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Kaempferol alleviates neurodegenerative disorders induced by *Naja nigricollis* venom via mechanisms of antioxidants, anti-inflammatory, dopaminergic and neuronal functions

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ABSTRACT

Naja nigricollis venom (NnV) contains neurotoxins that influence neurological functions. Kaempferol is a bioactive compound present in edible plants with numerous pharmacological activities. This study investigated the ameliorative potential of kaempferol against NnV-induced neurotoxicity in rats. Fifty male Wistar rats were randomized into five groups ($n = 10$). Group 1 rats were the control while 1.0 mg/kg^{-1} (LD_{50}) of NnV was injected intraperitoneally into rats in groups 2–5 to observed neurotoxicity. Group 2 was untreated post envenomation, while groups 3–5 were treated with polyvalent antivenom, 4 and 8 mg/kg of kaempferol, respectively. The biochemical analysis, neurotoxicity, and pathomorphological defects were assessed in the brain of the envenomed treated rats. Envenomation with NnV elevated oxidative and inflammatory biomarkers, and induced neurotoxicity accompanied with neurobehavioral deficits, and severe pathohistological defects were seen in the brain of untreated envenomed rats. However, treatment with kaempferol significantly ($p < 0.05$) decreased malondialdehyde (MDA) levels and upregulated levels of reduce glutathione (GSH) antioxidant including superoxide dismutase (SOD) and glutathione peroxidase (GPX) antioxidant enzymes, while inflammatory biomarkers, nitric oxide (NO) levels and myeloperoxidase (MPO) activity significantly decreased in envenomed treated groups. Kaempferol upregulated dopamine concentration with significant suppression of acetylcholinesterase (AchE) activity, and restored neurobehavioral and locomotor activities in envenomed treated rats. Also, severe pathomorphological alterations observed in the cortex of the brain were attenuated after kaempferol treatment. The underlaying ameliorative mechanisms of kaempferol are linked to its antioxidant activity, lipid peroxidation inhibition, anti-inflammatory activity, acetylcholinesterase suppression, and alleviation of dopamine system and neurobehavioral abilities.

Abbreviations
NnV Naja nigricollis venom

1. Introduction

Neurotoxic effects are a prominent manifestation observed in cases of envenomation by elapids including cobras (Naja spp.) as their venoms are composed of neurotoxins (Khandelwal et al., 2007). This neurotoxicity has also been extensively documented in other snake species belonging to the Viperidae family and a subfamily of Crotalinae (Johnston et al., 2012; Richardson et al., 2007). Neurotoxins exhibit a vast range of toxic actions that alters processes and functions with damaging effects on the neurological system. One of the mechanisms underpinning the neurotoxicity induced by snake venom is via competitive inhibition of the nicotinic acetylcholine receptor (nAChR) to alter normal neurotransmission which thus results in body paralysis and respiratory failure by α-neurotoxins, a peptide toxin abundantly present in elapids venoms (Nys et al., 2022).

Neurotoxic components found in snake venoms exhibit a

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multifaceted impact on the neuromuscular junction. The predominant fraction of these neurotoxins exerts their effects either at the presynaptic motor nerve terminals or on the postsynaptic nicotinic acetylcholine receptors located at the motor end-plate. Presynaptic toxins initiate a sequence of events, commencing with synaptic vesicle depletion and culminating in structural impairment of the motor nerve terminals (Logonder, 2008; Nys et al., 2022). This particular form of injury is inclined to exhibit resistance to therapeutic interventions, with successful recuperation hinged upon the intrinsic capacity for nerve terminal regeneration. This phenomenon is substantiated by empirical investigations employing presynaptic toxins derived from the venoms of kraits and vipers (Logonder, 2008; Prasarnpun, 2005).

Snake venom toxins, specifically postsynaptic neurotoxins, engage in competitive interactions with the agonist-binding sites of nicotinic acetylcholine receptors located at the motor end-plate. These interactions are characterized by a pronounced affinity, albeit with limited reversibility, resulting in the inhibition of neuromuscular signal transmission (Nys et al., 2022). Certain neurotoxic snake venoms, such as those found in kraits, comprise a combination of diverse toxin classes (Rusmili, 2014). Numerous constituents found in snake venom demonstrate a proclivity for targeting distinct ion channels or exerting influence over the enzymatic activity of acetylcholinesterase within the neuromuscular synapse (Harris, 2009).

N. nigricollis is a cobra (Naja spp.) with a high abundance of cytotoxins, cardiotoxins and neurotoxins present in the venom which contribute to cell destruction, tissue necrosis and systemic neurotoxicity after envenomation (Bala et al., 2022; Chong et al., 2020; Gomez et al., 2023). Neurotoxins such as phospholipase A2 (PLA2) is well known presynaptic neurotoxic enzyme present in *N. nigricollis* venom (NnV) which distorts muscle contraction processes by blocking the binding of acetylcholine to its receptors and disrupting the transmission of nerve impulses to the muscles consequently leading to malfunctioning of major organs of the central nervous system (Ajisebiola et al., 2022; Al-Mamun et al., 2015). Furthermore, neurotoxic venoms have been reported to cause detrimental effect by inducing oxidative damage and inflammation in important organ of the nervous system resulting in neurological toxicities and other severe pathophysiological alterations after NnV envenomation *in vivo* (Adeyi et al., 2020; Ajisebiola et al., 2022).

