Drug Discovery

Beetroot juice ameliorates neurotoxicity in the cerebellum following combined ingestion of coffee and *Garcinia kola* (bitter kola)

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ABSTRACT

Over the years, the influence of diet on neurological health has been extensively studied, especially regarding the effect of plant materials and beverages on the morphology and function of the brain. This study explored the impact of beetroot juice, coffee, and *Garcinia kola* (bitter kola) on the cerebellum of adult Wistar rats. Fifty rats weighing 180 - 250 g were divided into ten groups of five animals each (n = 5). Group 1 served as the normal control group. Groups 2 to 10 served as the experimental groups receiving low and high doses (362.3 mg/kg body weight and 1086.8 mg/kg body weight) of coffee; low and high doses (273.9 mg/kg body weight and 821.6 mg/kg body weight) of *Garcinia kola* and beetroot juice (3.5 mL/kg body weight) for 28 days. This study evaluated body weight changes and conducted histological analysis of the cerebellar tissues. Significant weight loss with severe cerebellar damage was recorded among rats administered high doses of coffee and *Garcinia kola*. Combination of both substances intensified the neurotoxic effects. However, the addition of beetroot juice showed neuroprotective effect. Rats that received beetroot juice along with coffee and *Garcinia kola* showed improved body weight changes and better histological features. In conclusion, excessive intake of coffee and *Garcinia kola* can harm cerebellar health and impair motor function, while beetroot juice appears to mitigate these effects.

Keywords: Coffee, *Garcinia kola*, beetroot juice, cerebellum.

1. INTRODUCTION

Neurotoxicity refers to the adverse effects of chemical, biological, or physical agents on the structure or function of the nervous system. This becomes a significant issue since it has the potential to cause permanent nervous system impairment, leading to neurological disease (Pellacani and Eleftheriou, 2020). Acrylamide, a compound with neurotoxic and carcinogenic tendencies, is found in caffeinated beverages such as coffee (Naous *et al*., 2018).

Coffee is a highly popular beverage consumed by people all over the world. It is notable for its ability to stimulate the central nervous system as well as its appealing taste and aroma. Next to water and tea, coffee is the most popular beverage in the

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world, and it is the most regularly consumed caffeine-containing beverage. The content of caffeine in coffee is highly variable, ranging between 30 mg and 350 mg per cup of coffee or 150 milliliters of home-made coffee (Wachamo, 2017). Even as a psychostimulant, the consumption of caffeine is both legal and unregulated as compared to other psychoactive compounds in nearly all parts of the world (Okoli *et al*., 2012). Many researchers, including Ludwig *et al*. (2014) and Patay *et al*. (2016), have recorded the toxicity potentials of coffee on multiple organ systems, including the nervous system.

Many studies have noted that Garcinia kola (*G. kola*) possesses several pharmacological potentials such as anti-inflammatory, anti-atherogenic (Etukudoh *et al*., 2021), antidiabetic (Omage *et al*., 2011; Etukudoh *et al*., 2021), analgesic (Nwaehujor *et al*., 2015), antioxidant (Omage *et al*., 2011; Farombi and Owoeye, 2011), antimicrobial effects (Antwi-Boasiako and Abubakari, 2011), and hepatoprotective properties (Joshua *et al*., 2017), which is attributed to some of its phytochemical compositions.

In a study by Yakubu and Quadri (2016), the authors concluded that the administration of the seed of *G. kola* at doses of 25, 50, and 100 mg/kg body weight induced functional toxicity. Obi and Nwoha (2016) also reported that rats that were fed a diet containing *G. kola* showed retarded growth. The authors also noted that kolaviron (which is a significant constituent of *G. kola*) altered the histology of the hypothalamus, pituitary gland, and testis of the rats.

