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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, tes-

ticular 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 (ZnSO4) both have demonstrated health benefits. There is, however, a dearth of information in the literature describing the therapeutic effectiveness of co-administering NAC and ZnSO4 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 (ZnSO4).

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.

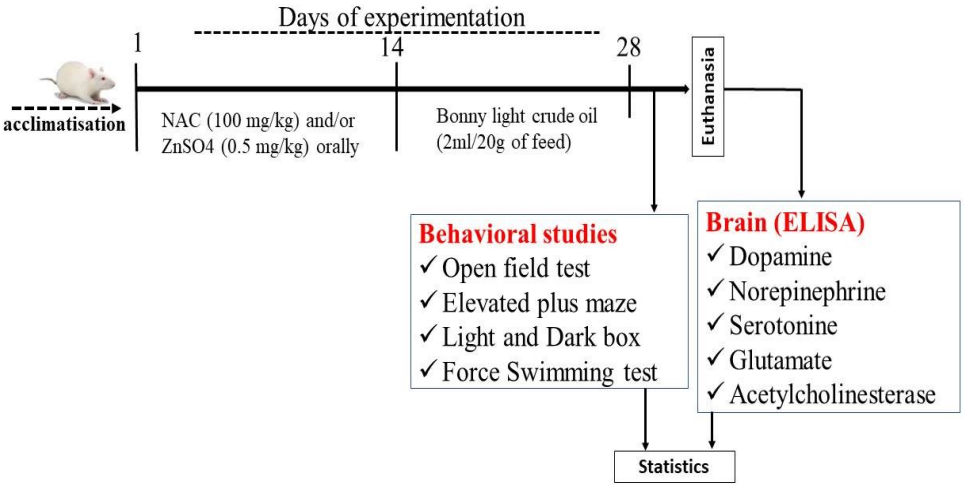

Figure 1: Study design

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.

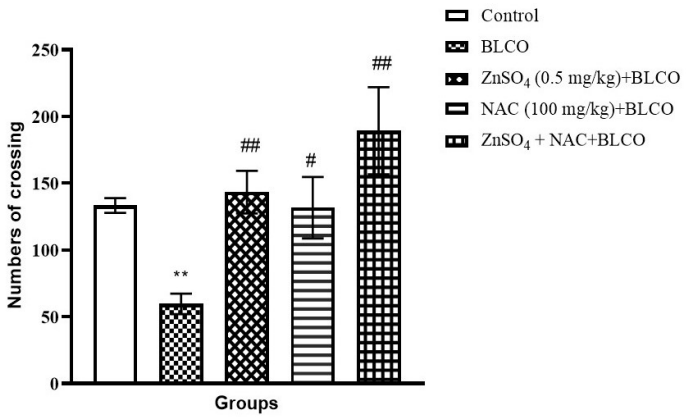

Figure 2: *Effects of ZnSO4 or NAC co-administration in BCLO-enhanced locomotive decline on open field test (OFT). Locomotive activities of rats were assessed by estimating the number of line crossing. Data expressed in mean±SEM, n=5. **p<0.01 vs control, #p<0.05, ##p<0.01 vs. BLCO (one-way ANOVA, Tukey's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

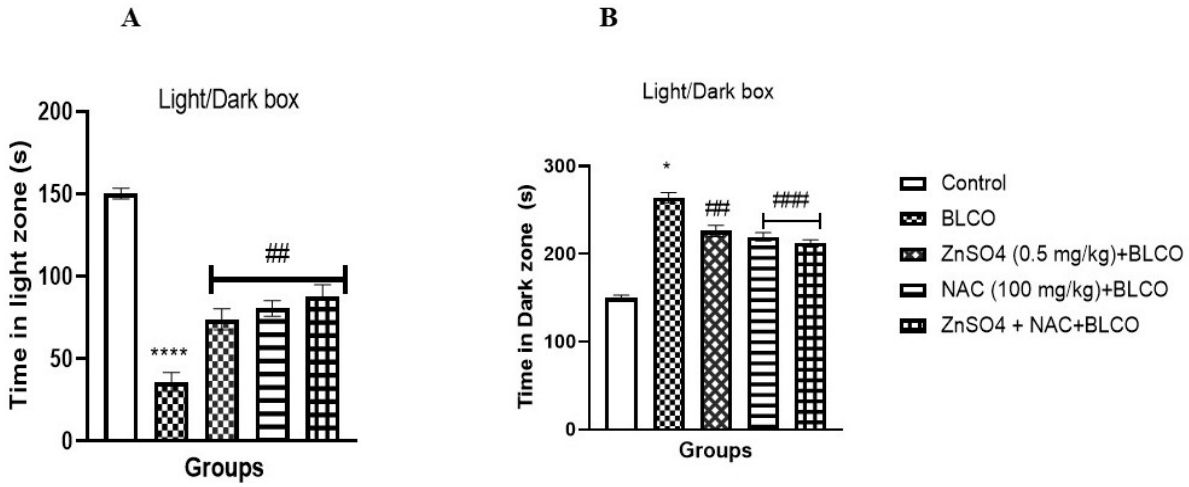

Figure 3: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behavior of rats were assessed by estimating the time spent in the light zone of the light and dark box test (LDB). Data expressed in mean±SEM, n=3rats/group. ****p<0.0001 vs control, ##p<0.01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

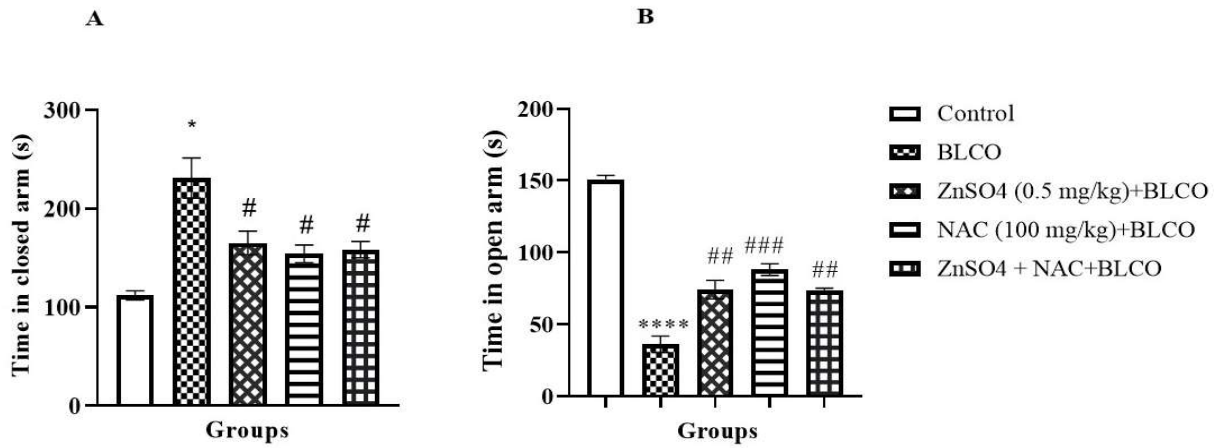

Figure 4: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behavior of rats were assessed by estimating the time spent in the open arm of elevated plus maze (EPM). Data expressed in mean±SEM, n=3.*p<0.05, ****p<0.0001 vs control,#p<0.05, ##p<0.01,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

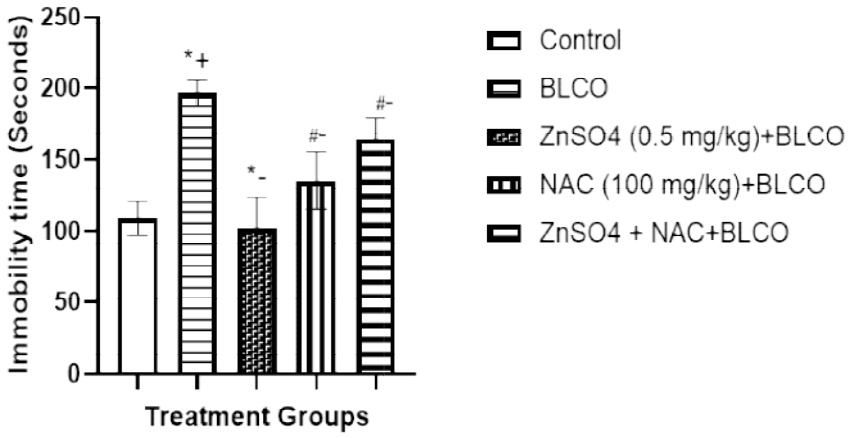

Figure 5: Effects of ZnSO4 or NAC co-administration in BCLO-enhanced depressive like behavior. *Depressive-like behaviors of rats were assessed by estimating the immobility time in forced swimming test (FST). Data expressed in mean±SEM, n=5. *p<0.05 vs. control, #p<0.05 vs. BLCO only group (one-way ANOVA,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

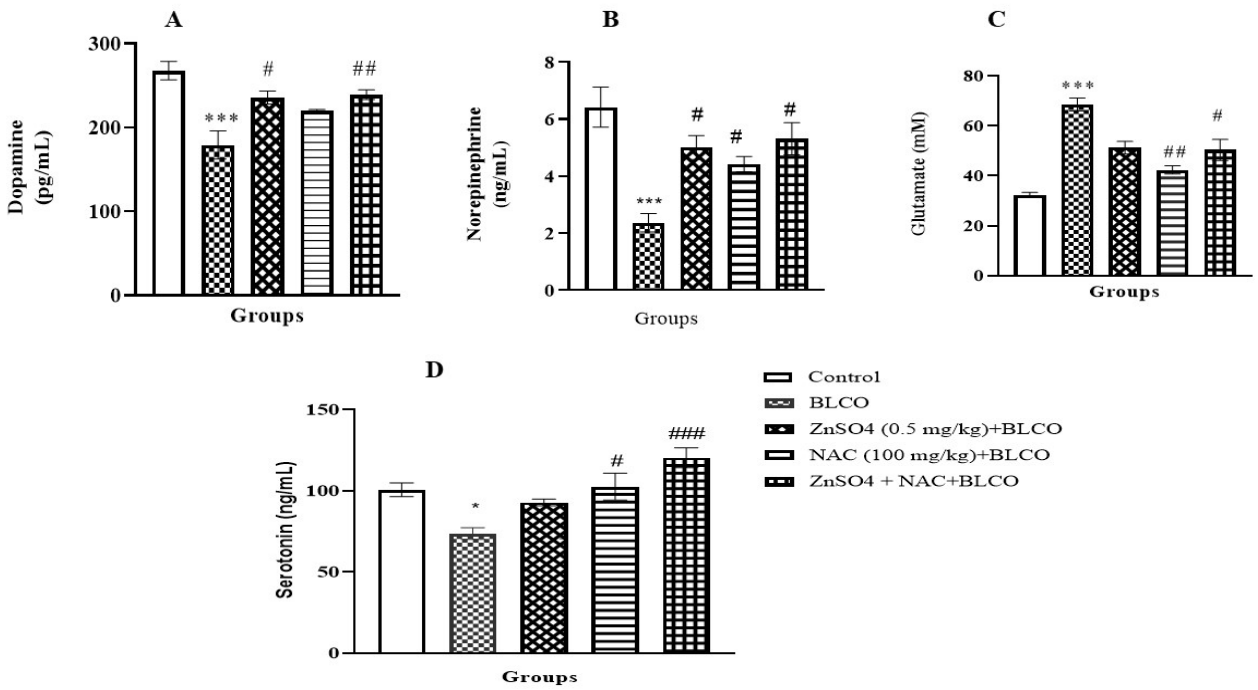

Figure 6: ZnSO4 or NAC co-administration modulates neurotransmitters in the brain of BLCO treated mice*(A) Dopamine (B) Norepinephrine (C) Glutamate (D) Serotonin. Data expressed in mean±SEM, n=5mice/group. ***p<0.001 vs. control, #p<0.05 vs. BLCO only group (one-way ANOVA, Tukey's multiple comparison).*

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.

