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

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ABSTRACT

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

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 (Chittrakarn 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 blockage of neuronal Ca^{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 antidiarrhreal activity and decreased

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body weight (Chittrakarn 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 Vacumn-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 ^{1}H and ^{13}C 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 (Bulbring, 1946). Isolated phrenic nerve–hemidiaphragm preparations were mounted vertically into an 80 mL organ bath containing Krebs solution (mM: NaCl 118.07, $NaHCO_3$ 25, KCl 4.69, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.52, glucose 10.09) and continuously aerated with 95% O_2 and 5% CO_2 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 isotonic 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% O_2 and 5% CO_2 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-

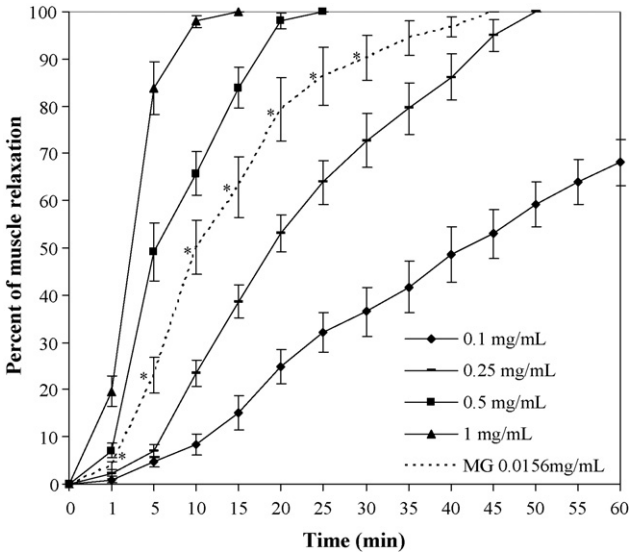

Fig. 1. The percentage relaxation (mean ± SEM, $n = 8$) of the twitch amplitude produced by the kratom extract present at 0.1, 0.25, 0.5 and 1 mg/mL and mitragynine (MG) present at 0.0156 mg/mL on the isolated phrenic nerve–hemidiaphragm preparation. *Statistical significance $p < 0.05$ vs. kratom extract present at 1 mg/mL.