Antivenom is the only effective drug for the treatment of snake envenoming but faced with clinical side effects after use, scares and very expensive (WHO, 2010). Victims of snakebite envenoming in rural communities rely on medicinal plants as alternatives to treatment of snakebite. Our studies have previously reported the antivenom activity of *Moringa oleifera* Lam. leaves which validated the traditional use of the plant against snakebite (Ajisebiola et al., 2021). In our recent study, kaempferol was identified from *M. oleifera* leaves and confirmed as the main bioactive antivenom compound against toxic enzyme activity and toxicities induced by snake venom (Ajisebiola et al., 2023). Kaempferol is a flavonoid that exhibits a vast range of therapeutic benefits notably anti-inflammatory, anti-lipid metabolizing, and anti-oxidative stress potentials in alleviating several diseases and ailments (Cao et al., 2023; Periferakis et al., 2022). Also, natural products from plants are known to possess outstanding pharmacological effects on neurological disorders (Cui et al., 2023). The venom of *N. nigricollis* have been documented to alter neurological functions in envenomed rats (Ajisebiola et al., 2022), and its amelioration using synthetic or natural products is yet to be reported. This current study built on our existing findings to investigate the ameliorative effect of kaempferol on neurotoxicity and its associated neurodegenerative disorders in *N. nigricollis* envenomed rats.

2. Materials and methods

2.1. Venom and antivenom

A lyophilized NnV was obtained from the serpentarium of the Department of Zoology, University of Ibadan, Nigeria. The venom sample was preserved at 4 °C in the laboratory until required. The drug used as the reference standard for this research is a polyvalent antivenom (EchiTAb-Plus ICP).

2.2. Kaempferol

Kaempferol (MW: 286.24 g/mol, MF: $C_{15}H_{10}O_6$) was procured from Sigma–Aldrich®, USA, St Louis, Missouri, USA.

2.3. Ethics statement

The experimental procedures adhere to the regulations of the University of Ibadan-Animal Care and Use Research Ethics Committee (UI-ACUREC), authorized under approval number UI-ACUREC/19/0030. Furthermore, the experiment was carried out in accordance with the current rules and guidelines that have been established for the care of laboratory animals (National Research Council, 2011).

2.4. Experimental animals

Fifty male albino Wistar rats with weights between 120 and 140 g were procured from the Central Animal Facility of Osun State University, Osogbo, Nigeria. The animals were kept under ambient standard conditions (25 ± 2 ℃ and relative humidity of 50 ± 15 %) in transparent well-ventilated plastic cages in the Department of Zoology Laboratory, Osun State University. They were acclimatized for two weeks with free access to standard feed and water daily.

2.4.1. Design, envenoming and treatment protocols

The animals were separated unbiased into groups of five containing ten rats each. Group 1 rats were injected with saline (control) while groups 2–5 rats were envenomed intraperitoneal with 1.0 mg/kg^{-1} (LD_{50}) of NnV (Adeyi et al., 2020). to observed neurodegeneration. Group 2 was untreated post envenomation, while Groups 3, 4, and 5 were treated with polyvalent antivenom, 4 and 8 mg/kg of kaempferol, respectively. The LD_{50} of NnV was dissolved saline (10 ml) and 0.2 ml of the reconstituted venom was injected into each rat intraperitoneally on day 1 of the experiment at exactly 8:00 am. The envenomed groups were treated 30 minutes post envenomation and for seven consecutive days, while clinical signs of toxicity and death were monitored.

2.4.2. Body weight changes

The animals were measured pre-envenomation and post treatment on day 7 to determine the body weight changes. Changes in body weight were calculated using the formula:

$$Body\ weight\ gain = \frac{Terminal\ weight\ of\ rats - Initial\ weight\ of\ rats}{Initial\ weight\ of\ rats}\ x\ 100$$

2.4.3. Blood and organs collection

The blood samples were collected from each experimental rats using heparinized capillary tubes into plain bottles through retro-orbital sinus punctuation. The blood was centrifuged at 380 g for 10 min to obtain serum for hormonal assays. The animals were then sacrificed by adhering to protocols (Rowett, 1997). The whole brain of the experimental were removed and weighed to determine the organ and relative organ weight. Thereafter, the brain of each animal was divided into two portions, a portion of the was used for biochemical assays, while other portions were fixed in 10 % formalin for histopathological investigations. The relative brain index was determined using the formula:

$$Relative\ organ\ weight = \frac{Organ\ weight}{Termimal\ body\ weight} x100$$

2.5. Biochemical analysis of the brain

2.5.1. Preparation of homogenates

The tissues of the brain were washed in KCl and weighed. The tissues were thereafter homogenized using a Teflon homogenizer in four separate homogenizing buffer (0.1 M Tris-KCl, pH 7.4) and centrifuged for 15 min at 12,500 g in a cold centrifuge (4 °C), to obtain the post mitochondrial fraction. The biochemical analyses were performed using the supernatants.

