Applied Sciences Research Periodicals
December 2023, Vol. 1, No. 9, pp. 01-13

Effects of Isolated Cola *nitida* (Kolanut) Caffeine on Metoclopramide-Induced Parkinsonism in Male Wistar Rat

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Abstract:
This study investigated the effect of isolated cola nitida caffeine on metoclopramide-induced parkinsonism in male Wistar rat. Thirty (30) male Wistar rat weighing between 140 - 160 grams (g) were used for the research. They were divided into six (6) groups of five (5) rats each. Parkinsonism was induced in the male Wistar rats by chronic administration of Metoclopramide (MCP) dissolved in saline and injected intraperitoneally at a dose of 20 mg/kg body weight once daily for 14 days. The normal control group (group A) received standard pellet diet and distilled water ad libitum. Group B was induced with parkinsonism and untreated. Groups C, D and E, were induced with parkinsonism and received 50mg/kg, 100mg/kg and 200mg/kg, respectively, of Cola nitida caffeine extract orally. Group F, also induced with parkinsonism, was treated with 100/25mg/kg Pardopa (levodopa-carbidopa) - the standard drug. This study showed a measurable amelioration of parkinsonian symptoms in the groups treated with Cola nitida caffeine. However, it was concluded in this study that though the potential utility of Cola nitida caffeine as a neuroprotective agent is established, there is need for further research into the implications of this Cola nitida caffeine in the aftermath. The efficacy of Cola nitida caffeine appears to be fairly modest and not without limitations. The standard drug remains a superior choice in managing parkinsonism as it presents with minimal side effects compared to Cola nitida caffeine.

Keywords: Parkinsonism, levodopa, caffeine.

INTRODUCTION

Parkinsonism is a clinical syndrome that manifests as varied degrees of stiffness as well as a number of classical motor symptoms such as bradykinesia, tremor, rigidity and unstable posture (Balestrino & Schapira, 2019). They are commonly due to primary neurodegenerative disease, resulting in the loss of dopaminergic nerve terminals along the nigrostriatal pathway, similar to idiopathic Parkinson's disease, dementia with Lewy bodies and other neurodegenerative disorders (Ogawa *et al.,* 2018). It is a constellation of neurological abnormalities, including sluggish or delayed movement, resting tremor, muscular stiffness, and issues with balance and coordination. It is important to note that Parkinsonism is not the same as Parkinson's disease but because it is the most common cause of Parkinsonism, it is often used in place of the term "Parkinsonism." Parkinsonism typically affects both sides of the body, whereas Parkinson's disease generally affects one side more than the other (Jankovic, 2018). Parkinsonism is characteristically present in Parkinson's disease. The symptoms can also result from other

neurodegenerative disorders, as well as specific brain lesions, head trauma, medications, metabolic conditions, and toxin exposure (Wang *et al.,* 2023). Development of Parkinsonism has been linked to certain factors; Age, the prevalence and risk of development increases with age, affecting 1–2% of the population over the age of 65 years, and 3% of those over the age of 85 years (Ostrem and Galifianakis, 2010). Environmental toxins; Chronic manganese (Mn) exposure has been shown to produce Parkinsonism-like illness characterized by movement abnormalities (Guilarte & Gonzales 2015). Other environmental toxins such as pesticides, herbicides (paraquat) have been associated with an increased risk of developing Parkinsonism (Liu *et al.,* 2021).

Parkinson's disease is considered a degenerative process, affecting the nigrostriatal dopaminergic system and manifests with Parkinsonism symptoms (Bonnet *et al.,* 2012). The most common cause of Parkinsonism is Idiopathic Parkinson's disease, a degenerative brain disorder primarily caused on by the loss of dopaminergic neurons within the substantia nigra of the midbrain that releases dopamine in the striatum, which reportedly accounts for about 80% of all cases. The motor symptoms of Parkinsonism result from the loss of the inhibitory influence of dopamine on the basal ganglia, a group of brain structures that are involved in the control of movement. The basal ganglia normally receive signals from the cerebral cortex, which passes through the striatum, a part of the basal ganglia which then sends signals to the substantia nigra, which produces dopamine that is sent back to the striatum (Bear *et al.,* 2023). This feedback loop helps to regulate movement and ensure that it is smooth and coordinated. There is a disruption of this feedback loop in Parkinsonism due to loss of dopamine-producing neurons. As a result, there is an inconsistency in the levels of dopamine in the basal ganglia, which leads to the characteristic motor symptoms of Parkinsonism such as tremors, stiffness, postural disturbances and difficulty with movement. Dopamine is a catecholamine neurotransmitter that has an important role in motor behavior and is implicated in numerous mental conditions and emotional states (Milyavsky *et al.,* 2020). The dietary source and precursor for dopamine is the amino acid tyrosine. Prior to storage within vesicles, Dopamine synthesis occurs at the synaptic terminal and as dopamine is synthesized, it is released by dopaminergic neurons, and it then travels across synapses to bind to dopamine receptors. This binding cause signals to be sent to the receiving cell, which has a variety of physiological and behavioral implications (Korshunov *et al*., 2020).

