ASSESSMENT STUDY OF THE PATTERNS OF SOME GROSS BEHAVIORAL ACTIVITIES IN WISTAR RATS IN SEPARATE ACUTE DOSES OF CAFFEINE AND *KOLA NITIDA* EXTRACT

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc) DEGREE

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work on "ASSESSMENT STUDY OF THE PATTERNS OF SOME GROSS BEHAVIORAL ACTIVITIES IN WISTAR RATS IN SEPARATE ACUTE DOSES OF CAFFEINE AND *KOLA NITIDA* EXTRACT" by ENOGHASE GLYNISS OSAREFE, with matriculation number BMS2101628 in partial fulfillment for the award of Bachelor of Science Degree (B.Sc.) in department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state, Nigeria.

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DEDICATION

This project work is dedicated to God Almighty, my mother and my brother for their support and love.

To my father Rev. K. E. Moses, I hope you are beaming with pride from heaven.

ACKNOWLEDGEMENT

I am grateful to God the Father, God the son, and God the Holy Spirit for provision, guidance and comfort during the course of my tertiary education and for the grace to complete this project work.

To my mother, PST. D. E. ENOGHASE for constantly checking on the progress of my work and her financial and spiritual support, I love you mother dearest.

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I can't forget my friends, AFUBA MUNACHIMSOAGA and PETER SARAH who were always

in my corner cheering me on. I love and cherish you guys.

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ABSTRACT

Caffeine belongs to the methylxathine class and is recognized as the most utilized psychoactive stimulant worldwide. *Kola nitida*, also known as kola nut, is widely consumed for its alkaloid properties. The aim of this study was to investigate the effect of caffeine and *kola nitida* extract on the gross behavioral patterns of wistar rats. Thirty (30) wistar rats of both sexes and of comparable size and weight were used for this study. The doses investigated ranged from 10mg/kg - 160mg/kg. The animals were grouped into 3, consisting of ten (10) animals each. GROUP 1 (control group) of ten 10) animals were administered 0.4ml of 0.9% saline solution, GROUP 2 (treatment group) of ten (10) animals each, were administered with 10-160mg/kg doses of caffeine orally, GROUP 3 (treatment group) of ten (10) animals each, were administered with 10-160mg/kg doses of *Kola nitida* extract administered orally. Gross behavioral parameters of climbing, line crossing, hinding, stereotype movement, drinking, rearing, scratching, aggression and grooming were studied using the open field test. Prior to the test, all the animals were acclimatized for 8 minutes in the open field apparatus. Frequency of each behavior was scored manually and statistical analysis was done using graph pad prism after the duration of the experiment. One-way analysis of variance (ANOVA) with Tuckey's post hoc test was used to check for the differences between the means. Results showed that caffeine significantly ($p < 0.05–0.0001$) increased locomotor activities, including line crossing, climbing, and hinding up to 40 mg/kg, after which activities declined at higher doses (80–160 mg/kg). Conversely, *kola nitida* induced a dose-dependent reduction in locomotor and exploratory behaviors at low to moderate doses, though mild increases were seen at 160 mg/kg. Both substances altered grooming, scratching, and aggression differently, suggesting dose-dependent and substance-specific modulation of central nervous system (CNS) excitability. The findings support previous evidence that caffeine acts as a potent CNS stimulant, while *kola nitida*, though containing caffeine, also possesses compounds (theobromine, tannins) that modulate or counteract pure caffeine’s stimulant effect.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring central nervous system (CNS) stimulant belonging to the methylxanthine class and is widely recognized as the most utilized psychoactive stimulant worldwide (Evans *et al*., 2024), it is an unselective A2A and A1 adenosine receptor antagonist (Ribero *et al*., 2010). Its primary natural sources include the seeds and leaves of plants such as coffee (Coffea spp.) and tea (Camellia sinensis) (Nehlig, 2010). Caffeine is commonly found in coffee, tea, and energy drinks and is known to influence locomotor activity, alertness, and behavior in both humans and animal models (Nehlig *et al*., 1992). Caffeine can also be consumed through prescription and over the counter medications such as aspirin and appetite suppressant, it is also used cosmetically (Vundrala *et al*., 2024). Following oral administration, caffeine is rapidly and fully absorbed and is primarily metabolized by the hepatic enzyme Cytochrome P450 1A2 (CYP1A2) into its main metabolite, paraxanthine (Grosso *et al*., 2017). The kola tree is native to the rainforests of tropical West Africa, and its cultivation has spread globally due to its commercial importance, Nigeria is often cited as the largest global producer (Opeke, 2005). *Kola nitida*, is chewed for it's alkaloid properties caffeine, theobromine and tannins, which dispel sleep, thirst and hunger (Russell, 1955). Kola nut (*Kola nitida*), a central nervous system stimulant has been shown to mediate some physiological effects that are similar to the action of caffeine (Carrillo and Bennitez, 2000). Kolanuts are chewed fresh and are also in the form of extracts, which is a common food flavouring found in energy drinks (Adejoke *et al*., 2020). In Africa Kola nut is commonly used individually, in groups or ceremonially where it is presented to tribal chiefs or guests (Lim, 2012). Kola nut (*Kola nitida*) is a significant cash crop and has deep cultural and traditional relevance in West Africa, where its seeds are consumed for their stimulant and anti-fatigue properties (Erukainure *et al*., 2017). The nut is a potent natural source of the methylxanthine,

with caffeine concentrations potentially reaching up to 3.0% (Dah-Nouvlessounon *et al*., 2014). Wistar rats, due to their well-characterized physiology and behaioral responses, serve as an ideal model for neuropharmacological and toxicological studies (Debnath *et al*., 2024). Behavioral Activities are the observable and measurable responses of an organism to internal or external signals, recorded as specific actions such as locomotion, feeding, or social activity (Gupta et al., 2022).

1.1. BEHAVIORAL ACTIVITIES IN RATS

Gross behavioral activities encompass the complete repertoire of an animal's spontaneous or evoked actions that reflect the functional state of its Central Nervous System (CNS). When assessing the effects of a drug or extract (like caffeine or *kola nitida*), researchers often monitor these behaviors to determine general toxicity, CNS depression, or CNS stimulation (Turner, 2011; Vogel, 2008).

The behavioral activities observed in this study include; climbing, line crossing, hinding, stereotype movement, drinking, rearing, scratching, aggression and grooming.