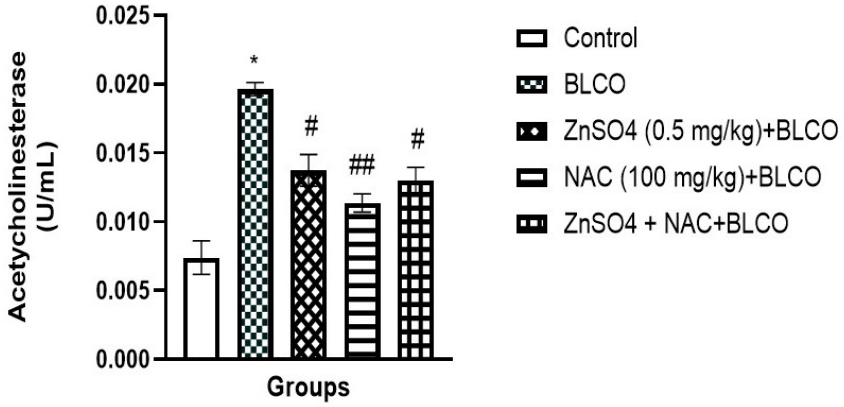

Figure 7: ZnSO4 or NAC co-administration reduces acetyl-cholinesterase activity in the brain of BCLO treated mice. *Data expressed in mean±SEM, n=5mice/group. *p<0.05 vs. control, #p<0.05, ##p<0.01 vs. BLCO (one-way ANOVA, Tukey's multiple comparison).*

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 re-

versed. 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
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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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Original Article

Haematoprotective effects of *Khaya senegalensis* stem bark extract in a Dextran Sulfate Sodium Induced Rat Model of Inflammatory Bowel Disease

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Abstract

Background: Inflammatory Bowel Disease (IBD) is a long-lasting disease of the gastrointestinal tract that continues to increase in prevalence around the world. Conventional treatments such as sulfasalazine are problematic in regions where resources are scarce, such as Nigeria, where they experience haematological toxicity, which includes anaemia. In this study, the haematoprotective effect of a traditional medicine, Khaya senegalensis, was investigated to mitigate blood-related pathological changes in a DSS-induced rat model of IBD.

Materials and Methods: Rats were induced with an IBD model with the use of Dextran Sulfate Sodium (DSS). The rats were further distributed into 6 groups: a normal control, an untreated DSS group, 2 groups treated with *Khaya senegalensis* (100 mg/kg and 200 mg/kg), a sulfasalazine group (500 mg/kg), and a combination group (250 mg/kg sulfasalazine + 100 mg/kg *Khaya senegalensis*). Hydro-ethanol extract was orally given, and haematological parameters were then determined.

Results: The experiment established that the 200 mg/kg treatment with *Khaya senegalensis* lowered the white blood cell count significantly as compared to the untreated DSS group ($p<0.05$). The doses of 200 mg/kg produced substantial adverse alterations in counts of granulocytes, respectively, with mid-sized cells responding to doses in a dose-dependent manner. Parameters of platelets, such as the platelet count, platelet crit (PCT), and platelet large cell ratio (P-LCR), also increased significantly in all the treatment groups ($p<0.001$ to $p<0.01$). There were no substantial changes in the level of red blood cells and other parameters.

Conclusion: These results suggest that the hydro-ethanol extract of *Khaya senegalensis* is haematoprotective in the DSS-induced rat model of colitis. The favourable effect of the extract on the main haematological indicators poses the hypothesis about its potential as a natural adjuvant to the treatment of Inflammatory Bowel Disease.

Keywords: *Khaya senegalensis*, DSS-induced colitis, Haematological parameters, Inflammation, Inflammatory bowel disease

1. Introduction

Crohn's disease and ulcerative colitis are included in Inflammatory Bowel Disease (IBD), a chronic inflammatory gastrointestinal ailment that is becoming a major worldwide health concern [1,2]. According to the Global Burden of Disease Study, there are an estimated 4.9 million cases globally; the frequency is lowest in underdeveloped nations and varies greatly throughout industrialized regions, including North America (721 instances per 100,000 in the US), [3–6]. In Nigeria, IBD has been considered rare, with only a few isolated case reports [7,8], but this likely reflects underdiagnosis due to limited colonoscopy access and low disease awareness [9,10]. With increasing recognition of IBD in sub-Saharan Africa, there is an urgent need for better epidemiological data and accessible diagnostic approaches [7,10].

IBD commonly presents with abdominal pain, diarrhoea, weight loss, and fatigue, and is frequently complicated by anaemia, which affects 20–30% of patients globally and up to 74% in some cohorts [11–16]. The anaemiain inflammatory bowel disease is multifactorial, arising from chronic intestinal blood loss, micronutrient malabsorption, and inflammation-driven iron sequestration [17–19], contributing significantly to impaired quality of life [18]. Although they are effective, the cost, availability, and side effects of current treatments, such as aminosalicylates, corticosteroids, immunomodulators, and biologics, limit their use [19]. For instance, sulfasalazine reduces inflammation by preventing the synthesis of proinflammatory cytokines and prostaglandins [20, 21], but can induce haematological toxicities such as haemolyticanaemia and neutropenia [20–23]. These risks are particularly concerning in IBD patients who are already predisposed to anaemia, and access to advanced therapies such as biologics remains restricted in resource-limited regions due to prohibitive costs. All of these limitations highlight the necessity of accessible, affordable, and safe substitutes, especially in areas with limited resources [7, 9].

Table 1: *Anaemia and IBD prevalence*

Region	IBD Prevalence (per 100,000)	Anaemia Prevalence in IBD (%)
Global	~64 (2019)	20–30
United States	721	16–74 (mean 17%)
Nigeria	Rare (under diagnosed)	Limited data

The medicinal plant *Khaya senegalensis*, often known as African mahogany, is used extensively in sub-Saharan Africa. Its bioactive limonoids are responsible for its anti-inflammatory, antioxidant, and antibacterial qualities [24, 25]. Traditionally employed across West and Central Africa for gastrointestinal and inflammatory conditions [26–28], the plant's availability and cultural acceptability make it a promising therapeutic candidate. This study aims to assess the impact of Khaya senegalensis hydro-ethanol stem bark extract on haematological parameters in a Wistar rat model of colitis induced by dextran sulfate sodium (DSS). By investigating its potential to improve haematological outcomes, this research seeks to provide evidence for *Khaya senegalensis* as a natural, accessible therapeutic option for IBD, particularly in regions with limited healthcare resources.

Table 2: *Anaemia Mechanisms in IBD*

Mechanism	Description
Iron Deficiency	Chronic blood loss from mucosal ulcerations leads to reduced iron stores.
Chronic Disease Anaemia	Hepcidin levels rise with inflammation, which hinders iron sequestration and absorption.
Nutrient Malabsorption	Reduced folate and vitamin B12 absorption as a result of intestinal inflammation.

2. Method

2.1 Plant Materials

The Iyamho settlement in Uzairue, located in the Etsako West Local Government Area of Edo State (7.1356° N, 6.3078° E), is where the stem bark of *Khaya senegalensis* was obtained. Prof. Akinnibosun Henry Adewale, a botanist from the University of Benin's Department of Botany and Biotechnology, Faculty of Life Sciences, Benin City, Edo State, identified the plant. After preparation, a voucher specimen (UBH-K478) was placed in the department's herbarium.

2.1.1 Preparation and Extraction of the Plant

After giving the plant materials a good rinsing, they were allowed to air dry at room temperature (25–28 °C) until their weight remained consistent. The plant material that resulted from pulverizing the dried samples with a mortar and pestle was then kept in an airtight container until it was needed for extraction. A solvent mixture consisting of 70% ethanol and 30% distilled water was used to extract 1365 grams of the plant material that had been transferred into a container. To improve extraction, a 1:10 w/v solution of the plant material was made and left to macerate for 72 hours while being intermittently stirred manually. Following the maceration period, the mixture was filtered to get rid of coarse particles using a sterile, clean muslin cloth. Then, Whatman No. 1 filter paper was used to get a clear filtrate. The filtrate was concentrated to dryness using a water bath set at a regulated temperature of 40°C to prevent thermal degradation of bioactive chemicals. The dried extract was weighed, and the percentage yield was calculated as follows:

Percentage Yield = (Weight of Dried Extract / initial weight of the Plant Powder) ×100

= (136g/1365g) ×100

The percentage yield was found to be 9.96%.

2.2 Ethical Approval

Ethical approval was obtained from the Research and Ethics Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka, with reference number RBC/FBMC/DELSU/25/650.

2.3 Experimental Animals

For this study, thirty (30) male Wistar rats were used. The animals, which weighed between 150 and 200 grams, were divided into six (6) groups, each consisting of five (5) animals (group 1 being the normal control, and groups 2 through 6 being the colitis-induced groups). They were then allowed to acclimatize for two (2) weeks.

2.4 Induction of Inflammatory Bowel Disease (colitis)

According to Kim *et al*., the Wistar rats were given unlimited access to water and fasted for 12 hours before the induction of inflammatory bowel disease (colitis) [29]. Experimental colitis was induced in rats (groups 2-6) by substituting their drinking water with a filter-purified 5% (w/v) DSS solution in graduated drinking tubes, to which the animals had unrestricted access for 7 days. As a standard control group, however, group one animals received distilled water devoid of DSS for seven days. Rats were weighed daily, and the daily volume of DSS intake was documented. To determine the estimated volume of DSS consumed per cage during induction, each tube was topped after the DSS solution levels were recorded.

