The neuromuscular blockade produced by pure alkaloid, mitragynine and methanol extract of kratom leaves (Mitragyna speciosa Korth.)

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\textbf{A B S T R A C T}

\textbf{Aim of the study:} The effects of pure alkaloid, mitragynine and a methanolic extract of kratom leaves were investigated on neuromuscular junction and compound nerve action potential.

\textbf{Materials and methods:} Wistar rats were killed by cervical dislocation and decapitated. The phrenic nerve–hemidiaphragms, hemidiaphragms and sciatic nerve were isolated.

\textbf{Results:} Kratom methanolic extract present at 0.1–1 mg/mL and mitragynine (0.0156 mg/mL) decreased the muscle twitch on the isolated phrenic nerve–hemidiaphragm and hemidiaphragm preparation. Muscle relaxation caused by kratom extract (1 mg/mL) was greater than the effect of mitragynine. Pancuronium and succinylcholine potentiated the effect of kratom extract. It also had a direct relaxation effect on the hemidiaphragm muscle. The muscle relaxation caused by kratom extract was not antagonized by neostigmine, tetraethylammonium and calcium chloride. High concentrations of kratom extract (10–40 mg/mL) and mitragynine (2 mg/mL) blocked the nerve conduction, amplitude and duration of compound nerve action potential.

\textbf{Conclusions:} The mechanism of action of kratom extract might not act as a competitive antagonist of acetylcholine yet its dominant effect was at the neuromuscular junction and not at the skeletal muscle or somatic nerve.

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\section{1. Introduction}

Kratom (Mitragyna speciosa Korth.) is a tall leafy tree in the family Rubiaceae, and is native to Southeast Asia. It grows in hot, wet tropical areas such as Thailand, where it is generally called kratom. It grows mostly in the southern regions of this country. The leaves have long been for “medicinal purposes” and as a narcotic drug. It was also classified in Category V of a five category classification of narcotics by the Thai government enacted the Narcotics Act B.E. 2522, placing kratom along with marijuana. This means that it is illegal to buy, sell, import, or growing and harvesting. This law makes planting the tree illegal and requires existing trees to be cut down. However it is not fully effective, since the tree is indigenous to the country and native people prefer to use them. Hence, kratom remains a popular drug in Thailand, especially in southern regions.

Kratom has been traditionally used in Thailand, and there are also reports of some use in Malaysia. There are two kinds of kratom, distinguished by the color of veins in the leaf, red or green. Local people preferred to use both of them. In addition to being used, in its own right, as a narcotic drug, it is often used as a substitute for opium when opium is unavailable, or to moderate opium addiction. Kratom has been reported to be a central nervous system stimulant, and also depressant. It helps to increase work efficiency and tolerance to hard work under a scorching sun (Suwanlert, 1975). It also uses to treat muscle ache and fatigue (Chucheun, 2005).

Over 25 alkaloids have been isolated from kratom leaves with mitragynine being the most dominant (Chitrakarn et al., 2005). Other alkaloids are mitraphylline, speciogynine, 7-hydroxymitragynine, etc. Mitragynine has an antinociceptive action through the supraspinal opioid receptors and descending noradrenergic and serotonergic systems (Matsumoto et al., 1996). Mitragynine inhibited the vas deferens contraction elicited by nerve stimulation, probably through its blockade of neuronal Ca\textsuperscript{2+} channels (Matsumoto et al., 2005). Mitragynine inhibits guinea-pig ileum contraction in vitro via the opioid receptor (Watanabe et al., 1997). 7-Hydroxymitragynine has a more potent analgesic activity than that of morphine (Matsumoto et al., 2004). In folk medicine, it has been used to treat diarrhea. It was found that methanolic extract of kratom had the antidiarrheal activity and decreased...
body weight (Chitrakarn et al., 2008). The fresh leaves are usually chewed, often continuously, by workers or manual laborers seeking a numbing, stimulating effect that helps to improve their tolerance to work and relieves muscle strains. From the traditional medicine use for relief muscle ache and strain, the effects of mitragynine and methanol extract of kratom leaves on neuromuscular junction and somatic nerves were investigated in this study.

