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Therapeutic Azithromycin Mitigated Monosodium Glutamate-Related Dysfunction in Rats' Body Weight and Serum, Liver, Kidney and Heart Antioxidant Defense Bioindicators

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Abstract

Monosodium glutamate (MSG) mediates body weight gain (BWG) and oxidative stress. Azithromycin (AZT), may be abused and co-consumed with MSG to present unknown outcomes on BWG and oxidative stress. This study evaluated the effect of AZT and MSG in rats' BWG and antioxidant bioindicators. Thirty rats assigned to five groups were orally exposed for seven consecutive days to groups A, control (distilled water, 1 ml/kg), B, MSG (MSG 8000 mg/kg), C, therapeutic AZT, TAZ (AZT 82.5 mg/kg), D, overdose AZT, OAZ (AZT 412.5 mg/kg) and E, TAZ + MSG (AZT 82.5 mg/kg + MSG 8000 mg/kg). MSG-treated rats exhibited a significantly ($p < 0.05$) increased BWG; serum, liver, kidney and heart reduced glutathione (GSH), glutathione peroxidase (GP_X), superoxide dismutase (SOD), and malondialdehyde (MDA) but decreased catalase (CAT) and zinc (Zn) levels compared to control. Co-treated TAZ + MSG rats significantly ($p < 0.05$) decreased BWG, GSH, GP_X, SOD, Zn; increased CAT and non-significantly ($p > 0.05$) decreased MDA compared to MSG and control. Thus, TAZ significantly mitigated BWG, and malfunction in the metabolism of antioxidant defense bioindicators in MSG rats *via* probable anorexigenic, anti-inflammatory and antioxidant responses. This suggests that TAZ could be useful in managing MSG-related dysfunction in BWG and metabolic activity of the antioxidant defense apparatus in rats.

Keywords: Monosodium glutamate; Azithromycin; Organ toxicity; Oxidative stress; Antioxidants; Body weight gain.

Abbreviations: ANOVA = Analysis of variance, AZT = Azithromycin, BWG = Body weight gain, CAT = Catalase, GSH = Reduced glutathione, GPX = Glutathione peroxidase, MDA = Malondialdehyde, MSG = Monosodium glutamate, OAZ = Overdose azithromycin, ROS = Reactive oxygen species, Rpm = rotor per minute, SD= Standard deviation, SOD = Superoxide dismutase, SPSS = statistical package for social sciences, TAZ = Therapeutic azithromycin, Zn = Zinc.

INTRODUCTION

In the peak of the outbreak of the pandemic disease (viral pneumonia) caused by the 2019-novel coronal virus (2019-nCoV, COVID-19), the management was based mainly on scanty information (Schünemann et al., 2019). Initial studies indicated good improvement following inclusion of AZT in the treatment of COVID-19 (Gautret et al., 2020) but some randomized clinical trials reported otherwise (Principle Trial Collaborative Group, 2021; Recovery Collaborative Group, 2021). Azithromycin, an azalide subclass of macrolides, is a broad spectrum antibiotic with antiviral and anti-inflammatory potencies that is recently exploited in the treatment of COVID-19 (Ismael and Elsamman, 2022). The broad-spectrum antibacterial activities of AZT is related to its mechanism of action that inhibits protein synthesis of bacteria (by binding to the 50S subunit of the bacterial ribosome to inhibit translation of mRNA) and prevent their growth (Dinos, 2017). Azithromycin is inexpensive, widely available, and with an excellent safety profile but is prone to selection for macrolide resistance following abuse (O'Brien et al., 2019). AZT has a long half-life duration and a high affinity to tissue penetration and accumulation (Fohner et al., 2017). The largest tissue concentration of AZT was observed in the liver, followed by the kidney and lastly the heart. And, the toxic manifestation in these organs due to AZT was associated to its direct effect and that of its metabolites on ribosomal functions and long duration of the derivatives in the organs (Ismael and Elsamman, 2022). It was reported that AZT can increase the risk of cardiovascular

complications leading to death (Bergami et al., 2023) while the AZT-induced nephrotoxicity can be reduced *via* antioxidant and anti-inflammatory pathways (Ismael and Elsamman, 2022).

