ผลของสารสกัดกะทกรกด้วยเอทานอลต่อการเสพติดมอร์ฟีนในหนูเมาส์และหนูแรท

นางสาว ชิรยา นิพัทธมานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF THE ETHANOLIC EXTRACT OF *PASSIFLORA FOETIDA* ON MORPHINE ADDICTION IN MICE AND RATS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ชิรยา นิพัทธมานนท์ : ผลของสารสกัดกะทกรกด้วยเอทานอลต่อการเสพติดมอร์ฟีนในหนูเมาส์และหนูแรท. (EFFECTS OF THE ETHANOLIC EXTRACT OF *PASSIFLORA FOETIDA* ON MORPHINE ADDICTION IN MICE AND RATS)
อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ร.ท.หญิง ดร.ภัสราภา โตวิวัฒน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร.ธงชัย สุขเศวต, 65 หน้า.

Passiflora foetida หรือกะทกรก เป็นพืชในวงศ์ Passifloraceae ในตำรับยาไทยแผนโบราณ มีการนำมาใช้ในการรักษา อาการไอ ไข้ ปวดศีรษะ รวมทั้งขับเสมหะ และขับปัสสาวะ เนื่องจาก มีการ รายงานถึงประ โยชน์ของ *Passiflora incarnata* ในการรักษาการติดยาเสพติด ดังนั้นงานวิจัยครั้งนี้จึงสนใจที่จะ ศึกษาถึงผลของ *P. foetida* ซึ่งเป็นพืชในสกุลเดียวกับ *P. incarnata* ต่อการเสพติดมอร์ฟีน โดยเริ่มต้นจากการศึกษาผลของสารส กัดกะทกรกด้วยเอทานอลต่อ การเคลื่อนไหว ในหนูเมาส์ จากผลการทดลองพบว่า สารสกัดกะทกรกด้วยเอทานอล ในทุกขนาด (25, 50, 100 และ 200 มก./กก.) โดยการป้อนไม่มีผลต่อการเคลื่อนไหว ทำการศึกษาฤทธิ์เสพติด ของสารสกัดกะทกรกด้วยเอทานอล และผลของสารสกัดกะทกรกด้วยเอทานอลต่อพฤติกรรมการชอบสถานที่แบบมีเงื่อนไขจากการ เหนี่ยวนำด้วยมอร์ฟีนด้วยแบบจำลองพฤติกรรมการชอบสถานที่แบบมีเงื่อนไข ในหนูแรท พบว่าสารสกัดกะทกรกด้วยเอทานอล ในทุกขนาด ไม่ทำให้เกิดพฤติกรรมการชอบสถาน ที่แบบมีเงื่อนไข แต่สามารถ ยับยั้งพฤติกรรมการชอบสถานที่แบบมีเงื่อนไขจากการเหนี่ยวนำด้วยมอร์ฟีนได้ ดังนั้นจึงทำการศึกษาผลของสารสกัดกะทกรก ด้วยเอทานอลต่อ อาการถอนยาของมอร์ฟีนในหนูเมาส์ พบว่าการได้รับสารสกัดกะทกรกด้วยเอทานอลในทุกขนาดและเมทาโดน (1 มก./กก.) ก่อนการเหนี่ยวนำให้เสพติดมอร์ฟีน สามารถลดการกระโดดและอาการหางชี้ได้อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม สารสกัดกะทกรกด้วยเอทานอล ในทุกขนาด ยกเว้นขนาดที่ต่ำที่สุด สามารถลดการกระโดด ของหนูที่เสพติดมอร์ฟีนได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม เช่นเดียวกับ เมทาโดน นอกจากนี้ยังพบว่าสารสกัดกะทกรกด้วยเอทานอลในทุกขนาดสามารถลดอาการหางชี้ได้อย่างมีนัยสำคัญทางสถิติ และสารสกัดกะทกรกด้วยเอทานอลขนาด 25 และ 50 มก./กก. สามารถลดการสะบัดตัวได้อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม

จากผลการศึกษาครั้งนี้แสดงให้เห็นว่าสารสกัดกะทกรกด้วยเอทานอลไม่มีฤทธิ์กระตุ้นหรือกดระบบประสาทส่วนกลาง และไม่มีฤทธิ์เสพติด แต่สามารถยับยั้งฤทธิ์เสพติดของมอร์ฟีนได้ นอกจากนี้สารสกัดกะทกรก อาจมีฤทธิ์ในการป้องกันและรักษาอาการถอนยาของมอร์ฟีนได้อีกด้วย ผล การศึกษาครั้งนี้แสดงให้เห็นว่า สารสกัดกะทกรกด้วยเอทานอลเป็นสมุนไพรที่มี ศักยภาพในกา รพัฒนาเพื่อ นำไปใช้ในการรักษาผู้ติดยาเสพติด กลุ่มโอปิออยด์ อย่างไรก็ตามควรมีการศึกษาถึงสารสำคัญของสารสกัดกะทกรกด้วยเอทานอลและกลไกการออกฤทธิ์ที่แน่ชัดต่อไป

สาขาวิชา เภสัชวิทยา ลายมือชื่อนิสิต .....

ปีการศึกษา 2553 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5187148120 : MAJOR PHARMACOLOGY

KEYWORDS : ADDICTION / *PASSIFLORA FOETIDA* / MORPHINE /CONDITIONED PLACE PREFERENCE / WITHDRAWAL

CHIRAYA NIPATTAMANON: EFFECTS OF THE ETHANOLIC EXTRACT OF *PASSIFLORA FOETIDA* ON MORPHINE ADDICTION IN MICE AND RATS. ADVISOR: ASST. PROF. FLG. OFF. PASARAPA TOWIWAT, Ph.D., CO-ADVISOR: ASSOC. PROF. THONGCHAI SOOKSAWATE, Ph.D., 65 pp.

Passiflora foetida, (Ka-tok-rok, Passifloraceae) has been used for the treatments of cough, fever, pain, head-ache, and also used as an expectorant and diuretic in Thai traditional medicine. Since *P. incarnata* has been proved to be useful in drug addiction therapy, therefore we were interested to investigate the effect of *P. foetida*, a plant in the same genus *Passiflora*, on morphine addiction. We initially evaluated the effect of the ethanolic extract of *P. foetida* (PF) on locomotor activity in mice. The results showed that all doses of PF (25, 50, 100 and 200 mg/kg, p.o.) produced no significant effects on locomotor activity. The reinforcing effect of PF and the effect of PF on morphine-induced conditioned place preference (CPP) were investigated using CPP paradigm in rats. All doses of PF did not show any significant effects on CPP but could suppress morphine-induced CPP. We then assessed the effects of PF on morphine withdrawal in mice. Pretreatment with of all doses of PF and methadone (1 mg/kg, i.p.) prior to morphine injection significantly (p<0.05) decreased naloxone-precipitated withdrawal jumping and straub tail behaviors compared to vehicle controls. All doses of PF tested except the lowest dose significantly (p<0.05) decreased naloxone-precipitated withdrawal jumping behavior in morphine dependence when compared to vehicle controls similar to methadone (1 mg/kg, i.p.). Additionally, all doses of PF also significantly (p<0.05) reduced withdrawal straub tail behavior and PF at the doses of 25 and 50 mg/kg significantly (p<0.05) reduced withdrawal wet dog shake behavior when compared to vehicle controls.

In conclusions, the present study demonstrated that PF did not have neither stimulating nor sedative effects, PF did not have reinforcing effect by itself but it could suppress the reinforcing effect of morphine. Moreover, PF may have prevention and treatment effects on morphine withdrawal. These results indicated that PF may have a potential to be developed for the treatment of opioid addiction. However, the active chemical constituents in PF and mechanisms of PF action may need to be further investigated.

Field of Study : Pharmacology ..... Student's Signature .....

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LIST OF ABBREVIATIONS

BZF	Benzoflavone
cm	Centimeter
^{0}C	Degree Celsius
CPP	Conditioned place preference
CMC	Carboxymethylcellulose
δ	Delta
e.g.	Exempli gratia (for example)
et al.	Et alii (and other)
g	Gram
GABA	Gamma-aminobutyric acid
hr	Hour
i.p.	Intraperitoneal
i.v.	Intravenous
κ	Kappa
LAAM	Levo-alphaacetylmethadol
LSD	Lysergic acid diethylamide
mg/kg	Milligram per kilogram
ml	Milliliter(s)
min	Minute
MO	Morphine
μ	Mu
N	Sample size
NAc	Nucleus accumbens
NSS	Normal saline solution
%	Percent
PF	The ethanolic extract of *Passiflora foetida*
ROD	Rapid opiate detoxification

sec	Seconds
S.E.M.	Standard error of means
UROD	Ultra rapid opiate detoxification
VTA	Ventral tegmental area

CHAPTER I

INTRODUCTION

Background and Rationale

Addiction to cannabinoids, cocaine, amphetamine and opioids represents significant burden to societies worldwide. The opiates and their synthetic analogues such as codeine, heroin and morphine, are commonly used in the clinical setting for pain relief and at the same time can produce tolerance, dependence, and lead to their abuse and ultimately may result in addiction.

Drugs of abuse affect the brain reward pathway either by directly influencing the action of dopamine within this pathway, or by altering the activity of other neurotransmitters that exert a modulatory influence over the mesocorticolimbic dopaminergic system—the dopaminergic neurones in the ventral tegmental area (VTA) of the midbrain and their projections to the Nucleus Accumbens (NAc) and thence to the Prefrontal Cortex. Opioid, gamma-aminobutyric acid (GABA), noradrenergic, cholinergic and serotonergic neurotransmitter pathways have all been shown to interact at various points along the mesolimbic dopaminergic pathway and to modulate its activity. Opioids direct binding to opioid receptors on NAc neurons. Moreover, opioids also indirectly act by inhibiting inhibitory GABA interneurones in the VTA to increase dopamine release. Increased activity of these dopamine neurons produces increases in extracellular dopamine levels in the NAc (Konipsky et al., 2002; Melichar et al., 2001; Nestler, 2005; Tomkins and Sellers, 2001).

At the present time, the strategies to treat opioid dependence consist of 2 approaches: detoxification and maintenance. Methadone maintenance has been the gold standard treatment in spite of its limitation. Other agonist medication such as levoalphaacetylmethadol (LAAM) has been examined, but none has gained significant acceptance. The newest medication treatment for opioid dependence is the partial mu-opioid agonist buprenorphine (Kraigher et al., 2005). It has been studied for the treatment of opioid dependence both in detoxification and maintenance phase. Clonidine, a non-opioid medication has been investigated for detoxification. Lofexidine

has fewer side effects than clonidine especially hypotension. More recently, "rapid" detoxification techniques is a new strategy for shortening the opiate withdrawal period in which opioid withdrawal is precipitated by opioid antagonists such as naloxone or naltrexone (Amato et al., 2004; Gowing et al., 2004; Ling and Compton, 2005). The standard methods of the detoxification and maintenance need to be further investigated.

Various species of plants in the genus *Passiflora* (family Passifloraceae) have been used extensively in the traditional medicine in many countries. One of the species which was proved to be useful in drug addiction therapy is *Passiflora incarnata*. The methanolic extract of *P. incarnata* was found to reduce the naloxone-precipitated withdrawal jumps in mice after morphine injection (Dhawan et al., 2002c). In this study, we considered it was interesting to investigate the effect of *Passiflora foetida* (Ka-tok-rok), a plant in the same genus which is found abundant in Thailand, on drug addiction. We then evaluated the effect of the ethanolic extract of *P. foetida* (PF) on locomotor activity, conditioned place preference (CPP) and morphine withdrawal.

Objective

1. To investigate the reinforcing effect of the ethanolic extract of *Passiflora foetida.*
2. To investigate the effects of the ethanolic extract of *Passiflora foetida* on morphine withdrawal.

Hypothesis

1. The ethanolic extract of *Passiflora foetida* does not produce reinforcing effect.
2. The ethanolic extract of *Passiflora foetida* can inhibit the reinforing effect of morphine.
3. The ethanolic extract of *Passiflora foetida* can be used for both prevention and treatment of morphine withdrawal.

Research design

Experimental research

Expected benefit and application

Results from this study about the effect of ethanolic extract of *Passiflora foetida* on morphine addiction may lead to the development of a novel anti-addictive agent from plants to be used clinically for drug addiction treatment.

Key words

Addiction

Passiflora foetida

Morphine

Conditioned Place Preference

Withdrawal

CHAPTER II

LITERATURE REVIEWS

Addiction

Addiction is defined as a chronic relapsing disorder that is characterized by three major elements: (1) compulsive drug seeking, (2) loss of control in limiting intake, and (3) emergence of adverse consequence. In 1968, The World Health Organization and the American Psychiatric Association used the term drug dependence replaced that of drug addiction (Cami and Farre, 2003; Nestler, 1992; Shippenberg and Koob, 2002). The term drug dependence in definition of The American Psychiatric Association requires a person who has at least three of the seven criteria listed in Table 2.1 (Cami and Farre, 2003).

Table 2.1 Definitions of Terms Used in Drug Addiction (Cami and Farre, 2003)

Craving (formerly called psychological dependence)	An intense desire to reexperience the effects of a psychoactive substance. Craving is the cause of relapse after long periods of abstinence.
Physical or physiological dependence	An outdated term that refers to physical tolerance and the withdrawal syndrome.
Priming	A new exposure to a formerly abused substance. This exposure can precipitate rapid resumption of abuse at previous levels or at higher levels.
Relapse	A resumption of drug-seeking or drug-taking behavior after a period of abstinence. Priming, environmental cues (people, places, or things associated with past drug use), and stress can trigger intense craving and cause a relapse.

Reward	A stimulus that the brain interprets as intrinsically positive or as something to be attained.
Sensitization	The increase in the expected effect of a drug after repeated administration (e.g., increased locomotor activation after the administration of psychostimulants). Sensitization also refers to persistent hypersensitivity to the effect of a drug in a person with a history of exposure to that drug (or to stress). Sensitization is one of the neurobiologic mechanisms involved in craving and relapse.
Substance abuse	Recurrent and clinically significant adverse consequences related to the repeated use of substances, such as failing to fulfill major role obligations, use of drugs in situations in which it is physically hazardous, occurrence of substance-related legal problems, and continued drug use despite the presence of persistent or recurrent social or interpersonal problems.
Substance dependence	A cluster of cognitive, behavioral, and physiological symptoms indicating that a person is continuing to use a substance despite having clinically significant substance-related problems. For substance dependence to be diagnosed, at least three of the following must be present: symptoms of tolerance; symptoms of withdrawal; the use of a substance in larger amounts or for longer periods than intended; persistent desire or

	unsuccessful attempts to reduce or control use; the spending of considerable time in efforts to obtain the substance; a reduction in important social, occupational, or recreational activities because of drug use; and continued use of a substance despite attendant health, social, or economic problems.
Withdrawal syndrome	A constellation of signs and symptoms that follows the abrupt discontinuation or reduction in the use of a substance or after blockage of the actions of a substance with antagonists (e.g., naloxone in heroin addiction). The syndrome can also be produced by cues associated with substance use (conditioned withdrawal). Symptoms tend to be the opposite of those produced after short-term exposure to a drug. Withdrawal is one of the causes of compulsive drug-taking behavior and short-term relapse.

Addiction Related Brain Regions

1) Ventral tegmental area (VTA)

VTA is part of the midbrain. It is rich in dopamine and serotonin neurons, and is part of two major dopamine pathways: 1) the mesolimbic pathway, which connects the VTA to the nucleus accumbens; 2) the mesocortical pathway, which connects the VTA to cortical areas in the frontal lobes. The VTA consists of dopaminergic neurons which respond to glutamate. These cells respond when stimuli indicative of a reward are present. The VTA supports learning and sensitization development and releases dopamine (DA) into the forebrain. These neurons also project and release DA into the nucleus accumbens, through the mesolimbic pathway. Virtually all drugs causing drug

addiction increase the dopamine release in the mesolimbic pathway, in addition to their specific effects (Hyman et al, 2006).

