UNIVERSITI PUTRA MALAYSIA

EFFECTS OF MITRAGYNINE ON LOCOMOTOR AND ANXIETY IN RATS SUBJECTED TO RESTRAINT STRESS

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By

NURUL RAUDZAH BINTI ADIB RIDZUAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

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DEDICATION

~to beloved ZZ and little ZN junior~

who always inspire me and make it all meaningful

- NRAR

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Masters of Science

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Chairman: Mohd. Taufik Hidayat Baharuldin, PhD

Faculty: Faculty of Medicine and Health Sciences

Mitragyna speciosa is an indigenous tree found in Southeast Asia particularly in Thailand and Malaysia. It is popularly known as 'kratom' in Thailand and 'ketum' in Malaysia. The medicinal properties of this plant were due to its opium-like effects and cocaine-like stimulant ability in combating fatigue and as hard work tolerance. The major alkaloid from this plant, mitragynine was extracted and isolated by solvent systems. Standard spectroscopic analysis was performed to identify the compound. Due to its potential properties as psychostimulant ability and anti-anxiety, this study was designed to determine the effects of mitragynine on locomotor activities and anxiety level in eustress and stress-induced rats. An effect of mitragynine on the corticosterone level was also determined. Locomotor and grooming activity in open-field test (OFT) and anxiety study in elevated plus-maze (EPM) were performed for the behavioral profiles. A hundred and sixty male Sprague-Dawley rats were used in the study and

divided into two groups which were eustress and stress-induced rats. Mitragynine of different dosages (1.0, 5.0, 10.0 and 30.0 mg/kg) were administered intraperitoneally to each rat. Stress-induced rats were restrained for 2 hours in the restrainer before the behavioral activities were conducted. The findings showed that locomotor activity statistically increased ($P<0.05$) in 5.0 and 30.0 mg/kg mitragynine treated rats compared to 1.0 mg/kg mitragynine in non-stressed rats. Locomotor activity also increased in stressed-rats in all doses of mitragynine, however the results were not statistically significant. Grooming activity increased significantly ($P<0.05$) in stressed rats treated with 1.0, 5.0, 10.0 and 30.0 mg/kg mitragynine compared to control group. In non-stressed rats, grooming activity also increased in all doses of mitragynine but the results were not statistically significant. In anxiety study, three parameters were performed on each rat including time spent in open arms, open arm entries and time spent in central platform. Thirty (30.0) mg/kg mitragynine increased the time spent in open arms and open arm entries in non-stress and stress-rats. However, 30.0 mg/kg mitragynine decreased the time spent in central platform in non-stress and stress groups. Time spent in central platform only increased in non-stress rats treated with 5.0 and 10.0 mg/kg mitragynine. Corticosterone levels in stress-rats treated with all doses of mitragynine increased significantly ($P<0.05$) compared to control group. Higher dosages of mitragynine are able to induce behavioural changes by increasing the locomotor, grooming activity and anxiety parameters. Thus, the study showed that mitragynine produced sensitivity towards the locomotor, grooming and anxiety by increasing the parameters and is highly correlated with corticosterone levels in stressed rats. In conclusion, mitragynine is able to exert the possible psychostimulant and anxiolytic

properties in non-stressed and stressed rats through the possible mechanism of action of mitragynine in rats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

