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NEUROPHARMACOLOGICAL EFFECTS OF THEMETHANOL SEED EXTRACT OF *COLA ROSTRATA* IN MICE

BY

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This is to certify that the thesis, **"Neuropharmacological Effects Of Methanol Seed Extract Of *Cola rostrata* In Mice"** submitted to the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria in partial fulfilment of the requirements for the award of Master of Science (Pharmacology) by Adeniyi Sabur Adewale is an authentic work carried out by him.

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DEDICATION

This work is dedicated to the glory of the ALMIGHTY ALLAH, who has made the completion of the work possible.

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LIST OF ABBREVIATIONS

CFS: Chronic Fatigue Syndrome

GABA: Gamma-amino butyric acid

DA: Dopamine

NA: Noradrenaline

5-HT: 5-hydroxytryptamine

Ach: Acetylcholine

GAD: Generalised Anxiety Disorder

PD: Panic Disorder

BDZs: Benzodiazepines

TCAs: Tricyclic Antidepressants

AEDs: Antiepileptic Drugs

CR: *Cola rostrata* seed extract

MeOH: Methanol extract of *Cola rostrata* seed

OFL: Open-Field Locomotion

NIB: Novelty Induced Behavior

NIR: Novelty-Induced Rearing

NIG: Novelty-Induced Grooming

FST: Force-Swimming Test

CPZ: Chlorpromizine

DZP: Diazepam

FXT: Fluoxetine

CYP: Cyproheptadine

NS: Normal Saline

LOS: Latency OfSleeep

DOS: Duration Of Sleep

SNRIs: Serotonin and Noradrenaline Reuptake Inhibitors

GPCRs: G protein-coupled receptors

LGICS: Ligand-Gated Ion Channels

NMDA: N-Methyl-D-Aspartate

AMPA: α-Amino-3-Hydroxyl-5-Methyl-4-isoxazole-propionate

BDNF: Brain-Derived Neurotrophic Factor

VEH: Vehicle

ABSTRACT

Cola nut is widely used in Nigeria and many West African countries as part of traditional hospitality, cultural and social ceremonies. It is known to contain caffeine, theobromine and some vitamins such as niacin and riboflavin. *Cola rostrata* (CR) seed has been grouped among the plants that possessed aphrodisiac effect. Comparative effect of chronic consumption of a specie "*Cola nitida*" and it active constituent, caffeine diet on locomotor behavior and body weights has been elucidated.This study investigated the neuropharmacological effect of the methanol extract of *Cola rostrata*seed in mice. This was with the objective of establishing the possible stimulant effects of the seed being a *Cola specie*.

The seeds obtained from ripe pulp of authenticated *Cola rostrata* plant, were air-dried, powdered and was extracted with pure methanol. The acute toxicity test using Lorke's method was carried out to determine oral LD_{50} of the plant in mice. The effects of the extract on novelty-induced behaviors (NIBs) such as open-field locomotion, rearing, and grooming in mice were evaluated. The exploratory and anxiolytic effect using hole board test were also investigated. The extract was also screened for anti-depressant and sedative activity using forced-swimming test (FST) and pentobarbitone-induced sleeping time models in mice respectively. The involvement of noradrenergic (NA) and serotonergic (5-HT) systems in the activities of the plant were also investigated by the use of NA antagonist (yohimbine) and 5-HTantagonist (cyproheptadine) to elucidate the mechanism(s) of action of the extract in depression.

The results obtained showed that methanol (MeOH) extract has LD_{50}(p.o) of 3807.89 mg/kg. The extract caused a significant ($p < 0.05$) inhibition of novelty-induced grooming (NIG) at 500 mg/kg and 1000 mg/kg, p.o, when compared to the vehicle control group (VEH; 10 ml/kg of 5% Tween 80). A significant ($p < 0.01$) reduction in the frequency of head dips was produced at 250

mg/kg in the same pattern as Chlorpromazine (0.01 mg/kg, i.p), and also a significant ($p< 0.05$) reduction was produced at 500 and 1000 mg/kg, p.o, compared to vehicle group. The extract at 250 mg/kg and 1000 mg/kg produced a significant ($p < 0.01$) reduction in latency of sleep (LOS) and significant ($p < 0.05$) increase in the duration of sleep (DOS) at 250 mg/kg and a significant ($p < 0.01$) increase in the duration of sleep at 500 mg/kg and 1000 mg/kg doses when compared to the vehicle group. which implicated that CR possessed a sedative activity at intermediate and high doses. CR at low dose produced a significant ($p < 0.05$) reduction of immobility time and more significant ($p < 0.01$) reduction when pretreated with fluoxetine (20 mg/kg,i.p) compared to vehicle group. However, pretreatment with cyproheptadine (3 mg/kg, i.p) significantly ($p < 0.001$) reduces the immobility time at all doses when compared to vehicle group. Pretreatment with yohimbine (1 mg/kg, i.p) also produced a significant ($p < 0.01$) reduction of immobility time only at low and intermediate doses compared to the vehicle group. These pretreatment with cyproheptadine or yohimbine only significantly reduced ($p < 0.05$) the immobility time at low dose when compared to the respective positive control groups.