2.5.2. Oxidative stress profiles

The measurement of malondialdehyde levels in the brain was carried out as described (Ohkawa et al., 1979). Superoxide Dismutase and glutathione peroxidase activities were measured as described by Marklund and Marklund (1974) and Rotruck et al. (1973), respectively. Also, reduced glutathione levels were assayed as described by Ellman (1959).

2.5.3. Inflammatory profiles

The levels of nitric oxide (NO) and myeloperoxidase (MPO) in the brain were assessed by procedures described by Green et al. (1982) and Granell et al. (2003), respectively.

2.5.4. Neurotoxicity profiles

2.5.4.1. Monoamine neurotransmitter estimation. Concentration of dopamine in the brain was determined by enzyme linked immunoassay using BioTech- ELISA reader- USA following the procedure previously described (Gaballah et al., 2016).

2.5.4.2. Acetylcholinesterase estimation. Acetylcholinesterase activity was determined in brain tissues homogenates following Ellman's protocol (Khan et al., 2012). The brain homogenate was incubated for 5 min with 2.7 ml of phosphate buffer and 0.1 ml of 5, 5-dithiobis (2-nitrobenzoate) (DTNB). Further, 0.1 ml of freshly prepared acetylcholine iodide (pH 8) was added, and the change in absorbance was recorded at 412 nm.

2.6. Neurobehavioral assessment

The animals' behavioral activities to assess the locomotory and exploratory behaviors including changes in neuromuscular abilities associated with cobra envenoming were evaluated. The effect of kaempferol treatment on envenomed rats was evaluated 24 h after the last day of treatment. The total distance travelled, total time spent, and average speed were assessed in open field and analyzed using video tracking software (EthoWatcher). The fore limb grip test was carried out as previously described (Farombi et al., 2019; Oladele et al., 2020)

2.7. Histopathological examination

Tissues of the brain were examined for structural defects using conventional techniques of paraffin-wax sectioning and hematoxylin-eosin staining (Carleton et al., 1980).

2.8. Data analysis

The results obtained were stated as mean ± Standard Error of values obtained. Statistical changes within the groups tested were determined using one-way Analysis of Variance and Duncan multiple range test. The values were considered significant at $P < 0.05$. Data were analyzed using statistical package for social sciences, version 25 produced by IBM Corp. Ltd.

3. Results

3.1. Clinical signs of toxicity, body weight gain, organ weight changes, and organo-somatic index of experimental rats

Post-envenoming toxicity signs include postural instability, dizziness, sluggishness and mortality. The group injected with saline (control) recorded no death while the group envenomed and not treated had 50 % mortality. The envenomed rats treated with antivenom and 4 mg/kg of kaempferol recorded 30 and 20 % mortalities, respectively, while group envenomed and treated with 8 mg/kg of kaempferol recorded no death (Table 1). The body weight gain of the envenomed untreated rats was significantly ($P < 0.05$) lower compared to the envenomed group treated with antivenom and different doses of kaempferol (Table 2). A significant decrease was observed in brain weight and index of envenomed untreated rats compared to the envenomed treated groups (Table 2).

3.2. Oxidative stress status

3.2.1. Malondialdehyde levels

The levels of malondialdehyde (MDA) in the brains of envenomed untreated rats were significantly ($P < 0.05$) higher compared to the control and envenomed treated groups. The envenomed group treated with 8 mg/kg of kaempferol recorded a significant ($P < 0.05$) lower value in the MDA levels compared to the envenomed groups treated with 4 mg/kg and antivenom, respectively (Fig. 1A).

3.2.2. Reduced glutathione levels

The glutathione (GSH) levels in the brain of group envenomed with NnV and untreated significantly ($P < 0.05$) decreased compared to the control. However, treatment with antivenom and varying concentrations of kaempferol significantly ($P < 0.05$) increased the GSH levels of the envenomed treated groups. The envenomed group treated with 8 mg/kg of kaempferol showed a significant ($P < 0.05$) increase compared to the envenomed groups treated with 4 mg/kg of kaempferol and antivenom, respectively (Fig. 1B).

3.2.3. Superoxide dismutase activity

The superoxide dismutase (SOD) activity in the brain of envenomed untreated rats significantly decreased relative to the control. However, antivenom and kaempferol treatment significantly ($P < 0.05$) elevated SOD activity in the brain compared to the envenomed untreated group. The envenomed group treated with 8 mg/kg of kaempferol recorded a significant ($P < 0.05$) higher value compared to groups treated with 4 mg/kg of kaempferol and antivenom (Fig. C).

3.2.4. Glutathione peroxidase activity

The glutathione peroxidase (GPX) activity decreased significantly ($P < 0.05$) in the brain of envenomed untreated rats compared to the

Table 1
Mortality of envenomed animals.

Number of death						
Groups	Envenomation	Day 1	Day 2	Day 6	Day 7	Mortality (%)
1	–	–	–	–	–	0.00
2	–	1	2	2	–	50.00
3	–	1	1	1	–	30.00
4	–	–	1	1	–	20.00
5	–	–	–	–	–	0.00

Number of rats per group ($n = 10$).
Legend.
Group 1: Injected with saline (Control), **Group 2:** Envenomed not treated (Venom control), **Group 3:** Envenomed and treated with 0.2 ml of antivenom, **Group 4:** Envenomed and treated with 4 mg/kg^{-1} of kaempferol, **Group 5:** Envenomed and treated with 8 mg/kg^{-1} of kaempferol.

