

**CENTRAL NERVOUS SYSTEM STIMULATING ACTIVITY
OF PHYTEXPONENT PREPARATION**

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DECLARATION

This research project is entirely my own work and has not been duplicated from any source or submitted for examination or award of any degree in any university for any other award.

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Signature

Date.....

SUPERVISOR APPROVAL

I can confirm that this project has been submitted for examination with my approval as university supervisor.

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DEDICATION

I dedicate my work to my parents and siblings for their unending support.

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I am forever grateful to the LORD JESUS CHRIST for His gift of life and perfect health, strength and wisdom throughout my degree course and my project exercise.

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ABSTRACT

Background: Disorders affecting the central nervous system are categorized as some of the chronic conditions that affect mankind. These have been managed by prescription of the central nervous stimulants that help in reducing the symptoms such as fatigue and as well as increasing the alertness and cognitive function. However, in many cases these stimulants have been abused by many as some of them are illicit substances such as cocaine. The abuse of these stimulants results into addiction and upon use over a long time they result into long term effects and even death. The abuse of these substances has been common among the young people of between 18-25 years of age. The statistics on abuse of the stimulants by these younger generation was at 406,000 by the year 2014. This has seen much control of these products making them only available under critical condition. Use of herbal stimulants is proving to be an alternative. The use of these products has been there for some quite a long time but less attention was granted to them. **Aim:** The current study aimed at evaluating the CNS stimulating effects of the phytexponent. **Methods:** The ability of the phytexponent to increase the motor activity was evaluated by the Rota rod and actophotometer methods. The Swiss albino mice were divided in five groups of five mice. Caffeine at dose level of 30 mg/kg bw was chosen as the standard CNS stimulant. The phytexponent was evaluated in three different doses of 50 %, 25% and 12.5%. Both caffeine and phytexponent was administered orally with the volume administered depending on the body weight of each mouse. The reduction in the number of counts and fall of time from the rod was taken as an indicator in the reduced stimulation of the central nervous system. **Results:** caffeine at dose of 30 mg/kg bw recorded fall of time of 281.0 ± 12.19 seconds while phytexponent at 50 %, 25% and 12.5% recorded fall off time of 240.0 ± 28.21 , 281.2 ± 18.21 and 300.0 ± 0.00 seconds respectively. The number of counts recorded in the actophotometer were 248.6 ± 25.68 , 204.6 ± 25.28 , and 231.0 ± 25.12 and 213.8 ± 35.00 counts for caffeine, phytexponent at 50 %, 25% and 12.5% respectively. In the actophotometer caffeine significantly increased the alertness of the mice hence increase in the locomotion that was indicated by high counts than the phytexponent. Phytexponent in the Rota rod test showed dose dependent CNS stimulating activity while in the actophotometer 25 % doses recorded the highest activity. **Conclusion:** phytexponent is a potential stimulant of the central nervous system.

TABLE OF CONTENT

DECLARATION.....	i
DEDICATION.....	ii
ACKNOWLEDGEMENT.....	iii
ABSTRACT.....	iv
TABLE OF CONTENT.....	v
LIST OF TABLES.....	viii
TABLE OF FIGURES.....	ix
LIST OF ABBREVIATIONS.....	x
CHAPTER ONE: INTRODUCTION.....	1
1.1 Background Information.....	1
1.2 Problem Statement and Justification.....	3
1.3 Objectives.....	4
1.3.1 General objective.....	4
1.3.2 Specific objectives.....	4
1.4 Research Question.....	4
CHAPTER TWO: LITERATURE REVIEW.....	5
2.1 Central Nervous System.....	5
2.2 Central Nervous System Stimulants.....	5
2.2.1 Mechanism of action of the central nervous system stimulants.....	6
2.2.2 Therapeutic uses of CNS stimulants.....	6
2.2.3 Side effects of CNS stimulants.....	7
2.3 Classification of the Central Nervous System Stimulants.....	8
2.3.1 Cerebral stimulants.....	8
2.3.1.1 Psychomotor Stimulants.....	8
2.3.1.2 Psychotomimetic Stimulants.....	8

2.3.2 Medullary stimulants.....	9
2.3.3 Spinal Cord stimulant	9
2.4 Complementary Management of CNS Disorders	9
2.4.1 <i>Bacopa monnieri</i>	10
2.4.2 <i>Aswagandha</i>	10
2.4.3 <i>Hypericum perforatum</i>	11
2.4.4 <i>Erythroxylum coca</i>	11
2.4.5 <i>Catha edulis</i>	11
2.5 Phytoexponent preparation.....	12
2.5.1 Composition of the phytexponent.....	12
CHAPTER THREE: MATERIALS AND METHODS	15
3.1 Study Design.....	15
3.2 Source Of Herbal Preparation.....	15
3.3 Study Animals	15
3.4 Animal Sorting And Grouping	15
3.4 The central nervous system stimulant effects of the phytexponent.....	16
3.4.1 Rota rod test	16
3.4.2 Actophotometer test.....	16
3.5 Data Management and Statistical Analysis	17
CHAPTER FOUR: RESULTS AND DISCUSSION	18
4.1 Results	18
4.2 Discussion.....	21
CHAPTER FIVE: CONCLUSION AND RECOMMENDATION.....	22
5.1 Conclusion	22
5.2 Recommendation.....	22