It was hypothesized that AZT could exert activity against the Covid-19-related disease (Oliver and Hinks, 2021) which may result to increased demand for, use and abuse of, AZT; and possibly co-consumed with MSG. Monosodium glutamate is a commonly used flavour enhancer worldwide (El-Gendy et al., 2023; Wuyt et al., 2023). It is known to mediate enhanced BWG, organ toxicity (El-Gendy et al., 2023; Joshi et al., 2023; Yang et al., 2023) and oxidative stress (Obi and Egbuonu, 2019). It was reported that MSG interfered with the function of the peptide hormone, leptin, in the hypothalamus to mediate orexigenic activity (increased appetite) that could lead to increased body weight and metabolic syndrome (Yang et al., 2023). Suppression of leptin expression in the hypothalamus decreased energy expenditure and spiked the drive to eat leading to increased energy balance and body weight gain (Martelli and Brooks, 2023; Obradovic et al., 2021). Oxidative stress is known to increase inflammation and to underlie the onset and development of many health challenges (Correia et al., 2023; Kiran et al., 2023; Wuyt et al., 2023) whereas increased BWG leading to obesity is frequently associated with inflammation and other diseases, including cancer, diabetes and non-alcoholic fatty liver (Casado et al., 2023). The body employs antioxidant defense mechanism to combat free radicals associated with oxidative stress and also to decrease inflammation (Ismael and Elsamman; 2022; Correia, et al., 2023). Oxidative stress contributed significantly to MSG-related toxic effects and eventual cellular and organ-functional damage in animals as assessed by antioxidant defense system markers, including GSH, GP_X, SOD, CAT, Zn and MDA (Egbuonu et al., 2021; Correia, et al., 2023). Concomitant intake of MSG and AZT may present with unknown effect on the BWG and antioxidants metabolism function of the animals which warranted this study. This study aimed to evaluate the effect of AZT and MSG on BWG and antioxidant defense bioindicators in rats' serum, liver, kidney and heart.

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 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 assigned to five groups of six rats each. Group A rats, control, were given distilled water (1 mL Kg^{-1}). Group B rats were fed therapeutic dose of AZT (82.5 mg Kg^{-1} body weight), whereas group C rats were fed overdose (therapeutic dose × 5) of AZT (412.5 mg Kg^{-1}). Group D rats were given intoxicating dose of MSG (8000 mg Kg^{-1}) according to Mariyama et al. (2009) whereas group E rats received MSG (8000 mg Kg^{-1}) with therapeutic dose of AZT (82.5 mg Kg^{-1} body weight). Treatment was by daily oral intubation for 7 successive days. The rats were housed in cleaned stainless steel cages at room temperature (28±2 ^{0}C); 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 with approval of the Animal Ethics Committee of the host department and institution (ACE-OFUS/16-960862021).

Blood and tissues collection and preparation

Blood samples of the rats sacrificed following cervical dislocation 24 h after 7 days treatment were collected individually using sterile capillary tubes into properly labeled plain polystyrene centrifuge tubes by cardiac puncture technique. The blood samples thus collected were allowed to clot. Then, 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, antioxidant status bioindicators namely GSH, GP_X, SOD, CAT, Zn and MDA. Tissue samples (liver, kidney and heart) of the rats as sacrificed above were excised individually and 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, kidney and heart homogenate antioxidant status indicators namely GSH, GP_X, SOD, CAT, Zn and MDA.