The main motor features of Parkinsonism (i.e., tremor, rigidity, and akinesia) are associated with a deficiency of dopamine in the posterior putamen and the motor circuit (Jeong *et al*., 2023). The loss of dopamine-producing neurons in the pars compacta region also leads to changes in other parts of the brain including the basal ganglia and cerebral cortex (Kochar *et al.,* 2022). This has been shown to significantly affect the quality of life of people with Parkinsonism, causing significant morbidity, and reducing life expectancy. It also has significant financial repercussions, such as loss of earnings, increased healthcare expenses, and increased cost of drugs globally. Drug-induced extrapyramidal illnesses are becoming more common, but certain drugs can cause Parkinsonism to show up by obstructing dopamine receptors in the brain. One of such drugs is metoclopramide, which will in the course of this study, be the inducer for Parkinsonism in a male Wistar rat.The Food and Drug Agency (FDA) has approved Metoclopramide to treat nausea and vomiting in patients with gastroesophageal reflux disease (in patients who fail to respond to established therapy) and diabetic gastroparesis by increasing gastric motility (Shakhatreh *et al*., 2019; Rettura *et al*., 2021). In simple terms, Metoclopramide is an anti-sickness medicine taken by prescription to help you stop feeling sick mostly after an operation or chemotherapy. It has therefore been developed as an anti-emetic for a variety of gastrointestinal diseases, such as nausea and vomiting, but it may also impact dopamine levels in the brain and by implication,

reduces dopamine availability in the brain, leading to a decrease in motor function and development of Parkinsonism symptoms. Older people and those who use Metoclopramide for a prolonged period are at a higher risk of developing Parkinsonism from the drug (Jankovic, 2018).

Presently, some available drugs do provide beneficial symptomatic relief, but with sustained use, they are frequently linked to manifestations of adverse effects. Levodopa, among others is one of the therapies for Parkinson's disease, and can improve motor function for years, but subsequently results in uncontrollable muscular spasms such as dyskinesia and dystonia (sustained muscle contractions). These prompted the quest for alternative measures that can be adopted to manage Parkinsonism. Some chemical compounds available as a supplement or in a person's diet also have been shown to have a neuroprotective effect for Parkinsonism. Caffeine (tea, kola) has been shown to reduce the loss of dopamine-producing neurons in the brain (Alasmari, 2020).

Caffeine, 1,3,7-trimethylxanthine, is a natural stimulant that is found in various plants, including coffee beans, tea leaves, and cocoa beans and is also added to some soft drinks and medications (Giada, 2019). It is both water- and lipid-soluble, it readily crosses the blood–brain barrier that separates the bloodstream from the interior of the brain. The caffeine molecule is structurally similar to adenosine, and therefore competes with adenosine for binding sites on the cell surface without activating them (Giada, 2019), Once in the brain, caffeine acts as an agent that reduces the effects of adenosine. The European Food Safety Authority (EFSA) reported that up to 400 mg of caffeine per day (around 5.7 mg/kg of body mass per day) does not raise safety concerns for non-pregnant adults, while intakes up to 200 mg per day for pregnant and lactating women do not raise safety concerns for the fetus or the breast-fed infants. It is possible for caffeine to be good for your health as well as bad. It may result in undesirable consequences including restlessness, anxiety, trembling, and an accelerated pulse. However, studies have shown that moderate caffeine use has been linked to a lower chance of developing certain conditions, including Parkinsonism. This further suggests that caffeine may have a protective effect against the development of Parkinson's disease, and that it may also have some potential as a treatment for the symptoms of Parkinson's disease, including Parkinsonism. One possible mechanism by which caffeine may reduce Parkinsonism is through its effects on the brain's dopamine system. Caffeine has been shown to increase dopamine release and block the activity of adenosine, a neurotransmitter that inhibits dopamine release. These effects may help to compensate for the loss of dopamine in the brain and reduce the severity of Parkinsonism symptoms. In addition to other therapeutic options, this study hopes to explore the potential protective and therapeutic effects of isolated Cola *nitida* caffeine in metoclopramide-induced Parkinsonism on Male Wistar rat.

