ASIAN J. EXP. BIOL. SCI. VOL 1 (2) 2010: 404 - 408

ISSN 0975-5845

AJEBS

http://www.ajebs.com

ORIGINAL ARTICLE

In-Utero Effects of the Crude Ethanolic Extract of the Leaves of *Mitragyna speciosa* on Neural Tube Formation in Rats

Bala Y. Muhammad*, Abubakar D. Abdullahi*, Siti Nur Sharida Abd Kadir*, Tariq Bn. Abdul Razak* and Qamar U. Ahmed**

Departments of Basic Medical Sciences* and Pharmaceutical Chemistry**
Kulliyyah of Pharmacy, International Islamic University, Kuantan Campus, Indera Mahkota, 25200, Kuantan, Pahang, Malaysia.
Email: balamuhd@yahoo.com

ABSTRACT

The developmental effects of in-utero administration of the crude ethanolic extract of the leaves of Mitragyna speciosa (MS) on neural tube in fetal rats were investigated. Pregnant Sprague-Dawley rats were dosed orally once daily by gavage, with graded (500, 1000 and 1500 mg/kg) doses of the extract between the 8th and 13th day prenatally. The control group received corn oil used as vehicle for the extract. On the 18th day of gestation, mothers were sacrificed and embryos removed and stained under established procedures. The embryos were then analyzed for the presence of neural tube defects (NTD) through measurements of the extent of vertebral arch closure and brain size. Results indicate that the medium (C) and high (D) (1000 & 1500mg/kg) doses but not the low (B) (500mg/kg) dose in comparison with control (A) group, significantly ($p \leq 0.001$) produced a widening of the vertebral arch in the thoracic, lumber and cervical regions of the spinal cord. The brain transverse diameter was also significantly ($p \leq 0.05$) increased by the high dose only. These effects were seen in the absence of any significant differences in litter size and other gross physical abnormalities. This study indicates that the crude extract of the leaves of MS is capable of selective neurotoxicity and producing spina bifida like NTD as characterized by altered brain size and neural tube formation, a finding that may have an important implication in the dependence liability associated with its use.

Key Words: *Mitragyna speciosa, neural tube, Prague-Dawley rats*

INTRODUCTION

Intervention with variety of pharmacological agents especially during sensitive period of the developing Central Nervous System (CNS) has been shown to result in potentially long lasting neurobehavioural and/or neurochemical abnormalities [1]. The leaves of *MS*, (family: Rubiaeae), locally named katum or kratom is a plant endemic to tropical Southeast Asia, used by natives for opium-like effects and stimulant ability to combat fatigue and enhance tolerance to hard work [2]. Its recreational use has been banned in many countries including Australia, Burma, Thialand and Malaysia, due to concerns over dependence liability potential. *MS* contains many alkaloids including mitragynine (once thought to be the primary active), mitraphylline, and 7-hydroxymitragynine (which is currently the most likely candidate for the primary active chemical in the plant). Although structurally related to yohimbine and other tryptamines, its pharmacology is quite different, acting primarily as a mu-opioid receptor agonist. It also shares some adrenergic receptor activity similar to that of yohimbine. *MS* also contains alkaloids found in uña de gato, which are thought to play a beneficial role on the immune system and lower blood pressure, as well as epicatechin, a powerful antioxidant also found in dark chocolate and closely related to the EGCG that gives green tea its beneficial effects. Other active chemicals in MS include raubasine (best known from *Rauwolfia serpentina*) and some yohimbe alkaloids such as corynantheidine [3]. The opioid-like effects, of MS alkaloids, the naloxone-induced withdrawal syndrome and cross tolerance with morphine [2,4,5], all point to a dependence liability potential. These findings also raise questions on the possibilities and potentials that these alkaloids have to other toxicological effects and mechanisms. The vulnerability of the developing central nervous system to a variety of neuroactive substances is well documented.

