**Reviewer’s Comments**

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

**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 dose AZT, TAZT, (AZT 82.5mg Kg−1), and 5, (TAZT 82.5 mg Kg−1 + MSG 8000 mg Kg−1). Liver function markers in the serum and in the liver tissue homogenate were determined and the histologic changes in the liver assessed by acceptable protocols. Numeric data were tested for the least significant difference in mean at p < 0.05 by one way analysis of variance (ANOVA) and the results presented as mean ± standard deviation (SD) for sample size, n =6 rats.

**Results:** MSG treatment significantly (p < 0.05) increased hepatic and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (T-BIL), and direct bilirubin (D-BIL) but decreased albumin (ALB)compared to control and others. TAZT treatment significantly (p < 0.05) decreased these effects compared to MSG and OATZ treatments while TAZT + MSG co-treatment significantly (p < 0.05) reversed these effects compared to MSG treatment. Rats’ liver sections rats’ groups 2 and 3showed 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 5showed 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; X

xenobiotic detoxifications

**INTRODUCTION**

Monosodium glutamate (MSG) is a common flavouring used the world over1,2. Itmediatesvaried toxic manifestations3-5 notably hepatotoxicity4. It also mediates inflammation*via* up-regulation in body weight gain2,6-8.Pro-inflammatory activities, including the release of pro-inflammatory cytokines, wererecentlyimplicated with the induction and progression of hepatotoxicity9, indicating the overriding importance of inflammatory responses in the molecular mechanism of hepatotoxicity.

Azithromycin (AZT),anazalide-based broad spectrum antibiotic,hasanti-inflammatory and antiviral activities that could be useful in managing the complications of corona viral diseases10,11. Azithromycin has an excellent safety profile. It is inexpensive, widely available and accessible12. Hence, AZT may be easily procured and abused or be co-consumed with MSG to present unknown outcomes on the liver. Outcomes following co-consumption of AZT with MSG may be significant. AZT has a long half-life and a high affinity to penetrate and accumulate in tissues, notably the liver tissue11,13.

The liver tissue functions in inflammatory responses14 implicated in the metabolism of MSG and AZT.The liver is also a major organ for the detection, detoxificationand elimination of xenobiotics, hence a prime target for toxicity manifestation following any xenobiotic assault14,15. AZT has anti-inflammatory activity10,11 and recently Finelli16 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 were of certified analytical grade.

**Animals and treatments**

The animals used in this study were thirty (30) adult male Wistar rats with average weight 101 – 170 g. The animals were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukkka. They were kept in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria for 1 week to acclimatize and randomly sampled to five groups of six rats each. Group 1rats were given distilled water (1 mL Kg−1) and served as the control group, whereas group 2 (MSG) rats were given intoxicating dose of MSG (8000 mg Kg−1)as in Egbuonu&Oriji17. Group 3 (OAZT) rats were fed overdose (therapeutic dose × 5) of AZT (412.5 mg Kg−1). Group 4 (TAZT) rats were fed therapeutic dose of AZT (82.5 mg Kg−1) whereas group 5(TAZT + MSG) rats received MSG (8000 mg Kg−1) with therapeutic dose of AZT (82.5 mg Kg−1). Treatment was by daily oral intubation for 7 successive days. The rats were housed in cleaned stainless steel cages at room temperature (28±2 0C), 12 h light/dark cycle and humid tropical conditions. Animals were provided with rat feed (Vital Feed Growers Marsh containing 20 % crude protein and 280 kcal 100−1 g metabolizable energy, manufactured by Vital Feed Industries Limited, Nigeria) and portable (tap) water *ad libitum* for the duration of the experiment. The study adhered to standard ethical practice guidelines of the National Research Council, USA18with approval of the Animal Ethics Committee of the host department and institution (ACE-OFUS/16-960862021).