2.5 Assessment of Disease Activity Index (DAI)

The clinical progression of the illness was evaluated using a disease activity index (DAI) score. The DAI was the aggregate score of weight reduction relative to initial weight, stool consistency, and rectal bleeding. The following was the definition of scores:

i. Stool consistency: 0 (normal), 2 (loose stool), and 4 (diarrhoea);
ii. Weight loss: 0 (no loss), 1 (1-5%), 2 (5-10%), 3 (10-20%), and 4 (>20%); and
iii. Haemorrhage via rectum: 0 indicates no blood, 1 indicates haemoccult positive, 2 indicates haemoccult positive with visible pellet bleeding, and 4 indicates severe haemorrhage and blood surrounding the anus.

For seven (7) days, DAI was scored every day while the DSS was being administered, and the animals were grouped as follows:

Group (1): Normal control group (distilled water)

Group (2): DSS-induced colitis rats (untreated)

Groups (3): DSS-induced colitis-treated rats received 100 mg/kg *K. senegalensis* extract once a day, orally, for seven (7) days

Groups (4): DSS-induced colitis-treated rats received 200 mg/kg *K. senegalensis* extract once a day, orally, for seven (7) days

Group (5): DSS-induced colitis-treated rats received 500 mg/kg sulfasalazine once a day, orally, as a positive control group for seven (7) days

Group (6): DSS-induced colitis-treated rats received 250mg/kg sulfasalazine once a day and 100mg/kg *K. senegalensis* extract once a day, orally for seven (7) days.

Doses were selected according to Lee *et al*. [30].The combination group received sulfasalazine at 250 mg/kg combined with *Khaya senegalensis* at 100 mg/kg. The sulfasalazine dose in the combination group was established at half the monotherapy dose to evaluate whether the potential of the extract from the plant could permit dose reduction of sulfasalazine while maintaining efficacy and potentially reducing haematological toxicity.

2.6 Sample Collection

A 5ml syringe (Monoject Pharmaceutical Ltd, Nigeria) was used to draw blood samples from the abdominal aorta into EDTA bottles (BD Vacutainer®, BD-Plymouth, Plymouth, U.K.), and the contents were carefully mixed by rolling the bottle gently for haematological tests [28]. An automated blood analyzer (Automated sysmex KX-21 haematology analyzer, Sysmex Corporation, Kobe, Japan) was used to analyze the following parameters: white blood cell (WBC), platelet count (PC), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), monocytes (MON), lymphocytes (LYM), and granulocytes (GRAN).

2.7 Statistical Analysis

The mean ± standard error of the mean (SEM) is how the data are presented. The outcomes were compared between groups using a one-way ANOVA, followed by Tukey's post hoc test. When $p < 0.05$, differences are deemed significant. To conduct the statistical analysis, GraphPad Prism (Version 10.0) was used.

3. Result

3.1. The impact of *Khaya senegalensis* stem bark hydro-ethanol extract on red blood cells (RBC) and white blood cells (WBC) in Wistar rats with DSS-induced inflammatory bowel disease.

The impact of *Khaya senegalensis*(KS) stem bark hydro-ethanol extract on white blood cells (WBC) in Wistar rats with DSS-induced inflammatory bowel disease is depicted in Figure 1. Comparing DSS to control, the white blood cell count was significantly higher ($p<0.0001$) while the red blood cell count was significantly lower ($p<0.001$) (Figures 1 and 2). The DSS + 100 mg/kg KS ($p<0.001$), DSS + 200 mg/kg KS ($p<0.001$), DSS + 500 mg/kg SULFA ($p<0.0001$), and DSS + 100 mg/kg KS + 250 mg/kg SULFA ($p<0.0001$) groups ad-

ministered for 7 days demonstrated a significant decrease in white blood cell count when compared to the DSS group, according to post hoc analysis using Tukey's post hoc test. Additionally, the DSS + 100 mg/kg KS ($p<0.001$), DSS + 200 mg/kg KS ($p<0.001$), DSS + 500 mg/kg SULFA ($p<0.01$), and DSS + 100 mg/kg KS + 250 mg/kg SULFA ($p<0.001$) administered for 7 days demonstrated a significant increase in Red Blood Cell (RBC) count in comparison to the DSS group, according to post hoc analysis using Tukey's post hoc test (Figure 2).

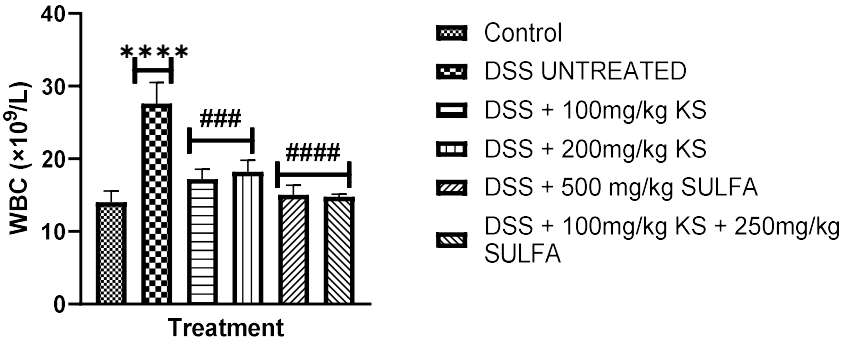

Figure 1: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on white blood cells (WBC) in Wistar rats with DSS-induced inflammatory bowel disease.*

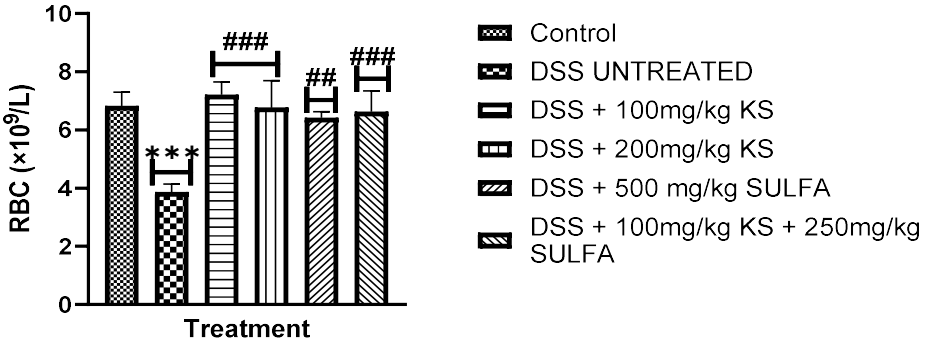

Figure 2: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on RBC in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.2. The Impact of *Khaya senegalensis* Stem Bark Hydro-Ethanol Extract on the Number of Neutrophils and Lymphocytes in Wistar Rats with DSS-Induced Inflammatory Bowel Disease

When compared to the control, Figure 3 showed that DSS significantly raised neutrophil counts ($p<0.01$). Additionally, compared to the DSS + 500 mg/kg SULFA group, the neutrophil counts of the DSS + 100 mg/kg KS and DSS + 200 mg/kg KS groups increased significantly ($p>0.05$). When compared to the DSS untreated group, therapy with 500 mg/kg Sulfasalazine was able to considerably ($p>0.01$) lower neutrophil numbers, as seen in Fig. 3. In contrast to the DSS + 500 mg/kg SULFA group, the DSS + 100 mg/kg KS + 250 mg/kg SULFA group significantly ($p<0.01$) raises neutrophil (%).

Comparing the DSS untreated groups to the control (group 1), Figure 4 showed a significant reduction ($p<0.0001$) in lymphocyte levels. When compared to the DSS untreated group, treatment with DSS + 100 mg/kg KS ($p<0.01$), DSS + 200 mg/kg KS ($p<0.001$), DSS + 500 mg/kg SULFA ($p<0.0001$), and DSS + 100 mg/kg KS + 250 mg/kg SULFA ($p<0.0001$) significantly boosts lymphocyte counts. Additionally, compared to the DSS + 500 mg/kg SULFA group, the DSS + 100 mg/kg KS group showed a significant reduction ($p>0.05$).

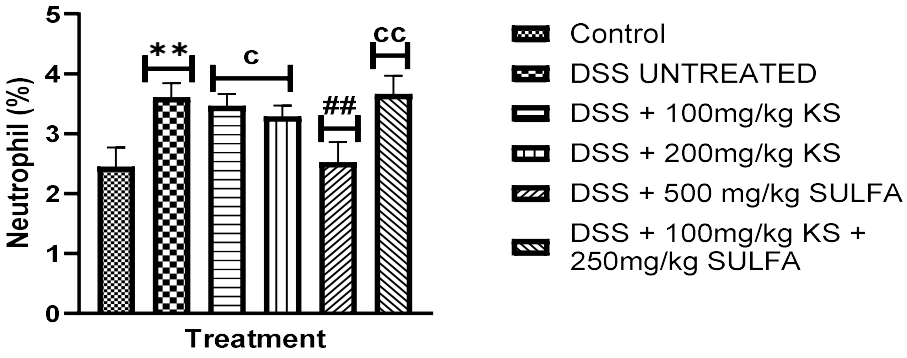

Figure 3: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on neutrophil counts in Wistar rats with DSS-induced inflammatory bowel disease*

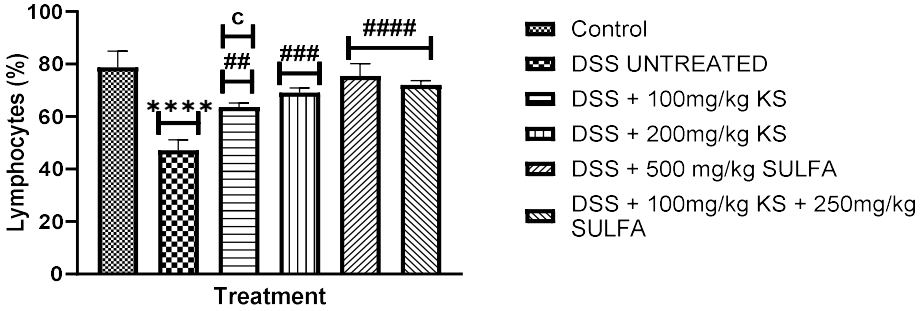

Figure 4: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on the number of lymphocytes in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.3. The Effect of Hydro-Ethanol Extract of *Khaya senegalensis* Stem Bark on Granulocyte Count in DSS-induced Inflammatory Bowel Disease in Wistar rats

Comparing DSS to the control, Figure 5 showed that granulocyte levels increased significantly ($p<0.001$). Additionally, when compared to the DSS group, treatment with DSS + 200 mg/kg KS ($p<0.05$) and DSS + 500 mg/kg SULFA ($p<0.001$) significantly decreased granulocyte levels. The DSS + 200 mg/kg KS granulocyte level was significantly lower ($p<0.01$) than that of 100 mg/kg KS. In contrast to the DSS + 500 mg/kg SULFA group, there was a significant increase in DSS + 100 mg/kg KS ($p<0.001$) as well as DSS + 100 mg/kg KS and 250 mg/kg.

