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Omega-3 attenuates codeine-induced hepatic injury in female wistar rats via its antioxidative, anti-inflammatory, and anti-apoptotic effects

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ABSTRACT

Codeine is widely used for managing chronic and intermittent pain, particularly in females experiencing menstrual cramps; however, prolonged use has been associated with hepatic injury mediated by oxidative stress and apoptosis. Despite this growing concern, limited data exist on effective strategies to protect the liver against opioid-induced damage. This study investigated the protective role of omega-3 fatty acid supplementation against codeine-induced liver injury in female Wistar rats. Twenty female rats were divided into four groups (n = 5 each): vehicle control, omega-3-treated (400 mg/kg BW, p.o.), codeine-treated (10 mg/kg BW, p.o.), and codeine + omega-3 co-treated (10 mg/kg BW of codeine + 400 mg/kg BW of omega-3, p.o.) for eight weeks. Chronic codeine exposure significantly ($p < 0.05$) elevated hepatic injury biomarkers (AST, GGT, and LDH), oxidative stress indices (MDA and 8-OHdG), and inflammatory mediators (MPO and NO), accompanied by ($p < 0.05$) increased caspase-3 activity. In contrast, omega-3 supplementation markedly ($p < 0.05$)reduced these alterations, enhanced antioxidant defense, and preserved hepatic architecture. Moreover, omega-3 improved hepatic metabolic function, as indicated by decreased lactate levels and lactate/pyruvate ratios. These findings demonstrate that omega-3 fatty acid effectively attenuates chronic codeine-induced hepatic injury by mitigating oxidative stress, inflammation, and apoptosis, thereby preserving liver function and cellular integrity in female rats. The study is relevant as it highlights omega-3 fatty acid as a potential dietary intervention for reducing opioid-related hepatotoxicity and promoting safer long-term pain management.

Introduction

Drug-induced liver injury (DILI) remains a significant global health concern due to the liver's central role in xenobiotic metabolism and detoxification [1]. The liver performs critical physiological functions—including metabolism, secretion, and storage making it a vital organ for maintaining homeostasis [2]. However, exposure to hepatotoxic drugs often leads to oxidative stress, lipid peroxidation, mitochondrial dysfunction, and apoptosis, all of which contribute to hepatic impairment [3].

Codeine, chemically known as 3-methylmorphine, is one of the most widely prescribed opioid analgesics for the management of pain, cough, and diarrhea [4]. While effective therapeutically, prolonged or excessive use of codeine-containing medications has been associated with severe adverse effects, including renal and hepatic damage, as well as gastrointestinal ulceration [5]. In Nigeria, codeine remains the most

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commonly abused opioid after cannabis, positioning opioids as the second most misused drug class in the country [6]. Despite government regulations restricting its availability, the misuse of codeine persists, particularly among adolescents and young adults [7].

Omega-3 fatty acids—comprising alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)—are essential long-chain polyunsaturated fatty acids (LC-PUFAs) that must be obtained from dietary sources such as flaxseed, canola, and fish oils [8,9]. These fatty acids possess well-documented anti-inflammatory, antioxidant, and lipid-regulating properties [10]. Evidence suggests that EPA and DHA can protect against hepatic steatosis and oxidative damage by modulating fatty acid metabolism, suppressing free radical formation, and reducing inflammatory cytokine activity [11].

Recent studies have shown that women are particularly vulnerable to codeine misuse, often initiating use for legitimate pain relief, such as menstrual cramps, and progressing to dependency or abuse [12–14]. Females also exhibit a higher tendency to self-administer larger doses of over-the-counter opioids over extended periods due to recurrent pain experiences [15–17]. Chronic codeine exposure has been implicated in hepatocellular injury mediated through oxidative stress and caspase-3-dependent apoptosis, ultimately resulting in functional hepatic impairment [18,19].

Despite these findings, few studies have explored the hepatotoxic effects of chronic codeine use specifically in females, and even fewer have examined the potential protective role of omega-3 fatty acids in mitigating such damage. Previous research has focused largely on other models of hepatotoxicity (e.g., alcohol- or acetaminophen-induced), leaving a critical gap in understanding the protective efficacy of omega-3 against opioid-induced liver injury.

Therefore, this study investigates the protective role of omega-3 fatty acid supplementation against chronic codeine-induced liver damage in female Wistar rats. The study evaluates key parameters including liver function, oxidative stress biomarkers, inflammation, apoptosis, and histological changes to provide novel insights into the hepatoprotective mechanisms of omega-3 fatty acids.

Materials and methods

Animals

Twenty female Wistar rats, with weights ranging 160–180 g, were acquired from Anchor Biomedical Research Institute in Ogbomoso, Nigeria. The animals were accommodated in clear plastic enclosures within the Department of Physiology's animal facility at Ladoke Akintola University of Technology (LAUTECH), Ogbomoso. Their diet consisted of a regular laboratory feed (Top feed Grower's Mash), and they had unlimited access to water. The rats were exposed to their normal environment and were isolated in the 10 days preceding the start of the research. The handling of all rats adhered to the normal code of practice for the care and use of animals in laboratory settings.

Ethical approval

The study protocol was submitted to the Faculty of Basic Medical Sciences Research Ethics Review Committee for approval and received ethical clearance with reference number ERCFBMSLAUTECH: 026/03/2024. Also, the study was conducted and reported in accordance with the ARRIVE guidelines.

Drugs and chemicals

Nigeria's National Drug Law Enforcement Agency generously provided codeine only for study purposes, whereas Omega-3 fatty acid was acquired from Pfizer, a pharmaceutical enterprise. Assays were conducted with conventional ELISA kits stated accordingly. Unless specified differently, all additional reagents used were of analytical quality.

Experimental protocol

The twenty female Wistar rats were randomly assigned into four groups, each comprising five animals. Group 1 served as the control and was administered 0.5 mL of distilled water; Group 2 was administered omega-3 fatty acid (400 mg/kg BW); Group 3 received codeine (10 mg/kg BW); while Group 4 was co-administered codeine and omega-3 fatty acid (10 mg/kg BW and 400 mg/kg BW, respectively). All administrations were carried out orally once daily, between 7:00 a.m. and 8:00 a.m., for a duration of eight weeks for sub-chronic modelling [19] using an oropharyngeal cannula. The selected doses of codeine and omega-3 fatty acid were adopted from the studies of Akhigbe et al. [20] and Espírito Santo et al. [21], respectively.

