

EFFECTS OF *CUCUMEROPS IS MANNI* SEED OIL ON NEURONAL BIOMARKERS IN THE CORTEX OF RATS EXPOSED TO CALCIUM CARBIDE

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Abstract

Calcium carbide, widely used in industrial applications and ripening of fruits, poses significant health risks, including neurotoxic effects. This study investigated the neuroprotective potential of *Cucumeropsis mannii* seed oil (CMSO) against calcium carbide-induced neurotoxicity in the cortex of rats. The experiment involved twenty-eight albino male rats divided into seven groups, including a normal control, a positive control, a standard control, and four treatment (test) groups receiving varying concentrations of CMSO. The primary objective was to assess the impact of CMSO on key neuronal biomarkers: monoamine oxidase (MAO), adenosine deaminase (ADA), acetylcholinesterase (AChE), and Na⁺/K⁺-ATPase. Results indicated that calcium carbide exposure significantly decreased the activities of these enzymes, highlighting its neurotoxic impact. However, treatment with CMSO led to a significant restoration of these biomarkers, suggesting its neuroprotective efficacy. This study revealed the potential of CMSO as a therapeutic agent in mitigating calcium carbide-induced cortical neurotoxicity.

Introduction

Neurotoxicity results due to the ability of certain substances to disrupt the delicate balance of neuronal biomarkers, which are crucial for normal brain function (Ouma *et al.*, 2022). Increasing in environmental pollutants and toxic chemicals has sparked enormous concerns about their detrimental effects on human health especially the brain. Among these chemicals is calcium carbide, widely used as an artificial means to activate fruit ripening and in various industrial applications poses serious health risks (Okeke *et al.*, 2022). Specifically, when applied to ripen fruits, calcium carbide releases acetylene gas, which can trigger the formation of harmful substances within the body.

Consequently, prolonged exposure to calcium carbide has been linked to so many of health challenges such as, skin irritation, respiratory abnormalities and ultimately, neurotoxic problems (Ouma *et al.*, 2022). Neuronal biomarkers such as monoamine oxidase (MAO), adenosine deaminase (ADA), acetylcholinesterase (AChE), and Na⁺/K⁺-ATPase play pivotal roles in maintaining neuronal health and function (Olasehinde *et al.*, 2022). Therefore, any alterations in the levels of these biomarkers may signal neuronal damage and are often associated with the development of neurodegenerative diseases (Toader *et al.*, 2023). The cortex is particularly vulnerable to damage from toxic substances due to both its intricate structure and its critical functions. This region is responsible for key higher-order brain activities, including cognition, sensory perception, and motor control. Therefore, any damage to cortical neurons can result in severe neurodegenerative conditions and significant cognitive impairments (Jobson *et al.*, 2021).

Cucumeropsis mannii, commonly known as egusi and melon, is indigenous to West Africa and has long been a staple in traditional diets and medicine. More importantly, *Cucumeropsis mannii* is valued for its medicinal properties, including its antioxidant, anti-inflammatory, and neuroprotective potential (Enujiugha *et al.*, 2023). Research suggests that its seed oil may contain bioactive compounds capable of protecting neurons from oxidative stress and inflammation, which are common pathways of neurotoxicity (Enujiugha *et al.*, 2023). Therefore, this study seeks to explore the neuroprotective effects of *Cucumeropsis mannii* seed oil by examining its impact on the critical neuronal biomarkers in the cortex of rats exposed to calcium carbide. Moreso, despite the widespread industrial use of calcium carbide and its known health risks, there remains a significant lack of effective interventions to mitigate its neurotoxic effects. *Cucumeropsis mannii* seed oil is recognized for its antioxidant and anti-inflammatory properties as a result of its rich content of essential fatty acids and vitamins. Therefore, this study aims to fill this knowledge gap by exploring the therapeutic potential of *Cucumeropsis mannii* seed oil on neuronal biomarkers in the cortex of rats exposed to calcium carbide.

