# ANTIANXIETY EFFECT OF VINPOCETINE ON CAFFEINE-INDUCED ANXIETY IN RATS

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology)

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OCTOBER 2018

# ABSTRACT

Anxiety disorders (AD) are increasing in all societies, especially developed ones. Caffeine (CAF) consumption in coffee, tea, and other beverages may aggravate and increase the frequency of anxiety symptoms. Vinpocetine (VP) is a synthetic derivative of the lesser periwinkle plant alkaloid vincamine and is used as a neuroprotective agent. The study was conducted to evaluate the capacity of VP to alleviate anxiety symptoms and to compare its effectiveness to that of lorazepam (LZP). The anxiety model in rat was induced via CAF injection (i.p.). Behavioural evaluation of the anxiety level in animal groups with and without treatment (oral LZP and VP) after a period of one month of treatment was done through elevated plus maze (EPM) apparatus. Additionally, neurochemical assessment of CAF, VP and LZP effects on rat brain neurotransmitters were done by using Ultra-performance liquid chromatography (UPLC). The results demonstrated the anxiogenic action of caffeine in rats receiving a high dose of CAF (100 mg/kg), as indicated by a significant increase ($p < 0.001$ ): in the number of entries (29 ± 5.5) and the duration of stay (292 ± 67) in the closed arms of the EPM compared to CONT group (4 ± 1.4) and (22 ± 5), respectively. After 30 days of treatment, VP led to anxiolytic effects in EPM behaviour. A pronounced anxiolytic effect was observed in rats administered with different doses of VP (10 mg [VP1], 20 mg [VP2] and 30 mg [VP3] /kg/day; p.o.), as compared to CONT and LZP groups. The concentration of serotonin (5-HT) in cingulate gyrus of VP2 (5.45 ± 0.05), VP3 (6.06 ± 0.02) and LZP (5.1 ± 0.01) groups was significantly decreased ($p < 0.001$) compared to the CAF group (9.45 ± 0.18) which may indicate that the VP at moderate and high doses has a negative correlation with the concentration of 5-HT and acts as anxiolytic agent. In the hippocampus, VP has significantly increased GABA concentration in VP1 (1010.14 ± 9.4), VP3 (1043.92 ± 18.73) and LZP (1093 ± 3.35) compared to CAF group (910.56 ± 18.32, $p < 0.01$). The concentrations of EPI in VP2 (8.64 ± 0.79) & LZP (8.41 ± 0.03) in frontal cortex were significantly decreased ($p < 0.001$) compared to the CAF group (10.33 ± 0.03), which may explain the anxiolytic effects of VP and LZP on a behavioural study. On the basis of the results obtained in the present study, it is concluded that long-term administration of VP at different doses presented anxiolytic-like activity on EPM test at least via 5-HT, GABA and EPI.