Measurement of body and organ weights

The rats' initial body weight was determined before the initiation of drug administration and recorded as the preliminary body weight (in gram). After the last treatment, the body weight was measured again 24 h later and recorded as the final body weight (in gram) using an automated weighing scale. The rats were anaesthetized by administering ketamine (40 mg/kg/BW) and xylazine (5 mg/kg/BW) via intraperitoneal injection [22]. After measuring the final body weight was measured and recorded. The body weight change was expressed as $\textit{Final Body Weight}(g) - \textit{Initial body weight}(g)$.

Sample collection

The abdominal region of the animals were opened up to the thoracic region and blood was collected via cardiac puncture into sterile heparinized containers and then spun at 3000 rpm for 15 min to isolate the plasma. Meanwhile, the liver was harvested, weighed, and prepared for further analysis. A 1 g portion of the liver was then sliced and homogenised in 5 mL buffered solution containing phosphate. The serum and homogenate were utilised for biochemical testing, while a separate liver lobe was dissected and immersed in a 10% formaldehyde solution for histological processing.

Biochemical assay

Liver function assessment and damage risk evaluation

Plasma enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were determined using the colorimetric technique, following the manufacturers' standard protocols. These analyses were conducted with commercially prepared diagnostic reagents (Agappe Diagnostics Ltd., India), known for their high specificity and reproducibility in biochemical assays. The colorimetric method is based on the principle that enzymatic reactions generate chromogenic products whose absorbance can be quantitatively measured with a spectrophotometer, thus reflecting enzyme activity in plasma [23].

The selection of these enzymes was based on their established roles as biochemical markers of hepatic function and integrity. ALT and AST serve as sensitive indicators of hepatocellular damage, as they are released into the circulation following membrane leakage or necrosis of hepatocytes [24]. ALP and GGT, on the other hand, are membrane-bound enzymes that reflect cholestatic injury or biliary obstruction, providing complementary information on hepatic and biliary health [24].

Additionally, lactate dehydrogenase (LDH) activity was measured using a commercial LDH assay kit (Agappe Diagnostics Ltd., India) according to the manufacturer's instructions. LDH catalyzes the interconversion of lactate and pyruvate, with the concomitant reduction of NAD^+ to NADH. The rate of NADH formation, monitored

spectrophotometrically, corresponds to LDH activity. Measurement of LDH provides further insight into cellular integrity and nonspecific tissue injury, as its release into plasma often accompanies membrane damage in multiple organ systems [25].

All enzymatic activities were expressed in international units per liter (IU/L), and analyses were performed in accordance with standard clinical biochemistry practices to ensure accuracy and reproducibility.

Determination of redox markers

The evaluation of oxidative stress and antioxidant defense mechanisms in hepatic tissues was conducted through the measurement of key redox biomarkers. These parameters provide insight into the balance between reactive oxygen species (ROS) generation and the antioxidant defense capacity of the liver. Specifically, malondialdehyde (MDA) was assessed as an index of lipid peroxidation, reduced glutathione (GSH) as a non-enzymatic antioxidant marker, and superoxide dismutase (SOD) and catalase (CAT) as major enzymatic antioxidants involved in the detoxification of superoxide and hydrogen peroxide, respectively. Myeloperoxidase (MPO) activity was determined as an indicator of neutrophil infiltration and inflammation, while 8-hydroxydeoxyguanosine (8-OHdG) was quantified to assess oxidative DNA damage. Additionally, hepatic nitric oxide (NO) levels were measured. All analytical procedures were performed using validated colorimetric methods described in previous studies [25–28].

Malondialdehyde (MDA) assay

Lipid peroxidation was determined by measuring MDA levels using the thiobarbituric acid reactive substances (TBARS) method. Briefly, 200 µL of liver homogenate was deproteinized with 500 µL of trichloroacetic acid (TCA) (Sure-Chem Products Ltd., England) and centrifuged at 3000 rpm for 10 min. Then, 1 mL of 0.75% thiobarbituric acid (TBA) (Loba Chemie Pvt. Ltd., India) was added to 0.1 mL of the supernatant. The mixture was heated in a boiling water bath (100 °C) for 20 min and rapidly cooled in ice water. The resulting pink chromogen was read spectrophotometrically at 532 nm, and MDA concentration was extrapolated from a standard calibration curve.

Superoxide dismutase (SOD) activity

SOD activity was assayed based on its ability to inhibit the autooxidation of adrenaline. One milliliter of liver homogenate was diluted with 9 mL of distilled water. An aliquot of 0.2 mL of the diluted sample was added to 2.5 mL of 0.05 M sodium carbonate buffer (pH 10.2) (Loba Chemie Pvt. Ltd., India). The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline (Central Drug House Ltd., India), and the absorbance was recorded at 480 nm at 30-second intervals for 150 s. The reference cuvette contained buffer, substrate, and distilled water. SOD activity was expressed as units per milligram of protein [29].

Catalase (CAT) activity

Catalase activity was determined using the dichromate–acetic acid method. One milliliter of the supernatant was diluted with 19 mL of distilled water. The reaction mixture contained 4 mL of hydrogen peroxide solution (800 µmol) (Guangdong Guanghua Chemical Factory Co. Ltd., China) and 5 mL of phosphate buffer in a 10 mL flat-bottom flask. The reaction was initiated by adding 1 mL of the diluted enzyme preparation, and the mixture was gently swirled at 37 °C. At 60-second intervals, 1 mL aliquots were withdrawn and added to 2 mL of dichromate–acetic acid reagent (Sure-Chem Products Ltd., India) to terminate the reaction. The residual hydrogen peroxide was determined spectrophotometrically at 653 nm, and catalase activity was calculated using a certified catalase standard [29].