Materials and Methods

The *Cucumeropsis mannii* seeds were sourced from a peasant farmer in Ndingwuta Amachi-Ndebor Izzi, Abakaliki L.G.A., Ebonyi State. They were authenticated by a plant taxonomist at the Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti, Nigeria.

Extraction of *Cucumeropsis Mannii* Seed Oil (CMSO)

Approximately 10 kg of seeds of *C. mannii* were dehulled manually, then hand-picked to remove shells and dirt. A manual grinding machine was washed thoroughly to remove dirt, and then, rinsed with distilled water. The seeds were ground three times with the grinding equipment to get a fine powdered texture. Sample was extracted by

pressing on a smooth stainless surface gently inclined to the horizontal. Approximately fifty (50) centiliter of lukewarm water was gently added at intervals (approximately 4 drops) to facilitate the release of the oil, due to the fact that water helps to rupture the cell membrane. A significant amount of the added water to aid release of oil was decanted immediately after the oil was extracted while water molecules attached to hydrocolloids (mucilage and gum) expressed by the broken cells was left uninterrupted to sediment (Dror *et al.*, 2006). The oil was left undisturbed in a closed container for about seven days to sediment before it was separated by the method of decantation to get a purified and clearer form of the oil which was put in storage in a different sterilized bottle (Hamed and Bahareh, 2012).

Experimental Animals

Experimental animals used to carry out this study were a total of twenty-eight (28) male albino rats of about eight (8) weeks old. They were obtained from the Animal House of Department of Biochemistry, Ekiti State University (EKSU) Ekiti State, Nigeria. Throughout the duration of the research the rats were guaranteed unceasing access to standard or regular rodent feed (vital food, Grand Cereals Ltd., Nigeria) and clean water while being kept in wooden cages in a properly ventilated animal house, in department of Biochemistry, Federal University, Oye-Ekiti, Ekiti State, Nigeria. They were acclimatized (12hours light/dark cycle, room temperature) for fourteen (14) days. The procedures for this research were carried out in compliance with the National Institute of Health Guidelines for the Care and Use Laboratory Animals (NIH Publications No. 80- 23, revised in 1996).

Experimental Design

The experiment was done using a total of twenty-eight rats of about eight (8) weeks old. Animals were divided into seven groups with each comprising of four rats per group for the study. Calcium carbide was administered to the experimental animals (rats) intraperitoneally while CMSO and ascorbic acid were administered by oral intubation.

Animal Grouping

Group one: Normal control received feed and water only.

Group two: Positive control received 100 mg/kg calcium carbide daily for 14 days.

Group three: Standard control received 100 mg/kg body weight of calcium carbide + 200 mg/kg body weight of ascorbic acid daily for 14 days.

Group four: Test group 1 received 1.5 mL of CMSO daily for 14 days.

Group five: Test group 2 received 100 mg/kg body weight of calcium carbide + 1.5 mL of CMSO daily for 14 days.

Group six: Test group 3 received 100 mg/kg body weight of calcium carbide + 2.5 mL of CMSO daily for 14 days.

Group seven: Test group 4 received 100 mg/kg body weight of calcium carbide + 5.0 mL of CMSO daily for 14 days.

The choice of CMSO dosage was established from our previous preliminary studies, though data not shown, dosage of calcium carbide and vitamin C was according to the previous studies of Ibeachu and Nwidobie, 2022.

Biochemical Analysis

Preparation of Organ Homogenates

Approximately 0.5 grams of brain tissue from the cortex was homogenized in 50 mM sodium phosphate buffer containing 1 mM EDTA and 0.5% Triton-X (pH 7.5). The mixture was then centrifuged at 12,000 rpm for 12 minutes at 4°C. The supernatants were collected and stored at -4°C for subsequent biochemical analysis.

