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Research Article
Artemether-lumefantrine Improved Superoxide Dismutase Co-regulation Related Antioxidant Defense in Monosodium Glutamate-induced Oxidative Stress in Rats

[1]Anthony Cemaluk C. Egbuonu, [1]Ebere Mercy Chukwu, [1]Wisdom Chibuzor, [1,2]Emmanuel Obi, [1]Nnaemeka Raymond Ezenwafor, [1]Cynthia Uchechi Simon and [1]Justina Utodinachi Oleghibe

[1]Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria
[2]Department of Biochemistry, Federal University of Technology Owerri, Nigeria

Abstract

Background and Objective: Oxidative stress, co-regulated by Superoxide Dismutase (SOD) with its cofactors (Zn and Mg), could be synergistic when its respective Mediators Monosodium Glutamate (MSG) and Artemether-Lumefantrine (AL) are co-taken. Thus, the study estimated SOD activity, zinc and magnesium concentration variations in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' serum and liver homogenate. **Materials and Methods:** Thirty rats acclimatized for two weeks were randomly grouped into six (n = 5) and for seven days exposed to feed and water (Group A, control). In addition, rats in Groups B, C, D, E and F, respectively received AL therapeutic dose, AL overdose (therapeutic dose ×5), MSG, 8000 mg kg^{-1} b.wt., AL therapeutic dose plus MSG and AL overdose plus MSG. Data obtained from spectrophotometric the estimation of the studied parameters were subjected to analysis of variance (ANOVA) set at 95% confidence level. **Results:** The higher serum and liver homogenate zinc concentration (µg dL^{-1}) in all groups relative to MSG was particularly marked (264.91±1.89, 174.04±1.33; $p<0.05$) in AL overdose plus MSG-intoxicated rats. Similarly, AL overdose plus MSG-assaulted rats had a markedly higher ($p<0.05$) serum (6.88±0.10) Mg concentration (Mg dL^{-1}) compared to others, including control and MSG-intoxicated rats. Amid an unclear-cut change, SOD activity (IU L^{-1}) in the rats' liver homogenate was highest (1.14±0.00, $p<0.05$) in MSG-treated rats relative to control. **Conclusion:** The study confirmed oxidative stress that overwhelmed the SOD-related antioxidant defense in MSG-assaulted rats unlike in rats exposed to AL irrespective of dose or co-MSG-intoxication. This perhaps implicated SOD as reserved antioxidant defense arsenal in possible AL or AL with co-MSG-intoxication adversity in rats' serum and liver, provoking further studies.

Key words: Artemisinin-based, therapeutic dose, serum, liver homogenate, superoxide dismutase, magnesium, zinc

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Corresponding Author: Anthony Cemaluk C. Egbuonu, Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria
Tel: +23480-3636-6565

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It is a common knowledge that food, by increasing bioavailability of xenobiotics, could elicit a substantial effect on the absorption of drugs when taken either alone or together. And, monosodium glutamate (MSG), a widely used food additive, enhances the flavor of food[1-3]. However, MSG elicited varied toxic effects on major tissues including the liver and the reported implications of oxidative stress in the pathogenesis of diverse diseases[3-16]. Artemether-Lumefantrine (AL) is a commonly used antimalarial in developing countries[17-19]. Monosodium glutamate and Artemether-Lumefantrine (AL) respectively mediated oxidative stress which is fundamental in diseased conditions[15,20-22]. Oxidative stress underlies numerous pathological conditions, as earlier reported and reviewed [21,23].

The body is known to employ antioxidant defense mechanisms in attempts to counteract the deleterious effects of free radicals under physiological condition. In particular, superoxide dismutase activity mitigates oxidative stress in concert with trace metals, zinc and magnesium, acting as co-factors and up-regulators of antioxidant defense mechanisms and has been of great interest to health researches[23-28].Thus, deficiencies of zinc and magnesium aside being pathological[29] could in particular down-regulate the antioxidant defense mechanisms, underscoring the importance of this study. Zinc functions in the structural development and catalysis of enzymes and as an antioxidant by various mechanisms including the enhancement of superoxide dismutase catalytic activity and diminution of inflammatory effects that could result to the generation of free radicals or augment oxidative stress[23]. Magnesium mitigates oxidative stress via antioxidant role by participating as a cofactor to regulate superoxide dismutase activity[25]. Magnesium deficiency correlated with the development of oxidative stress exist and at cellular level promoted apoptosis in rat hepatocyte primary culture and consequent accumulation of malondialdehyde, a marker of oxidative stress[29]. Low serum magnesium by reducing the antioxidant response capacity in cells allows the accumulation of free radicals, resulting in oxidative stress and tissue damage[24].