1.2. NEUROTRANSMITTER SYSTEM IN THE REGULATION OF BEHAVIORS

Acetylcholine

Acetylcholine (ACh) is a neurotransmitter (chemical messenger) and an ester of acetic acid and choline. It is a major component of the cholinergic system (Purves *et al.*, 2001; Cleveland Clinic, 2022). In learning and memory it is vital for the encoding of new memories and is involved in

synaptic plasticity, particularly in the hippocampus and cortex. Impairment of this system is a hallmark of Alzheimer's disease (Hasselmo, 2006; Huang *et al*., 2022). Acetylcholine is essential for maintaining wakefulness and vigilance, enabling selective attention to sensory stimuli, it is the the primary neurotransmitter at the neuromuscular junction, mediating muscle contraction and voluntary movement (Purves *et al*., 2001).

GABA(Gamma-Aminobutyric Acid)

GABA's main function is to slow down brain activity, producing a calming effect (Cleveland Clinic, 2023). It plays a major role in controlling the nerve cell hyperactivity associated with anxiety, stress, and fear (Cleveland Clinic, 2023). Diminished GABA levels or activity are associated with conditions like anxiety disorders, depression, epilepsy/seizures, and schizophrenia (Cleveland Clinic, 2023). Many common anxiolytic and sedative medications, like benzodiazepines, work by enhancing GABA's inhibitory effects at its receptors (StatPearls, 2024).

Biogenic Amines

Biogenic amines (also known as monoamines) include several important neurotransmitters that act as neuromodulators, significantly impacting cellular and synaptic properties and thus modifying network responses and complex behaviors (Frontiers, 2018). Dopamine is critical for the reward system, motivation, executive function, and the control of voluntary movement (Neuroscience Online, 2024).

Norepinephrine and Epinephrine (neurohumoral agents in the periphery) are associated with the body's "fight-or-flight" response, regulating arousal, vigilance, and attention (Khan Academy, 2023; Neuroscience Online, 2024).

1.3. JUSTIFICATION OF STUDY

Given the popularity and widespread use of both caffeine and Kola nitida as stimulants, there is a need for scientific research to evaluate their comparative effects on behavior. While caffeine is a globally consumed psychoactive drug and Kola nitida is heavily relied upon in traditional settings for alertness, their full behavioral effects and comparative actions remain poorly understood. This study uses Wistar rats to compare their effects on behavioral activities.

1.4. AIM OF STUDY

To assess and compare the patterns of some gross behavioral activities in Wistar rats after separate acute doses of caffeine and *Kola nitida* extract.

1.5. RESEARCH QUESTION

What are the effects of separate acute doses of caffeine and *Kola nitida* extract on some gross behavioral activities in Wistar rats.

1.6. RESEARCH OBJECTIVES

1. To assess the behavioral responses of Wistar rats after acute administration of caffeine.
2. To evaluate the behavioral effects of Kola nitida extract in Wistar rats.
3. To compare the differences in behavioral activities between caffeine and Kola nitida extract treated groups.
4. To determine any statistically significant behavioral alterations such as changes in locomotion, exploration and anxiety-like behavior.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. CAFFEINE

Caffeine (1,3,7-trimethylxanthine) stands as the world's most ubiquitous psychoactive compound, with billions of servings consumed daily (Cappelletti *et al*., 2015; Nehlig, 2010). Its pervasive use stems from its potent ability to modulate the central nervous system (CNS), offering

temporary relief from fatigue and enhancing cognitive function (Alasmari, 2020; Nehlig, 2010). A critical examination of scholarly literature reveals a robust understanding of its chemistry, plant origins, and complex neurological mechanisms.

2.1.1. CHEMISTRY AND SOURCES OF CAFFEINE

Caffeine is a naturally occurring methylxanthine alkaloid with the chemical formula C8H10N4O2 (Arnaud, 1987). Its structural similarity to purine bases, specifically the endogenous nucleoside adenosine, is the key determinant of its pharmacological activity (Alasmari, 2020; Faudone and Arifi, 2021). Once ingested, caffeine is rapidly and almost completely absorbed across the gastrointestinal tract, reaching peak plasma concentration in approximately 30 to 75 minutes (Temple, 2009). Its relatively low molecular weight and moderate lipophilicity allow it to efficiently cross the blood-brain barrier (Faudone and Arifi, 2021; Temple, 2009). In healthy adults, the average plasma half-life of caffeine is approximately five hours, though this exhibits large inter-individual variability (Arnaud, 1987; Nehlig, 2010). Metabolism primarily occurs in the liver via the cytochrome P450 enzyme CYP1A2, which demethylates caffeine into its main active metabolites: paraxanthine (about 85%), theobromine (about 10%), and theophylline (less than 5%) (Faudone and Arifi, 2021; Temple, 2009). Caffeine is synthesized by over 60 plant species, likely as a natural defense mechanism against herbivores (Cappelletti *et al*., 2015).

2.1.2. EFFECTS OF CAFFEINE ON THE CENTRAL NERVOUS SYSTEM

Caffeine's classification as a psychostimulant is rooted in its highly specific and potent interaction with neural receptors (Nehlig, 2010).

Primary Mechanism: Adenosine Receptor Antagonism

Caffeine exerts its key CNS effects by acting as a non-selective competitive antagonist at all four adenosine receptor subtypes A1, A2A, A2B, and A3, though its highest affinity is typically for A1 and A2A receptors (Fredholm *et al*., 1999; Nehlig, 2010). Adenosine functions as an endogenous inhibitory neuromodulator that accumulates in the brain during prolonged wakefulness, binding to its receptors to induce sedation, vasodilation, and a general depressant effect (Basheer *et al*., 2004).

By blocking these receptors, caffeine prevents adenosine from binding, thereby reversing its inhibitory effects (Alasmari, 2020). The antagonism of A2A receptors, which often form functional heteromers with dopamine D2 receptors, is considered the main driver of caffeine's wakefulness and psychomotor stimulant effects (Ferré *et al*., 1991; Fredholm *et al*., 1999).

2.2. *KOLA NITIDA*

The kola nut, predominantly derived from the species *Kola nitida*, is a cash crop and cultural icon indigenous to West Africa, known for its powerful stimulating properties (Ajaiyeoba and Durosaro, 2021; Akpan *et al*., 2018).

2.2.1. BOTANY AND TRADITIONAL USES OF *KOLA NITIDA*

Kola nitida belongs to the Malvaceae family (formerly Sterculiaceae) and is cultivated across the tropical regions of Africa (Adesina *et al*., 2023). The tree produces fleshy, star-shaped pods containing several seeds the kola nuts (Gadzama *et al*., 2016). *Kola nitida* is generally favored commercially over other species due to its higher yield and superior preservation characteristics. Botanically, its seed is identified by its common presentation as a two-cotyledonous nut (Ajaiyeoba and Durosaro, 2021). The nut is a fundamental component of social and ceremonial

life in countries like Nigeria, where its sharing signifies acceptance, respect, and is obligatory during traditional ceremonies (Akpan *et al*., 2018). Ethnopharmacologically, the primary use of chewing the kola nut is as a powerful stimulant to suppress sleep and combat fatigue during long journeys or tasks requiring sustained vigilance (Adesina *et al*., 2023). Beyond stimulation, it is traditionally applied as a folk remedy for various conditions, including fever, nausea, headaches, and intestinal disorders (Akpan *et al*., 2018; Gadzama *et al*., 2016).

