EVALUATION OF SKELETAL MUSCLE RELAXANT ACTIVITY OF METHANOLIC LEAF EXTRACT OF Moringa oleifera

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ABSTRACT

In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. Skeletal muscle relaxant activity of Moringa oleifera was studied on the frog (Rana tigrina) by the rectus abdominus muscle preparation. The Moringa oleifera was diluted with distilled water in different concentrations.

When the skeletal muscle relaxant property compared with that of standard acetylcholine, the leaf extract of Moringa oleifera, significantly shows skeletal muscle relaxant property. Present study, 1µg, 2µg and 4 µg of Moringa oleifera leaf extracts significantly inhibit the action of acetyl choline contractions. Based on height of contractions, Moringa oleifera inhibits the acetyl choline contraction. That indicates Moringa shows neuromuscular blocking activity. From above concentrations 2µg significantly inhibits the contractions of rectus abdominus muscle compare with other concentrations hence 2µg shows good skeletal muscle relaxant property compare with other doses.

KEYWORDS: Skeletal muscle activity, Moringa oleifera, Rana tigrina, Pancuronium and Acetylcholine.

INTRODUCTION

Spasticity and spasms are distinct etiologies, and each condition responds differently to certain medications. Spasticity is a disorder of motor neurons that manifests as increased muscle tone and stiffness. Spasms are involuntary localized muscle contractions that arise from acute trauma or muscle strain. Although antispasmodics and antispasticity agents
generally are not interchangeable, diazepam (Valium) is approved by the Food and Drug Administration (FDA) for both conditions.\(^1\)

Neuromuscular diseases form an heterogeneous group of illnesses. These diseases are rare and studies concerning their anesthetic management are difficult. Patients with neuromuscular disease represent a challenge for the anaesthesiologist because of the frequent preoperative complications. Cardiac and respiratory functions are often involved, and the severity of the lesions is difficult to estimate. The possible interactions of different drugs necessitate a good knowledge of the drugs’ actions and pathophysiological mechanisms.\(^2\) The drugs covered are carisoprodol, chlorphenesin carbamate, chlorzoxazone, cyclobenzaprine hydrochloride, diazepam, metaxalone, methocarbamol, and orphenadrine citrate. The mechanism of action of these agents is not well defined, and their effects are measured mainly by subjective responses.\(^3\) Muscle relaxants block neuromuscular transmission, acting at nicotinic acetylcholine receptors of the neuromuscular junction. Suxamethonium (succinylcholine) is a depolarizing agent, whereas all other relaxants in clinical use are non depolarising. The desired neuromuscular block results from the structural similarity of muscle relaxants to acetylcholine, enabling the interaction with receptors at the neuromuscular junction. Adverse effects of suxamethonium are generally related to its agonist mode of action. Autonomic cardiovascular effects may result.\(^4\) Loss of muscle mass after stroke has implications for strength and functional ability and may also contribute to impaired glucose metabolism. Therefore, prevention of muscle loss is desirable.\(^5\) Prolonged recovery may be related to effects of the blocking drug that are pharmacologic (drug overdoses or accumulations of active metabolites), physiologic (changes in the neuromuscular junction with critical illness), or potentially toxic (effects that are secondary to drug–drug interactions or to drug–muscle or drug–nerve interactions).\(^6\) Neuromuscular blocking agents have found widespread use as an adjunct to intubation and to mechanical ventilation in patients with ARDS, IRDS, pulmonary edema, status asthmatics, status epileptics, tetanus, neonatorum, poisoning, hypothermia, and spinal shock. Numerous physicochemical changes, drug interactions, and pathologic states can affect these drugs. Awareness of these factors and of the pharmacology of these drugs allows the physician to use them safely in acute and critical care medicine.\(^7\) While all neuromuscular blocking agents (NMBAs) effectively interrupt neuromuscular transmission, it must be emphasized that these drugs are completely devoid of analgesic, sedative, or amnestic properties.\(^8\)
Muscle strains and other musculoskeletal disorders (MSDs) are a leading cause of work absenteeism. Muscle pain, spasm, swelling, and inflammation are symptomatic of strains. The precise relationship between musculoskeletal pain and spasm is not well understood. The dictum that pain induces spasm, which causes more pain, is not substantiated by critical analysis. The painful muscle may not show EMG activity, and when there is, the timing and intensity often do not correlate with the pain. Clinical and physiologic studies show that pain tends to inhibit rather than facilitate reflex contractile activity. The decision to treat and choice of therapy are largely dictated by the duration, severity of symptoms, and degree of dysfunction. Trigger point injections are sometimes used with excellent results in the treatment of muscle spasm in myofascial pain and low-back pain.\[^9\] Skeletal muscle relaxants are a heterogeneous group of medications used to treat two different types of underlying conditions: spasticity from upper motor neuron syndromes and muscular pain or spasms from peripheral musculoskeletal conditions. Although widely used for these indications, there appear to be gaps in our understanding of the comparative efficacy and safety of different skeletal muscle relaxants.\[^10\] Skeletal muscle relaxants are a heterogeneous group of medications commonly used to treat two different types of underlying conditions: spasticity from upper motor neuron syndromes and muscular pain or spasms from peripheral musculoskeletal conditions. Spasticity from the upper motor neuron syndrome (a complex of signs and symptoms that can be associated with exaggerated reflexes, autonomic hyperreflexia, dystonia, contractures, paresis, lack of dexterity, and fatigability, in addition to spasticity) can result from a variety of conditions affecting the cortex or spinal cord.\[^10\]