REFERENCES.....	23
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LIST OF TABLES

Table 4. 1 CNS stimulating effects of phytexponent on the locomotor activity on
Rota rod.....18

Table 4. 2 CNS stimulating effects of phytexponent using Actophotometer20

TABLE OF FIGURES

Figure 4. 1 CNS stimulating effects of phytexponent on the locomotor activity on Rota rod.....	19
Figure 4. 2 CNS stimulating effects of phytexponent using Actophotometer	20

LIST OF ABBREVIATIONS

CNS	Central nervous system
CS	cardiovascular system
ADHD	Attention deficit hyperactivity disorder
FDA	Food and drug administration
NMU	Non-medical use
ADF	Abuse Deterrent formulation
BWT	body weight

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The term central nervous stimulants covers a broad class of drugs that alters activity within the central and peripheral nervous systems and tend to increase behavioral alertness, agitation, or excitation(Avois et al., 2006). The CNS stimulants are classified according to their structural similarities, their site of therapeutic action in the central nervous system and their therapeutic usage(*Medical Pharmacology*, n.d.).

Their classification according to their mode of action and the target site include the following groups: cerebral stimulants, medullary stimulants and spinal cord stimulants(Campbell & Young, 2018). The cerebral stimulants also known as the cortical stimulants act on the central nervous system and produce a temporary sense of alertness and well-being(Davis, 2019). The psychomotor and psychotomimetic stimulants (hallucinogenic drugs) are the subclasses of the cerebral stimulants. The psychomotor Stimulants include: methylxanthines (caffeine, theobromine and theophylline), nicotine, cocaine, amphetamines, methylphenidate. These molecules produce excitement and euphoria, increase motor activity and reduce fatigue.The psychotomimetic stimulants also best referred to as delusionary agents, affect cognition and produce hallucinations by altering the perception of the surrounding environment. As example they include lysergic acid, psilocybin, and mescaline. The medullary stimulants also referred as brain stem stimulants or analeptics act on the medulla oblongata.

This class includes the following molecules as examples: picrotoxin, doxapram, pentylenetetrazol, cardiazole etc. The spinal cord stimulants are marked by the elicitation of spinal cord functional activity by increasing the reflex excitability or sedative action when consumed in limited quantities but when given in overdose causes tonic convulsions. As example this class includes: Strychnine, brucine, and thebaine.

Classifying stimulants according to their chemical structures is difficult, because of the large number of classes the drugs occupy, and the fact that they may belong to multiple classes. According to chemical structural similarities the following classification is used: amphetamines, serotonin agonists, sympathomimetics, and xanthines. The CNS stimulants are also classified according to their major therapeutic uses: anti-attention deficit disorder, anti-narcoleptic, anorexiants, antimigraine, and analeptic drugs

CNS stimulants are used clinically and therapeutically. They are used in the treatment of attention deficit hyperactivity disorder (ADHD) that occurs in children between 4-17 years of age and in a small percentage of adults(Davis, 2019). ADHD presents as inattention, hyperactivity, difficulty controlling behavior, sadness and anxiety; hyper somnolence and narcolepsy which is characterized by excessive sleepiness and episodes of sleep attacks; binge eating disorder; exogenous obesity(Management, 2019).

Just as other drugs, CNS stimulants have several side effects and they include: CVS effects like increased heart rate and increased blood pressure that may result in cardiac arrhythmias, CNS effects like wakefulness, alertness, decreased sense of fatigue, elevated mood, anxiety, insomnia, irritability, the drugs administered transdermally e.g. cause dermatological effects like contact dermatitis due to allergic sensitization(Davis, 2019). Other effects include; growth suppression associated with weightloss, accommodation and visual disturbances, social disability, tolerance and dependence is a major concern associated with CNS stimulant use (Avois et al., 2006).

Central nervous system stimulants are used clinically and by prescription only to treat conditions like attention deficit disorder, narcolepsy etc. These drugs when used appropriately and as advised by health professionals, they confer therapeutic activity and are helpful to the patient. Despite their usefulness therapeutically, central nervous system stimulants are widely abused due to their action. Amphetamine and cocaine are the most commonly abused due to their effects eg euphoria, increased reflexes especially for athletes, decreased sense of exhaustion

and increased alertness. Cocaine is more addictive than amphetamine and its euphoric effects also are higher. It causes an increase in motor activity, talkativeness and a sense of wellbeing. This feeling wears off with time and often leaving the user feeling more depressed than before. This down feeling leads the addict to use more cocaine, sometimes just to feel “normal”. Over a period of time, the amount of cocaine needed and the frequency of use to achieve a “high” have to be increased, this leads to dependency and addiction(Avois et al., 2006).

Due to the high dependency and addictive nature of this class of drugs, the US Food and Drug Administration has seen the need of researchers, health professionals, academicians, pharmaceutical experts and all other stakeholders to develop and evaluate abuse deterrent formulations of central nervous stimulants to curb the issue of abuse and misuse of this class of drugs. In the same regard, it has issued federal register notice to interested parties so as to receive insight on the same. Drug developers have started working on prescription stimulant formulations that are aimed at deterring abuse and misuse. In developing Phytexponent we follow this FDA recommendation: "Drug developers have started work on novel formulations of prescription stimulants with properties intended to deter abuse."