2. Materials and methods

2.1. Plant material

Kratom leaves (red vein type) were collected from Satoon province, in the southern part of Thailand during the months of February–May 2005. Specimens of this plant have been deposited at the PSU Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand with the specimen voucher number PSU 012821.

2.2. Preparation of the methanolic extract

Air-dried leaves were pulverized by grinding and then macerated, at room temperature, with absolute methanol for 7 days, twice, while stirring 2–3 times/day. The extracts were mixed, filtered and concentrated using a rotary evaporator (BUCHI, B 169 Vacuum-System, Switzerland). Then they were freeze-dried (Corrosion Resistant Freezer Drier, FTS System, Inc., USA). The yield was 7.92% (w/w).

2.3. Isolation of mitragynine

The dried product from the methanolic extract of kratom leaves was dissolved in 10% acetic acid solution. This solution was shaken and left overnight. The acidic filtrate was washed with petroleum ether, adjusted to pH 9 with 25% ammonia solution, and then extracted with chloroform. The chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated to yield a dry crude alkaloid extract. According to the isolation procedure, the yield of crude alkaloid extract was approximately 0.25% based on fresh weight of *Mitragyna speciosa*. An aliquot (2.5 g) was then subjected to silica gel column chromatography, eluted with 5% methanol in chloroform to obtain a major alkaloid (1.25 g), which appeared as a single spot on TLC analysis (four different solvent systems). It was found to be a pure compound upon spectroscopic analysis (including 1H and 13C NMR, IR, and mass spectrometry), and identified to be mitragynine by comparing the obtained spectra data with the published data (Shellard et al., 1978; Houghton et al., 1991). Over all, the yield of mitragynine in the methanolic extract was approximately 1.56%.

2.4. Experimental animals

Wistar rats of either sex, weighing 200–250 g, were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University. They were housed in a temperature controlled room at 25 ± 2 °C with a relative humidity at 50 ± 5% and a 12 h light/12 h dark cycle. They were fed with animal food pellets and water *ad libitum*. The study protocol was approved by the Ethics Committee for Experimental Animals, Prince of Songkla University.

2.5. Isolated phrenic nerve–hemidiaphragm preparation

Rats were killed by cervical dislocation and decapitated. The chest was opened and the diaphragm was divided into right and left parts. Each part of the diaphragm with its attached phrenic nerve was removed (Bubbring, 1946). Isolated phrenic nerve–hemidiaphragm preparations were mounted vertically into an 80 mL organ bath containing Krebs solution (mM: NaCl 118.07, NaHCO3 25, KCl 4.69, MgSO4·7H2O 1.18, KH2PO4 1.18, CaCl2 2.52, glucose 10.09) and continuously aerated with 95% O2 and 5% CO2 at a temperature of 30 °C. The muscle tension was maintained at 2 g and attached to an FT-03 force transducer (Grass Instrument Co., Quincy, MA, USA), connected to a Grass 79D polygraph (Grass Instrument Co., Quincy, MA, USA) for recording isometric contraction. The phrenic nerve was gently drawn through the loops of a bipolar platinum stimulating electrode connected to a Grass S88 stimulator via a SIU5 stimulus isolation unit (Grass Instrument Co., Quincy, MA, USA). The nerve–muscle preparation was left in the organ bath for 30 min to reach equilibrium before carrying out experiments. The neurally evoked twitch was recorded by stimulation with electrical pulses of supramaximal voltage at a frequency of 0.4 Hz and duration of 0.6 ms throughout all experiments.

2.6. Isolated hemidiaphragm preparation

The preparation was established as previously mentioned for the isolated phrenic nerve–hemidiaphragm. The phrenic nerve was removed from the hemidiaphragm. The fan shaped hemidiaphragm was then transferred into the organ bath containing 80 mL Krebs solution, aerated with 95% O2 and 5% CO2 at a temperature of 30 °C. The muscle tension was set at 2 g. The needle platinum electrode was passed through the basement of hemidiaphragm. The direct muscle twitch was recorded by electrical stimulation of supramaximal voltage at a frequency of 0.4 Hz and duration of 0.6 ms. The preparation was completely curarized by adding 0.005 mM pancuronium (Chongrak, 1985). Thus, the contractile response was due only to the skeletal muscle response.