Reduced glutathione (GSH) assay

Hepatic GSH levels were quantified using Ellman's reagent. An aliquot of the homogenate was deproteinized with an equal volume of 4% sulfosalicylic acid (Loba Chemie Pvt. Ltd., India) and centrifuged at 4000 rpm for 5 min. Subsequently, 0.5 mL of the supernatant was added to 4.5 mL of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid)) (Central Drug House Ltd., India). A blank was prepared using 0.5 mL of the precipitating agent and 4.5 mL of Ellman's reagent. The absorbance was measured at 412 nm, and GSH concentration was calculated using a standard curve [29].

Myeloperoxidase (MPO) activity

MPO activity was measured according to a previously described method [29]. The reaction mixture contained 3 mL of assay reagent composed of 50 mM potassium phosphate buffer, 100 mM guaiacol (Loba Chemie Pvt. Ltd., India), and 0.0017% (w/w) hydrogen peroxide. To this mixture, 0.035 mL of MPO enzyme solution (Loba Chemie Pvt. Ltd., India) and 200 µL of hepatic homogenate were added. The rate of guaiacol oxidation was monitored spectrophotometrically at 470 nm, and MPO activity was expressed as units per milligram of protein.

8-Hydroxydeoxyguanosine (8-OHdG) assay

Oxidative DNA damage was evaluated by quantifying 8-hydroxydeoxyguanosine (8-OHdG) levels using a commercial ELISA kit (Elabscience Biotechnology Inc., USA), following the manufacturer's protocol. The assay provides a sensitive and specific estimation of oxidative DNA modification and genotoxicity [30].

Apoptosis status

Caspase 3 activities were assessed to determine the degree of apoptosis in the liver. To achieve this, ELISA kits from Elabscience Biotechnology Co., Ltd., USA were employed to quantify the levels of these biomarker. The ELISA kits were used to perform assays in accordance with the manufacturers' instructions.

Histology

Liver tissues from each animal were fixed in 10% neutral-buffered formalin for 24 h, processed through graded alcohols, cleared in xylene, and embedded in paraffin. Blocks were sectioned at 5 µm with a rotary microtome and mounted on glass slides. For each animal, three non-consecutive sections were prepared and stained with haematoxylin and eosin (H&E). From each section, five random, non-overlapping fields were captured using a light microscope fitted with a digital camera at two magnifications: 100 × for overall architecture and 400 × for cellular details. Representative photomicrographs were taken and all images included a scale bar were quantified using Image-J software. All histological evaluations were performed blind to treatment group by two independent observers; disagreements were resolved by consensus [31].

Statistical analysis

Data analysis was performed using GraphPad Prism 10.0 software due to its reliability and precision in handling biomedical data. A one-way analysis of variance (ANOVA) was employed to assess statistical differences among the experimental groups. When significant variations were detected, Tukey's multiple comparison post-hoc test was applied to determine specific group differences while minimizing Type I error. Results are expressed as mean ± standard deviation (SD), and statistical significance was considered at $p < 0.05$.

Results

Effects of codeine and omega-3 on body weight change and relative liver weight

The statistical analysis of weight-related parameters revealed that codeine administration exerted a distinct effect on body weight gain and liver weight. As shown in Fig. 1A, rats treated with codeine exhibited a significant reduction ($p < 0.05$) in body weight gain compared with the control, omega-3-treated, and codeine plus omega-3-treated groups. In contrast, Fig. 1B indicates that the treatment produced no significant change in relative liver weight across the experimental groups.

Effects of codeine and omega-3 on hepatic enzyme activities and liver function indices

Table 1 summarizes the effects of codeine, omega-3 fatty acids, and their co-administration on key hepatic enzyme activities and liver function indices in rats. These biomarkers ALT, AST, ALP, GGT, LDH, Liver Damage Index (LDI), Hepatic Risk Ratio (HRR), GGT/ALT ratio, and De Ritis ratio serve as sensitive indicators of hepatocellular integrity, membrane stability, and overall hepatic function.

Alanine transaminase (ALT) activity remained relatively unchanged across all groups, with no statistically significant difference between codeine-treated and control rats. However, aspartate transaminase (AST) activity increased significantly ($p < 0.05$) in the codeine-treated group compared with the control, Notably, rats co-administered omega-3 or treated with omega-3 alone exhibited AST levels comparable to control values (Table 1).

Similarly, alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activities were markedly elevated ($p < 0.05$) in codeine-exposed rats. There was restoration of these enzyme levels toward normal in the omega-3 and codeine plus omega-3 groups (Table 1).

The activity of lactate dehydrogenase (LDH), a nonspecific marker of cellular damage, was also significantly increased in the codeine group. This elevation was substantially attenuated in the omega-3 and combination groups. The Liver Damage Index (LDI) showed a similar trend, being markedly higher in the codeine group, while omega-3 treatment significantly reduced this index, both independently and in co-administration. In contrast, the Hepatic Risk Ratio (HRR) remained statistically unchanged across all groups, indicating that overall hepatocellular risk was not significantly altered under the treatment conditions (Table 1).

Table 1
Effects of Omega-3 on the Liver function enzymes and Liver Damage Indices.

	Control	Codeine	Omega-3	Codeine + Omega-3
ALT (U/L)	4.28 ± 0.132	4.25 ± 0.230	3.87 ± 0.165	3.94 ± 0.254
AST (U/L)	5.94 ± 0.953	11.89 ± 0.449 [a]	6.23 ± 1.444 [b]	6.30 ± 0.847 [b]
ALP (U/L)	8.98 ± 0.964	15.84 ± 0.66[a]	8.046 ± 0.863 [b]	9.23 ± 0.895 [b]
GGT (U/L)	7.11 ± 1.49	13.12 ± 0.58[a]	5.34 ± 1.01 [b]	8.08 ± 1.30 [b,c]
LDH (U/L)	15.31 ± 0.80	28.51 ± 0.54[a]	13.68 ± 0.76[b]	13.89 ± 0.51[b]
Liver Damage Index (LDI) (U/L)[3]	246.1 ± 51.5	1368 ± 125.7[a]	181.3 ± 42.62[b]	340.9 ± 126.5[b]
Hepatic Risk Score (HRR)	1.016 ± 0.17	1.016 ± 0.43	0.983 ± 0.09	1.21 ± 0.09
GGT/ALT	0.921 ± 0.08	0.822 ± 0.03	0.571 ± 0.12[a,b]	0.891 ± 0.07[c]
De Ritis Ratio	0.586 ± 0.005	0.745 ± 0.03[a]	0.637 ± 0.05	0.566 ± 0.08[b]

Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine; c = significantly ($P < 0.05$) when compared to Omega-3.