1.2 Problem Statement and Justification

Prescription drug abuse cases are on the rise among Americans of all age groups especially adults and adolescents, education status and economic levels, ethnicity and gender. Statistics show that different classes of prescription medicines like pain medications, tranquilizers, sedatives, and stimulants are abused, misused, malingered and used for non-medical purposes. CNS Stimulants are abused due to their ability to increase alertness and concentration, euphoric effect. This class of drugs associated with abuse, misuse and diversion. Abuse is defined as “the intentional, nontherapeutic use of a drug product or substance, even once, to achieve a desirable psychological or physiological effect.” Misuse is “the intentional therapeutic use of a drug product in an inappropriate way and specifically excludes the definition of abuse. “Diversion is when medication is prescribed and given to one individual but it is taken by another person. Non-

medical use (NMU) encompasses abuse and misuse and is defined as the use of a prescription drug without a prescription or in any other way other than as directed. CNS stimulants are abused and misused and are associated with side effects (Factors et al., 2020).

The non-medical usage of CNS stimulants is a major public health concern. The development and evaluation of abuse deterrent formulations (ADF) is a promising approach to mitigate these concerns. ADF is a reformulation of a medication whose formulation process aims at decreasing side effects, abuse and misuse while still achieving its intended therapeutic goals. This can be achieved by making the abuse of the drug less rewarding, hindrance of extraction of the active component or reducing its bioavailability (Naloxone et al., 2018). If Phyteponent works it will be safe, cheap and an abuse deterrent formulation.

1.3 Objectives

1.3.1 General objective

To evaluate the CNS stimulating activity of phytexponent.

1.3.2 Specific objectives

- I. To evaluate the CNS stimulating activity of phytexponent using Rota rod model.
- II. To evaluate the CNS stimulating activity of phytexponent using actophotometer model.

1.4 Research Question

- I. Does phytexponent have CNS stimulating activity?

CHAPTER TWO: LITERATURE REVIEW

2.1 Central Nervous System

The central nervous system is made up of two major portions; the brain and spinal cord that work together in harmony (Taylor & Hardcastle, 2020). The two structures are tasked with the function of integrating the information from both the peripheral nervous system and the sensory organs and channeling the output signals to the skeletal muscles and to the autonomic nervous system which controls the internal body organs (Taylor & Hardcastle, 2020). The central nervous system acts as the command center of the body and its failure results into negative effects that affect the proper functioning of the body from day to day.

Function wise the two structures of the central nervous have specialized sub functions. The spinal cord is responsible for conveying of information from the peripheral nervous system to the brain and also sends the motor information from the brain to the somatic and autonomic motor systems. On the other hand, the brain regulates almost the entire functions of the body including; cognition, body movements and organ functions (Taylor & Hardcastle, 2020).

The central nervous system disorders have the ability to effect either of the two major parts of the central nervous system that is the brain and the spinal cord. When these two parts are affected many neurological or psychiatric disorders develop. Many factors contribute immensely on pathogenesis of the central nervous system diseases. These include trauma infections, degeneration autoimmune disorders, structural defects, tumors and stroke, neurodegenerative diseases, mood disorders and autism (Srineraja, 2017).

2.2 Central Nervous System Stimulants

The central nervous system stimulants comprises of the agents mostly drugs that affect the mind. These agents usually offer an improvement in both mental and physical function and in the process enhance the activity of the central nervous system. The CNS stimulants improves the mental and physical functions by speeding up the processes in the brain and spinal cord (Srineraja, 2017). These agents are of great help in management of many mental disorders such as attention

Deficit Hyperactivity Disorder (ADHD). However, even though they have been of help, are widely abused by many people as illicit substances.

Amphetamine and Methylphenidate are prescribed in Attention Deficit Hyperactivity

Disorder (ADHD) for children but National Department of Health reported that these drugs

are used by 2-3.5% of adults in USA. It produces generalized action which on higher doses

may produce convulsions.

2.2.1 Mechanism of action of the central nervous system stimulants

The CNS stimulants achieve their main aim of ensuring the alertness and adequate functions of the brain and the spinal cord in humans through various mechanisms.

The stimulants elicit their beneficial role by increasing the levels of dopamine, serotonin and norepinephrine in the brain (Brown et al., 2012). Dopamine is a neurotransmitter that is associated with concentration, attention and feelings of reward and pleasure. On the other hand norepinephrine is vital in ensuring alertness.

Other stimulants show their mode of action by increasing the levels of glutamate which is a neurotransmitter that helps in behavioral control and inhibition. The low levels of glutamate is mostly detected in patients suffering from attention deficit hyperactivity (ADHD) (Wolraich et al., 2011).

2.2.2 Therapeutic uses of CNS stimulants

The prescription of the central nervous stimulants is done by professional medical personals. This s done under certain conditions that include managing of the central nervous system disorders. Central nervous stimulants is prescribed in treating the attention deficit hyperactivity (ADHD) which affects both children and adults (Wolraich et al., 2011). The attention deficit hyperactivity (ADHD) has been ranged as the most common reason for prescription of the CNS stimulants. This disorder is estimated to be about 8 % in children and about 2-5% in adults. The stimulants helps the individuals with this condition to be able to manage their urge

and behaviors. In children and other adults they results into total calmness and increased concentration in school and work (Wolraich et al., 2011).