# خلاصة البحث

إنَّ أمراض القلق تزداد في جميع المجتمعات وخاصة المتقدمة منها. إنَّ الكافيين (CAF) الموجود في القهوة والشاي ومشروبات الطاقة وغيرها هي من المواد التي تؤدي الي تفاقم وزيادة وتيرة أعراض القلق. القنبوستين(VP) هو مشتق اصطناعي من نبتة البرونكل والتي تستخدم بشكل واسع في علاجات الأمراض العصبية .حاولت هذهِ الدراسة قبل السريرية أثبات أنَّ مادة القنبوستين قد تقلل من أعراض القلق أو أنَّ فعاليته تشبه الى حد كبير مادة اللورازيبام (LZP) . وقد استخدم نموذج الفئران القلقة عن طريق حقنها بمادة الكافيين في الغشاء البريتوني. وبالإضافة الى ذلك قد اجريت دراسة لتقييم تأثير القنبوستين على تراكيز النواقل العصبية في الدماغ باستخدام تقنية الكروموتوغرافيا السائلة فائقة الدقة. لقد أظهرت النتائج أنَّ أستخدام الكافيين في جرعة ١٠٠ ملغم /كيلو يُسبب القلق في النموذج الحيواني كما هو واضح من الزيادة الكبيرة في عدد الدخول (29 ± 5.5) والبقاء (292 ± 67) في الذراع المغلق من المتاهة ($p<0.001$) مقارنة مع مجموعة السيطرة (4 ± 1.4) و (22 ± 5) ، على التوالي . بعد ثلاثين يوم من العلاج بمادة القنبوستين كانَ هُناكَ تأثير واضح على جهاز المتاهة العالية. ولوحظ وجود تأثير مزيل للقلق واضح في الفئران التي عولجت بجرعات مختلفة من VP (10 [VP1]، 20 [VP2] و 30 [VP3] ملغم /كغ / يوم) ، مقارنة مع مجموعات السيطرة و LZP. فيما يخص قياس تراكيز النواقل العصبية ،كانَ تركيز هرمون السيروتونين في مجموعة VP2 (5.45 ± 0.05) ، VP3 (6.06 ± 0.02) و LZP (5.1 ± 0.01) في منطقة التلفيف الحزامي قد قلَّ بشكل واضح ($p<0.001$) مقارنةً مع مجموعة CAF (9.45 ± 0.18) والتي قد تشير إلى أن القنبوستين في جرعات معتدلة وعالية له ارتباط سلبي مع تركيز 5-HT ويعمل كعامل مزيل للقلق. زاد القنبوستين تركيز GABA في قرن آمون في المجموعات المعالجة VP1 (9.4± 1010.14) ، VP3 (1043.92 ± 18.73) و LZP (1093 ± 3.35) مقارنة بمجموعة CAF (910.56 ± 18.32). بينما انخفضت تراكيز EPI في VP2 (8.64 ± 0.79) و LZP (8.41 ± 0.03) في القشرة الأمامية بشكل ملحوظ ($p <0.001$) مقارنة مع مجموعة CAF (10.33 ± 0.03) ، مما قد يفسر تأثيرات مزيل القلق من VP و LZP على دراسة السلوك. واستنادا الى النتائج التي تم الحصول عليها في هذهِ الدراسة نستنتج ان الاستخدام المزمن للقنبوستين في جرعات مختلفة أظهرت تأثير مضاد للقلق على جهاز المتاهة وعلى الأقل عن طريق تأثيره على السيروتونين وحامض الغاماامينوبتيورك أسد والأبنفرين.

# APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

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# DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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# ACKNOWLEDGEMENTS

First of all, I thank Allah, the Almighty, for giving me the strength to carry on this project and for blessing me with many great people who have been my greatest support in both my personal and professional life.

An utmost pleasure to dedicate this work to my dear parents, my husband (Sinan) and my kids (Abdul Rahman, Mariam, Mayar and Abdul Aziz), who granted me the gift of their unwavering belief in my ability to accomplish this goal: thank you for your support and patience.

Respected thanks to Asst. Prof. Dr. Wan Mohd. Azizi Bin Wan Sulaiman for his continuous support, encouragement and leadership, and for that, I will be forever grateful. Very thankful to my co-supervisors Assoc. Prof. Dr. Marwan Saad Azzubaidi for his guidance, advice and motivation.

A Special gratitude to Sinan Mohammed Abdullah Pharmacy (Iraq) which funded this research, Ref: PG 1/1/2013.

