EVALUATION OF THE PHARMACOKINETICS OF KETAMINE FOR THE TREATMENT OF MAJOR DEPRESSIVE DISORDER

BY

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EVALUATION OF THE PHARMACOKINETICS OF KETAMINE FOR THE TREATMENT OF MAJOR DEPRESSIVE DISORDER (MDD)

Vivian Campbell Naidoo

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A thesis submitted to the School of Health Sciences, College of Health Sciences, University of KwaZulu-Natal, for the degree of Master of Pharmacy in Pharmaceutical Chemistry.

This is the thesis in which the chapters are written as a set of discrete research publications that have followed the Journal of Pharmaceutical and Biomedical Analysis format with an overall introduction and final summary. Typically, these chapters will be published in internationally recognized, peer-reviewed journals.

This is to certify that the contents of this thesis is the original research work of Miss Vivian Campbell Naidoo, carried out under supervision, at the Catalysis and Peptide Research Unit, Westville campus, University of KwaZulu-Natal, Durban, South Africa.

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ABSTRACT

Recent reports have demonstrated ketamine's potential use in the treatment of major depressive disorder (MDD), as it elicits potent antidepressant effects via a different mechanism compared to conventional antidepressants. Ketamine's hypothesized antidepressant effect is elicited by a neurochemical cascade involving the antagonization of the N-methyl-D-aspartate (NMDA) receptors and the subsequent activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors; resulting in the disinhibition of glutamate signalling due to the suppression of tonic glutamate input into the GABAergic interneurons, providing rapid symptomatic relief as opposed to the two-week delay with conventional treatments. There is a large escalation in the number of individuals being diagnosed with treatment resistant depression (TRD) even after numerous trials on conventional antidepressant therapy. Health care professionals are now resorting to unconventional treatments, such as ketamine's off-label use, to achieve therapeutic outcomes and provide symptomatic relief. MDD's increasing prevalence has been associated with significant public health costs and morbidity rates and therefore alternative, effective treatments are now essential. Many reports have been published on the intranasal (IN) efficacy of ketamine in the treatment of major depressive disorder, however there have been no studies investigating the effects on the route of administration in drug delivery to the brain.

The purpose of this study was to investigate pharmacokinetics of ketamine following oral, intraperitoneal and intranasal administration. A dose of 15mg/kg (body weight) was administered to healthy male Sprague-Dawley rats, and ketamine concentrations were quantified in both plasma and brain tissue homogenates at time intervals of 5, 15, 30, 60, 120, 240 minutes post-treatment.

The results showed that with intraperitoneal administration, concentrations of 524,58 ng/mL and 352,06 ng/mL, were achieved in plasma and brain tissue, respectively. Surprisingly, IN administration which is believed to favour drug delivery to the brain only exhibited moderate levels post administration; whereas, oral administration produced significantly lower levels due to extensive first-pass metabolism of ketamine in the liver and intestines.

These results show that parenteral administration should be used for the administration of ketamine in the treatment of MDD. The findings of the study provide a platform for future investigations assessing alternative routes of administration of ketamine; and its use in clinical practice for the treatment of MDD. This paves the way forward to optimize treatment and

provide symptomatic relief were conventional antidepressants have failed those suffering with MDD.

DECLARATIONS

DECLARATION 1- PLAGIARISM

I, **Vivian Campbell Naidoo** declare that

1. The research reported in this thesis, except where otherwise indicated, is my original work.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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    a. Their words have been re-written but the general information attributed to them has been referenced.
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5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

LIST OF PUBLICATIONS

Evaluation of the intranasal Pharmacokinetics of Ketamine as a potential route of administration for the treatment of Major Depressive Disorder (MDD)

Vivian C. Naidoo, Sipho Mdanda, Sphamandla Ntshangase, Tricia Naicker, Hendrik G. Kruger, Thavendran Govender, Panjasaram Naidoo, Sooraj Baijnath

Submitted to:

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Contributions:

Vivian C. Naidoo: developed and validated a liquid chromatography-mass spectrometry method for the simultaneous quantification of Ketamine. Evaluated the *in vivo* pharmacokinetics of Ketamine in plasma and brain tissue of male Sprague dawley rats through oral, intranasal and intraperitoneal administration; and also contributed in the writing this manuscript.

Sphamandla Ntshangase, and Sipho Mdanda: provided technical supported and assisted with the writing of the manuscript

Sooraj Baijnath: provided assistance technically and experimentally and supervised the overall study.

Panjasaram Naidoo, Tricia Naicker and Hendrik Kruger co-supervised this study.

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LIST OF ABBREVIATIONS

MDD	Major Depressive Disorder
DLPFC	dorsolateral prefrontal cortex
TRD	Treatment Resistant Depression
TCAs	Tricyclic Antidepressants
CYP450	Cytochrome P450
MAOIs	Monoamine Oxidase Inhibitors
SSRIs	Selective Serotonin Reuptake Inhibitors
SNRIs	Serotonin Noradrenaline Reuptake Inhibitors
NMDARs	N-methyl-D-aspartate receptors
AMPARs	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid Receptors
LTP	long-term potentiation
LTD	long-term depression
PFC	Prefrontal cortex
GABA	Gamma-Aminobutyric Acid
BDNF	Brain-derived Neurotrophic factor
Mtor	Mammalian Target of Rapamycin
IN	Intranasal
IM	Intramuscular
PO	Oral
MS	Mass Spectrometry
IP	Intraperitoneal
IV	Intravenous
ESI	Electrospray Ionization
MALDI	Matrix Assisted Laser Desorption/Ionization
HPLC	High-Performance Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
MSI	Mass Spectrometry Imaging
TOF	Time-Of-Flight
NE	Norepinephrine
5-HT	Serotonin

THESIS OUTLINE

This project consists of:

- **Chapter One**, the introduction to the use of ketamine for the treatment of major depressive disorder and theory behind the instrumentation used for this study.
- **Chapter Two**, the results of this study which was submitted for publication, and demonstrate the pharmacokinetics of ketamine through intranasal, oral and intraperitoneal administration. The LC-MS method for the simultaneous quantification of ketamine in rat plasma that was developed.
- **Chapter Three,** A summary of the work done, which is inclusive of limitations and conclusions of the potential use of ketamine in major depressive disorder.

CHAPTER 1: INTRODUCTION

1.1 MAJOR DEPRESSIVE DISORDER (MDD)

Major Depressive Disorder (MDD) is an incapacitating mental disease, with a lifetime incidence of 16.2% [1, 2]. It is predicted to be amongst the leading causes of the global disease burden in the industrialized world by 2020 , compromising the overall quality of everyday life in affected individuals [1,2]. The symptoms of MDD are hypothesized to arise from a combination of environmental, inherent and genetic factors [4, 5]. Major Depressive Disorder is characterized by abnormalities in mood and affect, contracted interest, impaired cognition, vegetative symptoms such as insomnia and weight loss, in addition to diminished psychomotor activity; with episodes usually lasting for two weeks or more [1, 4, 5]. Diminished cognitive capacity is a fundamental endo-phenotype of MDD [8]. The disruptions in cognition have been attributed to well documented abnormalities in the dorsolateral prefrontal cortex (DLPFC), hippocampus and medial temporal lobe of MDD patients [9]. In a study conducted on animals subjected to various stressors for a period of time, atrophy of the hippocampal regions of the brain were observed which is similar to neurological changes seen in patients with depression [10]. Impairment of hippocampal functioning, as well as the capability for neuroplasticity could contribute to several of the pathophysiological characteristics of MDD [11, 12].

A small population of patients with MDD may experience manic episodes which are characterized by euphoria, hyperactivity and pleasure seeking in comparison to unipolar depression [10, 11]. Treatment approaches consist of both psychological and pharmacological interventions, as well as social and lifestyle modifications [15]. The current diagnostic criteria for MDD involves clinical and historical background on the more prominent symptoms and signs experienced by the patient, however affected individuals exhibit an extensive variety of clinical symptoms and signs making diagnostic conventions subjective [16]. Major Depressive Disorder is a heterogenous psychological disease with no definitive causation; however, gender, specific personality characteristics, stressful life circumstances, genetics and negative childhood experiences are risk factors closely associated with the incidence of MDD [4, 9, 11]. The correlation between a dysregulated stress response or chronic stress accompanied by behavioral and molecular changes that exhibit a depressive state has become increasingly more evident [11].

A significant percentage of patients with MDD experience their first episode during childhood and adolescence [16]. In these circumstances these individuals continue to experience recurring episodes during adulthood [16]. Approximately 20%-25% of individuals with MDD

experience a chronic unremitting course and may also exhibit significant deficits in memory, necessitating the need for long-term prophylactic treatment [4, 9].