Beetroot (Beta vulgaris L.) is notable for its juice value and medicinal potential (Kale *et al*., 2018). It is rated among the ten most potent vegetables because of its antioxidant properties (Chawla *et al*., 2016). Beetroot helps to prevent hepatitis or cirrhosis, which are major diseases of the liver. Consumption of beetroot also helps to reduce inflammation of the gastrointestinal tract, thereby supporting the healing process in the body (Guldiken *et al*., 2016) and also possesses neuroprotective potential (Szymański *et al*., 2023). In a study conducted by Petrie *et al*. (2016), the motor structure in the brain was more consistent in the beetroot juice (BRJ) supplementation group as compared with the placebo group. The authors concluded that BRJ, when used together with exercise, promotes brain plasticity of somatomotor regions of the brain. Many other authors, including Wightman *et al*. (2015), Olasehinde *et al*. (2020) and Shaban *et al*. (2021), agreed that beetroot possesses enormous neuroprotective abilities and therefore, can be used in the management of various nervous system disorders.

2. MATERIALS AND METHODS

2.1. Materials

Coffee was purchased from provisions store in Uyo, South-South Nigeria. Beetroot plants and fresh seeds of *G. kola* were obtained from a local market in Uyo, South-South Nigeria.

2.2. Animal care and handling

A total of fifty (50) adult male Wistar rats of body weight 180 - 250 g were used for the study. They were procured from the Animal House of the Faculty of Pharmacy, University of Uyo. They were allowed to acclimatize for two weeks under standard housing conditions (ventilated room with 12/12 hour light/dark cycle at 25-28 ℃). The rats were fed standard rat chow with free access to water. A non-aversive method was used to handle the animals throughout the experiment.

2.3. Preparation of Extracts

For the coffee diet, the coffee powder was diluted in distilled water, as described by Andrade *et al*. (2013). The ethanol extraction of the *G. kola* seed was done according to the methods of Atsukwei *et al*. (2015). The *G. kola* seeds were peeled, chopped into smaller pieces, and oven-dried at 40 °C to a constant weight using laboratory oven. The dried pieces were pulverized with an electric blender to obtain a smooth powder. Three hundred grams (300 g) of the powder was extracted in 1 L of 70 % ethanol for 72 hours at room temperature with continuous agitation. The extract was filtered with Whatman No. 1 filter paper. Removal of ethanol was done using a rotary evaporator at 40 °C and the resulting filtrate was further dried using a steam bath. The dried down extract was stored in the refrigerator until used.

The bulb of the beetroot was washed, chopped into pieces, and emptied into the juice extraction machine. The beetroot juice was then extracted with the juice extractor. This fresh preparation was done daily.

2.4. Determination of Mass/Volume (m/v) Concentration of Beetroot Juice

To calculate the mass/volume concentration of the beetroot juice, the beetroot juice was freshly prepared, and 10 mL of the juice was collected in a beaker and dried in the oven at 60 ^{0}C until a constant weight was achieved. The dried matter was weighed using an

electronic weighing balance. The volume of 10 mL of beetroot juice yielded 1100 mg of dried matter. Therefore, the mass/volume ratio was gotten as:

$$\frac{1100\ mg}{10\ mL} = 110 \text{ mg/mL}$$

2.5. Median Lethal Dose Studies (LD_{50})

Lorke's method (Lorke, 1983) was used to calculate the LD_{50} of the extracts. The result showed 3622.8 mg/kg for coffee, 2738.6 mg/kg for *G. kola*, and 17.3 mL/kg for beetroot juice.

2.6. Research Design

Group 1 served as the normal control group while groups 2 and 3 received low and high doses of coffee, respectively. Groups 4 and 5 received low and high doses of *G. kola*, respectively. Group 6 received a combination of low doses of coffee and *G. kola,* while Group 7 received a combination of high doses of coffee and *G. kola*. Group 8 was treated with combined low doses of coffee and *G. kola* plus beetroot juice, while Group 9 received combined high doses of coffee and *G. kola* plus beetroot juice. Group 10 received beetroot juice (3 mL/kg). The experiment lasted for 28 days (Table 1).