Assessment of Monoamine Oxidase Activity in the Cortex

Monoamine oxidase (MAO) activity was determined using a Kynuramine assay method, which involved the use of the substrate kynuramine. The substrate was incubated with the cortical supernatant under controlled conditions. The enzyme-substrate reaction was monitored, and the production of the reaction product was measured with the aid of a spectrophotometer at a wavelength of 580 nm. The change in absorbance was proportionate to MAO activity (Reis and Binda, 2023)

Assessment of Adenosine Deaminase Activity in the Cortex

Adenosine deaminase (ADA) activity was measured using adenosine deaminase assay method, typically involving the conversion of adenosine to inosine. The reaction was performed in a buffer with a known concentration of adenosine. The ADA activity was assessed by measuring the amount of inosine produced. This was done using spectrophotometric methods to quantify inosine levels and absorption read at 550 nm (Lee *et al.*, 2019).

Assessment of Acetylcholinesterase Activity in the Cortex

AChE activity was measured using a specific colorimetric assay called Ellman's Assay. The assay typically involves the hydrolysis of acetylcholine to choline and acetic acid. The change in absorbance is proportional to AChE activity. Acetylcholine was added to the sample and incubated under optimal conditions for AChE activity. After incubation, the Ellman's reagent was added to measure the release of thiocholine, which is proportional to AChE activity. Activity of AChE was calculated centered on the adjustment in absorbance at 412 nm over a period of 15mins at 15 second interval. The activity is typically expressed in units per milligram of protein (Friedman and Robbins, 2022).

Assessment of Na⁺/K⁺-ATPase Activity in the Cortex

Na⁺/K⁺-ATPase activity was assayed using a colorimeter. The assay typically involves the hydrolysis of ATP to ADP and inorganic phosphate (Pi). The amount of Pi released is proportional to the activity of the enzyme Na⁺/K⁺-ATPase. The supernatant was incubated with ATP and specific reagents that react with the released inorganic phosphate. The colorimetric reaction, often using a reagent like the malachite green or ammonium molybdate, allowed for quantification of phosphate. The reaction mixture absorbance at the definite wavelength of 850 nm was taken, and Na⁺/K⁺-ATPase activity was obtained based on the amount of inorganic phosphate released, expressed in units per milligram of protein (Kushkevych *et al.*, 2021).

Results

Effect of *Cucumeropsis mannii* seed oil on Monoamine oxidase (MAO) activity in Rats Exposed to Calcium Carbide.

Result showing the effect of CMSO on the activity of monoamine oxidase in the cortex of albino rats exposed to calcium carbide is shown in Figure 1. A significant ($p < 0.05$) reduction monoamine oxidase activity was observed in the untreated (CAC) group, when compared to the normal control group. Monoamine oxidase activity in the test groups showed considerable ($p < 0.05$) increase compared to CAC group. The standard control (Vit.C + CAC) showed a considerable ($p < 0.05$) increase in monoamine oxidase activity when compared to the untreated animals.

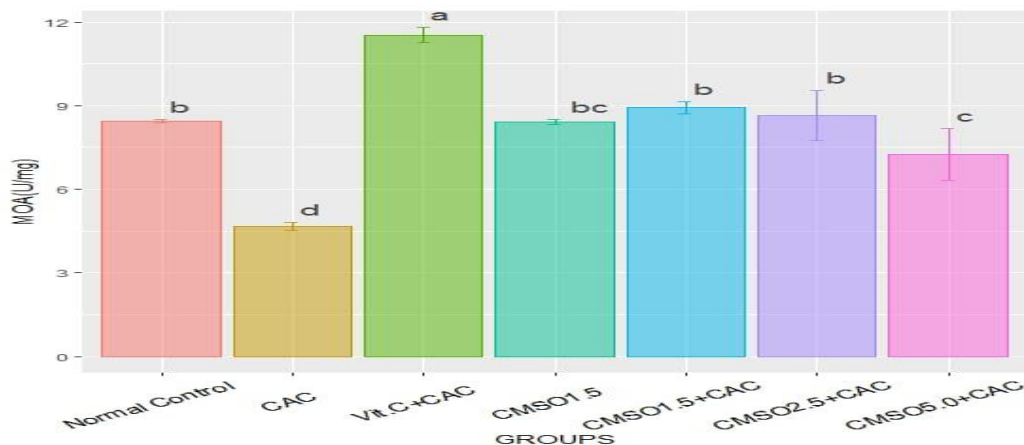


Figure 1: Effect of CMSO on monoamine oxidase activity in the cortex of albino rats exposed to calcium carbide. Data are shown as mean $\pm$ S.D (n=4). Bars with different alphabets are significantly different $p < 0.05$. Whereas bars with double letters are significantly different from bars that does not have any of the two alphabet it contains. CAC (calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

Effect of *Cucumeropsis mannii* seed oil on Adenosine deaminase (ADA) activity in Rats Exposed to Calcium Carbide.