**Blood and liver tissues collection and preparation**

The rats were sacrificed following cervical dislocation 24 h after 7 days treatment. Their blood samples were collected individually using sterile capillary tubes into properly labeled plain polystyrene centrifuge tubes by cardiac puncture technique. The collected blood samples were allowed to clot. The serum was removed by centrifugation at 3000 rotor per minute (rpm) for 5 minutes, collected individually and stored in a deep freezer for determination of the serum bioindicators of liver function namely ALT, AST, ALP, T-BIL, D-BIL and ALB.Liver tissue samples of the rats as sacrificed above were excised individually and shared into two parts. A part was homogenized. In brief, 10 % of the respective organ homogenate was obtained by separately grinding a 0.5 g of respective organ sample in 5 ml of phosphate buffer saline (pH 7.2), using mortar and pestle. The supernatant was removed by centrifugation at 1000 g for 10 minutes, collected individually and stored in a deep freezer for determination of liver homogenate bioindicators of liver function namely ALT, AST, ALP, T-BIL, D-BIL and ALB. The other part was fixed in 10 % phosphate buffered formalin (formal-saline) for 48 hours until prepared for histologic evaluation according to the method described by Egbuonu & Ejike19.Photomicrographs were taken using a Motic 9.0 megapixels microscope camera at × 400 magnifications.

**Determination of changes in rats’ serum and liver homogenate bioindicators of liver function (ALT, AST, ALP, T-BIL, D-BIL and ALB)**

The ALT, AST, ALP, T-BIL and ALB levels were respectively determined with Randox commercial Kits based on methods described as referenced earlier20. The D-BIL level was determined based on the principle that D-Bil couples with diazo reagent in the presence of sulfanilic acid to form a colouredazo bilirubin with absorbance as measured at 580 nm directly proportional to the concentration of the direct bilirubin21.

**Statistical analysis**

All analyses were performed by one way analysis of variance (ANOVA), using the statistical package for social sciences (SPSS) for windows version 16.0 package. The least significant difference test was used for the *post-hoc* multiple comparison of means. Differences in mean were considered significant at p < 0.05. The results were presented as mean ± standard deviation (SD) for 6 rats.

**RESULTS AND DISCUSSION**

The results of the effect of AZT and MSG on the biochemical markers of liver function in rat’ serum and in the liver tissue homogenate were as shown in Figures 1 to 6. The results revealed that MSG treatment significantly (p < 0.05) increased hepatic and serum ALT, AST, ALP, T-BIL, and D-BIL but decreased (p < 0.05) ALB compared to control and others. TAZT treatment significantly (p < 0.05) decreased these indicators (except ALB) compared to MSG and OATZ treatments. TAZT + MSG co-treatment significantly (p < 0.05) decreased hepatic and serum ALT, AST, ALP, T-BIL, and D-BIL but increased (p < 0.05) ALB compared to MSG treatment.

**Figure 1: Effect of azithromycin and MSG on hepatic and serum ALT activity (IU/L) (Each bar represents Mean + SD; n = 6)**

**Figure 2: Effect of azithromycin and MSG on hepatic and serum AST activity (IU/L) (Each bar represents Mean + SD; n = 6)**

**Figure 3: Effect of azithromycin and MSG on hepatic and serum ALP activity (IU/L) (Each bar represents Mean + SD; n = 6)**

**Figure 4: Effect of azithromycin and MSG on hepatic and serum T-BIL (µmol/L) (Each bar represents Mean + SD; n = 6)**

**Figure 5: Effect of azithromycin and MSG on hepatic and serum D-BIL (µmol/L) (Each bar represents Mean + SD; n = 6)**

**Figure 6: Effect of azithromycin and MSG on hepatic and serum ALB (mg/dl) (Each bar represents Mean + SD; n = 6)**

The histologic changes in rats’ liver were as presented in Figure 7.Sections of the liverof rats in group 1 (control)showed the normal hepatic histomorphology for laboratory rodents. They have numerous normal hepatic lobules which contain normal hepatocytes. These hepatocytes were arranged in radiating interconnecting cords around the central veins (V). Hepatic cords were separated by normal sized sinusoidal spaces and normal structures of the portal triads (hepatic vein, hepatic artery and bile ducts)] (slide 1A) but with mild periportal infiltration of mononuclear inflammatory cells in the portal area (P) (slide 1B).