2.2.2. PHYTOCHEMISTRY OF *KOLA NITIDA*

Xanthine Alkaloids

The primary compounds responsible for the nut's psychoactive properties are the methylxanthine alkaloids (Okocha and Olayinka, 2017).

Caffeine: Constituting up to 3% of the nut’s dry weight, caffeine is the most abundant and pharmacologically active constituent, providing the immediate stimulatory effect (Sadiq *et al*., 2019).

Theobromine and Theophylline: Present in lower concentrations, these metabolites contribute auxiliary effects, including diuresis and mild smooth muscle relaxation (Asongalem *et al*., 2005).

Polyphenols and Antioxidants

The nut possesses a rich profile of polyphenolic compounds that confer potent antioxidant and anti-inflammatory properties (Ekalu and Habila, 2020; Erukainure *et al*., 2020).

Tannins and Proanthocyanidins: These abundant compounds give the nut its characteristic astringent taste and contribute to its traditional use in managing diarrhea (Amadi and Nwachukwu, 2020; Iwu, 1993).

Catechins and Flavonoids: Compounds like (-)-epicatechin and procyanidins B1 and B2 are present, supporting the plant's efficacy in scavenging free radicals and protecting against oxidative stress, an effect studied in the context of hepatic toxicity (Erukainure *et al*., 2017; Sanni *et al*., 2021).

Other constituents include glycosides, cardiac glycosides, saponins, and various vitamins and minerals, which collectively contribute to the nut's total biological activity (Ekalu and Habila, 2020).

2.2.3. EFFECTS OF KOLA NITIDA ON THE CENTRAL NERVOUS SYSTEM

The impact of *Kola nitida* on the CNS mirrors that of its principal alkaloid, caffeine, but is potentially modulated by the presence of other psychoactive and non-psychoactive compounds (Ekalu and Habila, 2020).

Mechanism of Stimulation

The CNS stimulating action is rooted in the competitive antagonism of adenosine receptors A1 and A2, specifically by caffeine (Ajaiyeoba and Durosaro, 2021). Adenosine, a key inhibitory neuromodulator, promotes sleep and reduces neuronal firing (Fredholm *et al*., 2004). By blocking these receptors, the kola nut effectively inhibits the inhibitory brake on the CNS, leading to:

Increased Arousal and Vigilance: This results in reduced drowsiness and enhanced mental clarity, justifying its traditional use (Akpan *et al*., 2018).

Modulation of Excitatory Systems: The antagonism indirectly facilitates the release of excitatory neurotransmitters, including dopamine and norepinephrine, which contributes to the psychomotor activation, elevated mood, and mild reinforcing effects of consumption (Adesina *et al*., 2023).

Safety and Toxicological Findings

While moderate consumption is traditional, high-dose or chronic exposure to kola nut extracts has prompted toxicological investigation, particularly concerning developmental impacts (Adebayo *et al*., 2017).

Neurotoxicity: Studies in juvenile rats administered high doses of kola nut extract demonstrated oxidative stress and histopathological damage within the cerebral cortex and cerebellum, suggesting that excessive exposure may compromise the integrity of sensitive CNS structures (Okonji *et al*., 2018).

Behavioral Effects: The stimulant properties can be dose-limiting; acute high-dose consumption is associated with adverse effects like insomnia, agitation, tremors, and anxiety symptoms characteristic of methylxanthine overdose (Adebayo *et al*., 2017). These findings underscore the need for caution, especially among sensitive populations.

2.3. GROSS BEHAVIORAL ACTIVITIES IN WISTAR RATS

The assessment of gross behavioral activities in Wistar rats is a fundamental methodology in behavioral neuroscience, serving as a rapid, quantitative measure of central nervous system

(CNS) function, anxiety, motor coordination, and response to pharmacological agents (Ajayi *et al*., 2017; Omoniyi *et al*., 2021). These observable behaviors, typically recorded in the Open Field Test or similar novelty arenas, are categorized to interpret the animal's psychological and physiological state (Lister, 1987; Snout Contact Fixation, 1982).

CHAPTER THREE

3.0. RESEARCH DESIGN AND METHODOLOGY

3.1. MATERIALS USED

The materials used for this study include:

- Wistar rats

- Kola nut seeds
- Caffeine
- Ethanol
- Distilled water
- Perspex cages
- Cotton wool
- Surgical gloves
- Pelleted feed
- Clean water
- Wood shavings
- Syringe
- Oral gastric tube
- Open field apparatus

3.2. EXPERIMENTAL ANIMALS AND MANAGEMENT

This study involved the use of Wistar rats. They all received proper animal care in line with international guidelines for experimental animal handling and Ethical approval was obtained from the College of Medical Sciences ethics board. The Wistar rats were housed in a clean, cool and sterile environment and they were kept in cages where they had access to food and water *ad libitum* throughout the period of the study. The animals were allowed to acclimatize for 2 weeks for proper adaptation to their new environment and were weighed weekly using a Metler Toledo digital weighing balance.

3.3. DURATION OF STUDY

The study was conducted for a period of 15 weeks

3.4. PREPARATION OF ETHANOIC EXTRACT OF *KOLA NITIDA*

The seeds of *Kola nitida w*as peeled, air-dried, and ground into fine powder using a laboratory blender. Two hundred grams (200 g) of each powdered seed were macerated in 1 L of 70% ethanol for 72 hours with intermittent stirring. The extracts were filtered through Whatman No.1 filter paper and concentrated in a rotary evaporator under reduced pressure at 40°C to yield the crude ethanolic extracts. The extract was then reconstituted by dissolving 1 g of the extract in 10 ml of distilled water, followed by a secondary dilution of 1 ml of this mixture in 9 ml of distilled water to produce a working concentration of 10 mg/ml. The extracts were stored in opaque airtight bottles until use.

3.5. EXPERIMENTAL DESIGN

The rats were divided into three (3) major groups with 10 rats (n=10) in each group.

GROUP 1 (control) (n=10) received 0.4ml of 0.9% saline solution

GROUP 2 (n=10) received acute doses of 10mg/kg - 160mg/kg of Caffeine

GROUP 3 (n=10) received acute doses of 10mg/kg - 160mg/kg of *Kola nitida* extract

All the behavioral studies were observed in the open field apparatus, studying two animals at a time for an hour. The observation was carried in a quiet environment.

3.6. ETHICAL CONSIDERATION

Animal management and experimental protocol were carried out in accordance with the recommendation of the 1996 guide for the care use of laboratory animals (Clark *et al.*, 1996).