The pathophysiology of depression remains an elusive topic; however, studies suggest the involvement of serotonergic, dopaminergic and noradrenergic pathways [4]. Serotonergic and noradrenergic receptor systems are located deep in the brain and spread out over almost the entire brain [13]. These systems are hypothesized to be responsible for modulating thought, behavior and emotions [13]. Inhibition of the reuptake of these neurotransmitter pathways at the synapse results in an increase in postsynaptic neuron stimulation; resulting in the development of antidepressants targeting either one or all monoamines [19]. These findings led to the construction of the monoamine-deficiency hypothesis (Figure 1), which suggests that the primary symptoms of depression are due to insufficient levels of monoamine neurotransmitters, notably norepinephrine (NE) and serotonin (5-HT) in the brain [9, 12, 13] . These depletions could result from abnormalities in synthesis, metabolization, storage and reuptake processes; or receptor abnormalities irrespective of normal monoamine levels [1]. While this theory has provided extensive insight in the treatment of MDD; studies suggest that MDD surpasses monoaminergic depletion as its primary pathophysiological mechanism [1]. Therefore, recent studies suggest other mechanisms that contribute to the development of MDD; such as neurotrophins, gene expression and synaptic plasticity[1, 2]. Synaptic plasticity is the dynamic ability of the synapse generation and retraction processes; therefore modulating synaptic communication and strength [22]. Decreased synaptic plasticity and neurogenesis has a significant role in the hypothesized pathophysiology of MDD [22].

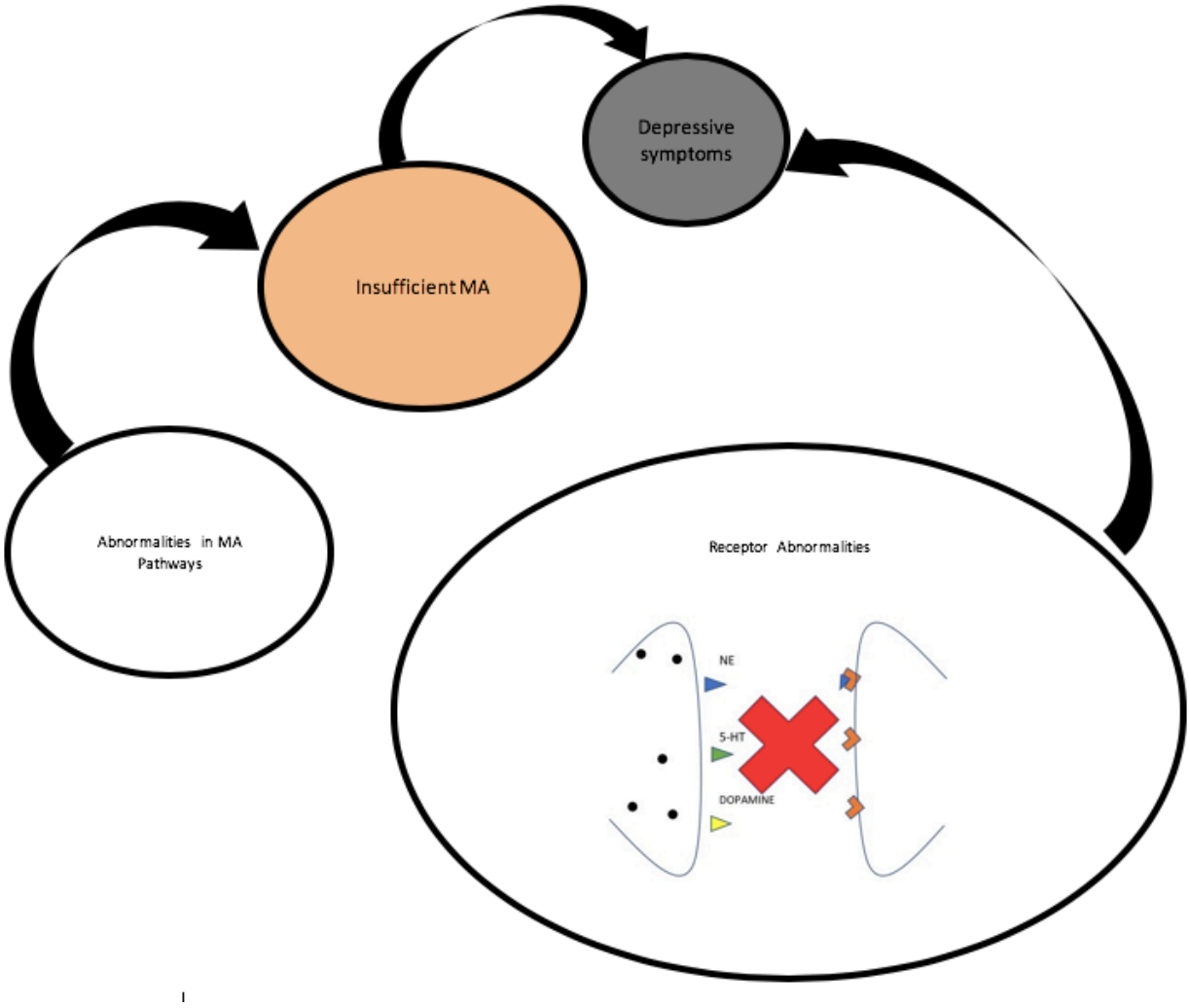


Figure 1: Depression caused by Neurotransmitter Deficiency. NE:norepinephrine, 5-HT: Serotonin, Dopamine neurotransmissions is hindered due to receptor abnormalities preventing post-synaptic binding.

1.2 CURRENT ANTIDEPRESSANT TREATMENTS

Successful treatment of depressive symptoms in patients with treatment resistant depression [TRD] has been a challenge for almost 100 years, in which many treatment regimens and other practices have been employed in an attempt to provide sufficient relief and regulations of depressive symptoms [1]. The somatic treatment of depression began in the 1920s and since then many advancements have contributed to treating depression [1].

1.2.1 Tricyclic Antidepressant (TCAs)

Tricyclic Antidepressants were founded in the 1950s and named, based on their structures (Figure 2); TCAs has provided a platform for the development of later drugs for the treatment of depression [1]. The mechanism of action of TCAs is complex but its therapeutic action is primarily by inhibition of the serotonergic, dopaminergic and noradrenergic pathways reuptake [1,13]. However, they do have anticholinergic activity, α1-adrenoceptor blockade and

antagonism of the histamine (H1) receptor [23]. A comprehensive understanding of the exact neurochemical mechanism remains elusive; as TCAs produce a variety of biochemical effects [24]. TCAs are rapidly absorbed following oral administration and bind extensively to plasma albumin at therapeutic concentrations [25]. They also bind to extravascular tissue which may account for its relatively large distribution volumes [25]. The bioavailability varies among the different TCAs, with amitriptyline, imipramine and protriptyline ranging between 30-60%, 30-75%, 76-90% respectively [26]. The use of TCAs for the treatment of depression is associated with low acceptability and tolerability due to their side effects and that is why they are less frequently prescribed nowadays [27]. Common side effects experienced with the use of TCAs is sexual dysfunction, dizziness, drowsiness, cardiovascular effects and more rarely behavioral (pacing, confusion, memory impairment, agitation) or cognitive toxicity [1, 9] Some TCAs such as amitriptyline are employed in the management of chronic pain syndromes; such as post-herpetic neuralgia, diabetic neuropathy, atypical facial pain and fibromyalgia [24]. Growing evidence of interaction with other drugs and especially those involving cytochrome P450 (CYP450) inhibition with TCAs necessitates careful consideration of the usefulness of TCAs in the treatment of depression, especially in relation to newer drugs [25]. However, findings in a meta-analyses states that TCAs are more effective in the treatment of severe depression than SSRI's due to their dual action on serotonin and norepinephrine receptors as opposed to the selective nature of SSRI's [28]. Therefore, TCAs still have their place in treatment of depression, the disadvantages and risks associated with their use have been exaggerated when compared to newer drugs [25].

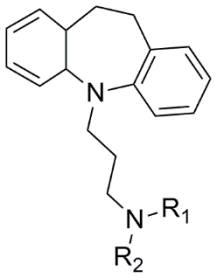


Figure 2: General Structure of Tricyclic Antidepressants

1.2.2 Monoamine Oxidase Inhibitors

Monoamine Oxidase is an enzyme that is found in two isoforms, A and B; with isoform Subtype A being involved in affective disorders due to its higher affinity for noradrenaline and serotonin [29]. Monoamine Oxidase Inhibitors are classified based on their selectivity for an isoform; and the reversibility of enzyme inhibition [30]. Significant side effects are associated

with the use of MAOIs as they cause inhibition of enzyme activity resulting in non-specific increases in synaptic monoamines, therefore caution is necessary with their use [31].With older drugs strict dietary restrictions are required especially with tyramine or tryptophan rich foods and circumvention of serotonergic drugs, as concomitant use with MAOIs may cause serotonin syndrome or a hypertensive crisis ('cheese reaction') , due to the sudden increase in monoamine transmitter availability and impaired synaptic clearance with the enzyme inhibition [32]. Reversible MAOIs such as Moclobemide (Figure 3), selectively inhibit isoform A of the enzyme; resulting in an increase in serotonin and noradrenaline without markedly affecting the degradation of tyramine [30]. Non-selective MAOIs such as Tranylcypromine act by irreversible inhibition of the enzyme; resulting in an increase in biogenic amines such as noradrenaline, serotonin and dopamine in both the periphery and brain [30]. This type of MAOIs are not used frequently because of its higher affinity of leading to a hypertensive crisis when combined with certain foods and medicines [30]. Monoamine Oxidase Inhibitors are therefore reserved for the treatment of TRD, atypical depression and specific forms of bipolar depression [33].