MATERIALS AND METHODS

Drugs and Chemicals

The drugs used in this study were Reglan (Metoclopramide), Pardopa (levodopa and carbidopa drug combination). Reglan was manufactured by ANI Pharmaceuticals Inc, U.S.A and Pardopa was manufactured by Schwarz Pharmaceuticals, U.S.A. They were both purchased from Donaville Pharmacy, Chime Avenue, New Haven, Enugu Nigeria. The chemicals used in this study were ethanol, dichloromethane, hexane, nitric acid, ammonium molybdate, formalin, ethyl acetate, silica gel, sodium sulfate and standard solutions of caffeine. All chemicals were of analytical grade and obtained from Ogbete Main Market, Enugu, Nigeria.

Plant Collection, Identification and Authentication

Fresh Cola *nitida* seeds were locally sourced and obtained from new market in Enugu, Nigeria and used for this study. It was identified and authenticated as a viable cola nut fruit (Cola *nitida*) by Mr. J.I. Enyi at the Department of Crop Science, University of Nigeria, Nsukka.

Sample Preparation (Aqueous Extraction) of Cola *nitida*

Approximately 3.5kg of the Cola *nitida* seeds were weighed using a weighing balance. The seeds were properly washed and then transferred into a thermostatic oven maintained at 80°C for 48 hours. At the end of 48 hours, it was removed and reweighed to obtain the dry weight of the Cola *nitida* as 1.86kg. The 1.86kg of dry Cola *nitida* was transferred into an electric kenwood blender and pulverized to further increase the surface area. The powdered specimen was soaked in 250mL of 70% ethanol for 72 hours with occasional shaking. The ethanol extract was filtered using a Whatman filter paper grade 1 (with pore size of 11 microns and a filtration rate of 150 seconds/100 mL) and evaporated to dryness at temperature 40-45 °C and stored at 4 °C until further use. (Ani et al., 2020)

Extraction of Caffeine from Aqueous Cola- nitida Extract Using Column Chromatography:

The extraction of caffeine from aqueous Cola *nitida* extract using column chromatography was performed as following the method of column chromatography by Ani et al., 2022. The aqueous extract was dissolved in a small amount of water and transferred to a separator funnel. Dichloromethane was added and the funnel was shaken gently. The layers were allowed to separate and the lower organic layer was collected. The extraction was repeated with fresh dichloromethane until no more caffeine was extracted. The organic layers were combined and dried over anhydrous sodium sulfate. The solvent was filtered and evaporated to obtain a yellowish solid, which was the crude caffeine. A glass column was packed with silica gel and wetted with hexane. The crude caffeine was loaded on top of the column and eluted with a mixture of hexane and ethyl acetate at a ratio of 9:1. The fractions were collected and monitored by TLC (Thin layer Chromatography) using a UV lamp. The fractions that contained pure caffeine, as indicated by a single spot on the TLC plate, were combined and the solvent was evaporated to obtain white crystals of caffeine (Nyamien *et al.*, 2015).

Recovery Procedure:

The recovery procedure was performed to determine the percentage yield and the purity of the extracted caffeine. The percentage yield of the extract was calculated by dividing the mass of the extracted caffeine by the mass of the original extract and multiplying by 100. The purity was determined by measuring the peak area of the extracted caffeine and comparing them with those of authentic caffeine standard (Umeda & Puyate, 2020).

Thin Layer Chromatography:

Thin layer chromatography (TLC) was used to monitor the progress of the extraction and purification of caffeine from Cola *nitida* extract. TLC was also used to identify any other compounds that may be present in the extract, such as tannins and phenols.

Tests for identification of Caffeine:

Tannic Acid Test:

This test is based on the formation of a white precipitate between caffeine and tannic acid. A small amount of the caffeine extract is dissolved in water and a few drops of tannic acid are added. The formation of a white precipitate indicated the presence of caffeine (Umeda & Puyate, 2020).

Murexide Color Reaction Test:
This test is based on the formation of a purple-colored complex between caffeine and ammonium molybdate in the presence of nitric acid. A small amount of the caffeine extract is dissolved in water and a few drops of nitric acid are added. The solution is heated until it becomes yellow and then cooled. A few drops of ammonium molybdate were added and the solution was heated again. The formation of a purple color indicated the presence of caffeine (Umeda & Puyate, 2020).

Experimental Animals
A total of thirty (30) male Wistar rats weighing between 140 - 160 grams (g) were used in this experiment. These animals were purchased from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, Enugu State University College of Medicine (ESUCOM), Enugu, Nigeria. The rats were accommodated in the Animal House after purchase and they were kept in cages (27 × 30 × 42 cm) in a well-ventilated space at room temperature 25 ± 1 °C. They were fed with standard pellet diet produced by Guinea Feed Nigeria Limited and water given was given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced. Proper hygiene was maintained by constant cleaning and removal of feces and spilled feed from the cages daily.