In particular, magnesium deficiency may result from administration of some drugs[29]. In addition to this, possibility exists of concomitant intake of AL and MSG which may elicit synergistic influence on zinc and magnesium metabolism and overall superoxide dismutase-related antioxidant apparatus and responses in the liver of non-malarial-infected-hosts. A substantial quantity of MSG could be consumed together with food, prior to artemether-lumefantrine medication. And, as an over the counter drug a possibility of abuse (overdose and even prolonged intake) of AL exists. Self-medication with antimalarials is common[4,30]. Also, possibility of co-intake of artemether-lumefantrine with monosodium glutamate-flavour enhanced fast foods, even at an overdose and in absence of malaria infection, exists.

Not withstanding the foregoing, there is paucity in literature as regards possible effect on the superoxide dismutase activity, magnesium and zinc concentration in the serum and liver homogenate following use or abuse of AL either alone or with co-monosodium glutamate intoxication particularly in absence of malaria parasite infection. Recently, normal rats exposed to overdose of AL together with MSG had a significantly compromised liver morphology and antioxidant capacity[4,30] but the studies did not include the contribution of superoxide dismutase-related activity, warranting this study. Metabolic abnormalities could be detected in the associated bio-indicators in the serum and in high metabolic organs (including liver) homogenate. Thus, this study, by standard procedures involving spectrophotometric estimation, aimed to determine superoxide dismutase activity, zinc and magnesium concentration variations in artemether-lumefantrine co-treated monosodium glutamate-compromised rats' serum and liver homogenate.

MATERIALS AND METHODS

Study area: The study was carried out in the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria, from June-August, 2017 as a group degree research study for 2016/2017 academic session.

Chemicals: Monosodium glutamate (99% purity) and artemether-lumefantrine (20:120 mg) combination were respectively purchased from appropriate sale outlets in Umuaha, Abia State, Nigeria.

Research procedure: Male Wistar rats weighing between 89 and 183 g were, (after two weeks acclimatization in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria,) randomly grouped into 6 (sample size, n = 5 rats) and allowed free access to vital feed pellets and portable tap water. The rats in the respective groups were exposed orally (using a gavage) to nothing (Group A, control), AL therapeutic dose (Group B), AL overdose, calculated as therapeutic dose for 70 kg man multiplied by 5, (Group C), MSG (Group D), AL therapeutic dose plus MSG (Group E) and AL overdose plus

MSG (Group F). The artemether-lumefantrine administration was twice each day after 8 hrs interval[4,30]. Rats' intoxication with MSG was achieved at 8000 mg kg^{-1} b.wt. and by daily exposure for 7 days as described earlier[31-34].

All rats used in this study were housed at 25°C in stainless steel cages under normal daylight/cycle and humid tropical conditions and were conditioned throughout the process according to the guidelines approved by the College of Natural Science Research Ethics Committee, Michael Okpara University of Agriculture, Umudike (CREC, MOUAU) on use and handling of experimental animals (Reference number: CREC/003/19). After 7 days, the rats were sacrificed the next day following overnight fast by ocular puncture. The blood sample of the respective rats was collected into a clean non-anticoagulated polystyrene tube, allowed to clot and centrifuged at 3000 rpm for 5 min. The resultant respective serum collected was separately stored in a refrigerator until used. The liver of the respective rat was excised, rinsed in iced-cold sucrose and a 10% w/v homogenate was prepared from it using 0.15 M KCl as buffer to obtain the supernatant sample after centrifugation[32].

Estimation of superoxide dismutase activity, zinc and magnesium concentration: The Superoxide dismutase activity was determined by the method of Misra and Fridovich[35]. Zinc concentration in the samples was estimated by the method of Johnsen and Eliasson[36]. Magnesium concentration in the samples was estimated by the method of Farrell[37] based on the principle that magnesium reacts with Calmagite in alkaline medium/solution forming a purple-coloured complex with colour intensity proportional to the magnesium concentration in the sample, hence can be measured using a spectrophotometer at 520 nm. The widely used calcium-selective chelator, ethylene glycol tetraacetic acid (EGTA), was included in the reagent to selectively remove or reduce calcium interference from the samples[38].

Calculation of change relative to groups: The calculation of change relative to any group was based on the formula:

$$\text{Change relative to K (\%)} = \frac{V - K}{K} \times 100$$

where, K represents the constant group hence constant value and V represents the variable group hence variable values. The formula was developed and severally used in previous studies[14,21,31-34,39-44].