The result showing the effects of CMSO on adenosine deaminase activity in the cortex of albino rats exposed to calcium carbide is shown in Figure 2. A considerable ($p < 0.05$) decrease in adenosine deaminase activity in the untreated animals was observed, when compared to animals in the normal control. Adenosine deaminase activity in the test groups significantly ($p < 0.05$) increased compared to the positive control (CAC) group. Animals that received vit.C +CAC (standard control) revealed a major ($p < 0.05$) increase in adenosine deaminase activity when compared to the positive control group.

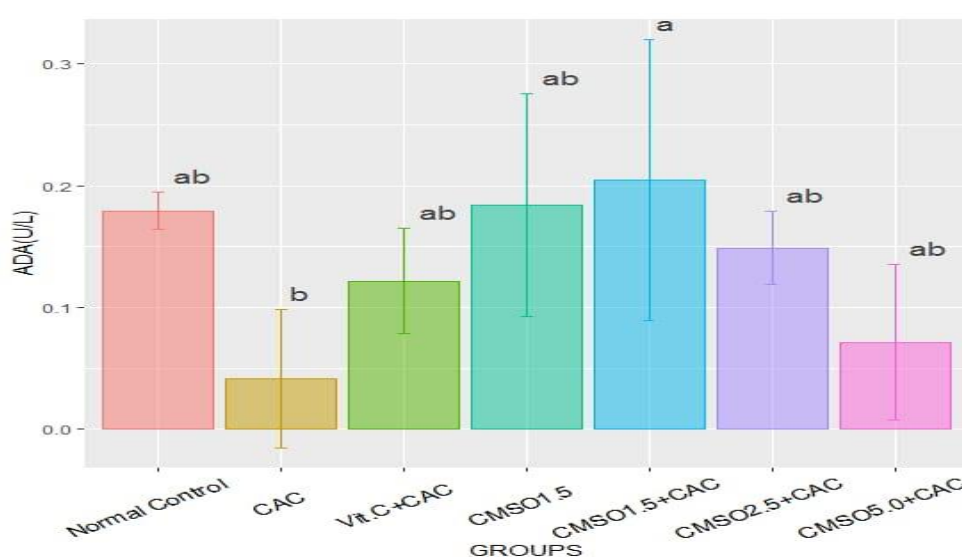


Figure 2: Effect of CMSO on adenosine deaminase activity in the cortex of albino rats exposed to calcium carbide. Data are shown as mean $\pm$ S.D ($n=4$). Bars with different alphabets are significantly different $p < 0.05$. Whereas bars with double letters are significantly different from bars that does not have any of the two alphabet it contains. CAC (calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

Effect of *Cucumeropsis mannii* seed oil on Acetylcholinesterase activity in Rats Exposed to Calcium Carbide.

Figure 3 shows the effects of CMSO on acetylcholinesterase activity in the cortex of albino rats exposed to calcium carbide. A significant ($p < 0.05$) decrease in acetylcholinesterase activity was observed in the untreated group, when compared to the normal control group. Acetylcholinesterase activity in the test groups, especially animals that received 1.5 mL CMSO, revealed a considerable ($p < 0.05$) increase when compared to the CAC group. The standard control group showed a significant ($p < 0.05$) improvement in acetylcholinesterase activities when compared to the positive control group.