3.7. STATISTICAL ANALYSIS

Frequency of each behavior was scored manually and the data were analyzed using graph pad prism and results reported as mean ± SEM. A one-way analysis of variance (ANOVA) Tuckey's post hoc test was used to check for differnces between the means. The significant data set was set at $p< 0.05$, $p< 0.01$ and $p< 0.0001$

CHAPTER FOUR

4.0. RESULTS

Table 1: Comparing the mean values of gross behavioral activities in Wistar rats following a graded dosage administration of caffeine.

Parameters	Climbing	Line Crossing	Hinding	Stereotype movement	Drinking
Control	18.40 ± 1.08	228.2 ± 21.98	33.20 ± 2.54	0	9.400 ± 2.06
10 mg/kg	11.00 ± 1.58**	503.6 ± 35.82***	78.20 ± 7.28***	0	29.60 ± 7.53*
20mg/kg	61.50 ± 3.30***	523.2 ± 62.61**	90.00 ± 11.29**	0	28.20 ± 4.78**
40mg/kg	34.20 ± 8.62	515.6 ± 49.38***	82.80 ± 11.62**	0	18.00 ± 3.32
80mg/kg	10.60 ± 3.46	308.2 ± 37.31	62.40 ± 7.56**	2.000 ± 1.00	34.60 ± 3.36***
160mg/kg	23.40 ± 3.74	397.4 ± 14.53***	44.80 ± 5.17**	4.250 ± 1.03	20.20 ± 1.99**

*P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences.

Table 2: Comparing the mean values of gross behavioral activities in Wistar rats following a graded dosage administration of caffeine.

Parameters	Rearing	Scratching	Aggression	Grooming
Control	0	52.20 ± 3.64	7.00 ± 1.16	50.60 ± 4.74
10mg/kg	0	73.40 ± 5.03**	4.667 ± 3.18	64.60 ± 7.05

20mg/kg	0	79.00 ± 8.44*	5.000 ± 4.00	76.20 ± 8.33*
40mg/kg	0	139.2 ± 17.43**	3.000 ± 1.00	69.00 ± 8.80
80mg/kg	0	53.20 ± 3.89	1.667 ± 0.33*	52.80 ± 4.91
160mg/kg	0	41.60 ± 4.23	5.000 ± 2.31	63.80 ± 7.32

*P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences.

Table 3: Comparing the mean values of gross behavioral activities in Wistar rats following a graded dosage administration of *kola nitida.*

Parameters	Climbing	Line crossing	Hinding	Stereotype movement	Drinking
Control	18.40 ± 1.08	228.2 ± 21.98	33.20 ± 2.54	0	9.400 ± 2.06
10mg/kg	9.200 ± 1.43***	246.6 ± 4.51	38.80 ± 1.53	0	29.40 ± 0.87***
20mg/kg	3.000 ± 0.95***	128.0 ± 2.47**	8.800 ± 1.16***	0	21.60 ± 1.47**
40mg/kg	5.400 ± 1.44***	108.6 ± 3.01***	19.40 ± 1.44**	0	13.20 ± 1.86
80mg/kg	9.000 ± 1.58**	190.2 ± 3.56	27.40 ± 2.73	0	20.80 ± 2.29**
160mg/lg	23.80 ± 2.52	230.8 ± 7.68	53.20 ± 3.65**	0	21.80 ± 2.89**

*P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences.

Table 4: Comparing the mean values of gross behavioral activities in Wistar rats following a graded dosage administration of *kola nitida.*

Parameters	Rearing	Scratching	Aggression	Grooming
Control	0	52.20 ± 3.64	7.00 ± 1.16	50.60 ± 4.74
10mg/kg	0	36.80 ± 1.88**	1.667 ± 0.33*	43.20 ± 2.35
20mg/kg	0	21.40 ± 0.75***	1.00 ± 0.0	40.00 ± 0.84
40mg/kg	0	31.60 ± 1.50***	1.667 ± 0.67*	35.40 ± 1.50*
80mg/kg	0	50.00 ± 2.70	2.000 ± 1.00*	58.20 ± 1.20
160mg/kg	0	51.40 ± 1.17	2.250 ± 0.63*	53.00 ± 2.41

*P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences.

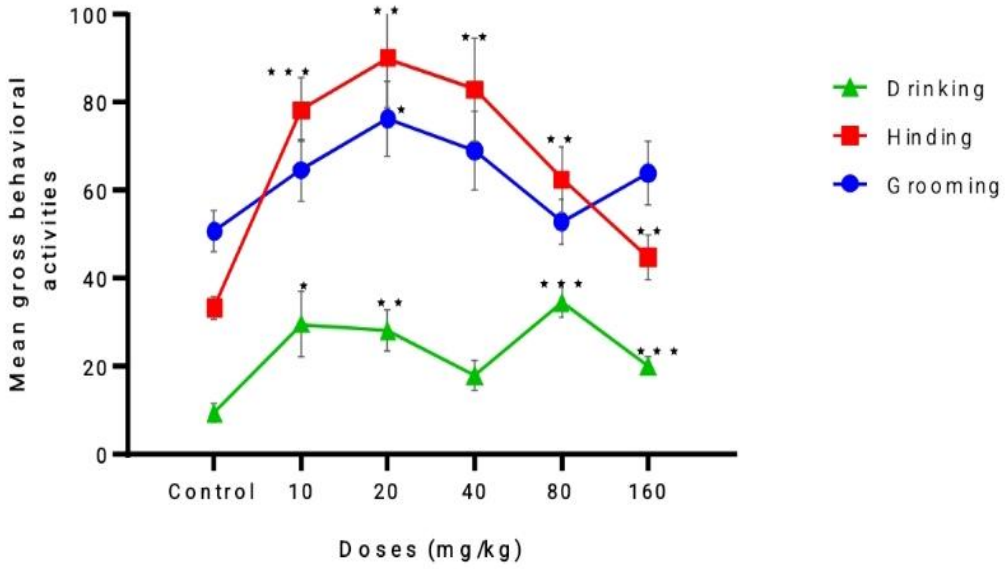

Figure 1: Mean gross behavioral activities (Drinking, Hinding and grooming) in doses of caffeine in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