Determination of changes in rats BWG, and serum, liver, kidney and heart antioxidant defense bioindicators (GSH, GPX, SOD, CAT, Zn and MDA)

The rats' body weight gain (body weight change) was calculated as the difference in the measured initial and

final body of the rats. The percentage body weight gain (body weight change) was computed as a fraction of the corresponding initial body weight multiplied by 100. The GSH concentration was determined with Randox kit based on the method of Goldberg and Spooner (1983) as described recently (Egbuonu and Elendu, 2021). The GP_X activity was determined by the method of Paglia and Valentine (1967) reported recently (Egbuonu et al., 2021). This was based on the principle that glutathione peroxidase catalyzes the oxidation of reduced glutathione. As described recently in Egbuonu and Elendu (2021), the SOD activity was assayed by the method of Madesh and Balasubramanian (1998); the CAT activity was assayed by the method of Johansson and Borg (1988) while the MDA concentration was determined by the method of Wallin et al. (1993). The Zn concentration was estimated by the method of Johnsen and Eliasson (1987) as reported recently (Egbuonu et al., 2021).

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

Rats in MSG group had the highest while those in OAZ group had the least followed by TAZ and MSG + TAZ ($p < 0.05$) body weight increase compared to control and the other groups (Figure 1).

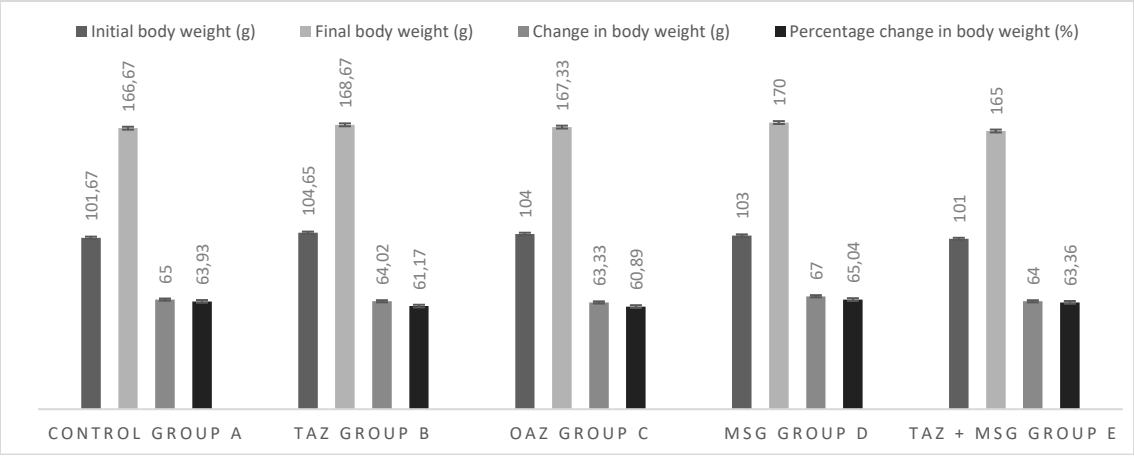


Figure 1. Effect of AZT and MSG in rats' BWG (Each bar represents Mean + SD; n = 6).

As shown in Figure 2, GSH concentration in the serum liver, kidney and heart was highest ($p < 0.05$) in MSG group and least in these organs and in the serum in TAZ + MSG group compared to control and others. Similarly, GP_X activity in the serum, liver, kidney and heart was highest ($p < 0.05$) in MSG group and least in these organs and in the serum in TAZ + MSG group compared to control and others (Figure 3).