Similarly stimulants are prescribed in treatment of narcolepsy. The individuals with narcolepsy are characterized by sudden attack of extreme sleepiness and impairment of the basic functioning of the body. This results into the affected people to be unable to perform simple tasks such as driving and reduced concentration at work places. Stimulants are prescribed in this case to help the individuals maintain alertness throughout the day(Brown et al., 2012). In other instances such as exogenous obesity stimulants such as amphetamines are prescribed and incase of headache, caffeine is used in combination with some analgesics to relieve pain, stress, tension and fatigue (Brown et al., 2012).

2.2.3 Side effects of CNS stimulants

The use of CNS stimulants has to be keenly monitored for potential side effects on the users. The undesirable side effects of the stimulant negatively impact the various parts of the person's health and functioning. The use of amphetamines has effects on the cardiovascular system. These stimulants results into side effects such as valculopathy (a disorder of the heart valves), syncope, arrhythmias, hypertension, angina pain, and circulator collapse. Others such as chills, sweating, and headache may occur as well. Cardiomyopathy and myocardial infarction with sudden death, are very rare and very dangerous side effects that have been reported with large doses.

On the central nervous system Amphetamines results into side effects such as insomnia, weakness, dizziness, tremors, hyperactive reflex, and confusion. On use of high doses of amphetamines seizure and coma are sometimes observed. In pregnant and lactating women, amphetamine causes severe teratogenic effect. Also the metabolites of these stimulants are distributed in breast milk. Finally, other effects associated with the CNS stimulants include, nausea, vomiting, abdominal cramps, constipation and diarrhea. In other circumstances, blurred vision, glaucoma, rashes, urticaria and hypersensitivity.

2.3 Classification of the Central Nervous System Stimulants

The classification of the central nervous system is majorly done based on criteria such as the mode of action and target site. Based on the site of action criteria, three major groups of central nervous stimulants,. These are cerebral stimulants, medullary stimulants and spinal cord stimulants. The cerebral stimulants further contain other two subgroups; Psychomotor and Psychotomimetic stimulants (Agarwal et al., 2019).

2.3.1 Cerebral stimulants

These stimulants are as a well identified as the cortical stimulants and act on the central nervous system producing temporary sense of alertness and well-being. They are broadly grouped into two major groups Psychomotor and Psychomimetic stimulants(Agarwal et al., 2019).

2.3.1.1 Psychomotor Stimulants

These stimulants affect the central nervous system by boosting the release of certain brain chemicals (Heal et al., 2013). The drugs in this category include methylxanthines that comprises of caffeine, theobromine and theophylline, nicotine, cocaine, amphetamines and methylphenidate. The amphetamine were the first to be introduced in this group and were used as potential treatment for depression, suppressing appetite and managing nasal congestion (Badisa et al., 2018). Similarly, these drugs had positive effects during the Second World War in reducing fatigue as well as increasing alertness and narcolepsy during the later stages (Agarwal et al., 2019).

2.3.1.2 Psychotomimetic Stimulants

This group of the cerebral stimulants are also known as delusionary agents. These drugs are associated with sate of anesthesia upon use. They are characterized with production of hallucinations as resulting of interfering with the perception of the surrounding environment. This category of the stimulants include drugs such as lysergic acid, psilocybin, and mescaline (Rang et al., 2012).

2.3.2 Medullary stimulants

These are the central nervous system stimulants with the medulla oblongata as the site of action (Agarwal et al., 2019).. These agent are as well recognized as the brain stem obtaining this name from the fact that the medulla oblongata constitutes the brain stem. Similarly this set of drugs are as well-known as analeptics. Examples of these drags include picrotoxin, doxapram, pentylenetetrazol and cardiazole. Medulla oblongata plays a role in regulating respiration hence these drugs have an effect on respiration (Agarwal et al., 2019). Every stimulant in this category has distinct mode of action. For instance, Doxapram stimulates the carotid chemoreceptors while Picrotoxin and Bicuculline are GABAA report antagonist (Agarwal et al., 2019).

2.3.3 Spinal Cord stimulant

These stimulants act on the spinal cord. To elicit their action they cause the elicitation of spinal cord functional activity by increasing the reflex excitability or sedative action when used in limited quantities. However, upon overdose they result into tonic convulsions. This category consist of drugs such as Strychnine, brucine, and thebaine which have are able to both show and enhance the excitatory activities(Agarwal et al., 2019).

2.4 Complementary Management of CNS Disorders

The complementary management of the central nervous disease /disorders /conditions involves use of the herbal medicines which have CNS stimulating properties (Ernst, 2005). The use of herbal medicines from plaints has been with us for quite some time. The affinity towards these form of medicine has been growing each time and its utilization in treating and managing critical conditions/diseases has greatly resulted into tremendous outcomes. These herbal medicine are as well in great demand in developed countries where they are being incorporated in the health sector as a result of their high efficacy, safety and laser side effects. These herbal products offer therapeutics for various chronic disorders such as cognitive impairment, immune disorders and mental disorders whose modern medicine is not available.