The "brain growth spurt", a period of heightened neuronal development which in the rat is around day 10 prenatally, is especially sensitive to the toxic effects of many neuroactive agents. In this study, we investigate the effect of in utero administration of the crude ethanolic extract of the leaves of *MS* on neural tube development of fetal rat.

MATERIALS AND METHODS

Preparation of plant extracts: Authenticated plant material (leaves of *MS*) was air-dried at room temperature under shade and grinded into powdered form. Two kilograms material was extracted in soxhlet extractor three times (in order to ensure maximum yield) for about 2 h every time with 90% ethanol (EtOH). The extract was meticulously filtered and solvent evaporated to dryness under reduced pressure below 50°C to yield a purified EtOH extract. Dry extract were stored in a deep freezer at –30°C until reconstituted for use..

LD_{50} (Safety profile): Oral acute lethal dose study was carried out (to determine the safety profile) using the revised Up and Down (UPD) procedure according to recommendations [6,7].

Animal dosing: Pregnant prague-Dawley rats (weighing 156-246g each) were randomly assigned groups, caged individually and were fed rat chow and water ad libitum under 12:12 light: dark cycle. The rats were dosed orally by gavage between day 8 and 13 of gestation with corn oil (control) or graded doses (500, 1000 and 1500 mg/kg) of the crude extract of *MS*.

Sample processing and staining: At 18 days gestation the females were euthanized with chloroform, the abdomen opened through laparotomy and the uterus and uterine contents removed. Fetuses were "milked out" and their abdomens opened to allow adequate penetration of 10% formalin in which they were immersed. Fetuses were stained for bone and cartilages after 4 days of fixation as described previously [8]. Briefly, the fetuses were eviscerated and skinned. Cartilage was stained with Alcian blue followed by bone staining with alizarin red. The fetuses were then cleared in KOH and graded concentrations of glycerol [8].

Measurement of vertebral canal diameter: After clearing the fetuses were inspected with a dissecting microscope and the width of the vertebral arch gap was measured with computer built-in micrometer gauge, from T-9 to S-4 [8].

Measurement of the brain diameter: After fixation of the fetuses the brain was dissected out of the head through the use of knife and dissecting forceps. The brains were weighted and processed for histology preparation. Briefly the fetal brains were processed over 24 hours in a manual tissue processing system that includes graded ethanol, xylene and paraffin. The brains were embedded and sectioned and then stained with haematoxilin and eosin.

Measurement of the width of gray and white matter was carried out. This is to correlate with the width of the mantle and marginal layers.

Data analysis: Data was analyzed using one-way ANOVA and a Bonferonni post-hoc test for comparison between groups, with SPSS version 12.0. Values are expressed as mean ± sem. Significant differences was considered when $P \leq 0.05$.

RESULTS

LD_{50} (Safety profile): None of the 5 rats died nor showed any sign of toxicity at the limit dose of 3000mg/kg/oral OD per rat, and no evidence of toxicity was noted during 5 days of observation. The LD_{50} of *MS* in rats was therefore taken as above 3000 mg/kg/oral. Graded doses (500, 1000 and 1500mg/kg) of the crude extract of *MS* were chosen to study the effect of *in utero* administration on neural tube development in fetal rats.

Measurement of neural tube diameter: Neural tube diameter of fetuses treated in utero with graded doses of *MS*, indicates that the medium (C) and high (D) (1000 & 1500mg/kg) doses but not the low (B) (500mg/kg) dose in comparison with control (A) group, significantly ($p \leq 001$) produced a widening of the vertebral arch in the thoracic, lumber and cervical vertebral regions of spinal cord (fig.1).

Measurement of the brain diameter: Brain diameter was significantly ($p \leq 001$) increased transversely (table 1) in the high dose treatment group in comparison with the control.

Other abnormalities: There were no other gross physical abnormalities any significant differences in litter size observed in the rat pups. The mean daily weight changes of pregnant rats over the treatment period, day 8- 13 of gestation was also not significantly different between treatment groups (fig 2).

However, the mean litter weight of the low dose (500mg) was significantly lower, while that of medium dose (1000mg/kg) group was significantly higher than the control group (table 2).

