Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 10, Issue 1, 2022

Responses of Artemether-Lumefantrine in Rats' Heart Histomorphology and Antioxidant Status Compromised by Monosodium Glutamate

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ABSTRACT

Monosodium glutamate (MSG), a common food flavouring, and the easily assessed antimalarial, artemether-lumefantrine could be consumed together with unknown effects on animals' heart histology and antioxidant metabolism. This study evaluated the responses of artemether-lumefantrine in rats' heart histomorphology and antioxidant metabolism compromised by monosodium glutamate. Monosodium glutamate and artemether-lumefantrine were co-exposed to 30 male Wistar rats orally and daily for 7 days by standard protocols. Results revealed that MSG administration significantly ($p \leq 0.05$) reduced the level of albumin, total protein, catalase and superoxide dismutase but increased malondialdehyde, zinc, magnesium and glutathione peroxidase compared to normal control. Respective administration of therapeutic artemether-lumefantrine (TAL), high artemether-lumefantrine (HAL) and concomitant administration of MSG plus either TAL or HAL altered ($p<0.05$) these biological indicators compared to MSG administration. Photomicrograph of heart section of rats in the normal group showed normal cardiac muscle fibers. Rats in the TAL and HAL groups showed mild oedema within the cardiac muscle fibers, while those in the MSG group and TAL+MSG or HAL+MSG groups showed mild hypertrophy. Thus, MSG administration compromised the heart histomorphology and antioxidant status. Concomitant administration of either TAL or HAL with MSG did not modulate, but may spike MSG-induced effects, in the rats.

Keywords: Albumin, catalase, glutathione peroxidase, magnesium, superoxide dismutase, therapeutic dose

INTRODUCTION

Artemether-Lumefantrine (AL) is an easily assessed antimalarial just as monosodium glutamate is a globally used food flavouring additive. When consumed, each mediates oxidative stress [1].

Oxidative stress results from excessive generation of oxidants without commensurate antioxidants to scavenge the oxidants through antioxidant defense reactions [2-4]. Oxidative stress damages and impairs the proper functioning of cellular components and has been implicated in varied unhealthy state of animals [1,2]. Varied and complex antioxidant defense systems exist in animals. These include biochemical interaction of enzymes (superoxide dismutase, SOD, catalase, CAT, glutathione peroxidase, GP_x and non-enzymes (malondialdehyde, MDA), albumin, total protein and metal enzyme cofactors, including magnesium and zinc [1,2].

Concomitant consumption of artemether-lumefantrine and (even intoxicating concentration of) monosodium glutamate is possible. Outcome of previous similar studies in a simulated animal model indicated that simultaneous administration of AL and MSG affected the histology and antioxidant status of the studied organs [1, 5, 6]. The heart is among the high metabolic organs. It functions in the physiologically important systemic blood circulation. Thus, it is important to know the oxidative stress status of the heart organ following simultaneous consumption of xenobiotics especially artemether-lumefantrine and monosodium glutamate known to separately mediate oxidative stress.

These warranted this study aimed at determining the responses of artemether-lumefantrine in rats' heart histomorphology and antioxidant status compromised by monosodium glutamate. The study objectives included the evaluation of the heart histomorphology and determination of selected antioxidant biological indicators in the heart homogenate of the experimental rats.

MATERIALS AND METHODS

Sample collection and preparation

A brand of monosodium glutamate (99.9% purity) was bought from a market whereas that of artemether-lumefantrine tablets (80:480 mg) was bought from a Pharmacy shop, in Umuahia, Nigeria. Chemicals used in this study were purchased with the kits manufactured by Randox Limited, and were used without further purification.

Experimental animals

The thirty (30) male Wistar rats used in this study which weighed between 80 - 120 g, were obtained from a reputable animal breeder. They were kept in metal cages for one-week

acclimatization. The rats were randomly assigned into six groups of five (5) rats each. Throughout the animal study, the rats were kept under laboratory conditions (25 ^{o}C, twelve (12) hours normal daylight/day cycle and tropical humidity).

Experimental design

Rats in the various groups were respectively exposed freely to feed on commercial growers mash (product of Top feed limited, Sapele, Nigeria) and portable tap water with no other exposure. The assigned doses with groups were: Normal Control (Group A), therapeutic dose of AL, TAL (Group B), overdose of AL, HAL (Group C), MSG (Group D), therapeutic dose of AL plus MSG (Group E) and overdose of AL plus MSG (Group F). The exposure was through gavages-assisted oral cavity. Artemether-lumefantrine overdose was calculated as therapeutic dose of AL for 70 kg man multiplied by 5. The administration of artemether-lumefantrine and monosodium glutamate was as described recently [1, 5, 6].

Ethical consideration, sacrifice, sample collection and sample preparation

The ethical consideration was based on the ethical guidelines by National Research Council, NRC, USA [7]. Sacrifice, heart organ collection and homogenization were as described recently [1].