Table 1. Experimental Design

Groups (n = 5)	Treatment/Dosage	Duration
1	Normal Control	28 days
2	362.3 mg/kg body weight of coffee	28 days
3	1086.8 mg/kg body weight of coffee	28 days
4	273.9 mg/kg body weight of *G. kola*	28 days
5	821.6 mg/kg body weight of *G. kola*	28 days
6	362.3 mg/kg body weight of coffee + 273.9 mg/kg of *G. kola*	28 days
7	1086.8 mg/kg body weight of coffee + 821.6 mg/kg of *G. kola*	28 days
8	362.3 mg/kg body weight of coffee + 273.9 mg/kg body weight of *G. kola* + 3.5 mL/kg body weight of Beetroot juice	28 days
9	1086.8 mg/kg body weight of coffee + 821.6 mg/kg body weight of *G. kola* + 3.5 mL/kg body weight of Beetroot juice	28 days
10	3.5 mL/kg body weight of Beetroot juice	28 days

2.7. Determination of Changes in Body Weight

The body weight of the animals was recorded before administration and weekly on days 8, 15, 21, and 28, using an electronic weighing balance. Weight change was calculated by subtracting the final body weight (on day 28) from the initial body weight. Analysis of the change in body weight was done using student paired t- test.

2.8. Termination of Experiment

On the 30th day, the animals were sacrificed. The animals were anaesthetized through injection of 50 mg/kg of ketamine intraperitoneally. The animals were perfused with phosphate buffered saline (for cleansing) and phosphate buffered formalin (for fixing). The cerebellum was isolated and fixed immediately in 10% buffered formalin for histological tissue processing.

3. RESULTS

3.1. Effect of G. Kola, Coffee, and Beetroot Juice on Body Weight

The extracts used in this study showed varying effects on body weight changes across the different groups (Table 2). Groups 8 (LDCGkB), 9 (HDCGkB), and 10 (BRJ) had the highest weight gain. On the other hand, Groups 3 (HDC) and 5 (HDGk), showed slight weight loss which were significant when compared to the Control group. When compared to the control group, the most significant weight loss was observed in Group 7 (HDCGk).

Table 2. Effect of *G. Kola*, coffee, and beetroot juice on the body weight

Groups	Group ID	Initial weight (g)	Final weight (g)	Weight Difference (g)
1	NC	209.07 ± 1.91	221.54 ± 2.54	12.47 ± 1.12
2	LDC	215.27 ± 1.50	220.64 ± 1.75	5.37 ± 1.30
3	HDC	208.40 ± 3.88	205.87 ± 2.30	-2.53 ± 3.60[a]
4	LDGk	222.23 ± 2.21	232.06 ± 3.66	4.83 ± 2.98
5	HDGk	218.70 ± 2.20	215.57 ± 3.62	-3.13 ± 4.64[a]
6	LDCGk	207.07 ± 0.44	207.87 ± 1.07	0.80 ± 4.43[a]
7	HDCGk	223.30 ± 2.82	217.23 ± 2.90	-6.07 ± 1.66[a]
8	LDCGkB	211.83 ± 3.18	222.80 ± 3.36	10.97 ± 2.90[c,e,f,g]
9	HDCGkB	224.63 ± 4.77	231.93 ± 3.28	7.30 ± 5.23[e,g]
10	BRJ	210.10 ± 1.88	229.10 ± 1.02	19.00 ± 6.34[c,e,g] P = 0.009 F = 18.25