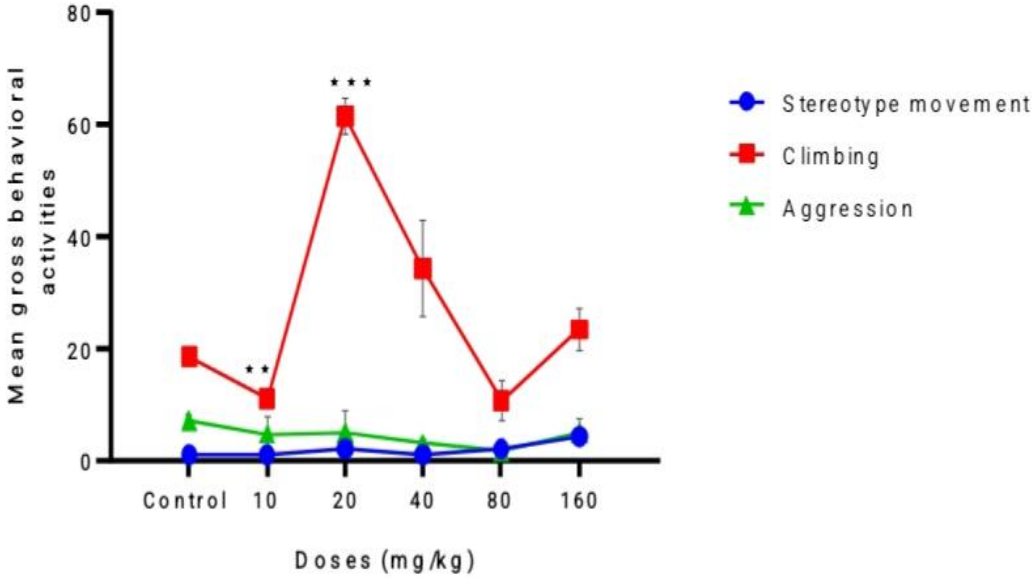

Figure 2: Mean gross behavioral activities (stereotype movement, climbing and aggression) in doses of caffeine in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

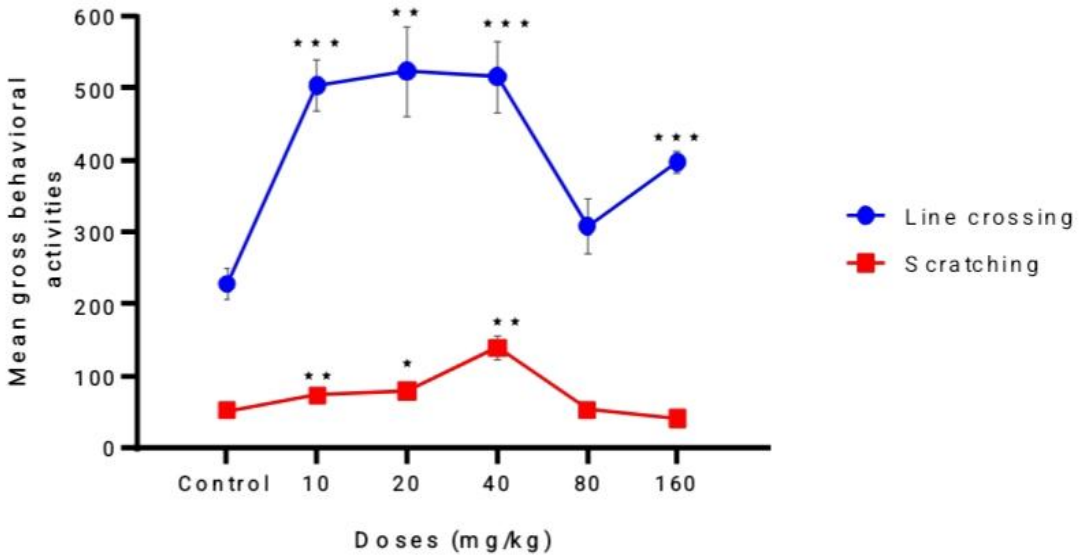

Figure 3: Mean gross behavioral activities (line crossing and scatching) in doses of caffeine in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

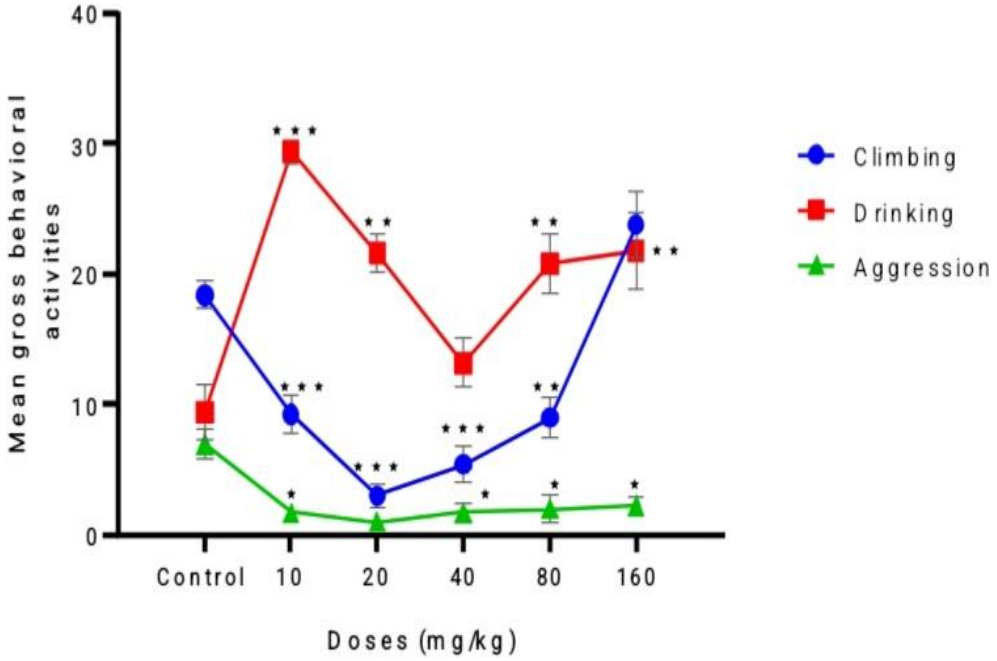

Figure 4: Mean gross behavioral activities (Drinking, climbing and aggression) in doses of *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