This study shows that the methanol extract of *Cola rostrata*was moderately toxic in mice and possessed a significant ($p < 0.05$) anti-depressant effect at low dose which could be mediated partly by serotonergic and partly by adrenergic system. It also concluded that CR possessed anxiogenic effect prominent at low dose a CNS depressant and sedative effect at intermediate and high dose.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cola nut is the seed of the cola nut plant. It is the largest genus of the family Sterculiaceae (Cheek, 2002), which are groups of flowering plants and some of them are used in folk medicine in the environment where they are found (Jobstl 2004, Watson 1994). Cola nut is widely used in Nigeria and many West African countries as part of traditional hospitality, cultural, and social ceremonies. It is known to contain caffeine, theobromine and some vitamins such as niacin and riboflavin (Dades Obed, 2013). Cola nut is used for short-term relief of fatigue, depression, chronic fatiguesyndromes (CFS), melancholy, lack of normal muscle tone (atony), exhaustion, dysentery (Morton, 1992), atonic diarrhea, weight loss, and migraineheadaches(Seitz *et al.,* 1992).

The previous study carried out on the comparative effects of chronic consumption of kola nut (*Cola nitida*), 25% wt/wt of rodentchow and its active constituent, caffeine(0.66% wt/wt of rodent chow)on locomotor behaviour and body weights of mice has been elucidated showing that chronic consumption of kola nut and caffeine diets caused a decrease in food intake and body weight (Umoren *et al.,* 2009).Consumption of caffeine-diet significantly decreased water intake and locomotor activity while kola nut-diets caused no significant change in the two parameters. Hence, this suggested that the stimulatory effect produced by most cola species could not be linked to the caffeine-content of the plant. (Umoren *et al.,* 2009). The stimulatory or

inhibitory activity possessed by someplants can give a clue on the possibility of the plant in mediating some neurological disorders. Thus, the Neuropharmacology of the plants can be explored.

Neuropharmacology is the study of how drugs affect the cellular functions of the nervous system. It is divided in two main branches: behavioural and molecular. Behavioural neuropharmacology focuses on the study of how drugs affect human behaviour (Neuropsychopharmacology). This includes the study of how drug dependence and addiction affect human brain (Everitt and Robbins, 2005). On the other hand, molecular neuropharmacology involves the study of neurons and their neurochemical interactions, with the overall goal of developing drugs that have beneficial effects on neurological function (Narahashi, 2000).

1.2 Literature review

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being. (Salmans and Sandra, 1997).Depression is a major psychiatric disorder affecting nearly 17% of the world population and imposes a substantial health burden on the society (Nemeroff, 2007; Yu *et al.,* 2002). The World Health Organization estimates that unipolar depression will be the second most prevalent cause of illness-induced disability by the year 2020. Depressed people can feel sad, anxious, empty, hopeless, helpless, worthless, guilty, irritable, ashamed or restless. They may lose interest in activities that were once pleasurable, experience over-eating or loss of appetite, have problems concentrating, remembering details or making decisions, and may contemplate, attempt or commit suicide.

Insomnia, excessive sleeping, fatigue, aches, pains, digestive problems or reduced energy may also be present. Adversity in childhood, such as bereavement, neglect, unequal parental treatment of siblings, physical abuse or sexual abuse, significantly increases the likelihood of experiencing depression over the life course (Linder*et al*., 2014; Christine Heim *et al*., 2008; Pillemer Karl *et al.,* 2010).Three quarters of the patients experience more than one episode of depression and the risk of recurrence is higher if the first episode occurs at a younger age and if there is a family history of depression (Hollon*et al*, 2006). The risk of recurrence increases with each new episode and as the number of depressive episodes increases, the influence of life stress on recurrence wanes(Kendler*et al.,* 2000).It is generally accepted that the major neurochemical process involved in depression is impairment of monoamine functions and decrease of serotonin, noradrenalin and dopamine levels (Delgado, 2000). The role of anti-depressant is to increase the availability of these monoamines at the synapse which may modulate a long-term adaptive changes in modulating monoamine functions and promote nuerogenesis (Elhwuegi, 2004; Daily *et al.,* 2004).

Serotonergic, noradrenergic and dopaminergic pathways represent the major target of current therapeutic treatment and drug development. Yet, there are few major issues with conventional antidepressant drugs;

- Low remission rate experienced by some patients with about 50% individual with depression showing full remission (Berton and Nestler, 2006).

Side effects: There are some anti-depressants drugs that are not tolerable by some depressed individual because of their side effects which can be predicted by the receptor selectivity and site of action and ranges from one class of drug to another (Feighner, 1999). For example, GI

disturbances are often associated to SSRIs which is more prominent with fluvoxamine. Agitation, anxiety, and insomnia are mostly reported in patient taken sertraline and fluoxetine. Other side effects include sexual