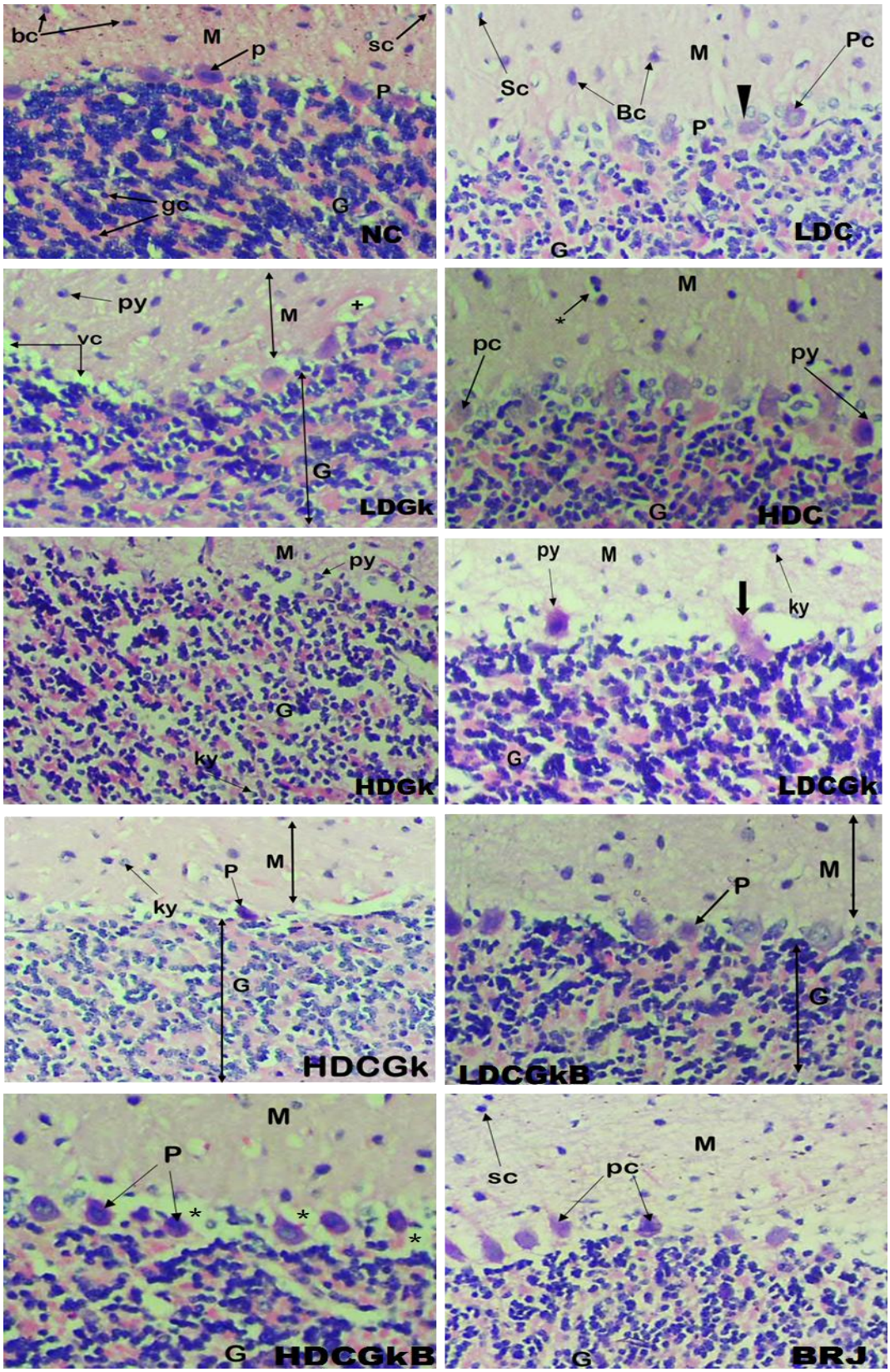
NC = Normal Control
LDC = Low dose of coffee (362.3 mg/kg bw)
HDC = High dose of coffee (1086.8 mg/kg bw)
LDGk = Low dose of *G. kola* (273.9 mg/kg bw)
HDGk = High dose of *G. kola* (821.6 mg/kg bw)
LDCGk = Low dose of coffee (362.3 mg/kg bw) + Low dose of *G. kola* (273.9 mg/kg bw)
HDCGk = High dose of coffee (1086.8 mg/kg bw) + High dose of *G. kola* (821.6 mg/kg bw)
LDCGkB = Low dose of coffee (362.3 mg/kg bw) + Low dose of *G. kola* (273.9 mg/kg bw) + beetroot juice (3.5 mL/kg bw)
HDCGkB = High dose of coffee (1086.8 mg/kg bw) + High dose of *G. kola* (821.6 mg/kg bw) + beetroot juice (3.5 mL/kg bw)
BRJ = Beetroot juice (3.5 mL/kg bw)

3.2. Histological Analysis using Haematoxylin and Eosin

The cerebellar cortex of NC group showed normal histological features across all layers. LDC group indicated pyknotic and anucleated Purkinje cells. HDC (Group 3) showed molecular layer having cells surrounded by perineural spaces and Purkinje cell layer with pyknotic cells while LDGk group showed molecular layer with pyknotic cells, vacuolated Purkinje cell layer having very scanty hypertrophied Purkinje cells, granular layer having karyorrhectic granular cells, and vacuolated neuropil. HDGk group had very scanty Purkinje cells, granular layer showed hyperplasia with pyknotic, karyorrhectic, and pale-stained granular cells. Both LDCGk and HDCGk groups revealed molecular layer having karyorrhectic cells, Purkinje cell layer having very scanty, shrunken, anucleated, and pyknotic Purkinje cells. Amelioration of the cerebellar histological features was seen in LDCGkB and HDCGkB groups (Figure 1).

