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Original Article

Co-administration of N-acetylcysteine and Zinc Sulfate prevents Bonny light crude oil-induced neurobehavioral alterations in Mice via modulation of serotonergic / glutamatergic signaling

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Abstract

Background: The possibility of preventing the neurotoxicity brought on by exposure to bonny light crude oil (BLCO) by co-administering N-acetylcysteine and ZnSO4 is still unknown. Therefore, this study looked at how N-acetylcysteine and ZnSO4 exposure together could protect against the neurotoxicity caused by bonny light crude oil.

Methods: Forty male mice were randomly assign to 5 groups (n=10). Group 3 to 5 were pretreated with NAC (20 mg/kg, p.o.), ZnSO4 (20 mg/kg, p.o.) and NAC+ZnSO4, respectively for 14 days followed by BLCO (2ml in 20g feed) from day 15 to day 28. Group 1, served as normal control and received normal saline (1ml/kg)and normal diet throughout the duration of the study while group 2 served as negative control and was fed BLCO (2ml in 20g feed) after 14 days of receiving normal saline. Between days 27 and 29, tests to measure locomotor activity, the anti-depressive and anxiolytic effects of NAC and/or ZnSO4 on BLCO-induced neurobehavioral derangement were assessed, and then animals were euthanized.

Results: Our findings demonstrated that administering ZnSO4 and/or NAC before BLCO exposure significantly increased the time spent in open-arm on the EPM test, increased the time spent in the light chamber on the LDB test, and decreased immobility time on the FST, thereby reducing the behavioral deficit caused by BLCO. By pretreating with NAC and/or ZnSO4, neurochemicals such as glutamate, serotonin, noradrenaline, as well as acetylcholinesterase activity, were regulated.

Conclusion: N-acetylcysteine and Zinc Sulfate co-administration protects mice from Bonny Light crude oil-induced neurobehavioral changes through modifying serotonergic/glutamatergic transmission.

Keywords: Neurochemicals, N-acetylcysteine (NAC), Zinc sulphate, Neurobehavior, Depression, Anxiety

1. Introduction

According to Ekpenyong and Asuquo [1], Nigeria depends heavily on crude oil as a source of foreign exchange and energy for running machinery. However, due to exploratory activities, exposure to crude oil might happen orally, through skin contact, or by inhalation [2]. Studies have shown that exposure to petroleum fractions and products may be linked with hepatotoxicity, nephrotoxicity, haematotoxicity, genotoxicity, immuno toxicity, testicular toxicity, neurotoxicity [3-8]. It's interesting to note that bonny light crude oil (BLCO) is allegedly well-known for its folklore uses as a source of medicine for a variety of maladies, including topical application for burns, foot rot and leg ulcers, poisoning and witchcraft, and ingestion for the treatment of gastrointestinal disorders [4]. A growing body of research has connected neurotoxicity to a number of causes, such as decreased antioxidant status, an increase in pro-inflammatory cytokines, apoptosis, and a decrease in acetyl cholinesterase activity, which may affect neural and functional processes. The molecular layer, granular layer, and density of Purkinje cells of the cerebellum are all reportedly affected by the overproduction of reactive oxygen species (ROS) and activation of apoptotic signaling [8-10]. According to studies, exposure to spilled oils can cause symptoms including anxiety, depression scores, worse mental health, and self-reported headache, sore eyes, and throat [11-13]. Research on neuronal degeneration has been prompted by mounting data, and this has led to the development of potential treatments that could reverse the neuronal damage brought on by prolonged exposure to bonny light crude oil (BLCO) and its chemical constituents.

N-acetylcysteine (NAC) is a thiol molecule that offers sulfhydryl groups and functions as a direct ROS scavenger as well as a precursor of reduced glutathione, hence regulating the redox status in cells [14]. According to research by Sadowska et al. [15], NAC has been demonstrated to disrupt signaling pathways that control inflammatory responses, angiogenesis, apoptosis and cell proliferation. Due to NAC's antioxidant, anti-inflammatory, mucolytic, ant-mutagenic, and anti-carcinogenic capabilities, several disorders have been treated with it [16, 17]. According to studies, methamphetamine organophosphate insecticides and heavy metals create behavioral abnormalities (acute hyperlocomotion and development of behavioral sensitization) that NAC has been shown to be effective in treating [17, 18].