Table 2
Body weight gain and organo-somatic index of experimental rats.

Groups	Body weight gain (g)	Brain Weight (g)	Brain index (%)
1	8.10 ± 1.01^{c}	2.32 ± 0.11^{a}	3.62 ± 0.10^{a}
2	4.42 ± 0.12^{b}	2.09 ± 0.10^{b}	2.82 ± 0.12^{c}
3	6.13 ± 0.16^{a}	2.21 ± 0.02^{ab}	3.24 ± 0.08^{ab}
4	6.32 ± 1.10^{a}	2.25 ± 0.12^{a}	3.32 ± 0.02^{ab}
5	7.84 ± 0.21^{a}	2.27 ± 0.15^{a}	3.58 ± 0.16^{a}

Data are represented as mean ± SE ($n = 10$). Values in the same column with different superscript are considered significant ($p < 0.05$).
Legend.
Group 1: Injected with saline (Control), **Group 2:** Envenomed not treated (Venom control), **Group 3:** Envenomed and treated with 0.2 ml of antivenom, **Group 4:** Envenomed and treated with 4 mg/kg^{-1} of kaempferol, **Group 5:** Envenomed and treated with 8 mg/kg^{-1} of kaempferol.

control. However, kaempferol at 4 and 8 mg/kg enhanced GPX activity in the envenomed treated groups compared to the group treated with antivenom (Fig. 1D).

3.3. Inflammatory status

3.3.1. Nitric oxide levels

The nitric oxide (NO) level was more intense in the brain of the envenomed untreated group compared to the control and envenomed group rats treated with kaempferol and antivenom. However, treatment of envenomed rats resulted in significant ($P < 0.05$) repression of NO levels in the brain with a significant decrease in the group treated with 8 mg/kg of kaempferol compared to other treated groups (Fig. 2A).

3.3.2. Myeloperoxidase activity

The myeloperoxidase (MPO) activity increased significantly ($P < 0.05$) in the brain of envenomed untreated rats compared to the control and envenomed treated groups. Kaempferol demonstrated significant ($P < 0.05$) dose dependent decrease in the MPO activity in the brain of envenomed treated rats (Fig. 2B).

3.4. Neurotoxicity status

3.4.1. Dopamine concentration

Envenomation in untreated rats was associated with a prominent decrease in monoamine neurotransmitter levels in the brain. The dopamine levels decreased significantly ($P < 0.05$) in envenomed untreated rats as compared to the control. However, treatment with antivenom and different concentrations of kaempferol resulted in a significant upregulation in the dopamine levels of envenomed treated rats as compared to the envenomed untreated rats. The dopamine level of the groups treated with 4 and 8 mg/kg of kaempferol showed a dose dependent increase compared to the group treated with antivenom (Fig. 3A).

3.4.2. Acetylcholinesterase activity

Envenomation with NnV in untreated rats resulted in a significant increase in acetylcholinesterase (AChE) activity in the brain compared to the control. Meanwhile, different concentrations of kaempferol significantly ($P < 0.05$) decreased AChE activity with a dose dependent effect relative to envenomed untreated rats. The results showed kaempferol suppressed the activity of AChE compared to the reference drug (Fig. 3B).

3.5. Neurobehavioral status

The envenomed untreated rats displayed a significant ($P < 0.05$) decline in the total distance travelled, total time mobile, and average speed when compared with the control. However, treatment of