Statistical analysis: The data obtained from this study 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 Error of Mean (SEM). Difference was accepted as significant at $p< 0.05$ and supported by a relative change of up to 10% and above[43].

RESULTS

Compared to other groups, rats exposed to MSG had a markedly significant reduction ($p<0.05$) in serum Zn concentration compared to the control. The observation which was higher in the other groups relative to MSG was marked and significant ($p<0.05$) in rats exposed to overdose of AL and MSG (Group F) (Table 1). Aside rats exposed to MSG that had significant reduction, rats in the other groups had non-significant change in Zn concentration in the liver homogenate compared to the control. The observation in the other groups, including the control was higher and significant ($p<0.05$) relative to rats in MSG group (Table 2).

Compared to other groups, rats exposed to overdose of AL plus MSG had a marked and significant increase ($p<0.05$) in serum Mg concentration compared to the control and relative to rats in the MSG group (Table 3). Rats exposed to MSG had significant ($p<0.05$) and marked reduction in the liver homogenate Mg concentration compared to control while rats in the other groups had a higher ($p<0.05$) Mg concentration in the liver homogenate relative to rats in the MSG group (Table 4).

Table 1: Variations in the Zinc concentration (μg dL^{-1}) in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' serum

Groups	Zinc (μg dL^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	179.16±2.17	0.00	+20.13*
B: Therapeutic dose of AL	159.72±3.85*	-10.85*	+7.09
C: Overdose of AL	161.58±2.90	-9.81	+8.34
D: MSG (8000 mg kg^{-1} b.wt.)	149.14±2.03*	-16.76*	0.00
E: Therapeutic dose of AL+MSG	166.03±1.44	-7.33	+11.32*
F: Overdose of AL+MSG	264.91±1.89*	+47.86*	+77.63*

Values are Mean±SEM for n = 5, +: Denotes higher by, -: Denotes lower by, *Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD: Superoxide dismutase, MSG: Mediators monosodium glutamate, AL: Artemether-lumefantrine

A clear cut change was not observed in the serum and liver homogenate SOD activity of rats among the groups, including the control (Table 5 and 6). In particular, compared to control, superoxide dismutase activity (IU L^{-1}) in the serum was essentially similar and non-significant ($p>0.05$) in rats in the different groups (Table 5). However, compared to control, superoxide dismutase activity (IU L^{-1}) in the liver homogenate significantly ($p<0.05$) increased in rats in all groups but lowered ($p< 0.05$) in control rats relative to rats in MSG-treated group (Table 6).

Table 2: Variations in the Zinc concentration (μg dL^{-1}) in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' liver homogenate

Groups	Zinc (μg dL^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	171.33±2.92	0.00	+49.45
B: Therapeutic dose of AL	185.31±1.70	+8.16	+61.65
C: Overdose of AL	165.07±0.91	-3.65	+43.99
D: MSG (8000 mg kg^{-1} b.wt.)	114.64±1.15	-33.09	0.00
E: Therapeutic dose of AL+MSG	153.45±1.74	-10.44	+33.85
F: Overdose of AL+MSG	174.04±1.33	+1.58	+51.81

Values are Mean±SEM for n = 5, +: Denotes higher by, -: Denotes lower by, Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD: Superoxide dismutase, MSG: Mediators Monosodium Glutamate, AL: Artemether-lumefantrine

Table 3: Variations in the Magnesium concentration (mg dL^{-1})in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' serum

Groups	Magnesium (mg dL^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	5.52±0.20	0.00	-0.36
B: Therapeutic dose of AL	4.63±0.19	-16.12	-16.43
C: Overdose of AL	4.68±0.13	-15.22	-15.52
D: MSG (8000mgkg^{-1} b.wt.)	5.54±0.11	+0.36	0.00
E: Therapeutic dose of AL+MSG	5.47±0.03	-0.91	-1.26
F: Overdose of AL+MSG	6.88±0.10	+24.64	+24.19

Values are Mean±SEM for n = 5, +: Denotes higher by, -: Denotes lower by, Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD : Superoxide dismutase, MSG: Mediators monosodium glutamate, AL: Artemether-lumefantrine

Table 4: Variations in the Magnesium concentration (mg dL^{-1}) in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' liver homogenate