Many plants that are used as CNS stimulants are available and have been studied through various investigations. These include *Bacopa monnieri*, *Acacia catechu*, *Aswagandha*, Neem tree, *Catha edulis*, *Hypericum perforatum* and *Erythroxylum coca* (Mestry et al., 2016; Srineeraja, 2017)

2.4.1 *Bacopa monnieri*

Bacopa monnieri, is as well identified by the common name; Water Hyssop (Brahmi). This herbal plant is mostly used in the Ayurveda medicinal system, originating from India (Srineeraja, 2017). This plant has an ability to enable the body to withstand resistance to physical and emotional stress (Anju, 2011). This plant as well aids in blood purification and is as well used for chronic skin diseases such as leprosy and syphilis and eczema and psoriasis.

It's CNS stimulating properties are seen through its ability to improve and build the mental performance. The plant has both long and short term improvement of the memory. Additionally, it increases the intelligence, longevity and circulation in the brain. This in turn reduces the senility and ageing. It as well improves the immune system, both cleansing and feeding (Ghédira & Goetz, 2017).

2.4.2 *Aswagandha*

The *Aswagandha* commonly known as the “Indian Winter cherry” or “Indian Ginseng” belongs to the family Solanaceae. This herb is as well used in the Indian medicinal system of Ayurveda (Srineeraja, 2017). It has wide use in treating various kinds of disease processes and more so as a nervine tonic (Tantra, 2019).

This herb improves the function of both brain and the nervous system. Additionally, it improves the memory and these properties makes it be used as the CNS stimulant. As a potent adaptogen it enables the body respond positively to stress. Its protective role in the body is ensured by the ability of this herb to improve the cell mediated immunity. As a potent antioxidant, it helps protect the body against cellular damage due to free radicals (Singh et al., 2011).

2.4.3 *Hypericum perforatum*

This a perennial herb commonly referred to as St. John's wort. It is characterized by yellow flowers and is native to Europe, West Asia and North America. From previous studies this plant has been suggested to be effective in managing other diseases such as cancer, inflammatory disorders, microbial diseases and oxidative stress related disorders (Klemow et al., 2011) as well as its neuroprotective role (Mestry et al., 2016). This herb is as well used as an antidepressant (Melzer et al., 2010) and this property is due to the Hypericin (Klemow et al., 2011). This is the active ingredient in St. John's wort and is responsible for inhibiting monoamine oxidase enzyme which is responsible for degrading amine neurotransmitters and in the process it increases the levels of neurotransmitters (Mestry et al., 2016).

2.4.4 *Erythroxylum coca*

This herbal contain active ingredient called cocaine which possess CNS stimulating properties. This product is isolated from the herbal by extraction process where the final extract is inform of a paste (Mestry et al., 2016). Due to the instability of this paste is later on converted into a salt similar to hydrochloride or sulphate. The salt is further processed into other forms that entirely depend on the administration rout such as intravenous injection or inhalation. As a stimulant cocaine has been widely in central and South America (Wood et al., 2014). Cocaine is thought to elicit its central nervous system stimulation by blocking the reuptake of NA, and adrenaline and dopamine back into the adrenergic nerve endings. This later on results into increased concentration s of the transmitter around the receptors hence causing CNS stimulation (Colzato et al., 2008; Wood et al., 2014). However, the ability of cocaine to block the reuptake of dopamine has been associated with its reinforcing and addictive properties (Colzato et al., 2008).

Even though cocaine has its origin from herbs, the prolonged use has been linked to cause cardiotoxic and neurovascular complications (Tamrazi & Almast, 2012).

2.4.5 *Catha edulis*

This herb produces is commonly known as Khat which is a CNS stimulant. It mainly consist of the young shoots of this plant and the major active ingredient that

is used as a CNS stimulant known as cathinone (Mestry et al., 2016). Cathinone is an alkaloid whose structure is analogue to amphetamine (Rojek et al., 2014). Studies have shown similarities in the effect on metabolism and appetetic suspression that is shown by both cathinone and amphetamine (Alshagga et al., 2017). The continuous use of khati is addictive and results into decreased felling of hunger and increase in the feeling of activeness (Kelly, 2011)

2.5 Phytoexponent preparation

Phytexponent preparation is an herbal preparation of German origin that comprises of five different medicinal plants. The plants are first extracted with ethanol by soaking individual plant material into an extraction vessel for two days and then filtered on the third day. The different extracts solution of the respective plants is then mixed in specific percentages. The mixing is as follows; *Viola Tricolor* - 3.77% *Echinacea purpurea*- 26.42% *Allium sativum*- 11.32% *Triticum repens*- 26.42% *Matricaria chamomilla*- 32.08% (swanstrom, 2007). The final product then comprises of 62.1 % ethanol with the rest of the percentage representing the various plant extract.

2.5.1 Composition of the phytexponent

Phytexponent comprises of five different medicinal plants that are mixed in different proportions to make the final product.

Viola tricolor also commonly known as Heartsease, Johnny Jump up, Call-me-to-you, or Bird's Eye (Hellinger et al., 2015). It's taxonomically placed in the family Violaceae. This plants has been used as anti-inflammatory agent and skin remedy in Europe (Hellinger et al., 2015). These plants contain various phytochemicals such as flavonoids, polysaccharides, phenylcarbonic acids, coumarins, catechins, and salicylic acid derivatives. The plant has as well been reported to have macrocyclic peptides such as cyclotides that are potent immunosuppressive peptides that have the ability to inhibit T-cell proliferation(Hellinger et al., 2015).