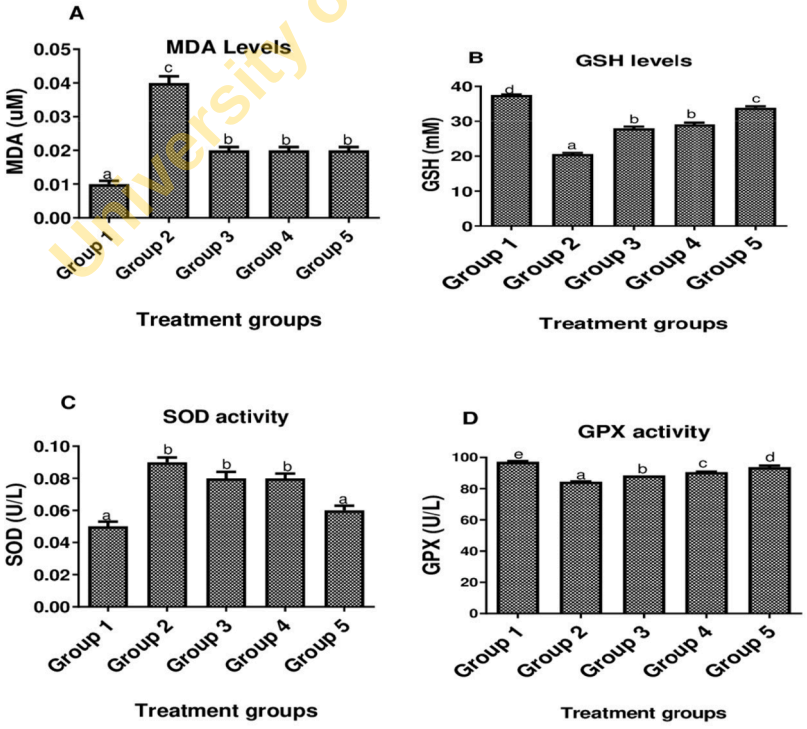

Fig. 1. Oxidative stress profiles of the brain of envenomed rats treated with kaempferol. Data are represented as mean ± SE ($n = 10$). Bar with different lower-case letter represent significant difference in stress profiles of the brain among the groups at $p < 0.05$ using DMRT. **Group 1:** Injected with saline only (control), **Group 2**: envenomed and not treated, **Groups 3:** envenomed and treated with antivenom, **Groups 4:** envenomed and treated with kaempferol (4 mg/kg), **Groups 5:** envenomed and treated with kaempferol (8 mg/kg).
MDA: Malondialdehyde, GSH: Reduced Glutathione, SOD: Superoxide Dismutase, GPX: Glutathione Peroxidase

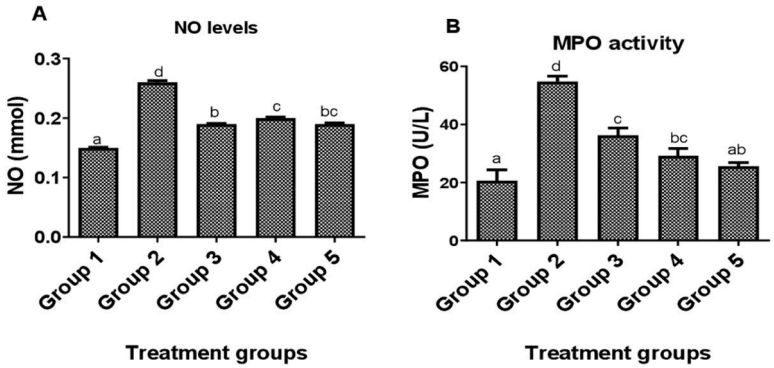

Fig. 2. Inflammatory profiles of the brain of envenomed rats treated with kaempferol. Data are represented as mean ± SE ($n = 10$). Bar with different lower-case letter represent significant difference in inflammatory biomarkers of the brain among the groups at $p < 0.05$ using DMRT. **Group 1:** Injected with saline only (control), **Group 2**: envenomed and not treated, **Groups 3:** envenomed and treated with antivenom, **Groups 4:** envenomed and treated with kaempferol (4 mg/kg), **Groups 5:** envenomed and treated with kaempferol (8 mg/kg).
NO: Nitric Oxide, **MPO:** Myeloperoxidase

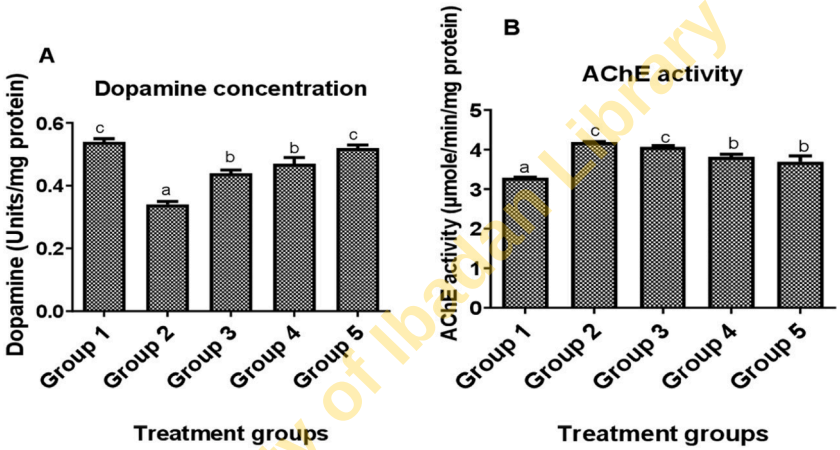

Fig. 3. Neurotoxicity profiles of envenomed rats treated with kaempferol. Data are represented as mean ± SE ($n = 10$). Bar with different lower-case letter represent significant difference in dopamine levels and acetylcholinesterase activity of the brain among the groups at $p < 0.05$ using DMRT. **Group 1:** Injected with saline only (control), **Group 2**: envenomed and not treated, **Groups 3:** envenomed and treated with antivenom, **Groups 4:** envenomed and treated with kaempferol (4 mg/kg), **Groups 5:** envenomed and treated with kaempferol (8 mg/kg).
AChE: Acetylcholinesterase

envenomed rats with varying doses of kaempferol significantly improved the motor and locomotor activities dose dependently as significant improvement was recorded in the total distance travelled, and total time mobile compared with untreated envenomed rats. Also, the motor and locomotor activities of the envenomed group treated with antivenom significantly improved as compared with the envenomed untreated rats. Moreover, envenomed untreated rats exhibited significant lesser time in the forelimb grip test than the control. Treatment of envenomed rats with antivenom and different concentrations of kaempferol significantly enhanced the forelimb grip activities of the treated groups (Table 3).