Zinc (Zn) is the trace element found in the body in the greatest concentration after iron. It is important for the development of the cell cycle, apoptosis, and aging as well as for biochemical pathways and physiological activities such oxidative stress response, homeostasis, immune responses, DNA replication and DNA damage repair. The fact that it can bind to more than 300 enzymes and more than 2000 transcriptional factors makes it a versatile trace element. The development of pathogenic diseases could, however, be caused by dysregulation of Zn homeostasis [19]. According to studies, zinc is a cofactor for the metabolism of several neurotransmitters, including those that may modulate fast excitatory transmission [19, 20], suppress the increase in extracellular glutamate, inhibit NMDA receptors, and promote the release of Gamma-Amino Butyric Acid (GABA) [21]. However, Zn supplementation may act as an effective adjuvant agent in the therapy [22]. Reports have linked a reduction in serum Zn level in individuals with neurodegenerative illnesses. According to reports, Zn supplements have been used to treat a variety of ailments associated with Zn deficiency states, including diarrhea, age-related macular degeneration, and wound healing. According to studies, a zinc deficiency causes a rise in the rate of apoptosis, a decrease in cell viability, and neuronal damage, all of which are indicators of neurotoxicity [10]. Zink is claimed to have antidepressant properties and has been used successfully to treat attention deficit hyperactivity disorder (ADHD) in children and adolescents in a number of experimental and some clinical investigations. N-acetylcysteine (NAC) and zinc sulphate ($ZnSO_4$) both have demonstrated health benefits. There is, however, a dearth of information in the literature describing the therapeutic effectiveness of co-administering NAC and $ZnSO_4$ against neurotoxicity linked to exposure to Bonny light crude oil. Therefore, this study examined the neurobehavioral changes caused by Bonny light crude oil (BLCO) and the preventive efficacy of co-administration of N-acetylcysteine (NAC) and/or Zinc Sulphate ($ZnSO_4$).

2. Materials and Methods

2.1 Drugs and chemicals

N-acetylcysteine was acquired from Sigma Aldrich in the United States (catalog number A7250-10G) and according to previous study of Butt et al., [23], it was administered at a dose of 100 mg/kg. Pure Zinc sulfate was acquired from a neighborhood pharmacy in Delta State and administered at a rate of 0.5 mg/kg/day following the protocol of Carlucci et al. [24]. Bonny light crude oil (BLCO) was gotten from the Nigerian National Petroleum Corporation (NNPC), Warri, Nigeria, and 2.0mL of bonny light crude oil was mixed with 20g of rat meal according to earlier studies of [25]. Using the computer program Omni dose calculator, the daily chemical dosage was calculated based on the animal weight. Zinc sulfate as well as N-acetylcysteine was dissolved in normal saline according to their respective doses and administered orally between the hours of 7 am and 10 am in a weight/volume ratio.

2.2 Laboratory animals

In accordance with the regulations, guidelines, and policies governing the use of animals in research as described in the public health service policy on laboratory animals and the National Guideline for Laboratory Animal Care (NIH Publication No. 85-23), forty (40) adult male Swiss mice with an average weight of 30 g were purchased from an accredited dealer at Ogbomosho. They were housed and maintained in the animal holding facility of the University of Medical Sciences, Ondo under convectional laboratory condition of temperature, humidity and 12 hours light/dark cycle.

2.3 Laboratory design

Male Swiss Mice were acclimatized for 7 days before being divided into five groups at random (n=10). To develop the preventive regimen outlined by Monte-Silver, et al. [26], Groups 3-5 underwent pretreatment with NAC (100 mg/kg, p.o.), ZnSO4 (0.5 mg/kg, p.o.), and NAC+ZnSO4 for fourteen days, respectively before being exposed to Bonny light crude oil (2ml in 20g of feed) until the 28th day. For 14 days, Groups 1 and 2 respectively received Bonny light crude oil (2 ml in 20 g of feed) and normal saline (10 mL/kg, p.o.). NAC and ZnSO4 were co-administered at a 45-minute intertreatment interval, and all treatments were given once daily between 7:00 and 8:30 a.m. Between days 27 and 29, neurobehavioral characterization in open field test, elevated plus maze, light and dark box test and force swimming test were conducted to determine the locomotive activity, anti-depressive-like effect as well as anxiolytic effect of NAC, ZnSO4, or their co-administration on BLCO-induced neurobehavioral despair. For biochemical tests, all animals were euthanized on day 29 via cervical dislocation (Figure 1). Each animal's removed brain tissue was weighed, thoroughly cleaned and homogenized in sodium phosphate buffer (0.1 M, pH 7.4). Then, it was centrifuged at 5400 g for 15 min. at 4ºC, and the supernatant was kept at -20ºC for biochemical tests.

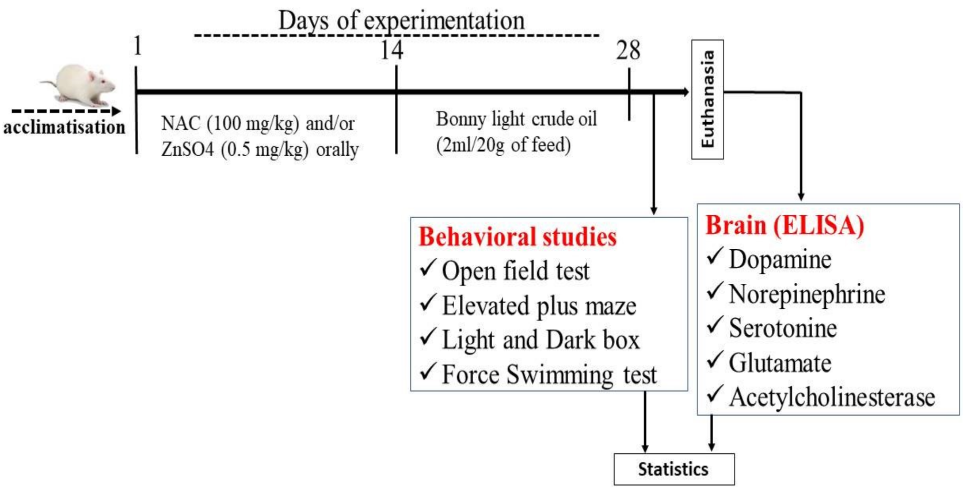

Figure 1: Study design

2.4 Behavioral assessment

Utilizing various test models, including the open field test, light dark box, raised plus maze, and force swimming test, depressive-like behavior was evaluated.