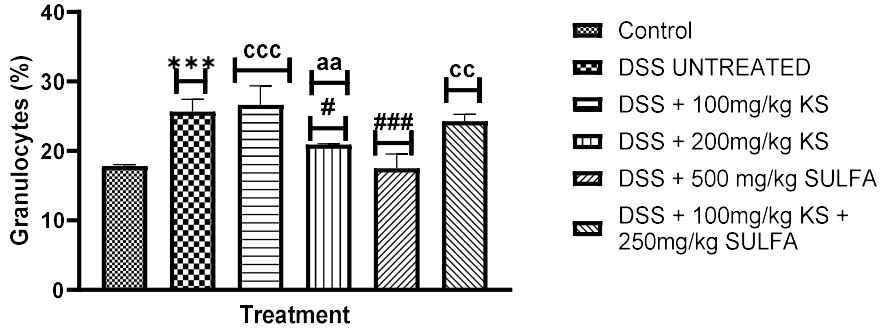

Figure 5: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on granulocyte count in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.4. The Impact of *Khaya senegalensis* Stem Bark Hydro-Ethanol Extract on the Number of Medium-Sized Cells (MID) in Wistar Rats with DSS-Induced Inflammatory Bowel Disease

Comparing DSS to the control, Figure 6 showed that the medium-sized cell count increased significantly ($p<0.01$). Additionally, compared to the DSS group, there was a significant reduction after treatment with DSS+200 mg/kg KS ($p<0.05$) and DSS+500 mg/kg SULFA ($p<0.001$). Comparing the DSS + 500 mg/kg SULFA group to the DSS + 100 mg/kg KS and 250 mg/kg groups, however, also revealed a significant rise ($p<0.001$). Additionally, compared to the DSS+500 mg/kg SULFA group, there was a significant increase ($p<0.05$) after treatment with DSS+100 mg/kg KS and DSS+100 mg/kg KS + 250 mg/kg SULFA.

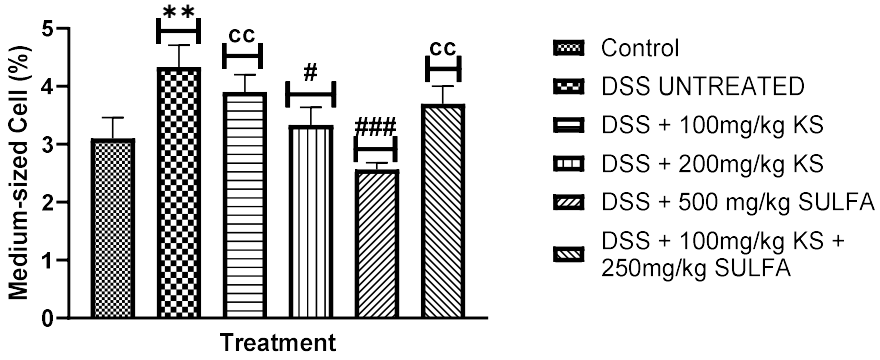

Figure 6: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on the medium-sized cell count in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * $p<0.05$, ** $p<0.01$, **** $p<0.001$, and **** $p<0.0001$ in comparison to the control group, # $p<0.05$, ## $p<0.01$, ### $p<0.001$, and #### $p<0.0001$ in comparison to the DSS Untreated group, a $p<0.05$, aa $p<0.01$, aaa $p<0.001$, and aaaa $p<0.0001$ in comparison to the DSS + 200 mg/kg KS group, b $p<0.05$, bb $p<0.01$, bbb $p<0.001$, bbb $p<0.001$, bbbb $p<0.001$, bbbb $p<0.0001$ in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c $p<0.05$, cc $p<0.01$, cc $p<0.001$, cccc $p<0.0001$ in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.5. The Impact of *Khaya senegalensis* Stem Bark Hydro-Ethanol Extract on Plateletcrit (PCT) Levels in Wistar Rats with DSS-Induced Inflammatory Bowel Disease

When compared to the control, Figure 7 showed that DSS significantly increased the number of mid-sized cells ($p<0.0001$). However, when comparing the treated groups to the DSS untreated group, there was no significant difference ($p>0.05$).

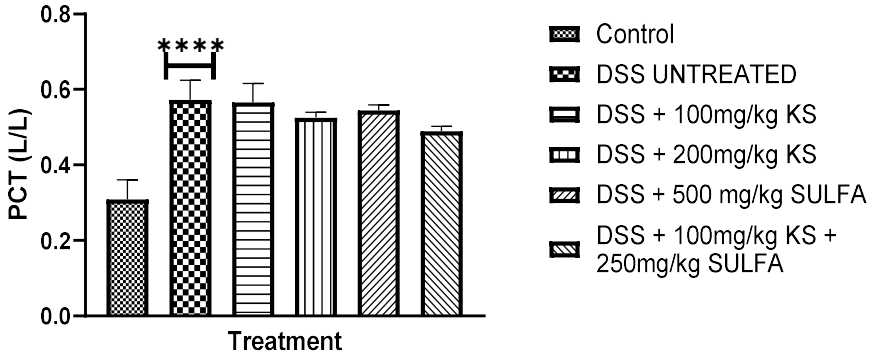

Figure 7: *The impact of a hydro-ethanol extract of the stem bark of Khaya senegalensis on the number of platelet-forming cells (PCT) in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.6. The Impact of *Khaya senegalensis* Stem Bark Hydro-Ethanol Extract on Platelet Count.

Figure 8 showed that, in comparison to the control, DSS considerably (p<0.001) raises platelet count. In comparison to the DSS group, the platelet count decreased significantly by DSS + 100 mg/kg KS (p<0.05) and DSS + 200 mg/kg KS (p<0.001). The platelet count was significantly lower in the DSS + 500 mg/kg SULFA and DSS + 100 mg/kg KS + 250 mg/kg SULFA groups (p<0.0001) than in the DSS group. Additionally, the DSS + 100 mg/kg KS and DSS + 200 mg/kg KS platelet counts were significantly elevated in comparison to the DSS + 500 mg/kg SULFA group, and the DSS + 100 mg/kg KS platelet count significantly increased in comparison to the DSS + 100 mg/kg KS + 250 mg/kg SULFA group.

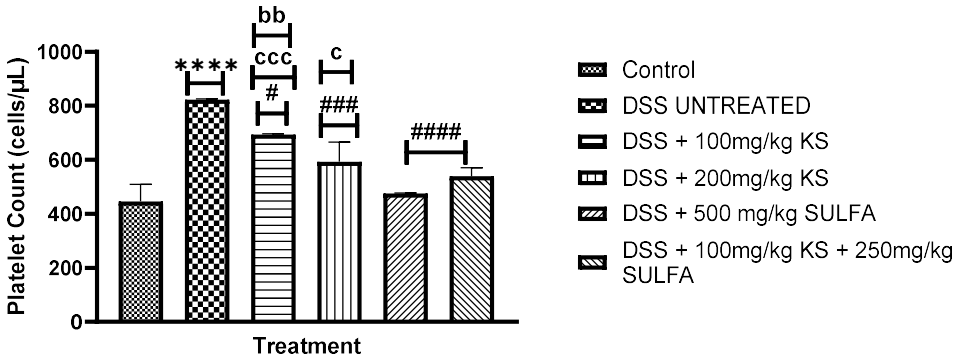

Figure 8: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on platelet counts in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

3.7. The Impact of *Khaya senegalensis* Stem Bark Hydro-Ethanol Extract on Platelet Large Cell Count (P-LCC) and Platelet Large Cell Ratio (P-LCR) in Wistar Rats with DSS-Induced Inflammatory Bowel Disease

The findings showed that, in comparison to control, DSS significantly reduced (p>0.01) platelet large cell count (P-LCC) but increased (p<0.05) platelet large cell ratio (P-LCR). DSS + 200 mg/kg KS and DSS + 500 mg/kg SULFA both markedly reduced the platelet large cell ratio (P-LCR) in comparison to the DSS group (p<0.001 and p<0.05, respectively). Additionally, compared to the DSS group, the platelet large cell ratio (P-LCC) was significantly higher (p<0.01) for DSS + 500 mg/kg SULFA and DSS + 100 mg/kg KS + 250

mg/kg SULFA (p<0.001). As illustrated in Figures 9 and 10, respectively, P-LCR levels demonstrated a significant decrease in DSS + 200 mg/kg KS, while P-LCC decreased in 100 and 200 mg/kg KS in comparison to DSS + 100 mg/kg KS + 250 mg/kg SULFA. However, 100 mg/kg KS + 250 mg/kg SULFA demonstrated a significant increase (p<0.01) in P-LCR when compared to 500 mg/kg SULFA. P-LCR was reduced by treatment with 200 mg/kg KS as opposed to 100 mg/kg KS.

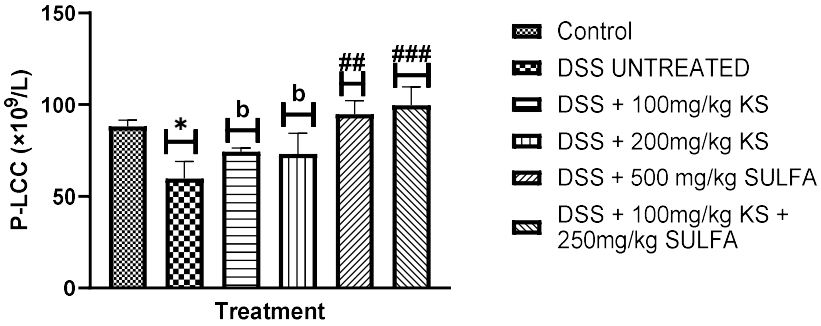

Figure 9: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on (a) platelet large cell count (P-LCC) in Wistar rats with DSS-induced inflammatory bowel disease.*

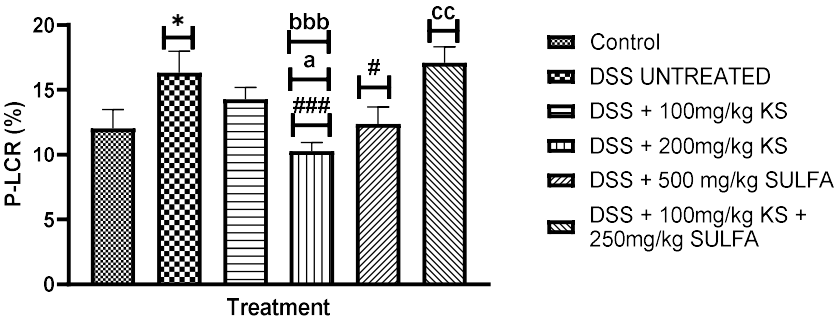

Figure 10: *The impact of Khaya senegalensis stem bark hydro-ethanol extract on the platelet large cell ratio (P-LCR) in Wistar rats with DSS-induced inflammatory bowel disease.*

*The mean ± S.E.M. for five animals per group is represented by the values. * p<0.05, ** p<0.01, **** p<0.001, and **** p<0.0001 in comparison to the control group, # p<0.05, ## p<0.01, ### p<0.001, and #### p<0.0001 in comparison to the DSS Untreated group, a p<0.05, aa p<0.01, aaa p<0.001, and aaaa p<0.0001 in comparison to the DSS + 200 mg/kg KS group, b p<0.05, bb p<0.01, bbb p<0.001, bbb p<0.001, bbbb p<0.001, bbbb p<0.0001 in comparison to the DSS +100 mg/kg KS + 250 mg/kg SULFA group, and c p<0.05, cc p<0.01, cc p<0.001, cccc p<0.0001 in comparison to the DSS + 500 mg/kg SULFA group (one-way ANOVA followed by Tukey's post hoc test).*

4. Discussion

In a Wistar rat model of inflammatory bowel disease (IBD) induced by dextran sulfate sodium (DSS), this study investigated the potential therapeutic benefits of hydro-ethanol Khaya senegalensis stem bark extract by assessing changes in white blood cell (WBC) and red blood cell (RBC) counts, differential leukocytes (neutrophils, lymphocytes, granulocytes, and mid-sized cells), and platelet indices (platelet count, plateletcrit [PCT], platelet large cell count [P-LCC], platelet large cell ratio [P-LCR]). It was shown that the extract significantly affects the factors causing haematological alterations, which may be mediated by a number of different methods.