Echinacea purpurea is a perennial medicinal herb that is commonly identified as eastern purple coneflower or generally the purple coneflower and its native to North America. It has been reported to be an anti-inflammatory and an

immunostimulatory agent (Manayi et al., 2015). Additionally, it has as be used in managing of cold symptoms and as well it has shown antimutagenicity, cytotoxicity, antidepression, and antianxiety effects. The presence of caffeic acid derivatives (Manayi et al., 2015) as one of its components suggests that it can be used as a central nervous system stimulator. However, no studies on its central nervous system stimulation has been conducted. Due to its multiple uses it has gained much attention from many researchers. However it has been reported to have certain side effects such as urticaria, erythema, rash, pruritus, nausea, dyspnea, angioedema, and abdominal pain (Manayi et al., 2015).

Allium sativum also known as garlic is the most widely used spices in most of the countries and the most sold herbal product globally (Majewski, 2014). It is as well used as an aphrodisiac and belongs to the family alliaceae in the onions are the members (Moutia et al., 2018). Various bioactive compounds summing up to 200 have been identified garlic. The aqueous garlic extracts have been reported to have antioxidant activity by inhibiting reactive oxygen species (ROS) and enhancing enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (Arreola et al., 2015). No researchs are available on its central nervous stimulating effects.

Matricaria chamomilla is a medicinal plant found in the family *Asteraceae*. The compounds isolated from this plant have been used in about 26 drugs. It has as well been used as antiinflammatory and antispasmodic drug. It has as well been used in treating pain that results from the disturbance from the stomach. It has as well been used extensively as a tea or tonic and treating hysteria, anxiety, insomnia, and nightmares (Satyal et al., 2015). Its central nervous system stimulating effects has not been evaluated in any study.

Tricum vulgare commonly identified as couch grass is an invasive weed whose roots and leaves are used in medicine. It is used in managing constipation, swollen bladder, cough, fever, hypertension, kidney stones and inflammation (Sanguigno et al., 2018). It has as well been incorporated in some pharmaceutical formulations for treatment of burns, skin lesions and decubitus ulcers. The antiinflammatory

properties of this weed has been an area of concern. No data is available on its ability to stimulate the central nervous system.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Design

This study was entirely a laboratory-based study.

3.2 Source Of Herbal Preparation

The phytextponent poly herbal preparation was sourced from Maendeleo pharmacy in Nairobi. This product was transported to Mount Kenya university pharmacology laboratory as per the instruction outlined by the manufacturer. Here this product was kept until the study day.

3.3 Study Animals

The Swiss albino mice 5 to 6 weeks old of weight range of 20 to 30 grams and male and female were used in this study. These animals were sourced from the animal breeding house located in the Centre of biotechnology Muguka. They were transported to the Mount Kenya university animal laboratory where they were housed under standard conditions. This involved a maintained natural light and dark cycle of 12 hours each and free access to the rodent pellets and clean water. The animals were allowed two to three days acclimatization period and the cages were layered with dry wood shavings. The wood shavings were changed each day to prevent dampness. Prior and during the test, all the protocols, directions and guidelines about the use of animals in an experiment were adhered to in this study.

3.4 Animal Sorting and Grouping

All mice of respective genders were kept in different cages to preventing mating and pregnancy during the study period. The five study groups consisted of five mice per group (n =5). The five study groups included the negative control (Group I), positive control (Group II) and three treatment groups (Group III, IV and V). All mice in each group were weighed and marked on the tail using non-toxic permanent marker to indicate mice number one to mice number five. On the other hand, each group was marked on different body point to prevent mixed up and confusion. Mice in each group were labeled on the tail to indicated mice number one to mice number five. Group I (negative control) was marked on the head while group II (positive

control) was marked on the bark. Groups III, IV and V (treatment groups) were marked on the tail tip, left hind leg and right hind leg respectively (table 3.1).

Group	Mark	Treatment
I	Head	Negative control
II	Back	Positive control
III	Tail tip	Treatment (50%)
IV	Right hind leg	Treatment (25%)
V	Left hind leg	Treatment (12.5%)

3.4 The central nervous system stimulant effects of the phytexponent.

The CNS stimulant effects of the phytexponent were evaluated by adopting two methods. The Rota rod test and Actophotometer test.

3.4.1 Rota rod test

The effect of the phytexponent on neurological depression in Swiss albino mice were evaluated by Rota rod test as described by (Rayapureddy et al., 2017) modifications. The Rota rod (labtech model) apparatus equipped with two panels and a timer were used. All mice in all the groups were trained and during the pretest, only the mice with ability to remain on the revolving rod at speed of 25 rpm for all the cut off period of 5 minutes were selected for this study. The selected mice were then treated with the respective phytexponent and reference drug 30 minutes prior to the test. Phytexponent preparation at the three dose levels was administered to the three experimental groups. The positive control group mice were administered the standard CNS stimulant (Caffeine) at selected dose while the negative control group were administered normal saline. The mice were then placed on the rod bar 30 minutes later and the time taken by each mouse before it falls off from the rod within the cut time of 5 minutes recorded.