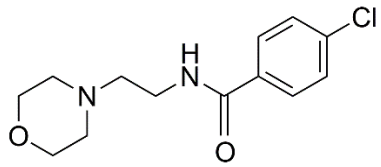


Figure 3: Molecular Structure of Moclobemide

1.2.3 Selective Serotonin Reuptake Inhibitors (SSRIs)

The development of SSRIs has transformed psychiatric therapy; making them the most frequently prescribed antidepressants of today [1]. Its working mechanism involves the blocking of serotonin re-uptake at pre-synaptic neurons by inhibition of membrane serotonin transporter proteins [30]. Upon repeated dosing SSRIs are able to preserve their selective nature and potent inhibition of serotonin reuptake [34]. SSRIs effectiveness in the treatment of depression does not vary to that of TCAs; however, they are preferentially used due to their unequivocally superior tolerability and safety, due to fewer anticholinergic and cardiovascular effects [19, 22]. Their advantageous tolerability and safety profile plays a significant role in the improvement of compliance and is consequently cost effective in the long run [36]. However, even though SSRIs exhibit superior clinical benefits the structural diversity of this group results in a variety of potential side effects such as sleep deprivation, sexual dysfunction and more seriously suicide ideation, drug interactions and variations in pharmacological and pharmacokinetic profiling [1, 33]. Likewise, Fluoxetine (Figure 4) has a much longer half-life

when compared to other SSRIs, resulting in side effects and drug interactions that may potentiate, long after, treatment has ceased [36].

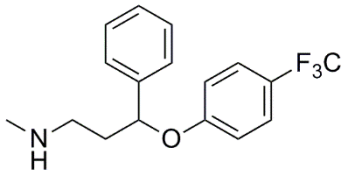
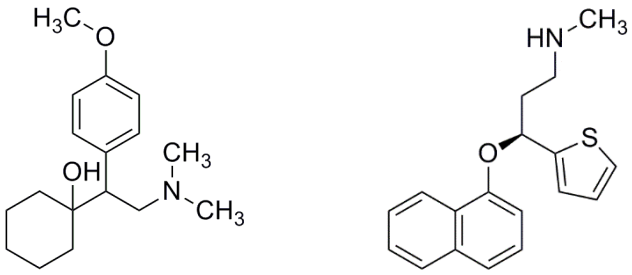


Figure 4: Molecular Structure of Fluoxetine

1.2.4 Alternative treatments

Serotonin Noradrenaline Reuptake Inhibitors (SNRIs) such as Venlafaxine and Duloxetine (Figure 5), activity is based on dual reuptake inhibition as it blocks the reuptake of noradrenaline and serotonin by inhibiting their transporter proteins [37]. These agents have a higher tolerability and safety profile that is comparable to that of SSRIs due to their chemical specificity [37].



Venlafaxine Duloxetine

Figure 5: Serotonin Noradrenaline Reuptake Inhibitors

Tetracyclic antidepressants such as Maprotiline and Mianserin (Figure 6) are closely related to tricyclic antidepressants; sharing a lot of properties as well as limitations with their use in the treatment of depression, especially cardiovascular complications [38]. However, Mianserin does not inhibit the reuptake of noradrenaline when compared to their tricyclic counterparts; but works by blocking presynaptic alpha receptors, enhancing the turnover of noradrenaline in the brain [30].

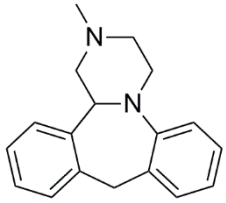


Figure 6: Molecular Structure of Mianserin

Noradrenergic and specific serotonergic agents such as Mirtazapine (Figure 7), act centrally by causing presynaptic alpha antagonization; subsequently increasing central noradrenergic and serotonergic neurotransmission [30].

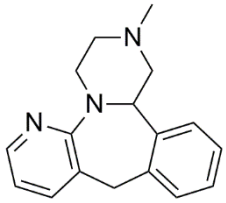


Figure 7: Molecular Structure of Mirtazapine

Serotonin Re-uptake Inhibitors and receptor antagonist (Trazodone (Figure 8)) does not influence the reuptake of noradrenaline peripherally but rather indirectly facilitates neuronal release [30]. Its primary activity is based on the inhibition of the reuptake of presynaptic serotonin as well as serotonin 5HT1 activity [30].

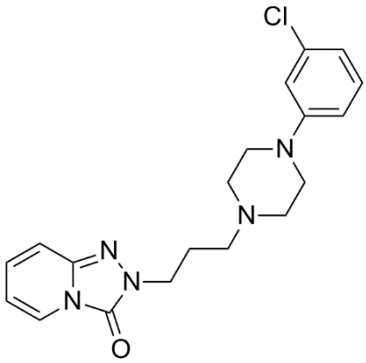


Figure 8: Molecular Structure of Trazodone

Dopamine Re-uptake Inhibitors (Bupropion (Figure 9)) mechanism of action is primarily by inhibition of dopamine reuptake with weak serotonin and noradrenaline reuptake inhibition. Bupropion is more commonly utilized to aid smoking cessation [30].

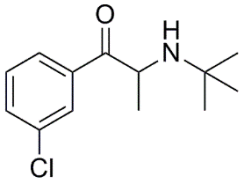


Figure 9: Molecular Structure of Bupropion

1.3 KETAMINE

Ketamine (RS-2-2-Chlorophenyl-2-methylamincyclo-hexanone) is clinically approved to be used as an anesthetic and analgesic; however, there is growing off-label use of ketamine in the treatment of depression at a sub-anesthetic dose, particularly in TRD [7,8]. Ketamine (Figure 10) is primarily utilized in veterinarian pharmacotherapy and occasionally in paediatrics [1]. It can be administered intranasally via snorting or inhaling as a powder, orally, and by intravascular or intramuscular injection [1]. A single infusion of ketamine sufficiently relieves patients that are symptomatic within hours; and is effective for an extensive period, however, the duration of the antidepressant effects of ketamine varies across studies with most patients experiencing relief for 72 hours or longer [7, 10]. In a trial on the antidepressant effects of ketamine, most patients experienced mood elevation approximately 120 minutes post- infusion administration of ketamine [40].

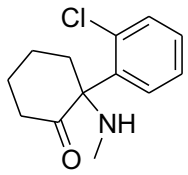


Figure 10: Molecular Structure of Ketamine

1.3.1 Ketamine's use in Depression

Approximately two-thirds of patients with MDD do not receive sufficient relief from conventional antidepressant treatments; and as many as one-third of these patients remain untreated after numerous trials of antidepressants [15]. Patients diagnosed with Treatment Resistant Depression (TRD) are defined as being unresponsive to two or more trials of antidepressants [22]. Given the heterogenous pathophysiology of MDD a number of environmental, patient specific characteristics, and the nature of the depression, contributes to how the patient responds to treatment [40]. Patients with TRD experience extensive debilitation in social, economic and personal aspects, and are more likely to exhibit suicide ideation [15]. Moreover, the two week delay that is associated with the use of conventional antidepressants is of particular concern in patients with continuous suicidal ideation [22]. Thus, the pressing need for the development of novel treatment regimens which provide effective relief of

symptoms and at the same time maintain antidepressant therapeutic effects; this partly accounts for the use of ketamine in the treatment of depression [15].

The current hypothesized mechanism of action of ketamine in the treatment of depression is based on a neurochemical cascade. Ketamine antidepressant effect is produced via the antagonization of the N-methyl-D-aspartate receptors (NMDARs) and the subsequent activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs). N-methyl-D-aspartate receptors are widely distributed throughout the cortical and subcortical areas in the brain [1]. Synaptic plasticity and neurogenesis depletion have a significant influence on the pathophysiology of MDD. Synaptic plasticity is a biological process in which specific patterns in synaptic activity results in the modifying of the communication and strength of the synapse based on its ability to retract and form [22]. Plasticity is most commonly mediated by long-term potentiation (LTP) and long-term depression (LTD) mechanisms that involve extensive alterations in both pre- and post-synaptic glutamate receptors (primarily glutamatergic receptors such as NMDA and AMPA) and scaffolding proteins [22]. Long-term potentiation is associated with the GluR1, GluR4,and GluR2 subunits of the AMPA receptor whereas the GluR2, GluR3, and GluR4 subunits are involved in internalization of the receptor, required for LTD [22]. During the LTD the NMDA receptors at the excitatory synapses are significantly reduced and subjected to trafficking [22].