Sections of the liver of rats in group 4 (TAZT) (Slides 4A and 4B) showed the normal hepatic histo-architecture as those in group 1 (control), but with a few multifocal areas hepatcellular necrosis and varying amounts of mononuclear cellular infiltration (arrow). Central vein (V); Portal area (P).

Compared to others, sections of the liver of rats in group 2 (MSG) showed hepatocellular degeneration and necrosis, involving the hepatocytes in the centrilobular and mid-zonal areas of the hepatic lobules (arrow) (slides 2A and 2B). Central vein (V); Portal area (P). Similarly, sections of the liver of rats in group 3 (OAZT) (slide 3A) showed hepatocellular degeneration and necrosis, involving the hepatocytes in the centrilobular areas of the hepatic lobules (arrow). Central vein (V).

Secions of the liver of rats in group 5 (TAZT+ MSG) showed marked cellular swelling involving only the cells in the centrilobular areas of the hepatocytes (white arrow) (slide 5A) and individual hepatocellular necrosis with leukocytic infiltration as well as moderate periportal infiltration of mononuclear leukocytes (black arrow). Central vein (V); Portal area (P) (slide 5B).

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**Figure 7: Effect of azithromycin and MSG on rats’ hepatichistoachitecture (H & E × 160)**

**DISCUSSION**

Pro-inflammatory activity was implicated with the induction and progression of hepatotoxicity9.Monosodium glutamate (MSG) mediates hepatotoxicity4 and inflammation2,6-8 while AZThas anti-inflammatory activity10,11. The liver organ functions in inflammatory responses14 and is prone to intoxication following any xenobiotic assault14,15. Thus, this study evaluated the effect of AZT and MSG on histologic and biochemical changes in rats’ liver. MSG treatment significantly (p < 0.05) increased hepatic and serum ALT, AST, ALP, T-BIL, and D-BIL but decreased ALB compared to control and others. These demonstrated adverse outcomes on the biochemical markers of liver function in the rats following MSG induction, suggesting that MSG hampered the functions of the rats’ liver. Concordantly, Omogbiya*et al*.22 concluded onset of hepatotoxic event in MSG-treated mice which recorded increased ALT, AST, ALP, T-BIL and D-BIL but decreased ALB as in this study.Also, increased ALT and AST enzymes indicated hepatotoxicity in a rat model23while increased total bilirubin and direct bilirubin but decreased albumin indicated hepatocellular damage resulting from impaired liver uptake, conjugation, metabolism and excretion of bilirubin (bound by albumin) leading to decreased albumin but increased bilirubin recorded herein24. 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. Pathological damage to the hepatocytes releases these liver markers into the blood circulation, leading to their increase in the serum also observed in this study which is consistent with recent report25. The MSG-induced hepatotoxicity herein may be *via*, or accompanied by, inflammation as suggested recently9-8 warranting further studies.

In a recent review, Finelli16 highlighted the implication of inflammation in the etiology of hepatotoxicity as well as the associated liver diseases and the possible therapeutic efficacy of anti-inflammatory drugs against hepatotoxicity. This study therefore explored the possibility of TATZ in modulating MSG-induced effects. Results revealed that TAZT treatment significantly (p < 0.05) decreased these effects compared to MSG and OATZ treatments while TAZT + MSG co-treatment significantly (p < 0.05) reversed these effects compared to MSG treatment. These suggested the hepatoprotective activity of TATZ evidenced by the attenuation of MSG-induced deregulations of liver enzymes (ALT, AST and ALP) and proteins (T-BIL, D-BIL and ALB) in both the liver homogenate and serumsamples22. Thus, it is probable that the anti-inflammatory activity of TAZT overwhelmed the pro-inflammatory potential of MSG leading to the observed mitigation of MSG-induced effects in the rats. 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 groups 2 and 3 showed severe hepatocellular degeneration and necrosis compared to others which further confirmed the hepatotoxicity effect of high doses of MSG treatment (and also by OATZ treatment)in rats 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, suggesting active restorative responses by TATZ against MSG-induced hepatocellular degeneration. This hepatoprotective activity of TAZT may be related in part to its acclaimed anti-inflammatory activity, long half-life as well as high affinity to penetrate and accumulate in the liver tissue11,13. This study was acute (lasting for only seven days) warranting more in-depth and longer duration studies to confirm the restorative activity of TAZT against MSG intoxication effect in rats’ liver.