Method of Induction of Parkinsonism:
Parkinsonism was induced in the male Wistar rats by chronic administration of metoclopramide (MCP), which is an antiemetic drug that blocks dopamine receptors in the brain and induces Parkinsonism-like symptoms in rats. MCP-induced Parkinsonism is a well-established animal model that mimics the motor symptoms seen in movement disorders like in Parkinson's disease. MCP was dissolved in saline and injected intraperitoneally at a dose of 20 mg/kg body weight once daily for 14 days. This dose and duration were selected based on previous studies that reported optimal induction of Parkinsonism by MCP (Vaidya *et al.*, 2022).

Experimental Design:
- Group 1 (n=5): Normal control (NC), received saline orally
- Group 2 (n=5): Negative control received Metoclopramide (MCP) 20mg/kg + saline orally
- Group 3 (n=5): 20mg/kg MCP+ 50mg/kg Cola *nitida* caffeine extract orally
- Group 4 (n=5): 20mg/kg MCP + 100 mg/kg Cola *nitida* caffeine extract orally
- Group 5 (n=5): 20mg/kg MCP + 200 mg/kg Cola *nitida* caffeine extract orally
- Group 6 (n=5): Levodopa-carbidopa (LC) group, 20mg/kg MCP + Pardopa 100/25 mg/kg of LC respectively orally

The treatments were given once daily for 14 days after induction of Parkinsonism by MCP. The doses of Cola *nitida* caffeine extract was selected based on previous studies that reported its pharmacological effects. The dose of levodopa-carbidopa was selected based on previous studies that used it as a standard drug for anti-Parkinsonism (Erukainure *et al.*, 2017).

Measurement of Serum Dopamine Levels
Sample Collection and Storage:
In other to measure serum dopamine levels, blood samples were collected through the orbital sinus of the control and experimental rats. Anesthesia (Ketamine Hydrochloride Injection, USP) was administered intraperitoneal before the blood collection and, alcohol swabs were placed around the site of puncture to reduce bleeding after collection. The blood samples were collected

using serum separator and was allowed to clot overnight. After which, it was centrifuged for 20minutes at approximately 1000xg. The freshly prepared serum samples were assayed immediately at room temperature.

Reagents and Materials Provided:

1. Pre-coated, ready-to-use well strip plate
2. Plate sealer
3. Standard (2)
4. Standard Diluent (1x20mL)
5. Detection Reagent A (1x120uL)
6. Assay Diluent A (1x12mL)
7. Detection Reagent B (1x120uL)
8. Assay Diluent B (1x12mL)
9. TMB (Tetramethylbenzidine) Substrate (1x9mL)
10. Stop Solution (1x6mL)
11. Microplate reader with 450nm filter
12. Absorbent paper for blotting the microplate
13. Wash Buffer (30x concentrate, 1x20mL)

Dopamine Assay Procedure:

1. Prepare wells for diluted standard, blank, and samples. Use a microplate shaker for gentle mixing.
2. Add dilutions of standard, blank, and samples, then Detection Reagent A and cover with plate sealer, Incubate at 37°C for 1 hour.
3. Wash wells thoroughly and remove residual liquid.
4. Add Detection Reagent B and cover with plate sealer, incubate at 37°C for 30 minutes.
5. Repeat washing process.
6. Add Substrate Solution, incubate at 37°C for 10-20 minutes. Protect from light and the liquid turns blue on application of substrate.
7. Add Stop Solution and the liquid turns yellow, mix gently by tapping.
8. Ensure no water, fingerprints, or bubbles on the plate. Run microplate reader at 450nm immediately

Test Principle:

The assay employs a competitive inhibition enzyme immunoassay, involving a microplate pre-coated with a monoclonal antibody specific to dopamine. A competitive reaction occurs between biotin-labeled and unlabeled dopamine (standards or samples). After incubation, unbound conjugate is washed off. Avidin-HRP (Horseradish Peroxidase) is added and incubated. Bound HRP conjugate inversely correlates with dopamine concentration. Substrate solution produces color intensity inversely proportional to dopamine concentration.

Calculation of Results:

The assay follows a competitive inhibition enzyme immunoassay principle, where dopamine concentration inversely correlates with assay signal intensity. Calculate averages of duplicate readings. Create a standard curve (log-log or semi-log) with dopamine concentration vs. absorbance. The dopamine concentration is then determined via regression analysis or plot software.