Table 1: The effect of maternal treatment of crude ethanolic extract of *Mitragyna speciosa* on Brain Size Measurement in Fetal Rats.

Treatment Group	AnteriorPosterior Diameter (μm) ± sem	Transverse Diameter (μm) ± sem
Control	5924.6 ± 832	3642.7 ± 253
M. speciosa 500mg/kg/day	6212.1 ± 769	4895.7 ± 452
M. speciosa 1000mg/kg/day	4491.2 ± 655	3464.9 ± 476
M. speciosa 1500mg/kg/day	8750.6 ± 800	7244.1 ± 696**

** = P≤0.001

Table 2: The effect of maternal treatment of crude ethanolic extract of *Mitragyna speciosa* on Litter weight and size of fetal rats.

Treatment Group	Mean litter weight (g) ± sem	Mean litter Size ± sem	Number of fetal rats (n)
Control	1.49 ± 0.09	9.75 ± 1.3	39
M. speciosa 500mg/kg/day	1.27 ± 0.9*	10.60 ± 1.7	53
M. speciosa 1000mg/kg/day	2.04 ± 0.05**	8.00 ± 1.0	24
M. speciosa 1500mg/kg/day	1.45 ± 0.59	7.50 ± 1.5	45

* =P ≤ 0.05; ** = P ≤ 0.001

DISCUSSION

Our studies have shown that the crude ethanolic extract of the leaves of *Mitragyna speciosa* (MS) is capable of altering neural tube formation and causing a Spina Bifida-like neural tube defects, characterized by failure of neural tube of the developing fetus to close properly [8]. There were no observable physical abnormalities or effect on litter size although a reduction and corresponding increase in litter weight was seen in the low and medium dose groups respectively. Effect on litter weight after chronic MS in utero has been reported previously [9]. Considerable amount of literature from human and animal studies exist showing a greater vulnerability of the developing nervous system particularly during critical period, to a variety of environmental toxicants and neuroactive agents. Such exposure if coincident with neural developmental processes including proliferation, migration, differenciation, synaptogenesis, myelination and apoptosis, may cause enduring functional and behavioural abnomalities in the offspring later in life [1]. Pharmacological but not structural association has been made between morphine, a prototype opioid agonist and the alkaloids of MS. Further, the development of tolerance and cross-tolerance as well as mu-opioid receptor selectivity of morphine and MS [10,2], may suggest commonality in the biochemical and molecular mechanisms of opioid-induced dependence effects. The report of Fazel & Jalali [11] demonstrating a high incidence of CNS defects including exencephaly and spina bifida in fetal mice exposed in utero to morphine on days 10 and 11 of gestation, period that are critical in CNS development, is an attraction to our study. Indeed, CNS disorders including some seemingly peripheral diseases, like chronic pain and disorders in major organs have clear pathological basis in the CNS. Pathological insults and long-term plastic changes within neural circuits may lead to maladaptive behaviours. Drug addiction is a chronic brain disease characterized by compulsive drug-seeking, craving and taking behaviour. Experimental evidence suggests that following exposure to drugs of abuse, neurones within the mesolimbic

dopamine system undergo a series of plastic changes that may lead to compulsive emotional and motivational states [12]. In support of this, Antony *et al.*, [13] have demonstrated an association