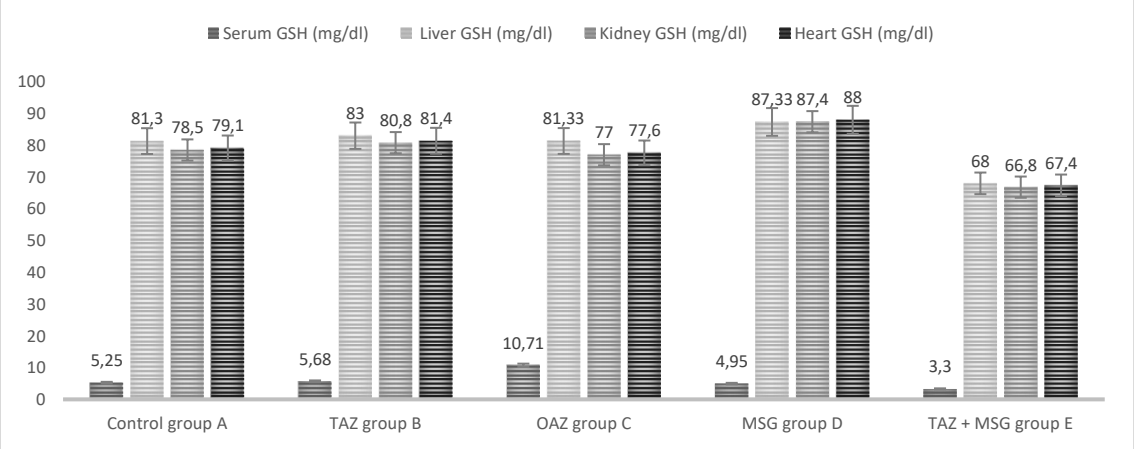
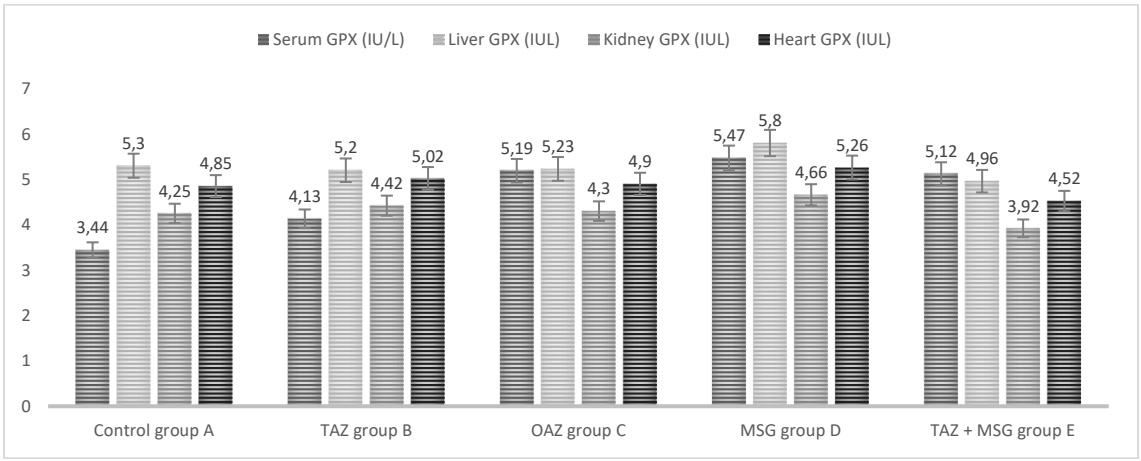
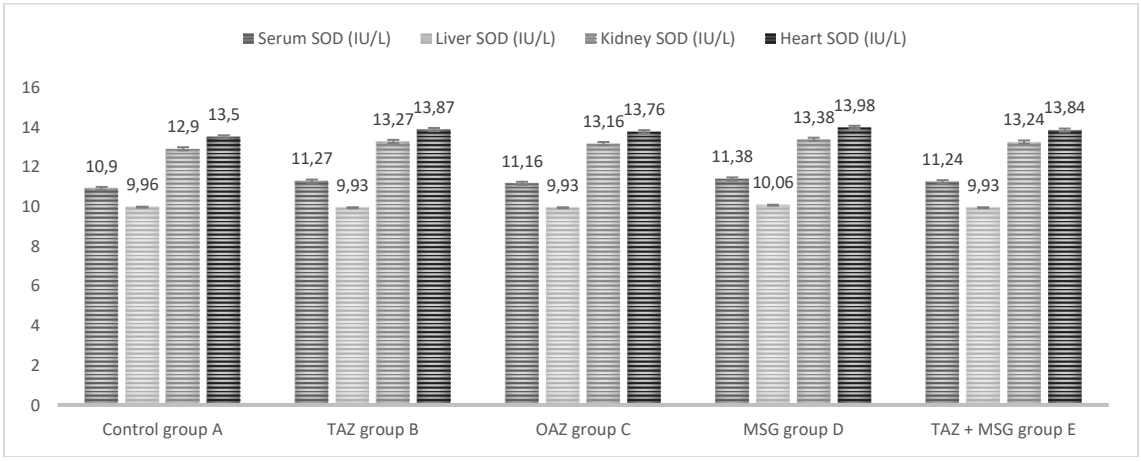