Histological Analysis

Rats from group A and group B were euthanized and perfused with a fixative solution- formalin, to preserve the tissue and prevent degradation. Their brains were removed from the skull and post-fixed in the same fixative solution before the analysis began. The brain was sliced into thin sections (about 20-40 µm thick) and collected in a buffer solution and stored at 4°C until further processing. The sections were stained with hematoxylin and eosin (H&E) stain to show the general morphology and nuclei of cells. The stained sections were mounted on glass slides and cover slipped with a mounting medium, such as glycerol or xylene. The slides are then ready for examination and analysis with the aid of an electronic microscope.

Ethical Approval

All experimental procedures were conducted in accordance with National Institute of Health Guide for the care and use of Laboratory Animals as stated in the "guide to the care and use of Laboratory Animals Resources". Ethical approval was obtained from the Research and Ethics committee of the College of Medicine, University of Nigeria Enugu Campus, Enugu State.

Statistical Analysis

The data obtained from the study were expressed as mean ± standard deviation (SD). The differences between the groups were analyzed by using one-way analysis of variance (ANOVA) and Tukey Post- hoc test for multiple comparisons. The level of significance was set at $p < 0.05$. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 25.

RESULTS

Table 4.1: Effects of isolated Cola *nitida* (Kolanut) caffeine on serum dopamine concentration (pg/ml) in male Wistar rat

Groups	Day 1	Day 14	Day 28
A	19.53 ± 1.58	19.84 ± 1.66	19.91 ± 1.71
B	20.08 ± 2.03	16.12 ± 1.77*	11.90 ± 1.12*
C	20.65 ± 0.14	16.50 ± 1.10$^{*\beta}$	17.52 ± 1.16$^{*\beta}$
D	19.76 ± 0.16	15.56 ± 0.91$^{*\beta}$	17.25 ± 0.74$^{*\beta}$
E	20.61 ± 0.17	16.43 ± 1.20$^{*\beta}$	18.62 ± 1.36$^{*\beta}$
F	20.50 ± 0.15	16.46 ± 1.68$^{*\beta}$	20.18 ± 2.06$^{*\beta}$
P-values	0.8420	0.0031	0.0027

Results were expressed as Mean ± Standard Deviation. $^{*\beta}$ $P<0.05$ showed a statistically significant difference compared with groups A and B respectively using Tukey Post-hoc test for multiple comparisons.

Keys: Group A= Normal control and received standard pellet diet and distilled water *ad libitum;* Group B= metoclopramide-induced Parkinsonism untreated; Group C, D and E= metoclopramide-induced Parkinsonism + 50, 100 and 200 mg/kg respectively and Group F= metoclopramide-induced Parkinsonism + Parcopa (levodopa-carbidopa) 100/25 mg/kg orally.

Table 4.1 shows a typical result of the antiparkinsonian effect of isolated Cola *nitida* caffeine on dopamine concentrations in male wistar rats. There was a statistical increase in the concentrations of dopamine in groups C, D, E and F indicating a recovery of dopaminergic function by treatments while group B has a significant decrease in dopamine concentrations when

compared to the normal control group A. The increase in the concentrations of dopamine followed a regular incremental pattern and therefore suggests that the increase is dose-dependent. Group F had a significantly higher dopamine concentration than group C, D and E, indicating a superior effect of Pardopa- the standard drug over isolated Cola *nitida* caffeine.

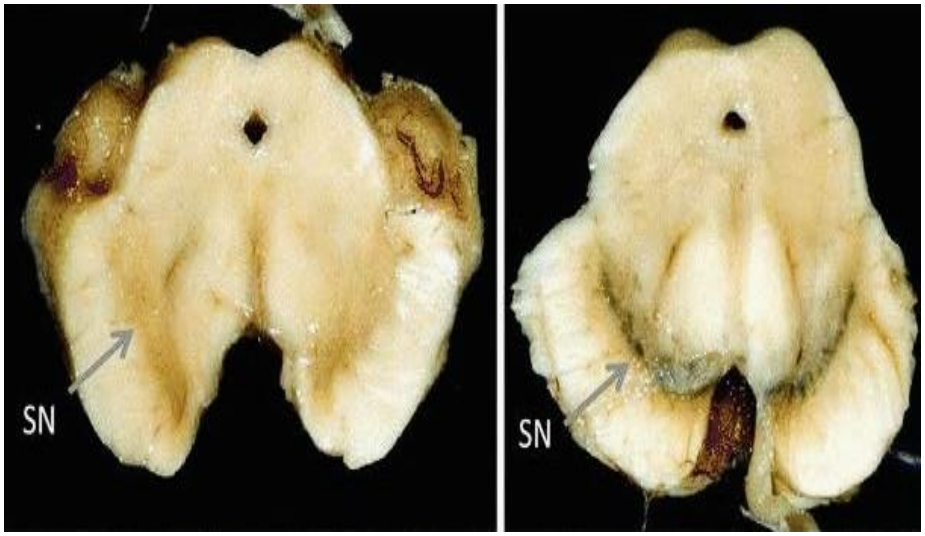

Fig 1: Cross section of mid brain showing the substantia nigra (SN) of rats with metoclopramide-induced parkinsonism vs normal control rats