2) Nucleus accumbens (NAc)

Nucleus accumbens is a collection of neurons located where the head of the caudate and the anterior portion of the putamen meet just lateral to the septum pellucidum. The nucleus accumbens, the ventral olfactory tubercle, and ventral caudate and putamen collectively form the ventral striatum. This nucleus is thought to play an important role in reward, pleasure, and addiction. It is part of the ventral continuation of the dorsal striatum, and shares general principles of connectivity with the striatum. The nucleus accumbens is also called ventral striatum. The principal neuronal cell type found in the nucleus accumbens is the medium spiny neuron. The neurotransmitter produced by these neurons is Gamma-Amino Butyric Acid, GABA, the main inhibitory neurotransmitter of the central nervous system (Hyman et al, 2006; Koob and Nestler, 1997).

3) Prefrontal cortex

Prefrontal cortex (PFC) is the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. This brain region has been implicated in planning complex cognitive behaviors, personality expression and moderating correct social behavior (Bardo, 1998).

4) Amygdala

Amydala is an almond-shaped set of neurons located deep in the brain's medial temporal lobe. Amygdala is a key role in the processing of emotions, the amygdala forms part of the limbic system. In humans and other animals, this subcortical brain structure is linked to both fear responses and pleasure. Conditions such as anxiety, autism, depression, post-traumatic stress disorder, and phobias are suspected of being linked to abnormal functioning of the amygdala, owing to damage, developmental problems, or neurotransmitter imbalance (Konipsky, 2002).

5) Locus ceruleus (LC)

Locus ceruleus is a nucleus in the brain stem responsible for physiological responses to stress and panic. The locus ceruleus resides on the dorsal wall of the upper pons, under the cerebellum in the caudal midbrain, surrounded by the fourth

ventricle. This nucleus is one of the main sources of norepinephrine in the brain, and is composed of mostly medium-sized neurons. The projections of this nucleus reach far and wide, innervating the spinal cord, the brain stem, cerebellum, hypothalamus, the thalamic relay nuclei, the amygdala, the basal telencephalon, and the cortex. The norepinephrine from the LC has an excitatory effect on most of the brain, mediating arousal and priming the brain's neurons to be activated by stimuli. It has been said that a single noradrenergic neuron can innervate, via its branches, the entire cerebral cortex (Nestler, 1992).

6) Hippocampus

Hippocampus is a part of the brain located inside the temporal lobe (humans have two hippocampi, one in each side of the brain). It forms a part of the limbic system and plays a part in memory and navigation (Konipsky, 2002; Nestler, 1992).

Mechanisms of addiction

The brain circuits of addiction involve reward pathways. A key component of the reward pathway is the mesocorticolimbic dopaminergic system in the ventral tegmental area (VTA) of the midbrain and their projections to the Nucleus Accumbens (NAc), Amygdala and thence to the Prefrontal Cortex (Ballantyne et al., 2007; Melichar et al, 2001). Some of the major elements in the brain reward circuit are illustrated in Figure 2.1 (Tomkins and Sellers, 2001). Drugs of abuse such as opioids, alcohol, cocaine and amphetamine act by stimulating this system, which is usually activated by natural rewards (food, water and sex). Addictive drugs are able to exert influence over the brain reward pathway either by directly influencing the action of dopamine within the system, or by altering the activity of other neurotransmitters that exert a modulatory influence over this mesolimbic dopaminergic pathway (Table 2.2). Gamma-Aminobutyric acid (GABA), opioid, serotonergic, cholinergic and noradrenergic neurotransmitter pathways have all been shown to interact at various points along the mesolimbic dopaminergic pathway and to modulate its activity. For example, Opioids act by inhibiting inhibitory GABA interneurones in the VTA to increase dopamine release, whereas cocaine increases the amount of intra-synaptic dopamine by inhibiting the dopamine transporter (Melichar et al, 2001).

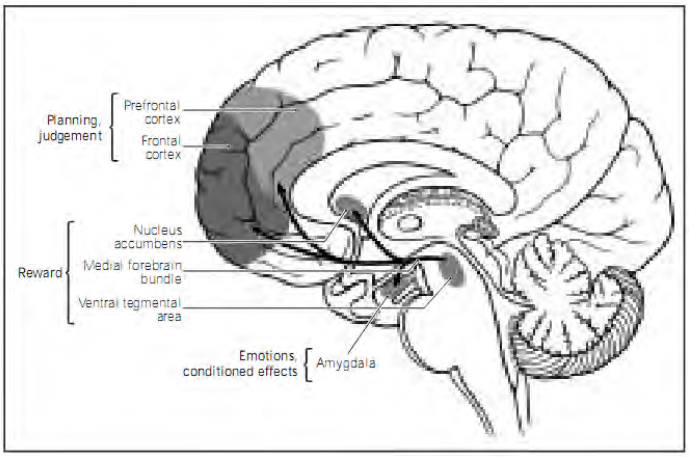

Figure 2.1 Schematic diagram of the human brain that highlights some of the main brain areas and neurotransmitter pathways implicated in reward processes (Tomkins and Sellers, 2001).

Table 2.2 Initial targets of drugs of abuse (Nestler, 2004)

Drugs	Properties
Opiates	Agonist at mu, delta and kappa opioid peptide receptors
Cocaine	Indirect agonist at dopamine receptors by inhibiting dopamine transporters
Amphetamine and related stimulants	Indirect agonist at dopamine receptors by stimulating dopamine release
Ethanol	Facilitates $GABA_A$ and inhibits NMDA glutamate receptor function
Nicotine	Agonist at nicotinic acetylcholine receptors
Cannabinoids	Agonist at cannabinoid CB_1 and CB_2 receptors
Hallucinogens	Partial agonist at 5-HT_{2A} receptors

Neurotransmitters involved in brain reward

1) Dopamine

Dopamine is the neurotransmitter used by the reward pathway (also called the mesolimbic pathway, which is closely associated with the mesocortical pathway).

Mesolimbic/mesocortical pathways originate in the ventral tegmental area and project to cortical structures considered to be crucial for cognitive function and motivation. There are two other important pathways in the brain that utilize dopamine: the nigrostriatal pathway and the tuberoinfundibular pathway. The nigrostriatal pathway originates in the substantia nigra projects to the striatum, and is involved in extrapyramidal motor function. The tuberoinfundibular pathway originates in the hypothalamus projects to the hypothesis, and is involved in neuroendocrine regulation. Generally, drugs that affect dopamine levels in the brain affect all three of these dopamine pathways (Hyman et al, 2006).

2) Serotonin

Serotonin is another neurotransmitter that is affected by many of the drugs of abuse, including cocaine, amphetamines, LSD, and alcohol. Serotonin is produced by neurons in the Raphe nuclei. Raphe nuclei neurons extend processes to and dump serotonin onto almost the entire brain, as well as the spinal cord. Serotonin plays a role in many brain processes, including regulation of body temperature, sleep, mood, appetite and pain. Problems with the serotonin pathway can cause obsessive-compulsive disorder, anxiety disorders, and depression. Most of the drugs used to treat depression today work by increasing serotonin levels in the brain (Bardo, 1998).

3). Glutamate and GABA (gamma-aminobutyric acid)

Glutamate and GABA (gamma-aminobutyric acid) are the brain's major neurotransmitters. Over half of all brain synapses release glutamate, and 30-40% of all brain synapses release GABA. Since GABA is inhibitory and glutamate is excitatory, both neurotransmitters work together to control many processes, including the brain's overall level of excitation. Many of the drugs of abuse affect either glutamate or GABA or both to exert tranquilizing or stimulating effects on the brain (Koob and Nestler, 1997).

Animal models of drug addiction

In recent conceptualizations of drug reinforcement, the positive reinforcing properties of drugs have been thought to play an important role in drug dependence. It is amply clear that animal and humans will readily self-administer drugs in the nondependent state and that drugs have powerful reinforcing properties in that animals

will perform many different tasks to obtain drugs. The drugs that have positive reinforcing effects correspond well with the drugs that have high abuse potential in humans

1) Conditioned place preference

Conditioned place preference (CPP) paradigm is used routinely as a measure of the rewarding properties of drugs. A variety of drugs including opiates and stimulants have been shown to produce CPP (Kim et al., 1996). Conditioned place preference is a classical conditioning procedure in which administration of a drug is paired with one distinct environment and administration of placebo with another. In a simple version of the conditioned place preference paradigm, animals experience two distinct neutral environments that are subsequently paired spatially and temporally with distinct drug states. The animal is later given an opportunity to choose to enter and explore either environment, and the time spent in either environment is considered an index of the reinforcing value of the drug. Animals exhibit a conditioned preference for an environment associated with drugs that function as positive reinforcers (e.g. spend more time in the drug-paired compared to placebo-paired environment) and avoid those that induce aversive states (e.g. conditioned place aversion). This procedure permits assessment of the conditioning of drug reinforcement and can provide indirect information regarding the positive and negative reinforcing effects of drugs (Shippenberg and Koob, 2002). Other important methodological procedures that should be considered in CPP research include the sensory modalities used to discriminate between environments. Sensory modalities should be appropriate for species being used. For example, visual cues are poor choice for albino rats, whereas olfactory cues are an excellent choice for these rats. Tactile and auditory cues are also good choice when using rodents (Hoffman, 1989).

2) Animal models of withdrawal

Drugs of abuse are usually classified as producing physical dependence, psychological dependence, or both and physical psychological dependence. With physical dependence, pronounced signs of withdrawal are seen when chronic drug use

is terminated. The magnitude of these signs (e.g. vomiting, convulsions, hypertension and cardiovascular collapse) can be striking, and their avoidance has been cited frequently as one reason why drug users continue chronic administration of drug. Animals were made dependent on morphine by implantation of a pellet and withdrawal was precipitated by the injection of naloxone. Withdrawal was assessed by scoring each of the following signs individually: chewing, licking, teeth chattering, facial tremor, grooming, writhing, diarrhea, weight loss, wet dog shakes, head shakes and hypothermia (Emmett-Oglesby, 1990).

3) Locomotor activity test

Locomotor activity measurement is commonly used in mice to study sensitivity to the locomotor activating or depressing effects of a drug. It can be used for investigating the locomotor effects of drugs from all pharmacological classes. The doses required to produce locomotor stimulant or depressant effects are dependent on the drug and on the genotype of mouse. It measures exploration in response to a novel environment, habituation rate (as the environment become familiar), and baseline spontaneous ambulation. This test is conducted for various time intervals, but typically 1 hr in 5 min intervals. The test is fully automated. Test chambers are consist of infrared photo-detectors. The system records horizontal activity, total distance (cm), vertical movement, repetitive movements, and time in different zones (Oades et al., 1986).

Opioid

Opium, the dried juice of the seedpod of the opium poppy (*Papaver somniferum*), has been used for the relief of pain and suffering for several thousand years. Many opioid drugs are still derived from opium, and the opium poppy is cultivated for both legitimate and illegitimate purposes (Ballantyne et al., 2007). The alkaloid content of opium is approximately 10-20% with more than 40 individual alkaloids having been isolated. Only five of these alkaloids account for virtually all of the quantitative alkaloid content in opium, including: morphine (about 10% by weight), noscapine (about 6%), papaverine (about 1%), codeine (about 0.5%) and thebaine

(about 0.2%). Morphine and codeine are effective pain relievers, but are also abused as drugs for non-medical purposes (Kalant, 1997; Schiff, 2002).

Opioids are a class of drugs that are commonly prescribed for their analgesic, or pain-killing properties. They include substances such as derivatives of opium (opiates) or endogenous or synthetic opioid peptides. Opioids may be classified as natural, semi-synthetic, fully synthetic, or endogenous. Natural opioids such as codeine and morphine are derived from opiate alkaloids contained in the resin of the opium poppy. Semi synthetic opioids such as oxycodone and hydrocodone are created by chemically altering the natural opioids (Paice, 2007). Fully synthetic opioids such as methadone are synthesized from non-opioid substances in laboratories. Endogenous opioids are naturally produced by the body and include substances such as endorphins.

Morphine

Morphine and like narcotic agonists have agonistic actions at the mu (μ), kappa (κ), and delta (δ) receptors that are members of the G protein-coupled receptor family. Morphine is used therapeutically in the relief of moderate to severe acute and chronic pain. The drug may be given orally, parenterally (intramuscular, intravenous), or rectally in addition to epidurally and intrathecally. Nociceptive effects result from actions at the level of both the brain and the spinal cord, the former being responsible for the attenuation of impulse spread and the inhibition of pain perception, while the latter is responsible for inhibition of transmission of nociceptive impulses. Although morphine is accepted as an irreplaceable tool in pain management, it has several side effects. Adverse effects produced by morphine include among others nausea, vomiting, sedation, euphoria, miosis, drowsiness, constipation and respiratory depression. In addition, prolonged use of morphine can cause tolerance to its analgesic effect as well as psychological and physical dependence. However, the appropriate dosage and proper administration intervals allow avoiding dependence (Schiff, 2002).

Opioid receptors

There are three distinct opioid receptor types: μ, δ, κ (Table 2.3). Morphine and other morphine-like opioid agonists produce analgesia primarily through μ-receptor

activation, which also produces respiratory depression, miosis, reduced gastrointestinal motility, and feelings of well-being (euphoria). The supraspinal mechanisms of analgesia produced by μ-opioid agonist drugs are thought to involve the μ_1 receptor, whereas spinal analgesia, respiratory depression, and the effects of opioids on gastrointestinal function are associated with the μ_2 receptor. The μ_3 receptor (another splice variant of the receptor) binds opioid alkaloids such as morphine, but has exceedingly low or no affinity for the naturally occurring endogenous opioid peptides. The μ_3 receptor occurs in macrophages, astrocytes, and endothelial cells and may be involved in immune processes (Bovill, 1997). The μ_3 receptor is believed to be coupled to constitutive nitric oxide (NO) release. The endogenous ligand for this receptor may be morphine or codeine, which have been found to be present in vertebrate tissues, including the nervous system. There are three κ receptor subtypes. Dynorphin A is the natural ligand for the κ_1 receptor, which elicits spinal analgesia. The role of the κ_2 receptor is unknown. The κ_3 receptor is the dominant brain opioid receptor. Selective κ receptor agonists continue to produce analgesia in animals who have been made tolerant to μ agonists (Piros et al., 1996).

Table 2.3 Opioids (Silberstein and McCrory, 2000)

Receptor	Endogenous ligand	Agonist	Antagonist
μ (μ_1, μ_2, μ_3)	Beta-endorphin	Morphine	Naloxone
δ (δ_1, δ_2)	Enkephalin		Naloxone
κ (κ_1, κ_2, κ_3)	Dynorphin	Butorphanol	Naloxone

Physical opioid dependence

Physical dependence is a state of neurophysiologic adaptation, manifested as rebound symptoms or withdrawal signs and symptoms if the opioid is abruptly stopped, if the dose is precipitously reduced, or if a pharmacologic antagonist is given. Rebound symptoms are an exacerbation of the symptoms for which the drug was initially given (e.g. pain). Withdrawal signs and symptoms are drug-specific and may overlap with rebound symptoms but may include new ones, such as lacrimation, hypertension, and abdominal cramps (Way, 1982).

Opioid tolerance

The effect of an opioid analgesic may diminish over time, so that higher and higher doses are needed to produce the initial level of pain relief, an effect called tolerance. Rather than increase the dose, one can switch to another drug. Tolerance may be due to pharmacokinetic adaptations (e.g. increased metabolism or clearance of the drug) or, more importantly, pharmacodynamic adaptations. Pain-facilitating systems such as the N-methyl-D-aspartate (NMDA) receptors, nitric oxide, and cyclo-oxygenase (COX) may play important roles in opioid tolerance without enhancing morphine's antinociceptive effects. Tolerance develops more readily when large doses are given at short intervals, particularly parenterally. Tolerance to the sedative, euphoric, and respiratory depressant effects of opioids usually develops more quickly than tolerance to the emetic and urinary effects. Patients may never develop tolerance to the miotic, convulsant, or constipating effects of opiates (Hsu and Wong, 2000).

Opioid action in the mesocorticolimbic dopaminergic system

The opiates enhance NAc dopamine release by increasing the activity of VTA dopamine neurons. It is postulated that this is achieved via activation of μ-opioid receptors located on GABA neurons within the VTA, which play an important role in regulating the activity of VTA dopamine neurons, results in dopamine release in the NAc. Moreover, it binds directly to opioid receptors in the NAc, an action that is independent of dopamine. Increased activity of these dopamine neurons produces increases in extracellular dopamine levels in the NAc (Figure 2.2) (Konipsky and Hyman, 2002; Tomkins and Sellers, 2001).