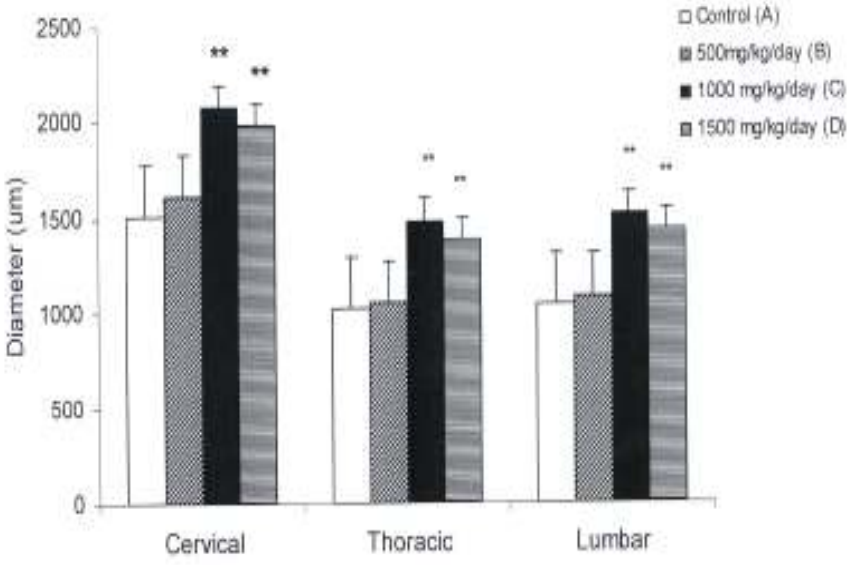

Figure 1: Effect of crude ethanolic extract of *Mitragyna speciosa* on neural tube diameter of fetal rats. Histogram represent mean ± sem (24-53 pups per treatment group) neural tube diameter of fetal rats of control and *Mitragyna speciosa* (graded doses) treated mothers. * =P ≤ 0.05; ** = P ≤ 0.001

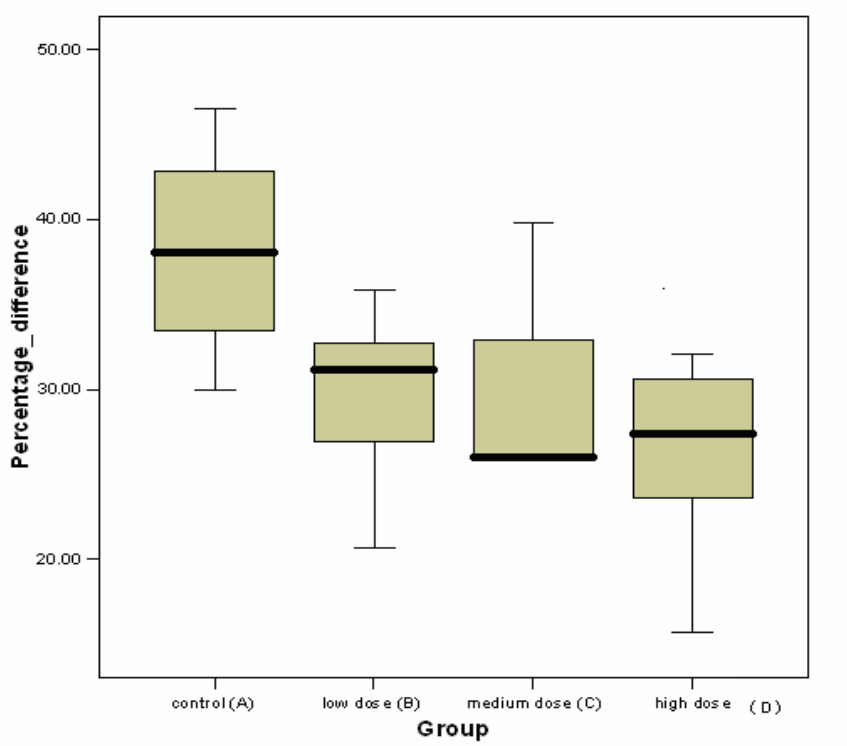

Figure 2: Percentage weight difference (Stem-and-Leaf Plots) of control and *Mitragyna speciosa* treated Pregnant Rats, (n = 4-6). No significant difference between control and *Mitragyna speciosa* treated groups.

between risk of alcoholism and developmental defects of specific brain structures, in which children having strong family loading for alcoholism were found more vulnerable to developing alcoholism than those having no history of alcohol dependence. These subjects were shown to have significantly lower volumes of certain brain structures such as the left and right amygdala and hippocampus (due to dysregulation of brain reward system), areas implicated in the aetiology of drug and alcohol addiction [14].