Here, I wish to express my appreciation and thanks to those who provided their time, effort and support for this project. To the members of my thesis committee, thank you for sticking with me.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| APA | American Psychiatric Association |
| AS | Acute stress |
| 5-HT | Serotonin |
| AOO | Age-of-onset |
| CAF | Caffeine |
| cGMP | Cyclic guanosine monophosphate |
| $C_{max}$ | Maximum observed concentration |
| CRF | Corticotrophin-releasing factor |
| DOPAC | Dihydroxyphenyl acetic acid |
| EPI | Epinephrine |
| EPM | Elevated plus maze |
| FDA | Food and Drug Administration |
| GABA | Gamma-aminobutyric acid |
| GAD | Generalised anxiety disorder |
| i.p. | Intraperitoneal injection |
| LZP | Lorazepam |
| MAOI | Monoamine oxidase inhibitor |
| MHPG | 3-methoxy-4-hydroxyphenethyleneglycol |
| MMP | Mitochondrial membrane potential |
| NAS | Neuroactive steroids |
| NTs | Neurotransmitters |
| OCD | Obsessive-compulsive disorder |
| PD | Phobic disorder |
| PTSD | Post-traumatic stress disorder |
| QoL | Quality of life |
| SEM | Standard error of the mean |
| SNRIs | Serotonin-norepinephrine reuptake inhibitors |
| SP | Substance P |
| SSRIs | Selective serotonin reuptake inhibitors |
| TCAs | Tricyclic antidepressants |
| $t_{max}$ | Time of maximum observed concentration |
| UPLC | Ultra-performance liquid chromatography |
| VP | Vinpocetine |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

Anxiety disorders, compounded by modern-day sedentary and high-stress lifestyles, are becoming ever more prevalent. All around the world, the burden of anxiety disorders, such as acute stress (AS), generalised anxiety disorder (GAD), obsessive-compulsive disorder (OCD), panic disorder, phobic disorder (PD) and post-traumatic stress disorder (PTSD) are on the rise. The affected person finds the worries to control, and this can result in decreased occupational and social functioning (Cape et al., 2011). Besides, it has long been known that stress increases the risk of drug abuse (Steckler, Kalin, & Reul, 2005).

Anxiety and depression are common in youths, and each is associated with substantial functional impairment and future mental health problems (e.g., substance use, bipolar disorders). Anxiety and depression often co-occur, and their comorbidity is common in children and adolescents. Among youths with depression, anxiety disorders are the most common comorbid mental health disorders, with comorbidity estimates ranging from 15% to 75% (Cummings, Caporino, & Kendall, 2014).

With anxiety, various neurotransmitter and hormone levels change immediately. In particular, monoamines, such as epinephrine, norepinephrine and serotonin (5-HT), which are involved in mood, stress and other physical homeostasis. In the mammalian brain, 5-HT and norepinephrine mainly regulate stress and negative mood and their dysfunctions cause various mood disorders, such as social anxiety disorder and depression (Yamada, Yamada, Okano, Terashima & Yokogoshi, 2009). The

GABAergic system represents a promising future target of new pharmacological strategies to treat anxiety. The GABAergic system has been widely demonstrated to regulate cognitive function and emotional behaviour. Consequently, most of the anti-anxiolytic effects of benzodiazepines are due to increases in GABAergic neurotransmission. In rodents, direct modulation of the GABAergic system reduces anxiety-like reactivity (Ibarra, Feuillere, Roller, Lesburgere & Beracochea, 2010).

Since caffeine (CAF), the world's most commonly consumed psychostimulant, stimulates the CNS, it has been postulated that the rise of excessive coffee-drinking cultures has contributed to the rise of anxiety disorders (Wang, Woo, & Bahk, 2015). For the majority of people, the appeal for caffeine is most likely due to the caffeine-induced increase in alertness and arousal. They experience arising from caffeine's ability to block adenosine receptors. Adenosine through $A_1$ and $A_{2A}$ receptors exert anxiolytic effect through its facilitatory influence on release of Gamma-amino butyric acid (GABA) in the septum and hippocampus (Maximino, Lima, Olivera, Picanço-Diniz, & Herculano, 2011). Caffeine displays high affinity for A1 and $A2_A$ adenosine receptors, which antagonises the effects of endogenous adenosine (Daly & Fredholm, 1998). In those people who are sensitive to caffeine, high or even moderate doses can result in dysphoric and anxiety experiences (Childs, Hohoff, Deckert, Xu, Badner & De Wit, 2008).

During the late 1960s, the alkaloid vincamine was isolated from the leaf of Vinca minor plants, from which the synthetic derivative, vinpocetine (VP), was created. Since 1978, the compound has been marketed as Cavinton and has been available in Europe, where its use in managing cerebrovascular based pathologies is widespread (Bereczki & Fekete, 1999).