Open field Test (OFT):

In an open-field chamber, the effects of co-administration of NAC and ZnSO4 on mice's BCLO-induced locomotor activity were evaluated. The OFT apparatus consists of a wooden box with dimensions of 35 x 30 x 23 cm that is put in a sound-free space with visible lines drawn to divide the floor into 25 squares measuring 7 cm by 7 cm. Animals were maintained in the test room for at least one hour prior to the open-field test for each test to prevent environmental stress from affecting the results. Mice were positioned one by one in the center square and permitted to roam the room. For five minutes, the number of squares crossing was watched and counted. After each evaluation, the observation cage was cleaned with 70% ethanol to get rid of the preceding animal's odor cues.

Elevated Plus Maze (EPM):
The tested device has two open (25x5x0.25 cm) and two closed (25 5 16 cm) arms that extend from a central platform (5x5x0.5 cm), which is positioned 50 cm above the ground. The entire contraption was constructed from a dark polyvinyl plastic. Mice were given 5 minutes to freely explore the device after being put on the center platform facing one of the closed arms. When the mouse inserted all four paws into the arm, it was considered to have entered the arm. The amount of time spent in the open and closed arms was noted and examined. Prior to testing, mice were kept in the silent laboratory for at least 30 minutes. Furthermore, two researchers who were blind to the treatment circumstances personally monitored every testing session on the video system. To remove smell cues from the preceding animal, 70% ethanol was used to sanitize all arms and the middle space after each trail.

Light-dark box (LDB):
The LDB test was conducted in a two-sectioned apparatus (21x42x25 cm) with a restricted opening that was 3 cm high and 5 cm wide. The dark area was covered with a lid to keep it dark (0 lux) while the bright area was illuminated (50 lux). After being placed in the light chamber, the rats were watched for five minutes. An evaluation of anti-anxiety behavior used the length of time spent in the light chamber as a criterion. To get rid of olfactory cues left behind after measuring each animal, 70% ethanol was used to clean the device.

Forced Swimming test (FST):
Force swimming test (FST) was used to measure behavioral despair. As previously reported by Porsolt et al. [27], the FST was used to measure the anti-depressive-like effects of co-administering NAC and ZnSO4 on BCLO-enhanced behavioral despair in mice. At each FST stage, each mouse was submerged vertically for a total of six minutes in a 25-cm-high, 10-cm-diameter container of water that was kept at room temperature (25°C). Additionally, the total amount of time each mouse was immobile over the course of the previous 4–6 minutes was noted as immobility time, which is a sign of behavioral despair.

2.5 Tissue preparation
The animals were euthanized by cervical dislocation at the conclusion of the experiment after fasting for an overnight period and the brain was taken, weighed, and homogenized in phosphate buffer saline in order to prepare it for all biochemical examination utilizing ELISA techniques.

2.6 Estimation enzyme acetyl cholinesterase (AChE)
The method described earlier by Ellman et al. [28] was used to measure the activity of the brain's acetylcholinesterase (AChE), a marker of cholinergic function that catalyzes the breakdown of acetylcholine to acetate and choline. 5',5′dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholine iodide, and sodium phosphate buffer (0.1 M, pH 7.4) were combined in an aliquot. The rate of AChE activity was directly proportional to color formation as a result of the reaction between thiocholine and DTNB, which was measured using a UV spectrophotometer (752P UV-VIS spectrophotometer, Searchtech, UK) for 10 min at 2-min intervals against blanks. This activity's unit of measurement is M of AChE/min/mg protein.

2.7 Neurotransmitter concentrations measurement
Enzyme immunoassay was used in accordance with the manufacturer's instructions to measure the amounts of Dopamine, Norepinephrine, Serotonin (5-HT), and Glutamate in the brain supernatant. Prior to use, all reagents, reference materials, and samples were cooled to room temperature. Dopamine, 5-HT, and glutamate concentrations were represented as ng/g tissue and ng/g tissue, respectively.

2.8 Statistical evaluation
The software GraphPad Prism 9.0 (GraphPad Software, Inc. La Jolla, CA 92037, USA) was used for all data analysis. One-way analysis of variance (ANOVA) was used to express the data as mean±SEM (standard error of the mean), and then Tukey's post-hoc test was used for multiple comparisons.Pearson linear regression r and regression coefficient used to test for relationship and P-value of 0.05 or lower was regarded as statistically significant.

3. Results

3.1 Effects of co-administration of NAC and ZnSO4 on BCLO-induced locomotive decline on open field test

Result obtained from this effect of ZnSO4 or NAC on behavioral despair, was based on the number of crosses using OFT. As shown in Figure 2, BLCO fed mice (2ml in 20g diet) significantly ($p<0.05$) decreased the locomotive activity in the preventive treatment protocol in comparison with saline-treated group [$F(4, 10)=15.34$, $p=0.0003$, $R2=0.8598$]. However, preventive study with ZnSO4 or NAC elicited a significant ($p<0.001$) increase number of crosses when compared with BLCO treated group.