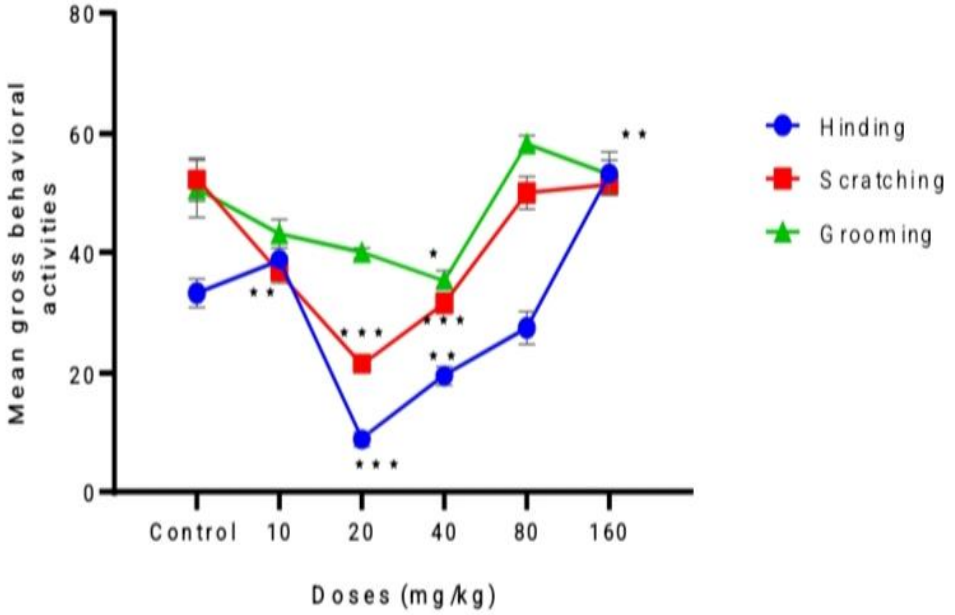

Figure 5: Mean gross behavioral activities (Hinding, scratching and grooming) in doses of *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

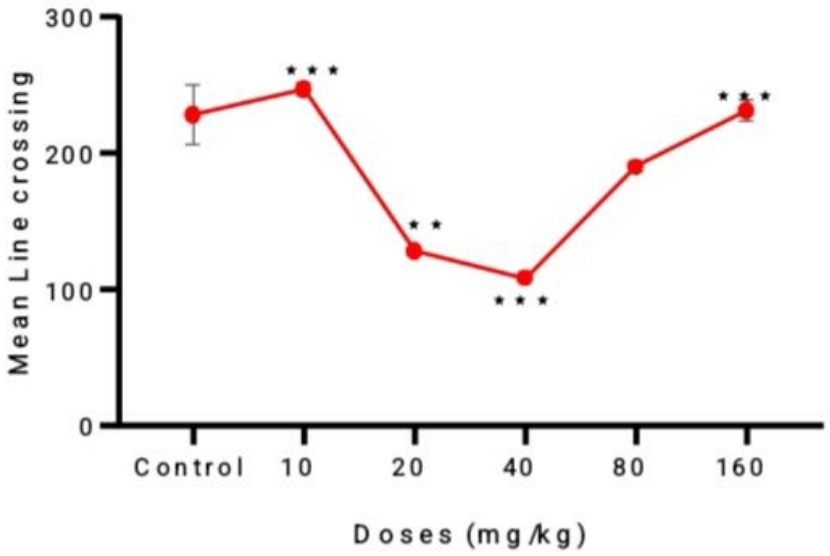

Figure 6: Mean gross behavioral activities (line crossing) in doses of *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significant differences relative to control (n= 5).

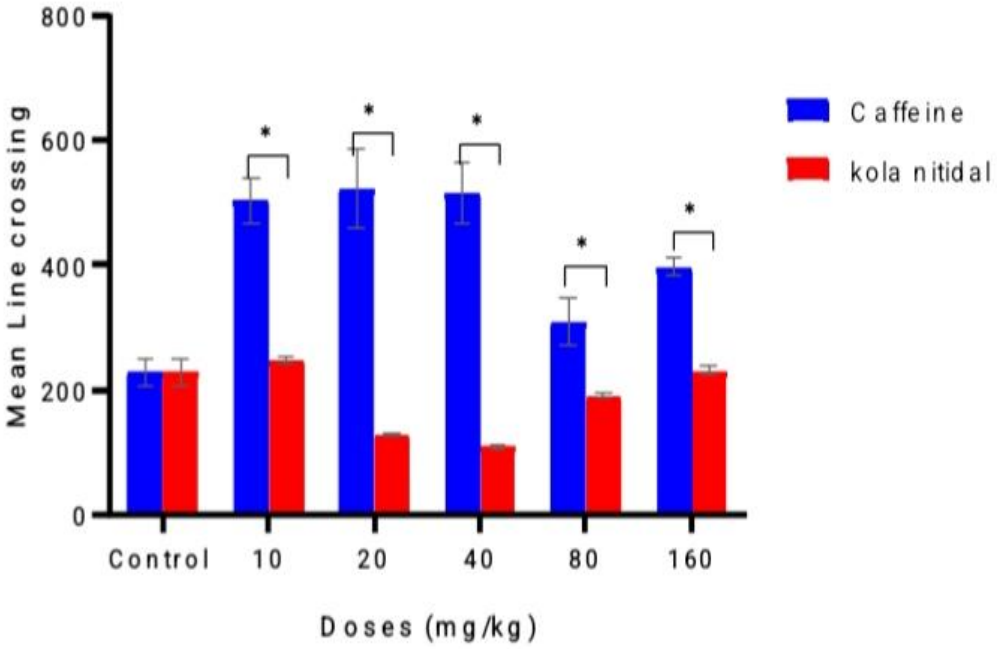

Figure 7: Comparing mean line crossing in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

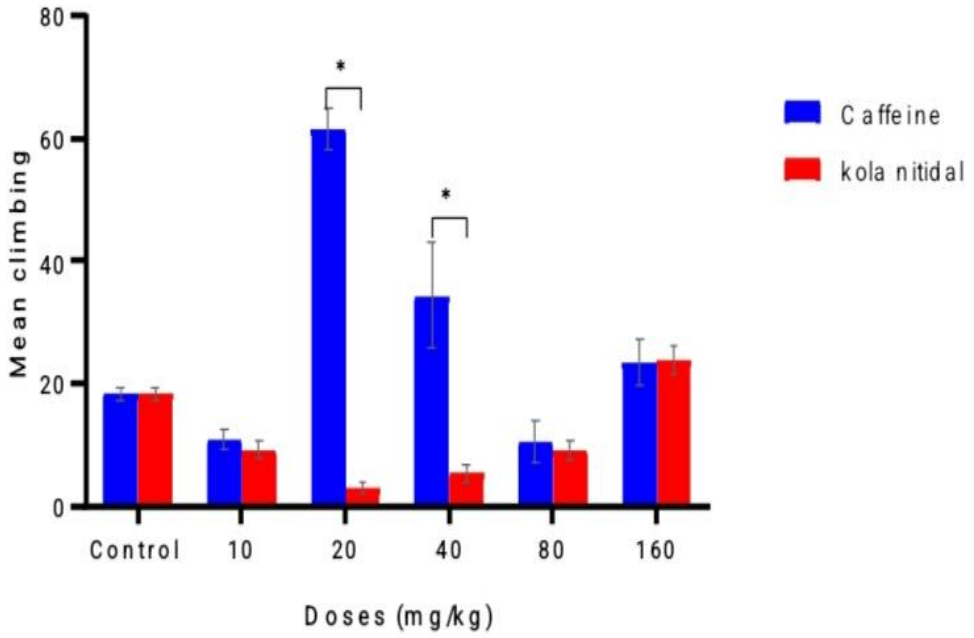

Figure 8: Comparing mean climbing in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