Vinpocetine's mechanism of action in central nervous system-related diseases is by selectively blocking the voltage-sensitive sodium ($Na^{+}$) channels, leading to a dose-dependent drop in the evoked extracellular calcium ($Ca^{2+)}$ at the terminals of striatal nerves (Sitges, Galván & Nekrassov, 2005). The compound also selectively inhibits $Ca^{2+}$-calmodulin-dependent cGMP-phosphodiesterase; this results in the intracellular levels of cGMP in vascular smooth muscle to become elevated, which in turn lowers the resistance in cerebral blood vessels, promoting cerebral blood circulation (Hagiwara, Endo & Hidaka, 1984).

## 1.2 STATEMENT OF THE PROBLEM

Problems that justify the study are:

- Anxiety disorders are increasing in all societies, especially developed ones.
- Caffeine consumption in coffee, tea and other beverages may aggravate and increase the frequency of anxiety symptoms.
- There are several significant disadvantages associated with the anxiolytic medications currently available:
    1- They can result either in retrograde or anterograde amnesia.
    2- They can produce tolerance as a major problem during the course of treatment, which necessitates increasing the dose to obtain the same effect.
    3- They result in physical dependence, as with most benzodiazepines.
    4- Long-term studies of vinpocetine did not present symptoms of physical dependence. Promoting the prospect of vinpocetine being a safe anxiolytic alternative to benzodiazepines, especially for those who are most vulnerable to abusing drugs.

Therefore, it is still important to look for alternative drugs that can solve anxiety problems without creating the adverse effects outlined above.

## 1.3 RESEARCH OBJECTIVES

General Objective:

To evaluate the anti-anxiety effects of vinpocetine in the rat model.

Specific Objectives:

1- To evaluate the anxiolytic effect of lorazepam and vinpocetine on behavioural changes in rats.
2- To assess neurochemical changes in anxiolytic rats in response to vinpocetine.
3- To compare the behavioural and neurochemical effects of vinpocetine with that of lorazepam in rats.

## 1.4 RESEARCH QUESTIONS

Is it possible for vinpocetine antagonises caffeine-induced anxiety?

If it does, which CNS neurotransmitters might be involved in vinpocetine's action?

## 1.5 RESEARCH HYPOTHESES

1- Caffeine i.p. injection will stimulate anxiety in rats and will be detected experimentally on EPM apparatus and through brain tissue analyses.
2- Vinpocetine may antagonise (pharmacologically or physiologically) caffeine-induced anxiety.

## 1.6 SIGNIFICANCE OF THE STUDY

Although vinpocetine is well known for its perceived memory boosting capability, this study represents the first research testing the potential anxiolytic effects of the drug. Should this preclinical study successfully demonstrate that vinpocetine alleviates anxiety symptoms or that its effectiveness is sufficiently similar to lorazepam, then the following important advances to treating anxiety will be considered:

1. A compound that simultaneously minimises anxiety and promotes memory will be important (because present anxiolytics are prone to causing anterograde or retrograde amnesia).
2. The literature indicates that the long-term use of vinpocetine does not lead to tolerance. This advantage is considerable, as the majority of benzodiazepines produce significant tolerance issues during the course of treatment.
3. In contrast to benzodiazepines, long-term studies of vinpocetine did not present symptoms of physical dependence. Promoting the prospect of vinpocetine being a safe anxiolytic alternative to benzodiazepines, especially for those who are most vulnerable to abusing drugs.

## 1.7 DEFINITIONS OF TERMS

### 1.7.1 Anxiety

This is a broad term that covers a number of disorders that manifest as apprehension, fear, nervousness and worry. The psychological and physiological symptoms associated with anxiety influence behaviour and feelings; at best, mild anxiety is unsettling but severe presentations can be enormously debilitating and significantly impinge upon a person's day-to-day life (Zafar & Majid, 2016).

### 1.7.2 Anxiolytic agent

This category of drugs is used to treat anxiety and manage disorders related to anxiety. Typically, they are fast-acting drugs but have the disadvantage of being abused, as they are habit-forming. Consequently, they are generally prescribed for short-term use only and are unsuitable for anyone with a history of drug addiction or substance abuse (Lader, 2015).

