**UNIVERSITI PUTRA MALAYSIA**

***ANTINOCICEPTIVE EFFECT OF 3-(2, 3 DIMETHOXYPHENYL)-1 (METHYLFURAN-2-YL) PROP-2-EN-1-ONE IN MICE AND ITS MECHANISMS OF ACTION***

**NUR IZZATI BINTI ISMAIL**

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# ANTINOCICEPTIVE EFFECT OF 3-(2, 3 DIMETHOXYPHENYL)-1 (METHYLFURAN-2-YL) PROP-2-EN-1-ONE IN MICE AND ITS MECHANISMS OF ACTION

**By**

**NUR IZZATI BINTI ISMAIL**

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

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**ANTINOCICEPTIVE EFFECT OF 3-(2, 3 DIMETHOXYPHENYL)-1 (METHYLFURAN-2-YL) PROP-2-EN-1-ONE IN MICE AND ITS MECHANISMS OF ACTION**

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**NUR IZZATI BINTI ISMAIL**

**February 2015**

**Chairman : Professor Mohd Roslan bin Sulaiman, PhD**
**Faculty : Medicine and Health Sciences**

**The objective of the present study is to** evaluate the antinociceptive activity of the new chalcone derivative, **the 3-(2,3 dimethoxyphenyl)-1 (methylfuran-2-yl) prop-2-en-1-one (DMPF)**, using the chemical- and thermal-induced nociception test together with the mechanisms of actions. It was demonstrated that, administration of DMPF given by the i.p route produced a significant dose-dependent inhibition on acetic acid-induced writhing test, increased in response latency time for the hot plate test and reduction in time spent licking the injected paw for both neurogenic and inflammatory phases in formalin-induced paw licking test. Furthermore, the antinociceptive effect of DMPF in the acetic acid-induced writhing and the hot plate test was not reversed by non-selective opioid receptor antagonist, naloxone, that shown the non-involvement of the opioidergic system in antinociceptive activity of DPMF. Contrarily, administration of DMPF produced significant dose-dependent inhibition in capsaicin-induced paw licking test, glutamate-induced paw licking test, and PMA-induced paw licking test proven the involvement of TRPV1 receptor, glutamatergic system and PKC system. Antinociception caused by DPMF in the acetic acid test reversed the treatment with L-NOARG (nitric oxide synthase inhibitor) and charybdotoxin (a large-conductance $ca^{2+}$-sensitive $K^+$ channels blocker). However, antinociception of DPMF was enhanced by ODQ (a specific guanylyl cyclase inhibitor). To sum up, the results indicate that DPMF produced pronounced central and peripheral antinociception by inhibition on glutamate receptors, TRPV1 receptors and protein kinase C. It is also strongly suggested that the L-arginine/NO/cGMP/$BK_{ca}$ channel pathway plays a significant role in DPMF-induced antinociception.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai Memenuhi keperluan untuk ijazah Master Sains

**KESAN ANTINOSISEPTIF YANG DIARUH OLEH 3-(2, 3 DIMETHOXYPHENYL) 1(METHYLFURAN-2-YL) PROP-2-EN-1-ONE (DPMF) IAITU DERIVASI CHALCONE KE ATAS MENCIT DAN MEKANISMA-MEKANISMANYA**