Some of the more common conditions associated with spasticity include multiple sclerosis, spinal cord injury, traumatic brain injury, cerebral palsy, and post-stroke syndrome.\[^12\] In many patients with these conditions, spasticity can be disabling and painful, with a marked effect on functional ability and quality of life.\[^13\] Common musculoskeletal conditions causing tenderness and muscle spasms include fibromyalgia, tension headaches, myofascial pain syndrome, and mechanical low back or neck pain. If muscle spasm is present in these conditions, it is related to local factors involving affected muscle groups. These conditions are commonly encountered in clinical practice and can cause significant disability and pain in some patients. Skeletal muscle relaxants are one of several classes of medications frequently used to treat these conditions 8–10 Drugs classified as skeletal muscle relaxants include baclofen, carisoprodol, chlorzoxazone, cyclobenzaprine, dantrolene, metaxalone, methocarbamol, orphenadrine, and tizanidine. Only Pain is the most common reason people
enter the health care system. The presence of low back pain remains among the top 5 reasons for primary physician visits. Three of the skeletal muscle relaxants, carisoprodol, cyclobenzaprine and metaxalone comprise 45% of all prescriptions for lower back pain.\[14\] These heterogeneous groups of agents are useful in the treatment of musculoskeletal disorders associated with spasticity resulting from upper motor neuron disorders or in the treatment of peripheral musculoskeletal disorders associated with muscle pain or spasm. No clinical practice guideline suggests using a skeletal muscle relaxant as first-line therapy for the treatment of pain or spasm. These agents are indicated as adjunct or alternative therapy after first or second line therapies have failed. Virtually every guideline points out the adverse effect profile (including abuse potential) of these agents and suggests caution in patient selection. For the treatment of spasticity baclofen is considered a first line agent with dantrolene and tizanidine generally recommended second line.\[15\] Many initial clinical trials were published in the 1960’s to 1970’s and were of poor quality. Trials often did not define the use of concomitant medications, pre-study treatment, many were not double-blind, and among other issues, the term muscle-spasm was not well defined. The data supporting many of these agents is weak. Data supporting the use of combination products is rare.\[16\] Efficacy appears to decline over time particularly for muscle pain and spasm. No data clearly demonstrated an advantage over non-steroidal anti-inflammatory drugs or when used in combination with them. The agents with the least clinical evidence supporting their use include chlorzoxazone, methocarbamol, dantrolene and baclofen for pain and spasm. Carisoprodol and cyclobenzaprine offer more evidence supporting their use for muscle pain and spasm. Only tizanidine, dantrolene and baclofen have evidence supporting their use in spasticity. In general, these agents are associated with significant side effects.\[17\] They are not considered first-line therapy in the indications for which they have approval and most carry the 4 risk potential for significant adverse events and/or abuse potential.

Hence, there is a need for new drug research with wide therapeutic index and good skeletal muscle relaxant activity, and by this aim, we have chosen Moringa oleifera plant leaves.

