

# EVALUATING THE EFFECTS OF *Erythroxylum cuneatum* FORMA *CUNEATUM* KURZ (CHINTA MULA) IN MORPHINE ADDICTED RATS.

Mohd Ilham Adenan<sup>1,2</sup>, Anee Suryani Sued<sup>2</sup>, Muhd Haffiz Jauri<sup>2</sup>, Siti Syarifah Mohd Mutalip<sup>2</sup>, Nurhanan Murni Yunos<sup>2</sup>, Asiah Osman<sup>2</sup>, Norhayati Ismail<sup>2</sup> & Lili Sahira Husin<sup>2</sup>

<sup>1</sup>Bio-screening Division, Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPharm), Ministry of Science, Technology & Innovation (MOSTI), Sains@USM, Blok A, 10 Persiaran Bukit Jambul, 11900 Bayan Lepas, Penang

<sup>2</sup>Drug Discovery Centre, Forest Research Institute of Malaysia (FRIM), 52109 Kepong, Selangor Darul Ehsan

## INTRODUCTION

The substance abuse situation in our country is quite worrying. Despite the Government spending up to RM50 million a year on its drug rehabilitation programme at 28 government-run drug rehabilitation centers throughout the country, the number of drug addicts detected each year has not shown any sign of abating. In fact, anecdotal reports say that up to 70% of drug users who left the centers went back to drugs again. Heroin (36%), morphine (30%), ganja (23%) and metamphetamine or syabu (7%) constituted the most frequently abused drugs. Even though the research on Malaysian plants in Malaysia has been in existence for the past few decades, there are still gaps in the understanding the potential Malaysian plants used for the treatment of drug addiction in this country. Fundamental research in terms of bio- and chemical profiling of selected Malaysian plants species with addiction therapy properties will be able to contribute to the knowledge advancement and scientific validation of the ethnobotanical uses of phytotherapeutics for the development of herbal remedies for alternative addiction therapeutic agents. *Erythroxylum cuneatum* forma *cuneatum* Kurz (family; Erythroxylaceae) locally known as chinta mula has been chosen in this study solely based on information gathered from our traditional practioners who have had some experienced in using this plant when treating heroin addicted juvenile.

## OBJECTIVES

- To produce standardised extract of *E. cuneatum* (chinta mula)
- To evaluate the effects of *E. cuneatum* extract and its active fractions in morphine addicted rats, especially their effect on morphine withdrawal symptoms
- To identify the active fractions of the extract
- To obtain the protein profiles in response to exposure to morphine and after the administration of the plant extract

## METHODOLOGY

Male *Sprague Dawley* rats were made dependent to morphine through intra-peritoneal administration of increasing doses of morphine starting from 10 to 100 mg/kg body weight for seven days. The extracts of *E. cuneatum*, 300 and 100 mg/kg body weight respectively, were fed orally into three different treatment groups of morphine addicted rats as follows: (a) single dose

of extract 24 hrs prior to first dose of morphine; (b) multiple doses of extract together with morphine twice daily for seven days; and (c) single dose of extract 24 hours after last dose of morphine. The withdrawal signs exhibited by the morphine addicted rats 24 hours after the last dose of morphine were quantified by 18 counted and checked signs parameters. Three counted signs (head shaken, exploring and wet dog shakes) and six checked signs (chewing, squeaking on touch, hostile while handling, diarrhoea, ptosis, and eye twitching) were observed in the morphine addicted rats in these three treatment groups.

Protein serum was separated in two dimensions; iso-electric focussing using immobilised pH gradient strip (17 cm) with narrow range pH 4-7 (linear); followed by separation according to their molecular mass with polyacrylamide gel electrophoresis [gel gradient concentration (7.5%-15%)]. The 2-DE gels obtained were stained with silver nitrate.

## RESULTS

The results showed that administration of both aqueous and methanolic extracts of *E. cuneatum* caused significant reduction ( $p \leq 0.05$ ) in the frequency of counted and checked withdrawal signs observed, especially in group (b) and (c). The cumulative withdrawal scores of both aqueous and methanolic extracts of *E. cuneatum* are 66.51 and 67.26 respectively in group (a); 24.64 and 38.38 respectively in group (b); and 34.26 and 45.27 respectively in group (c)]. These scores were significantly lower when compared to total scores of the base-line group of morphine addicted rats (79.39), and to morphine addicted rats treated with pure opioid antagonist, naloxone (150.13).

Image analysis on the 2-DE profiles using *The Discovery Series PDQuest*<sup>TM</sup> version 7.2.0 software (BioRad, USA) gave the lowest total protein spots (861 spots) in the serum of negative control rats and the highest number of protein spots (893 spots) in morphine addicted rats treated with *E. cuneatum* aqueous extracts. Thirteen protein spots were found unique to the serum of morphine addicted rats; ten protein spots were up-regulated ( $p < 0.001$ ), and five protein spots were down-regulated ( $p < 0.001$ ) when compared to negative control rat serum. Image analysis between morphine addicted rats and morphine addicted rats treated with aqueous extracts *E. cuneatum*, showed 20 protein spots were up-regulated ( $p < 0.001$ ), and nine spots were down-regulated ( $p < 0.001$ ) in the morphine addicted rat serum. The analysis of serum protein profiles showed specific pattern of 2-DE serum protein profiles related to morphine dependence and to administration of the aqueous extract *E. cuneatum* in morphine addicted rats.

## CONCLUSION

The effects of the aqueous and methanolic extracts of and *Erythroxyllum cuneatum* forma *cuneatum* (Miquel) Kurz (family Erythroxyllaceae) on the withdrawal symptoms of morphine addicted rats and their serum protein were evaluated. Studies on the effect of both aqueous and methanolic extracts from the leaf part of *Erythroxyllum cuneatum* forma *cuneatum* on the withdrawal symptoms in morphine-dependent rats have shown the ability of these extracts to attenuate the withdrawal symptoms exhibited by morphine addicted rats 24 h after cessation of morphine. The withdrawal symptoms are namely head shaken, exploring and wet dog shakes, chewing, squeaking on touch, hostile while handling, diarrhoea, ptosis, and eye twitching.

Withdrawal symptoms observed in morphine-dependent rats administered with aqueous extract of *E. cuneatum* was lower compared to methanolic extract; however the difference was not significant when tested with post-hoc Dunnett's. From these findings it can be concluded that extracts from the leaf of *E. cuneatum* has the ability to reduce significantly withdrawal symptoms exhibited by morphine addicted rats; and can be develop further as an alternative remedy in the treatment of abused drug addiction.