The GGT/ALT ratio was significantly decreased ($p < 0.05$) in the omega-3-treated rats compared with control and codeine groups. The De Ritis ratio (AST/ALT) was significantly elevated in the codeine group while the omega-3 co-treated group displayed near-normal values (Table 1)

Effects of codeine and omega-3 on hepatic redox status

Codeine administration led to a significant increase ($p < 0.05$) in

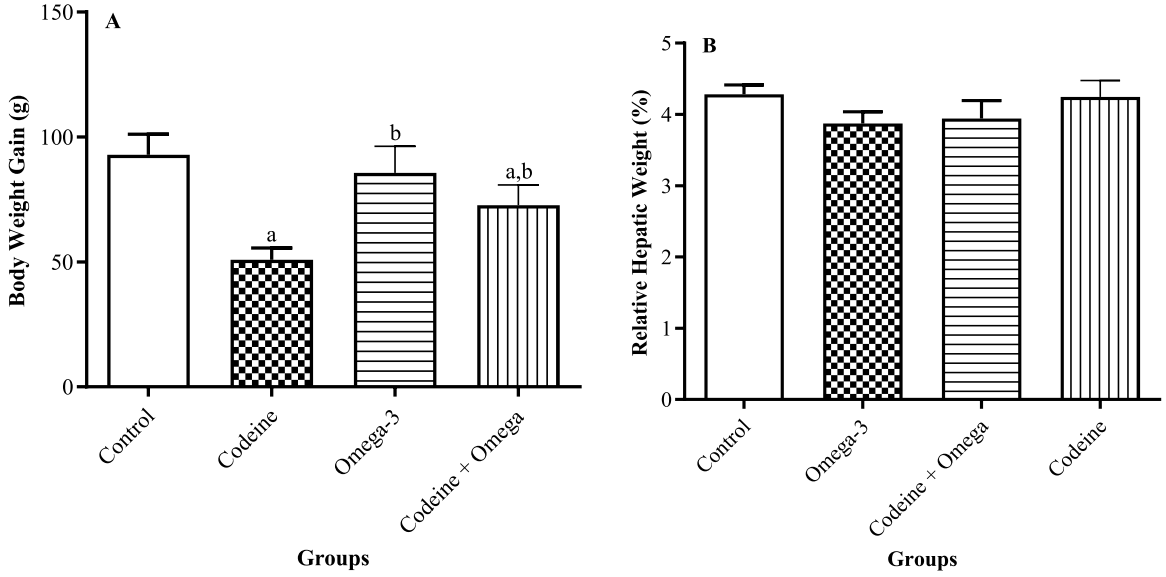

Fig. 1. Effect of Omega-3 fatty acid on the (A) Body weight gain and (B) relative hepatic weight of codeine exposed female rats. Values are means of five replicates measurements ± SD; bars carrying a = significantly ($P < 0.05$) when compared to control, b = significant ($P < 0.05$) when compared to Codeine.

malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentrations in hepatic tissues compared to all other experimental groups (Figs. 2A and 2B). In contrast, co-administration of omega-3 fatty acids with codeine significantly ($p < 0.05$) reduced MDA and 8-OHdG levels relative to the codeine-only group (Figs. 2A and 2B).

Furthermore, codeine treatment in female Wistar rats caused a marked reduction ($p < 0.05$) in the activities of key hepatic antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) compared to the control, omega-3, and omega-3 plus codeine groups (Fig. 3A–C). Notably, omega-3 co-administration significantly ($p < 0.05$) enhanced SOD activity relative to codeine-only treatment (Fig. 3A), further supporting its role in ameliorating oxidative enzyme depletion and restoring hepatic redox balance.

Effects of codeine and omega-3 on liver tissues inflammatory markers

Administration of codeine to female rats resulted in a significant increase ($p < 0.05$) in hepatic myeloperoxidase (MPO) activity and nitric oxide (NO) levels compared with all other treatment groups (Fig. 4A - B). Conversely, omega-3 supplementation significantly ($p < 0.05$) attenuated the codeine-induced elevations in MPO activity and NO levels (Figs. 4A and 4B).

Effects of codeine and omega-3 on liver energy metabolism

Codeine treatment in female rats resulted in a significant elevation ($p < 0.05$) of hepatic lactate levels and the lactate-to-pyruvate ratio compared with all other treatment groups (Figs. 5A and 5B). Conversely, co-administration of omega-3 fatty acids with codeine significantly ($p < 0.05$) reduced both hepatic lactate levels and the lactate/pyruvate ratio (Figs. 5A and 5B).

Effects of codeine and omega-3 on liver apoptotic marker

Rats treated with codeine alone exhibited a marked and significant ($p < 0.05$) increase in hepatic caspase-3 concentration compared with the control group. In contrast, omega-3 supplementation, whether administered alone or in combination with codeine, resulted in a significant ($p < 0.05$) reduction in caspase-3 levels relative to the codeine-only group. Notably, the codeine + omega-3 group maintained caspase-3 levels comparable to the control (Fig. 6).

Effects of codeine and omega-3 on liver histology

The liver sections stained with hematoxylin and eosin show distinct histological patterns across the different experimental groups. In the control group and omega-3-treated group, the liver architecture is well preserved and the hepatocytes appear polygonal with clearly visible with centrally placed nuclei. The hepatic portal vein and bile ducts are normal in appearance, showing no evidence of congestion or dilation. The sinusoids are uniformly distributed and open, indicating healthy microcirculation and intact hepatic structure (Fig. 8).