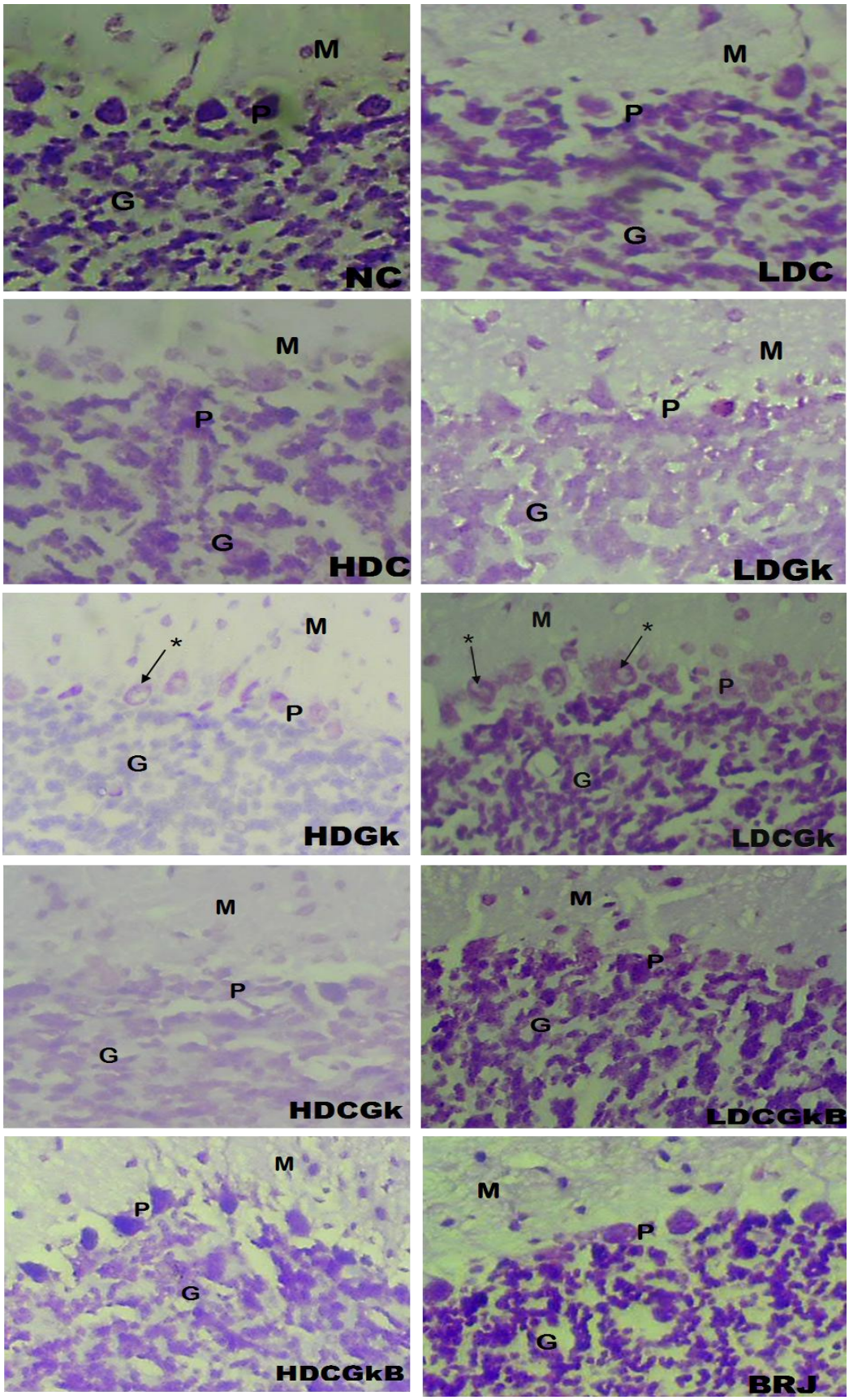
3.3. Histological Analysis using Cresyl Fast Violet (CFV) Stain

LDC group indicated moderate staining intensity of Nissl substances across the three layers. In addition to the poor staining intensity seen across all the three layers of the cerebellum in HDGk group, the Purkinje cells showed chromatolysis. HDCGk group showed the poorest staining intensity of Nissl substance across the layers of the cerebellum among all the groups. LDCGk and HDCGk groups showed improvement in the staining intensities of Nissl substance (Figure 2).