Ketamine antagonizes the pre-synaptic NMDARs signalling which results in the disinhibition of glutamate signalling due to the suppression of tonic glutamate input into the GABAergic interneurons [22]. A decrease in GABAergic inhibitory feedback of the pyramidal neurons that are in the Layer V of the Prefrontal cortex (PFC) mediates the subsequent increase in glutamate due to the antagonization of the NMDARs [22]. Glutamate is the most prevalent excitatory neurotransmitter that is found in the subcortical and cortical parts of the brain; it is an amino acid that is produced from glutamine via the glutaminase enzyme and released usually from voltage-gated calcium channels [1, 38]. Glutamic acid decarboxylase converts glutamate into an inhibitory neurotransmitter known as Gamma-Aminobutyric Acid (GABA) whereas glutamine synthetase reduces glutamate to glutamine [1]. Glutamate effects are antagonized by the activity of glutamate transporter proteins at the synapse; which triggers the reuptake and metabolism of the amino acid, maintaining extracellular homeostasis [1]. Glutamate homeostasis plays a significant role in maintaining synaptic plasticity hence the necessity for the pathway to be closely regulated [1]. Too little glutamate results in an impairment in synaptic plasticity, where as too much glutamate could result in excito-toxicity and oxidative

damage[1]. Glutamate is involved in many processes regarding emotion and cognition [42]. This enhanced glutamate signaling due to pre-synaptic NMDARs antagonization results in the subsequent activation of the AMPARs and the consequential cell depolarization activates the voltage sensitive calcium channels [43]. This results in the influx of calcium and Brain-derived Neurotrophic factor (BDNF) exocytosis. BDNF stimulates the Tropomyosin Receptor Kinase B (TrkB) as well as other signalling pathways downstream such as the MEK-Erk1/2 PI3K-Akt pathways. These pathways lead to the activation of the Mammalian Target of Rapamycin (mTOR) complex 1 via phosphorylation [43]. Subsequently this activates the Ribosomal protein S6 kinase (p70S6K) which results in the inhibition of Eukaryotic elongation factor-2 kinase (eEF2K), stunting eEF2 phosphorylation and consequentially inhibiting it [43]. Simultaneously translation repressor protein 4E-binding protein 1 (4E-BP1) is hyperphosphorylated by mTOR, diminishing the interaction with Eukaryotic translation initiation factor 4E (eIF4E) [43]. The combination of the release of eIF4E from 4E-BP1 and decreased phosphorylation of eEF2 results in the disinhibition of protein translation resulting in enhanced production of synaptic proteins such as PSD95, Arc, synapsin I, BDNF and GluR1 [43]. This enhances synaptogenesis and dendritic spine density in the PFC and hippocampus as these proteins are significantly involved in the function, formation and maturation of new spine synapses; therefore producing anti-depressant-like behaviour [43]. Ketamine also regulates other neurotransmitter pathways, such as the striatal and nucleus accumbens release, GABAergic activity, serotonin and noradrenaline transporter proteins and dopamine [1]. This neurochemical cascade leading to the antidepressant effects of ketamine is illustrated in Figure 11.

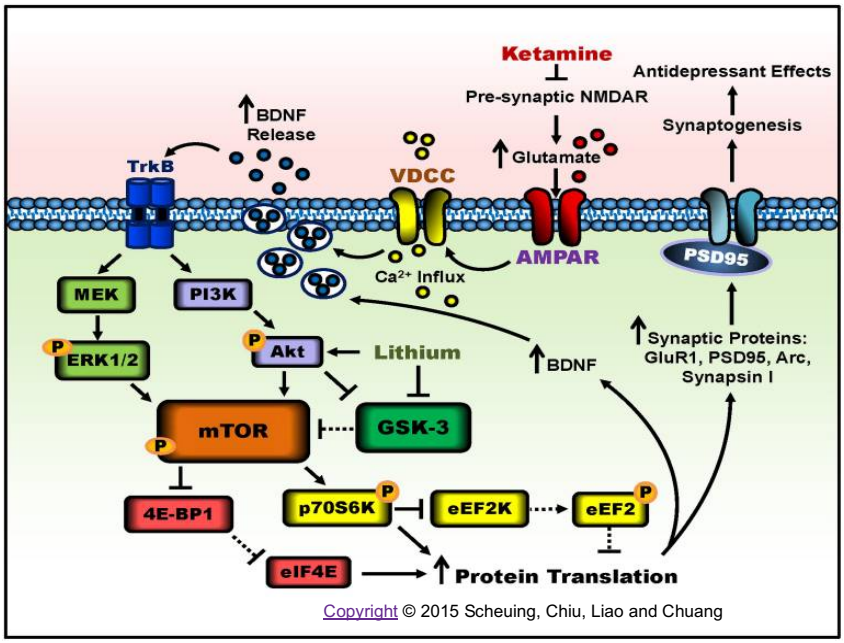


Figure 11: Diagrammatic representation of the mechanism of action of Ketamine. Putative signalling pathways results in NMDAR signalling resulting in subsequent glutamate release which activates post-synaptic AMPAR resulting in a neurochemical cascade eliciting antidepressant activity.

1.3.2 Pharmacokinetics and bioavailability of ketamine

Numerous factors can influence the pharmacokinetic profile of a drug, including age, gender, nutritional status, environmental conditions and disease states [41, 42]. Ketamine exhibits a chiral structure that comprises of two optical isoforms [46]. The S(+)-enantiomer is the preferred form due to it being four times more potent that the R(-)-enantiomer; and twice as much more effective than ketamine's racemic mixture [46].

Ketamine is metabolised primarily to Nor-ketamine in the body by cytochrome P450, 3A and 2B6 enzymes via oxidative metabolism; with approximately 90% excreted *via* the kidneys after extensive hepatic metabolism [30–32].

Oral bioavailability of ketamine is reduced significantly due to the extensive first-pass metabolism of ketamine in the body; therefore, other routes of administration such as sublingual and intranasal formulations are being developed [46]. Intranasal (IN) and intramuscular (IM) formulations of ketamine produce maximum plasma concentrations rapidly post-administration, with comparatively high bioavailability [30, 32]. In a randomized controlled trial of intranasal administration of ketamine it was found that patients had significant improvements in symptoms and tolerability after 24-hours of ketamine administration [49]. Findings in a study on healthy human volunteers at an analgesic dose, showed that the absorption of ketamine following IM administration is rapid with a

bioavailability of 93% [50]; whereas only 17% was absorbed following oral administration, due to extensive first pass metabolism [50].

Ketamine is well distributed and rapidly absorbed in the central nervous system [44]. The half-life of ketamine varies in different species and is also dependant on age and route of administration. In a study conducted on Sprague-Dawley rats the half-life in young rats was approximately 1.3 hours whereas in aged rats 8.5-13 hours following IP administration of 125mg/kg in three divided doses; indicating that drug clearance is markedly reduced with age [44]. Subsequently, the stagnation in clearance results in enhanced drug availability which is reflected by the AUC [44].

1.4 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Following the initial studies conducted by J.J. Thomson (1912), Mass Spectrometry (MS) has undergone numerous improvements; and has revolutionized the analysis of non-volatile and volatile compounds [48, 49]. Mass spectrometry's distinctive characteristics has made it one of the most exceptional and important analytical techniques used today for the determination of elemental concentrations, surface and isotope analysis and for the analysis of organic and bioorganic compound structures, due to its diversity of applications, speed, unsurpassed sensitivity, small sample volume analysis and low detection limits [35, 36].

Mass Spectrometry was limited to thermostable and smaller compounds for a long time due to the deficiency of effective methods to softly ionize molecules and transfer these molecules from the condensed phase to the gaseous phase without unnecessary fragmentation [54]. In the late 1980s the development of two techniques, Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption/Ionization (MALDI) for the systematic and general construction of molecular ions of intact biomolecules made it possible to analyze polypeptides by mass spectrometric methods [55]–[57].

This mainstream chemical analysis techniques uniqueness is attributed to its ability to directly detect molecules based on the mass-to-charge ratio as well as compound fragmentation patterns [58]. Firstly, the analyte of interest is introduced into the system and transferred to the source, in which ionization takes place [59]. Mass Spectrometry is more commonly associated with the separation of species that are electrically charged (ions) when produced by the ion source in a gas phase [41, 42]. However, in some instances the ion source further assists the transfer of liquid-phase or solid-phase analytes into the gaseous phase [58]. Once in the gaseous phase the ions are successively transferred into the mass analyzer; in which they are sorted (in time

or space) based on their mass-to-charge ratios (m/z) [36, 42]. The ion detector subsequently detects the separated ions in the relative domain (space or time), producing electrical signals that are processed to generate a mass spectrum which may correspond to the original analyte, their fragments and other species produced during the ionization process [58].