The induction effect of DSS led to significant thrombocytosis with dysregulated indices (increased platelet count, PCT, and P-LCR; p<0.001–p<0.05 vs. control; decreased P-LCC; p>0.01 vs. control), anaemia (reduced RBC; p<0.001 vs. control), and leukocytosis (elevated WBC, neutrophils, granulocytes, and medium-sized cells; p<0.0001–p<0.01 vs. control). Treatments involving 100 or 200 mg/kg of Khaya senegalensis, 500 mg/kg of sulfasalazine, or 100 mg/kg of *Khaya senegalensis* with 250 mg/kg of sulfasalazine resulted in dose-dependent reversals. Notably, 200 mg/kg *Significantly, Khaya senegalensis reduced WBC* (p<0.001 vs. DSS), neutrophils (p>0.05 vs. sulfasalazine), granulocytes (p<0.05 vs. DSS), and medium-sized cells (p<0.05 vs. DSS); elevated RBC (p<0.001 vs. DSS) and lymphocytes (p<0.001 vs. DSS); and normalised platelets (reduced count and P-LCR,

p<0.001–p<0.05 vs. DSS; increased P-LCC, p<0.01 vs. DSS). The combination was effective as monotherapies in WBC suppression (p<0.0001 vs. DSS) and platelet normalisation, but proved inferior for granulocytes and mid-sized cells (p<0.001 increase vs. 500 mg/kg sulfasalazine).

These results clearly show the anti-inflammatory and haematoprotective properties of Khaya senegalensis on the DSS model, which replicates actual IBD by destabilising colonic epithelium and triggering an overreaction of the immune system [31, 32]. While increasing RBC promotes prevention of inflammation-driven erythropoiesis and blood loss, direct dose-dependent decreases in WBC and granulocytes show direct suppression of inflammatory infiltration of innate immunity.[33, 35]. Platelet index normalisation also counteracts thrombotic propensity, a DSS-revived sequela that resembles thrombocytosis of IBD [36–38].Among the potential causes are bioactive limonoids and flavonoids that decrease cytokines (IL-6, TNF-α), which are not measured here, by blocking pro-inflammatory pathways (e.g., NF-κB, p38 MAPK/Nrf2/HO-1). Modification of the gut flora is likely to increase these effects. DSS leads to dysbiosis, increasing permeability, microbial translocation, and inflammation mediated by ROS, which maintains leukocytosis and prevents iron/folate uptake (p<0.001 RBC decrement) [31, 41 -43]. *Khaya senegalensis* (9.96% yield) is rich in polyphenols and can break the eubiosis through short-chain fatty acid producers (e.g., Lactobacillus, Bifidobacterium), thus strengthening barrier integrity, IL-10 secretion, and downregulation of hepcidin to boost erythropoiesis [44-48]. This dose (200 mg/kg and above) microbiota-haematology axis ought to be taken into account in future models of 16S rRNA/metagenomic profiling.

Combination therapy's discordant efficacy equates WBC/platelet amelioration yet granulocyte/mid-sized cell persistence (p<0.001 vs. sulfasalazine monotherapy), which implies pharmacodynamic antagonism. Halved sulfasalazine dosing (250 mg/kg) may inadequately saturate NF-κB inhibition [20, 21], while 100 mg/kg *Khaya senegalensis*yields subthreshold polyphenols for microbiota reshaping or antioxidant synergy [47], perpetuating innate responses. Alternatively, *Khaya senegalensis* flavonoids could competitively attenuate sulfasalazine's metabolite-driven effects or quench its ROS intermediates, blunting additive suppression without compensatory immunomodulation [21, 50]. To dissect this, proposed experiments include: (i) isobolographic analysis of dose-response curves for granulocyte endpoints to quantify interaction indices; (ii) co-incubation assays in LPS-stimulated RAW 264.7 macrophages assessing NF-κB/p38 phosphorylation and cytokine profiles; (iii) pharmacokinetic profiling via LC-MS to evaluate bioavailability and CYP-mediated interactions; and (iv) microbiota transfer from combination- vs. monotherapy-treated rats into germ-free DSS recipients, tracking haematological readouts.

Relative to sulfasalazine, 200 mg/kg *Khaya senegalensis* excelled in RBC/lymphocyte restoration (p<0.001–p<0.0001 vs. DSS; superior magnitude for RBC, p<0.01 vs. sulfasalazine for lymphocytes), evincing enhanced antioxidative/immunomodulatory potency withouthaematotoxicity (e.g., haemolysis, agranulocytosis) [20, 23, 51]. *Khaya senegalensis* ethnopharmacological salience [24, 26–28] foreshadows its use as a feasible adjuvant in Nigeria in a resource-constrained environment characterised by underdiagnosis and non-accessibility of IBD therapies [7-10, 52].

5. Conclusion

Khaya senegalensis phytochemicals contain antioxidant and anti-inflammatory properties that, taken together, help reduce colitis pathophysiology and reverse haematological alterations. These findings support the *Khaya senegalensis'* potential as a natural, accessible adjunct therapy for IBD, especially in resource-limited settings, and warrant further

mechanistic and clinical investigation. Additional investigation is necessary to elucidate its mechanisms and validate its clinical potential.

Declarations

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

Data availability statement: On reasonable request, the corresponding author will make the datasets created and/or analyzed during the current study available.

Competing Interests: The authors affirm 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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Authors' Contributions: KMA and OMO authors conceptualized the work, IOG and EOD authors contributed to the literature review, while the IIO author contributed to the haematological assays.

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Review Article

Collaborative impact of diabetes education by International Diabetes Federation and national health systems on prevalence of diabetes amongst the indigenous people of Africa

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Abstract:

Background: Diabetes mellitus (DM) is becoming a serious health problem in Africa, affecting about 24 million adults, with more than half of them undiagnosed. Indigenous communities are affected the most because of poverty, poor healthcare, and inequality. This narrative review examines how diabetes education from the International Diabetes Federation (IDF) and national health systems is helping to combat this growing concern among indigenous Africans.

Methods: A total of 39 relevant articles were used for this review covering up to 2025, using search engines and databases including PubMed, Scopus, Web of Science, and Google Scholar.

Main content: The IDF has set up various education programs, like the IDF School of Diabetes and other initiatives, often working with host governments, while national health systems have used community-based approaches, such as training health workers, running radio campaigns, and school programs. These efforts have helped improve diabetes control with self-care. Although, problems like lack of funding, cultural differences, weak health monitoring, and poor infrastructure still limit the impact of these education programs.

Conclusion: This review highlights how collaborative diabetes education initiatives by the IDF and national health systems have improved diabetes awareness and control among indigenous Africans.

Keywords: Africa, Community health, Diabetes education, Diabetes mellitus, International Diabetes Federation (IDF)

Introduction

Diabetes mellitus is a metabolic disorder characterized by persistently high blood glucose and its prevalence globally is constantly rising [1]. This poses a significant global health burden [2]. In 2021, the International Diabetes Federation (IDF) reported that 537 million adult people have diabetes globally, which has been projected to rise to 643 million in 2030 and 783 by 2045 [3,4]. In Africa, diabetes mellitus is also a growing problem as about 19 million people are affected [5]. This number is predicted to increase by 143% by the year 2045 [6]. As at 2021, an estimate of 24 million adults were living with diabetes mellitus and about fifty-four percent of these people were undiagnosed [7]. Diabetes mellitus

is now regarded as a crisis in Africa especially due to the alarming cases. Factors contributing to the fast rising cases of diabetes mellitus in Africa include poverty, limited access to good healthcare and social inequality [8].

The impact of diabetes mellitus extends far beyond its prevalence as it can lead to several health complications such as kidney failure, stroke, lower limb amputation, blindness and heart attack [9]. Since there are a lot of undiagnosed cases of diabetes mellitus, these complications can be exacerbated as most people do not receive timely treatment. The economic and social burden of diabetes mellitus in Africa cannot be overlooked as the healthcare expenditure was about 10 billion USD in 2024 [10].

In regions with limited resources such as Africa, diabetes education plays an important role in the prevention and management of diabetes mellitus. It also helps to improve the quality of life of patients and prevent the development of associated complications [11]. According to the American Diabetes Association (ADA), patient education is regarded as a standard of care to improve knowledge and proficiency in diabetes self-care skills [12]. Diabetes self-management education and support (DSMES) interventions is cost-effective by reducing emergency department visits and hospital admissions and readmissions [13]. This review seeks to evaluate the impact of diabetes education organized by the IDF and national health systems in African countries with a focus on how these collaborative initiatives have impacted diabetes prevalence and control among the African indigenous populations.

Methodology

Major research databases and search engines were searched for this review article. These included PubMed, Scopus, Web of Science, and Google Scholar and ResearchGate, for a period up to 2025. Articles directly addressing diabetes education, and prevalence among indigenous people of Africa were selected and analyzed for this study. Only 39 articles were adopted for this review. Excluded articles included those outside the scope of this study, not in English, or whose full texts were unavailable and not retrievable. Keywords search included community health, diabetes education, diabetes mellitus, indigenous people of Africa, and International Diabetes Federation (IDF).