Figure 2. Effect of AZT and MSG on GSH (mg/dl) concentration in rats' serum, liver, kidney and heart (Each bar represents Mean + SD; n = 6).



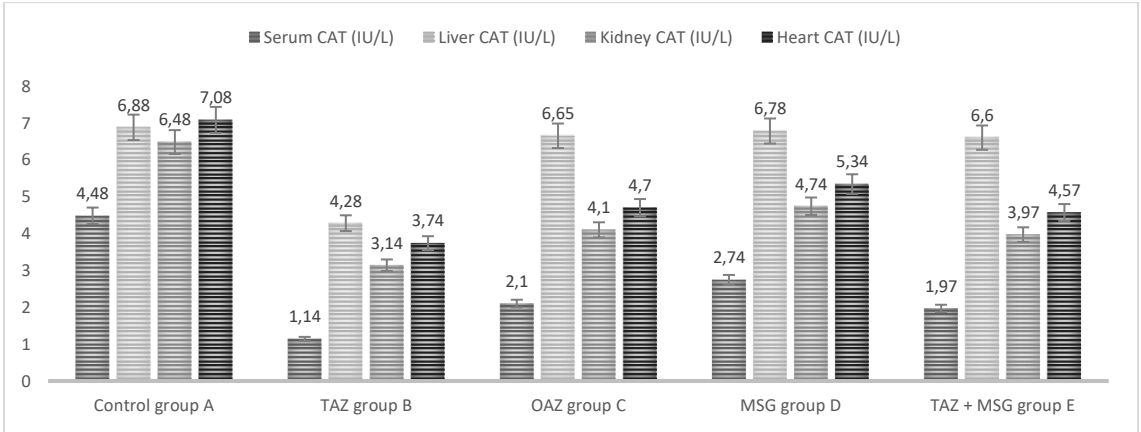
(Each bar represents Mean + SD; n = 6)
Figure 3. Effect of AZT and MSG on GP_X (IU/L) activity in rats' serum, liver, kidney and heart.

As depicted in Figure 4, SOD activity in the serum, liver, kidney and heart was highest ($p < 0.05$) in MSG group but least in TAZ, OAZ and TAZ + MSG group compared to control.

