>81%</td>
</tr>
</tbody>
</table>

C = Control (Distilled water- 10 ml/kg), S = Standard (Diazepam-10 mg/kg), T1 = AEBCV (50 mg/kg), T2 = AEBCV (100 mg/kg). All values are expressed as mean±SD, *P<0.05 when compared to control. **P<0.05 when compared to standard.

DISCUSSION
The present study was conducted to assess the muscle relaxant property of CV bark on Swiss albino mouse, comparing its efficacy to the standard drug diazepam.

In rotarod test, AEBCV (50 mg/kg, 100 mg/kg) showed significant reduction in the time spent by the animals on the revolving rod when compared to control (P<0.05). Maximum muscle relaxation was observed with 100 mg/kg of CV. The standard drug also showed a highly significant effect when compared to control and the muscle relaxant activity of AEBCV was comparable to the standard drug.

In actophotometer test, two different doses of AEBCV (50, 100 mg/kg) showed a highly significant reduction (P<0.05) in the
locomotor activity when compared to control, that is 74 and 81%. There was also significant reduction in the locomotor activity of AEBCV when compared to the standard. Maximum muscle relaxation was observed with 100 mg/kg of CV. The percentage reduction in the locomotor activity with diazepam showed highly significant decrease in the locomotor activity that is 93% when compared to control.

In a study conducted by Tirumalasetty J et al. [12], the actophotometer test and rotarod test showed similar findings i.e., the extract significantly reduced the motor coordination of the tested animals [11]. In another study, Sahu M et al. [2013], demonstrated that the extract possesses skeletal muscle relaxant property [12].

The bark of different cinnamon species holds significant importance and widespread popularity globally, serving not just as a culinary ingredient but also as a staple in both traditional and medicinal practices. Cinnamaldehyde and trans-cinnamaldehyde, found in the essential oil of cinnamon, are its key components, imparting both its aroma and contributing to the diverse range of biological activities associated with cinnamon. Other bioactive compounds are eugenol, phenolic compounds, terpenoids, gum, mucilage, resin, starch and sugar, all contributing to its beneficial properties [13]. The type-A procyanidin polyphenols found in CV exhibits anti-asthmatic and anti-inflammatory effects [14]. The bark harbors proanthocyanidins in dimeric, trimeric, and higher oligomeric forms, characterized by doubly linked bis-flavan-3-ol units within the molecule which have anti-inflammatory properties [15]. In previous studies it was found that cinnamaldehyde possesses anti-pyretic activity, astringent activity, antimicrobial activity, hepatoprotective activity, cytotoxic, anti-inflammatory and also have significant sedative and analgesic activity [16]. Hence the muscle relaxant activity of CV may be attributed to the biological activities of cinnamaldehyde and trans-cinnamaldehyde.

CONCLUSION

According to our findings, the AEBCV demonstrates properties conducive to skeletal muscle relaxation. The extract significantly impaired the motor coordination of the animals subjected to the extract. While cinnamon is primarily known for its culinary uses and potential health benefits, such as anti-inflammatory and antioxidant properties, there is limited scientific evidence to support its direct effectiveness as a muscle relaxant. As this study established the muscle relaxant property of CV, the exact mechanism and the active phytocultural constituent is still unknown and hence, further research is needed to validate these claims and to understand the mechanisms by which cinnamon may influence muscle relaxation.

ACKNOWLEDGEMENT

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AUTHORS CONTRIBUTIONS

The study protocol was designed and supervised by Dr. Pallavi Bordoloi. Data collection, data analysis, and preparation of the manuscript were done by Dr. Anamika Hazarika. Editing of the overall research work was done by Dr. Geetamoni Dutta and Dr. Dipjyoti Deka.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

REFERENCES