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RESEARCH ARTICLE

OUTCOME OF AZITHROMYCIN AND MONOSODIUM GLUTAMATE ON HISTOLOGIC AND BIOCHEMICAL ALTERATIONS IN RATS' LIVER

Anthony Cemaluk Chinedum Egbuonu*, Prince Ogochukwu Alaebo, Obioma Benedeth Eze, Ngozi Kalu Achi, Chinomso Juliet Njoku
Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria.

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***Address for Correspondence:**
Dr. Anthony Cemaluk Chinedum Egbuonu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State Nigeria. Tel-+23480-3636-6565.
E-mail: *egbuonu.anthony@mouau.edu.ng*

Abstract

Background: Monosodium glutamate (MSG), a commonly used flavouring, mediated hepatotoxic and pro-inflammatory responses. Azithromycin (AZT), an antibiotic with anti-inflammatory activity, may be co-consumed with MSG to present unknown outcomes on the liver, a major organ for the detoxification of xenobiotics.
Aim: This study evaluated the effect of AZT and MSG on histologic and biochemical changes in rats' liver.
Methods: Thirty rats in five groups were for seven successive days orally exposed to groups 1, (distilled water 1 mL Kg^{-1}), 2, (MSG 8000 mg/kg), 3, overdose AZT, OAZT (AZT 412.5 mg Kg^{-1}), 4, therapeutic concentration AZT, TAZT, (AZT 82.5mg Kg^{-1}), and 5, (TAZT 82.5 mg Kg^{-1} + MSG 8000 mg Kg^{-1}). Liver function markers in therats' serum and liver tissue, and changes in the liver histologic were assessed by acceptable protocols. The mean of numeric data were tested for significance at *p* value less than 0.05 by analysis of variance (ANOVA).
Results: MSG treatment significantly ($p<0.05$) increased hepatic and serum alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), alkaline phosphatase activity (ALP), total bilirubin concentration (T-BIL), and direct bilirubin concentration (D-BIL) but decreased albumin concentration (ALB) compared to control and others. TAZT treatment caused a significant ($p<0.05$) decrease in these effects unlike MSG and OATZ treatments while TAZT + MSG co-treatment significantly ($p<0.05$) reversed these effects compared to MSG-treated rats. Rats' liver sections for rats in groups 2 and 3 showed severe hepatocellular degeneration and necrosis compared to others while those in group 4 (TAZT) showed the normal hepatic histo-architecture comparable to those in group 1 (control). Those in group 5 showed marked cellular swelling and leukocytic infiltration in only the centrilobular areas, suggesting active restorative responses.
Conclusion: Thus, TAZT significantly mitigated the compromised histologic and biochemical integrities of liver function due to MSG treatment in rats *via* probable normalization of their enzymatic and non-enzymatic indicators of liver function. This suggests that TAZT could be useful in managing histologic and biochemical malfunction of rats' liver due to MSG assault.
Keywords: Azithromycin, liver histology, monosodium glutamate, pro-inflammatory, xenobiotic detoxifications.

INTRODUCTION

Monosodium glutamateis a common flavouring used worldwide[1,2]. It mediates varied toxic manifestations, including diarrhea, headache, nausea, nephrotoxicity and notably hepatotoxicity[3-5]. It also mediates inflammation *via* up-regulation in body weight gain and activities that could promote inflammation[2,6-8]. Proinflammatory activities, including the release of cytokines known to promote the onset of inflammation, were recently implicated with the induction and progression of hepatotoxicity[9]. These suggest the overriding importance of inflammatory responses in the molecular mechanisms leading to hepatotoxicity.
Azithromycin (AZT) is an azalide-based antibiotic with broad activity. It can reduce inflammation. It can also reduce viral activities. These two properties exhibited by AZT could be useful in managing the complications of corona viral diseases[10,11]. Azithromycin has an excellent safety profile. It is inexpensive, widely available and accessible[12]. Hence, AZT may be easily procured. This could increase the chances of abusing

AZT. It could also increase the possibilities of co-consuming AZT with MSG to present unspecified outcomes on the liver. Outcomes following possible co-consumption of AZT with MSG may be significant. AZT is stable and lasts long in systemic circulation. It can easily cling to and accumulate in tissues, notably the liver tissue[11,13].