In contrast, the codeine-treated group reveals marked histopathological alterations. The portal vein appears congested and dilated, with prominent accumulation of red blood cells in the lumen. The hepatocytes show varying degrees of disorganization, cytoplasmic vacuolation, and loss of normal architecture. The sinusoids are irregular and less defined with signs of mild inflammatory infiltration around the portal area (circled area).

Interestingly, the group co-treated with codeine and omega-3 shows improved histological features compared to the codeine-only group. Although some degree of vascular congestion persists within the portal vein, it is noticeably reduced. The hepatocyte morphology is largely preserved, and the sinusoids are more distinct and open compared to the codeine group. Only mild disorganization remains (Fig. 8).

Histomorphometric analysis of liver sections stained with hematoxylin and eosin demonstrated intact hepatic architecture in both the control and omega-3–treated groups. In contrast, the codeine-treated group exhibited pronounced hepatocellular degeneration relative to the control. Notably, co-administration of omega-3 with codeine resulted in significant histological improvement, evidenced by the restoration and preservation of normal hepatocyte morphology (Fig. 8).

Discussion

Previous experimental studies have demonstrated that prolonged codeine administration in rats leads to liver damage, possibly through mechanisms involving caspase 3 activation, increased free radical production and TNF-α levels, and reduced antioxidant defenses in liver cells [19, 32]. This current study aims to explore the potential protective effects of Omega-3 fatty acid supplementation on codeine-induced liver damage, considering individual variations in participants' backgrounds.

Research has indicated that it may be possible to evaluate organ

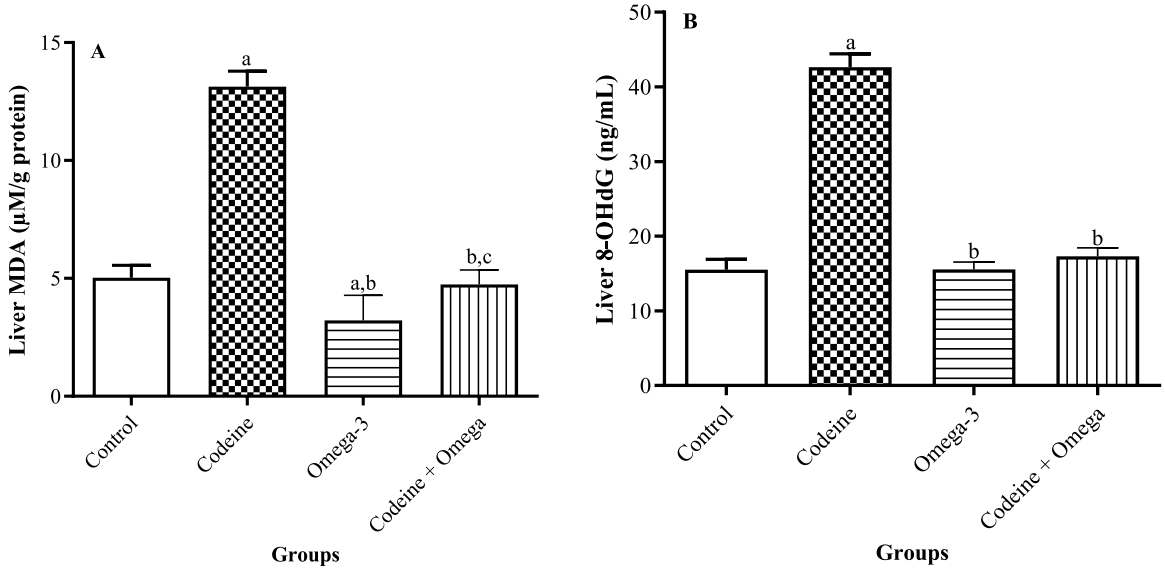

Fig. 2. Effect of Omega-3 fatty acid on the Liver tissues (A) MDA and (B) 8-OHdG levels in codeine exposed female rats. Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine; c = significantly ($P < 0.05$) when compared to Omega-3.