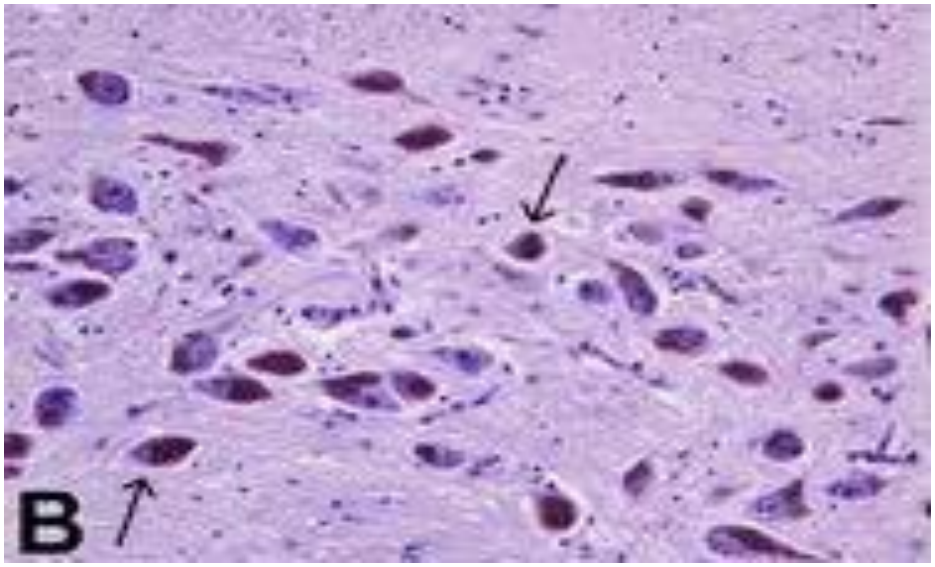

Fig 2*: Histological section of the Substantia nigra of the normal control group (H & E stain, 400× magnification).* ↑

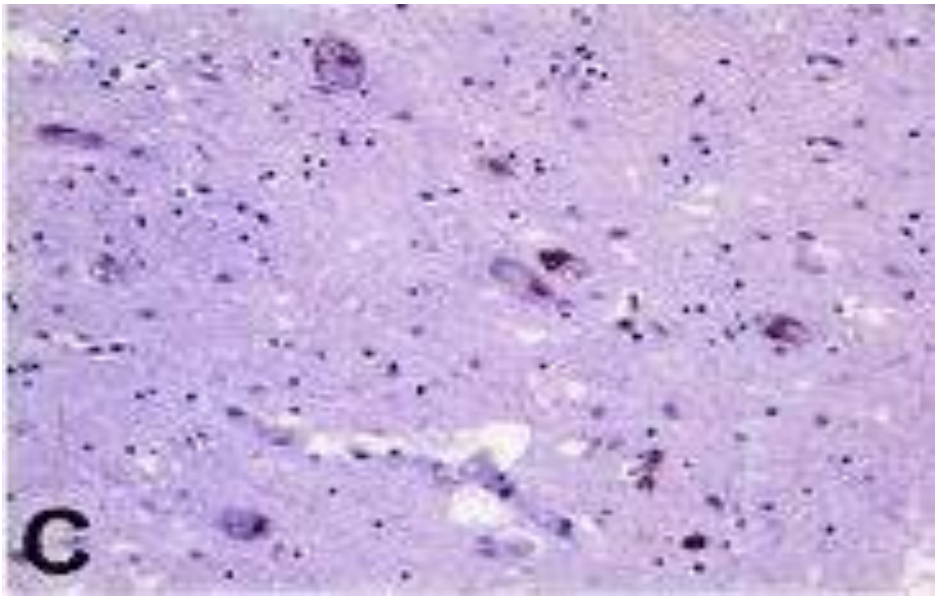

Fig 3: Histological section of the Substantia nigra of rats with metoclopramide-induced Parkinsonism (H & E stain, 400× magnification).