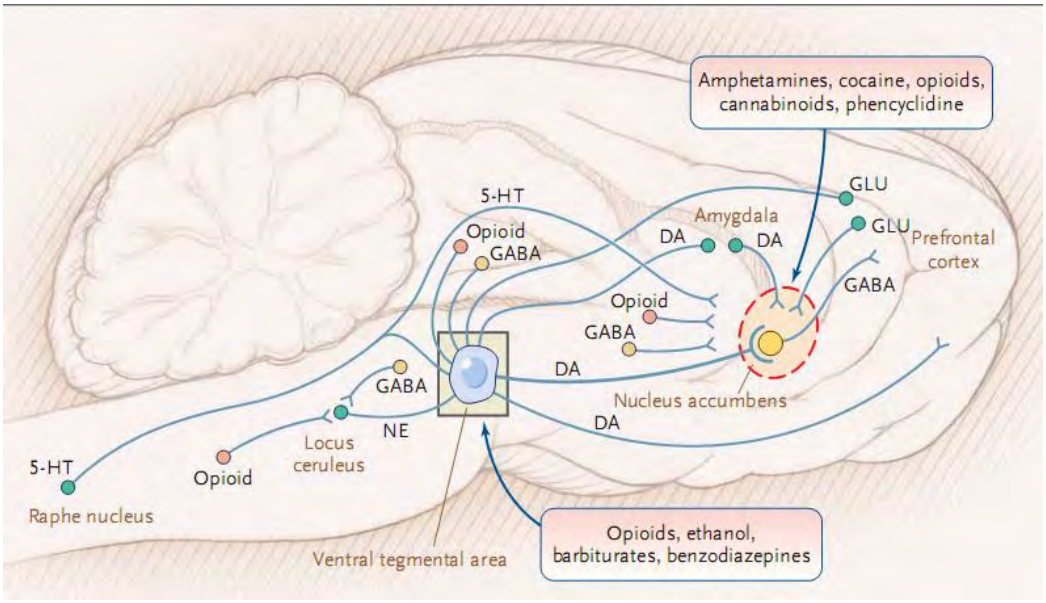

Figure 2.2 Neural reward circuits important in the reinforcing effects of drugs of abuse (Cami and Farre, 2003)

Treatment of opioid dependence

Opioid dependence is characterized by physical dependence, medical and psychological problems, and social dysfunction. Treatment strategies include counseling and pharmacotherapy. Pharmacotherapies for opioid dependence include detoxification and maintenance. Opioid detoxification involves the use of medications to bring a patient from an opioid dependent to an opioid-free state. Maintenance therapy involves the substitution of an abused opioid such as heroin or narcotic analgesics, which are often used intravenously or intranasally several times a day. Opioid agonists and partial agonists are commonly used for both maintenance and detoxification. Opioid antagonists are used to accelerate the detoxification process and prescribed post-detoxification to assist in preventing relapse. Alpha-2-adrenergic agonist medications are primarily used to enhance detoxification outcomes (Krantz and Mehler, 2004; Stotts et al., 2009).

1) Agonist for opioid dependence treatment

Opioid agonist treatment has been demonstrated to most effectively decrease craving and drug use and improve health and social outcomes; furthermore, sustained medication maintenance is much more effective than short-term detoxification (O'Connor, 2010). Methadone maintenance has been the gold standard treatment.

Other agonist medication is levo-alphaacetylmethadol (LAAM) (David and Gastpar, 2004; O'Connor, 2010).

1.1) Methadone

Methadone is a full μ-opioid receptor agonist, introduced in the 1960s, after being developed in Germany at the end of World War II. It is typically used as a replacement therapy for heroin or other opioid dependence (Stotts et al., 2009).

1.1.1) Methadone maintenance

Methadone is the most inexpensive and well-validated agent for opioid maintenance. Methadone maintenance resulted in greater treatment retention and lower rates of illicit opioid use than did detoxification. It has an onset of action within 30 minutes when taken orally and long elimination half-life (24 – 36 h) allows it to be used as either a maintenance therapy or detoxification agent. Its oral bioavailability is excellent and approaches 90%. One of the most important advantages of methadone is that it relieves narcotic craving, which is the primary reason for relapse. Methadone is metabolized extensively in the liver and its excretion rate can be accelerated by urinary acidification. Elimination is slower in women. Mild adverse effects observed include decreased libido, sweating, constipation, weight gain, and irregular menstrual periods. Most adverse effects occur during the initial stabilization process (Table 2.4). As with any potent narcotic, serious consequences such as fatal overdose and debilitating sedation may occur (Krantz and Mehler, 2004; Stotts et al., 2009).

Table 2.4 Adverse Effects of Methadone (Krantz and Mehler, 2004)

Common	Constipation, Sweating, Diminished libido, Mild nausea
Less common	Flushing of the face, Pruritus, Euphoria/dysphoria, Insomnia, Urinary retention or hesitancy, Bradycardia
Rare	Biliary spasm, Urticaria, Syncope, Death from overdose, Torsade de pointes

1.1.2) Methadone detoxification

Methadone is frequently used because it can be given once daily. Initially, methadone hydrochloride is given in a dosage range of 10 to 30 mg/d, depending on

the size of the opioid habit. Additional methadone may be necessary if signs of abstinence appear. A slower rate of reduction may be associated with decreased illicit opioid use (Krantz and Mehler, 2004).

1.2) Levomethadyl acetate (LAAM)

Levomethadyl acetate is a longer-acting derivative of methadone and full mu-opioid agonist substitute. It was approved by the US FDA for maintenance therapy in 1993, has been demonstrated to be effective in retention of patients in maintenance programs as well as in reducing illicit heroin use. Levomethadyl actually has very little opioid effect in its parent form and is functionally a "prodrug." It is extensively metabolized by the liver into 4 major metabolites. Norlevomethadyl and dinor-levomethadyl are the major active metabolites. It is rapidly absorbed from the gastrointestinal tract, although its oral bioavailability is somewhat lower than that of methadone. Because of these properties, the opioid effect of levomethadyl is somewhat slower in onset than that of methadone (90 minutes), but it has a much longer duration of action (48-72 hours) and is therefore able to be dispensed 3 times per week unlike methadone, which must be administered daily (Bhiman and Purkayastha, 2009). Levomethadyl has had very limited availability in opioid treatment programs because of its higher cost and requirement for electrocardiogram monitoring. Levomethadyl is metabolized primarily by the hepatic P450 isozyme CYP3A4. In addition, methadone and levomethadyl share the same protein binding sites in plasma. Because of this, methadone and levomethadyl when taken concurrently may have additive effects. Therefore, patients generally receive one or the other agent, but not a combination, for maintenance therapy. Other potential advantages of levomethadyl's longer duration of action include reduced dispensing time and less opportunity for illegal diversion. Similar to methadone, it suppresses symptoms of withdrawal and produces cross tolerance. Adverse effects of levomethadyl are infrequent and, when they occur, are the same as those for methadone. The average daily dose is 75 to 115 mg given 3 times per week. As with methadone, there have been a small number of reported cases of torsade de pointes in patients receiving levomethadyl. Because of this, the manufacturer has recommended that a baseline electrocardiogram be obtained to exclude significant prolongation of the QT segment before levomethadyl therapy is initiated. A follow-up

electrocardiogram should be obtained between 2 and 4 weeks after initiation, when steady-state dosing has been attained (Krantz and Mehler, 2004). LAAM is not as widely available internationally as methadone, and may be withdrawn from the market following ten cases of life threatening cardiac arrhythmias and an association with QT prolongation (Clark et al., 2002).

2) Partial agonist for opioid dependence treatment

New possibilities have arisen with respect to a differentiated therapy through the availability of new substances such as buprenorphine for the substitution treatment of patients addicted to opioids.

2.1) Buprenorphine

Buprenorphine was first suggested by Jasinski et al. (1978) as an alternative in the oral substitution therapy of opiate addiction. Buprenorphine is a semi-synthetic opiate derivative made from thebaine, an alkaloid that occurs naturally in opium poppy and is structurally related to morphine (Vocci and Sorer, 1992). It classified as a partial opioid agonist (David and Gastpar, 2004). It is the newest medication treatment for opioid dependence. It was approved by the US Food and Drug Administration (FDA) in 2002 (O'Connor, 2010). It has a high affinity, but low efficacy at the mu receptor where it yields a partial effect upon binding. Moreover, it also has the properties of kappa receptor antagonist resulting in it useful not only as an analgesic, but also in opioid abuse, detoxification, and maintenance therapies.

2.2.1) Buprenorphine maintenance

Buprenorphine has some advantages over methadone, including milder withdrawal symptoms after abrupt cessation, lower risk of overdose, and a longer duration of action, which allows alternate-day dosing. Because buprenorphine has a very low oral bioavailability, sublingual administration is the primary route of delivery for treating opioid dependence (Krantz and Mehler, 2004). When used alone, or in combination with naloxone in a sublingual tablet formulation, buprenorphine has been shown to be as effective as methadone with an improved safety profile (Helm et al., 2008; Melichar et al, 2001). The naloxone component is not significantly absorbed sublingually but is included to block opioid effects if intravenous use is attempted. Buprenorphine has a poor bioavailability with extensive first pass effect by the liver.

Buprenorphine is primarily metabolized by P450 CYP3A4 (Bhiman and Purkayastha, 2009). Conversely, because of high lipid solubility, it has an excellent sublingual bioavailability. Adverse effects of buprenorphine may include sedation, nausea and/or vomiting, dizziness, headache, and respiratory depression (Helm et al., 2008).

2.2.2) Buprenorphine detoxification

Buprenorphine has also been used in several experimental studies of opioid withdrawal. Most studies have found it to be equivalent to methadone when tapered over 4 to 6 weeks (Amass et al., 1994; Gowing et al., 2006; Kosten and Kleber, 1988)

3) Alpha-2-adrenergic agonist for opioid detoxification

Several alpha-2-adrenergic agonist medications have been investigated and found to facilitate positive opioid withdrawal outcomes. One process underlying opioid withdrawal is noradrenergic hyperactivity. Alpha-2-adrenergic agonists moderate the symptoms of noradrenergic hyperactivity by acting centrally. Clonidine was the first alpha-2 agonist discovered to ameliorate some signs and symptoms of withdrawal (Bhiman and Purkayastha, 2009). Because it is not a drug of abuse or dependence, clonidine has gained widespread use as a non-opioid alternative for managing withdrawal. Clonidine was described in the late 1970s. The purported explanation was that clonidine blocked activation of the noradrenergic locus ceruleus nucleus, which is involved with opioid withdrawal. Clonidine in initial dosages of 0.1 to 0.2 mg every 4 hours with careful monitoring of blood pressure eliminates most commonly reported withdrawal symptoms. Some withdrawal symptoms such as anxiety and myalgias are resistant to clonidine; benzodiazepines and nonsteroidal anti-inflammatory agents may be necessary adjuncts to treat these symptoms. Clonidine has been combined with the opioid antagonist naltrexone, in a dose of 12.5 to 50 mg, as a successful detoxification regimen of even shorter duration. Unfortunately, clonidine is associated with significant hypotension, which has limited its use (Bhiman and Purkayastha, 2009). This finding led to a search for alternative alpha-2 agonist medications without significant side effects. Lofexidine, another alpha-2-adrenergic agonist, has been used experimentally with some success. It may not have the hypotension side effect like clonidine (Veilleux et al., 2010). It is likely to replace clonidine as the leading opioid withdrawal treatment in this drug class. Initial studies of clonidine reported reduction or elimination of lacrimation,

rhinorrhea, muscle pain, joint pain, restlessness, and gastrointestinal symptoms, suggesting significant potential for managing opiate withdrawal. The primary adverse side effects were dizziness, sedation, and lethargy attributed to orthostatic hypotension and dry mouth. Lofexidine can be prescribed up to about 2 mg/day and appears to be associated with fewer adverse effects (Krantz and Mehler, 2004; Stotts et al., 2009).

4. Antagonist for opioid dependence treatment

Clonidine and lofexidine assist in reducing some of the symptoms of opiate withdrawal; however, they do not alter the duration of withdrawal. The rapid approach shortens the detoxification process to 3 to 5 days by precipitating withdrawal through the administration of opioid antagonists such as naloxone or naltrexone. They are competitive antagonists at the mu, kappa, and sigma receptors with a higher affinity for the mu receptor and lacking any mu receptor efficacy. Naloxone and naltrexone act centrally and peripherally, but have differing pharmacokinetic profiles favoring different therapeutic uses. Due to its extensive first-pass metabolism in the liver, the oral bioavailability of naloxone is less than 1% (Lobmaier et al., 2010). Naloxone is added to sublingual buprenorphine to prevent the intravenous abuse of buprenorphine (Helm *et al.*, 2008). Naltrexone is an oral, long-acting opioid antagonist with high affinity to mu-opioid receptors (Veilleux et al., 2010). Naltrexone is orally effective with a long duration of action making it useful in abuse deterrent, detoxification, and maintenance treatment modalities (Lobmaier et al., 2010). Neither tolerance nor dependence develops with naltrexone. Oral naltrexone is approved for relapse prevention of alcohol and opioid dependence in several countries, although its effectiveness for the latter (Kleber, 1985; Navaratnam, 1994). The common side effects are abdominal pain, nausea, dyspepsis and skin rash (Bhiman and Purkayastha, 2009). Use of the opioid antagonist naltrexone, typically combined with an alpha-2-adrenergic agonist, has been investigated as a method for rapid opiate detoxification (ROD) and has been purported to shorten the duration of withdrawal without significantly increasing patient discomfort, and initiating treatment more quickly with naltrexone maintenance combined with suitable psychosocial interventions (Krantz and Mehler, 2004; O'Connor, and Kosten, 1998). Controlled studies comparing naltrexone plus clonidine to clonidine alone or to methadone tapering have found that the former approach was well tolerated and

reduced the withdrawal period while improving retention (Gerra et al., 1995; Gowing *et al*., 2000; Kleber et al., 1987). ROD with naltrexone and clonidine is safe and effective in the management of opiate withdrawal. While the duration of detoxification is shortened, effectiveness in facilitating continued use of antagonist treatments post detoxification has yet to be established. Ultra-rapid opiate detoxification (UROD) is a variant of this technique in which patients undergo opioid antagonist-precipitated withdrawal under general anesthesia or sedation (O'Connor, and Kosten, 1998). Most of the rapid protocols use clonidine along with an opioid antagonist, as well as adjuvant benzodiazepines and antiemetics to treat the withdrawal syndrome (Krantz and Mehler, 2004; Stotts et al., 2009).

Passiflora

Passiflora, comprising about 500 species, is the largest genus in family Passifloraceae. The species of this genus are distributed in west USA, Australia, tropical Africa and Asia (Dhawan et al., 2004). Various species of plants in the genus *Passiflora* have been used extensively in the traditional medicine in many countries. One of the species which was proved to be useful in drug addiction therapy is *Passiflora incarnata*.

Passiflora incarnata

Various reports mentioned the usefulness of benzoflavone (BZF) phyto-moiety isolated from the methanol extract of *Passiflora incarnata* on drug addiction. BZF significantly prevented the expression of withdrawal effects of nicotine, cannabinoid, alcohol and morphine in mice (Dhawan et al., 2004).

*Passiflora foetida (*Ka-tok-rok*)*

One of the species which is found abundant in Thailand is *Passiflora foetida* (Ka-tok-rok) (Figure 2.3). Traditionally, the dried or fresh whole plants and their preparations from some *Passiflora* plants are used as ingredient of herbal remedies. Moreover, they are accepted for medicinal use for the treatment of nervous anxiety in many countries such as America, France and Germany (Mohanasundari et al., 2007). The pharmacological investigation of *Passiflora* have anxiolytic, sedative, antispasmodic and

hypotensive activity (Akhondzadeh et al., 2001; Dhawan *et al*., 2001a,b; Dhawan et al., 2004).

Figure 2.3 *Passiflora foetida*

Table 2.5 Systematic classification of *Passiflora foetida*

Classification	Name
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Violales
Family	Passifloraceae
Genus	*Passiflora*
Species	*Foetida*

Botany

The stems of *Passiflora foetida* are covered with sticky yellow hairs. The leaves are three- to five- lobed and viscid-hairy. The flowers are white to pale cream colored, about 5-6 cm diameter. The fruits are yellowish-orange to red when ripe, and have numerous black seeds embedded in the pulp, the fruit are 2-3 cm diameter.