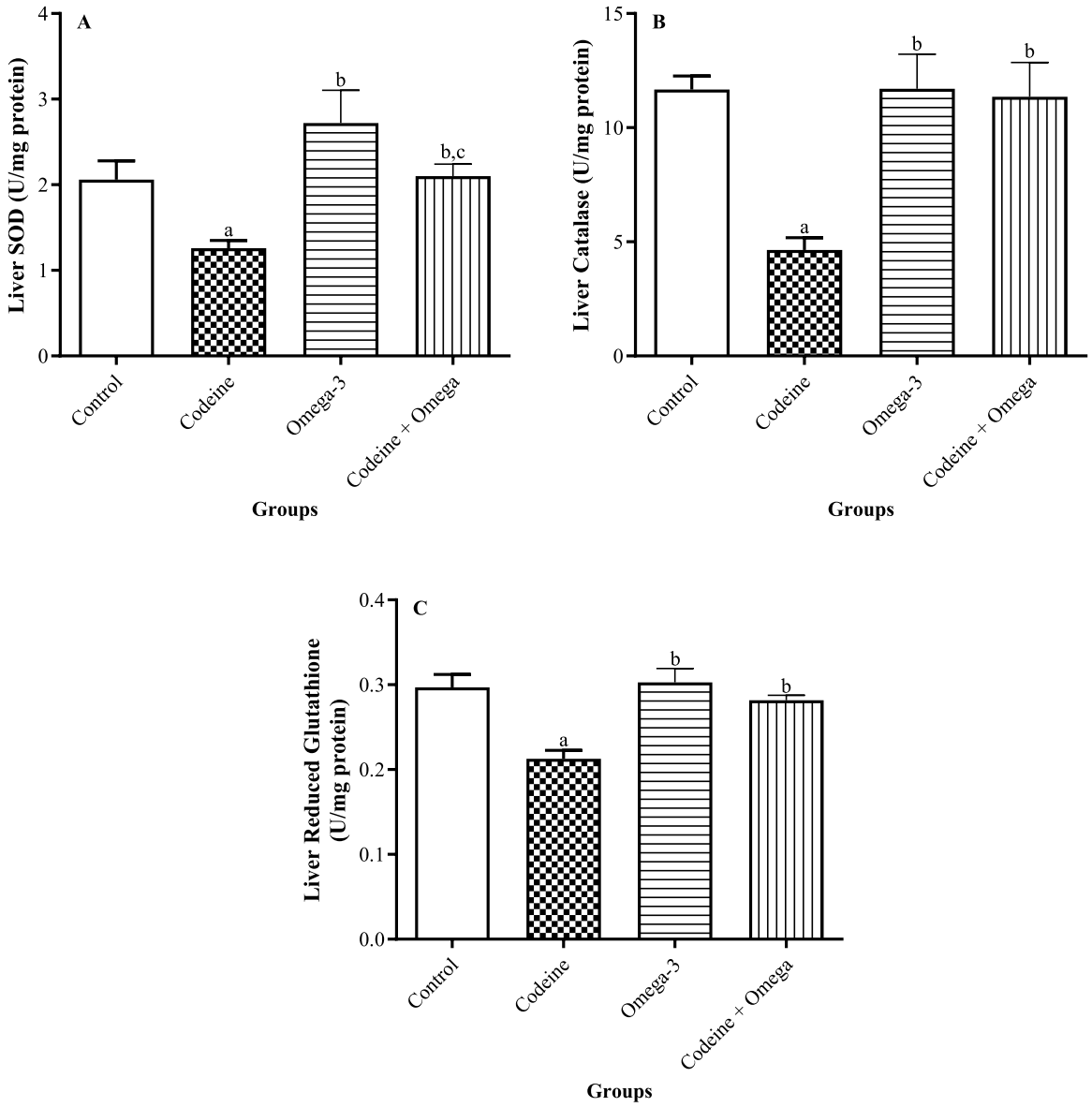

Fig. 3. Effect of Omega-3 fatty acid on the Liver tissues antioxidants (A) Super-Oxide Dismutase (SOD) (B) Catalase and (c) Reduced Glutathione (GSH) activities in codeine exposed female rats. *Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine; c = significantly ($P < 0.05$) when compared to Omega-3.*

toxicity by measuring the weight of the organs [33]. The decrease in body weight gain of the rats treated with codeine, as found in this study may be attributed to codeine-induced metabolic suppression, loss of appetite (anorexigenic effect), and gastrointestinal motility inhibition, all of which can impair nutrient absorption and energy utilization, suggesting that codeine may cause changes in metabolic processes that might lead to excessive tissue breakdown [34,35]. Meanwhile codeine influenced overall body metabolism and energy balance, it did not induce overt hepatomegaly or hepatic atrophy detectable at the organ weight level within the duration of treatment [36]. The administration of omega-3 fatty acid supplements to rats treated with codeine resulted in a considerable improvement in their metabolic process, as seen by an increase in body weight gain compared to untreated rats implying a protective metabolic effect of omega-3 fatty acids, likely through their anti-inflammatory, antioxidant, and lipid-regulating properties [37].

Since Alanine transaminase (ALT) activity remained relatively unchanged across all groups between codeine-treated and control rats, a suggestion that codeine administration did not markedly affect cytosolic enzyme leakage from hepatocytes [38]. However, codeine treatment leads to a significant elevation in hepatic enzyme activity, namely AST, indicating hepatocellular injury following mitochondrial enzyme leakage [38], GGT, and ALP reflecting cholestatic following biliary dysfunction [38]. The activity of lactate dehydrogenase (LDH), a nonspecific marker of cellular damage was also significantly increased indicating tissue necrosis or cellular injury [24]. This has a severe and immediate impact on liver function, as reported by Kalas et al. [39]. This discovery is consistent with the research by Akhigbe et al. [32], which showed that codeine can cause liver damage, as well as the study by Atici et al. [1], which established the hepatotoxic (liver-damaging) impact of morphine. Supplementing with omega-3 fatty acids provides protection to liver function, suggesting a hepatoprotective effect of omega-3 fatty acids and its ability to mitigates codeine-induced disruption of bile flow and membrane integrity. The protective effect of Omega-3 may rely on its ability to regulate the metabolism of lipids in the liver. This is accomplished by enhancing the breakdown of fatty acids and suppressing the activity of two key transcriptional regulators, Sterol regulatory element binding protein-1c (SREBP-1c) and Carbohydrate-responsive element-binding protein (ChREBP), which

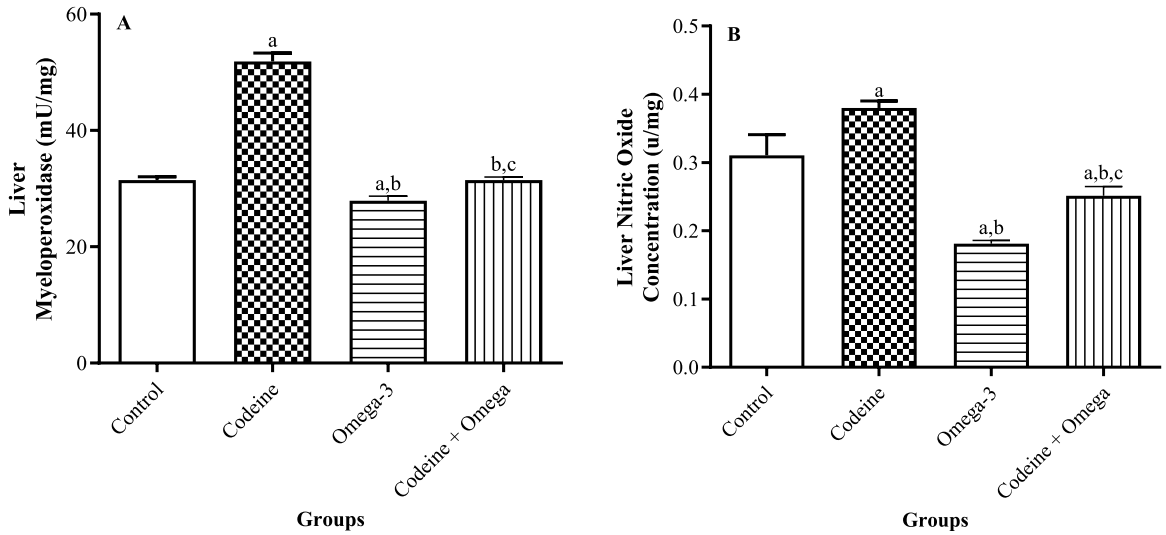

Fig. 4. Effect of Omega-3 fatty acid on the Liver inflammatory markers (A) myeloperoxidase (MPO) activities (B) Nitric Oxide (NO) level in codeine exposed female rats. *Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine; c = significantly ($P < 0.05$) when compared to Omega-3.*