NC: Normal control group showing normal histological features.
LDC: Low dose coffee group showing pyknotic Purkinje cells (py) and anucleated Purkinje cells (arrowhead).
HDC: High dose coffee group showing perineural spaces (*) and pyknotic Purkinje cells (py).
LDGk: Low dose *Garcinia kola* group showing pyknotic cells (py); vacuolated Purkinje cell layer (vc) with very scanty hypertrophied Purkinje cells with karyorrhectic granular cells (ky) and vacuolated neuropil (+).
HDGk: High dose *Garcinia kola* group showing very scanty Purkinje cells; hyperplasia of granular cells with pyknosis (py), karyorrhectic and pale-stained granular cells (*).
LDCGk: Low dose coffee + low dose *G. kola* group showing karyorrhectic cells (ky); scanty, shrunken, anucleated Purkinje cells (thick arrow) and pyknotic Purkinje cells (py).
HDCGk: High dose coffee + high dose *G. kola* group showing karyorrhectic cells (ky); very scanty pyknotic Purkinje cells (P); karyorrhectic granular cells (ky).
LDCGkB: Low dose coffee + low dose *G. kola* + beetroot juice group showing pyknotic cells (py) and other normal histological features.
HDCGkB: High dose coffee + high dose *G. kola* + beetroot juice group showing vacuolations (*) with other normal histological features.
BRJ: Beetroot juice (3.5 mL/kg) group showing normal histological features.

Figure 1. Photomicrograph of the cerebellum indicating molecular layer (M) with stellate cells (sc) and basket cells (bc); Purkinje cell layer (P) with Purkinje cells (pc); granular layer (G) with granular cells.



NC: Normal control group showing highly stained nissl substance.
LDC: Low dose coffee group showing moderately expressed nissl substance
HDC: High dose coffee group showing moderately expressed nissl substance
LDGk: Low dose *Garcinia kola* group showing poorly expressed nissl substance
HDGk: High dose *Garcinia kola* group showing poorly expressed nissl substance
LDCGk: Low dose coffee + low dose *G. kola* group showing moderately expressed nissl substance
HDCGk: High dose coffee + high dose *G. kola* group showing poorly expressed nissl substance
LDCGkB: Low dose coffee + low dose *G. kola* + beetroot juice group showing moderately expressed nissl substance
HDCGkB: High dose coffee + high dose *G. kola* + beetroot juice group showing moderately expressed nissl substance
BRJ: Beetroot juice (3.5 mL/kg) group showing highly expressed nissl substance.

Figure 2. Photomicrograph of the cerebellum showing the expression of Nissl substances in the molecular layer (M); Purkinje cell layer (P) and granular layer (G). * = chromatolysis. CFV (X400).

4. DISCUSSION

The relationship between choices in diet and neurological health has raised so much scientific concern, particularly in the study of how the effect of various plant materials and beverages on the function and structure of the brain (Moise *et al*., 2024). This study assessed the effect of beetroot juice on the cerebellum of adult male Wistar rats when combined with the ingestion of coffee and *Garcinia kola* (bitter kola). The cerebellum is an important part of the nervous system and is responsible for the control and coordination of motor activities in the body and specific cognitive functions, and it serves as an essential indicator of the general neurological health of the body (Habas, 2021).