**Limitations of the study**

**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.

**Authors’ Contributions:** Egbuonu A.C.C. and Alaebo P.O. designed and supervised the study. Njoku C.J. and Eze O.B., assembled the draft manuscript. All authors read and approved the final version of the manuscript.

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**REFERENCES**

1. El-Gendy MS, El-Gezawy ES, Saleh AA, Alhotan RA, Al-Badwi MAA, Hussein EOS, El-Tahan HM, Kim IH, Cho S, Omar SM. Investigating the chemical composition of lepidiumsativum seeds and their ability to safeguard against monosodium glutamate-induced hepatic dysfunction. Foods 2023; 12; 4129. https://doi.org/10.3390/foods12224129.

2. Wuyt AK, Nguelefack-Mbuyo EP, Fofie CK, Nguelefack TB. The methanol extract of *Ceibapentandra*reverses monosodium glutamate-induced cardiometabolic syndrome in rats *via* the regulation of dyslipidemia, inflammation, oxidative stress, and insulin sensitization. Heliyon 2023; 9(2023) e13689: 1-16.

3. Obi E, Egbuonu ACC. Changes in the liver histomorphology, catalase and glutathione peroxidase activity in the serum and liver homogenate of normal and monosodium glutamate-intoxicated rats co-treated with artemether-lumefantrine. International Journal of Molecular Biology Open Access 2019; 4(2): 67-73. doi: 10.15406/ijmboa.2019.04.00099.

4. Joshi DM, Dhurvey VT, Katke SR, Pawar HB, Mohurle PM. Effect of monosodium glutamate on hepatotoxicity and nephrotoxicity: A mini review. World Journal of Biology Pharmacy and Health Sciences 2023; 15(01): 152-159. doi: https://doi.org/10.30574/wjbphs.2023.15.1.0318.

5. Yang L, Gao Y, Gong J, Peng L, El-Seedi HR, Farag MA, Zhao Y, Xiao J. A multifaceted review of monosodium glutamate effects on human health and its natural remedies. Food Materials Research 2023; 3: 16. https://doi.org/10.48130/FMR-2023-0016.

6. Casado ME, Collado-Pérez R, Frago LM, Barrios V. Recent advances in the knowledge of the mechanisms of leptin physiology and actions in neurological and metabolic pathologies. Int. J. Mol. Sci. 2023;24: 1422. https://doi.org/10.3390/ijms24021422.

7. Correia AS, Cardoso A, Vale N. (2023). Oxidative stress in depression: the link with the stress response, neuro-inflammation, serotonin, neurogenesis and synaptic plasticity. Antioxidants 2023; 12: 470. https://doi.org/10.3390/antiox12020470.

8. Kıran TR, Otlu O, Karabulut AB. Oxidative stress and antioxidants in health and disease. Journal of Laboratory Medicine 2023; 47(1): 1-11. doi:10.1515/labmed-2022-0108.

9. Luo G, Huang L, Zhang Z. The molecular mechanisms of acetaminophen-induced hepatotoxicity and its potential therapeutic targets. Exp. Biol. Med. 2023; 248: 412-424. doi: 10.1177/15353702221147563.

10. Oliver ME, Hinks TSC. (2021). Azithromycin in viral infections. Rev Med Virol. 2021; 31(2): e2163. doi:10.1002/rmv.2163.

11. Ismael ZM, Elsamman WN. Evaluation of the effects of azithromycin on the kidney of adult albino rats and the possible protective role of vitamin c using histological and immuno-histochemical studies. SVU-International Journal of Medical Sciences 2022; 5(2): 484-500.