### 1.7.3 Elevated plus maze (EPM)

The EPM is a behavioural assay that has been devised for rodents. The assay has been validated and considered suitable for assessing anxiolytic drugs and steroid hormones. It has also been validated for defining the regions of the brain that are involved in anxiety-related behaviours and their supporting mechanisms, which include limbic regions, hippocampus, amygdala and dorsal raphe nucleus (Walf & Frye, 2007).

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 INTRODUCTION

It has become widely accepted that anxiety disorders become more common in recent years. With that acknowledgement has come the recognition that the burden of illness presented by anxiety disorders is frequently significant. The aetiology is complex and incorporates numerous factors including biological, psychological and social, each of which is modulated by various protective and risk factors (Spence & Rapee, 2016).

Epidemiological cross-cultural studies provide a rich supply of information about how these factors interact with each other. Despite being a topic of on-going research and the availability of effective interventions for anxiety disorders, the challenge anxiety presents to presenting public health programmes and services is enormous. Comparative epidemiology research has a critical role in informing health policy as it applies to anxiety; to be able to provide adequate services it is necessary to have empirical knowledge of the prevalence of anxiety disorders within a region. This knowledge also enables the most effective treatment interventions to be determined (Somers, Goldner, Waraich & Hsu, 2006). In Malaysia, more than 80 million USD is the estimated annual economic burden of anxiety. However, evidence suggests that primary care doctors in Malaysia struggle to diagnose and manage anxiety disorders. In addition, some studies show that psychological problems continue to be stigmatised in Malaysia (Wong, Shah, Teng, Lin, Majeed & Chan, 2016). Whilst there is a significant body of data available regarding anxiety disorders in Malaysia, there has yet to be a

coherent aggregation of the studies to identify the prevalence of anxiety disorders as a whole (Hanafiah & Van Bortel, 2015).

### 2.2 Epidemiology and prevalence

The global prevalence of anxiety disorders varies extremely between published epidemiological reports. Recent research has expanded its focus to Asian countries, an increasingly greater number of physical and psychiatric conditions, and traumatic events associated with anxiety. There is appreciably less variation with all anxiety disorders than there is with individual disorders. The rate of anxiety disorders is greater in women than in men. A number of features have been associated with heterogeneity of these rates, including the country of concern, diagnostic criteria, instruments used for diagnosis, response rate and sample size (Remes, Brayne, Linde & Lafortune, 2016).

Among the anxiety disorders recognised by the most recent version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) is GAD, OCD, PTSD, agoraphobia, panic disorder, social phobia and specific phobia. In classifying these diagnoses, the nosology recognises variants (e.g., panic disorder without agoraphobia) and subclinical presentations, such as anxiety disorders that do not meet specified diagnostic criteria. Whilst there are differences between these disorders in terms of severity and course, often the clinical presentations intersect and involve adrenergic symptoms, prominent anxiety, apprehension and fear (APA, 2013).

Studies in the general population of Malaysia showed that the prevalence of generalised anxiety disorder was 0.4–5.6%, mixed anxiety and depression were 3–5%, panic without agoraphobia 0.4%, phobia unspecified 0.5–1%, and anxiety not-otherwise-specified 0.3–6.5%. In addition, there is a significant variability in anxiety disorders in the selected population groups. The variability may be affected by

methodological factors within each study for example questionnaire design and sample size (Wong et al., 2016).

GAD is linked to a notable reduction in function and is associated with a range of comorbid medical and psychiatric conditions, which can be severe. The degree of debilitation attributed to GAD is comparable to major depressive disorders and other psychiatric disorders. Whilst less common than GAD, the extent of impairment presented by anxiety disorders, such as agoraphobia and OCD, can be considerable. It is thought that a sizeable portion of the population experience subclinical anxiety disorder or symptoms of anxiety. Whilst typically mild, the level of disruption to normal function and quality of life is still detectable. These 'worried well' patients (those with subclinical anxiety) can present a considerable strain on health care resources as they seek support in the primary care setting (Grigsby, Anderson, Freedland, Clouse & Lustman, 2002; Patriquin & Mathew, 2017).