Overview of Diabetes among Indigenous African Populations

Indigenous people of Africa refer to group of people who are native to Africa. Indigenous people are practitioners of unique culture and they possess cultural, social and economic characteristics that are quite different from the dominant societies in which they stay [14]. In Africa, indigenous communities are faced with high rates of diseases and challenges such as poor healthcare infrastructure, systemic discrimination, forced displacement and limited access to care. According to the International Diabetes Federation (IDF), about 70% of all reviewed studies reported the prevalence of type 2 diabetes mellitus to be over 10% in indigenous adult population [15]. Africa is estimated to have about 15.9 million adults living with DM which is a regional prevalence of 3.1% [16]. Africa has the largest amount of people living with undiagnosed diabetes mellitus and more than half of the people living with diabetes mellitus in Africa are undiagnosed [7]. Some factors responsible for undiagnosed diabetes mellitus for several years include poor healthcare systems, slow onset of the disease presentation and lack of awareness among the community [17].

In Africa, the prevalence of undiagnosed diabetes mellitus is not consistent across different countries as a result of disparities in social, economic and genetic variations. In North Africa, the prevalence of undiagnosed diabetes mellitus ranged from 18% to about 75% of all cases of diabetes [18]. Additionally, the incidence of undiagnosed diabetes mellitus in several African regions was displayed as follows: 2.6% and 5.97% in North

Sudan, 7.2%, 11.5%, 5%, 2.3%, 3.8%, and 2.13% in Ethiopia, and 9% in Tanzania (East African studies); 3.19% in Guinea, 6.3% in Cameroon, 4.77% in Mauritius, 4.64% in Senegal, and 7% and 4.6% in Nigeria (West African studies); 18.1% in South Africa; and 4.2% in Egypt (North Africa) [18]. The pooled prevalence of undiagnosed diabetes mellitus among adults was 3.85%, according to a systematic analysis of African nations; this translates to 4.43% in Eastern Africa, 4.72% in Western Africa, 4.27% in Northern Africa, and 1.46% in Southern Africa, respectively [19].

Diabetes Education Initiatives by the International Diabetes Federation (IDF) in Africa

The International Diabetes Federation (IDF) is focused on diabetes mellitus education targeting healthcare professionals and people living with DM. They are aimed at improving diabetes care and management. They offer educational resources, advocacy programs and online courses. One of the key IDF program in Africa is the IDF School of Diabetes which was launched in 2016 [20]. Currently, the IDF School of Diabetes provides three short courses (Diabetic Retinopathy, Diabetes and Cardiovascular Disease, and Type 2 Diabetes Prevention) and three Certified Online Courses (for Diabetes Educators, Specialists, and Primary Care Physicians). The Collaboration Centre is an additional feature that allows users to ask questions, start discussions, or participate in topics that have been started by others [20].

The International Diabetes Federation (IDF), in partnership with the International Society for Paediatric and Adolescent Diabetes (ISPAD) and Sanofi, created the Kids and Diabetes in Schools (KiDS) project, an educational initiative that is another important IDF activity in Africa. Its primary objective is to prevent type 2 diabetes by promoting healthy lifestyles in schools and fighting the stigma associated with diabetes. The program's main goal is to teach parents, teachers, and students about diabetes management and good lifestyle choices. Other diabetes education initiatives organized in Africa by IDF include world diabetes day campaigns and training of healthcare workers in diabetes management.

IDF Collaboration with Host National Health Systems

In Africa, the International Diabetes Federation (IDF) collaborates with national diabetes associations, including health authorities in the host country to lead diabetes education programs. One of such can be seen in sub-Saharan Africa where the IDF Africa region collaborated with World Diabetes foundation to create a standard Diabetes Education Training Manual which was intended for patients and also for healthcare workers [21]. This curriculum was distributed among 11 African countries. It was translated into various languages such as French, Portuguese and Kiswahili so as to ensure a unified culturally sensitive teaching framework [21]. In addition, IDF and its various partners have produced type 2 diabetes guidelines which is much applicable in the African context and this has been disseminated remotely and also among indigenous communities in Africa.

Indigenous and rural communities access IDF's healthcare provider training programs through multiple channels. For example, clinicians in rural and indigenous areas can access free online courses organized by the IDF School of Diabetes [22]. In 2024, a training program was organized by the IDF–Sanofi Global Health Unit collaboration for primary healthcare workers across Africa. This program provided online diabetes education to healthcare providers. It also provided in-person courses across African countries like Malawi, Chad, Togo and Uganda [23]. This program was beneficial because it enabled local nurses and doctors in indigenous communities to learn practical skills which facilitate the early diagnosis and treatment of diabetes. Another notable case to consider is the Tanzanian Diabetes Association which is an IDF member in collaboration with the national ministry of health. This initiative helped in providing training and educational

support in 44 diabetes clinic which was established nationwide [23]. This type of host government-IDF partnership integrated diabetes education into the existing health system, extending reach into indigenous and rural communities.

In indigenous and rural communities, IDF networks organize awareness campaign program and also education at the grass-root level [23]. Currently, IDF Africa network is now made up of 32 member associations in 27 countries and each of them organizes local outreach in cities and remote areas. Just in 2022, IDF Africa member association organized screening campaigns and public awareness programs in several places such as in schools, markets, prisons and workplaces [22]. Educational materials which help in sensitizing the public on diabetes were distributed. Also, school based programs like the global Kids and Diabetes in Schools (KiDS) initiative which was launched in 2013 takes place in some African countries to enlighten school children and their teachers on diabetes and healthy lifestyles to prevent diabetes.

Effectiveness of IDF diabetes education initiatives

Studies show that well-organized education in African settings can improve diabetes outcomes, but effectiveness is lopsided and there are significant barriers. A meta-analysis of diabetes self-management education (DSME) trials in the WHO African Region showed that educational interventions resulted in significantly lower HbA1c as compared to normal care [24]. This implies that there were moderate improvements in the blood sugar control in patients receiving DSME. Also, a few randomized trials in South Africa have shown that group education sessions boost self-care and patient adherence [25]. For instance, people who went to a one-day education program or several group sessions took their medications more days a week and took better care of their feet [25]. The consumption of starchy foods by patients was considerably decreased by another intervention that combined weekly group classes with teaching about community gardening [25]. These findings suggest that African patients, including those from underprivileged communities, can benefit from culturally tailored education that promotes healthier habits and improved glycemic control.

Challenges of IDF diabetes education initiatives

One of the challenges encountered is poor infrastructure facilities and workforce. One scoping review in West Africa significantly noted that there are inadequate clinical spaces and limited avenue for diabetes education [26]. Many primary healthcare facilities do not have reserved space for diabetes education or adequate staffing to carry out the task. When this is absent, educational campaigns cannot penetrate deep into remote villages. Shortages of qualified healthcare professionals also impede program reach [26].

Another challenge faced is cultural and socioeconomic restrictions. Diabetes education system in indigenous communities must tally with local beliefs and culture. Most times, conventional western-style advice often clashes with traditional views [26]. Experts recommend redesigning diabetes education that is rooted in local sociocultural structures such as partnering with local healers or combining nutritional advice with traditional African food [26].

In practice, most of these indigenous patients first consult traditional healers, so purely biomedical education can be restricted. In addition, funding is another significant challenge. This is because the government prioritizes funding for infectious diseases such as HIV and malaria and neglects others like diabetes. Due to this reason, IDF-affiliated initiatives may therefore be project-based or have a limited capacity for expansion. The IDF–Sanofi training hubs, for instance, show promise, but their long-term viability depends on governments and donors continuing to fund.

Diabetes Education by National Health Systems

Health Policy Frameworks

The increasing outbreak of diabetes in sub-Saharan Africa has led to the need for comprehensive diabetes education policies. Countries like Nigeria, Ghana, South Africa, and Kenya have developed policies which aim to raise public awareness, strengthen primary health care, and provide diabetes education within the available frameworks. However, these policies are not homogenous in their implementation due to the constraints of limited resources and chronic inefficiencies in the health care systems. Where and how these policies are implemented varies widely [27].

In Nigeria, the Federal Ministry of Health has launched the National Strategic Plan of Action on Non-Communicable Diseases for 2019-2025 which lays out goals for the prevention, early diagnosis, and health promotion of diabetes. It places emphasis on media and community outreach campaigns, though has little to no details on funding pathways to achieve these goals [28]. In parallel, the Ghana Health Service reported that the Ministry of Health integrated diabetes in the Non-Communicable Diseases (NCDs) Strategy 2022-2026 and focuses on community health education, training, and improving the detection of diabetes. Despite facing the dual burden of communicable and non-communicable diseases, South Africa has one of the most advanced diabetes frameworks on the continent. South Africa's Integrated Chronic Disease Management (ICDM) model has both a facility-based and community-based components with established programs employing trained community health workers (CHWs) [29]. On the other hand, Kenya has prepared the Kenya National Diabetes Strategy 2010-2015 and the National Strategic Plan for the Prevention and Control of NCDs (2021-2025) which aim to utilize mobile health units, radio programs, and school-based programs for health promotion and education [30].

Community Level Interventions

The majority of national diabetes education initiatives focus on community-based tactics. Community health workers are trained to provide health messages, conduct basic screenings, and refer patients to health centers. They are frequently hired from within indigenous communities. According to [31], this strategy has been successful in raising awareness and promoting lifestyle modifications among rural populations. Radio campaigns and mobile clinics are also frequently used, especially in underserved and rural areas. Important information regarding symptoms, diet, and foot care is provided by government-sponsored radio programs in local languages in Ghana and Kenya. These campaigns are particularly crucial in low-literate communities.

In sub-Saharan Africa, school-based health education initiatives have emerged as essential instruments for early intervention. In South Africa, primary schools in the Western Cape's socioeconomically disadvantaged communities implemented the HealthKick program, a randomized school-based nutrition and physical activity intervention. Teachers worked with students and parents using an action-planning framework to increase diabetes awareness, physical activity participation, and food diversity. According to de Villiers et al. [32], the process evaluation revealed that more than 54% of the planned activities pertaining to diabetes and chronic disease awareness were carried out successfully, indicating their viability and acceptability in school environments with limited resources.

Evidence from Nigeria, as well as South Africa, highlight the difficulties and possibilities of diabetes education in schools. A cross-sectional study conducted in Delta State revealed that while around 88% of secondary school students were aware of diabetes, their understanding of risk factors, symptoms, and preventive measures was alarmingly deficient, with overall knowledge scores averaging merely 3.4% [33]. These results indicate

an immediate necessity for organized educational interventions in educational environments to enhance awareness. School-based programs in both countries work together to get important messages across about diabetes risk factors, healthy eating, and exercise. These interventions also have an indirect effect on parents' behavior by getting kids and teens involved, which increases the reach and effectiveness of health education [32, 33].

Challenges Faced by National Health Systems

In spite of these efforts, national healthcare systems continue to grapple with significant challenges in effectively implementing educational programs aimed at diabetes management. There is a lack of funding as most NCD strategies rely on donor funding and lack sustainable domestic investment. The WHO notes that in many African countries, less than 2% of the national health budgets allocated to NCDs, which includes diabetes [34].