3.4.2 Actophotometer test

The locomotor activity of mice was monitored using the actophotometer (Jaswinder Kaur, Rajmeet Singh , Parminder Singh, 2017). The actophotometer (labtech

model) is equipped with six photocells that have infrared bulbs. This equipment is made in a manner that the animal blocks only one beam as it moves around. Set of five groups of mice were used and administered the respective study agent and reference drug respectively. The negative control group was administered normal saline while positive control group was administered the reference CNS stimulant drug (Caffeine). The three experimental groups will be administered the phytexponent reconstituted in normal saline to get the three doses. The administration of the drug and extract was done by the oral route. The locomotor activity was evaluated 30 minutes later and involved placing only one mouse in the actophotometer. The actophotometer was then be turned on and the monitored for a period of 5 minutes. On elapse of the cut off period, the count for each mouse for all the groups was recorded.

3.5 Data Management and Statistical Analysis

All the data was tabulated in the laboratory note book and then entered in the excel spread sheet. This data was imported in the graph pad software version 9.1.1 for analysis. Descriptive statistics was performed and the data expressed as Mean \pm SEM. The data was analyzed statistically by two-way anova by graph pad software. The level of significance was taken at $p < 0.05$.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Results

The central nervous system Stimulating effects of phytexponent was evaluated by monitoring the locomotor activity using Rota rod and actophotometer.

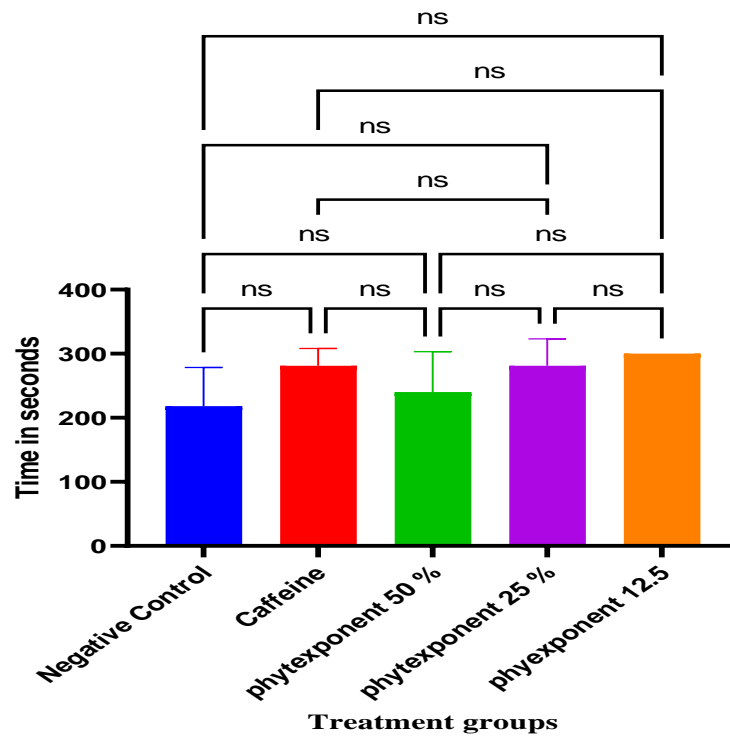
4.1.1 CNS stimulating effects of phytexponent using Rota rod

Table 4.1 summarizes the results for the effect of the phytexponent on the fall off time in all the groups. The oral administration of caffeine standard CNS stimulant increased the time taken by each mouse in the positive control group to fall off from the rotating rod (figure 4.1). When the positive control was compared to the negative control that was only administered normal saline, the fall off time was found to be higher. The administration of the phytexponent at the three different doses of 50%, 25% and 12.5% increased the locomotor activity of the mice in the reverse order. The higher dose of 50% recorded lower activity while the lower dose of 12.5 % recorded the highest activity. The phyexponent at 12.5 % recorded more activity indicated by the higher time mouse spent on the rotating rod as compared to caffeine at 30 mg/kg bw. The time taken by the mice in the experimental group administered with phytexponent at 25 % was similar to the group administered with caffeine (table 4.1).

Table 4. 1 CNS stimulating effects of phytexponent on the locomotor activity on Rota rod

Treatment groups	Fall off time
Negative control	218.0± 27.11
Positive control	281.0 ±12.19
Phytexponent 50%	240.0 ±28.21
Phytexponent 25%	281.2 ±18.21
Phytexponent 12. 5%	300.0± 0.00

Figure 4. 1 CNS stimulating effects of phytexponent on the locomotor activity on Rota rod