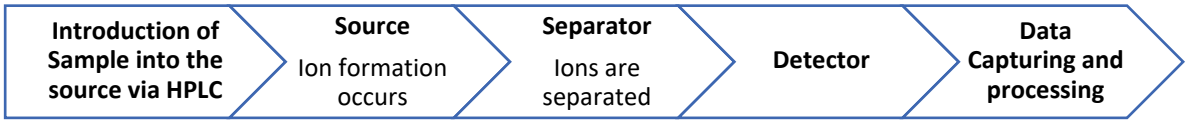


Figure 12: Diagram illustrating the workflow of MS (Adapted from [53] *)*

The development of electrospray ionization mass spectrometry (ESI-MS) as an ion source has contributed significantly to quantitative MS [41, 44]. Combined with High-Performance Liquid Chromatography (HPLC) it has become a useful tool for the analyses of various molecules in biological samples [61]. The ESI utilizes electrical energy to aid in the transfer of ions from the liquid phase into the gaseous phase prior to mass spectrometric analysis [62]. The process of the transfer of the ions into the gas phase occurs by (1) the dispersion of a fine spray of charged droplets which is followed by (2) solvent evaporation and (3) ion ejection from the charged droplets under high voltage relative to the enclosing chambers walls [61]. A fine mist of highly charged droplets is generated with a polarity that is the same to the capillary tube. The utilization of a nebulizing gas such as nitrogen enhances the sample flow rate [62]. The highly charged droplets exit the electrospray tip and move down a gradient (potential and pressure) towards the mass analyzer [62]. The charged droplets undergo size reduction when subjected to the ESI source temperature and nitrogen drying gas resulting in an increase in surface charge density and a reduction in the droplet radius. The generated electric field strength within the droplets make it possible for ions to be discharged into the gaseous phase [62]. However, ESI, is just one of the ionization techniques utilized in analytical techniques.

Liquid Chromatography-Mass Spectrometry (LC-MS) is a technique that is now routinely used, especially when coupled with ESI [63]. This technique can be applied to a variety of biological samples and with the use of tandem MS and stable internal standards it enables the development of accurate and extremely sensitive assays, accompanied with method optimization [63]. The LC systems coupling with MS has always been desirable due to the high sensitivity and specificity nature of MS in comparison to other chromatographic detectors [63].

A typical LC-MS system is the coupling of HPLC with MS; the sample is initially separated by LC, these separated species are thereafter introduced into the MS system [64]. In the HPLC system, solvent is pumped under high pressures through a column that is filled with solid adsorbent materials, allowing for separation of the analyte into different constituents and degrees based on its affinity for the columns material [65]. The sample is introduced into the system either by the autosampler or direct injection into the mobile phase stream that permeates through the column [64]. The different constituents of the sample travel at different speeds; as the velocity of the analyte is dependent on the compound's nature and the composition of the mobile phase [48, 49]. The time at which the analyte elutes or emerges from the column is called the retention time. Various columns are available, loaded with various absorbents that vary based on molecule size, nature of their surface and chemical constituents [66]. The composition of the mobile phase may remain constant (isocratic elution mode) or changed (gradient elution mode) during the analysis. The choice of the mobile phase is dependent on the partiality of the analyte with the mobile phase and the stationary phase within the column [65].

Components of a LC-MS system

The solvent reservoir contains the mobile phases that are used during the analysis. In HPLC the mobile phase is a mixture of non-polar liquid components and polar liquid components, depending on the chemical composition of the sample [65]. The sample injector injects the analyte into the mobile phase stream, in which a pump, suctions the mobile phase from the reservoir and forces it towards the column under high operating pressures. The pressure at which the pump operates is dependent on the column dimensions, flowrate and composition of the mobile phase and particle size. [46, 48] The sample is thereafter introduced into the column which is typically stainless steel, loaded with a stationary phase. Separation can be either in the reverse phase or normal phase mode of adsorption [64]. Normal phase separation involves a polar stationary phase and a non-polar mobile phase; whereas, reverse phase separation involves a non-polar stationary phase and a polar mobile phase [64]. The analyte is introduced into the mass spectrometric detection system in which it is ionized and thereafter a data collection device or integrator, receives signal from the detector producing a chromatogram [65].

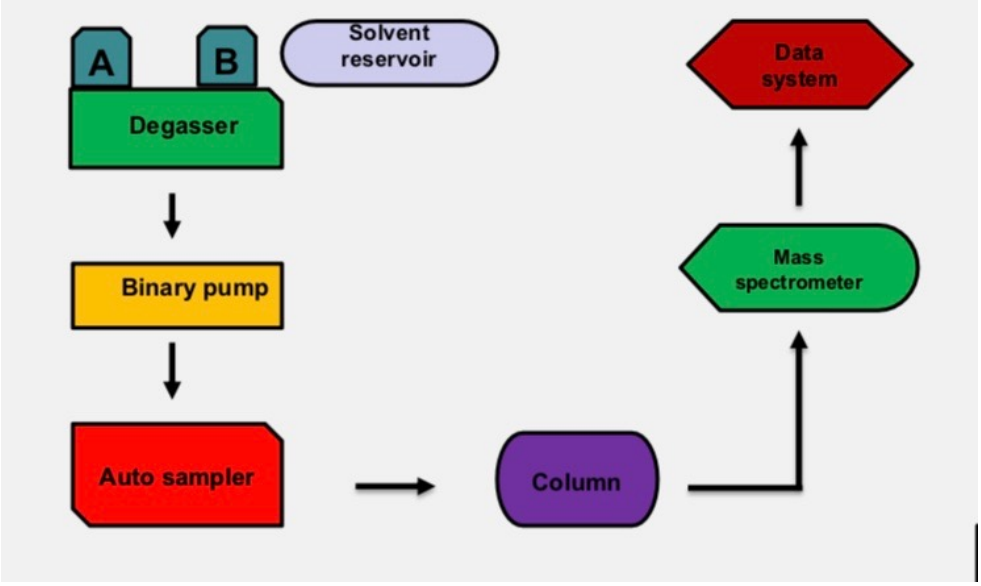


Figure 13: LC-MS Instrumentation ***(Adapted from*** [64] ***)***

Rationale for the Study

Quantification of ketamine in brain tissue and plasma enables health care professionals to develop treatment regimens that promote symptomatic relief, especially in patients with treatment resistant depression (TRD). The suffocating impact MDD has on the public health system and the progression to other mental conditions necessitates the development of more effective treatment regimens. The findings of this study will improve the understanding on the use of Ketamine for the treatment major depressive disorder for future clinical use.

1.5 AIMS AND OBJECTIVES

The aim of this project was to quantify ketamine concentrations in rat plasma and brain tissue using LC-MS in order to understand it's clinical use for the treatment of Major Depressive Disorder.

The following objectives of the study were:

1. The fundamental objective of the study is the quantification of ketamine in brain tissue and plasma using LC-MS.
2. To determine tissue specific (brain) pharmacokinetics of Ketamine using LC-MS after intranasal, intraperitoneal and oral administration to determine the best route for effective treatment of MDD.
3. To establish if these concentrations are adequate to enable more effective treatment of MDD and are sufficient for adequate symptom relief and regulation.

CHAPTER 2

EVALUATION OF THE INTRANASAL PHARMACOKINETICS OF KETAMINE AS A POTENTIAL ROUTE OF ADMINISTRATION FOR THE TREATMENT OF MAJOR DEPRESSIVE DISORDER (MDD)

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ABSTRACT

Ketamine is approved by the FDA to be used as an anesthetic however, recent reports have exhibited its success in the treatment of major depressive disorder (MDD). Studies have suggested that a sub-anesthetic dose produces rapid antidepressant activity providing significant symptomatic relief particularly in patients with a history of treatment resistant depression (TRD). Many reports have been published on the intranasal (IN) efficacy of ketamine in the treatment of depression, however studies that have investigated the effects of the route of administration on drug delivery to the brain appear to be absent in literature. Therefore, in this study, a single dose (15mg/kg body weight) was administered *via* different routes of administration [oral (PO), intranasal (IN) and intraperitoneal (IP)] to healthy male Sprague-Dawley rats in order to determine the brain tissue pharmacokinetics of ketamine. A novel validated liquid chromatography-mass spectrometry (LC-MS) method was developed using a fused core column for the determination of ketamine in plasma and brain homogenates. While IP administration resulted in favorable concentrations in the brain and plasma; IN administration, which is supposed to favour drug delivery to the brain, exhibited moderately low drug levels post administration. PO administration produced significantly lower levels due to extensive first-pass metabolism in the liver and intestines. These results have implications for future studies exploring the use of ketamine for the treatment of MDD in order to optimize treatment regimens and suggest that parenteral administration of ketamine should be used in the treatment of depression.

Keywords: Major Depressive Disorder, Ketamine, Antidepressant, Liquid Chromatography

1| INTRODUCTION

In the 1960s, ketamine (RS-2-2-Chlorophenyl-2-methylamincyclo-hexanone) was discovered to have analgesic and anesthetic effects with the potential to produce hallucinogenic and dissociative symptoms in individuals; however, recent studies have exhibited intranasal ketamine's rapid and long lasting anti-depressive effects in patients with major depressive disorder (MDD) and more especially in those diagnosed with treatment resistant depression (TRD) [7], [39], [67], [68]. MDD's increasing prevalence has been associated with significant public health costs and morbidity rates [4], [69]; the heterogeneity of this clinical disorder results in variations in symptoms experienced by individuals ranging from pervasive, low moods and loss of interest accompanied with more classical symptomology such as loss of appetite, sleep pattern alterations and diminished psychomotor and cognitive functions [1], [7],

[70]. The suffocating impact on the public health system arises as a result of the disorder having a greater propensity to originate in adulthood and continue as a chronic or recurrent condition into the later stages of life; diminishing the overall quality of life of those that are affected; and often resulting in devastating outcomes such as suicide ideation [70].