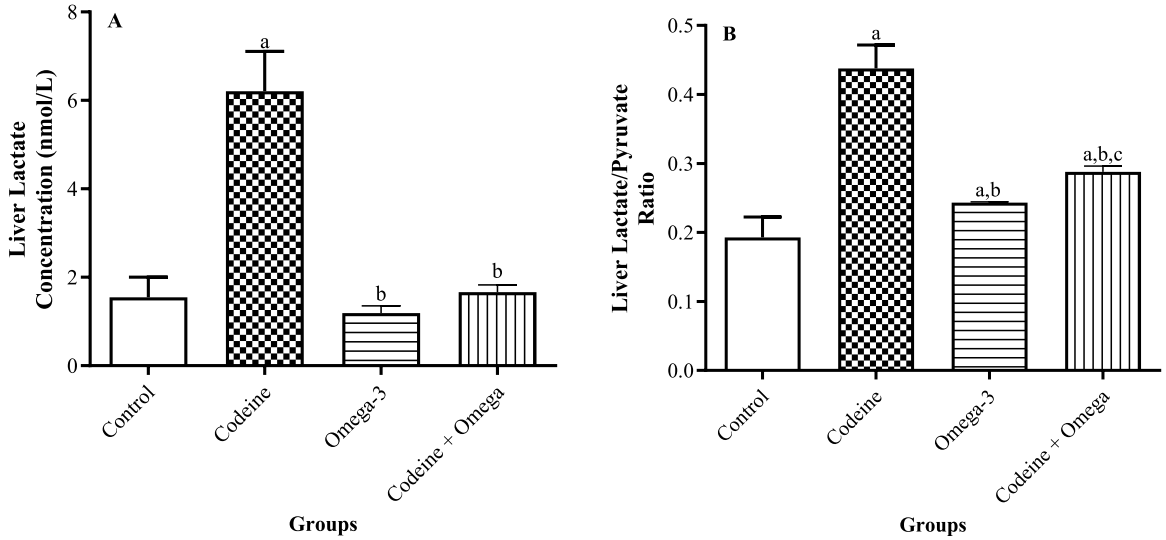

Fig. 5. Effect of Omega-3 fatty acid on the Liver energy metabolism (A) lactate Concentration (B) Lactate/pyruvate ratio in codeine exposed female rats. *Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine.*

play crucial roles in lipid and glucose metabolism. These regulators are responsible for promoting the production of lipids in the liver through the activation of key enzymes involved in lipogenesis [40].

The liver damage index (LDI) is a straightforward and precise index that is based on bio-mathematical and patho-physiological principles. It is useful for monitoring the progression of liver diseases, regardless of the cause and stage of liver injury. The LDI allows for a quantitative assessment of the severity of the disease and the improvement or deterioration in its course [41]. The hepatic risk score provides an indication of potential liver damage [42], whereas the De Ritis ratio assesses the advancement of liver illness [43]. The long-term use of codeine in the research resulted in increased levels of liver damage indices (such as liver damage index, hepatic risk score, GGT/ALT, De Ritis ratio), suggesting an imbalance in the immune response and the development of numerous liver failures. The potential modulation of hepatic lipid metabolism by omega-3 fatty acid is proposed as the method utilised to avoid liver damage in rats treated with codeine for an extended period. This regulation leads to the decrease in hepatic damage indicators.

This study demonstrates that long-term administration of codeine leads to an increase in hepatic lipid peroxidation and oxidative DNA damage, as indicated by the elevated concentrations of MDA and 8-OHdG. This aligns with the prior research conducted by Akhigbe et al. [32]. During oxidative stress, the compound nitric oxide quickly interacts with an excess of superoxide, leading to inflammation of the tissue [44]. Omega-3 fatty acid supplementation reduced the intricate interaction between inflammatory mediators and indicators for oxidative stress in hepatic cells generated by long-term codeine ingestion. Prior research has established that the addition of omega-3 fatty acids reduces oxidative stress, as demonstrated by Avramovic et al [44]. and Heshmati et al. [45]. The potential method by which omega-3 fatty acids reduce oxidative stress may be attributed to their antioxidant properties, which in turn inhibit lipid peroxidation [46].

The pattern of elevated hepatic enzymes in codeine-treated rats correlates strongly with increased oxidative stress biomarkers observed in the study, such as elevated malondialdehyde (MDA) and reduced antioxidant enzyme activities (SOD, CAT, and GSH). These changes

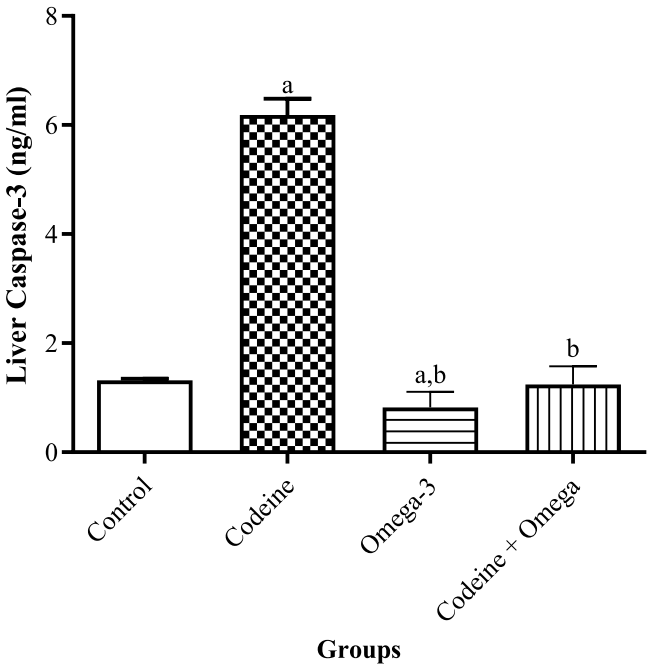

Fig. 6. Effect of Omega-3 fatty acid on the liver Caspase-3 activities in codeine exposed female rats. Values are means of five replicates measurements ± SD; bars carrying: a = significantly ($P < 0.05$) when compared to Control; b = significantly ($P < 0.05$) when compared to Codeine.

suggest that codeine-induced hepatotoxicity is mediated through oxidative damage, lipid peroxidation, and compromised antioxidant defense mechanisms. Conversely, the normalization of hepatic enzyme activities in omega-3-supplemented groups corresponds with improved redox status, reinforcing the protective antioxidant and membrane-stabilizing properties of omega-3 fatty acids in mitigating codeine-induced hepatic injury.