**MATERIALS AND METHODS**

**Standard Drug:** Pancuronium

**Test drug:** Methanolic extract of leaves of *Moringa oleifera*

**Physiological solutions:** Ringer Solution

**Animal:** Frog (*Rana tigrina*)
**Instruments:** Sherrington Rotating Drum, Frontal writing lever.

**Preparation of extract:** The leaves of *Moringa oleifera* was collected from houses at hanamkonda, Warangal district, Telangana, India. It was authenticated by B.Raju Kakatiya University Warangal district. One specimen was preserved in Department of Pharmacognosy of our institute for the reference. The leaves were washed thoroughly to remove adhered material and fine powder was made by using hand grinder. 1gm of powder was mixed with 100ml distilled water with the help of magnetic stirrer for half an hour. The material was filtered through Whatman filter paper no.40 and filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportions.

**Preparation of Pancuroium solution:** Pancuroium ampoules (Sun Pharma Ltd.) were purchased from local pharmacy. Various different dilutions were made with distilled water.

**Evaluation of skeletal muscle relaxant activity:** Frogs weighing 20-25 g were used in this study. The frog was stunned and decapitated and the spinal cord was destroyed. A frog was pithed and the skin of the anterior and abdominal wall was cut by a midline incision and then it was cut laterally to expose the anterior abdominal wall. The two rectus were seen running from base of sternum. The muscles were cut across just above the sternum at its base and the pair of muscles attached to it were dissected and transferred to a dish containing frog ringer solution at room temperature. The muscles were then carefully cleaned and one of them was trimmed to the desired size and mounted in an organ bath filled with ringer solution at room temperature and aerated by stream of fine bubbles emerging near the bottom of the bath. Isotonic contractions were recorded using gimbel lever with a sideways writing point. The lever was balanced for a tension of approximately 2-5g. An extra load of approximately 1g on the long arm was supplied because sometime the lever may not return to the base line after washing. The drug period allowed for stabilization was 30 minutes during which the muscle was subjected to 1g stretch. At 0th min - the kymograph was started after raising the extra load; in the 1st min- the drug was added and in the 2nd min- the kymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5th min- the lever point was brought to the base line and the next cycle was started. After recording the graded responses to different log dose of acetylcholine, the test drug (moringa olifera) was added and their effects upon acetylcholine induced contractions as well as the effect of its own in the tissue was studied.
RESULTS

Table 1: Effect of moringa olifera on frog’s rectus abdominus muscle.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (microgram)</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ach</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Ach</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ach</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>Ach</td>
<td>8</td>
<td>1.8</td>
</tr>
<tr>
<td>Standard pancuronium</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Test+ach</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Test+ach</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Test+ach</td>
<td>4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Figure 1: Effect of Ach on frog’s rectus abdominus muscle.

Figure 2: Effect of Moringa olifera leaf extract on frog’s rectus abdominus muscle.
DISCUSSION

B. S. SHEELAA…etal, skeletal muscle activity of milky latex from Calotropis gigantea was studied in the green frog (Rana hexadactyla) by the rectus abdominus muscle preparation. The milky latex was diluted with distilled water T1 [1: 100], T2 [1:500] and T3 [1:1000] concentrations. The result indicated that the treatment of milky latex alone does not produce skeletal muscle activity. But significant result was produced when the milky latex was tested along with acetyl choline.

In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. When the skeletal muscle relaxant property compared with that of standard acetylcholine, the leaf extract of *moringa oleifera*, shows more skeletal muscle relaxant property than that of standard acetylcholine.

Present study, 1µg, 2µg and 4 µg of *moringa oleifera* leaf extracts significantly inhibit the action of acetyl choline (see in table-1). Doses of 1µg, 2µg and 4 µg of acetyl choline alone gives height of contraction sequently 1.2, 2, 2.1 and 1.8 cm. When administration of 1µg, 2µg and 4 µg of *moringa* in presence of acetyl choline, muscle contractions are 0.9,1.4 and 1.9 cm. From above results of height of contraction, 1µg 2µg and 4 µg of *moringa oleifera* leaf extracts significantly inhibit the action of acetyl choline, from based on that 2µg significantly inhibits the contractions of rectus abdominus muscle compare with other concentrations (showed in graph no 2). From above discussion 2µg shows good skeletal muscle relaxant property compare with other doses.

CONCLUSION

The results of this study provide support for the traditional use of herbal leaf extracts shows skeletal muscle relaxant property, there is need to treatment for muscle spasm disorders like low back pain or neck pain, fibromyalgia, tension headaches and myofascial pain syndrome. In market skeletal muscle relaxant drugs limitedly used, so there is a need to discover new drugs. However, further studies are necessary to find the exact mechanism of skeletal muscle relaxant effect and to isolate the active compound responsible for this pharmacological activity.

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