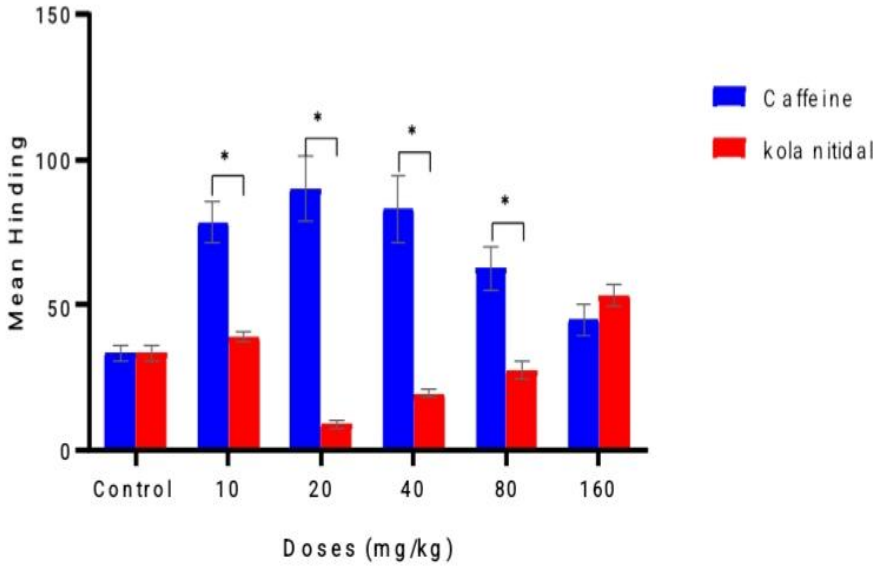

Figure 9: Comparing mean hinding in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

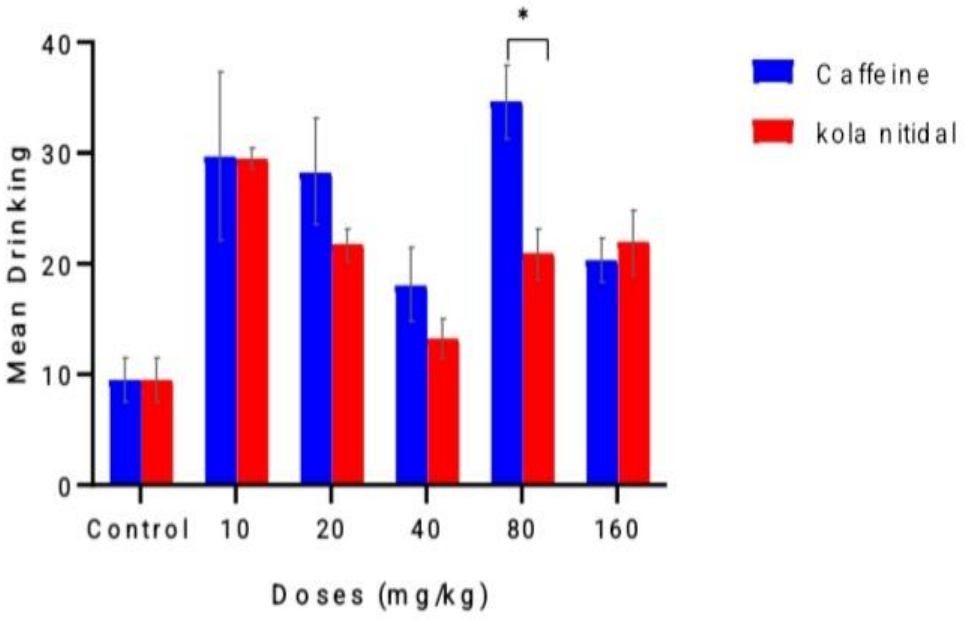

Figure 10: Comparing mean drinking in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

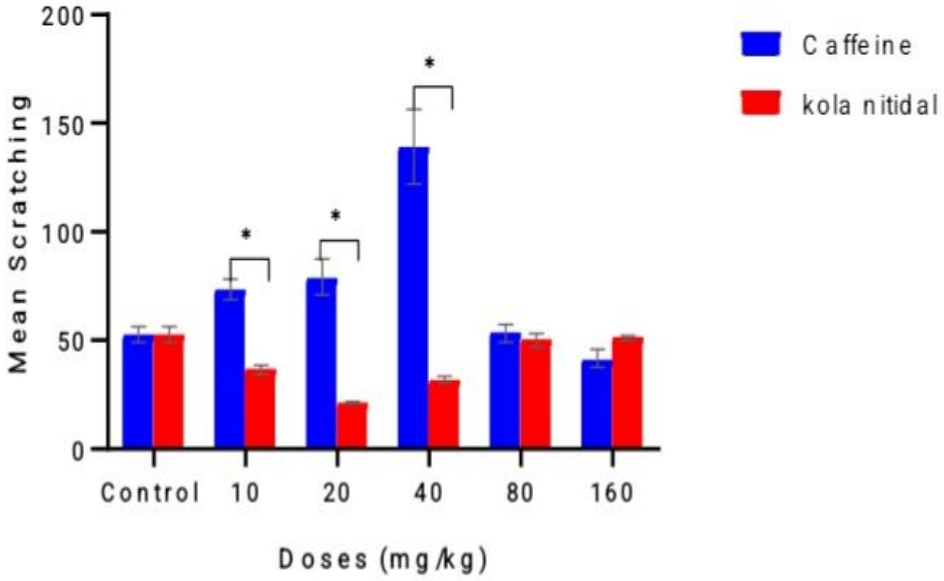

Figure 11: Comparing mean scratching in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

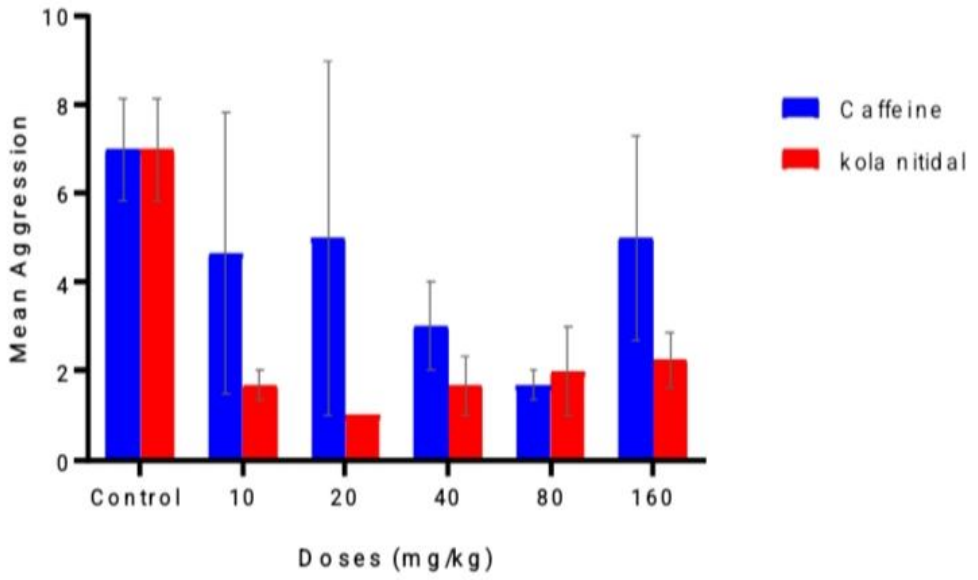

Figure 12: Comparing mean aggression in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