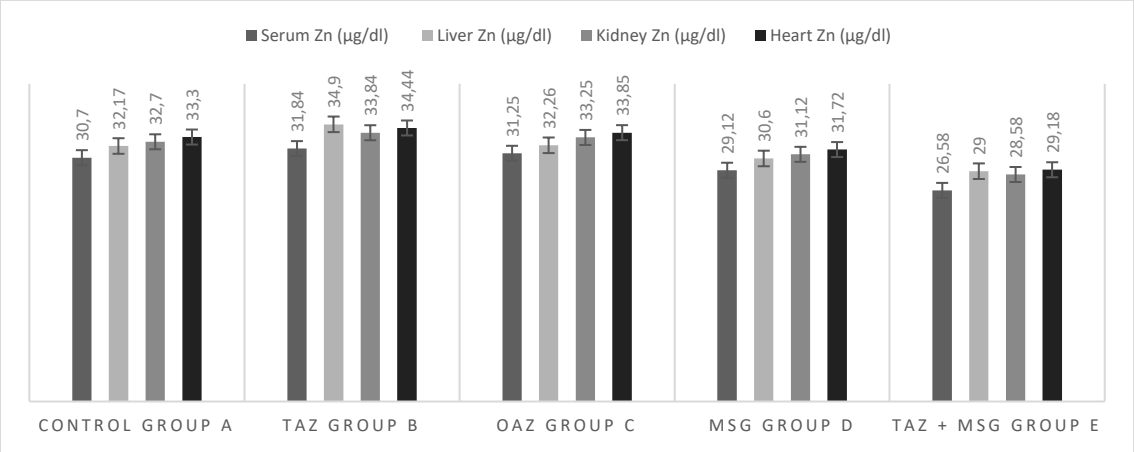


(Each bar represents Mean + SD; n = 6)
Figure 4. Effect of AZT and MSG on SOD (IU/L) activity in rats' serum, liver, kidney and heart.

CAT activity in the serum, liver, kidney and heart decreased ($p < 0.05$) the least in MSG group but most in TAZ group followed by TAZ + MSG group compared to control and others (Figure 5) while Zn concentration in the serum, liver, kidney and heart decreased ($p < 0.05$) in the TAZ + MSG group followed by MSG group compared to control and others (Figure 6).

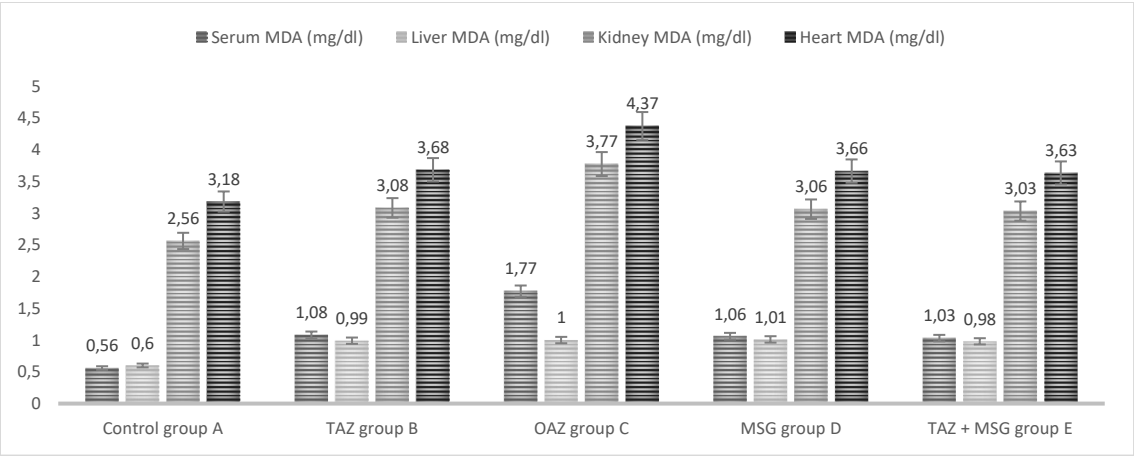


(Each bar represents Mean + SD; n = 6)
Figure 5. Effect of AZT and MSG on CAT (IU/L) activity in rats' serum, liver, kidney and heart.



(Each bar represents Mean + SD; n = 6)
Figure 6. Effect of AZT and MSG on Zn (µg/dl) concentration in rats' serum, liver, kidney and heart.

As shown in Figure 7, serum, liver, kidney and heart MDA concentration in all the treated groups increased ($p < 0.05$) and consistently highest ($p < 0.05$) in MSG group but least ($p < 0.05$) in TAZ + MSG group as compared to the control.



(Each bar represents Mean + SD; n = 6)
Figure 7. Effect of AZT and MSG on MDA (mg/dl) concentration in rats' serum, liver, kidney and heart.