12. O’Brien KS, Emerson P, Hooper PJ.et al. (2019). Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review. Lancet Infect. Dis. 2019; 19(1): e14-e25. doi:10.1016/S1473-3099(18)30444-4.

13. Fohner AE, Sparreboom A, Altman R.B, Klein TE. PharmGKB summary: Macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics. Pharmacogenet. Genomics 2017; 27(4): 164-167.

14. Barouki R, Samson M, Blanc EB, Colombo M, Zucman-Rossi J, Lazaridis KN, Miller GW, Coumoul X. The exposome and liver disease - how environmental factors affect liver health, Journal of Hepatol. 2023; 79(2): 492-505. https://doi.org/10.1016/j.jhep.2023.02.034.

15. Gu X, Jose E. Manautou JE. Molecular mechanisms underlying chemical liver injury. Expert Rev. Mol. Med. 2012; 14 e4: 1-25. doi:10.1017/S1462399411002110.

16. Finelli C. Molecular mechanisms and mediators of hepatotoxicity resulting from an excess of lipids and non-alcoholic fatty liver disease. Gastrointestinal Disorder 2023; 5: 243–260. https://doi.org/10.3390/gidisord5020020.

17. Egbuonu ACC, Oriji SO. Pulverized *Mangiferaindica* (mango) seed kernel mitigated monosodium glutamate-intoxicated rats’ kidney histology and bio-functions. Journal of Nutritional Health and Food Science 2017; 5(2): 1-7. doi: http://dx.doi.org/10.15226/jnhfs.2016.00192.

18. National Research Council, NRC. Guide for the Care and Use of Laboratory Animals. 8th Edition, Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011; Washington DC, USA: National Research Council, National Academies Press.

19. Egbuonu ACC, Ejike GE.Effect of pulverized *Mangiferaindica*(mango) seed kernel on monosodium glutamate-intoxicated rats’ serum antioxidant capacity, brain function and histology. EC Pharmacology and Toxicology 2017; 4(6): 228-243.

20. Egbuonu A.C.C. Opara CI, Akachukwu D, Onyedikachi UB. Effect of ethanolic extract of avocado pear (*Perseaamericana*) seed on normal and monosodium glutamate-compromised rats’ hepatic histo-morphology and serum bio-functional parameters. Research Journal of Environmental Sciences 2018; 12(2): 53-62. doi: 10.3923/rjes.2018.53.62.

21. Egbuonu ACC. Effect of some antihypertensives on the serum bilirubin concentration of male Wistar rats. *Journal of Pharmacy and Pharmacological Research 2010;* 1(1): 009-012.

22. Omogbiya AI, Ben-Azu B, Eduviere AT, Eneni AO, Nwokoye PO, Ajayi AM, Umukoro S. Monosodium glutamate induces memory and hepatic dysfunctions in mice: ameliorative role of Jobelyn® through the augmentation of cellular antioxidant defense machineries. Toxicol. Res. 2021; 37: 323-335. https://doi.org/10.1007/s43188-020-00068-9.

23. Obasi CN, Maagbo LJ, Mgbahurike AA. Hepatotoxicity effects of Paracetamol-Denik Cleanser® co-administration. Universal Journal of Pharmaceutical Research 2022; 7(6):46-50.

doi: https://doi.org/10.22270/ujpr.v7i6.869.

24. Guerra-Ruiz AR, Crespo J, Martínez RML, Iruzubieta P, Mercadal GC, Garcés ML, Lavin B, Ruiz MM. (2021). Measurement and clinical usefulness of bilirubin in liver disease. Adv. Lab. Med. 2021; 2(3): 352-361. https://doi.org/10.1515/almed-2021-0047.

25. Johra FT, Hossain S, Jain P, Bristy AT, Emran T, Ahmed R, Sharker SM, Bepari AK, Reza HM. Amelioration of CCl4‑induced oxidative stress and hepatotoxicity by *Ganodermalucidum*in Long Evans rats. Sci. Rep.2023; 13:9909: 1-13. https://doi.org/10.1038/s41598-023-35228-y.