The liver tissue functions in inflammatory responses[14] and it is implicated in the metabolism of MSG and AZT. The liver is also a major organ for the detection, detoxification and elimination of xenobiotics, hence a prime target for toxicity manifestation following any xenobiotic assault[14,15]. AZT has anti-inflammatory activity[10,11] and recently Finelli[16] highlighted the implication of inflammation in the etiology of liver diseases related to hepatotoxicity and possible therapeutic efficacy of anti-inflammatory drugs against hepatotoxicity and associated liver diseases. Thus, this study evaluated the effect of AZT and MSG on histologic and biochemical changes in rats' liver.

MATERIALS AND METHODS

Chemicals and Drug

Azithromycin tablets (500 mg) were obtained from a reputable pharmaceutical company (Achina Foundation Pharmaceuticals Limited), *Ariaria* market Aba, Abia State, Nigeria. MSG was procured from foodstuff section of the market. Other chemicals used were obtained from acceptable sources and were certified to be analytical grade.

Animal study designs

The study used thirty (30) male rats (Wistar strain) with average weight 101 – 170 g. The rats were bought from the Department of Veterinary Medicine, University of Nigeria, Nsukkka and were kept to acclimatize for 1 week in the rats facility in the Department of Biochemistry of the host institution and randomly sampled to five groups each comprising six rats. Rats in group 1served as the control and received 1 mL Kg^{-1} of distilled water. Rats in group 2 (MSG) were given 8000 mg Kg^{-1} of MSG to induce toxicity as justified in a recent study[17]. Rats in group 3 (overdose of AZT, OAZT) were fed 412.5 mg $Kg^{-1}$of AZT (obtained as the product of therapeutic dose of AZT × 5). Group 4 (TAZT) rats were fed therapeutic dose of AZT (82.5 mg Kg^{-1}). Rats in group 5(therapeutic dose of AZT (TAZT) + MSG) received MSG (8000 mg Kg^{-1}) with therapeutic dose of AZT (82.5 mg Kg^{-1}). Treatment was *via* oral intubation daily for 7 successive days. The rats were maintained in cleaned rat cages under room temperature, daylight-dark cycle and tropical conditions. Rats were freely fed clean water and rat feed (Vital Feed Grower Marsh a product of Vital Feed Industries Limited, Nigeria.

Ethical issues and approval

The study adhered to standard ethical practice guidelines of the National Research Council, USA[18] with approval of the Animal Ethics Committee of the host department and institution (ACE-OFUS/16-960862021).

Blood collection and preparation

After overnight fast on expiration of the 7 days exposure period, rats were sacrificed by cervical dislocation. The rats' respective blood sample was collected into labeled plain centrifuge tubes *via* cardiac puncture procedure. The serum was removed from the blood sample clot *via* centrifugation after five minutes at rotor speed of 3000 per minute and preserved in a refrigerator pending measurement of the studied bioindicators of liver function.

Liver tissues collection and preparation

The rats' respective liver tissue sample was excised. A part was homogenized(using mortar and pestle) to grind a 0.5 g of the liver tissue sample in 5 ml of phosphate buffer saline (pH 7.2) and removing the supernatant after 10 minutes centrifugation at a rotor speed of 1000 g. The supernatant obtained was preserved in a refrigerator pending measurement of the studied bioindicators of liver function.

Tissue preparation for histological study

The other part of the liver tissue sample was embedded in 10 % phosphate formalin buffer for 48 hours until prepared for histologic evaluation according to the method described in a previous study[19]. Photo-micrograph of the liver histology was taken *via* a Motic 9.0 megapixels microscope camera which was at a magnifiction of × 400.

Determination of changes in measured rats' serum and liver tissue bioindicators of liver function

The ALT activity, AST activity, ALP activity, T-BIL concentration and ALB concentration were determined with Randox commercial Kits based on methods described as referenced earlier[20]. The D-BIL concentration was determined based on the principle that D-BIL couples with diazo reagent in the presence of sulfanilic acid to form a coloured azobilirubin with absorbance (as measured at 580 nm) in direct proportion with the concentration of the direct bilirubin[21].