3.6. Histological examination

The brain tissues of the control rats (Fig. 4, plate 1) showed normal features of the cortex and large pyramidal neurons with long axons that extend well from the soma to adjacent neurons within the neuropil. Whereas, the brain tissues of envenomed untreated rats (Fig. 4, plate 2) revealed observable degenerative changes in the cortex characterized by clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within the soma. Also, a perineural spaces can be seen surrounding degenerating neurons with axons and dendrites scarcely appreciable around the neurons. However, the

Table 3
Effect of kaempferol treatment on neurobehavioral activities of envenomed neurodegenerative rats.

Behavioral activities	Group 1	Group 2	Group 3	Group 4	Group 5
Total distance travelled (cm)	37.74 ± 0.10^{c}	17.34 ± 1.00^{b}	24.74 ± 1.21^{ab}	27.38 ± 0.23^{a}	29.37 ± 0.12^{a}
Total time mobile (sec)	127.33 ± 3.32^{c}	75.43 ± 2.24^{b}	107.74 ± 2.04^{ab}	118.45 ± 1.23^{a}	125.18 ± 3.26^{a}
Average speed (cm/sec)	0.29 ± 0.01^{a}	0.23 ± 0.02^{b}	0.23 ± 0.01^{b}	0.23 ± 0.03^{b}	0.24 ± 0.02^{ab}
Fore-limb grip	62.10 ± 1.21^{a}	30.44 ± 1.11^{c}	50.13 ± 1.41^{b}	58.18 ± 1.32^{a}	60.22 ± 1.32^{a}

Mean ± S.E in the same column having similar superscript are not significantly different at $P < 0.05$.
Legend.
Group 1: Injected with saline (Control), **Group 2:** Envenomed not treated (Venom control), **Group 3:** Envenomed and treated with 0.2 ml of antivenom, **Group 4:** Envenomed and treated with 4 mg/kg^{-1} of kaempferol, **Group 5:** Envenomed and treated with 8 mg/kg^{-1} of kaempferol.

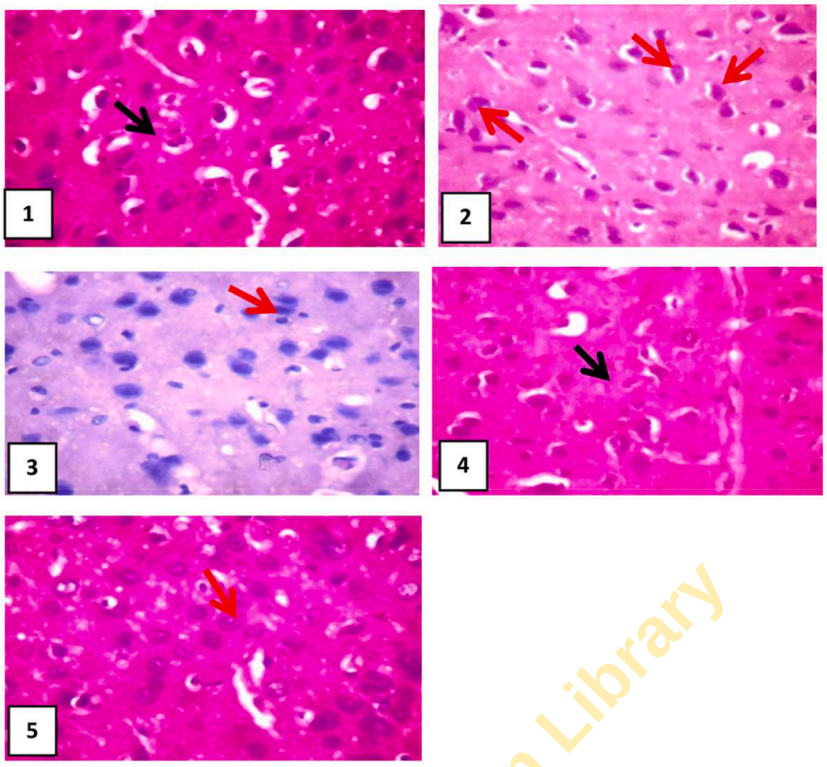

Fig. 4. Photomicrographs showing panoramic views of brain histomorphology. **Group 1 (control):** showed normal features of the cortex and large pyramidal neurons (black arrow) with long axons that extend well from the soma to adjacent neurons within the neuropil, **Group 2** (venom control): revealed observable degenerative changes in the cortex characterized by clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrows), **Groups 3 (venom/antivenom):** showed normal cytoarchitecture of the cortex with no marked lesion (red arrow), **Groups 4 (venom/4 mg/kg kaempferol):** showed normal cytoarchitecture of the cortex with no marked histological alteration (black arrow), **Groups 5 (venom/4 mg/kg kaempferol):** showed normal cytoarchitecture of the cortex with no marked structural defects (red arrow).

antivenom and kaempferol attenuated these tissues lesions. The envenomed group treated with antivenom (Fig. 4, plate 3) showed normal cytoarchitecture of the cortex with no marked lesion, while the envenomed group treated with 4 and 8 mg/kg of kaempferol (Fig. 4, plate 4 and 5) showed normal cytoarchitecture of the cortex with no marked histological alteration.