Groups	Magnesium (mg dL^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	7.43±0.17	0.00	+52.25
B: Therapeutic dose of AL	6.44±0.15	-13.22	+31.97
C: Overdose of AL	7.80±0.07	+4.98	+59.84
D: MSG (8000mgkg^{-1} b.wt.)	4.88±0.35	-34.32	0.00
E: Therapeutic dose of AL+MSG	7.24±0.17	-2.56	+48.36
F: Overdose of AL+MSG	7.47±0.16	+0.54	+53.07

Values are Mean±SEM for n = 5, +: Denotes higher by, -: Denotes lower by, Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD: Superoxide dismutase, MSG: Mediators monosodium glutamate, AL: Artemether-lumefantrine

Table 5: Variations in the Superoxide dismutase activity (IU L^{-1}) in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' serum

Groups	SOD (IU L^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	1.14±0.00	0.00	0.00
B: Therapeutic dose of AL	1.14±0.00	0.00	0.00
C: Overdose of AL	1.13±0.00	-0.88	-0.88
D: MSG (8000 mg kg^{-1} b.wt.)	1.14±0.00	0.00	0.00
E: Therapeutic dose of AL+MSG	1.14±0.00	0.00	0.00
F: Overdose of AL+MSG	1.14±0.00	0.00	0.00

Values are Mean±SEM for n = 5, +: Denotes higher by, -: Denotes lower by, Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD: Superoxide dismutase, MSG: Mediators monosodium glutamate, AL: Artemether-lumefantrine

Table 6: Variations in the Superoxide dismutase activity (IU L^{-1}) in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' liver homogenate

Groups	SOD (IU L^{-1})	Changes relative to the control (%)	Changes relative to the MSG group (%)
A: Control (feed+water only)	0.97±0.15	0.00	-14.91
B: Therapeutic dose of AL	1.12±0.01	+15.46	-1.75
C: Overdose of AL	1.12±0.01	+15.46	-1.75
D: MSG (8000 mg kg^{-1} b.wt.)	1.14±0.00	+17.53	0.00
E: Therapeutic dose of AL+ MSG	1.12±0.01	+15.46	-1.75
F: Overdose of AL+MSG	1.11±0.02	+14.43	-2.63

Values are Mean±SEM for n = 5, +: Denotes higher b, -: Denotes lower by, Difference considered statistically significant at $p<0.05$ as supported by a relative change of up to 10% and above (Egbuonu[43]), SOD: Superoxide dismutase, MSG: Mediators monosodium glutamate, AL: Artemether-lumefantrine

DISCUSSION

Oxidative stress, co-regulated by superoxide dismutase (SOD) with its cofactors Zn and Mg[23-25], could be synergistic when its respective mediator's monosodium glutamate (MSG) and artemether-lumefantrine are co-taken[21,22]. Recently, normal rats exposed to overdose of AL together with MSG had compromised liver morphology and some indices of antioxidant capacity[4,30]. Thus, SOD activity, zinc and magnesium concentration variations in artemether-lumefantrine co-treated monosodium glutamate-assaulted rats' serum and liver homogenate were spectrophotometrically estimated.

The recorded higher serum and liver homogenate zinc concentration (μg dL^{-1}) in all groups relative to MSG was particularly marked ($p<0.05$) in AL overdose plus MSG-intoxicated rats. This could be indicative of non-adverse response of AL irrespective of dose or together with intoxicating dose of MSG on the serum and liver homogenate zinc concentration and perhaps zinc-mediated superoxide dismutase enzyme activity, of rats. Zinc aside functioning in enzymes structural development and catalysis particularly acts as an antioxidant by enhancing superoxide dismutase activity and by suppressing anti-inflammatory responses related to onset and progression of oxidative stress[23]. Thus, the observation herein could be a plausible basis to preclude zinc-mediated superoxide dismutase enzyme activity as possible mechanism for the adverse influence from overdose of AL and MSG on the liver morphology and antioxidant capacity of rats reported previously[4,30]. Zinc could up-regulate superoxide dismutase activity and the marked increase in the other groups relative to MSG could explain the apparent mitigation of the oxidant effect caused by MSG in the AL plus MSG co-treated groups. This is an important observation that could be explored in the prevention and management of MSG-induced adverse effects warranting a follow-up.