Discussion

The antibiotic, AZT may be abused and co-consumed with MSG to present unknown outcomes on BWG, organ function and oxidative stress which warranted this study aimed to evaluate the effect of AZT and MSG in rats' BWG and antioxidant defense bioindicators. Monosodium glutamate mediates BWG, organ toxicity and oxidative stress (Joshi et al., 2023; Yang et al., 2023). Expectedly, MSG-treated rats significantly ($p < 0.05$) increased BWG; increased GSH, GP_X, SOD and MDA but decreased catalase (CAT) and zinc (Zn) in the serum, liver, kidney and heart of rats compared to control and others. These indicated adverse effect due to MSG on BWG that could be pathologic in the rats. Increased BWG leading to obesity is frequently associated with inflammatory responses and many diseases (Casado et al., 2023). MSG-induced enhancement in BWG of rats could be owing to orexigenic potential of MSG (Yang et al., 2023) which involves down-regulation in the expression of leptin hormone in the hypothalamus leading to decreased energy utilization, whetted appetite, increased energy balance and consequently increased BWG as reported herein (Obradovic et al., 2021; Martelli and Brooks, 2023). The results of the effect of MSG on the antioxidant defense bioindicators indicated MSG-induced overall toxicity in the rats following dysregulation in the metabolism and collapse of the antioxidant defense mechanisms leading to oxidative stress in the rats' serum, liver, kidney and heart. MSG-induced enhancement in BWG leading to obesity was linked with other pathologies (El-Gendy et al., 2023) which were enhanced by onset of oxidative stress (Wuyt et al. 2023). Concordantly, increased MDA concentration and decreased CAT activity as in this study indicated onset of oxidative stress (Ayoola et al., 2019; El-Gendy et al., 2023) whereas increased SOD but decreased Zn as

reported herein reflected extent of defensive role of SOD and its metal co-factor, Zn against oxidative stress (Sciskalska et al., 2020). Earlier, it was reported that oxidative stress contributed significantly to MSG-related cellular and organ toxicity in animals (Egbuonu et al., 2021; Correia, et al., 2023). In line with the results of the present study also, generation of reactive oxygen species (ROS) evidenced by increased GSH content as in this study was ascribed to a collapse in antioxidant defense system due to onset of oxidative stress (Elwahab et al., 2020). And, MSG especially at high dose as used herein mediated enhanced BWG, organ toxicity and oxidative stress (Obi and Egbuonu, 2019; Joshi et al., 2023; Yang et al., 2023). Collapse in the antioxidant defense mechanism to effectively combat free radicals resulted to oxidative stress and increased inflammatory responses (Ismael and Elsamman; 2022; Correia, et al., 2023) while oxidative stress and increased inflammatory responses due to increased BWG were associated with the onset and development of varied ailments (Kiran et al., 2023; Correia, et al., 2023). Intriguingly, GP_X activity increased due to MSG treatment. However, in a recent study, Handy and Loscalzo (2022) reported active involvement of GP_X in the activation of pro-inflammatory pathways. It is probable that the enzyme activity increased because it was channeled only to the activation of pro-inflammatory response by MSG without being depleted instead of being depleted by combating the ROS generated due to MSG assault in the rats. This is an important deduction on the response of MSG on GP_X role in animals, suggesting selective activity of GP_X in MSG metabolism and warranting further studies. Thus, the study demonstrated MSG-induced enhancement in BWG and significant liver, kidney and heart tissues toxicity evidenced by a compromised antioxidant defense system following onset of oxidative stress. Further studies are required to ascertain whether the present observation has pathologic effect in the rats, especially the studied organs.