Codeine treatment in female rats resulted in elevation of hepatic lactate levels and the lactate-to-pyruvate ratio suggesting a metabolic shift toward anaerobic glycolysis, likely reflecting mitochondrial dysfunction and impaired oxidative phosphorylation in hepatic tissues. The accumulation of lactate indicates a disruption in the NAD^+/NADH redox balance, which may arise from codeine-induced oxidative stress and compromised mitochondrial enzyme activity [47]. The supplementation of Omega-3 fatty acids prevents the negative effects of codeine exposure on mitochondrial functions. The physiological redox buffering capability of cells involves the uptake of pyruvate and the secretion of lactate. Primarily, glycolysis ending in lactate is redox balanced. The absorption of pyruvate and the release of lactate lead to the overall removal of electrons. Physiologically, the kidneys detoxify lactate electrons produced by the liver while releasing pyruvate and thus maintain an oxidized circulating lactate to pyruvate ratio [48]. The lactate/pyruvate (L/P) ratio is considered a useful although not the final diagnostic tool in the evaluation of patients with possible disorders of mitochondrial metabolism [49].

The results of this study indicate that omega-3 fatty acid supplementation can mitigate liver cell death caused by codeine. Interestingly, omega-3 fatty acids or their metabolites have been shown to suppress cellular proliferation in other studies [50]. Furthermore, research has demonstrated a direct correlation between DHA treatment and reduced apoptosis, characterized by decreased caspase-3 activity [51], suggesting a potential mechanism for the protective effects of omega-3 fatty acids.

This study is limited by its use of a rat model, short treatment duration, and focus on biochemical parameters without detailed molecular or long-term analyses, which may restrict the direct translation of findings to humans. Additionally, the use of only female rats and the absence of extended post-treatment assessments may overlook possible sex-related and chronic effects of codeine and omega-3 co-administration.

Conclusion

Co-administration of omega-3 fatty acids effectively mitigated codeine-induced hepatic injury by enhancing antioxidant defense, suppressing inflammatory responses, and inhibiting apoptotic pathways. These findings suggest that omega-3 supplementation may provide a hepatoprotective advantage during prolonged opioid therapy, supporting hepatic metabolism and maintaining adipose tissue function while preventing oxidative and inflammatory liver damage. Further studies are warranted to elucidate the molecular signaling pathways through which omega-3 exerts these protective effects, particularly in relation to mitochondrial function, lipid metabolism, and gene expression. Additionally, long-term clinical and translational studies should be conducted to determine whether omega-3 supplementation can be safely and effectively integrated into chronic pain management protocols involving opioid medications.

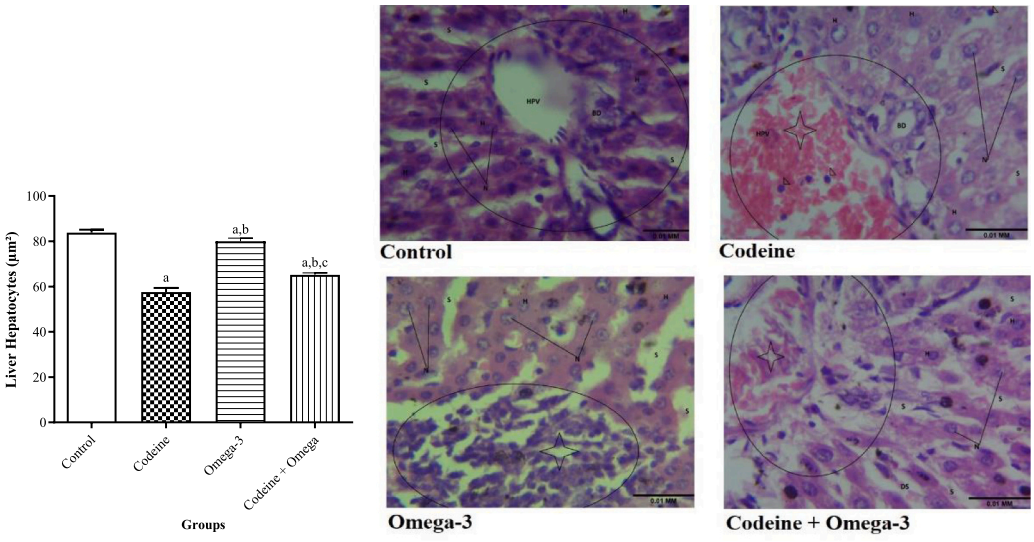

Fig. 8. Histomorphometric and Photomicrograph of the Liver Tissue (H & E stains) at × 400.

CRediT authorship contribution statement

Oladipupo Samuel Ebiwonjumi: Visualization, Methodology, Investigation, Formal analysis. **David Tolulope Oluwole:** Writing – review & editing, Writing – original draft, Software, Data curation, Conceptualization. **Hamed Moses Agbomhere:** Investigation, Formal analysis. **Ajayi Lydia Oluwatoyin:** Supervision, Project administration, Data curation. **Oke Deborah Adeola:** Writing – review & editing, Investigation, Conceptualization. **Ayodeji Folorunsho Ajayi:** Writing – review & editing, Validation, Supervision, Conceptualization. **Lawal Alimat Toyin:** Writing – original draft, Resources, Investigation.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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