There is a lack in workforce training. Most primary care clinicians receive little to no training in diabetes education. This is worsened by high turnover rates in staffing, particularly in rural regions. Additionally, inadequate surveillance systems restrict determining the outcomes of educational programs. Few countries have reliable, comprehensive data on the prevalence of diabetes and rates of patient adherence which hampers the evaluation of the impact of educational initiatives.

Impacts of Diabetes Education

The International Diabetes Federation (IDF) role has involved the development of solid frameworks for healthcare workers and the certification of educational programs that comply with standard guidelines. Programs certified by the IDF offer standardized educational materials and training methods which can be adjusted to local needs while keeping global quality standards. The accreditation process guarantees that educators have current evidence-based knowledge and teaching skills. Additionally, IDF supports the inclusion of multidisciplinary teams such as diabetologists, dietitians, physicians, nurses, pharmacist, diabetes educators, psychologists, and other relevant professionals to enhance patient engagement and tailor education delivery [35]. This educational structure helps provide consistent and scalable diabetes self-management education (DSME) programs around the world, hence, improving the overall effectiveness of diabetes care initiatives.

Despite certain challenges, some national health systems have made progress toward integrated care models that include clear pathways for diabetes education. Some systems are trying centralized education centers and community outreach to improve access and consistency. New strategies, like using opinion leaders and feedback for healthcare providers, show promise in overcoming resistance to change [36].

Research on IDF-certified education programs shows clear improvements in important health outcomes. Studies report lower HbA1c levels, meaning better blood sugar control, along with positive changes in body mass index (BMI) and blood pressure after participating in these structured programs [37]. Patients also become more active and stick to monitoring their blood sugar more closely. The lasting effects suggest that IDF programs successfully encourage behavior changes that lead to real health benefits. Results from national health systems (NHS) diabetes education programs revealed many national programs reported better blood sugar control, although differences still exist, often related to location, healthcare settings, or socioeconomic status [38].

IDF-endorsed integrated education and care models have shown promising cost- effectiveness by lowering consultation costs and delaying the onset of costly diabetic complications [38]. For example, studies comparing integrated outpatient clinics that deliver

IDF-aligned education with traditional care models reveal significant cost savings, especially for type 2 diabetes patients, through more efficient use of resources and lower complication rates [38]. These models use multidisciplinary teams and structured education to improve care quality while managing costs. The savings help ease financial pressures on health systems and also improve patient quality of life by preventing costly hospital stays and interventions in advanced stages of disease.

Gaps in Diabetes Education among Indigenous Africans

Across sub-Saharan Africa, diabetes education for indigenous communities faces many challenges. Cultural differences, low literacy levels, remote locations, and weak links with traditional health systems all make it harder to reach these populations. Often, educational materials are not adapted to fit local cultures, so many indigenous people do not engage with them because their health beliefs are quite different from Western medicine [34]. Another big obstacle is low literacy. Most indigenous people in rural areas have little formal schooling, making it hard for them to understand written materials. Health messages on posters or pamphlets do not work well when they are not shared through channels people actually use or in local languages [15].

Geography and poor infrastructure make things worse. Many indigenous groups live far from health centers, and outreach efforts like mobile clinics or radio programs are often inconsistent due to lack of funding. Also, community health workers often do not get enough training, which makes these programs less effective [29]. Another major issue is that formal diabetes education often does not connect with traditional healers or community elders, who many indigenous people trust first for health advice. Without working together with these trusted figures, educational programs risk being ignored or seen as untrustworthy by the communities.

Weaknesses in surveillance systems and health policy implementation make it difficult to build effective educational programs. Without reliable data on diabetes rates among indigenous populations, it is difficult to allocate resources properly or design interventions that fit the needs of specific communities [39]. To improve diabetes education for indigenous Africans, we need culturally relevant materials, simple interventions for those with low literacy, strong community involvement, and connections with traditional health systems. Without these efforts, health gaps will continue, and diabetes education programs will not reach their full potential.

Conclusion

The increasing prevalence of diabetes among indigenous Africans shows the urgent need for targeted education. The collaborative efforts of the International Diabetes Federation and national health systems in employing the use of diabetes education has ultimately led to the beneficial outcomes of diabetes control among indigenous Africans. Furthermore, increased successes can be achieved with the programs tailored to be more culturally sensitive and community-focused, with proper training of indigenous community health workers, integration of traditional healers, and provision of educational materials need in local dialect to address literacy challenges. Also, governments must invest in robust data-gathering practices to monitor diabetes trends in indigenous groups and ensure long-term funding by incorporating education programs into national NCD strategies.

Declarations
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Original Article

Ethanol Extract of *Spathodea campanulata* and its component Ellagic Acid Modulate β-haematin Metabolism, Mitochondrial Membrane Potential, and Calcium Homeostasis in Plasmodium Falciparum

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Abstract

Background: Malaria remains a global health threat, with increasing resistance to frontline drugs driving the search for novel therapies. Spathode acampanulata is traditionally used in African medicine against malaria, yet its molecular mechanisms are not fully defined. This study assessed the ethanol extract of *S. campanulata* leaves and its phytoconstituent ellagic acid for their ability to modulate β-haematin metabolism, calcium homeostasis, and mitochondrial membrane potential in Plasmodium falciparum.

Materials and Methods: Ellagic acid was isolated by preparative HPLC and confirmed using analytical HPLC. Hemolytic activity was evaluated in human O^+ erythrocytes, while β-haematin inhibition, calcium homeostasis, and mitochondrial potential disruption were measured in P. falciparum (3D7 strain) using standard assays, data were analyzed using SPSS version 25.0 and one-way ANOVA with significance at $p < 0.05$.

Results: The ethanol extract (IC_{50} = 4.80 ± 0.22 µg/mL) and ellagic acid (3.70 ± 0.80 µg/mL) exhibited low hemolytic activity relative to chloroquine (3.60 ± 0.82 µg/mL). The extract demonstrated superior β-haematin inhibition (0.35 ± 0.12 µg/mL) compared to ellagic acid (1.30 ± 1.00 µg/mL), whereas ellagic acid strongly disrupted mitochondrial potential (0.54 ± 0.21 µg/mL) and calcium signaling.

Conclusion: HPLC analysis confirmed ellagic acid content at 2.5 % w/w of crude extract. These findings suggest complementary mechanisms between extract and ellagic acid, supporting their potential as scaffolds for antimalarial drug development.

Keywords: *Spathodea campanulata*, ellagic acid, β-haematin, calcium homeostasis, mitochondrial potential, antimalarial

1. Introduction

Malaria remains a major global health challenge, responsible for more than 240 million cases and over 600 000 deaths annually, with Plasmodium falciparum being the most virulent causative species [1]. Resistance to chloroquine, artemisinin derivatives, and partner drugs continues to compromise control strategies [2, 3]. This underscores the need for novel agents, particularly from medicinal plants, which are rich in structurally diverse bioactive molecules[4]. *Spathodea campanulata* (African tulip tree), widely used in African ethnomedicine for fever and infections, has shown antimicrobial, antioxidant, and antiparasitic activities [4-7]. Its phytochemical profile includes phenolics, flavonoids, and ellagic acid, compounds with reported antiparasitic and mitochondrial effects [8, 9]. Ellagic acid, a polyphenol, interferes with haemozoin formation, disrupts calcium sig-

naling, and induces mitochondrial dysfunction in protozoa [10, 11]. The parasite relies on haem detoxification, mitochondrial electron transport, and Ca^{2+} signaling for survival. Disruption of haem detoxification by inhibiting β-haematin formation results in toxic heme accumulation [12]. Parasite mitochondria sustain energy metabolism and pyrimidine biosynthesis, making mitochondrial potential an essential target [13]. Calcium signaling regulates invasion, egress, and intracellular development [14]. Thus, targeting these processes provides a multi-pronged strategy against malaria. This study evaluated the ethanol extract of *S. campanulata* and ellagic acid in modulating β-haematin formation, mitochondrial potential, and Ca^{2+} homeostasis in P. falciparum, while assessing haemolytic safety.

2. Materials and Methods

2.1 Collection of Plants and Authentication

Fresh leaves of *S. campanulata* were harvested from their natural habitats in Abraka, Ethiope East LGA of Delta State, Nigeria (5.9051° N, 6.0314° E). The plant was identified using the Leaf Snap App and authenticated by Mr. Micheal Ozioma Emmanuel at the Delta State University Herbarium (voucher no. DELSU042). Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethical Committee (RBC/FBMC/DELSU/25/662).

2.2 Isolation and Purification of Ellagic Acid

Ellagic acid was isolated from the crude extract by solvent partitioning (ethyl acetate fraction), followed by preparative HPLC (Agilent 1200, C18 column, acetonitrile:water 40:60, detection 254 nm). Purity was confirmed by analytical HPLC (> 95 %), UV λmax (254, 366 nm), and co-injection with standard ellagic acid. Quantitative HPLC revealed ellagic acid content as 2.5 % w/w of crude extract.

2.3 Parasite and Erythrocyte Source

Plasmodium falciparum 3D7 strain (chloroquine-sensitive) was obtained from MR4/BEI Resources, USA, and cultured in human O^+ erythrocytes using RPMI-1640 medium supplemented with 10 % human serum.

2.4 β-Haematin Inhibition Assay

The assay followed Deharo and colleagues [15]. Haematin chloride was incubated with treatments under acidic conditions; β-haematin formation was quantified spectrophotometrically at 405 nm.

2.5 Mitochondrial Membrane Potential

Mitochondrial potential was evaluated with JC-1 dye (Thermo Fisher, USA) as described by Painter and co-workers [13].

2.6 Calcium Homeostasis

Ca^{2+} signaling was assessed using Fluo-4 AM dye (Invitrogen, USA) as outlined by Gazarini and team [14].

2.7 Haemolysis Assay

Washed O^+ erythrocytes were incubated with treatments; hemoglobin release was measured at 540 nm [16].

2.8 Statistical Analysis

All assays were performed in triplicate. Data are expressed as mean ± SD. One-way ANOVA with Tukey's post-hoc test was applied using GraphPad Prism version 9.