AZT caused a dose dependent decrease in the BWG of rats in TAZ, indicating a consistent BWG slimming potentials of AZT which suggests its capacity to mitigate MSG-induced enhancement in BWG in the rats. The noted body weight reducing property of AZT could be *via* anorexigenic activity (Yang, et al., 2023) and anti-inflammatory response (Ismael and Elsamman, 2022) to counter the orexigenic potential of MSG and the attendant inflammatory responses in the rats. However, the overriding significant body weight reduction caused by AZT in OAZ-treated rats as compared to control warrants detailed investigation as this may be a notable shortcoming due to overdose consumption of AZT. The possibility of the role of TAZ in mitigating the effect of MSG was explored further. Results revealed that co-treated TAZ + MSG rats significantly ($p < 0.05$) decreased BWG; decreased GSH, GP_X, SOD, Zn, MDA and increased CAT in the serum, liver, kidney and heart of rats compared to MSG treated group. The observations herein were important indicators of wellbeing in animals. In line with the current observations, comparatively decreased BWG (Okereke et al., 2023), GSH (Elwahab et al., 2020) and MDA (Ayoola et al., 2019; El-Gendy et al., 2023), but increased CAT (Ayoola et al., 2019) indicated beneficial responses on the metabolic, functional and overall health capacities in animals. Herein, anti-inflammatory response was adduced as a possible mechanism of AZT action against MSG assault, so a decrease in GP_X activity as reported herein is also a beneficial response in the rats. Active involvement of GP_X is necessary for the activation of pro-inflammatory pathways (Handy and Loscalzo, 2022), implying that the decrease in GP_X could be an indirect indication of the extent of anti-inflammatory response of AZT in counteracting the apparent pro-inflammatory response mediated following MSG assault in the rats. This is in line with the selective activity of GP_X in MSG metabolism suggested in this study. Similarly, decreased SOD and its metal co-factor, Zn relative to MSG treatment as recorded herein is reflective of TAZ-mediated active participation of SOD in anti-inflammatory response against MSG-induced pro-inflammatory response in the rats. In support of this suggestion, Sciskalska et al. (2020) confirmed the anti-inflammatory role of SOD. Thus, these results indicated significant capacity of TAZ to mitigate MSG-induced enhancement in BWG and compromised metabolic activity of the antioxidant defense bioindicators in the rats *via* probable anorexigenic activity (Yang, et al., 2023), anti-inflammatory and antioxidant responses (Ismael and Elsamman, 2022). This is intriguing as AZT can induce organ toxicity (Bergami et al., 2023), has long lasting half-life and capacity to penetrate and accumulate in organs (Fohner et al., 2017) whereas the AZT-induced organ toxicity can be mitigated through antioxidant and anti-inflammatory responses (Ismael and Elsamman, 2022). However, at therapeutic dose as used herein AZT showed an excellent safety profile (O'Brien et al., 2019). And, aside antibiotic and antiviral activities, AZT exhibits anti-inflammatory activity (Ismael and Elsamman, 2022). It is therefore plausible that in the interaction of TAZ and MSG, AZT expressed anorexigenic, antioxidant and anti-inflammatory responses in order to mitigate the MSG-induced effects observed in this study. To our knowledge, this is the first study reporting the concomitant effect of AZT and MSG on BWG and these antioxidant defense bioindicators in rats. Thus, this is a novel finding that deserve follow-up in subsequent studies aimed at providing further insights on the biochemistry, pharmacology and mechanistic responses of MSG and AZT in rats.

CONCLUSIONS

Thus, TAZ significantly mitigated BWG, and malfunction in the metabolism of antioxidant defense bioindicators in MSG rats *via* probable anorexigenic, anti-inflammatory and antioxidant responses. This suggests that TAZ could be useful in managing MSG-related dysfunction in BWG and metabolic activity of the antioxidant defense apparatus in rats. The outcome of this study deserves follow-up in subsequent studies aimed at providing further insights on the biochemistry, pharmacology and mechanistic responses of MSG and AZT in rats.

Authors' Contributions: Egbuonu A.C.C., Alaebo P.O. and Onuoha U.N. designed and supervised the study. Njoku C.J. and Eze O.B., assembled the draft manuscript. Odoemelam F.U., Edum M.E., Obi O.B., Ukaegbu M.J., Nwaogwugwu S.U., Orji M.C., Ndukwe C.N., Opara P., Oyoyo C., Joe-Eme C.B. and Okwoigwe C.J., carried out the laboratory work under supervision. All authors read and approved the final version of the manuscript.

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