Oleh

**NUR IZZATI BINTI ISMAIL**

**Februari 2015**

**Pengerusi : Profesor Mohd Roslan bin Sulaiman, PhD**
**Fakulti : Perubatan dan Sains Kesihatan**

Objektif kajian yang dijalankan baru-baru ini adalah untuk mengkaji kesan aktiviti antinosiseptif yang diaruh oleh derivatif chalcone iaitu 3-(2,3 dimethoxyphenyl)-1 (methylfuran-2-yl) prop-2-en-1-one (DMPF) dan mekanisma-mekanismanya dengan menggunakan model nosiseptif kimia dan haba. Kajian menunjukkan bahawa pemberian DPMF secara intraperitoneum menunjukkan perencatan signifikan yang berkadar selari dengan dos ke atas kajian ujian penggeliatan abdomen aruhan asid asetik, kajian ujian plat panas dan kajian ujian jilatan tapak kaki aruhan formalin. Kajian juga menunjukkan aktiviti antinosiseptif DPMF dalam kajian ujian penggeliatan abdomen aruhan asid asetik dan kajian ujian plat panas adalah tidak berbalik oleh antagonis reseptor opioid yang tidak selektif, naloxone, sekaligus menunjukkan bahawa tiadanya penglibatan sistem opioidergik di dalam aktiviti antinosiseptif DPMF. Namun, perawatan dengan DPMF menghasilkan rencatan yang signifikan bersandarkan dos bagi ujian nosisepsi aruhan kapsaisin, ujian jilatan tapak kaki ransangan glutamat, dan ujian jilatan tapak kaki ransangan FMA didalam mencit di mana ujian-ujian tersebut menunjukkan kesan rencatan DPMF terhadap reseptor TRPV1, sistem glutamatergik, dan dalam sistem PKC. Dalam ujian seterusnya, antinosiseptif yang ditunjukkan oleh DPMF dalam kajian ujian penggeliatan abdomen aruhan asid asetik menghasilkan perbezaan bilangan penggeliatan abdomen yang signifikan dengan pemberian rawatan oleh L-NOARG (perencat enzim nitrik oksida) dan “charybdotoxin” (perencat saluran K+ sensitif). Walaubagaimanapun, kajian mendapati kesan antinosiseptif oleh DMPF telah ditingkatkan dengan pemberian ODQ (perencat spesifik “guanylyl cyclase). Hasil kajian di atas menunjukkan bahawa laluan L-arginin/NO/cGMP/ $BK_{ca}$ memainkan peranan yang signifikan dalam membolehkan DPMF menjalankan kesan antinosiseptif.

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I certify that a Thesis Examination Committee has met on 27 February 2015 to conduct the final examination of Nur Izzati binti Ismail on her thesis entitled "Antinociceptive Effect of 3-(2,3 Dimethoxyphenyl)-1 (Methylfuran-2-YL) Prop-2-EN-1-One in Mice and its Mechanisms of Action" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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

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Signature: _____
Name of Member of Supervisory Committee: Dr. Daud Ahmad bin Israf Ali

Signature: _____
Name of Member of Supervisory Committee: Dr. Md Nordin bin Hj Lajis

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| | |
|---|---|
| μ | Mu |
| ADR | adverse drugs reaction |
| AMPA isoxazolepropionate) | α-amino-3-hydroxy-5-methyl-4- |
| ATP | Adenosin triphosphate |
| $BK_{ca}$ | large conductance $ca^{2+}$-activated $K^+$ channel |
| cGMP | cylic guanyl monophosphate |
| COX-2 | cyclooxygenase-2 |
| DAG | diacylglycerol |
| DMPF 2- | 3-(2,3 dimethoxyphenyl)-1(methylfuran-2-yl)pro- |
| EAAs | excitatory amino acids en-1-one |
| EPSP | excitatory postsynaptic potential |
| GABA | γ-amino butyric acid |
| GC | Guanylyl cyclase |
| GluRs | Glutamate receptors |
| IASP Pain | The International Association for the Study of |
| IFN-ɣ | Interferon-ɣ |
| iNOS | inducible nitric oxide synthase |
| $K_{ATP}$ | ATP-sensitive $K^+$ channels |
| LPS | lipopolysaccharide |
| mGLUr | Metabotropic glutamate receptor |
| mV | membrane potential |
| NGF | nerve growth factor |
| NMDA | N-methyl-D- aspartate |
| NO | nitric oxide |