Although there have been studies showing the potential health benefits of both coffee and *G. kola*, there is also evidence suggesting that their consumption, particularly in high amounts, may lead to adverse neurological effects. For instance, while coffee consumption in moderation has been linked to various health benefits, excessive intake has been associated with smaller total brain volumes, indicating potential adverse effects on brain health (Pham *et al*., 2021). In light of these concerns, there arise a need to identify and study natural compounds that is capable of ameliorating neurotoxicity and protect the brain (Silva *et al*., 2021). Beetroot (*Beta vulgaris* L.) has noted to possess this neuroprotective potential. It has been on record that beetroot rich in bioactive compounds such as betalains, nitrates, and polyphenols (Clifford *et al*., 2015).

This study showed that the administration of coffee had an adverse dose-dependent effect on body weight. This finding is in line with earlier research findings showing that higher doses of coffee lead to weight loss (Gamboa-Gómez *et al*., 2023). This is probably due to the caffeine in coffee, which increases energy expenditure and fat oxidation (Haraguchi *et al*., 2022). Caffeine stimulates the central nervous system, leading to increased catecholamine secretion, which in turn enhances metabolic rate and lipolysis (Guest *et al*., 2021). The dose-dependent effect of *G. kola* on body weight aligns with previous studies that have reported varying impacts of *G. kola* on body weight. The flavonoids in bitter kola can alter a number of cell-signaling pathways that control fat deposition. Furthermore, alkaloids (contained in bitter kola) increase energy expenditure and suppress the function of pancreatic lipase and differentiation of adipocyte (Beyang *et al*., 2024). The anti-obesity role of bitter kola is attributed to the presence of hydroxyl citric acid (HCA) in *G. kola*, which inhibits adenosine triphosphate (ATP) citrate lyase, a key enzyme in the biosynthesis of fatty acids, cholesterol, and triacylglycerols (Konstantinidi and Koutelidakis, 2019).

The combination of coffee and *Garcinia kola* at low doses (Groups 6) and high doses (Group 7) both produced synergistic effect. The combination enhanced the weight loss effects observed with each substance individually. This suggests that there could be complementary mechanisms of action between components of coffee and the bioactive compounds from *Garcinia kola.* The addition of beetroot juice (BRJ) to the administration of coffee and *Garcinia kola* yielded ameliorative results. The weight-promoting effect of beetroot juice observed in this study agrees with some previous research that has suggested that the presence of various micronutrients in beetroot could contribute to increased caloric intake and improved nutrient utilization, potentially leading to weight gain (Erigbali *et al*., 2021). The authors also noted that the nitrates in beetroot juice, which are converted to nitric oxide, may influence blood flow and nutrient delivery to tissues, potentially affecting weight gain.

The histological evaluation from this study showed various histomorphological changes. The low-dose coffee group had pyknotic and anucleated Purkinje cells. This indicates cellular degeneration which may lead to neuronal death. A higher dose of coffee (HDC group) resulted in more severe histological alterations. The molecular layer had perineural spaces, indicating oedema or cellular shrinkage. Presence of pyknotic cells suggests increased cellular stress and potential neuronal loss. The effect of coffee on the cerebellum found in this study can lead to decreased connectivity between various brain regions. These findings agrees with the position of Magalhães *et al*. (2021), who noted that higher consumption of coffee and caffeinated products decrease brain functional connectivity at rest. This result also corroborates the position of Atallah *et al.* (2022), who noted that prenatal administration of moderate and high doses (400 mg/kg and 600 mg/kg, respectively) of green coffee extract results in severe histological distortion in the cerebellum.

Administration of low dose of *G. kola* resulted in significant histological distortions. These distortions got worse at high dose. Some authors like Omotoso *et al*. (2019) and Ahidjo *et al*. (2024) have reported the neuroprotective effects of *Garcinia kola*. Contrary to these reports, the present study provides evidence suggesting that *Garcinia kola* may exert neurotoxic effects rather than neuroprotective effects under the conditions investigated. These findings indicate that while *Garcinia kola* has been widely regarded as beneficial, its impact on the cerebellum may be more complex than previously understood.