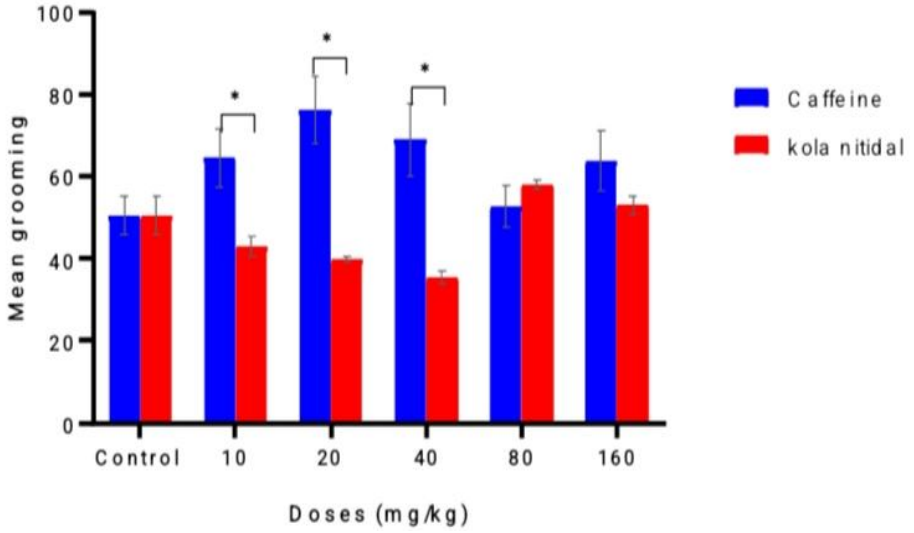

Figure 13: Comparing mean grooming in doses of caffeine and *kola nitida* in rats. *P< 0.05, **P< 0.01 and ***P <0.0001 indicate significance (n= 5).

CHAPTER FIVE

5.0. DISCUSSION AND CONCLUSION

5.1. DISCUSSION

Caffeine Treated Groups (Tables 1 and 2, Figures 1–3)

Caffeine produced a significant increase in climbing and line crossing behaviors up to 40 mg/kg ($p < 0.01–0.0001$), indicating enhanced locomotor and exploratory activity due to CNS stimulation. This aligns with previous findings showing caffeine enhances dopaminergic transmission and arousal (Nehlig *et al*., 1992; Ferré, 2016). At higher doses (80–160 mg/kg), however, these activities declined, possibly due to CNS over-excitation leading to fatigue or anxiety-like behavior, consistent with dose-dependent biphasic effects reported by (Fredholm *et al*. 1999). The hinding behavior increased notably between 10–40 mg/kg, reflecting heightened anxiety or restlessness, which has been linked to caffeine's adenosine receptor antagonism (Evans and Griffiths, 1992). Drinking behavior increased significantly across all caffeine doses ($p < 0.05–0.0001$), likely reflecting increased metabolic rate and dehydration, corroborating findings by (Lara 2010). Stereotype movement was observed only at higher doses (80–160 mg/kg), suggesting possible hyperlocomotion or dopaminergic over-stimulation, as seen in high-dose caffeine models (Garrett and Griffiths, 1998). Scratching increased significantly up to 40 mg/kg, possibly indicating enhanced sensory responsiveness, while aggression decreased at higher doses, showing CNS imbalance or stress responses. Grooming peaked at 20 mg/kg, signifying moderate stimulation, before dropping at higher doses, suggesting that optimal caffeine levels enhance alertness and exploratory cleaning, while excess causes discomfort (Nehlig, 2018).

***Kola nitida* Treated Groups** (Tables 3 and 4, Figures 4–6)

Kola nitida extract caused a reduction in climbing and line-crossing behaviors at low and moderate doses (10–40 mg/kg), showing CNS depressant-like effects. This differs from caffeine's response, despite kola containing caffeine, implying that other phytochemicals (theobromine, tannins) might counteract the stimulant effect (Eisner *et al*., 2016; Odebunmi *et al*., 2020). At 160 mg/kg, slight increases in locomotor activity were seen, suggesting biphasic responses similar to caffeine but with lower potency. Hiding significantly decreased at 10–40 mg/kg but increased at 160 mg/kg, suggesting low-dose sedation and high-dose activation, possibly due to the combined action of alkaloids. Drinking behavior increased steadily across doses, possibly due to mild metabolic stimulation or dry-mouth effect from tannins. No stereotyped movement was recorded, implying kola's CNS effect is less excitatory than pure caffeine. Scratching and aggression reduced significantly ($p < 0.05–0.0001$) at lower doses, supporting kola's calming or anxiolytic-like properties, possibly from flavonoids (Ogutuga, 1975). Grooming decreased at 20–40 mg/kg, confirming reduced alertness and activity, but increased again at 80–160 mg/kg, consistent with dose-dependent biphasic modulation of behavior.

Comparative Behavioral Analysis (Figures 7–13)

Direct comparison of caffeine and *kola nitida* revealed marked differences in behavioral outcomes: Line crossing and climbing: Caffeine consistently produced higher activity levels than *kola nitida*, confirming stronger psychostimulant action (Ferré, 2016). Hinding and scratching: *Kola nitida* showed lower scores, suggesting reduced anxiety and reactivity compared to caffeine. Drinking: Both increased consumption, but caffeine induced greater thirst, likely due to its

higher metabolic activation. Aggression and grooming: Caffeine increased grooming at moderate doses, while *kola nitida* reduced aggression, indicating different neurotransmitter interactions—caffeine enhancing dopaminergic tone, kola modulating GABAergic or serotonergic balance (Nehlig, 2018).

5.2. CONCLUSION

Overall, caffeine acts as a potent stimulant enhancing motor and exploratory behavior, whereas *kola nitida* exerts milder and partly depressive effects at comparable doses, likely due to complex phytochemical interactions.

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