4. Discussion

The venom of cobra contains neurotoxins such as three finger toxins (3FTXs) and phospholipases (PLA2s) which are important constituents of NnV, mainly responsible for neuromuscular paralysis and respiratory failure through binding to nicotinic acetylcholine receptors in skeletal muscles resulting in death after envenoming (Chippaux et al., 2017; Khourcha et al., 2023). Also, these neurotoxins, in addition to their role in neurotoxicity, exhibit a wide variety of pharmacological activity through interfering with normal physiological processes with detrimental effect in the body (Khourcha et al., 2023). In this study, neurotoxins in NnV caused high mortality in the envenomed untreated group which was consistent with previous studies (Adeyi et al., 2020; Ajisebiola et al., 2022). However, kaempferol protected the envenomed rats treated with high dose and no mortality was recorded indicating the compound neutralized the venom toxins and averted fatalities. This finding supported our previous findings in rats envenomed with viper venom (Ajisebiola et al., 2023).

There was a significant reduction in brain weight and index in the envenomed untreated rats, which is evidence of brain toxicity (Ajisebiola et al., 2022; Zhu et al., 2020). The decline in brain weight and index of envenomed untreated rats could be attributed to nutrients depletion, disruption of metabolic activities or pathways and inhibition of protein synthesis (Oladele et al., 2020). However, there was significant improvement in the organs weight and organo-somatic index of the envenomed groups treated with 4 and 8 mg/kg of kaempferol suggesting the ability of the compound to improve metabolic activities and enhance protein synthesis in the brain as previously observed (Oladele et al., 2020).

Envenomation is often accompanied by the activation of oxidative stress and inflammatory processes and its cascade of events play a vital role in the clinical pathogenesis after snakebite envenoming (Megale et al., 2021). Oxidative stress arises from an upsurge of reactive oxygen species (ROS) which are chemical reactive molecules implicated in the pathogenesis of neurodegeneration. ROS play a critical role as high levels of oxidative stress are commonly observed in the brain of patients with neurodegenerative disorders. NnV caused oxidant damage and inflammation via modulation of oxidative and inflammatory biomarkers with a significant elevation of levels of MDA, NO and MPO activities in the brain of the envenomed untreated animals indicating that the ROS suppressed the endogenous antioxidant enzymes in this group resulting in oxidant damage which was in tandem with previous study (Ajisebiola et al., 2022). Cells of neurological organs are vulnerable to oxidative stress and inflammation and thus, need sufficient potent antioxidants to eliminate free radical damage produced by the lipid peroxidation chain reaction (Oladele et al., 2020). Kaempferol repressed the oxidative and inflammatory biomarkers and significantly elevated endogenous antioxidant enzymes of GSH, GPX and SOD in the brains of treated envenomed rats which substantiated the antioxidant and anti-inflammatory properties of kaempferol as earlier reported (Hoffer et al., 2020). This indicated that kaempferol inhibited the lipid peroxidation chain

reaction and ameliorated neuro-inflammation induced by NnV in the brain of envenomed treated rats.

Acetylcholine in the cholinergic system is a neurotransmitter that is very important to neurological activities such as muscular movement, memory, learning, control and modulation of cerebral blood flow (Jolitha et al., 2008). Acetylcholinesterase (AchE) is an enzyme that controls acetylcholine levels and hydrolyses the neurotransmitter hence, the most efficient target to treat neuro-disorders. Modulation of AchE activity may have detrimental effect on cholinergic transmission including progressive cognitive impairment which contribute immensely to neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease (Abdalla et al., 2013). NnV upregulated AchE activity in the brain of envenomed untreated rats, however, treatment with kaempferol suppressed the activity of this enzyme establishing the potentials of the compound to influence cholinergic neurotransmission by modulating acetylcholine level in the synaptic cleft. Inhibition of AchE is key in the treatment of neurodegenerative disorders and kaempferol proved to be acetylcholinesterase inhibitor that could improve neuronal viability and functionality. The mechanism of kaempferol may be associated with the modulatory effect of the compound on AchE activity as earlier reported (Akram et al., 2017). Studies have reported the suppression of AchE activity in the brain using natural products from medical plants (Hussein et al., 2020; Oladele et al., 2020).

Dopamine is a central nervous system neurotransmitters involved in a variety of neurological functions and plays roles in reward, cognition and locomotor control (Kaplan et al., 2016). In this study, neurochemical analysis of brain monoamines metabolites showed NnV depleted dopamine levels in the brain of the envenomed untreated rats affirming that venom toxins could manifest neurotoxicity in snake envenomed victims. Dopamine system dysfunction has been reported in response to chemically induced oxidative stress, and neurodegenerative disorder (Kaplan et al., 2016; Oladele et al., 2020). Neurodegenerative disorders are characterized by factors such as dopamine depletion, uncontrolled oxidative stress, elevated acetylcholinesterase (AChE) activity, genetic mutations, aggregation of toxic misfolded proteins, decrease acetylcholine (ACh), and brain-derived neurotrophic factor (BDNF) in the brain (Kaizer et al., 2005). However, treatment with 4 and 8 mg/kg of kaempferol upregulated the release of dopamine in envenomed treated groups compared to antivenom treated group.