| | |
|---|---|
| NOS | Nitric oxide synthase |
| NSAIDs | non-steroidal anti-inflammatory drugs |
| P | relative membrane permeability |
| PAG | periaqueductal grey |
| PG | Prostglandin |
| PGE2 | prostaglandin 2 |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| SG | Substantia gelatinosa |
| $SK_{ca}$ | small conductance $ca^{2+}$-activated $K^{+}$ channel |
| SP | Substance P |
| TRP | transient receptor potential |
| TRPV1 | transient receptor potential vanilloid 1 |
| VGCCs | voltage-gated calcium channels |
| VGSCs | voltage-gated sodium channels |
| WHO | World Health Organization |
| δ | Delta |
| κ | Kappa |

# CHAPTER 1

## INTRODUCTION

To begin the chapter, the application of noxious stimuli for instance a thermal, mechanical or chemical injury may result in the perception of pain by a conscious individual. There are many complex factors that control the way in which the peripheral nociceptive stimulus undergoes transduction into electrochemical impulses and then onward transmission to reach the central nervous system particularly the cerebral cortex where the actual perception of pain is managed and interpreted. Above all, there is a debate among intelligences saying that pain is not a primary sense unlike vision, audition or hearing, somatosensation, and olfaction which are falling in that category (Price and Dubner 1977). Pain is described to be more of an emotional experience. However, most of the time pain is still considered to be produced by a complex noxious stimulation of the nervous system as explained previously. There are also possibilities that pain is more complicated than what had been studied until today. For instance, the setting of the tissues or nerve injury where the pain is persistent can certainly change the stimulus that evokes pain. These conditions are known as hyperalgesia and neuropathic pain. Under these conditions, sensitivity of pain is increased and the non-painful stimuli, allodynia, will cause production of pain.

The International Association for the Study of Pain (IASP) defines pain as ‘‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.’’ To put it in the simple way, pain always profound the emotional quality to the experience, hence the very discrete physiological and anatomical basis for the detection and transmission of signals that are interpreted as painful experience. Above all the unpleasant experience of pain, there is the reason or function of it. One of the important reasons is that pain served as protective function to protect our body as such withdrawal reflex of our body from the source of noxious simulation and immobility due to pain may serve to provide an environment where the rate of healing is increased and restoration of function can take place. Despite the benefit that pain had offered, it always become prolonged or chronic and when these happened with the addition of tissue damage, central nociceptors pathways are sensitized and reorganized (Figure 1). In such to manage the pain, analgesic drugs is the highly reliant current treatment it acts through weather the central or/and peripheral nervous systems; which includes opioid drugs and non-steroidal anti-inflammatory drugs (NSAIDs) such as morphine and salicylates respectively.

Currently, NSAIDs and opioids are the highly effective treatments that are used widely all over the world as pain management since they are very effective in the treatment of acute nociceptive pain, yet are poor treatment for other several types of pain for example neuropathic pain. Unfortunately, these analgesic drugs can cause several adverse reactions when they are taken in a long period of time. As for example, NSAIDs can cause two major adverse drugs reaction (ADRs) which are gastrointestinal and renal failure while opioids can cause tolerance, addiction and

respiratory depression. In this situation, it occurs that herbal medicine has captured much attention through its benefits to posses antinociceptive, anti inflammatory, anti fungal and in fact anti cancer. Due to all benefits, it positively opens new doors in order to search for the natural products that may take actions as similar as the known analgesic but with either less or no side effects.