### 2.3 Persistence

In epidemiological studies, Age-of-onset (AOO) and prevalence tend to receive much more attention than progression. Disorder persistence can frequently be determined by computing the ratios of recent-to-lifetime prevalence; this is possible because the estimates of recent and lifetime prevalence are frequently reported together. Usually the range of 12-month to lifetime prevalence ratios are 4–6 with GAD having the lowest ratios and specific phobias the highest (Kringlen, Torgersen & Cramer, 2001). The implication of high ratios is that anxiety disorders are fairly persistent throughout an individual's lifespan. The findings of the limited long-term longitudinal studies available indicate that the persistence of anxiety in sufferers runs an erratic, waxing and

waning course, with different comorbid anxiety disorders following a recurrent-intermittent course (Bruce et al., 2005; Wittchen, Kessler, Pfister, Höfler & Lieb, 2000).

### 2.4 Aetiology of anxiety disorders

The first stage in determining the cause of anxiety disorders is to consider whether it is attributable to pre-existing or unrecognised conditions. A common diagnosis that is overlooked is substance-induced anxiety, brought on by over-the-counter medications, herbal remedies or illicit substance abuse. It appears there may be a genetic predisposition, with particular gene factors increasing the risk of developing a number of anxiety disorders (Tambs et al., 2009). As for example, a highly significant association between panic disorder, phobias, and a duplication at chromosomal region 15q24-26 is one of the most exciting findings to date (Morris-Rosendahl, 2002). On the other hand, panic disorder appears to be a genetically inherited neurochemical dysfunction that may involve autonomic imbalance; decreased GABA-ergic tone (Zwanzger et al., 2009).

### 2.5 Role of neurotransmitters in anxiety (pathophysiology)

The neuroanatomic circuits that support anxiety and fear responses are modulated by a number of neurotransmitters, including GABA, adenosine, melatonin and neuroactive steroids, all of which have an anxiolytic function. In contrast, acetylcholine, cholecystokinin, corticotrophin, glutamate and serotonin are anxiogenic. Cannabinoids and substance P have both anxiety promoting and anxiety relieving capabilities (Kaur & Singh, 2017).

### 2.5.1 Gamma-aminobutyric acid (GABA)

The GABAergic system represents a promising future target of new pharmacological strategies to treat anxiety (Domschke & Zwanzger, 2008). The GABAergic system has been widely demonstrated to regulate cognitive function and emotional behaviour (Menzies, Kamath, Suckling, McKenna, Fletcher & Stephenson, 2007). Consequently, most of the anti-anxiolytic effects of benzodiazepines are due to increases in GABAergic neurotransmission. In rodents, direct modulation of the GABAergic system reduces anxiety-like reactivity (Ibarra et al., 2010 ; Zhang & Cranney, 2008)

Of all the various NTs that inhibit the CNS, the most abundant and significant is gamma-aminobutyric acid (GABA). This neurotransmitter has long been considered key to regulate anxiety, hence is the prime target of benzodiazepines and other anxiolytic medications (Lydiard, 2002).

Inhibition shapes brain activity, with GABA being the main modulator of excitability. Neuronal activity is determined by the aggregate of excitatory (largely glutamatergic) and inhibitory (mainly GABAergic) inputs. When GABA predominates, amnesia, ataxia and sedation ensue. Also, one animal study showed the mildest reduction in GABAergic activity compared to the control group led to arousal, anxiety, exaggerated reactions, insomnia and restlessness (Nemeroff, 2003).

Evidence for anxiety states being underpinned by GABA dysfunction comes from the anxiolytic action of drugs that act on GABA receptors. Benzodiazepines, gabapentin, pregabalin, tiagabine, valproate and vigabatrin have all demonstrated anxiolytic effects at the clinical level (Sandford, Argyropoulos & Nutt, 2000).