Figure 1: Loss of substantia nigra (SN) neurons causes Parkinsonism. In Fig 1, Pathological examination of a rat in normal control group reveals typical pigmented Dopaminergic, DA neurons in the Substantia nigra (arrows). In contrast, loss of Substantia nigra (SN) neurons leads to pigment disappearance in the brain of rats with Parkinsonism (arrows).

Magnification of the SN area reveals a dense network of melanin-pigmented SN neurons in the healthy brain (Fig 2) while most of SN neurons are lost in rats with Parkinsonism (Fig 3). The melanin-containing granules have a red-brown hue and are distributed in the cytosol of all Substantia nigra neurons (Fig 2-3).

Table 4.2: Differences in the body weight (g) of rats in the control and experimental groups.

Groups	Day 1	Day 14	Day 28
A	137.30 ± 6.78	142.02 ± 6.47	146.98 ± 6.36
B	138.46 ± 8.10	125.30 ± 9.30*	114.72 ± 6.56*
C	139.96 ± 5.98	122.52 ± 6.46$^{*\beta}$	120.20 ± 6.67$^{*\beta}$
D	137.20 ± 4.40	114.60 ± 3.70$^{*\beta}$	117.34 ± 4.67$^{*\beta}$
E	140.48 ± 7.30	116.48 ± 5.08$^{*\beta}$	122.32 ± 5.21$^{*\beta}$
F	142.12 ± 8.09	129.10 ± 7.53$^{*\beta}$	137.68 ± 8.30$^{*\beta}$
P-values	0.8460	0.0010	0.0021

Results were expressed as mean ± SD of percentage of body weight of six rats per group. $^{*\beta}$ P<0.05 showed a statistically significant difference compared to groups A and B respectively using Tukey Post-hoc test for multiple comparisons.

Keys: Group A= Normal control and received standard pellet diet and distilled water *ad libitum;* Group B= metoclopramide-induced Parkinsonism untreated; Group C, D and E= metoclopramide-induced Parkinsonism + 50,100 and 200 mg/kg respectively and Group F= metoclopramide-induced Parkinsonism + Pardopa (levodopa-carbidopa) 100/25 mg/kg orally.

Table 4.2: Differences in the body weight (g) of rats in the control and experimental groups.
The results from the table showed that there was a significant difference in the body weight of rats among the control and experimental groups. The normal control, group A, showed gradual and consistent increase in body weight throughout the study, indicating normal growth and development. The negative control, group B, showed a consistent decrease in body weight throughout the study, indicating adverse metoclopramide effect on health and metabolism of the Wistar rats. The C, D, and E groups showed a dose-dependent attenuation of weight loss throughout the study in a dose-dependent manner, indicating a reversal of malnutrition by the treatments but they were still lower than the normal control group. The administration of Pardopa (group E) also prevented or reversed the weight loss induced by metoclopramide, and it was statistically significant to the normal control group on day 28. The results suggest that caffeine intake may cause weight loss. This is in agreement with Uhuo *et al.,* 2021 who claimed that caffeine may cause weight loss by increased energy expenditure and metabolism, while Levodopa increases dopamine levels in the brain, reducing dyskinesia and improving mobility.

DISCUSSION AND CONCLUSION

This study investigated the possible neuroprotective effects of caffeine isolated from Cola *nitida* (Kolanut) with respect to parkinsonism in Wistar rat. This syndrome typically affects the dopaminergic neurons of the substantia nigra pars compacta thus reducing dopamine availability. In this study, outlined in Table 4.1, there was a measurable elevation of dopamine concentration in the treated groups following administration of Cola *nitida* caffeine. Dopamine levels were also observed to be low in the untreated group, to that effect. This indicates the active degradation of the dopaminergic neurons as a result of the disease. There was a relatively increased dopamine concentration in the group treated with a higher dose of caffeine. This study is in agreement with the research carried out by Soliman *et al*., 2016 which concluded that the effect of caffeine on parkinsonism is dose-dependent. However, there was a more significant increase in dopamine concentration in the group treated with the standard drug, Pardopa, suggesting that though caffeine may provide antiparkinsonian benefits by modulating the dopaminergic system and enhancing its function (Erukainure *et al*., 2017), the standard drug remains a superior choice; confirming the standard drug; levodopa-carpidopa as the 'gold standard' of treatment according to Pathan and Alshahrani, 2018.