3.2 Effects of ZnSO4 or NAC co-administration on time spent in light and dark box (LDB)

A significant decrease in the time spent in light chamber [$F(4, 10)=57.58$, $p<0.0001$, $R2=0.8998$] and increase in time spent in the dark chamber [$F(4,10)=68.58$, $p<0.0001$, $R2=0.9648$] in LDB test with BLCO when compared with the control. However, there was a significant increase in the time spent in the light chamber and decrease in time spent in the dark chamber among groups treated with ZnSO4 or NAC co-administration and exposed to BLCO when compared with the BLCO group as shown in Figure 3A-B.

3.3 Effects of ZnSO4 or NAC co-administration on time spent in Elevated plus maze (EPM)

The results showed there was a significant decrease in the time spent in the open arm [$F(4,8)=12.63$, $p=0.0016$, $R2=0.8633$] and increase in time spent in the closed arm [$F(4, 8)=76.07$, $p<0.0001$, $R2=0.9713$] among groups treated with ZnSO4 or NAC co-administration with BLCO when compared with the BLCO group as presented in figure 4A-B. In addition, there was a significant decrease in the time spent in open arm and decrease in time spent in the closed arm in EPM test with BLCO when compared with the control.

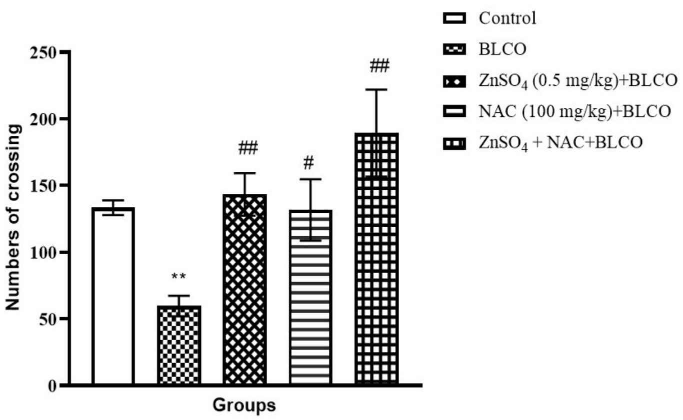

Figure 2: *Effects of ZnSO4 or NAC co-administration in BCLO-enhanced locomotive decline on open field test (OFT). Locomotive activities of rats were assessed by estimating the number of line crossing. Data expressed in mean±SEM, n=5. **p<0.01 vs control, #p<0.05, ##p<0.01 vs. BLCO (one-way ANOVA, Tukey's multiple comparison)*

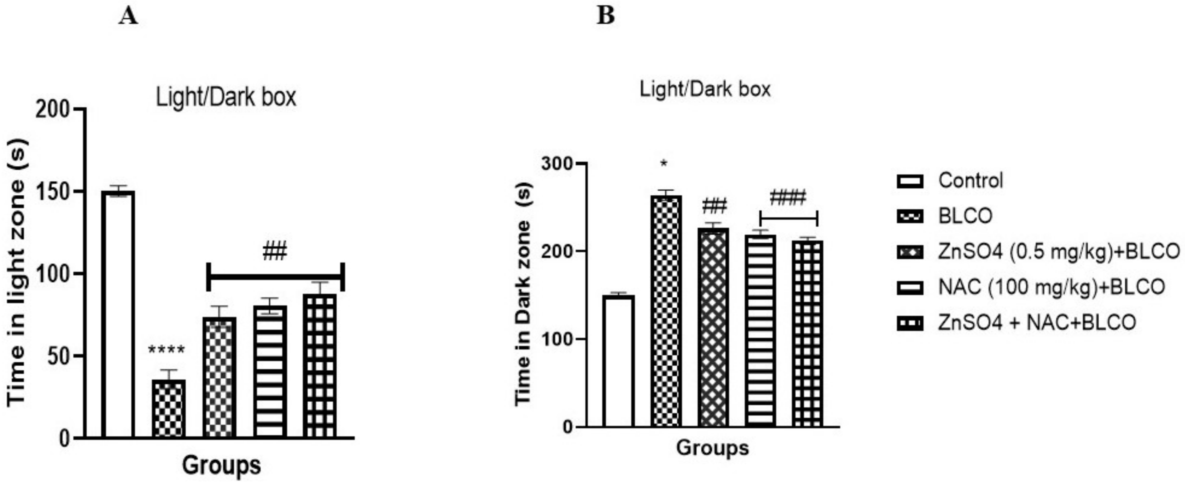

Figure 3: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behavior of rats were assessed by estimating the time spent in the light zone of the light and dark box test (LDB). Data expressed in mean±SEM, n=3rats/group. ****p<0.0001 vs control, ##p<0.01 vs. BLCO (one-way ANOVA, Tukey's multiple comparison)*