2.7. Isolated sciatic nerve preparation

The rat was anesthetized by intraperitoneal injection of sodium pentobarbital, 50 mg/kg body weight. Both the left and right sci-
atic nerves were dissected out. The nerve was placed in a three-compartment chamber which bipolar stimulating and recording platinum electrodes were placed under the nerve. The central compartment had a volume of 1 mL containing Tyrode's solution (mM: NaCl 140.31, NaHCO₃ 11.90, KCl 2.68, MgCl₂·6H₂O 1.08, NaH₂PO₄·2H₂O 0.38, CaCl₂ 1.8, glucose 5.05) or test drugs. The two sides of the chamber contained liquid paraffin to moisten the nerve (Chongrak, 1985). The nerve was stimulated with a supramaximal voltage at a frequency of 0.6 Hz and duration of 0.04 ms by a Grass S88 stimulator via a SIU5 stimulus isolation unit (Grass Instrument Co., Quincy, MA, USA). The compound action potentials were displayed on an oscilloscope (Tektronix TDS310, USA) via an AM502 Differential Amplifier (Tektronix TM502A, USA) for recording nerve conduction (ms), amplitude (mV) and duration (ms).

2.8. Standard drugs

Pancuronium, d-tubocurarine, succinylcholine, neostigmine, tetraethylammonium and sodium pentobarbital were purchased from Sigma–Aldrich (St. Louis, USA). Xylocaine was purchased from OLIC (Thailand) Limited.

2.9. Data analysis

Results were expressed as a mean ± SEM, n = 8 of the percentage muscle relaxation. The percent change of nerve conduction, amplitude and duration of the compound nerve action potential were also expressed as a mean ± SEM, n = 6. Significant differences were analyzed by SPSS 11 for windows followed by a Tukey test for comparison of more than two groups and independent-samples t test.

Fig. 2. Potentiative muscle relaxation of pancuronium (Panc 0.6 μM), succinylcholine (Suc 3.1 μM) and kratom extract (MS 0.1 mg/mL) on the isolated phrenic nerve–hemidiaphragm preparation.
for comparison of two groups. A probability level of less than 5% was considered significant.

3. Results

3.1. Isolated phrenic nerve–hemidiaphragm preparation

Kratom extract produced a decrease of the twitch contraction of the rat phrenic nerve–hemidiaphragm preparation. The degree of muscle relaxation depended on the concentration and time. Kratom extract present at 0.25, 0.5 and 1 mg/mL produced a complete relaxation in 50, 25 and 15 min, respectively (Fig. 1). Mitragynine present at 0.0156 mg/mL also produced a complete muscle relaxation in 45 min. The percent relaxation produced by mitragynine was significantly different from kratom extract in the concentration of 1 mg/mL at \( p < 0.05 \). A low concentration of pancuronium (0.6 \( \mu \)M) and also succinylcholine (3.1 \( \mu \)M) increased the effect of the kratom extract (Fig. 2). The concentration–response curve of kratom extract and kratom extract in the presence of pancuronium or succinylcholine was also shifted to the left (Fig. 3). Neostigmine (20 \( \mu \)M) and also succinylcholine (3.1 \( \mu \)M) antagonized the muscle relaxation caused by \( \alpha \)-tubocurarine (5 \( \mu \)M). However this standard drug did not reverse the effect of the kratom extract (Fig. 4). Muscle contraction was decreased in Krebs solution which lack off calcium chloride and calcium chloride (1.25 mM) could be increased muscle contraction in this condition (Fig. 5a). Tetraethylammonium (1 mM) reduced the effect of pancuronium (0.005 mM) (Fig. 5b). Calcium chloride and tetraethylammonium could not antagonize the effect of kratom extract (Fig. 5).