Approximately two-thirds of patients with Major Depressive Disorder do not receive satisfactory relief from conventional antidepressant treatments; and as many as one-third of these patients are categorized as treatment resistant; and remain untreated after numerous trials of antidepressants [15]. In an attempt to resolve this escalating resistance to conventional antidepressants, medical practitioners have resorted to the off-label use of ketamine for the treatment of MDD [40].

A single dose of ketamine provides symptomatic relief within hours; and is effective for an extensive period, however, the duration of the antidepressant effects of ketamine fluctuates across studies and is dependent on the route of administration [15], [40]. In a trial on the antidepressant effects of ketamine, most patients experienced mood elevation approximately 120 minutes post- infusion of ketamine [40]. Studies have also shown that a sub-anesthetic dose of ketamine (0.5mg/kg) over a 40-minute IV infusion produces rapid antidepressant activity in those diagnosed with TRD [71]. Ketamine's hypothesized antidepressant effect is elicited by a neurochemical cascade involving the antagonization of the N-methyl-D-aspartate (NMDA) receptors and the subsequent activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors; resulting in the disinhibition of glutamate signalling due to the suppression of tonic glutamate input into the GABAergic interneurons [1], [22].

The route of administration of ketamine significantly affects its efficacy in the treatment of MDD [71]. Oral bioavailability of ketamine is significantly reduced due to the extensive first-pass metabolism of ketamine in the body; therefore, other routes of administration such as sublingual and intranasal formulations are being investigated [46]. Intramuscular (IM) formulations of ketamine produce maximum plasma concentrations rapidly post-administration, with comparatively high bioavailability [46], [48]. In a randomized controlled trial of intranasal administration of ketamine it was found that patients had significant improvements in symptoms and tolerability after 24-hours of intranasal ketamine administration [49]. Ketamine is generally well tolerated, however, its use may be limited due

to its potential to cause psychedelic (panic attacks, hallucinations, memory defects), cardiovascular, neurological and hepatoxic side effects [72], [73].

Currently, several trials have been conducted to determine the analgesic, anaesthetic and antidepressant effect of ketamine; however, the quantification of ketamine in the brain in order to elicit it's antidepressant activity remains elusive [50], [67], [74]. In addition to several reports that have been published on the IN efficacy of ketamine in the treatment of depression, there have been no studies investigating the effects of the route of administration on drug delivery to the brain. Furthermore, considerable limitations occur in previously reported studies as methods employed for the determination of ketamine are costly and time-consuming; with extensively long analytical run-times [67], [75]. Therefore, the objectives of this study are to develop a rapid and sensitive method using a fused core column for the quantification of ketamine in rat plasma and brain tissue following to determine which route favours drug delivery to the brain.

2| MATERIALS AND METHODS

2.1| Chemicals and reagents

Ketamine HCL solution (100mg/ml, Fresenius Kabi) and Ketamine D-4 HCL (0.1mg/ml) (DLD Scientific, Durban, South Africa) were obtained. Ammonium Acetate (10mM, pH 9.5) and LC-MS grade methanol (MeOH) were all procured from Sigma Aldrich (Steinham, Germany). Analytical Grade formic acid was purchased from Merck Millipore, South Africa. Solid-Phase extraction cartridges were purchased from Supelco-Sigma (St. Louis, MO) Ultrapure water was purified using a Milli-Q water purification system (Bedford, MA, USA). All Chemicals utilized in the study were of analytical grade.

2.2| Chromatographic conditions

Separation was performed on an Ascentis® Express Biphenyl column (5cm x 2.1 i.d., 2.7 µm Particle size) with a Fused-Core® Particle design; and a gradient mobile phase changing from 50% Millipore water (0.1% v/v formic acid) (A) and 50% methanol (B). The flow rate was set at 0.4 mL/min with an injection volume of 5µL; and a total run time of 7 minutes.

2.3| Mass Spectrometry

An Agilent series 1100 (Agilent technologies, Waldbronn Germany) was coupled to a maXis 4G quadruple-time-of-flight Mass spectrometry instrument (Bruker Daltonics, Bremen, Germany). Results were analyzed on Bruker Data Analysis 4.0 SP 5.

Quantitative studies were conducted using MS via an ESI interface with the following settings: drying temperature, 200°C; nitrogen nebulizer gas 0.4 bar; end plate offset, -500V; capillary, 5500V and drying gas, 4.0 L/min. The collision energy was optimized to 12eV for Ketamine and the IS (ketamine D-4) to achieve the required selectivity and sensitivity for the method. The mass of Ketamine and the IS were m/z 238.1 and 242.1 respectively.

2.4| Animals

All experimental animal procedures involving handling and treatment were approved by the Institutional Animal Research Ethics Committee (AREC, UKZN) (approval Reference: AREC/003/018M). Fifty-four male Sprague-Dawley rats (weighing between 110-120g) were sourced from the University of Kwa-Zulu Natal Biomedical Resource Unit and were used to conduct the study. All animals where housed under appropriate ethical standards in a well-ventilated room with humidity control systems, a 12-hour light/dark cycle and the recommended enrichment.

2.5| Drug administration and sample collection

Animals (n=3 in each route of administration (ROA) per time point) were administered 15mg/kg body weight of ketamine-HCL via either intranasal (IN), oral (PO) or intraperitoneal route. Three animals per route were utilized as controls and received normal saline. Animals (*n*=3 per ROA) were euthanized at 0 (control|); 5; 15; 30; 60; 120; and 240-minutes post administration via IsoFor (Safeline Pharmaceuticals, South Africa) overdose. Blood samples were collected via cardiac puncture; and brain tissue surgically removed post termination. Brain tissue samples were frozen gradually with liquid nitrogen vapor; biological samples were stored at -80°C until analysis.

2.6| Method Validation

2.6.1| Calibration and quality control preparation

A primary stock solution of ketamine was prepared (10µg/10ml) in Ultrapure water and stored at -20°C. The lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC) and high-quality control (HQC) at 100ng, 250ng, 750ng and 2000 ng/ml were prepared from the stock; and spiked with IS at a concentration level of 750ng/ml. The calibration curve was prepared in triplicate (n=5) in a range of 100-2000 ng/mL.

2.6.2| Linearity and lower limit of quantification

The linearity of this method was evaluated by analyzing standard series of samples with concentrations ranging from 100 to 2000 ng/mL of ketamine in plasma and brain homogenates. Ketamine responses were established using internal standard peak area ratio and plotted against

the corresponding concentration of ketamine (expressed in ng/mL). The calibration curves were generated in Bruker QuantAnalysis (Bruker Daltonics, Bremen, Germany) using the least-square linear regression method. The benchmark for the calibration range includes, the accuracy and precision of ketamine's calibration to fall within ±15% deviation (n = 6) as stipulated in the EMA guidelines for Bioanalytical Method Development as well as a correlation coefficient of $r \geq 0.99$.

2.6.3| Accuracy, precision and recovery

Four QC levels were analyzed in order to determine the intra- and inter-day accuracy and precision parameters. The satisfactory percentage recovery standards and limit of variation on precision and accuracy should be within 15% of nominal values of the QC samples, LQC, MQC, HQC. In Accordance with the EMA guidelines the LLOQ (n = 6) should fall within 20% of the nominal value.

2.6.4| Stability Testing

Due to the delay in injection of extracted samples, the stability of ketamine was investigated at the LQC, MQC and HQC under different conditions i.e bench-top stability for 6 hours at room temperature, autosampler stability for 24 hours and after three freeze-thaw cycles (-80°C).

2.6.5| Sample Preparation

Samples were prepared using 100 µl of biological sample (plasma/ brain homogenate) that was obtained from treated animals and were spiked with 7.5 µl of IS. 892,5µl of Ammonium Acetate (10mM, pH 9.5) was thereafter added to the analyte mixture consisting of IS and biological sample. The samples were mixed vigorously by a vortex mixer for 1 minute and then centrifuged at 10 000 rpm for 15 minutes at 4°C. Solid phase extraction (SPE) was performed using a C_{18}-50mg, C_{18}-100mg, Hydrophilic-Lipophilic-Balance and Hybrid Phospholipid Cartridges (HLB) (Sigma Aldrich, Germany). Briefly, cartridges were preconditioned with 1ml of MeOH and ammonium acetate followed by the sample. The cartridges were washed with ultrapure water and analyte was collected with MeOH in an autosampler vial for analysis. The same procedure was followed in the construction of calibration curves of the biological matrices; in which recoveries were investigated at three-quality control (QC) levels; the low quality control (LQC), middle-quality control (MQC) and high-quality control (HQC). The QC levels were 100, 250, 750, 2000 (ng/ml or ng/g) for plasma and brain homogenate.

3| RESULTS AND DISCUSSION

This LC-MS method developed in this study was suitable for the quantification of ketamine in rat plasma and brain tissues. Chromatographic separation was achieved with a retention time of 1.0 min for the both the target analyte and the IS at m/z 238.1 and 242.1 respectively; with an optimized collision energy of 12eV for both Ketamine and the IS. Positive ion mode exhibited preferable signaling of the target analyte to that of the negative ion mode. Separation was performed on an Ascentis® Express Biphenyl column (5cm x 2.1 i.d., 2.7 µm Particle size) with a total run-time of 7 minutes. A recent study utilized a CHIRAL-AGP® MS (2.0 mm i.d x 15cm ., 5 µm Particle size) column for the chiral separation of ketamine and its enantiomers, however with significantly a longer run-time of 25 minutes [75].