CONCLUSION

In conclusion, the Spina bifida-like NTD induced by MS may bring about structural alterations in the course of neuronal developmental processes including alterations in biochemical and receptor mechanisms important in the genesis of opioid-induced dependency. Further studies will however be needed to determine possible association between these structural and behavioural alterations and vulnerability to opioid dependence liability potential.

ACKNOWLEDGEMENT

Authors are grateful to the International Islamic University Malaysia Research Centre for funding this research project. Also, they express their gratitude to all academic and technical staff of the Kulliyyah of Pharmacy for their support and assistance.

REFERENCES

[1] Rice, D. and Barone, S., (2002). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environ. Health Persp. Suppl.* 108 (**S3**): 1-31.

[2] Matsumoto, K. et al., (2005). Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa. Life Sciences,* 78 (**1**): *2-7.*

[3] Babu, K.M., McCurdy, Ch. R. and Boyer, E.W. (2008). Opioid receptors and legal highs: Salvia divi-norum and Kratom, *Clinical Toxicology* 46, 146-152.

[4] Matsumoto, K et al., (2006). Involvement of μ-opioid receptors in antinociception and inhibition of gastrointestinal transit induced by 7-hydroxymitragynine, isolated from Thai herbal medicine *Mitragyna speciosa. Eur. J. Pharmacol*., 549 (**1-3**): 63-70.

[5] Takayama H. et al, (2004). Chemistry and Pharmacology of Analgesic Indole Alkaloids from the Rubiaceous Plant, *Mytragyna speciosa*. *Chem. Pharm. Bull*., 52 (**8**):916-928.

[6] OECD. (1998). OECD Guideline for the Testing of Chemicals, Revised Test Guideline 425, Acute Oral Toxicity, Up-and-Down Procedure. OECD, Paris. Available: http://www.oecd.org//ehs/test/health.htm [cited October 1, 2001].

[7] U.S. EPA. (1998). Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity. Washington, DC, U.S. Environmental Protection Agency. Available:http://www.epa.gov /docs/OPPTS_Harmonized/870_ Health_Effects_Test_Guidelines/Series/ [cited October 1, 2001].

[8] Briner, W., (2001). The effect of GABA receptor ligands in experimental spina bifida occulta, *BMC Pharmacology*, 1:2, http://www.biomedcentral.com/1471-2210/1/2

[9] Kumarnsit, E. et al., (2006). Acute and long-term effects of alkaloid extract of *Mitragyna speciosa* on food and water intake and body weight in rats. *Fitoterapia*, 77(**5**): 339-345.

[10] Matsumoto, K. et al. (2004).Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of orally active opioid analgesic from the Thai medicinal herb Mitragyna speciosa, *Life Sciences*, 74 (**17**), 2143-2155,.

[11] Fazel, A and Jalali, M., (2002). Experimentally induced Exencephaly and Spina Bifida in Mice. *Arch Iranian Med*, 5 (**3**): 179-183.

[12] Zhen Ci Yan J., (2008). The pathological neural plasticity and its application in acupuncture research. http://www.ncbi.nlm.nih.gov/pubmed/18386644.

[13] Antony, G. Benegal, V. and Jayakumar, P.N. (2004). Risk for alcoholism and developmental defects of specific brain structures: A MR morphometric study. IEEE, 229- 232. http://ieeexplore.ieee.org/xpls/abs_ all.jsp?arnumber=1398516.

[14] Koob, G.F., 1999. The role of the striatopallidal and extended amygdale systems in drug addiction. *Annual New York Academy of Science*, 877: 445-460.

Corresponding Author: Dr. Bala Yauri Muhammad, Associate Professor Department of Basic Medical Sciences International Islamic University, Malaysia, Kuantan Campus, Indera Mahkota, 25200 Kuantan Pahang, Malaysia. Phone; +609 5716400 ext 6663 Fax number: +609 5716775 Mobile number: +60199068579 Email: balamuhd@yahoo.com