Statistical analysis

Data analyses were by analysis of variance (ANOVA) followed by *post-hoc* multiple comparison test using statistical software Windows SPSS (16.0). Significant difference in mean was set at $p<0.05$. Results obtained from the data were represented as the mean ± standard deviation (SD) for 6 rats.

RESULTS

The results of the outcome of AZT and MSG on biochemical markers of liver integrity in rats' serum and in the liver tissue homogenate were as shown in Figure 1 to Figure 6. It was observed that MSG administration caused a significant ($p<0.05$) increase in hepatic and serum ALT activity, AST activity, ALP activity, T-BIL concentration, and D-BIL concentration but a decrease ($p<0.05$) in ALB concentration on comparison with the control and others. TAZT treatment elicited a significant ($p<0.05$) decrease in all the other measured biochemical markers of liver integrity (except ALB) in contrast to MSG and OATZ treatments. TAZT + MSG co-treatment significantly ($p<0.05$) decreased hepatic and serum ALT activity,

AST activity, ALP activity, T-BIL concentration, and D-BIL concentration but increased (p<0.05) ALB concentration compared to MSG treatment.

The histologic changes in rats' liver were as presented in Figure 7. Rats liver sections from group 1 (control) rats presented characteristic hepatic histology for laboratory rodents. They have normal hepatic lobules with hepatocytes that were radially arranged over the central veins (labeled V) and separated by sinusoidal spaces (slide 1A). There were mild traces of mononuclear inflammatory cells infiltrating into the portal area (P) (slide 1B).

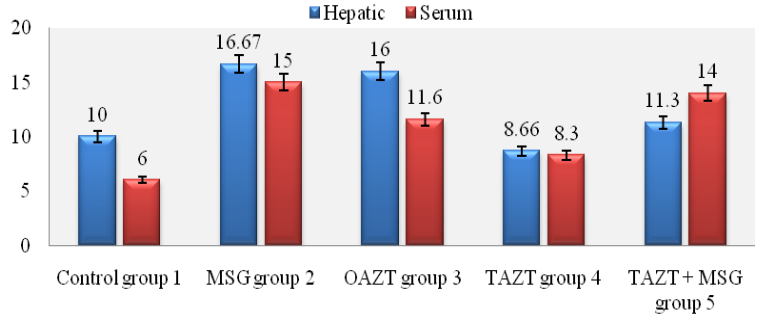

Figure 1: Effect of azithromycin and MSG on hepatic and serum ALT activity (IU/L).

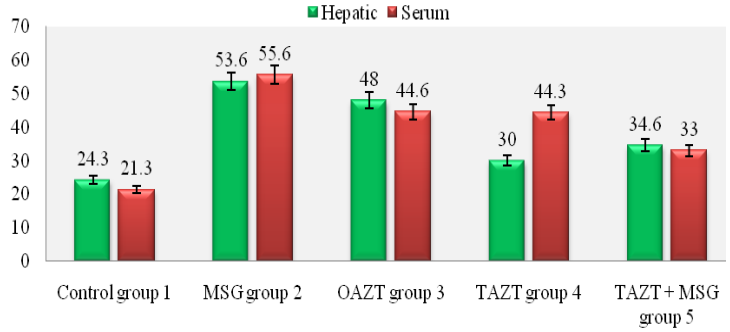

Figure 2: Effect of azithromycin and MSG on hepatic and serum AST activity (IU/L).

Liver sections from rats in group 4 (TAZT) as shown on slides 4A and 4Brepresented normal hepatic histo-architecture as those in group 1 (control), but with a mild hepatcellular necrosis and mononuclear cellular infiltration (arrow). In contrast to others, live histology for group 2 (MSG-treated) rats as on slides 2A and 2B, revealed hepatocellular degeneration of the liver lobules (depicted by arrow). Similarly, liver sections from rats in group 3 (OAZT) which were present on slide 3A, Figure 7, revealed hepatocellular degeneration of the hepatic lobules (arrow). Liver secions from rats in group 5 (TAZT+ MSG) revealed marked cellular swelling involving only the cells in the centrilobular areas of the hepatocytes (white arrow on slide 5A) and individual hepatocellular necrosis with leukocytic infiltration as well as moderate periportal infiltration of mononuclear leukocytes (black arrow on slide 5B).