Magnesium deficiency which may result from administration of some drugs correlated with the development of oxidative stress as evidenced in increased oxidative stress markers[29]. Thus, the magnesium concentration was determined in this study. Similar to the result of this study on zinc concentration, AL overdose plus MSG-fed rats had a markedly higher ($p<0.05$) serum and liver homogenate Mg concentration (Mg dL^{-1}) compared to others, control and MSG-intoxicated rats. This apparently supported the present result on zinc concentration. Thus, the observation could be indicative of non-adverse or negligible response from AL irrespective of dose or together with intoxicating dose of MSG on the rats' serum and liver homogenate Mg concentration and associated magnesium metabolism and bio-functions, including SOD-related antioxidant activity. Magnesium acts as a cofactor in the superoxide dismutase activity-related antioxidant mitigation of oxidative stress[25,29]. And, the high magnesium concentration as observed in this study could enhance the antioxidant response capacity leading to reduction of free radicals and as a consequence diminution of oxidative stress and tissue damage as suggested by Larsson *et al.*[24].

Amid an unclear-cut change, SOD activity (IU L^{-1}) in the rat's liver homogenate was highest ($p<0.05$) in MSG-treated rats relative to control. This could be indicative of non-adverse response of AL irrespective of dose or together with intoxicating dose of MSG on the serum and liver homogenate SOD activity in apparent support of the result of the present study on serum and liver homogenate zinc and magnesium concentration and the suggestions thereto. Howbeit, the likelihood of noise or extraneous factors, including perhaps undetected faulty determination of the SOD activity, beyond the control of this study is not precluded; warranting related further investigations for confirmation. The non-significant effect of MSG toxicity on superoxide dismutase activity (in contrast to that on zinc and magnesium concentration) on the serum and liver homogenate was not expected. This could be due to the short time frame of MSG administration which probably limited the overall expression of the observation on the zinc and magnesium concentration, on the superoxide dismutase activity of the rats' samples. This agrees with earlier reports of time-dependent expression of MSG induction of oxidative stress apparently indicated for 10 days[10,12,45] and 8 weeks[46]. The non-significant effect of MSG toxicity on superoxide dismutase activity within the time frame of this study may be an indirect pointer to the preclusion of SOD activity among the first line of antioxidant defense response in the rats' serum and liver, warranting further investigations. The lower ($p<0.05$) zinc and magnesium concentration but higher ($p<0.05$) SOD activity in the MSG-assaulted rats expectedly confirmed a successful oxidative stress induction that probably overwhelmed the SOD-related antioxidant defense and subsequent collapse in the MSG-assaulted rats unlike in rats exposed to AL irrespective of dose or co-MSG-intoxication. The observation and suggestion there to do agree with earlier reports of induction of oxidative stress attributable to oxidant effect of monosodium glutamate[47,21,4]. It is important to note that, in apparent disagreement of earlier similar studies[4,30], the results of this study did not implicate concomitant exposure of MSG and overdose of AL with a significant adversity on the determined rats' serum and liver homogenate bio-indicators of antioxidant status. This

apparent disagreement could be a pointer to a hitherto unknown pattern of antioxidant defense mechanism hence warrants follow-up studies. Increased dose and exposure time may make the effect of agent treatment in animals more obvious[48]. Thus, the noted non-significant ($p>0.05$) responses in some groups could be attributed to known negligible side response from therapeutic dose exposure of animals to AL and the time-dependent oxidative stress-induction capacity of MSG[30]. The observation and suggestion herein perhaps implicated SOD as reserved antioxidant defense arsenal in any AL or AL with co-MSG-intoxication adversity in rats' serum and liver, provoking further studies for clarification.

CONCLUSION

The study indicated that the oxidative stress induction in the rats exposed to monosodium glutamate at 8000 mg kg^{-1} b.wt. could not be mitigated by the SOD-related antioxidant defense mechanism in the rats. The study further indicated that AL, at the tested doses, did not induce oxidative stress in the rats owing probably to AL-associated efficient or enhanced SOD-related antioxidant mechanism in the rats. And, that the apparent AL-associated benefit on the antioxidant defense mechanism could significantly mitigate oxidative stress caused by MSG-intoxication in the rats. Thus, the study confirmed oxidative stress induction that probably overwhelmed the SOD-related antioxidant defense in MSG-assaulted rats unlike in rats exposed to AL irrespective of dose or co-MSG-intoxication. And that, artemether-lumefantrine improved superoxide dismutase co-regulation related antioxidant defense in monosodium glutamate-induced oxidative stress in rats.

SIGNIFICANCE STATEMENT

The study emphasized that oxidative stress induction following MSG-assault in rats could overwhelm the SOD-related antioxidant defense of the rats. This could be implicating SOD as a reserved antioxidant defense arsenal in possible AL or AL with co-MSG-intoxication adversity in rats serum and liver, provoking further studies for clarifications and are herein recommended.

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