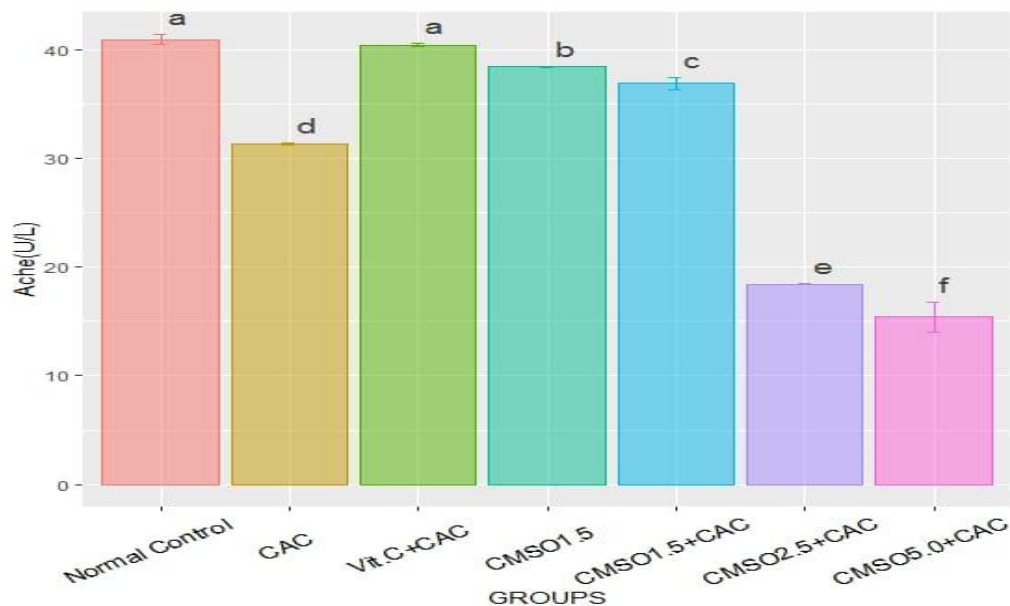


Figure 3: Effect of CMSO on acetylcholinesterase activity in the cortex of albino rats exposed to calcium carbide. Data are shown as mean $\pm$ S.D (n=4). Bars with different alphabets are significantly different $p < 0.05$. Whereas bars with double letters are significantly different from bars that does not have any of the two alphabet it contains. CAC (calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

Effect of *Cucumeropsis mannii* seed oil on Na⁺/K⁺-ATPase activity in Rats Exposed to Calcium Carbide.

The result showing the effects of CMSO on Na⁺/K⁺-ATPase activity in the cortex of albino rats exposed to calcium carbide is shown in Figure 4. A significant ($p < 0.05$) reduction in Na⁺/K⁺-ATPase activity was observed in the untreated group, when compared to the normal control group. Na⁺/K⁺-ATPase activity in the treatment groups, specifically treated with 1.5 mL of CMSO, revealed a considerable ($p < 0.05$) increase when compared to the untreated group.