3.2. Isolated hemidiaphragm preparation

The kratom extract and mitragynine were also investigated for any direct effect on the diaphragm skeletal muscle. Kratom extract and mitragynine decreased the muscle twitch response. Kratom extract produced complete muscle relaxation at a concentration of 1 mg/mL in 55 min time (Fig. 6). Although, mitragynine produced muscle relaxation significantly difference less than the effect of kratom extract.

3.3. Compound action potential on the isolated sciatic nerve

The kratom extract (10, 20 and 40 mg/mL) was tested on the isolated rat sciatic nerve. The nerve conduction, amplitude and duration of the compound nerve action potential were recorded. Kratom extract decreased nerve conduction, amplitude and duration. Kratom extract present at 10 and 20 mg/mL prolonged the duration of the compound action potential at the first period of time but at 40 mg/mL it blocked the compound action potential completely at the 55 min time (Table 1). Mitragynine present at 2 mg/mL caused a gradual decrease of the nerve conduction, amplitude and duration of the compound action potential. However, xylocaine present at 0.1% could block the compound action potential completely within 25 min.

4. Discussion

Kratom extract decreased muscle contraction on the isolated phrenic nerve–hemidiaphragm preparation. It caused complete muscle relaxation at a concentration of more than 0.1 mg/mL. Kratom extract at the highest concentration used (1 mg/mL) completely relaxed muscle contraction within 15 min. Electrical stimulation via the phrenic nerve causes acetylcholine liberation from the nerve ending. Acetylcholine binds to the nicotinic receptors of the motor endplates and causes diaphragm contraction (Silverthorn, 2007). Kratom extract may interfere with the neuromuscular junction so that the skeletal muscle could not contract. Low concentrations of pancuronium (0.6 \( \mu \)M) and succinylcholine (3.1 \( \mu \)M) potentiated the effect of 0.1 mg/mL kratom extract. Pancuronium is a non-depolarizing curare-mimetic muscle relaxant. It acts as a competitive antagonist to acetylcholine at nicotinic receptors. Succinylcholine acts as a depolarizing neuromuscular blocker. It imitates the action of acetylcholine at the neuromuscular junction and causes spontaneous depolarization upon association with the nicotinic receptors at the neuromuscular junction. Then it causes an endplate potential that is less than the action potential. The muscle undergoes flaccid paralysis (Taylor, 2006a). Kratom extract may act at the neuromuscular junction as does pancuronium and succinylcholine.

\( \alpha \)-Tubocurarine is a classical competitive antagonist acting at the neuromuscular junctions. This causes muscle paralysis, as does pancuronium. Neostigmine is a reversible cholinesterase inhibitor that prevents the breakdown of acetylcholine (Taylor, 2006b). Acetylcholine competes with \( \alpha \)-Tubocurarine at the nicotinic receptors and skeletal muscle increases its twitch contraction. Neostigmine reverses the effect of \( \alpha \)-tubocurarine, pancuronium
and competes with other competitive neuromuscular blocking drugs. Tetraethylammonium increases acetylcholine liberation from motor nerve endings (Stovner, 1958). Then it can antagonize the neuromuscular block caused by pancuronium. The muscle relaxation produced by kratom extract was not antagonized by neostigmine and tetraethylammonium. Thus kratom extract might not therefore act as a competitive or non-depolarized neuromuscular blocking drug.

The neuromuscular transmission can be interrupted by calcium deficiency. It would appear to be caused by a reduced transmitter output from presynaptic site. Calcium chloride did not reverse the effect of kratom extract. In this way the block may differ sharply from that produced by pancuronium or \(\text{d}-\text{tubocurarine. Kratom might not affect acetylcholine release.}

Kratom extract was also tested directly on the hemidiaphragm skeletal muscle. It caused muscle relaxation. The percentage relaxation of the kratom extract on the skeletal muscle was less than its effect on the isolated phrenic nerve–hemidiaphragm preparation. Complete relaxation of the muscle by kratom extract was only observed at a concentration of 1 mg/mL. The skeletal muscle contractions by the electrical impulse pass to the sarcoplasmic reticulum. The calcium gates in the membrane of the sarcoplasmic reticulum open. As a result, calcium diffuses out of the sarcoplasmic reticulum and among the myofilaments. Then the muscle fibers get contraction. The kratom extract may interfere these processes of muscle contraction.