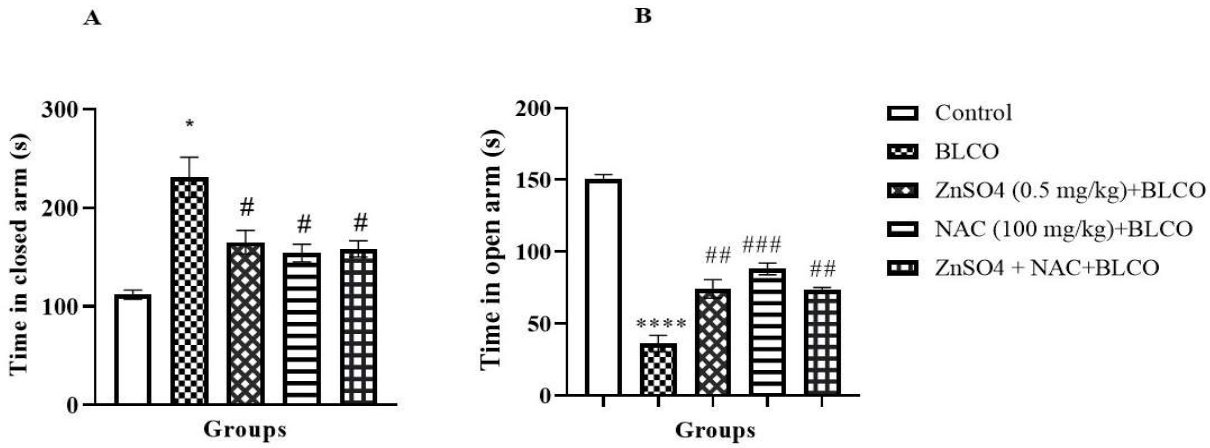

Figure 4: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behavior of rats were assessed by estimating the time spent in the open arm of elevated plus maze (EPM). Data expressed in mean±SEM, n=3.*p<0.05, ****p<0.0001 vs control,#p<0.05, ##p<0.01, ###p<0.001 vs. BLCO (one-way ANOVA, Tukey's multiple comparison)*

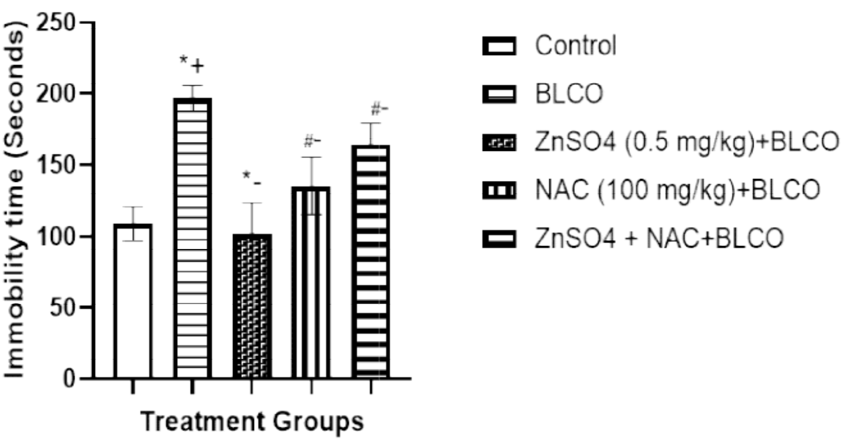

Figure 5: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behaviors of rats were assessed by estimating the immobility time in forced swimming test (FST). Data expressed in mean±SEM, n=5. *p<0.05 vs. control, #p<0.05 vs. BLCO only group (one-way ANOVA, Tukey's multiple comparison).*