According to World Health Organization (WHO), 80% of the world's population uses remedies based on the plants as their primary form of healthcare. In our country specifically, most of the traditional medicinal plants have been handed down from one generation to the next. There are many bioactive compounds that had been isolated from herb and were widely studied for instance chalcone (Sashidhara, Kumar et al. 2011), flavonoid (Kiplimo, Islam et al. 2011), triterpene (Longhi-Balbinot, Martins et al. 2011) and many others more. For this particular study, the play role here is chalcone. Through a number of study on synthetic compounds with chalcone backbone and these compounds proved to posses' antimicrobial (Prasad, Rao et al. 2008), antinociceptive (Correa, Pereira et al. 2001) , anti-inflammatory (Hsieh, Tsao et al. 2000), and antiulcerative activity (Murakami, Muramatsu et al. 1991). Previous studies have shown that chalcones bearing phenyl group with chloro and hydroxyl substituents inhibit nitric oxide (NO) synthesis as well as inducible nitric oxide synthase (iNOS) protein expression in RAW 264.7 cells (Ko, Tsao et al. 2003; Won, Liu et al. 2005). In the study done by our research group on a naturally occurring chalcone isolated from the fruits of *Alpinia rafflesiana*, cardamonin, we found that it suppressed both NO and PGE2 (prostaglandin 2) in interferon-ɣ (IFN-ɣ- and lipopolysaccharide (LPS)- induced RAW 264.7 cells (Ahmad, Israf et al. 2006; Israf, Khaizurin et al. 2007). Another chalcone compound is isolated from bark of *Toxicodendron vernicifluum* and this compound found to have anti-inflammatory effect (Kim, Moon et al.). It has been reported elsewhere that chalcone derivative isolated form root of *Glycyrrhiza uralensis* inhibited the synthesis of nitric oxide (NO), lipoxygenase as well as cyclo-oxygenase activities which constitute the major pro-inflammatory pathways and remain most targeted for anti-inflammatory and antinociceptive drug development (Kim, Park et al. 2008).

Since most pain originates from inflammation and the role of NO in pain is very important as it has been state that without the production NO there will be no generation of pain. Thus, with the support of the strong basis on the previous study, the group had done the investigation on antinociceptive activity DMPF using the chemical- and thermal-induced nociception models in mice.

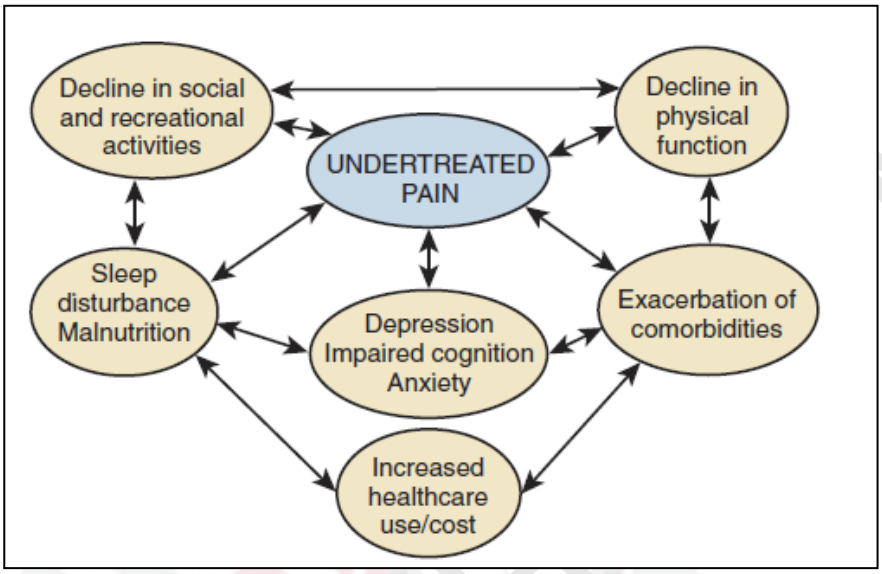


**Figure 1: Consequences of undertreatment of pain**
(Gureje, Von Korff et al. 1998).

## 1.1 Objectives

The objectives of the study are:

**General**

- To determine antinociceptive activity of DMPF compound and its possible mechanisms of action.

**Specific**

To determine :

- the involvement of peripheral and central antinociceptive activity of DMPF compound.
- the involvement of opioid receptors, TRPV1 receptor, glutamatergic system, NO-cGMP pathway, various types of potassium channels and protein kinase C system.

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