KESAN MITRAGININ TERHADAP LOKOMOTOR DAN KERESAHAN KEPADA TIKUS YANG DIDEDAHKAN STRES KURUNGAN

Oleh

NURUL RAUDZAH BINTI ADIB RIDZUAN

Oktober 2010

Pengerusi: Mohd. Taufik Hidayat Baharuldin, PhD

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Mitragyna speciosa ialah sejenis pokok tempatan didapati di Asia Tenggara terutamanya di Thailand dan Malaysia. Di Thailand, ia dikenali sebagai ‘kratom’ dan di Malaysia sebagai ‘ketum’. Ciri-ciri perubatan tumbuhan ini disebabkan kesan seperti opium dan perangsang seperti kokain dalam melawan kelesuan dan kerja berat. Alkaloid utama daripada tumbuhan ini, mitraginin telah diekstrak dan diasingkan melalui sistem pelarut. Analisis spektroskopik piawai telah dijalankan untuk mengenal pasti sebatian tersebut. Disebabkan ciri-ciri potensinya sebagai perangsang psiko dan anti-keresahan, kajian ini dijalankan untuk mencari kesan mitraginin terhadap aktiviti perlakuan lokomotor dan tahap keresahan tikus-stres dan tikus-tanpa stres (eustres). Kesan mitraginin terhadap tahap kortikosteron dalam tikus-stres juga turut dijalankan. Aktiviti lokomotor dan ‘grooming’ di dalam ‘open-field test’ (OFT) dan kajian keresahan dalam ‘elevated plus-maze’ (EPM) telah dilakukan untuk profil perlakuan. Seratus empat puluh (140) ekor

tikus Sprague-Dawley jantan digunakan dalam kajian dan dibahagikan kepada 2 kumpulan, iaitu tikus eustres dan tikus aruhan-stres. Empat dos mitraginin yang berlainan (1.0, 5.0, 10.0 dan 30.0 mg/kg) diberikan secara 'intraperitoneal' kepada setiap tikus. Tikus-tikus aruhan-stres telah dikurung selama 2 jam dalam kurungan sebelum aktiviti perlakuan dijalankan. Keputusan menunjukkan aktiviti lokomotor meningkat secara statistik ($P<0.05$) untuk tikus yang diberikan 5.0 dan 30.0 mg/kg mitraginin berbanding 1.0 mg/kg mitraginin dalam tikus tiada-stres. Aktiviti lokomotor turut meningkat dalam tikus-stres dalam semua dos mitraginin, namun keputusannya tidak nyata secara statistik. Aktiviti 'grooming' meningkat secara statistik ($P<0.05$) dalam tikus stres yang diberi 1.0, 5.0, 10.0 dan 30.0 mg/kg mitraginin berbanding kumpulan kawalan. Bagi tikus tiada-stres, aktiviti 'grooming' meningkat dalam semua dos mitraginin tetapi keputusannya tidak nyata secara statistik. Dalam kajian keresahan, tiga parameter dijalankan terhadap setiap tikus termasuk masa diambil dalam 'open arms', kemasukan ke dalam 'open arm' dan masa diambil dalam 'central platform'. Mitraginin (30.0 mg/kg) menambah masa diambil dalam 'open arms' dan kemasukan ke dalam 'open arm' dalam tikus-stres dan tiada-stres. Bagaimanapun, 30.0 mg/kg mitraginin mengurangkan masa diambil dalam 'central platform' dalam kumpulan stres dan tiada-stres. Masa diambil dalam 'central platform' hanya meningkat bagi tikus-stres diberikan 5.0 dan 10.0 mg/kg mitraginin. Tahap kortikosteron dalam tikus-stres yang diberikan semua dos mitraginin meningkat secara nyata ($P<0.05$) berbanding kumpulan kawalan. Dos mitraginin lebih tinggi mampu untuk mengaruhkan perubahan perlakuan dengan menambah lokomotor, aktiviti 'grooming' dan parameter keresahan. Oleh itu, kajian ini menunjukkan mitraginin membentuk sensitiviti terhadap aktiviti lokomotor, 'grooming' dan keresahan dengan meninggikan bacaan parameter bersama-sama dengan tahap

kortikosteron di dalam tikus aruhan stres. Kesimpulannya, mitraginin mampu untuk memberikan perangsang psiko dan ciri-ciri anti-keresahan bagi tikus stres dan tiada-stres melalui mekanisme tindakan mitraginin yang sesuai dalam tikus.

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I certify that an Examination Committee has met on 8th October 2010 to conduct the final examination of Nurul Raudzah binti Adib Ridzuan on her Master of Science (Anatomy) thesis entitled 'Effects of mitragynine on locomotor and anxiety in rats subjected to restraint stress' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Masters of Science (Anatomy).

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NURUL RAUDZAH BT ADIB RIDZUAN

Date: 8th October 2010

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