Phyto-constituents

The The phyto-constituents of *Passiflora foetida* contain flavonoids (pachypodol, 7,4'-dimethoxyapigenin, ermanin, 4',7-O-dimethyl-naringenin, 3,5-dihydroxy-4, 7-dimethoxy flavanone, C-glycosyl flavonoids chrysoeriol, apigenin, isovitexin, vitexin, 2"-xylosylvitexin, luteolin-7-β-D-glucoside, kaempferol), Cyanohydrin glycosides (tetraphyllin A, tetraphyllin B , tetraphyllin B sulphate, deidaclin, volkenin), Fatty acids (linoleic acid, linolenic acid) and alpha-pyrones (passifloricins) (Dhawan et al., 2004).

Traditional applications

The leaves of *Passiflora foetida* has been used for giddiness and headache, fruits are used to treat asthma and biliousness, leaves and root decoction to treat hysteria. In Brazil, this herb is used for treat erysipelas and skin diseases with inflammation in the form of lotion or poultices (Dhawan et al., 2004; Mohanasundari et al., 2007). In Central and South America, the leaf decoction is used to relieve urinary irritation and to stop vomiting during pregnancy. In addition, the decoction is used externally to treat inflamed eyes and to treat eczema, for washing wound and chronic ulcers (Puricelli et al., 2003).

Pharmacological studies

Passiflora foetida exhibits various pharmacological activities including:

1) Antidepressant effect

Antidepressant activity of crude extract of *Passiflora foetida* was tested using the open space swimming test. The results showed that the administration of crude extracts increased mobility time of mice. The subacute administration of *Passiflora foetida* extracts, subfraction PF 003-1 and PF003-2 at the dose 50 mg/kg (3 doses/day, for 3 consecutive days) significantly increased mobility time of mice. The antidepressant effect of *Passiflora foetida* extracts was compared with imipramine; a classical antidepressant drug. The results indicated that PF 003-1 has antidepressant effect in the same magnitude as imipramine. The mechanism of antidepressant activity of PF003-1 and PF003-2 was evaluated using SCH 23390 (dopamine D_1antagonist) and WAY 100635 (serotonin 5-HT_{1A}antagonist). The co-administration of SCH 23390 and WAY

100635 abolished the antidepressant effect of *Passiflora foetida*, subfractions PF003-1 and PF003-2 by prior IP administration of these antagonists. These results showed that antidepressant activity of *Passiflora foetida* may be mediated mostly via dopaminergic and serotoninergic mechanisms (Wijagkanalan, 2005).

2) Antibacterial effect

Antibacterial activities of the extract of *Passiflora foetida* were tested by well-in-agar method. The results showed that the acetone extract of *Passiflora foetida* exhibited an excellent antibacterial activity against the bacterial pathogens namely *V. cholerae* followed by *Ps. Putida, S. flexneri* and *St. pyogenes.* The ethanol extract showed moderate activity against *V. cholerae, Ps. Putida*, *St. pyogenes* and *S. flexneri*. From both of organic solvent extracts showed the leaf extracts exhibited better antibacterial activity than the fruits. The results of this study confirmed the uses of this plant to treat various infectious diseases caused by the microbes in the traditional system of medicine (Mohanasundari et al., 2007).

3) Anti-tumor effect

The fruit decoctions of *Passiflora foetida* var. *albiflora* and *Passiflora edulis* were determined the inhibition of activity of two metallo-proteases involved in the tumor invasion, metastasis and angiogenesis including gelatinase matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-2 (MMP-9). The results showed that the inhibitory activity of water extract of both species is dose-dependent on MMP-2 and MMP-9 and more efficient for MMP-2. The fruit decoctions of *Passiflora foetida* is more active compared to *Passiflora edulis* (Puricelli et al., 2003).

4) Antiparasite effect

The leishmanicidal effects of passifloricins A (polyketides alpha-pyrones from *Passiflora foetida* resin) and several hemisynthetic analogues were assayed using leishmanicidal assays. From this study, passifloricin A was the more active compound. Meanwhile, the activity against Leishmania amastigotes slight decreased when the hydroxyl substituents of passifloricin A were changed (Cardona et al., 2004).

CHAPTER III

MATERIALS AND METHODS

Experimental animals

Male ICR mice weighing 18-25 g and male Wistar rats weighing 200-250 g from the National Laboratory Animal Centre (Salaya campus, Mahidol University, Nakhonpathom, Thailand) were served as experimental subjects. Animals were housed under a 12 hr/12 hr light-dark cycle at a temperature of 25 ± 2 ^{o}C with free access to food and water and allowed for acclimatization for at least 1 week prior to testing. All procedures were approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Preparation of the ethanolic extract of *Passiflora foetida*

Passiflora foetida was collected from Nonthaburi province of Thailand. They were dried at 50 ^{o}C and grinded to powder. Then the powder was extracted with 95% ethanol using soxhlet apparatus for 8 hours/times. The ethanol extracts were evaporated to dryness using rotary evaporator. The ethanolic extract of *Passiflora foetida* (PF) was kindly supplied by Assoc. Prof. Dr. Rapepol Bavovada, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Drugs and Chemicals

- Morphine sulfate (Temad, Iran)
- 0.9% Normal saline solution (NSS)
- Carboxymethylcellulose (CMC; Sigma, USA)
- Methadone hydrochloride (Alchymars, Italy)
- Naloxone hydrochloride (Sigma, USA)
- Acetic acid (Sigma, USA)
- Ethanolic extract of *Passiflora foetida* (PF)

Instruments

- Locomotor activity cage (Ugo Basile, Comerio, Italy)
- Conditioned place preference apparatus
- Portable balances (Sartorius TE 612, Germany)
- Analytical balances (Mettler Toledo AG245, Switzerland)
- Vortex (Bohemia G-506E, NY USA)
- Syringes
- Needles
- Beaker
- Feeding tube
- Autopipette
- Pipette tip
- Glass cylinder (30 cm in diameter and 70 cm in height)

Methods

1. Locomotor activity

Locomotor activity test was used to evaluate the effect of drugs on the central nervous system. The effect of PF on locomotor activity in mice was tested using an activity cage (UGO Basile, Comerio, Italy; Figure 3.1). The drug administration took place with either intraperitoneal (i.p.) 0.9% normal saline solution (NSS; 0.3 ml/30 g), morphine (MO; 5 mg/kg) or oral administration of 0.5% carboxymethyl cellulose solution (CMC; 5 ml/kg) and various doses of PF (25, 50, 100 and 200 mg/kg). Immediately after administration of tested compound, a mouse was placed individually in the activity cage. The locomotor activity of the animal was continuously recorded at 5 min intervals for 75 min (Kuschinsky and Hornykiewicz, 1974).

Figure 3.1 Locomotor activity cage

2. Conditioned Place Preference

Conditioned place preference (CPP) paradigm has been used as a model for studying the reinforcing effects of dependence-liable drugs. The CPP apparatus was consisted of 3 different compartments. Two equal-sized compartments (length 25 cm, width 34 cm) were separated by guillotine doors from central compartment (Figure 3.2). One compartment was painted white on each wall, while the other was painted with black and white vertical stripes and had mesh floor. These lateral compartments offered distinct stimuli in odor, color and texture. Vinegar (2% acetic acid) was dropped onto the mesh floor before each rat was placed into the compartment (Swerdlow and Koob, 1984). The middle compartment (length 25 cm, width 11 cm) was painted with grey. Removal of the guillotine doors allowed animal's free access to all compartments. A video camera was placed over the apparatus and linked to a computer system. Time spent by animals in each of the two compartments was recorded for 15 min.

CPP test consisted of a 12 day schedule with three phases: preconditioning (3 days), conditioning (8 days) and testing phases (1 day). This protocol was described previously by Spyraki et al. (1982). Conditioned place preference was evaluated as the difference in pre-conditioning and post-conditioning time spent in the drug-paired compartment. An increase in the time spent in the drug-paired compartment after conditioning phase suggests the presence of the positive reinforcing effects.

Figure 3.2 Conditioned place preference apparatus

2.1) Effect of PF on conditioned place preference

Phase I (Preconditioning phase)

On day 1 and 2, the guillotine doors were raised and each rat was allowed to move freely through all compartments for 15 min. On day 3, the initial unconditioned preference was determined for less preferred vs. more preferred side for 15 min. Time spent by animals in each of the two compartments was recorded using video camera. Most of rats initially preferred the black and white striped compartment. Rats which preferred the white compartment were excluded from further analysis.

Phase II (Conditioning phase)

On day 1, 3, 5, and 7, each rat was received either intraperitoneal morphine (5 mg/kg) or orally administered 0.5% CMC or various doses of PF (25, 50, 100 and 200 mg/kg) before placing in the less preferred compartment (white compartment) for 30 min per day. Rats which received morphine were placed in the white compartment immediately after injection, while animals which received 0.5% CMC and various doses of PF (25, 50, 100 and 200 mg/kg) were placed in the white compartment at 30 min after oral administration. On day 2, 4, 6, and 8, all groups of rats were administered only NSS before were placed in the more preferred compartment (black and white striped compartment) for 30 min each day.

Phase III (Testing phase)

On day 12, neither tested compounds nor NSS was administered to rats. They were placed in the center compartment and the guillotine doors between the two

compartments were open to allow free access to the entire box for 15 min. The amount of time spent in each compartment was recorded. Conditioned place preference was evaluated as the difference in the time spent by the rats in the white compartment between preconditioning phase and test phase.

Phase	Preconditioning phase			Conditioning phase								Test phase
Day	1	2	3	4	5	6	7	8	9	10	11	12
Treatments	-			Drugs	NSS	Drugs	NSS	Drugs	NSS	Drugs	NSS	-

Drugs = Morphine 5 mg/kg, 0.5% CMC or various doses of PF

Figure 3.3 Diagram of the ethanolic extract of *Passiflora foetida* treatment on CPP

2.2) Effect of PF on morphine-induced conditioned place preference

Phase I (Preconditioning phase)

On day 1 and 2, the guillotine doors were raised and each rat was allowed to move freely through all compartments for 15 min. On day 3, the initial unconditioned preference was determined for less preferred vs. more preferred side for 15 min. Time spent by animals in each of the two compartments was recorded using video camera. Most of rats initially preferred the black and white striped compartment. Rats which preferred the white compartment were excluded from further analysis.

Phase II (Conditioning phase)

On day 1, 3, 5, and 7, rats were received 0.5% CMC or various doses of PF (25, 50, 100 and 200 mg/kg) 30 min prior to morphine (5 mg/kg) injection and placed in the white compartment for 30 min per day. On day 2, 4, 6, and 8, all groups of rats were orally administered only NSS 30 min prior to NSS injection before placing in the black and white striped compartment for 30 min each day.

Phase III (Testing phase)

On day 12, neither tested compounds nor NSS was administered to rats. They were placed in the center compartment and the guillotine doors between the two compartments were open to allow free access to the entire box for 15 min. The amount of time spent in each compartment was recorded. Conditioned place preference was

evaluated as the difference in time spent by the rats in the white compartment between preconditioning phase and test phase (Gilbert and Cooper, 1983).

Phase	Preconditioning phase			Conditioning phase								Test phase
Day	1	2	3	4	5	6	7	8	9	10	11	12
Pretreatment (30 min)	-			Drugs ↓ MO	NSS ↓ NSS	Drugs ↓ MO	NSS ↓ NSS	Drugs ↓ MO	NSS ↓ NSS	Drugs ↓ MO	NSS ↓ NSS	-

Drugs = CMC or various doses of PF, MO = morphine 5 mg/kg

Figure 3.4 Diagram of the ethanolic extract of *Passiflora foetida* (PF) treatment on morphine-induced CPP

3. Withdrawal effects

To study morphine dependence, withdrawal is frequently precipitated by naloxone since withdrawal signs can be observed within short time following its administration (Linseman, 1977). For morphine dependence induction, the mice were given morphine by intraperitoneal injection twice a day. The dose of morphine injected from day 1 to day 6, was 10, 20, 30, 40, 50, and 60 mg/kg, respectively. On day 7, only a single morning dose of morphine (70 mg/kg) was injected. Withdrawal signs were precipitated by injection of naloxone (3 mg/kg, i.p.) 2 hours after the last morphine injection on day 7. Immediately after naloxone injection, mice were placed individually in a glass cylinder (30 cm in diameter and 70 cm in height; Figure 3.3). Withdrawal signs of mice were recorded by video camera for a 30-min period. The incidence of the following behaviors was counted: jumping (leaping with all four feet off the ground at the same time); rearing (standing on hind legs); wet dog shake (brief episodes of rapid repetitive shaking of entire trunk while standing on their hind legs); straub tail (erection of the tail) (Blasig et al., 1973; Kest et al., 2002).

Figure 3.3 Glass cylinder

3.1) Effect of PF on induction of morphine dependence

To evaluate the effect of PF on induction of morphine dependence, mice were administered with 0.5% CMC (5 ml/kg, p.o.), methadone (1 mg/kg, i.p.) or various doses of PF (25, 50, 100 and 200 mg/kg, p.o.) 30 min prior to morphine injection from day 1 to day 7 twice daily. Withdrawal signs were precipitated by injection of naloxone (3 mg/kg, i.p.) 2 hr after the last morphine injection on day 7. Immediately after naloxone injection, mice were placed individually in a glass cylinder. Withdrawal signs of mice were recorded by video camera for a 30-min period.

3.2.) Effect of acute administration of PF on morphine dependence

To determine the effect of acute administration of PF on morphine dependence mice were administered with 0.5% CMC (5 ml/kg, p.o.), methadone (1 mg/kg, i.p.) or various doses of PF (25, 50, 100 and 200 mg/kg, p.o.) 30 min after the last morphine injection on day 7. Withdrawal signs were precipitated by injection of naloxone (3 mg/kg, i.p.) 2 hr after drug administration on day 7. Immediately after naloxone injection, mice

were placed individually in a glass cylinder. Withdrawal signs of mice were recorded by video camera for a 30-min period.

3.3 Statistical analysis

Results were expressed as mean ± S.E.M. Differences among means were tested by one-way ANOVA followed by Dunnett's test, $p<0.05$ was considered statistically significant.

CHAPTER IV

RESULTS

The effect of the ethanolic extract of *Passiflora foetida* (PF) on locomotor activity

To determine the effect of various doses of PF on the central nervous system, locomotor activity was investigated in mice after administration of the test substances. Morphine (5 mg/kg) produced no significant effect on locomotor activity as compared to NSS. All doses of PF also produced no significant effects on locomotor activity as compared to 0.5% CMC. No significant difference was found among all groups ($p<0.05$; Figure 4.1). From the results, neither stimulant nor sedative effects of PF on locomotor activity were found in any doses tested. Therefore, all doses of PF were further tested in CPP paradigm.

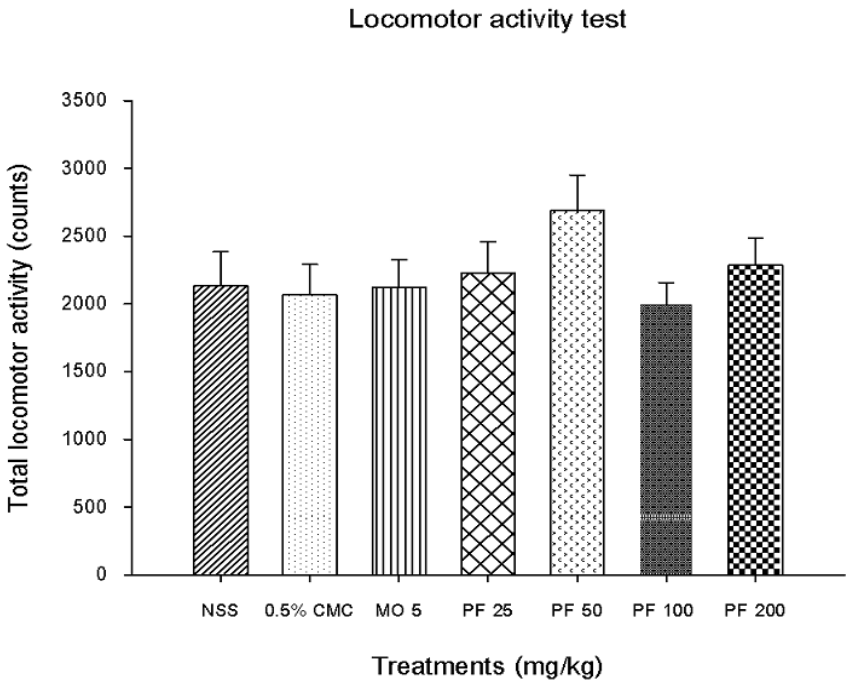

Figure 4.1 Locomotor activity of mice receiving NSS, 0.5% CMC, morphine (5 mg/kg, i.p.), and various doses of PF (25-200 mg/kg, p.o.). N=8 for all groups. Each value represents mean ± S.E.M.