The sciatic nerve was isolated to test the effect of kratom extract. Kratom extract, present at the high concentrations of 10, 20 and 40 mg/mL blocked the compound nerve action potential. It decreased nerve conduction, amplitude and duration of the compound action potential. The compound action potential was completely blocked at 40 mg/mL of kratom extract. The nerve action potential occurs when sodium ions enter the cell through sodium channels to induce depolarization. Then, the potassium channels open, causing potassium ions rush out to produce repolarization. Kratom extract may interrupt the influx or efflux of these ions. Kratom extract therefore had its dominant effect on the neuromuscular junction rather than on the skeletal muscle and the somatic motor nerve.

Mitragynine, a major alkaloid from Mitragyna speciosa Korth., was also tested on the isolated phrenic nerve–hemidiaphragm, hemidiaphragm and sciatic nerve preparation. Muscle relaxation caused by mitragynine present at 0.0156 mg/mL was significant difference less than the effect of kratom extract (1 mg/mL) both in the isolated phrenic nerve–hemidiaphragm and in the directed hemidiaphragm preparation. According to the isolation of mitragynine, the methanolic kratom extract contained 1.56\% mitragynine. Hence, kratom extract in the concentration of 1 mg/mL contained mitragynine 0.0156 mg/mL. Muscle relaxation produced by kratom may be effected by mitragynine and other alkaloids in kratom leaves. High concentrations of mitragynine (2 mg/mL) produced a blockage of the compound nerve action potential but it did not produce a complete blockage. Xylocaine solutions containing lidocaine hydrochloride is used as a common local anesthetic and antiarrhythmic drug. Lidocaine blocks the fast voltage gated sodium (Na\(^+\)) channels in the cell membrane. With sufficient blockage, the membrane of the presynaptic neuron will not depolarize and so fails to transmit an action potential, leading to its anesthetic effects (Catterall and Mackie, 2006). Xylocaine (0.1%) completely blocked the compound action potential within 15 min. A methanol extract of kratom leaf contained 1.6\% of the major alkaloid mitragynine. Hence the kratom extract added at 40 mg/mL contained only
Table 1

<table>
<thead>
<tr>
<th>Concentration of Mitragynine (mg/mL)</th>
<th>Nerve Conduction</th>
<th>Amplitude</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% xylocaine</td>
<td>-0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>0.2% xylocaine</td>
<td>-0.7 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.3% xylocaine</td>
<td>-0.8 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.4% xylocaine</td>
<td>-1.0 ± 0.5</td>
<td>0.7 ± 0.5</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.5% xylocaine</td>
<td>-1.2 ± 0.6</td>
<td>0.8 ± 0.6</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.6% xylocaine</td>
<td>-1.4 ± 0.7</td>
<td>0.9 ± 0.7</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.7% xylocaine</td>
<td>-1.6 ± 0.8</td>
<td>1.0 ± 0.8</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>0.8% xylocaine</td>
<td>-1.8 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>0.0 ± 0.2</td>
</tr>
</tbody>
</table>

0.61 mg/mL of mitragynine. Thus the inhibition of the compound action potential may be due to mitragynine and also other alkaloids present in the kratom leaf extract.

5. Conclusion

A methanol extract of kratom leaf and a major alkaloid, mitragynine produced skeletal muscle relaxation. Its mechanism of action was not by a competitive antagonism of acetylcholine binding. It had a synergistic effect with pancuronium and succinylcholine. Kratom extract and mitragynine also had a direct effect on skeletal muscle by decreasing the muscle twitch. The compound action potential was blocked by high concentrations of kratom extract and mitragynine. Thus, kratom extract had a greater effect at the neuromuscular junction than on the skeletal muscle or somatic nerve. It is possible that alkaloid components of the kratom extract other than mitragynine may have an effect on the compound action potential.

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References