Neurodegeneration is characterized by loss of brain functions resulting in motor deficits, tremors, and postural instability (Bernardes et al., 2018). The untreated envenomed rats demonstrated notable locomotor instability characterized by decrease in speed, total mobility time and total distance covered combined with significant decline in the forelimb grip and body rotation which are vital psychomotor coordination tools to assess neurodegenerative disorders. The observed depreciation of locomotor activities suggests cellular signaling dysfunction between the nervous system and muscular junctions (Adedara et al., 2016). Dopamine performs critical roles in motor functions and its depletion and consequent energy dysfunction of the mitochondria cascades affects the dopaminergic neurons, which requires greater energy for efficient function (Ayano, 2016). Also, the dopaminergic system plays an important role in neuromodulation such as motor control, and cognitive function. The decline in motor and locomotor activities of envenomed untreated rats could be linked to depletion in dopamine level and dopaminergic neuron degeneration in the brain which could result in chronic progressive neurodegenerative disease such as Parkinson's disease (Bernardes et al., 2018). Kaempferol remarkably improved these locomotor markers demonstrating its ability to reverse chemically mediated psychomotor dysfunctions compared to envenomed rats treated with antivenom. Report abounds that plant phytochemicals could serve as major precursors in the synthesis of neurotransmitters such as serotonin, acetylcholine and dopamine acetylcholine (Ebuehi, 2012).

The central nervous system controls all organs functions and activities of the body. Ultimately, the brain which is the primary coordinating organ of the central nervous system, controls neurological functions, which makes the brain an essential organ to consider when studying organ system-disrupting toxicants (Gore and Roberts 2003). Studies have reported that snake venom toxins can interact with the central nervous system mainly the brain, through direct or indirect *in vivo* interactions resulting in neurotoxicity (Osipov and Utkin, 2012). The toxins could penetrate the blood brain barrier (BBB) and influence the brain and other organs functions. In neurotoxic venoms, α-neurotoxins which are key constituents, interacts and binds neuronal nicotinic acetylcholine receptors (nAChRs) and brain capillary endothelial cells, a major component of the BBB which could in turn facilitate α-neurotoxins penetration through the BBB. The penetration of the toxins could result in brain toxicity which will definitely interrupt other systems functions such as the neurological and oxidant systems as observed in the envenomed untreated groups in this present study. Moreover, studies have reported brain toxicity due to snake venom toxins (Gomes et al., 2002; Osipov and Utkin, 2012).

The biochemical results corroborated the histopathological findings, as NnV caused severe histological lesions in the brain cortex region, which control motor activities and substantiated the changes in locomotor deficits of the envenomed untreated rats. Studies have revealed such histological degenerations in areas related to motor activities after exposure of animals to snake venom and toxicants (Ajisebiola et al., 2022; Oladele et al., 2020; Ruiz de Torrent et al., 2007). Furthermore, snake venom toxins have been reported to induced cellular morphological variations, and deep ganglioside level alterations in some regions of the brain which causes instability of righting reflex, posture and motor response in rats (Ruiz de Torrent et al., 2007). However, kaempferol ameliorated the structural lesions observed in the brain of envenomed treated rats compared to the group treated with antivenom.

Phytochemicals such as alkaloids, flavonoids, polyphenols and terpenes have been established to possess potent ameliorative effects on neuro-disorders (Garodia et al., 2023). Kaempferol is a flavonol class of plant-derived compounds that exhibit numerous health-related benefits. The compound has a three rings structure; a double bond in C2−C3, a hydroxyl moiety in C3, and a keto group in C4 of ring C. The compound shared the pattern of benzene ring hydroxylation in number and positions, as their structures include a hydroxyl group which is a major determinant of the ability of flavonoids to exert their bioactivity and antioxidant action by scavenging ROS (Speisky et al., 2023). In this study, kaempferol exert multidimensional mechanisms in ameliorating the pathophysiological features associated with neurodegeneration through antioxidant system strengthening, inhibition of lipid peroxidation, repression of acetylcholinesterase activity, and anti-inflammatory and neurobehavioral repairment properties. To further substantiate our reports, kaempferol have been reported to possess neuroprotective properties and mechanisms underlying the effectiveness of natural compounds on neurodegenerative disorders have been documented to include antioxidant, anti-inflammatory, and mitochondria protection (Cui et al., 2023; Xiao et al., 2023).

5. Conclusion

In light of the aforementioned findings, which showed convincing evidence that NnV could induced neurotoxicity accompanied by neurodegenerative disorders in cobra envenomed victims if proper and effective treatment is not administered. Also, results showed that kaempferol demonstrated potent ameliorative effect on the neuropathophysiology induced by NnV post envenoming. Our findings highlight the clinical significance and therapeutic potentials of this natural bioactive compound in the management of neurotoxicity and associated neurodegenerative disorders that could arise due to ineffective management of cobra envenoming most importantly in tropical rural areas where antivenom accessibility is a challenge.

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Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement

Babafemi Siji Ajisebiola: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Abdur-Rahman Kolawole Mustapha:** Resources, Supervision, Validation. **Omotayo Opemipo Oyedara:** Formal analysis, Software, Writing – review & editing. **Johnson Olaleye Oladele:** Conceptualization, Investigation, Methodology, Resources, Writing – original draft. **Akindele Oluwatosin Adeyi:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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