The effect of the ethanolic extract of *Passiflora foetida* (PF) on conditioned place preference (CPP)

In order to investigate the reinforcing effects of various doses of PF, the conditioned place preference test (CPP) in rats was used. All treatments were pretreated 30 min before starting the experiments. The time spent in the drug-paired compartment of morphine (5 mg/kg, i.p.) in the test phase significantly (p<0.05) increased when compared with the preconditioning phase (240.5±22.2 sec vs 127.8±13.4 sec; Figure 4.2). Moreover, the time spent in the drug-paired compartment of morphine (5 mg/kg, i.p.) in the test phase was also significantly (p<0.01) different when compared to 0.5% CMC (Figure 4.2). No significant difference was observed between the time spent in the drug-paired compartment in the test phase and the conditioning phase of all doses of PF tested (Figure 4.2). The results demonstrated that all doses of PF did not have reinforcing effect.

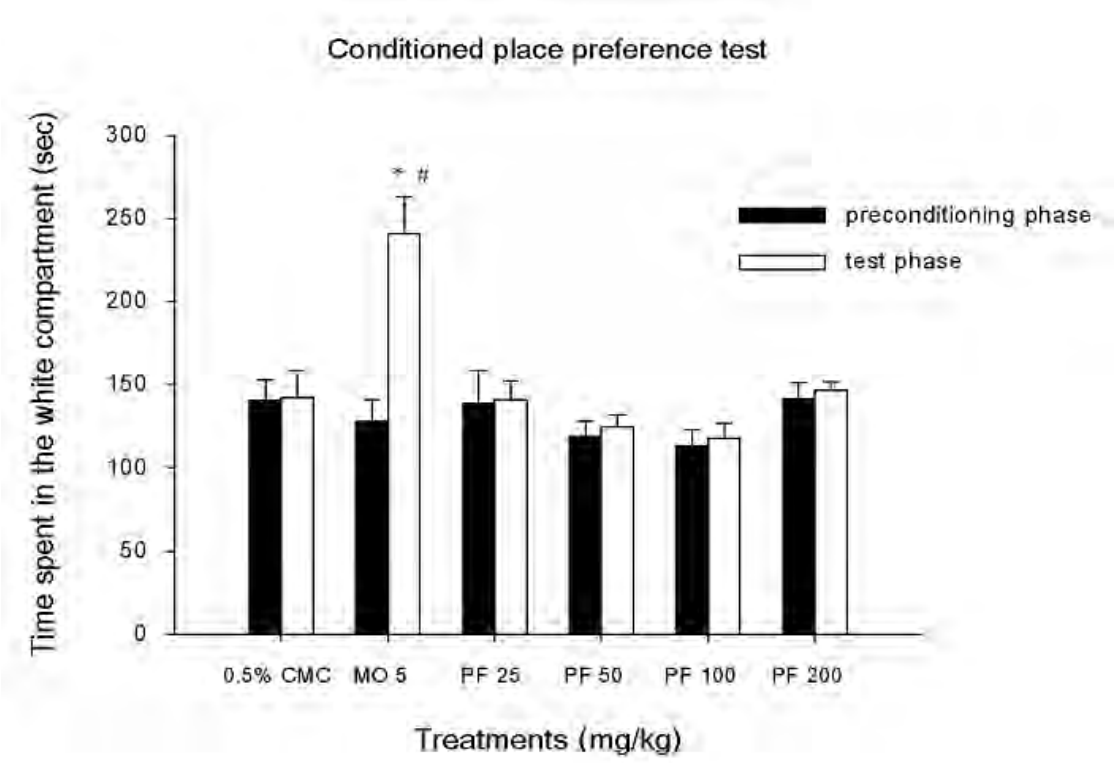

Figure 4.2 Conditioned place preference of rats receiving 0.5% CMC, morphine (5 mg/kg, i.p.), and various doses of PF (25-200 mg/kg, p.o.). N=8 for all groups. Each value represents mean ± S.E.M. *p<0.05 significantly different compared to preconditioning phase. $^{\#}p$<0.0 significantly different from 0.5% CMC.

The effect of the ethanolic extract of *Passiflora foetida* (PF) on morphine-induced conditioned place preference (CPP)

In order to investigate the effect of various doses of PF pretreatment on reinforcing effects of morphine, the conditioned place preference (CPP) test in rats was used. Pretreatment with methadone (1 mg/kg, i.p.), the positive control, significantly decreased morphine-induced CPP. Pretreatment with all doses of PF (25, 50, 100 and 200 mg/kg, p.o.) significantly decreased % change of place preference induced by morphine when compared to 0.5% CMC. Therefore, all doses of PF suppressed morphine-induced CPP when compared with 0.5% CMC (Figure 4.3).

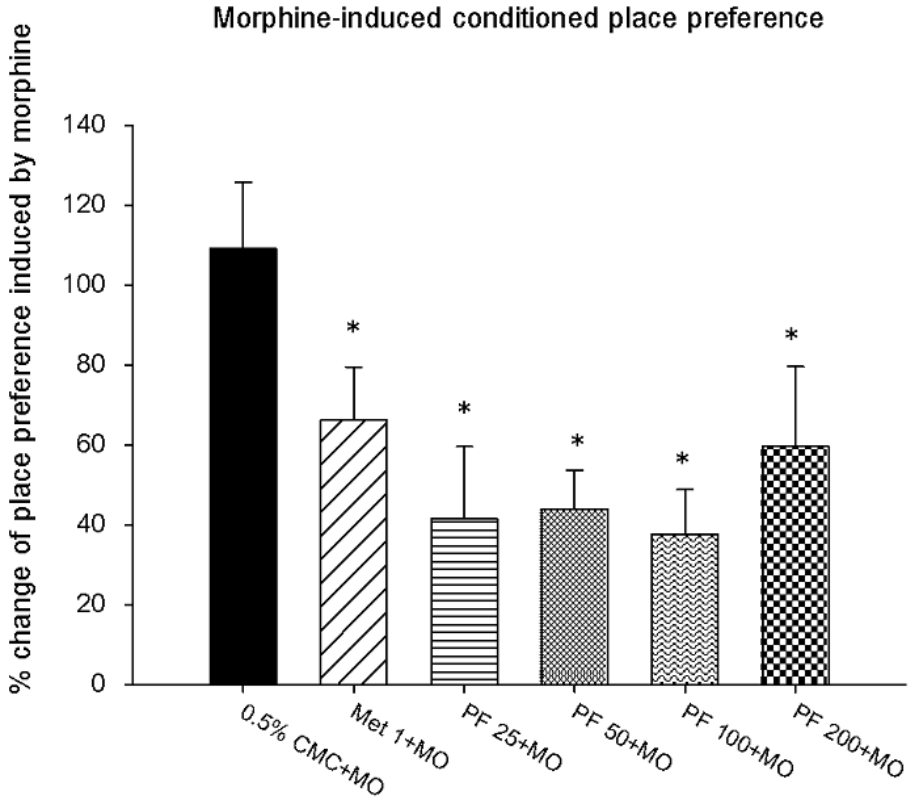

Figure 4.3 Morphine-induced CPP in rats receiving 0.5% CMC, and various doses of PF (25-200 mg/kg, p.o.) 15 min prior to morphine injection (5 mg/kg, i.p.). Percentage change of place preference equals the difference in time spent in the white compartment between preconditioning and test phases over the time spent in the preconditioning phase. N=8 for all groups. Each value represents mean ± S.E.M. *$p<0.05$ significantly different from 0.5% CMC.

Effect of the ethanolic extract of *Passiflora foetida* (PF) on naloxone-precipitated withdrawal

1) Effect of *Passiflora foetida* (PF) on induction of morphine dependence

To investigate the effect of pretreatment with various doses of PF on morphine dependence mice, withdrawal symptoms of morphine precipitated with naloxone were observed. Pretreatment with methadone (1 mg/kg, i.p.) significantly (p<0.05) decreased naloxone-precipitated withdrawal symptoms including jumping and straub tail behaviors when compared to 0.5% CMC (Figure 4.4 and 4.7). Pretreatment with all doses of PF (25, 50, 100, and 200 mg/kg) also significantly (p<0.05) decreased jumping and straub tail behaviors of morphine dependence mice in a dose-dependent manner when compared to 0.5% CMC (Figure 4.4 and 4.7).

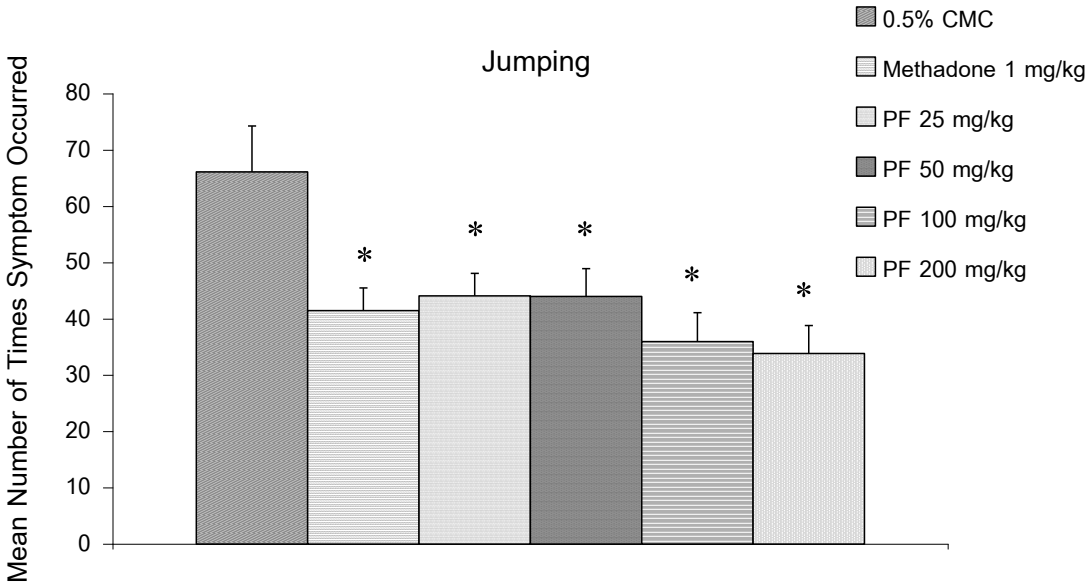

Figure 4.4 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on jumping behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M. *p<0.05 significantly different from 0.5% CMC group.

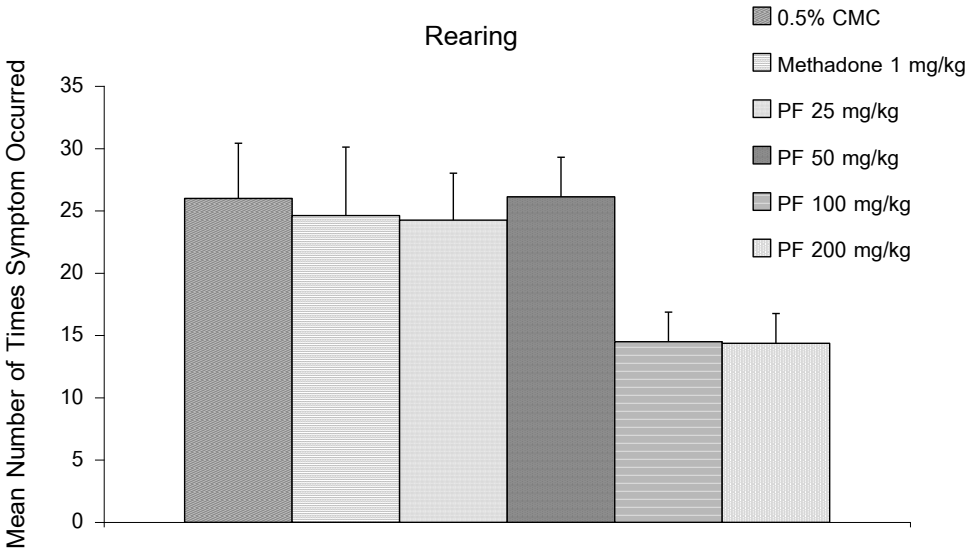

Figure 4.5 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on rearing behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M.

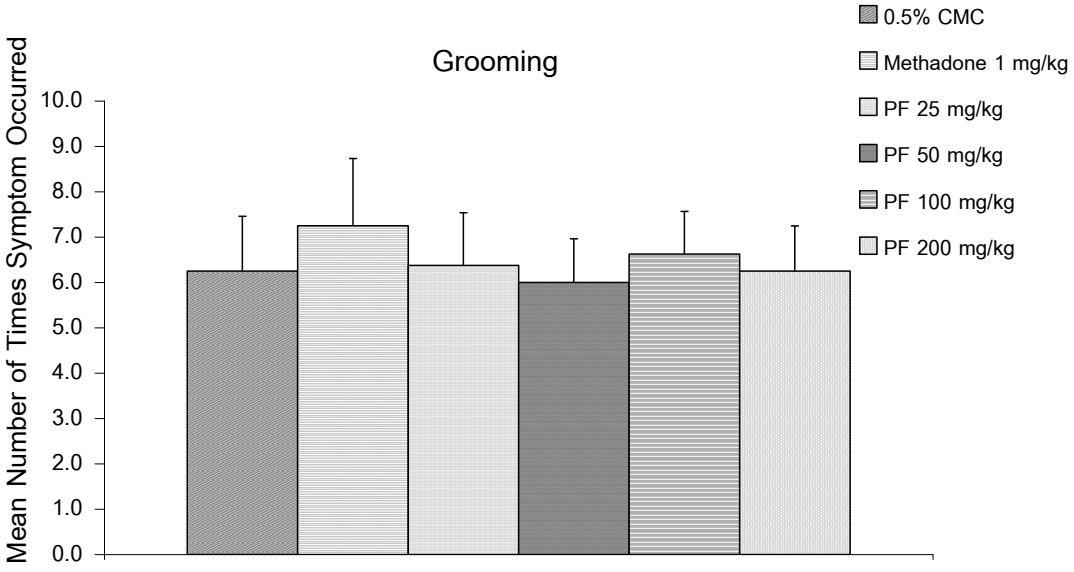

Figure 4.6 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on grooming behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M.

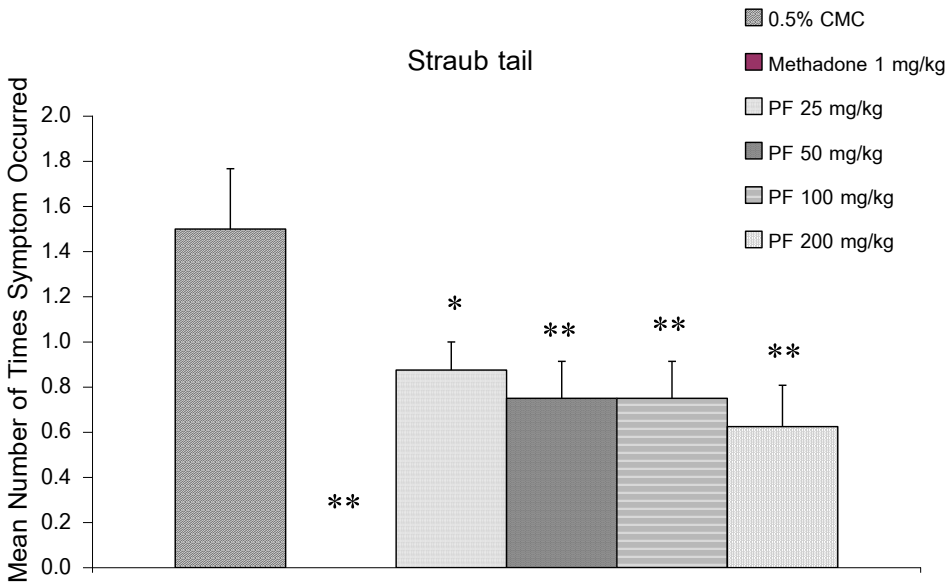

Figure 4.7 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on straub tail behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M. *p<0.05 significant differences from 0.5% CMC group, **p<0.01 significant differences from 0.5% CMC group.