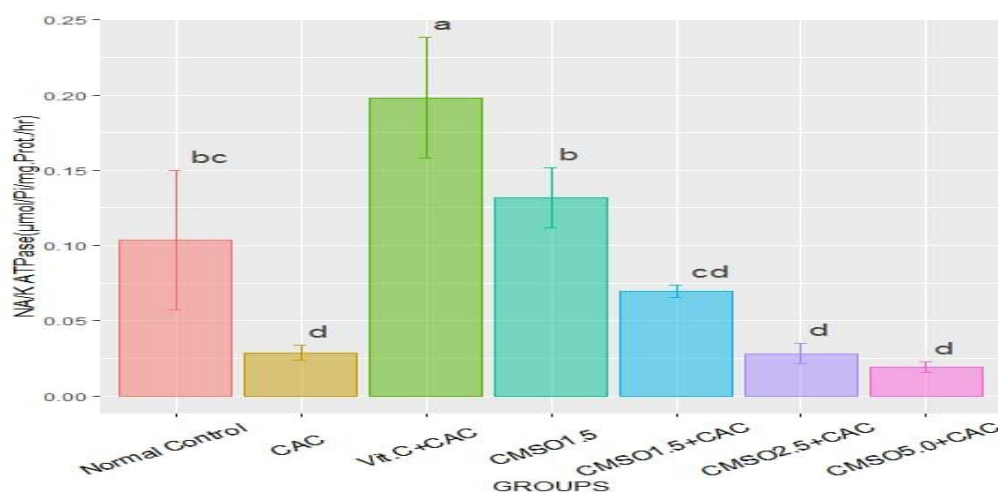


Figure 4: Effect of CMSO on Na⁺/K⁺-ATPase activity in the cortex of albino rats exposed to calcium carbide. Data are shown as mean $\pm$ S.D (n=4). Bars with different alphabets are significantly different $p < 0.05$. Whereas bars with double letters are significantly different from bars that does not have any of the two alphabet it contains. CAC (calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

Discussion

Cucumeropsis mannii seed oil which is highly abundant in unsaturated fatty acids, especially linoleic and oleic acids, as well as tocopherols and phenolic compounds. These constituents are known for their antioxidant properties (Agu *et al.*, 2022). Exposure of albino rats to calcium carbide was shown to decrease the activity of monoamine oxidase (MAO), adenosine deaminase (ADA), acetylcholinesterase (AChE) and Na⁺/K⁺-ATPase which are neuronal biomarkers in the cortex. The decrease in these biomarkers due to calcium carbide exposure can significantly disrupt normal neuronal functions. Reduced MAO activity may lead to neurotransmitter imbalances, ADA reduction can impair neurotransmission, decreased AChE activity affects muscle activation and cognitive functions, and lower Na⁺/K⁺-ATPase activity disrupts nerve impulse transmission (Behl *et al.*, 2021). Monoamine oxidase (MAO) is an enzyme involved in the breakdown of monoamines, which are neurotransmitters such as dopamine, norepinephrine, and serotonin. By regulating the levels of these neurotransmitters, MAO plays a critical role in maintaining normal neuronal function and mood regulation (Behl *et al.*, 2021). Abnormal MAO activity has been associated with various neurodegenerative disorders, including depression and Parkinson's disease. In this study, the activity of MAO decreased significantly in the untreated group compared to the normal control group, indicating neurotoxic effect occasioned by calcium carbide exposure (Tan *et al.*, 2022). However, the treatment groups

exhibited a significant increase in MAO activity compared to the untreated group. This increase may be attributed to the bioactive compounds in *Cucumeropsis mannii* seed oil, such as essential fatty acids, tocopherols, and phytosterols, which are known to support neuronal health and mitigate oxidative stress (Agu *et al.*, 2022). These compounds likely contribute to the restoration of MAO activity by reducing oxidative damage in the neurons and enhancing enzymatic function. This is in line with the work of Okwunduet al. (2021), who stated that bioactive compounds in *Cucumeropsis mannii* seed oil possess neuroprotective properties that can improve enzymatic regulation and reduce neurotoxicity. Similarly, Fajinmiet al. (2022) demonstrated that the antioxidant properties of the seed oil play a crucial role in scavenging free radicals and preventing neuronal damage, further supporting the observed increase in MAO activity in the treatment groups.

Adenosine deaminase (ADA) is an enzyme whose responsibility is the activate the metabolism of adenosine, a neuromodulator that influences various physiological processes, including neurotransmission, energy transfer, and immune function (Garcia-Gil *et al.*, 2021). ADA activity is crucial for maintaining brain function and immune responses, with abnormalities in ADA levels linked to neurological disorders and immune deficiencies. In this study, a significant decrease in ADA activity was observed in the untreated group compared to the normal control group, indicating compromised neuronal function due to calcium carbide exposure. However, treatment with *Cucumeropsis mannii* seed oil (CMSO) resulted in a significant increase in ADA activity in the treatment groups compared to the untreated group. This improvement may be attributed to the bioactive compounds in CMSO, such as essential fatty acids and antioxidants, which are known to reduce oxidative stress and support neuronal health (Agu *et al.*, 2022). This is tandem with the findings of Aja *et al.* (2023), who found that *Cucumeropsis mannii* seed oil has protective effects which can help restore normal enzyme functions and improve overall neuronal health, therefore supporting the observed increase in ADA activity in the treatment groups.