3. Results

HPLC Analysis of Ellagic Acid

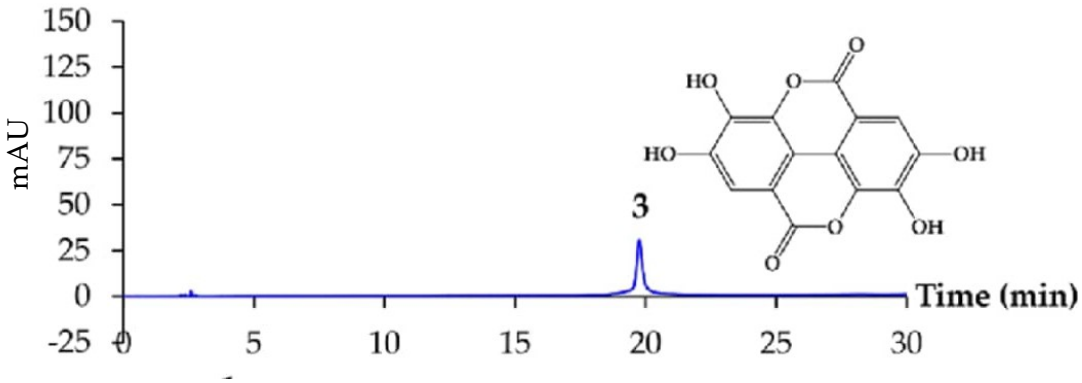

Figure 3.1: HPLC chromatogram of ellagic acid

Figure 3.1 presents a representative HPLC chromatogram, where the y-axis denotes detector response (mAU, −25 to 150) and the x-axis retention time (0–35 min). A distinct peak at ≈ 20 min corresponds to ellagic acid, confirming a clean separation and > 95 % purity. Such profiles are typical for phenolic compounds analyzed via reversed-phase HPLC[23].

Inhibition of β-Haematin Formation

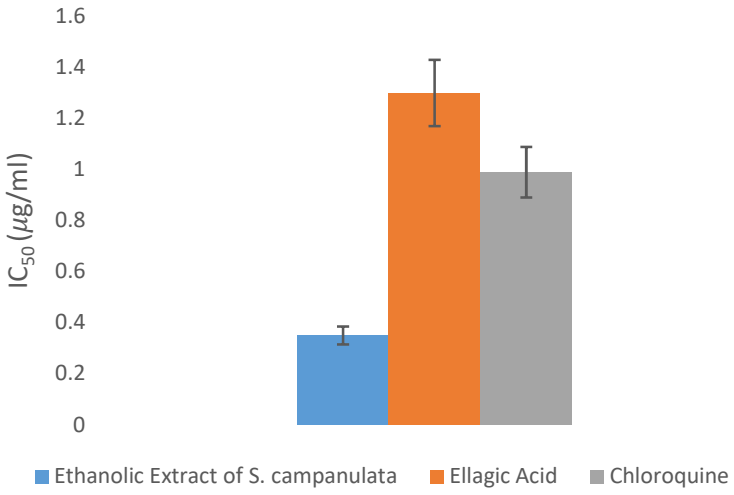

Figure 3.2: Inhibition of Beta Haematin formation

Results from this assay (IC50) is shown in Figure 3.2. The ethanol extract demonstrated superior β-haematin inhibition (IC_{50} = 0.35 ± 0.12 μg/mL) compared with ellagic acid (1.30 ± 1.00 μg/mL) and chloroquine (0.99 ± 0.85 μg/mL). Dose-response plots revealed a steep, concentration-dependent inhibition for the extract.

Disruption of Mitochondrial Potential

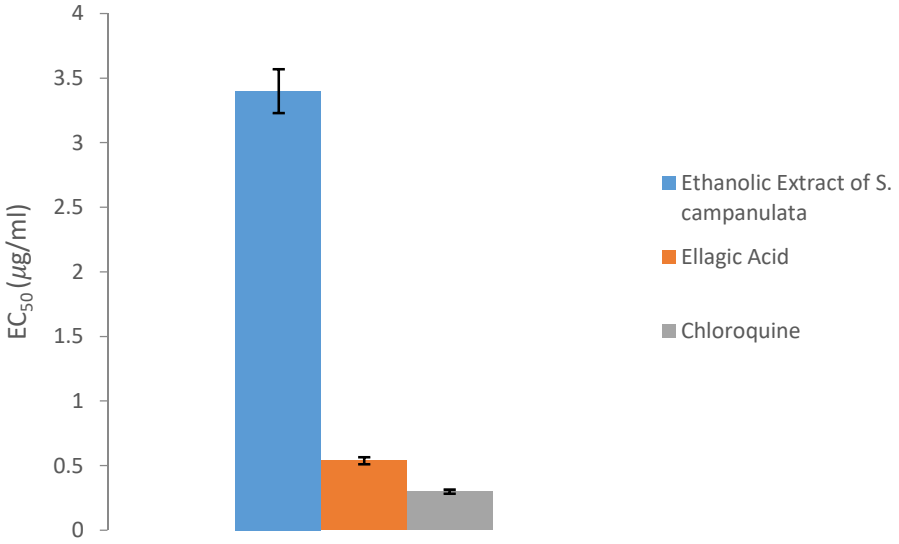

Figure 3.3: Changes in mitochondrial membrane potential.

Mitochondrial membrane potential (EC50) values obtained from this assay are displayed in Figure 3.3. Ellagic acid (0.54 ± 0.21 µg/mL) rapidly collapsed mitochondrial potential, comparable to chloroquine (0.30 ± 0.00 µg/mL). The ethanol extract exhibited a milder effect (3.40 ± 0.91 µg/mL), indicating that ellagic acid is the principal mitochondrial disruptor.

Disruption of Calcium Homeostasis

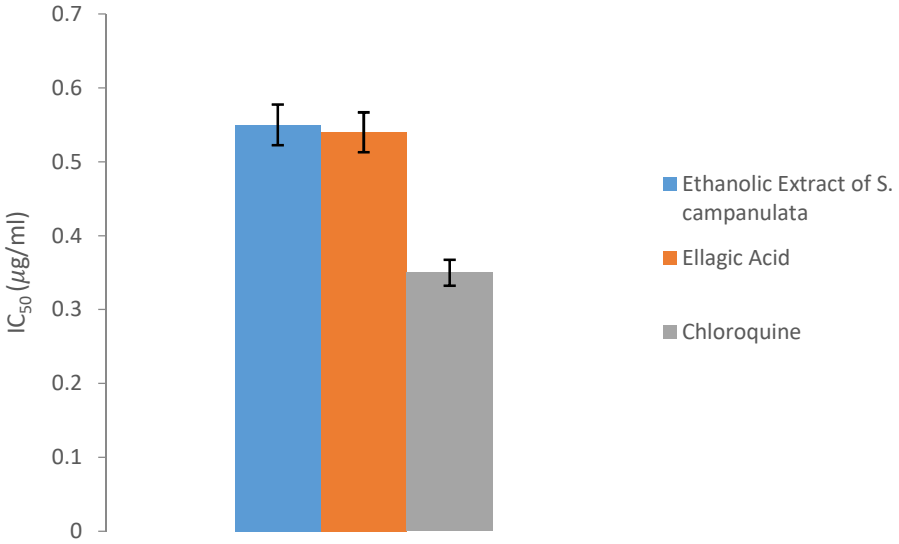

Figure 3.4: Disruption in P. falciparum calcium homeostasis induced by S. campanulata ethanol

Disruption of P. falciparum's Calcium Homeostasis (EC50) values from this assay are displayed in Figure 3.4. Both the ethanol extract (0.55 ± 0.34 µg/mL) and ellagic acid (0.54 ± 0.09 µg/mL) markedly elevated intracellular Ca^{2+}, whereas chloroquine caused minimal changes. The extract produced a sustained Ca^{2+} rise, while ellagic acid elicited a transient spike.

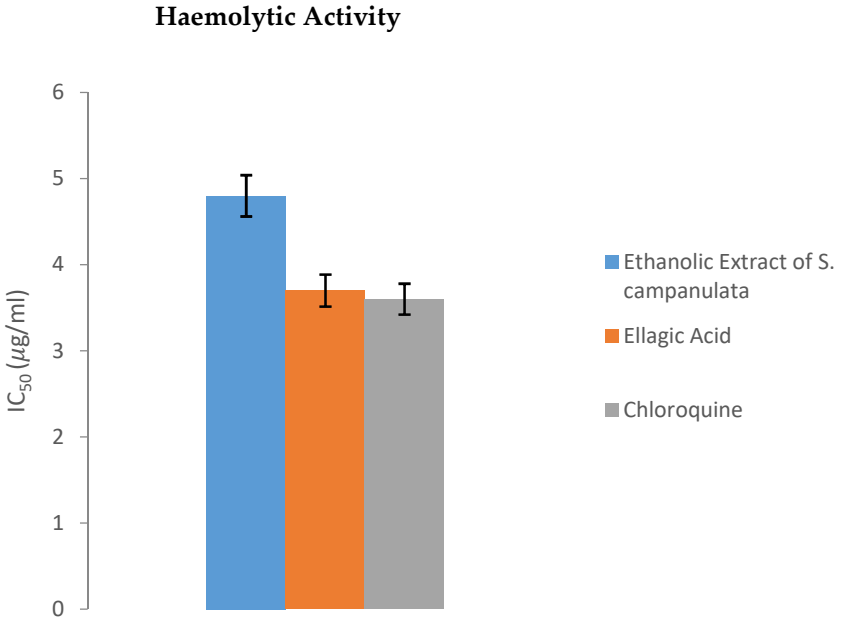

Figure 3.5: Haemolytic changes due to ellagic acid and ethanol extract of S. campanulata

Haemolytic (HC50) values obtained from this assay are displayed in Figure 3.5. Chloroquine induced the lowest haemolysis (3.60 ± 0.82 μg/mL), followed by ellagic acid (3.70 ± 0.80 μg/mL). The ethanol extract showed slightly higher haemolysis but < 10 % at therapeutic concentrations.

4. Discussion

Ellagic acid showed strong β-haematin inhibition, consistent with reports of polyphenols disrupting hemozoin polymerization [17]. The extract's higher potency suggests synergistic effects among its phytochemicals [18]. The collapse of mitochondrial potential by ellagic acid supports findings that natural polyphenols target protozoan mitochondria [19, 20]. Calcium dysregulation by both agents aligns with studies showing plant polyphenols interfere with parasite Ca^{2+} signaling [21]. Haemolysis data confirm ellagic acid's acceptable safety margin [22, 23].

5. Conclusion

This study demonstrates that the ethanol extract of *Spathodea campanulata* and ellagic acid exert anti-plasmodial activity through complementary mechanisms: inhibition of haem detoxification, disruption of mitochondrial potential, and interference with calcium signaling. Ellagic acid is the dominant bio-active compound, showing a favorable balance of potency and safety. These findings justify further in vivo evaluation and molecular modeling to develop ellagic acid as a multi-target antimalarial scaffold.

Declarations

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

Data availability statement: the corresponding author will make the datasets created and/or analyzed during the current study available.

Competing Interests: There is no known conflict of interest.

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Authors' Contributions: DCC conceptualize, drafted and submitted the manuscript, ODA and EKC authors contributed to the literature review. Authors OOC and OAO contributed to the biochemical analysis, while, OAO and OI supervised the study

Acknowledgments: Not applicable

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