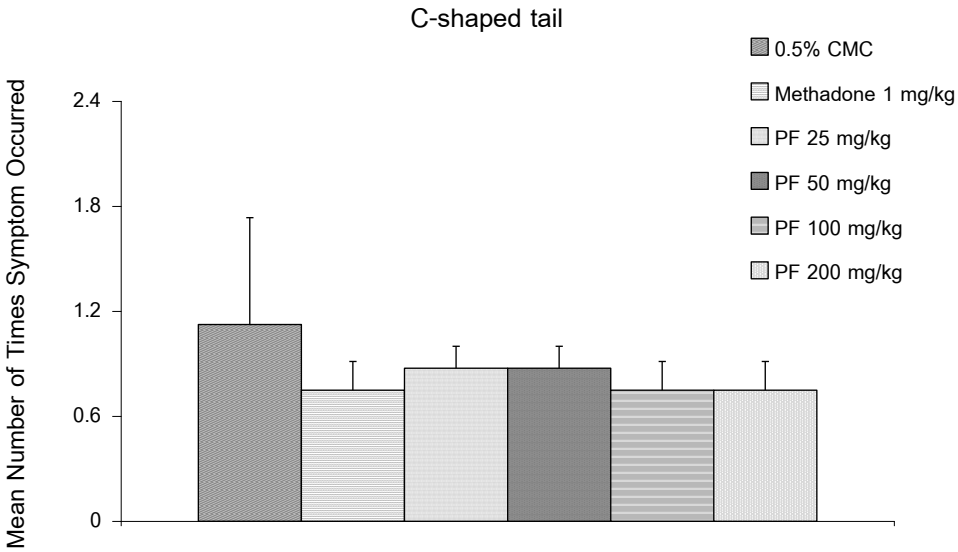

Figure 4.8 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on c-shaped tail behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M.

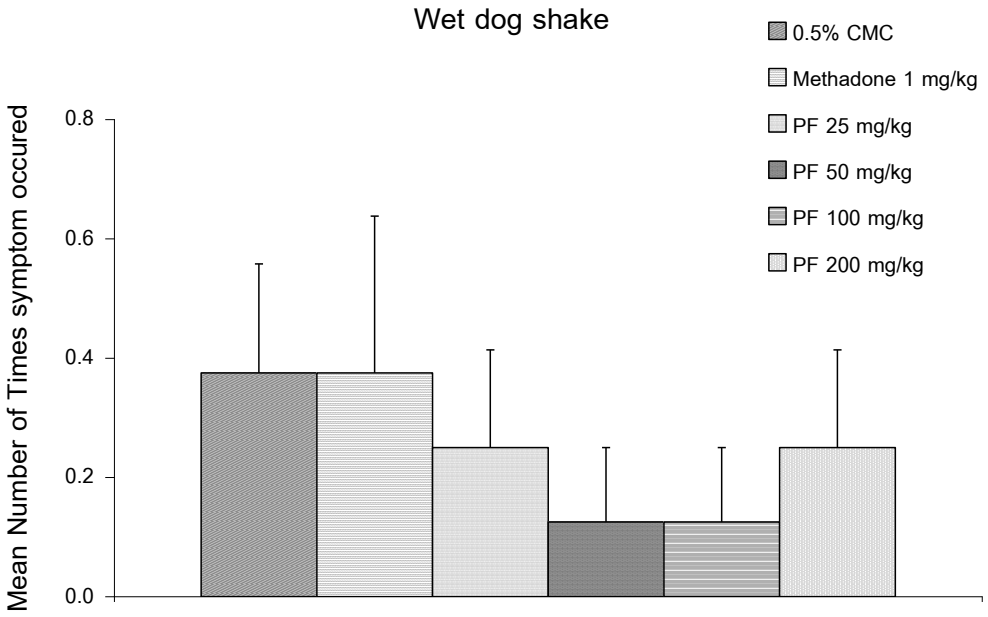

Figure 4.9 Effects of pretreatment with 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on wet dog shake behavior precipitated by naloxone in mice. N=8 for all groups. Each value represents mean ± S.E.M.

2) Effect of acute administration (post-treatment) of *Passiflora foetida* (PF) on morphine dependence

To investigate the effect of various doses of PF on morphine dependence mice, withdrawal symptoms of morphine precipitated with naloxone were observed. Methadone (1 mg/kg, i.p.) significantly (p<0.05) decreased only jumping behavior of morphine dependence mice when compared to 0.5% CMC (Figure 4.10). All doses of PF except 25 mg/kg significantly (p<0.05) decreased jumping behavior of morphine dependence mice when compared to 0.5% CMC (Figure 4.10). All doses of PF significantly (p<0.05) decreased straub tail behavior of morphine dependence mice when compared to 0.5% CMC (Figure 4.13). PF at the dose of 25 and 50 mg/kg decreased wet dog shake behavior of morphine dependence mice when compared to 0.5% CMC (Figure 4.15).

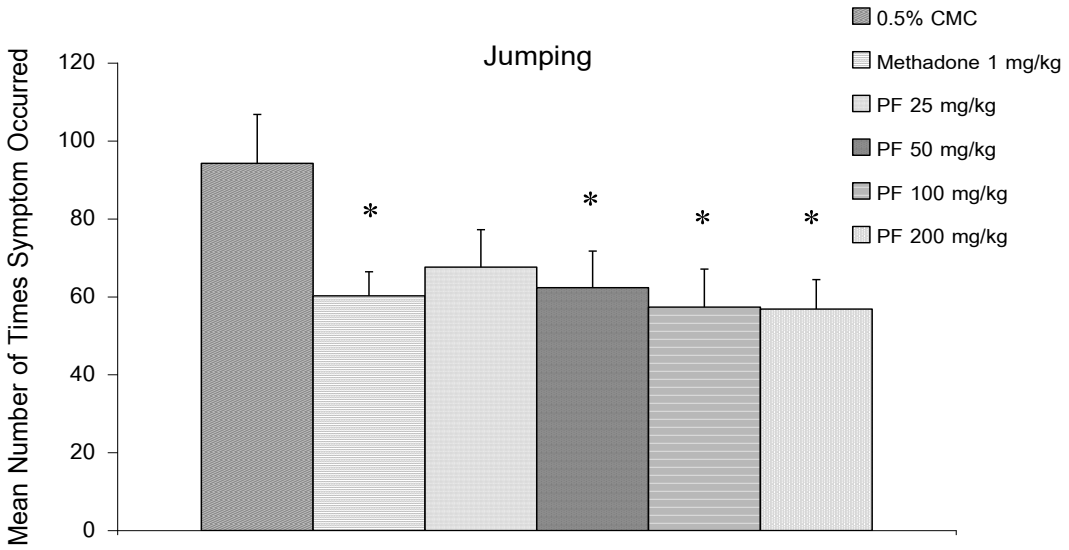

Figure 4.10 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on jumping behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups. *p<0.05 significantly different from 0.5% CMC.

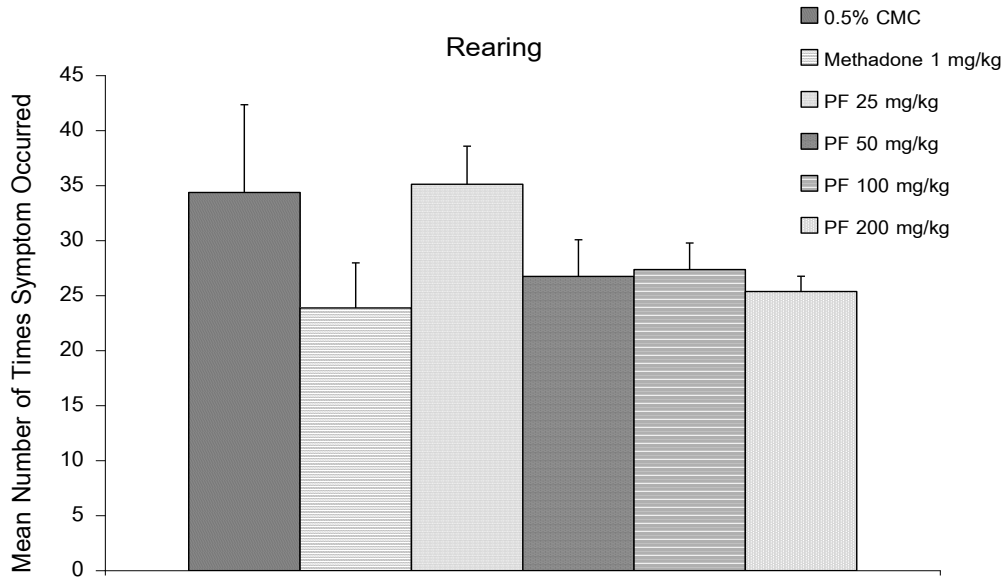

Figure 4.11 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on rearing behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups.

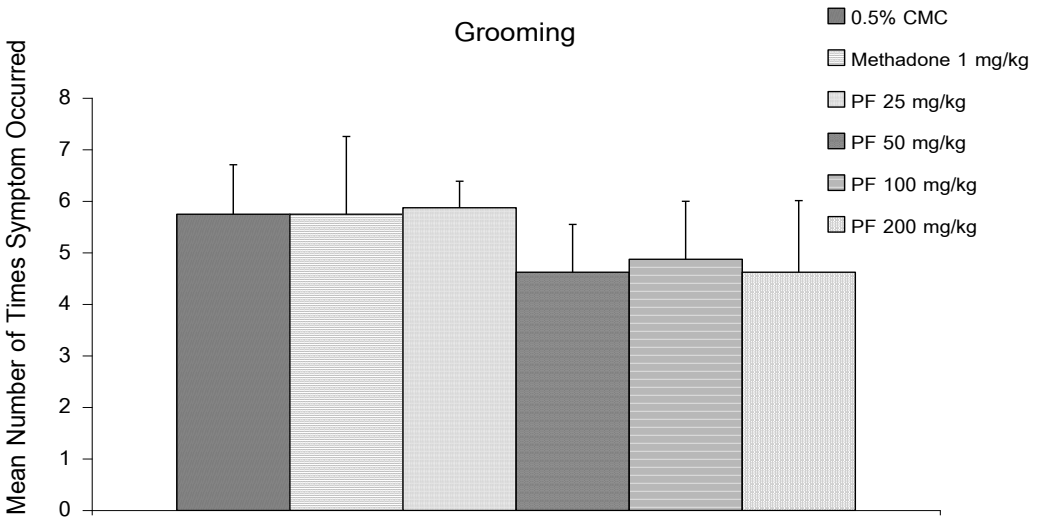

Figure 4.12 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on grooming behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups.

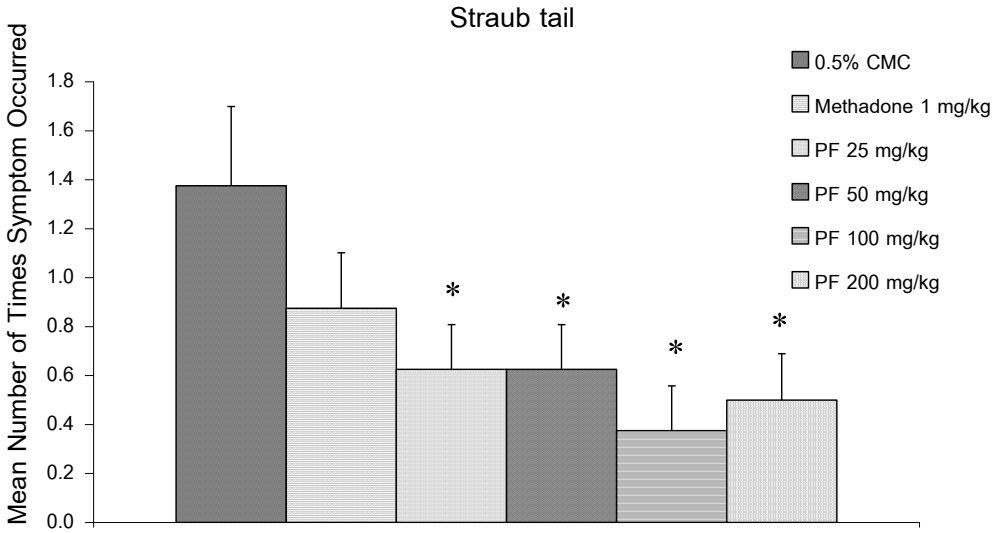

Figure 4.13 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on straub tail behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups. *p<0.05 significantly different from 0.5% CMC.

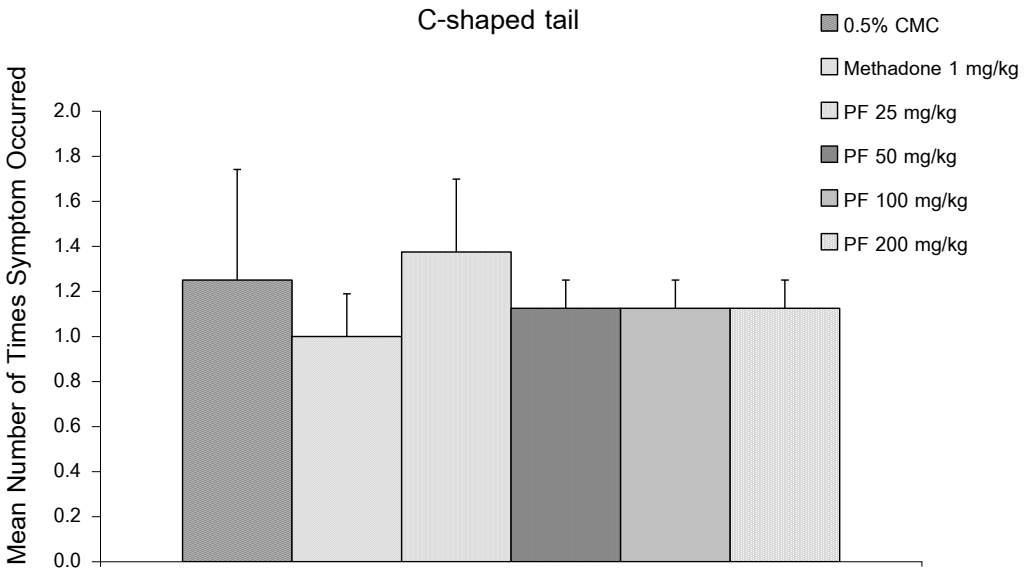

Figure 4.14 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on c-shaped tail behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups.

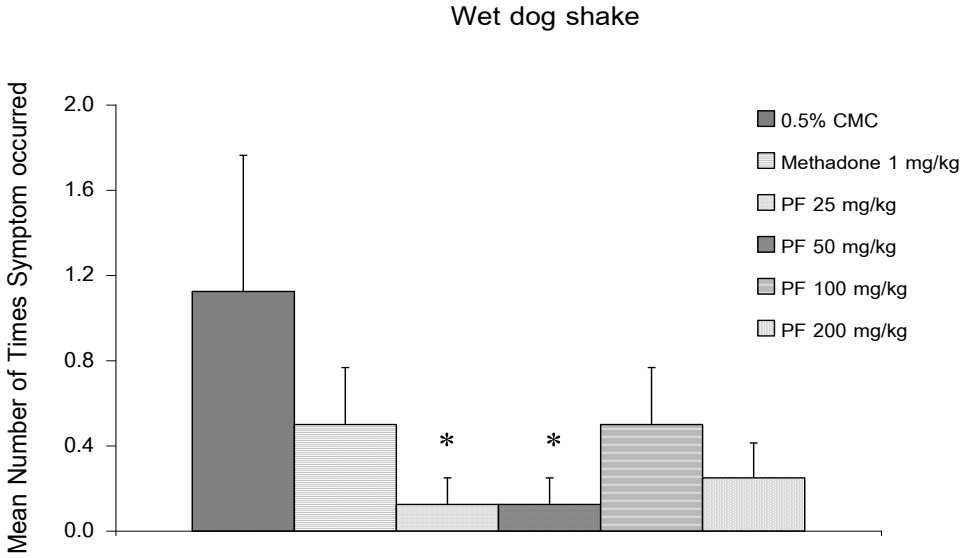

Figure 4.15 Effects of post-treatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on wet dog shake behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups. *p<0.05 significantly different from 0.5% CMC.