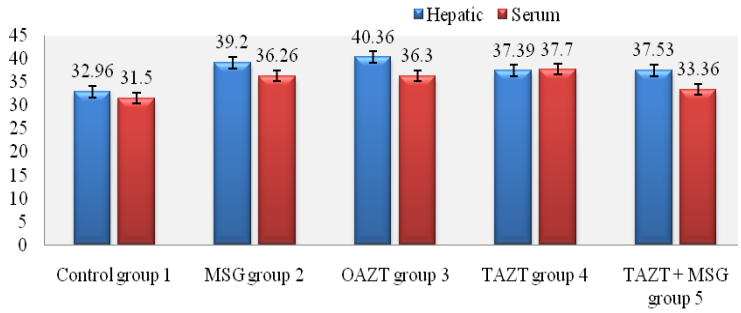

Figure 3: Effect of azithromycin and MSG on hepatic and serum ALP activity (IU/L).

DISCUSSION

Pro-inflammatory activity was implicated with the induction and progression of hepatotoxicity[9]. Monosodium glutamate (MSG) mediates hepato-toxicity[4] and inflammation[2,6-8] while AZT has anti-inflammatory activity[10,11]. The liver organ functions in inflammatory responses[14] and is prone to intoxication following any xenobiotic assault[14,15]. Thus, this study evaluated the effect of AZT and MSG on histologic and biochemical changes in rats' liver. MSG administration significantly (p<0.05) increased hepatic and serum ALT (Figure 1), AST (Figure 2), ALP (Figure 3), T-BIL (Figure 4), and D-BIL (Figure 5) but decreased ALB (Figure 6) compared to control and other.

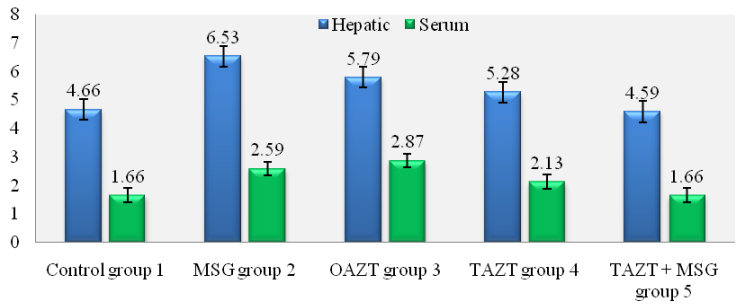

Figure 4: Effect of azithromycin and MSG on hepatic and serum T-BIL (µmol/L).

These demonstrated adverse outcomes on the biochemical markers of liver function in the rats following MSG administration and suggest that MSG hampered the functions of the rats' liver. In concordance, Omogbiya *et al.*[22], concluded onset of hepatotoxic event in MSG-treated mice which recorded an increase in ALT, AST, ALP, T-BIL and D-BIL levels but a decrease in ALB level as observed in this study. Also, in line with the present study increased activity of ALT (Figure 1) and AST (Figure 2) enzymes indicated hepatotoxicity in a rat model[23] while increased total bilirubin (Figure 4) and direct bilirubin (Figure 5) but decreased albumin (Figure 6) indicated hepatocellular damage resulting from impaired liver uptake, conjugation, metabolism and excretion of bilirubin (bound by albumin) leading to decreased albumin but increased (total and direct) bilirubin[24].

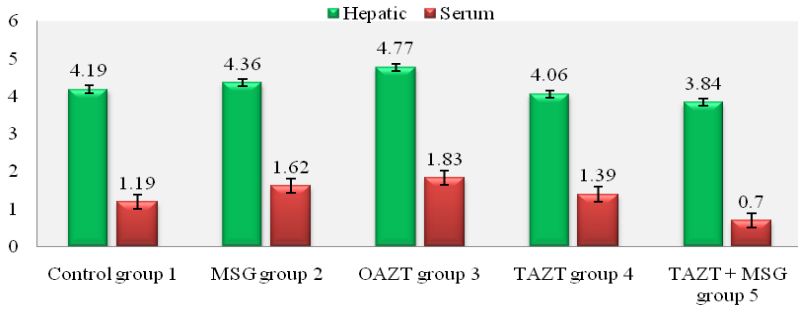

Figure 5: Effect of azithromycin and MSG on hepatic and serum D-BIL (µmol/L).