Combination of coffee and *G. kola* at low and high doses showed severe histological alterations, including karyorrhectic cells in the molecular layer, indicating severe nuclear damage. The Purkinje cell layer showed very scanty, shrunken, anucleated, and pyknotic

Purkinje cells, suggesting extensive damage and loss of these critical neurons. This shows there may be a synergistic neurotoxic effect when coffee and *G. kola* are co-ingested, due to the interaction of their bioactive compounds or cumulative damage. Presence of damage in the cerebellar cortex can result in problems of motor coordination, deficits in executive function, difficulty in spatial processing, and impaired language processing (Arleo *et al*., 2024 and Mastrangelo *et al.,* 2024). Cellular damage and death can trigger inflammatory responses in the brain, potentially worsening neurodegeneration and increasing the risk of neurodegenerative diseases (Adamu *et al*., 2024). Also, the loss of neurons and alterations in the neuropil can disrupt the cerebellar circuit, affecting its ability to process and integrate sensory and motor information (Mosconi *et al*., 2015).

Remarkably, the addition of beetroot juice (3.5 mL/kg) in LDCGkB and HDCGkB groups resulted in normal cerebellar morphology. This finding supports the growing body of evidence suggesting neuroprotective properties of beetroot juice, likely due to its high content of nitrates, betanin, and phenolic compounds (Szymański *et al*., 2023). The histopathological examination of the cerebellum of adult Wistar rats using cresyl fast violet (CFV) stain showed significant variations in Nissl substance staining intensity across different treatment groups.

The findings from this study suggest that coffee consumption may have a dose-dependent impact on neuronal metabolism and protein synthesis. This could be attributed to the action of caffeine on protein synthesis and degradation. Abd-El-Wahab and ElDakdoky (2013) had earlier reported the neurotoxic effect of caffeine in rat foetuses. The authors recorded that caffeine inhibits protein and nucleic acid synthesis and thus inhibits cell cycle; evidenced by reduced DNA, RNA, and protein contents. Egawa *et al*. (2016) also noted that caffeine inhibits protein synthesis by inhibiting Akt/mTOR/p70S6K signalling pathway, and it also upregulates protein degradation by stimulating the ubiquitin-proteasome system. The disruption in cellular proteostasis leads to neuronal death, which is responsible for the onset and progression of neurodegenerative diseases (Kurtishi *et al*., 2018).

Reduction in the staining intensity of Nissl substances across all cerebellar layers was noted in *G. kola* treatment group. In addition to this, the high dose of *G. kola* (821.6 mg/kg) also led to chromatolysis among the Purkinje cells. Chromatolysis, which involves the breakdown of Nissl bodies, is often a response to neuronal injury and can be a precursor to apoptosis if the neuron fails to recover (Kádár *et al*., 2009). This observation suggests that *G. kola* may have a notable impact on cerebellar neuronal metabolism and protein synthesis.

The combination of coffee and *G. kola* in low and high doses resulted in a dose-dependent decrease in Nissl substance staining. This finding indicates a potentially synergistic adverse effect - such as impaired protein synthesis and metabolic activity - of varying doses of coffee and *G. kola* on cerebellar neurons, which could lead to significant functional deficits in motor coordination and cognitive processes mediated by the cerebellum.

The addition of beetroot juice (3.5 mL/kg) to both low and high doses of coffee and *G. kola* combinations resulted in improved staining intensity for Nissl substances. This implies that beetroot juice possesses neuroprotective effect against the negative effect of coffee and *G. kola* on cerebellar neurons. This ameliorative tendency of beetroot juice could be attributed to the nitrates in beetroot juice, which are converted to nitric oxide (NO) in the body, may enhance blood flow and oxygen delivery to the brain, supporting neuronal health and function (Clifford *et al*., 2015).

4. CONCLUSION

It is concluded that high doses of coffee and *Garcinia kola* can have significant neurotoxic effects on the cerebellum. Conversely, beetroot juice demonstrated protective effects, ameliorating the toxicity caused by coffee and *Garcinia kola*. These findings show the importance of dietary considerations in managing the adverse effects of commonly consumed substances on brain health.

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Authors' Contributions

Idorenyin U. Umoh: Proposed the idea, supervised the research work, and edited the manuscript.

Akpanabasi A. Malachy: Conceived the idea, collected the data, performed the experiment and drafted the manuscript.

Iboro E. Edet: Supervised the research work, reviewed and edited the manuscript.

All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethical Approval

The ethical approval for this research was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee (FBMSREC), University of Uyo, Uyo, Nigeria (Ethical Approval number: UU_FBMSREC_2025_001).

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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