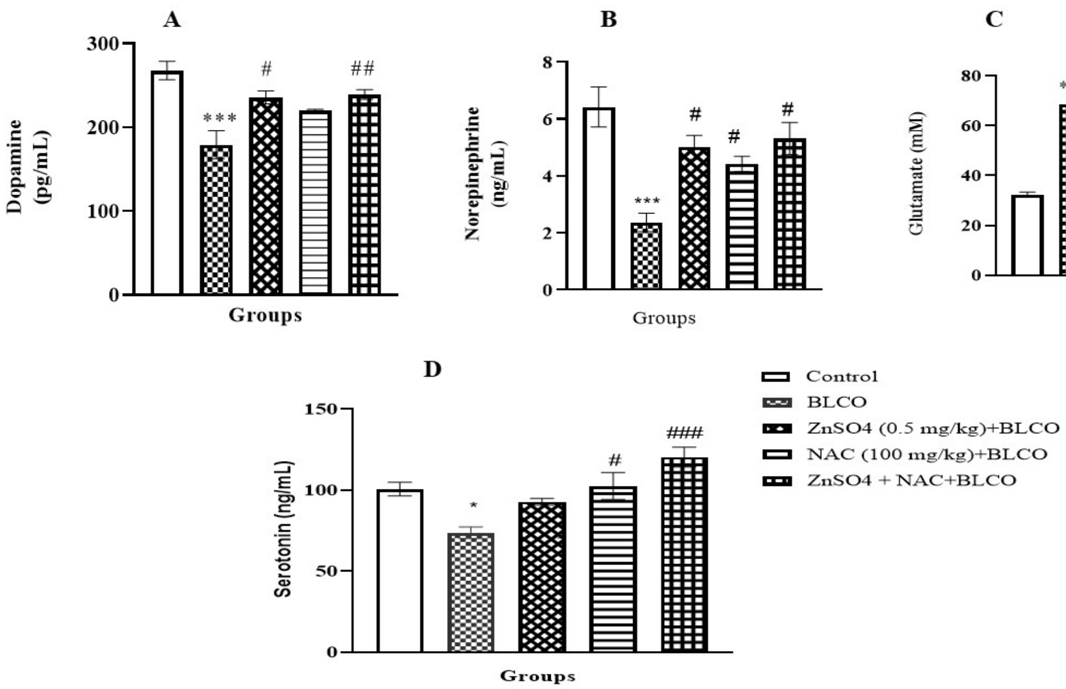

Figure 6: ZnSO4 or NAC co-administration modulates neurotransmitters in the brain of BLCO treated mice*(A) Dopamine (B) Norepinephrine (C) Glutamate (D) Serotonin. Data expressed in mean±SEM, n=5mice/group. ***p<0.001 vs. control, #p<0.05 vs. BLCO only group (one-way ANOVA, Tukey's multiple comparison).*

3.4 Effects of ZnSO4 or NAC co-administration and BLCO-enhanced immobility on forced swim test (FST).

Figure 5 showed the effect of ZnSO4 or NAC on behavioral despair, and was based on duration of immobility using forced swim test. BLCO only fed mice (2ml/20g diet) significantly ($p<0.05$) increased the duration of immobility in the FST in when compared with saline-treated group, which suggests behavioral despair indicative of in BLCO only fed group [$F(4, 8)=15.43$, $p=0.0018$, $R2=0.9761$]. However, preventive study with ZnSO4 or NAC co-treatment with BLCO groups elicited a significant ($p<0.001$) decrease in immobility time when compared with BCLO treated group.

3.5 ZnSO4 or NAC co-administration modulates neurotransmitters in the brain of BLCO treated mice.

The result presented in figure 6A-D, showed that BLCO significantly ($p<0.05$) decreased the level of Dopamine (DA) [$F(4,10)=11.95$, $p=0.0003$, $R2=0.7862$], Nor-epinephrine (NE) level [$F(4,10)=9.569$, $p=0.0019$, $R2=0.7929$] Serotonin (5-HT) level $F(4,10)=9.925$, $p=0.0012$, $R2=0.7830$], and increase in glutamate [$F(4,10)=18.59$, $p=0.0008$, $R2=0.9140$], when compared control animals. However, pretreatment with NAC and/or ZnSO4 showed a significant ($p<0.05$) increase in DA (Fig. 6A), NE (Fig. 6B), 5-HT levels (Fig. 6D), and a significant decrease in Glutamate (Fig. 6C), when compared with BLCO control animals. The activity of AchE [$F(4, 10)=15.80$, $p=0.0007$, $R2=0.8876$] in the brain was significantly increased when compared to the control mice. However, NAC and/or ZnSO4 significantly suppressed the AchE activity when compared with BLCO as shown in figure 7

3.6 Relationship between behavioral models and brain neurotransmitter

As presented in table 1, the result showed that there was a significantly strong positive relationship between serotonin level and time spent in the open arm of EPM and time spent in light chamber of LDB. In addition, AchE, NE and dopamine levels were negatively related with the time spent in the open arm of EPM and time spent in light chamber of LDB although not significant.

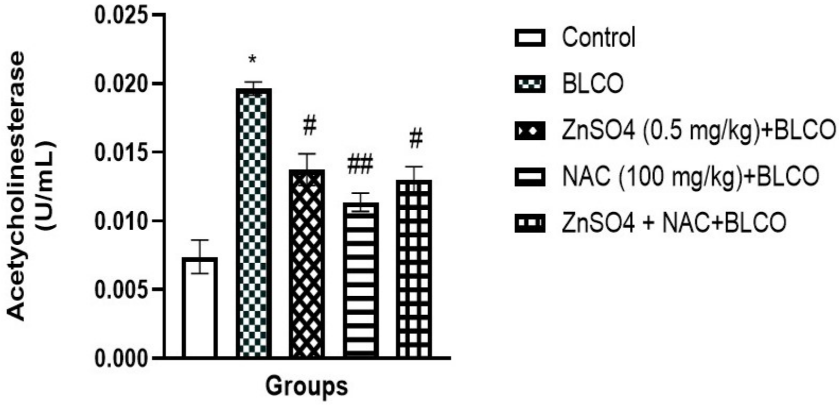

Figure 7: ZnSO4 or NAC co-administration reduces acetyl-cholinesterase activity in the brain of BCLO treated mice. *Data expressed in mean±SEM, n=5mice/group. *p<0.05 vs. control, #p<0.05, ##p<0.01 vs. BLCO (one-way ANOVA, Tukey's multiple comparison).*

Table 1. Relationship between behavioral models and brain neurotransmitter level in mice pretreated with ZnSO4 or NAC co-administration fed BLCO.