CHAPTER V

DISCUSSION AND CONCLUSION

The present study was aimed to determine the effects of the ethanolic extract of *Passiflora foetida* (PF) on morphine addiction in mice and rats utilizing locomotor activity, conditioned place preference (CPP) and precipitated withdrawal tests.

Locomotor activity was measured by determining the amount of distance traveled and observations of various horizontal behaviors in response to a novel environment. Locomotion was mediated mainly through dopaminergic pathway (Rang et al., 1999). A decrease in locomotor activity in rodents is suggestive of a possible central nervous system (CNS) depressant activity while an increase in locomotor activity is suggestive of a possible CNS stimulant activity. Locomotor activity test was then used for screening the effect of PF on the CNS. All doses of PF produced no significant effects on locomotor activity as compared to 0.5% CMC. The results demonstrated that all doses of PF did have neither stimulant nor sedative effects (Figure 4.1). These results are consistent with the earlier report of Wijagkanalan (2005). Therefore, all doses of PF were further tested in CPP paradigm.

Conditioned place preference (CPP) paradigm is a standard preclinical behavioral model that used for evaluating the reinforcing or rewarding effects of drugs. CPP was evaluated as the difference in pre-conditioning and post-conditioning time spent in the drug-paired compartment. An increase in the time spent in the drug-paired compartment (the white compartment) after conditioning phase suggests the presence of the positive reinforcing effects. Typically, drugs of abuse, including morphine, amphetamine and cocaine produced significant effect of CPP (Kim et al., 1996). Opiates are known to enhance mesocorticolimbic dopamine neuronal firing and ultimately release dopamine into the nucleus accumbens, which has been shown to be caused by disinhibition of dopamine neurons in the ventral tegmental area through attenuating gamma-aminobutyric acid (GABA) release in the ventral tegmental area (Phillips and LePiane, 1980; Van der Kooy, 1982). From the results, morphine also produced significant CPP (Figure 4.2). Moreover, the results showed that all doses of PF did not

produce any CPP (Figure 4.2). However, the withdrawal effects of acute and chronic administration of PF are needed to be further investigated to confirm that PF did not have reinforcing effect. The effect of PF on morphine-induced conditioned place preference was evaluated. The results were expressed as percent change of place preference induced by morphine. From the results, pretreatment with all doses of PF (25-200 mg/kg, p.o.) significantly decreased %change of place preference induced by morphine when compared to 0.5% CMC similar to methadone (1 mg/kg, i.p.). The results demonstrated that all doses of PF tested suppressed morphine-induce CPP (Figure 4.2), which suggested that PF could prevent the development of psychological dependence and inferred the possible application of PF to the prevention of positive reinforcement for opioids. PF itself did not produced CPP, which suggested that the effect of PF on morphine-induced CPP is not based on its own effect on spontaneous behavior, but related to the interaction between the morphine-opioid receptor system and PF and its action system. However, the mechanisms of PF action are needed to be further investigated.

The effect of PF on the induction of morphine dependence was also investigated. To study morphine dependence, withdrawal signs were precipitated by injection of naloxone (μ-opioid antagonist) since withdrawal signs can be observed within a short time following its administration (Linseman, 1977). In this study, methadone (a full μ-opioid receptor agonist) was used as the positive control because it is the gold standard treatment for opioid dependence (O'Connor, 2010). The results showed that pretreatment with PF (25-200 mg/kg; p.o.) and methadone (1 mg/kg, i.p.) before morphine administration during the induction of morphine dependence significantly decreased morphine withdrawal jumping and straub tail behaviors when compared to control group (Figure 4.4 and 4.7). The results demonstrated that PF has preventive effect on naloxone-precipitated morphine withdrawal similar to methadone. These results were in agreement with the previous study of Dhawan et al. (2002) which showed that the bioactive fraction (BZF) of the methanol extract of *Passiflora incarnata*, the plant in the same genus as *Passiflora foetida* could decrease the naloxone-precipitated withdrawal jumps in mice.

We then evaluated the effect of PF treatment on morphine withdrawal. The results demonstrated that treatment with PF at the doses of 50, 100, and 200 mg/kg significantly decreased jumping and straub tail and wet dog shake behaviors when compared to the control group, while methadone significantly decreased jumping behavior when compared to the control group (Figure 4.10). PF and methadone showed similar results only in attenuating jumping behavior whereas they showed different results in attenuating other withdrawal symptoms. These results are consistent with earlier report which stated that withdrawal jumping has been considered the most sensitive index of the intensity of withdrawal syndrome (Francis and Schneider, 1971). Previous study of Calvino et al. (1979) indicated that lesions of the amygdala and of the raphe nuclei blocked jumping behavior in dependent rats, but failed to alter other withdrawal signs. Tremblay and Charton (1981) revealed that local injection of naloxone to the amygdala, globus pallidus and medial thalamus selectively elicited wet dog shake and jumping. PF significantly decreased jumping and wet dog shake behaviors. These results suggest the involvement of amygdala, raphe nuclei, globus pallidus and medial thalamus in PF actions.

Some classical antidepressants such as fluvoxamine and sertraline (selective serotonin reuptake inhibitor) were found to reduce the severity of the naloxone precipitated opioid withdrawal syndrome (Gray, 2002). Additionally, venlafaxine (a novel serotonin and adrenaline reuptake inhibitor) was also demonstrated to attenuate morphine withdrawal (Lu et al., 2001). The crude extract of *Passiflora foetida* exhibited antidepressant-like activity in open swimming test in mice and the antidepressant activity of *Passiflora foetida* may be mediated mostly via dopaminergic and serotoninergic mechanisms (Wijagkanalan, 2005). Thus, we hypothesized that the possible mechanism of PF action in reducing withdrawal symptoms of morphine may be mediated via serotoninergic mechanisms. Previous study of Wiley and Downs (1979) indicated that methadone produced a pattern of naloxone-induced jumping which appeared to be dose and time-related. Pretreatment and post-treatment with methadone (1 mg/kg, i.p.) in this study attenuated jumping behavior in morphine dependence mice suggesting the use of methadone for the prevention and treatment of opioid dependence and withdrawal. These finding indicated that PF may have both preventive

and treatment effects on morphine withdrawal however, it is necessary to further investigate the effect of PF on morphine withdrawal using other animal models.

In conclusion, the present study demonstrated that PF did not have neither stimulating nor sedative effects. PF did not have reinforcing effect by itself but it could suppress the reinforcing effect of morphine. Moreover, PF may have prevention and treatment effects on morphine withdrawal. These results indicated that PF may have a potential to be developed for the treatment of opioid addiction. However, the active chemical constituents in PF and mechanisms of PF action may be needed to be further investigated.

REFERENCES

Akhondzadeh, S., Naghavi, H.R., Shayeganpour, A., Rashidi, A., and Khani M. Passionflower in the treatment of generalized anxiety: a pilot double blind randomized controlled trial with oxazepam. J Clin Pharm Therap 26 (2001): 363-367.

Amass,L., Bickel, W.K., Higgins, S.T., and Hughes, J.R. A preliminary investigation of outcome following gradual or rapid buprenorphine detoxification. J Addict Dis (13) 1994: 33-45.

Amato, L., Davoli, M., Ferri, M., Gowing, L., and Perucci, C. Effectiveness of interventions on opiate withdrawal treatment: an overview of systematic reviews. Drug Alcohol Depend 73 (2004): 219-226.

Ballantyne, J.C., and LaForge, K.S. Opioid dependence and addiction during opioid treatment of chronic pain. Pain 129 *(*2007): 235–255.

Bardo, M.T. Neurophrmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. Clin Rev Neurobiol 12 (1998): 37-67.

Benyhe, S. Morphine: New aspects in the study of an ancient compound. Life Sci 55 (1994): 969-979.

Bhiman, A., and Purkayastha, M. Pharmacological Treatment of Opioid Dependence JMHHB 14 (2009): 30-37.

Blasig, J., Herz A., Reinhold, K., and Zieglgansberger, S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats, Psychopharm (Berlin) 33 (1973): 19-38.

Bovill, J.G. Mechanisms of actions of opioids and nonsteroidal antiinflammatory drugs. Eur J Anesthesiol 14 (1997): 9-15.

Calvino, B., Lagowska, J., and Ben-ari, Y. Morphine withdrawal syndrome: Differential participation of structures located within the amygdaloid complex and striatum of the rat. Brain Res 177 (1979): 19-34.

Cami, J., and Farre, M. Drug addiction. N Engl J Med 349 (2003): 975-986.

Capasso, A., and Sorrentino, L. Pharmacological studies on the sedative and hypnotic

effect of *Kava kava* and Passiflora extracts combination. Phytomedicine 12 (2005): 39-45.

Cardona, W., Quinones, W., and Echeverri, F. Leishmanicidal activity of passifloricin A and derivatives. Molecules 9 (2004): 666-672.

Clark, N.C., and others. LAAM maintenance vs methadone maintenance for heroin dependence. Cochrane Database Syst Rev 2 (2002): CD002210.

Davids, E., and Gastpar, M. Buprenorphine in the treatment of opioid dependence. Eur Neuropsychopharmacol 2004*; 14:* 209–216.

Dhawan, K., Kumar, S., and Sharma, A. Anti-anxiety studies on extracts of aerial *Passiflora incarnata* Linneaus. J Ethnopharmacol 78 (2001a): 167-170.

Dhawan, K., Kumar, S., and Sharma, A. Anxiolytic activity of aerial and underground parts of *Passiflora incarnata*. Fitoterapia 72 (2001b): 922-926.

Dhawan, K., Kumar, S., and Sharma, A. Nicotine reversal effects of the benzoflavone moiety from *P. incarnata* Linneaus in mice. Addict Biol 7 (2002a): 435-442.

Dhawan, K., Kumar, S., and Sharma, A. Reversal of cannabinoids (delta9-THC) by the benzoflavone moiety from methanol extract of Passiflora incarnata Linneaus in mice: a possible therapy for cannabinoid addiction. J Pharm Pharmacol 54 (2002b): 875-881.

Dhawan, K., Kumar, S., and Sharma, A. Reversal of morphine tolerance and dependence by Passiflora incarnata – a traditional medicine to combat morphine addiction. Pharmaceut Biol 40 (2002c): 576-580.

Dhawan, K., Dhawan, S., and Sharma, A. *Passiflora*: a review update. J Ethnopharmacol 94 (2004): 1-23.

Dole, V.P., and Nyswander, M. A medical treatment for diacetyl- morphine (heroin) addiction. JAMA 193 (1965): 646-650.

Echeverri, F., and others. Passifloricins, polyketides alpha-pyrones from *Passiflora foetida* resin. Phytochemistry 56 (2001): 881-885.

Emmett-Oglesby, M. W., Mathis, D. A., Moon, R. T., and Lal, H. Animal models of drug withdrawal symptoms. Psychopharm 101 (1990): 292-309.

Francis, D.L., and Schneider, C. Jumping after naloxone precipitated withdrawal of chronic morphine in the rat. Br J Pharmac 41 (1971): 424-425.

Gerra, G., Marcato, A., and Caccavari, R. Clonidine and opiate receptor antagonists in the treatment of heroin addiction. J Subst Abuse Treat 12 (1995): 35-41.

Gilbert, D., and Cooper, S.J. beta-Phenylethylamine-, d-amphetamine-and l-amphetamine-induced place preference conditioning in rats. Eur J Pharmacol 95 (1983): 311-314.

Glanz, M., Klawansky, S., McAullife, W., and Chalmers, T. Methadone vs. L-alpha-acetylmethadol (LAAM) in the treatment of opiate addiction. A meta-analysis of the randomized, controlled trials. Am J Addict 6 (1997): 339-349.

Gonzalez, J.P., and Brogden, R.D. Naltrexone: a reveiw of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of opioid dependence. Drugs 35 (1988): 192-213.

Gowing, L., Ali, R., and White, J. Opioid antagonists and adrenergic agonists for the management of opioid withdrawal. Cochrane Database Syst Rev (Online) 2(2000): CD002021.

Gowing, L., Ali, R., and White, J.M. Buprenorphine for the management of opioid withdrawal. Cochrane Database Syst Rev 2 (2006): CD002025.

Gowing, L., Ali, R., and White, J.M. Opioid antagonists with minimal sedation for opioid withdrawal. Cochrane Database Syst Rev 4 (2009): CD002021.

Gowing, L., Farrell, M., Ali, R., and White, J. Alpha 2 adrenergic agonists for the management of opioid withdrawal. Cochrane Database Syst Rev 4 (2004): CD002024.

Gray A.M. The effect of fluvoxamine and sertraline on the opioid withdrawal syndrome: A combined in vivo cerebral microdialysis and behavioural study. Eur Neuropsychophamacol 12 (2002): 245-254.

Helm, S., Trescot, A.M., Colson, J., Sehgal, N., and Silverman, S. Opioid Antagonists, Partial Agonists, and Agonists/Antagonists: The Role of Office-Based Detoxification. Pain Phys 11 (2008): 225-235.

Hickey, M., and King, C. 100 Families of Flowering Plants. Cambridge University Press, Cambridge (1988):130-133.

Hoffman D.C. The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res Bull 23 (1989): 373-387.

Hsu, M.M., and Wong, C.S. The roles of pain facilitatory systems in opioid tolerance. Acta Anaesthesiol Sin 38 (2000):155–166.

Hyman, S. E., Malenka, R. C., and Nestler, E. J. Neural mechanisms of addiction: The role of reward-related learning and memory. Annu Rev of Neurosci 29 (2006): 565-598.

Kahn, A., Mumford, J.P., Rogers, G.A., and Beckford, H. Double-blind study of lofexidine and clonidine in the detoxification of opiate addicts in hospital. Drug Alcohol Depend 44 (1997): 57-61.

Kalant, H. Opium revisited: a brief review of its nature, composition, non-medical use and relative risks. Addiction 92 (1997): 267–277.

Kest, B., and others Naloxone-precipitated withdrawal jumping in 11 inbred mouse strains: evidence for common genetic mechanisms in acute and chronic morphine physical dependence. Neuroscience 115 (2002): 463-469.

Kleber, H.D. Naltrexone. J Subst Abuse Treat 2 (1985): 117-122.

Kleber, H.D., and others. Clonidine and naltrexone in the outpatient treatment of heroin withdrawal. Am J Drug Alcohol Abuse 13 (1987): 1-17.

Kraigher, D., and others. Use of slow-release oral morphine for the treatment of opioid dependence. Eur Addict Res 11 (2005): 145–151.

Krantz, M.J., and Mehler, P.S. Treating opioid dependence. Growing implications for primary care. Arch Intern Med 164 (2004): 277-288.

Konipsky, K.L., and Hyman, S.E. Molecular and cellular biology of addiction. Neuropsychopharmacol (2002):1367-1379.

Koob, G., and Nestler, E. The Neurobiology of Drug Addiction. J Neuropsychiatry Clin Neurosci 9 (1997): 482-497.

Kuschinsky, K., and Hornykiewicz, O. Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice, Eur J Pharmacol 26 (1974): 41–50.

Kosten, T.R., and Kleber, H.D. Buprenorphine detoxification from opioid dependence: a pilot study. Life Sci 42(1988): 635-641.

Lobmaier, P., Gossop, M., Waal, H., and Bramness, J. The pharmacological treatment of

opioid addiction – a clinical perspective. Eur J Clin Pharm 66 (2010): 537-545.

Ling, W., and Compton, P. Recent advances in the treatment of opiate addiction. Clin Neurosci Res 5 (2005):161-167.

Linseman, M.A. Naloxone-precipitated withdrawal as a function of the morphine-naloxone interval. Psychopharmacol (Berl) 54 (1977):159–164.

Lu, L., and others. Attenuation of morphine dependence and withdrawal in rats by venlafaxine, a serotonin and noradrenaline reuptake inhibitor., Life sciences 69 (2001): 37-46.

Melichar, J.K., Daglish, M.R., and Nutt, D.J. Addiction and withdrawal-current views. Curr Opin Pharmacol 1 (2001): 84-90.