Generally, bilirubin is a product of the degradation of hemoglobin which is bound by albumin and transported to the liver where it becomes conjugated with glucuronic acid and excreted. Pathology-related damage to liver cells results to the release of most of these measured markers of liver integrity into the blood stream, leading to their increase in the serum in concord with the present observation and with recent report[25]. The MSG-induced hepatotoxicity herein may be *via*, or accompanied by, inflammation as suggested recently[8,9] warranting further studies.

In a recent review, Finelli[16] highlighted the implication of inflammation in the etiology of hepatotoxicity; the associated liver diseases, and the possible therapeutic efficacy of anti-inflammatory drugs against hepato-toxicity. This study therefore explored the possibility of TATZ playing a role in modulating MSG-induced effects.

Results revealed that TAZT treatment caused a significant ($p<0.05$) modulation of the present study on the measured bioindicators of liver integrity in comparison to MSG and OATZ treatments while TAZT + MSG co-treatment significantly ($p<0.05$) reversed these effects in contrast to MSG mono-treatment. These suggested the hepatoprotective activity of TATZ which was evidenced by the attenuation of MSG-induced down regulations in the expression of the measured liver enzymes (ALT (Figure 1), AST (Figure 2) and ALP (Figure 3); proteins (T-BIL (Figure 4), D-BIL (Figure 5) and ALB (Figure 6)) in both the liver tissue homogenate and in the serum[22].

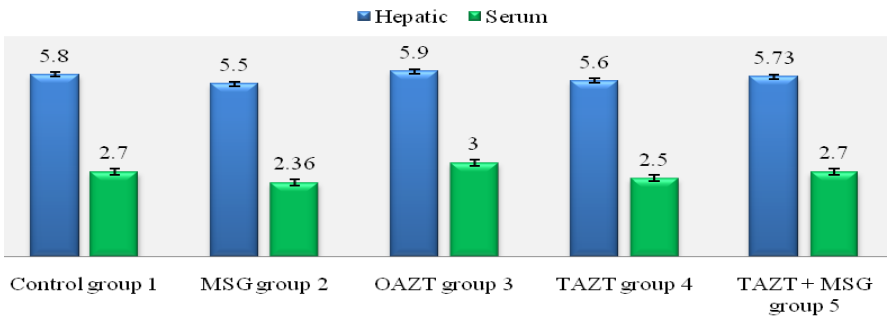

Figure 6: Effect of azithromycin and MSG on hepatic and serum ALB (mg/dl).