BLCO	line crossing (r,p)	Time in open arm (r, P)	Time in close arm (r,p)	Time in light zone (r, P)	Time in dark zone (r, P)
AchE	(-0.66, 0.54)	(-0.80, 0.40)	(-0.009, 0.99)	(-0.80, 0.40)	0.8073, 0.40
5-HT	(0.95, 0.18)	(0.99, 0.04)	(-0.51, 0.65)	(0.99, 0.04)	(-0.99, 0.04)
Norepinephrine	(-0.63, 0.56)	(-0.78, 0.42)	(-0.04,0.97)	(-0.78, 0.42)	(0.78, 0.42)
Dopamin	(-0.84, 0.35)	(-0.94, 0.21)	(0.27, 0.81)	(-0.94, 0.21)	(0.94, 0.21)
Glutamate	(0.82, 0.38)	(0.92, 0.24)	(-0.23, 0.84)	(0.92, 0.24)	(-0.92, 0.24)

Data was expressed in Mean ±SEM, n=5mice/group. Pearson linear regression r; regression coefficient, P; p-value significant at 0.05.

4. Discussion

Neurological disorders such mental retardation, behavioral impairments, nerve damage as well as neurodegeneration have been connected to the interaction between genetic and epigenetic variables [29, 30]. Intriguingly, our findings show that BLCO exposure caused behavioral impairments, decreased locomotor activity, and disruptions in serotonergic, glutaminergic, and dopaminergic activities. This work examined the protective effects of co-administering NAC and ZnSO4. NAC and ZnSO4 co-administration dramatically reduced the neurobehavioral alterations brought on by BLCO via altering the neurotransmitters, which enhanced behavioral and locomotor activity. In the current investigation, we used a variety of neurobehavioral models, including OFT, EPM, LDB, and FST, to evaluate the animals' behavioral function. The results of the neurobehavioral tests conducted in this study suggested that BLCO generates neurobehavioral deficits since they revealed a decrease in swimming time, an increase in immobility time, and a decrease in locomotor activity in the animals exposed to it. Additionally, across all the treatment groups that received BLCO, we noticed a substantial decrease in locomotor activity, and increase in decrease in time spent in the light chamber and immobility time. This study's findings suggest that BLCO may have produced a variety of behavioral effects on mice, including decreased motor activity and an increase in anxious or sad behavior. These behavioral effects were counteracted by pre-treatment with NAC or ZnSO4, however. Similar to earlier research, benzo-a-pyrene has been shown to impair spatial learning, memory and other behavioral problems, including gait abnormalities, loss of coordination, neuromuscular weakness, and reduced responses to sensory stimuli [31, 32]. Prior studies have shown a significant decrease in the spontaneous alteration and number of arm entries following the Y maze test across all dose groups that received BLCO; this reduction is, however, attributed to an increase in reactive oxygen species (ROS) in the brain tissue that causes neuronal derangement [30].

According to a growing body of evidence, biogenic amines including 5-HT, NE, and DA act as neurotransmitters in the brain that mediate a number of neuronal pathways and are discovered to be affected by neurotoxins. In addition to significant tissue destruction, BLCO may be linked to a variety of local biochemical alterations, including dopamine, serotonin and dysfunctional glutamate neurotransmission. According to the results of the current investigation, BLCO lowers levels of NE, 5-HT and DA in the brain. These findings are consistent with previous studies of Yan and Rein [33], Sanacora et al., [34] and Olowoparija et al., [10] who reported that a reduction in 5-HT level maybe linked with a decrease in its synaptosomal uptake, the inhibition of tryptophan hydroxylase activity as well as the decrease of tryptophan level in the neurotoxic model via deactivation of the serotonergic system [35]. Meanwhile, the enhanced monoamine oxidase activity and the decreased reuptake may be responsible for the decreased DA level in BLCO-fed mice [33].

The ability of NAC to scavenge free radicals in the brain may be responsible for the reversal impact of biogenic amine on pre-treatment with NAC and ZnSO4 [36]. This would control the synthesis, storage and metabolism of the monoamines. NE is a neuromodulator that also functions as an antidepressant. Dopamine-hydroxylase activity, the rate-limiting enzyme in NE production, may have decreased as a result of the fall in NE seen in this study [37]. The alterations in monoamine levels (NE, DA, and 5-HT) in the mice brain were dramatically reduced by pre-treating BLCO-exposed mice with either ZnSO4 or NAC. More specifically, the moderating effects of ZnSO4 and NAC on monoamines may be caused by their ability to prevent neuronal degeneration by reducing ROS-driven reactions, which are mediated by microglial activation and the production of inflammatory and oxygen intermediates that go along with it. Chopra, et al. [18] revealed that the injection of ZnSO4 and NAC has improved the biochemical abnormalities in the cerebral cortex in a rat seizure model, which is consistent with the findings of the current investigation. Similar to this, NMDA receptors are found in almost all glutamatergic synapses and are essential for the development of the brain, the regulation of emotion, and synaptic plasticity [32]. However, as discovered frequent among neurologic illnesses such depression, autism, schizophrenia, and Alzheimer's disease, dysregulation of glutamatergic synaptic transmission may be closely connected to social deficit, affective disturbance, cognitive deficit, and memory loss [33]. In accordance with the findings of the present study, mice treated with BLCO showed an increase in glutamate levels, a key excitatory neurotransmitter in the central nervous system. This finding suggests that glutamatergic neuronal imbalance may lead to excitotoxicity-mediated neuronal cell death, which is linked to a number of CNS diseases, including ischemia and neurodegenerative disease [38]. According to earlier research, excessive extracellular glutamate levels, particularly those caused by asynchronistic glutamate levels, can cause cellular damage when glutamatergic excitatory neurotransmission is sufficiently abnormal [39]. This may also be the result of over expressed excitatory amino acid transporter (EAAC1), a glutamate transporter [40].