Determination of heart homogenate antioxidant bio-indicators

Catalase activity in the heart homogenate of the rats was determined by the method as described by Sinha [8] while that of glutathione peroxidase activity was determined by the method of Paglia and Valentine [9]. The heart homogenate superoxide dismutase activity (IU/L) was determined using the method of Xin, et al. [10], while that of lipid peroxidation was determined by the method of Wallin, et al. [11]. Magnesium concentration in the heart homogenate samples was estimated by the method of Farrell [12], while zinc concentration was determined by the method of Johnsen and Eliasson [13]. Total protein content in the rats' heart homogenate was determined using Peterson's modifications of the Micro-Lowry method with a protein assay kit (Sigma Diagnostics, P 5656, Sigma, MO, USA) as explained earlier [1]. Albumin concentration in the rats' heart homogenate was estimated using bromcresol green (BCG) according to Doumas, et al. [14].

Histopathological evaluation

The respective heart section of the rats collected for histopathological evaluation was prepared and examined as reported recently [1].

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using Statistical package for social sciences (SPSS) version 20.0. Results were expressed as mean ± standard deviation (SD). Difference was accepted as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Monosodium glutamate (MSG) could be consumed simultaneously with easily assessed antimalarial, artemether-lumefantrine with unknown effects on animals' heart histology and antioxidant metabolism, warranting this study. MSG administration significantly ($p<0.05$) reduced the level of albumin, total protein, catalase and superoxide dismutase but increased malondialdehyde, zinc, magnesium and glutathione peroxidase compared to control. It caused mild hypertrophy in the rats' heart compared to normal control, TAL and HAL rat groups (Tables 1-3).

Table 1: Changes in albumin and total protein (TP) concentration in the heart homogenate of normal and monosodium glutamate intoxicated rats treated together with artemether-lumefantrine

Groups	Albumin (Mg/dl)	TP (g/dl)
Group A (Normal Control)	3.66 ± 0.39^{d}	$6.42+0.75^{c}$
Group B (TALfed)	3.30 ± 0.85^{c}	$6.20+0.70^{c}$
Group C (HALfed)	2.92 ± 1.63^{b}	$4.88+2.02^{b}$
Group D (MSGfed)	2.18 ± 1.22^{a}	$3.96+2.02^{a}$
Group E (MSG+TALfed)	2.72 ± 1.61^{b}	$4.76+2.01^{b}$
Group F (MSG + HALfed)	3.42 ± 0.29^{c}	$6.92+1.73^{d}$

Values of mean±SD for n = 5 rats. Difference in mean was accepted as statistically significant at $P \leq 0.05$. Means on a column with different superscript letters (arranged from a = least to f = highest) are significantly different at $P \leq 0.05$.

Table 2: Changes glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity in the heart homogenate of normal and monosodium glutamate intoxicated rats treated together with artemether-lumefantrine

Groups	GPx (IU/L)	CAT (IU/L)	SOD (IU/L)
Group A (Normal Control)	122.76+10.52^{a}	4.77 ± 0.41^{f}	11.19±0.31^{c}
Group B (TAL fed)	134.48+34.58^{b}	2.93 ± 0.84^{c}	11.19±0.27^{c}
Group C (HAL fed)	133.28+17.33^{b}	2.23 ± 0.92^{b}	10.11±1.15^{a}
Group D (MSG fed)	139.68+20.61^{b}	4.40 ± 1.04^{e}	10.79±1.01^{b}
Group E (MSG+TAL fed)	197.76+16.68^{c}	3.99 ± 1.53^{d}	11.39±0.08^{c}
Group F (MSG + HAL fed)	155.16+38.04^{d}	1.99 ± 0.48^{a}	11.29±0.36^{c}

Values of mean±SD for n = 5 rats. Difference in mean was accepted as statistically significant at $P \leq 0.05$. Means on a column with different superscript letters (arranged from a = least to f = highest) are significantly different at $P \leq 0.05$.

These demonstrated that the effect of MSG administration compromised the antioxidant metabolism and histology of the rats' heart. Albumin (including total protein) metabolism aids in maintaining antioxidant balance. In previous studies, the reduction of albumin as in the MSG group was attributed to its catabolism in response to oxidant stress [4, 15]. The likely increased free radical scavenging activity in the MSG-challenged rats may be responsible for the increased MDA, zinc, magnesium and glutathione peroxidase levels in the heart tissue of rats fed with the assault dose of MSG. In particular, malondialdehyde is a lipid peroxidation product that increased following injury related to oxidative stress as reported in a recent study [16]. Hypertrophy of the heart typically results from exertion on the heart organ as an adaptive response to stress [17,18], and as reported herein indicates pathological exertion on the rats' heart following MSG assault. Induction of oxidative stress in rats by administering MSG at 8000 mg/kg has been confirmed [1].