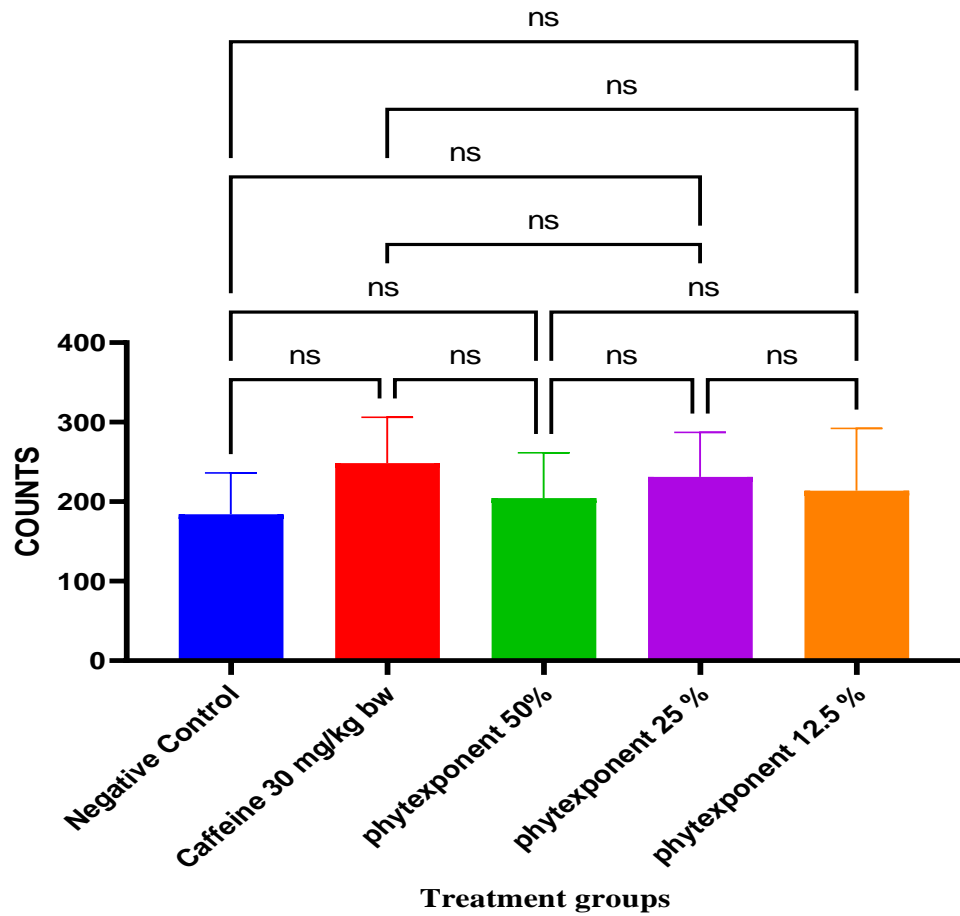
4.1.2 CNS stimulating effects of phytexponent using Actophotometer

The results for the CNS stimulating effects of phytexponent evaluated using actophotometer are presented in table 4.2 and figure 4.2. The results indicated an increase in the number of counts in mice administered with caffeine at dose level of 30 mg/kg bw as compared to the negative control group (table 4.2). Phytexponent increased the counts taken in the actophotometer with a reduction in concentration of the extract. However the increase in the counts was lower than that recorded in the positive (caffeine 30 mg/kg bw) group. The negative control group recorded the least counts.

Table 4. 2 CNS stimulating effects of phytexponent using Actophotometer

Treatment groups	Counts
Negative control	184.2±23.22
Positive control	248.6±25.68
Phytexponent 50%	204.6±25.28
Phytexponent 25%	231.0±25.12
Phytexponent 12. 5%	213.8±35.00

Figure 4. 2 CNS stimulating effects of phytexponent using Actophotometer



4.2 Discussion

Two commonly used methods were adopted to evaluate the CNS stimulating activity of the phytexponent. This study was important as it was aimed to validate if combining of other herbs with *Matricaria chamomilla* which has antidepressant activity will affect this property. Many agents have the ability to excite the function of the CNS by inhibiting calmness or sedation (drowsiness). Sedation usually indicates a reduction in activity, moderate excitement and drowsiness.

The results obtained from this study indicate that phytexponent has CNS stimulating activity. This has been shown by the ability of the phytexponent to increase the time taken by each mouse on the rotating rod and the high counts observed by the actophotometer. The administration of the phytexponent in the three different doses resulted into an increase in fall off time in reverse order. The high concentration 50% recorded the least time while the lower concentration 12.5% recorded the highest fall off time in the Rota rod test. The CNS stimulating activity of the phytexponent at this lower dose was more as compared to the conventional CNS stimulant, caffeine that was used. In the actophotometer test, phytexponent preparation increased the motor locomotor activity of the mice. This was indicated by the high counts observed in the groups administered with phytexponent at different doses. However, caffeine, a standard CNS stimulant increased the locomotor activity more as compared to the phytexponent.

The mechanism of action of phytexponent as CNS stimulant is not known. However, as it has shown similar effects as those shown by caffeine, its mode of action can be said to be similar to that of caffeine. Caffeine works by inhibiting the adenosine receptors and phosphodiesterase which increases the concentration of dopamine, nor epinephrine and serotonin at the receptors.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study revealed that phytexponent has CNS stimulating effects. The consumption of the phytexponent significantly increased the motor activity that saw the mice spend much time on the rotating rod. Also, the number of counts were observed to be very high an indication of CMNS stimulation. The CNS activity of the phytexponent was increasing with reduction in dose of the phytexponent.

5.2 Recommendation

Recommendations based on the outcomes of the present study include;

- I. Further studies targeting the exact mode of action of phytesponent as a CNS stimulant be done.

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