Mohanasundari, C., Natarajan, D., Srinivasan, K., Umaheswari, S., and Ramachandran, A. Antibacterial properties of *Passiflora foetida* L. - a common exotic medicinal plant. Afr J Biotechnol (2007): 2650-2653.

Narita, M., Funada, M., and Suzuki, T. Regulations of opioid dependence by opioid receptor types. Pharmacol Ther 89 (2001): 1-15.

Navaratnam, V., Jamaludin, A., and Raman, N. Determination of naltrexone dosage for narcotic agonist blockade in detoxified Asian addicts. Drug Alcohol Depend 34 (1994): 231-236.

Nestler, E.J. Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. Trends Pharmacol Sci 25 (2004):210-218.

Nestler, E.J. Molecular mechanisms of drug addiction. J Neurosci 12 (1992): 2439-2450.

Nestler, E.J. Is there a common molecular pathway for addiction? Nat Neurosci 8 (2005): 1445-1449.

Nwosu, M.O. Herbs for mental disorders. Fitoterapia 7 (1999): 58-63.

Oades, R.D., Taghzouti, K., Rivet, J.M., Simon, H., and Le Moal, M. Locomotor activity in relation to dopamine and noradrenaline in the nucleus accumbens, septal and frontal areas: a 6-hydroxydopamine study. Neuropsychobiol 16 (1986): 37–42

O'Connor, P.G. Advances in the Treatment of Opioid Dependence: Continued Progress and Ongoing Challenges, JAMA 304 (2010): 1612-1614.

O'Connor, P.G., and Kosten T.R. Rapid and Ultra-Rapid Opiate Detoxification

Techniques. JAMA 279 (1998): 229-234.

Paice, J.A. Pharmacokinetics, pharmacodynamics, and pharmacogenomics of opioids. Pain Manag Nurs 8 (2007): S2–5.

Petry, R.D., and others. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. Phytother Res 15 (2001): 162-164.

Phillips, A.G., and LePiane, F.G. Reinforcing effects of morphine microinjection into the ventral tegemental area. Pharmacol Biochem Behav 12 (1980): 965-968.

Piros, E.T., Hales, T.G., and Evans, C.J. Functional analysis of cloned opioid receptors in transfected cell lines. Neurochem Res 21 (1996): 1277-1285.

Puricelli, L., and others. Preliminary evaluation of inhibition of matrix-metalloprotease MMP-2 and MMP-9 by *Passiflora edulis* and *P. foetida* aqueous extracts. Fitoterapia 74 (2003):302-304.

Rang, H.P., Dale, M. M., and Ritter, J.M.Pharmacology 3rd Edition (1999): 491-665.

Schiff, P.L. Opium and its alkaloids. Am J Pharm Educ 66 (2002): 186-194.

Schottenfeld, R.S., Pakes, J.R., Oliveto, A., Ziedonis, D., and Kosten, T.R. Buprenorphine vs methadone maintenance treatment for concurrent opioid dependence and cocaine abuse. Arch Gen Psychiatry 54 (1997): 713-720.

Schottenfeld, R.S., Pakes, J.R., and Kosten, T.R. Prognostic factors in Buprenorphine versus methadone-maintained patients. J Nerv Ment Dis 186 (1998): 35-43.

Shippenberg, T., and Koob, G. Recent advances in animal models of drug addiction. Neuropsychopharmacol (2002): 1381-1397.

Silberstein, S.D., and McCrory, D.C. Opioids. Monogr Clin Neurosci 17(2000): 222-236.

Speroni, E., and Minghetti, A. Neuropharmacological activity of extracts from *Passiflora incarnata*. Planta Med 54 (1988): 488-491.

Spyraki, C., Fibiger, H.C., and Phillips, A.G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res 253 (1982): 185-193.

Stotts, A.L., Dodrill, C.L., and Kosten, T.R. Opioid dependence treatment: options in pharmacotherapy. Expert Opin Pharmacother 10 (2009): 1727-1740.

Swerdlow, N.R., and Koob, G.F. Restrained rats learn amphetamine-conditioned locomotion, but not place preference. Psychopharm 84 (1984): 163–166.

Tomkins, D.M., and Sellers, E.M. Addiction and the brain: the role of neurotransmitters in

the cause and treatment of drug dependence. Can Med Assoc J 164 (2001): 817-821.

Tremblay, E.C., and Charton, G. Anatomical correlates of morphine withdrawal syndrome: Differential participation of structures located within the limbic system and striatum. Neurosci Lett 23 (1981): 137-142.

Van der Kooy, D., Mucha, R., O'Shaughnessy, M., and Bucenieks, P. Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res 243 (1982): 107-117.

Veilleux, J.C., Colvin, P.J., Anderson, J., York, C., and Heinz, A. J. A review of opioid dependence treatment: Pharmacological and psychosocial interventions to treat opioid addiction. Clin Psychol Rev 30 (2010): 155-166.

Vocci, F.J., and Sorer, H. Pharmacotherapies for treatment of opioid dependence. JHCPU 3 (1992): 109-124.

Way, E.L. History of opiate use in the Orient and the United States. Ann N Y Acad Sci 398 (1982):12–23.

Wijagkanalan, P., Antidepressant activity of extracts of Passiflora foetida in mice and the involvement of D1 and 5-HT1A receptors. Bangkok: Chulalongkorn University (2005): 1-63 (T E26692).

Wiley, J.N., and Downs, D.A. Naloxone-precipitated jumping in mice pretreated with acute injections of opioids. Life Sciences 25 (1979): 797-802.

APPENDICES

APPENDIX A

Effect of PF on locomotor activity

Treatments	Total locomotor activity (counts/ 75 min)
NSS	2134±250
0.5% CMC	2067±224
Morphine 5 mg/kg	2123±204
PF 25 mg/kg	2226±232
PF 50 mg/kg	2687±263
PF 100 mg/kg	1999±157
PF 200 mg/kg	2284±200

Table 1: Total locomotor activity of mice that received NSS, 0.5% CMC, morphine (5 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.). The data is presented as mean ± S.E.M. (n=8).

Effect of PF on conditioned place preference

Treatments	Time spent in the white compartment of preconditioning phase (sec)	Time spent in the white compartment of test phase (sec)
0.5% CMC	140.25±12.77	142.38±16.01
Morphine 5 mg/kg	127.75±13.36	240.50±22.21 *#
PF 25 mg/kg	139.00±19.46	141.13±11.36
PF 50 mg/kg	118.75±9.05	124.75±7.14
PF 100 mg/kg	113.38±9.37	118.00±8.94
PF 200 mg/kg	141.50±9.89	146.88±4.4

Table 2: Time spent in the white compartment of preconditioning phase and test phase The data is presented as mean ± S.E.M. (n=8). *$p<0.05$ significantly different compared to preconditioning phase (Student's paired *t*-test). $^{\#}p<0.01$ significantly different from 0.5% CMC were performed by one-way ANOVA and Dunnett's test for comparison.

Effect of PF on morphine-induced conditioned place preference

Treatments	Time spent in the white compartment of preconditioning phase	Time spent in the white compartment of test phase
0.5% CMC + Morphine 5 mg/kg	130.63±16.41	252.63± 26.52
Methadone 1 mg/kg + Morphine 5 mg/kg	141.75±15.20	227.13±16.43
PF 25 mg/kg + Morphine 5 mg/kg	128.25±6.51	183.00±26.33
PF 50 mg/kg + Morphine 5 mg/kg	150.25±8.05	214.50±16.11
PF 100 mg/kg + Morphine 5 mg/kg	147.50±12.31	197.13±13.35
PF 200 mg/kg + Morphine 5 mg/kg	131.13±10.16	200.88±22.63

Table 3: Effects of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on morphine-induced conditioned place preference in rats. The data is presented as mean ± S.E.M. (n=8).

Treatments	% change of place preference
0.5% CMC + Morphine 5 mg/kg	109.02± 16.60
Methadone 1 mg/kg + Morphine 5 mg/kg	66.22±13.27*
PF 25 mg/kg + Morphine 5 mg/kg	41.45±18.08*
PF 50 mg/kg + Morphine 5 mg/kg	43.90±9.71*
PF 100 mg/kg + Morphine 5 mg/kg	37.56±11.26*
PF 200 mg/kg + Morphine 5 mg/kg	59.60±19.96*

Table 4: Effects of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg; p.o.) on morphine-induced conditioned place preference in rats. Each value represents mean± S.E.M. N=8 for all groups. *p<0.05 significantly different from 0.5% CMC were performed by one-way ANOVA and Fisher's LSD test for comparison.

Effect of PF on induction of morphine dependence

Treatments	Number of times symptom occurred					
	jumping	rearing	grooming	straub tail	c-shaped tail	wet dog shake
0.5% CMC	66.13±8.15	26.00±4.43	6.25±1.21	1.50±0.27	1.13±0.61	0.38±0.18
Methadone 1 mg/kg	41.50±4.01*	24.63±5.50	7.25±1.49	0.00±0.00**	0.75±0.16	0.38±0.26
PF 25 mg/kg	44.13±3.98*	24.25±3.77	6.38±1.16	0.88±0.13*	0.88±0.13	0.25±0.16
PF 50 mg/kg	44.00±4.95*	26.13±3.18	6.00±0.96	0.75±0.16**	0.88±0.13	0.13±0.13
PF 100 mg/kg	36.00±5.13*	14.50±2.37	6.63±0.94	0.75±0.16**	0.75±0.16	0.13±0.13
PF 200 mg/kg	33.88±4.98*	14.38±2.39	6.25±1.00	0.63±0.18**	0.75±0.16	0.25±0.16

Table 5: Effects of pretreatment of 0.5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on jumping, rearing, grooming, straub tail, C-shaped tail and wet dog shakes behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups. *p<0.05 significantly different from 0.5% CMC were performed by one-way ANOVA and Dunnett's test for comparison.

Effect of acute administration of PF on morphine dependence

Treatments	Number of times symptom occured					
	jumping	rearing	grooming	straub tail	c-shaped tail	wet dog shake
0.5% CMC	94.25±12.57	34.38±7.97	5.75±0.96	1.38±0.32	1.25±0.49	1.13±0.64
Methadone 1 mg/kg	60.25±6.17*	23.88±4.10	5.75±1.51	0.88±0.23	1.00±0.19	0.50±0.27
PF 25 mg/kg	67.63±9.61	35.13±3.45	5.88±0.52	0.63±0.18*	1.38±0.32	0.13±0.13*
PF 50 mg/kg	62.38±9.36*	26.75±3.33	4.63±0.92	0.63±0.18*	1.13±0.13	0.13±0.13*
PF 100 mg/kg	57.38±9.77*	27.38±2.41	4.88±1.13	0.38±0.18*	1.13±0.13	0.50±0.27
PF 200 mg/kg	56.88±7.53*	25.38±1.39	4.63±1.39	0.50±0.19*	1.13±0.13	0.25±0.16

Table 6: Effects of post-treatment of .5% CMC, methadone (1 mg/kg, i.p.) and various doses of PF (25-200 mg/kg, p.o.) on jumping, rearing, grooming, straub tail, C-shaped tail and wet dog shakes behavior precipitated by naloxone in mice. Each value represents mean ± S.E.M. N = 8 for all groups. *$p<0.05$ significantly different from 0.5% CMC were performed by one-way ANOVA and Dunnett's test for comparison.

APPENDIX B

Study Protocol Approval by the Institutional Animal Care and Use Committee,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☐ Original ☐ Renew
Animal Use Protocol No. 11-33-004	**Approval No. 11-33-004**
Protocol Title Effects of the ethanolic extract of *Passiflora foetida* on morphine addiciton in mice and rats	
Principal Investigator Pasarapa Towiwat, Ph.D.	
Certification of Institutional Animal Care and Use Committee (IACUC) This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.	
Date of Approval March 11, 2011	**Date of Expiration** March 11, 2012
Applicant Faculty/Institution Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330	
Signature of Chairperson	**Signature of Authorized Official**
Name and Title THONGCHAI SOOKSAWATE, Ph.D. Chairman	**Name and Title** PARKPOOM TENGAMNUAY, Ph.D. Associate Dean (Research and Academic Service)
The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals. *This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.*	

Appendix C

HPLC Chromatogram of the Ethanolic Extract of *Passiflora foetida*

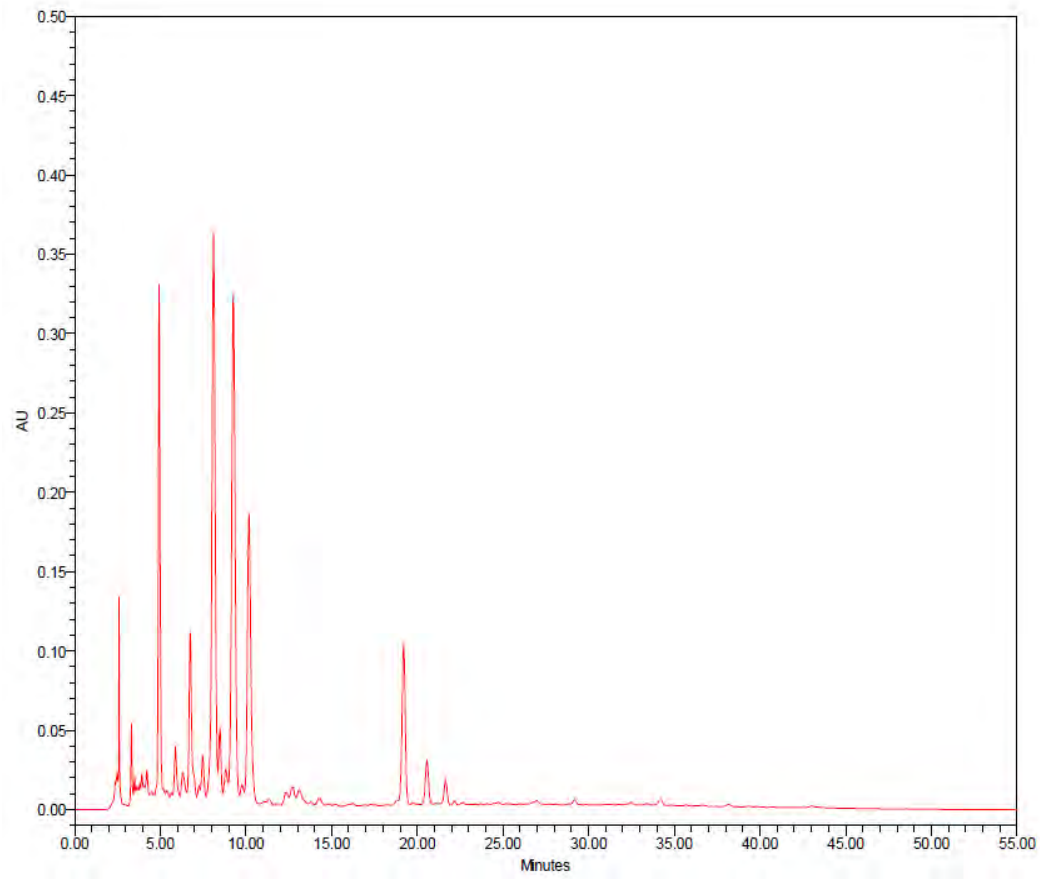

Figure 5.1 HPLC chromatogram of the ethanolic extract of *Passiflora foetida*

Wave length: 320 nm

Column: ACE® C18-AR column

Organic modifiers: methanol and acetonitrile (ACN)

Acid modifier: phosphoric acid

Rate of change ratio of carrier solvent

Time (min)	% solvent 0.3% Phosphoric acid	% Methanol	%ACN
0	75	13.5	11.5
10	75	13.5	11.5
15	70	17	13
30	60	18	22
40	60	18	22
50	75	13.5	11.5
55	75	13.5	11.5

BIOGRAPHY

Miss Chiraya Nipattamanon was born on July 12, 1986 in Trang province, Thailand. She graduated with Bachelor of Science in Chemistry in 2008 from Faculty of Science, Mahidol University.

Poster presentation entitled “Effect of the Ethanolic Extract of Passiflora foetida on Conditioned Place Preference” of Miss Chiraya Nipattamanon was presented in Thai Journal of Pharmacology Vol.33, Suppl.2, 2011, Poster presentation in 33rd Pharmacological and Therapeutic Society of Thailand Meeting 17-19 March 2011 at Diamond Plaza Hotel, Hat Yai, Songkla.