Acetylcholinesterase (AChE) is an enzyme that breaks down acetylcholine, a neurotransmitter crucial for muscle activation, memory, and cognitive function. Proper regulation of acetylcholine levels by AChE is essential for effective neuronal communication and cognitive performance (Trang *et al.*, 2023). Disruption of AChE activity is linked to neurodegenerative diseases such as Alzheimer's disease. In this study, a significant decrease in AChE activity was noted in the positive control group compared to the normal control group, indicating the neurotoxic impact of calcium carbide. Notably, the treatment groups, particularly those receiving 1.5mL of *Cucumeropsis mannii* seed oil (CMSO), demonstrated a significant increase in AChE activity compared to the positive control group. This improvement can be attributed to the bioactive compounds in CMSO, which likely enhance AChE function and mitigate the neurotoxic effects of calcium carbide. This observation aligns with the findings of Ooi *et al.* (2021), who reported that edible seed oil has neuroprotective properties that

support cognitive function and restore enzyme activity compromised by neurotoxic agents.

Na⁺/K⁺-ATPase is a crucial enzyme responsible for the maintenance of electrochemical gradient across the cell membrane by being adequately involved in the transportation of potassium and sodium ions. This gradient is essential for nerve impulse transmission, muscle contraction, and overall cellular homeostasis (Pivovarov *et al.*, 2019). Disruptions in Na⁺/K⁺-ATPase activity can impair neuronal function and contribute to various neurological disorders. In this study, a significant decrease in Na⁺/K⁺-ATPase activity was observed in the untreated group compared to the normal control group, indicating that calcium carbide exposure disrupted cellular homeostasis. However, the treatment groups, particularly those receiving 1.5 mL of *Cucumeropsis mannii* seed oil (CMSO), exhibited a major increase in Na⁺/K⁺-ATPase activities compared to the CAC group. This improvement may be attributed to the bioactive compounds present in CMSO, which likely support cellular function and restore enzyme activity disrupted by calcium carbide. This finding is consistent with the research by Singh *et al.* (2022), which demonstrated that *Cucumeropsis mannii* seed oil has protective effects on Na⁺/K⁺-ATPase activity, helping to counteract the adverse impacts of neurotoxic agents and support neuronal health.

From this study, it was observed that lower doses of CMSO had maximum potential in mitigating the effects of calcium carbide in the cortex of rats

Ascorbic acid, used as the standard control in this study, demonstrated significant neuroprotective effects against calcium carbide-induced neurotoxicity. The results revealed that Ascorbic acid significantly increased the activity of monoamine oxidase, adenosine deaminase, acetylcholinesterase, and Na⁺/K⁺-ATPase in the cortex of the rats in the standard control. This shows that Ascorbic acid helps to restore normal neuronal function by counteracting the toxic effects of calcium carbide. Its potent antioxidant properties play a crucial role in mitigating oxidative stress and preserving the integrity of neuronal cells, thus reversing the detrimental changes observed in the positive control group (Olufunmilayo *et al* 2023).

Conclusion

This study was able to show that *Cucumeropsis mannii* seed oil significantly restores the activities MAO, ADA, AChE, and Na⁺/K⁺-ATPase domiciled in the cortex of animals exposed to calcium carbide. This study investigated the neuroprotective potential of *Cucumeropsis mannii* seed oil (CMSO) against calcium carbide-induced neurotoxicity in the cortex of rats. Results indicated that calcium carbide exposure significantly decreased the activities of neuronal enzymes, highlighting its neurotoxic impact. However, treatment with CMSO led to a significant restoration of these biomarkers, suggesting its neuroprotective efficacy. Overall, the study suggests that CMSO could serve as a promising natural intervention to counteract the adverse neurotoxic effects of calcium carbide in the cortex.

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