This study implies that the behavioral loss brought on by BLCO may be related to glutamatergic transmission. A growing body of research has shown that glutamate plays a crucial part in the control of anxiety. Furthermore, research has indicated that the pathophysiology of anxiety and depression as connected to toxin-induced neurotoxicity is likely to be governed by an imbalance in 5-HT, NE, and/or DA neurotransmission [41]. Moreover, in line with the previous study of Goncalves et al., [42] our findings showed that ZnSO4 and NAC causes a significant increase in the levels of monoamine neurotransmitters (5-HT, NE, Dopamin levels) when compared with BLCO group the possible mechanism maybe via the inhibition of calcium-ATPase and phosphodiesterase, as well as the blocking of Ca2+/calmodulin binding, which play an important role in the release of the neurotransmitters. This study showed that mice treated with ZnSO4 and/or NAC had a number of biochemical alterations in their brains, including elevated levels of DA, NE, and 5-HT and decreased glutamate and acetylcholinesterase activity. When the rats exposed to the BLCO treatment groups received therapy with ZnSO4 or NAC, the altered biochemical parameters and behavioral despair were noted concurrently on the OFT, EPM, LDB, and FST. One of the highly active enzymes in the brain, acetylcholinesterase (AchE), is widely expressed in the brain and serves a number of purposes. By quickly hydrolyzing the neurotransmitter acetylcholine (Ach), it is an enzyme that stops cholinergic transmission. In our study, mice exposed to BLCO exhibited an increase in AchE levels; this may be related to an increase in ROS production as it has been found to enhance peroxidation of plasma membrane, which affects the integrity and functionality of the cholinergic system. However, when NAC and/or ZnSO4 were administered concurrently, this effect was reversed. It is possible that neurological and behavioral dysfunctions are influenced by an increase in AchE activity because it decreases Ach levels and consequently lack of cholinergic neurotransmission. According to Gonçalves et al. [42], the enzyme AchE can be used as a measure of cholinergic functions and changes in the activity of the enzyme may be a sign of changes in the availability of Ach at the receptor level. The pre-treatment of kindled rats with ZnSO4 or NAC, on the other hand, prevented the BLCO-induced increase in AchE activity. Additionally, ZnSO4 and NA's neuroprotective properties may be attributed to their antioxidant and free radical scavenging properties [43]. This study suggests that ZnSO4 and/or NAC pretreatment recovered neurochemical abnormalities and BLCO-induced decreased 5-HT tissue levels. Behavioral deficits brought on by BLCO were further correlated with decreases in dopamine (DA), norepinephrine (NE), and serotonin (5-HT), as well as increases in glutamate and AchE levels. Additionally, we found a significantly strong positive relationship between serotonin level and time spent in the open arm of EPM and time spent in light chamber of LDB. However, the lack of antioxidant assay in this study stands as a limitation to further elucidate the mechanism of neuroprotection. Meanwhile, neurotransmitter profile and robust neurobehavioral test in this study has demonstrated altered neurochemical signaling and behavioral deficits associated with BLCO exposure can be improved by ZnSO4 and/or NAC.

5. Conclusion

Finally, administration of ZnSO4 and/or NAC after BLCO exposure significantly increased the time spent in open-arm on the EPM test, increased the time spent in the light chamber on the LDB test, and decreased the immobility time on the FST, thereby reducing the behavioral deficit caused by BLCO. Therefore, injection of ZnSO4 and/or NAC was linked to anxiolytic-like effects in the EPM, LDB test, FST, and OFT, perhaps through altering the serotonergic/glutamatergic signaling system. These results suggested that the neurochemical reactions and behavioral deficits associated with BLCO exposure can be improved by ZnSO4 and/or NAC. In order to stop the neurobehavioral changes brought on by BLCO exposure, an alternative treatment may therefore be ZnSO4 and/or NAC.

Declarations

Consent for publication: All authors approved the publication of this manuscript

Data availability statement: All data associated with this study are included in this manuscript

Competing Interests: The authors declare that there have no conflicts of interest.

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Authors' Contributions: AON conceptualized the experiments, JNA, JC, OOI, AO, PTA and EJA managed the animal experiment, JNA,BO, DOO, EIE, OOE, AON and JC managed the laboratory assays, JNA and JC wrote the first draft of the manuscript. All authors read and approved the final draft and submission of the manuscript.

Ethical consideration: The study was approved by ethical committee of Faculty of Basic Medical Science, Delta State University, Abraka. The animal handling was according to the principles of National Guideline for Laboratory Animal Care (NIH Publication No. 85-23).

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