The calibration curves (n = 5) for quantification of ketamine maintained a linear range of 100 to 2000 ng/ml in both plasma and brain homogenates. The linear equation of ketamine in plasma was y=0.895679x + 0.324036 with a $R^2 \geq 0.998$; the brain homogenate linear equation was y=1.058604x + 0.462059 with a $R^2 \geq 0.999$. The lower limit of quantification was 100ng/mL which was determined by a S/N ≥5 and a limit of detection (LOD) of 10ng/mL for both brain homogenate and plasma.

The inter- and intra-day precision and accuracy in plasma and brain homogenates are presented in table 1 and 2. In plasma the intra- and inter-day precision ranged from 1,05 to 3,74%; whereas in brain homogenate it ranged from 0,65 to 2,20% with RSD values within the acceptable limits outlined by the EMA, with satisfactory percentage recovery standards and limit of variation on precision and accuracy falling within 15% of nominal values of the QC samples, LQC, MQC, HQC and the LLOQ falling within 20% of the nominal value.

Table 1: Intra- and inter-day precision and accuracy of ketamine in plasma and brain homogenate(n=3 days)

Plasma-Ketamine				
Quality control levels	**LLOQ**	**LQC**	**MQC**	**HQC**
Concentration ($ngmL^{-1}$)	**100**	**250**	**750**	**2000**
Mean	98,45	244,85	721,48	1906,34
Accuracy (%)	98,45	97,94	96,20	95,32
Inter-day precision (R.S.D., %)	2,03	2,05	1,28	2,32

Intra-day precision (R.S.D., %)	1,05	1,35	1,26	3,74
Brain-Ketamine				
Quality control levels	**LLOQ**	**LQC**	**MQC**	**HQC**
Concentration ($ngmL^{-1}$)	**100**	**250**	**750**	**2000**
Mean	95,94	241,95	730,73	1957,22
Accuracy (%)	95,94	96,78	97,43	97,86
Inter-day precision (R.S.D., %)	0,83	1,41	0,75	1,82
Intra-day precision (R.S.D., %)	2,20	1,35	0,65	1,80

Table 2: Recoveries of Ketamine (n=3) from plasma and brain homogenate

Plasma				
Concentration ($ngmL^{-1}$)	**100**	**250**	**750**	**2000**
	64,85	61,24	58,68	60,07
Recovery (%)	62,69	62,28	59,46	60,06
	65,71	62,90	59,98	59,45
Mean (%)	64,42	62,14	59,37	59,86
S.D. (%)	1,56	0,84	0,66	0,36
R.S.D. (%)	2,42	1,35	1,11	0,60
Brain Homogenate				
Concentration ($ngmL^{-1}$)	**5**	**60**	**500**	**1500**
	62,87	64,75	58,68	60,07
Recovery (%)	64,43	64,68	59,46	60,06
	65,38	64,70	59,98	59,45
Mean (%)	64,23	64,71	59,37	59,86
S.D. (%)	1,27	0,03	0,66	0,36
R.S.D. (%)	1,97	1,35	1,11	0,60

The autosampler, freeze-thaw and bench-top stability of Ketamine was evaluated at the LQC, MQC and HQC levels shown in Table 3. The results exhibit that the stability and recovery found in brain homogenate and plasma were within a range of 93,72-98,27% with a RSD of 0,49-4,24% for the evaluated conditions.

Table 3:Stability of Ketamine HCL in plasma and brain homogenate (n = 3)

Ketamine Concentration ng/mL						
Bench-top stability	added			**250**	**750**	**2000**
	Plasma	Found		241,35	737,06	1912,75
		Accuracy %		96,54	98,27	95,64
		RSD %		2,99	1,51	3,53
	Brain	Found		243,44	714,62	1926,72
		Accuracy %		97,37	96,34	96,34
		RSD %		2,07	3,25	3,25
Freeze-thaw stability	Plasma	Found		241,15	722,36	1896,22
		Accuracy %		96,46	96,31	94,81
		RSD %		3,24	3,30	3,62
	Brain	Found		234,29	715,43	1910,28
		Accuracy %		93,72	95,39	95,51
		RSD %		0,49	4,24	4,05
Autosampler stability	Plasma	Found		238,26	729,92	1895,71
		Accuracy %		95,30	97,32	94,79
		RSD %		0,78	2,47	2,29
	Brain	Found		238,81	712,38	1926,53
		Accuracy %		95,53	94,98	96,33
		RSD %		0,46	0,96	2,34

The Pharmacokinetic parameters of Ketamine (15mg/kg body weight), were determined post IN, PO and IP administration in rat plasma and brain homogenates (Table 1) and Figures 14 and 15. The pharmacokinetic parameters showed a C_{max} of 524,58; 121,07 and 109,11 ng/mL and an $AUC_{0\text{-}inf}$ of 13059,65; 6785,37 and 5436,58 in plasma within following IP, IN and PO administration respectively (Figure 14).

The purpose of this study was to compare the plasma and brain concentrations of ketamine following various routes of administration. The pharmacokinetic parameters showed a C_{max} of 352,06; 77,81 and 61,77 ng/g and an $AUC_{0\text{-}inf}$ of 10791,61; 2892,80 and 4060,70 in brain homogenates following IP, IN and PO administration, respectively (figure 15). Ketamine's

concentration in brain homogenate following PO and IN administration were below the limit of quantification of 100 ng/ml.

Pharmacokinetic profiles of ketamine demonstrated that absorption is rapid in both plasma and brain homogenates with IP administration producing significantly higher plasma and brain concentrations to that of PO and IN administration. Oral bioavailability of ketamine is low due to extensive first-pass metabolism in the liver and intestine which can be compared to previous studies [19, 20], therefore this effect can be largely responsible for the low levels of ketamine observed in plasma and brain samples. Malinovsky et al. (1996) found that ketamine's bioavailability in children was approximately 50% in plasma post IN administration and; therefore, the lowered ketamine levels following IN administration could be attributed to its moderate bioavailability [78]. This effect may be partly caused by substantial swallowing of relatively large intranasal depositions reducing its concentration in both the brain and plasma [76]. The C_{max} reached was higher and reached quickly in both plasma and brain followed by a rapid decline until undetectable levels of ketamine were present 120 minutes post-administration. It can therefore be concluded that IP administration exhibits more favorable concentrations in both brain tissue and plasma.

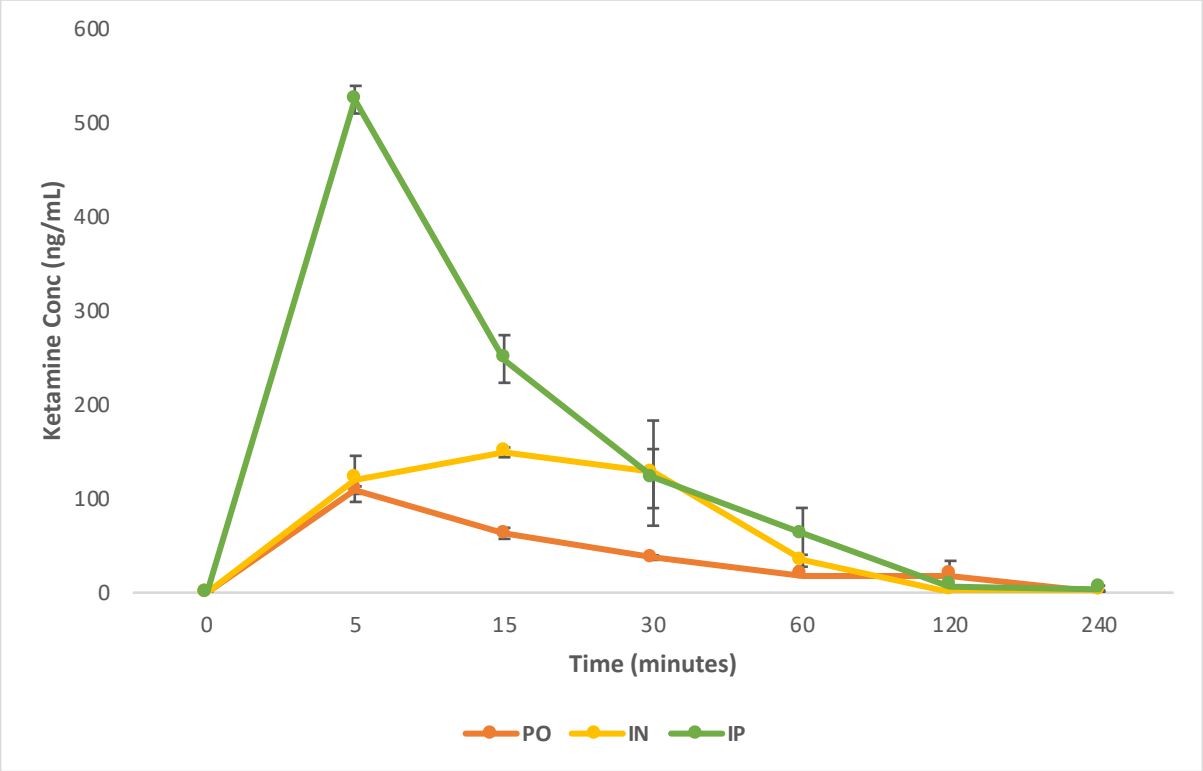

Figure 14:Concentration-time profile of Ketamine in plasma(ng/mL) following a single dose of 15mg/kg to male Sprague Dawley rats via PO, IP., and IN administration (n=3, mean ± SD)