Parkinsonism is characterized by the presence of at least two of four fundamental pointers; tremor, rigidity, bradykinesia, and postural instability (Faiz and Pihlstrøm, 2017) with its major cause being Idiopathic Parkinson's Disease or simply Parkinson's Disease. Metoclopramide was used to induce parkinsonism in the group of Wistar rats for the research. Metoclopramide, an antiemetic commonly used in patients with gastric motility disorders, acts as a dopamine receptor antagonist, thus following long term use; it can induce parkinsonian symptoms – bradykinesia, tremor, rigidity and postural instabilities.

The substantia nigra of the normal control group appears pigmented as a result of neuromelanin present in the dopaminergic neurons of the substantia nigra (Fig 1). As the degeneration of the dopaminergic neurons progresses in the groups induced with parkinsonism, the pigmentation in the substantia nigra is lost. Viewed under a microscope, the substantia nigra of the normal control group was heavily pigmented (Fig 2). This indicates the presence of neuromelanin. Histology of group B, examined under a microscope shows a scantier substantia nigra with reasonably low pigmentation as dopaminergic neurons degenerates and neuromelanin is lost (Fig 3).

Throughout the experiment, there were a lot of differences in the body weight of the Wistar rat. There was a gradual and statistically significant increase in the body weight of the normal control group as seen in Table 4.2. The untreated group exhibited consistent and statistically significant decrease in body weight throughout the experiment. Metoclopramide in high doses over a prolonged period of time leads to progressive decrease in body weight which is in agreement with the work of Wang *et al.,* 2016 which suggests that chronic administration of Metoclopramide for 14 days resulted in a significant decrease in body weight and food intake in rat. When treated with Cola *nitida* caffeine, rats in groups C, D, E, showed a little improvement in body weight and this is because caffeine has the tendency to increase metabolism in the body therefore influencing some level of weight loss. Group F treated with Pardopa displayed a measurable increase in body weight. The standard drug reverses the influences of metoclopramide on the rats and thus enhances weight restoration, and overall mobility improvements.

Essentially, parkinsonism as a result of Parkinson's Disease cannot be cured but can, in fact, be managed. So many approaches have emerged through the years to manage parkinsonian symptoms. The most common is the standard drug; Levodopa (Pardopa), which enhances dopamine delivery to the CNS and dopamine replacement. Caffeine has been studied widely because of the significant effects it has on the central nervous system; from mediating the release of certain neurotransmitters in the brain to sleep regulation and general cognition. Caffeine exerts some neuroprotective effects and can reduce the incidence of parkinsonism and increase the availability of dopamine in the substantia nigra of the brain. This is in agreement with previous studies carried out by the Honolulu Heart Program between 1965 and 1998 in the study of cardiovascular diseases, where caffeine from coffee was found to reduce the incidence of Parkinsonism. In this study, caffeine isolated from Cola *nitida* showed some ameliorative effects on the brain of the rats induced with parkinsonism. This sheds light on the possible therapeutic route that could transform our approach to preventing and managing parkinsonism. This study presents positive indications that Cola *nitida* caffeine treatment over a period of time improved motor and neurochemical impairments caused by metoclopramide-induced parkinsonism. However, though the potential utility of Cola *nitida* caffeine as a neuroprotective agent is established, there is need for further research into the implications of this Cola *nitida* caffeine in the aftermath. The efficacy of Cola *nitida* caffeine appears to be fairly modest and not without limitations. Caffeine can be harnessed for its positive attributes but must be used mindfully to prevent adverse effects. Administration of isolated Cola *nitida* caffeine over a longer period of time may cause varying physiological changes viz concomitant weight loss, loss of appetite and water imbalance. Therefore, at this point, it may not be the best choice when approaching Parkinsonism but it does present some neuroprotective benefits. This research suggests a balanced approach to the use of naturally occurring substances in the quest for effective treatment. The variation in the efficacy of isolated Cola *nitida* caffeine calls for further research to explore potential enhancements in its therapeutic application. Exploring alternative avenues and innovative strategies to managing Parkinsonism is important, especially in clinical practice.

Based on the findings of this study, the following recommendations are therefore suggested Since the standard drug Pardopa showed more significant benefits, it might be worthwhile to explore the potential of combining Cola *nitida (*kolanut*)* with other compounds which have neuroprotective effects and other existing medications to enhance therapeutic outcomes. Further research on the optimal dose, duration and frequency of Cola *nitida* caffeine administration for the treatment of parkinsonism and to compare its efficacy and safety with other sources of caffeine, such as tea, coffee, etc.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the effort of Dr. Ani Celestine and Mr. Nweke Maduka who initiated this project title as well did a thorough supervision. We also appreciate all the staff of Animal House Unit of the College of Medicine, Enugu State University of Science & Technology Enugu for the provision of a portable and much convenient environment for this study.

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