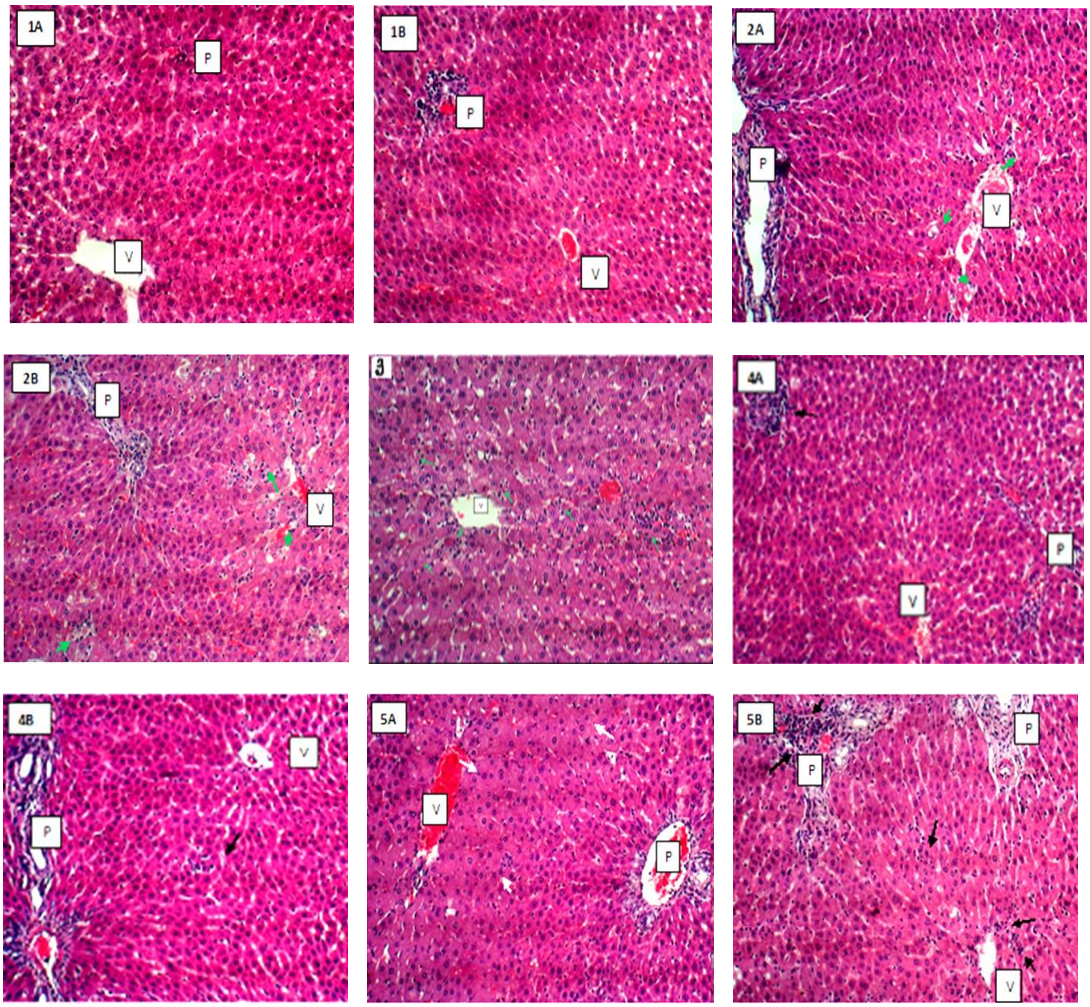

Figure 7: Effect of azithromycin and MSG on rats' hepatic histoarchitecture (H & E × 160).

Thus, it is probable that the anti-inflammatory activity of TAZT overwhelmed the pro-inflammatory potential of MSG. This is an important outcome that deserves further investigation to gain insight on the mechanistic roles of AZT in MSG intoxication in rats. Concordantly, liver sections of rats in group 2 and in group 3 (Figure 7) showed severe hepatocellular degeneration compared to others which confirmed the significant dysfunction in the liver integrity of rats exposed to 8000 mg Kg^{-1} of MSG, and to 412.5 mg Kg^{-1} a AZT in line with the report of Omogbiya *et al.*[22]. Sections of rats in group 4 (TAZT) showed the normal hepatic histo-architecture comparable to those in group 1 (control) highlighting the restorative capacity of TATZ on liver integrity while those in group 5 showed marked cellular swelling and leukocytic infiltration in only the centrilobular areas (Figure 7), suggesting active restorative responses by TATZ against MSG-induced hepatocellular degeneration. The apparent hepato-protective role of TAZT treatment against liver toxicity due to MSG mono-therapy reported in this study is as expected. This role may be related to the capacity of AZT to cling to and accumulate in the liver tissue where it last for a long time to exert anti-inflammatory activity and counteract pro-inflammatory activities and attendant liver damage caused by MSG mono-therapy[11,13]. This study was acute (lasting for only seven days) and was based on a sample size of six rats warranting more in-depth, larger sample sized and longer duration studies to confirm the restorative activity of TAZT against MSG intoxication effect in rats' liver.

Limitations of the study

The acute study design (seven days exposure) to align with the degree calendar limited this study. The high cost implications of using large number of rats also limited this study to a sample size of six rats.

CONCLUSIONS

Thus, TAZT significantly mitigated the compromised histologic and biochemical integrities of liver function due to MSG treatment in rats *via* probable normalization of their enzymatic and non-enzymatic indicators of liver function. This suggests that TAZT could be useful in managing histologic and biochemical malfunction of rats' liver due to MSG assault.

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AUTHORS' CONTRIBUTIONS

Egbuonu ACC: designed and supervised the study and reviewed the draft copy. **Alaebo PO:** co-supervised the study. **Achi NK:** drafting of manuscript. **Eze OB:** data analysis, report drafting. **Njoku CJ:** data analysis and interpretations. All the authors approved the finished version of the manuscript. Every author gave their approval to the manuscript's final draft.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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