Table 3: Changes in zinc, magnesium and malondialdehyde (MDA) concentration in the heart homogenate of normal and monosodium glutamate intoxicated rats treated together with artemether-lumefantrine

Groups	Zinc (Mg/dl)	Magnesium (Mg/dl)	MDA (µmol/ml)
Group A (Normal Control)	45.91±41.19^{b}	3.24 ± 0.78^{a}	1.70+0.29^{a}
Group B (TAL fed)	53.43±63.68^{d}	3.22 ± 0.40^{a}	1.66+1.09^{a}
Group C (HAL fed)	31.86±25.13^{a}	4.28 ± 1.61^{b}	1.94+0.90^{b}
Group D (MSG fed)	51.66±6.16^{c}	4.68 ± 1. 91^{b}	3.26+1.37^{c}
Group E (MSG+TAL fed)	51.30+10.31^{c}	5.32 ± 1.01^{c}	3.54+1.05^{d}
Group F (MSG + HAL fed)	51.38±39.47^{c}	3.80 ± 0.51^{a}	3.62+0.63^{d}

Values of mean±SD for n = 5 rats. Difference in mean was accepted as statistically significant at $P \leq 0.05$. Means on a column with different superscript letters (arranged from a = least to f = highest) are significantly different at $P \leq 0.05$.

The administration of TAL or HAL alone significantly ($p \leq 0.05$) and variously altered the level of albumin, total protein, superoxide dismutase, zinc, malondialdehyde, magnesium, catalase and glutathione peroxidase (Tables 1-3) without causing hypertrophy in the rats' heart, compared to MSG administration. These demonstrated benefit on the rats' heart histomorphology and antioxidant metabolism due to the administration of TAL (or HAL) as against MSG. In previous study, zinc metabolism mediated protective benefit [3].

The concomitant administration of MSG plus either TAL or HAL significantly ($p \leq 0.05$) and variously spiked the level of malondialdehye, albumin, total protein, glutathione peroxidase, superoxide dismutase and catalase with accompanying hypertrophy in the rats' heart histology as compared to MSG administration (Tables 1-3, Figure 1). These indicated adverse outcome in the rats' heart antioxidant metabolism and histology following concomitant administration of either TAL or HAL with intoxicating dose of MSG. Increased malondialdehyde level particularly indicated injury accompanying oxidative stress in earlier studies [16,19]. Overall, these observations demonstrated TAL (or HAL) related benefit on the rats' heart histomorphology and antioxidant status as against MSG but demonstrated that concomitant administration of MSG with either TAL or HAL could spike adverse outcome induced by MSG in the rats' heart tissue antioxidant status and histology.

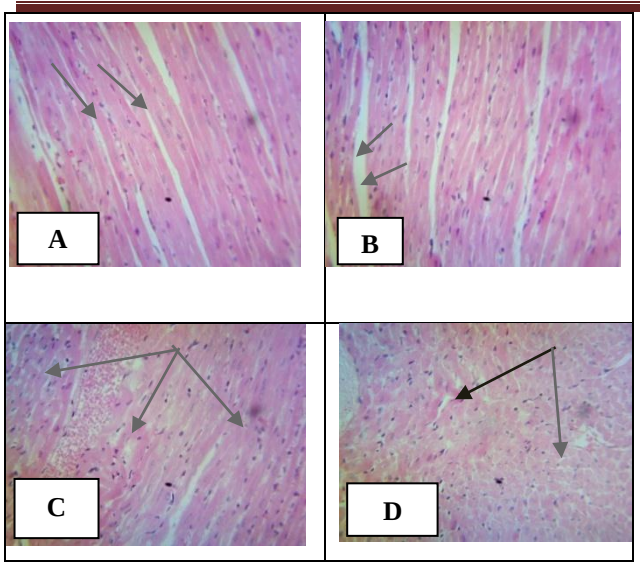

Figure1: Photomicrograph of Rats' Heart Section Hematoxylin and Eosin (H&E) stained ×400

[Photomicrograph of heart section of rats in the normal group showed the normal histologic architecture with normal cardiac muscle fibers (A). That of rats in the TAL and HAL groups showed mild oedema within the cardiac muscle fibers but without causing hypertrophy in the rats' heart (B). That of MSG group showed mild hypertrophy (C) just as that of TAL + MSG or HAL + MSG groups (D)].

CONCLUSION

The results from this study demonstrated that MSG administration compromised the rats' heart histomorphology and antioxidant status. The administration of either TAL or HAL elicited benefit on the rats' heart histomorphology and antioxidant status as against MSG. The concomitant administration of either TAL or HAL with MSG did not modulate, but may spike the effects by MSG in the rats. Thus, concomitant administration of either therapeutic or high dose of artemether-lumefantrine with assault dose of monosodium glutamate could aggravate the effects of MSG on the heart organ of rats *via* probable compromised antioxidant metabolism and histology.

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