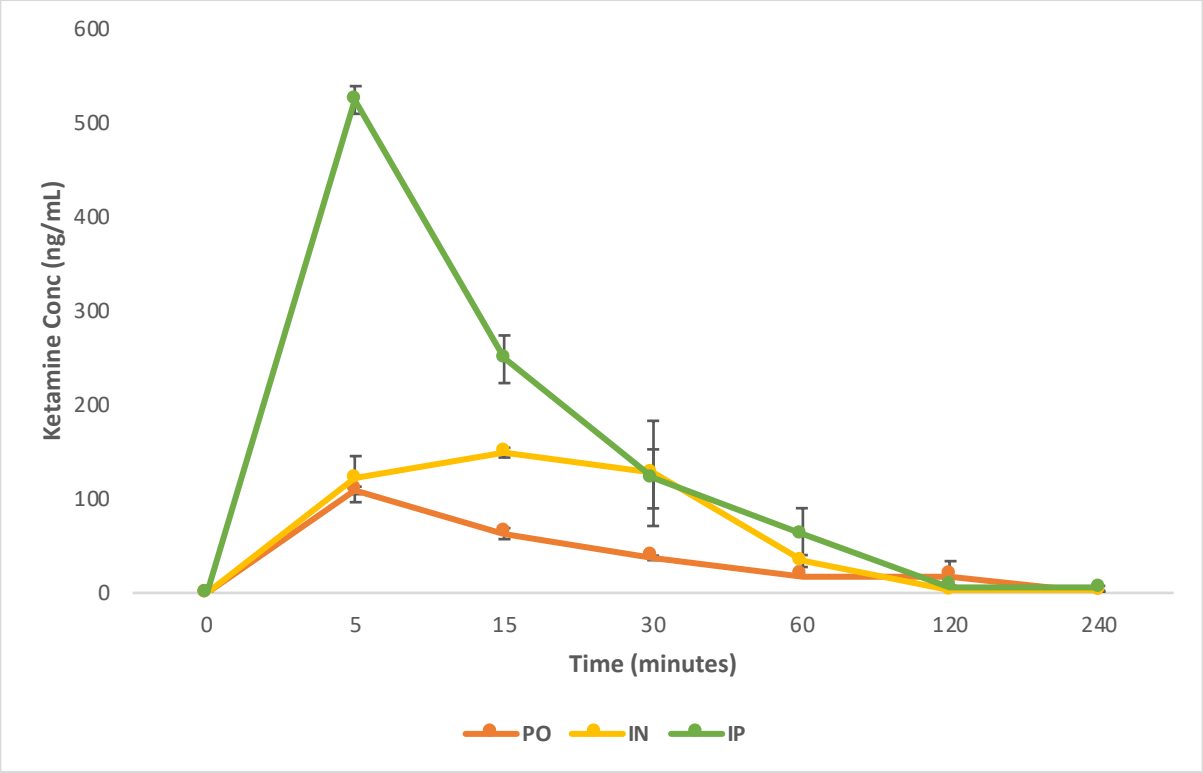

Figure 15:Concentration-time profile of ketamine in brain tissue(ng/g) following a single dose of 15mg/kg to male Sprague dawley rats via PO, IP, IN administration (n=3, mean±SD)

Table 4: A summary of the pharmacokinetic parameters of Ketamine following a single dose of ketamine of 15mg/kg b.w

Oral		
	Plasma	Brain
C_{max} (ng/mL or ng/g)	109,11	61,77
T_{max} (min)	5	5
$T_{1/2}$ (min)	2,5	2,35
K_{el}	0,0156	0,0060
$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	5436,576	4060,696
IP		
	Plasma	Brain
C_{max}±SD (ng/mL or ng/g)	524,58	354,06
T_{max} (min)	5	5
$T_{1/2}$ (min)	15	2,55
K_{el}	0,0126	0,0137
$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	13059,649	10791,611
IN		
	Plasma	Brain
C_{max}±SD (ng/mL or ng/g)	149,29	77,81
T_{max} (min)	15	15
$T_{1/2}$ (min)	2,85	2,6
K_{el}	0,0149	0,0083

$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	6785,367	2892,804

We developed a novel rapid and sensitive LC-MS method for the quantification of ketamine in rat plasma and brain homogenates in order to understand the effect of the route of administration on the brain concentration of ketamine for the treatment of major depressive disorder. We demonstrate that IN administration of ketamine is not be the ideal route of administration for drug delivery to the brain. However, future studies focused on the development of novel technologies that enhance its bioavailability may result in an easy to use IN formulation of ketamine for the treatment of MDD and TRD. Currently, parenteral administration of Ketamine exhibits more favorable brain drug delivery in order to produce satisfactory therapeutic outcomes when compared to IN and PO administration. Future investigations utilizing brain tissue imaging are required in determining the brain distributions of Ketamine and how this is beneficial in the treatment of MDD.

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Declaration of interest

The authors declare that they have no conflict of interest.

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CHAPTER 3: SUMMARY AND CONCLUSION

The purpose of this study was to investigate the pharmacokinetics of ketamine in rat plasma and brain following administration of the same dose via different routes (IN, PO and IP). Ketamine was recently found to have excellent antidepressant activity aside from its analgesic and anaesthetic use in clinical practice. Almost two-thirds of patients with MDD do not obtain sufficient relief from conventional antidepressant treatments; and as many as one-third of these patients are categorized as treatment resistant. These patients remain untreated after numerous trials of antidepressants. In an attempt to resolve this increasing resistance to conventional antidepressants, medical practitioners have resorted to off-label use of ketamine for the treatment of MDD. However, a noteworthy problem was identified, that the route of administration significantly affected the pharmacokinetics of ketamine, especially in the brain, and is therefore a crucial consideration that needs to be taken when stabilizing a patient on a treatment regimen in order to ensure that optimum therapeutic levels are attained at the drug desired site. Many reports have been published on the IN efficacy of ketamine in the treatment of depression, however there have been no studies investigating the effects of the route of administration on drug delivery to the brain.

In **Chapter 1**, it was emphasized that MDD is a debilitating mental disease, compromising the overall quality of everyday life of affected individuals. This also has a devastating impact on the public health system, based on the fact that this disorder has a more likely tendency to originate in adolescence and continue as a chronic or recurrent condition. MDD's increasing prevalence has been associated with significant public health costs and morbidity rates; therefore, the effective treatment of the mental disorder is crucial. The findings of this study implicate future decisions on the use of ketamine and approaches to treat MDD in clinical practice.

In **Chapter 2**, the study focused on the pharmacokinetics of ketamine by quantifying its concentration in plasma and the brain following a dose of 15mg/kg b.w after either oral, intranasal or intraperitoneal administration. Compared to previously reported studies of oral administrations, similar hindrances, due to low oral bioavailability were experienced due to extensive first pass metabolism in the liver and intestines. After oral administration ketamine's concentrations were found to be below the limit of quantification in the brain, which is not ideal for the treatment of MDD. Intranasal administration is an alternative approach to administering ketamine due to its more favorable tolerability that has been reported; however as exhibited in this study and in other studies, ketamine concentrations are lower when

compared to IM., IV and IP administration due to its diminished bioavailability. IP administration exhibited favorable concentrations as previously reported with a C_{max} of 524,58 and 352,06 ng/mL in plasma and brain tissue respectively.

It is imperative that future studies centered on the use of ketamine for the treatment of MDD take into consideration the highlighted pharmacokinetic parameters, paving the way forward for the effective treatment of MDD, especially in those that have TRD. Future ketamine studies should be inclusive of brain distribution of ketamine with dosage forms and routes of administration that provide steady-state distribution of the drug; so that treatment regimens are centered at achieving the desired therapeutic outcomes. Studies should also focus on the development of IN formulations that increase the drug's bioavailability in the brain.

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UNIVERSITY OF KWAZULU-NATAL
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25 May 2018

Ms Vivian Campbell Naidoo (218032375)
School of Health Sciences
Westville Campus

Dear Ms Naidoo,

Protocol reference number: AREC/003/018M
Project title: The use of mass spectrometry methods to investigate the brain distribution of Ketamine for the treatment of Major Depression Disorder (MDD) following various routes of administration

Full Approval – Research Application

With regards to your revised application received on 04 May 2018. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 25 May 2019.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

Prof S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Supervisor: Dr Sooraj Baijnath
Cc Registrar: Mr Simon Mokoena
Cc Academic Leader Research: Professor Pragashnie Govender
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