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Effect of Amlodipine on Some Serum Indicators of Oxidative Stress in Monosodium Glutamate-Challenged Wistar Rats

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ABSTRACT

Amlodipine (AML), a commonly used anti-hypertensive, could interact with common food flavouring, monosodium glutamate (MSG), to interfere with antioxidant balance. This study investigated the effect of amlodipine on some serum indicators and associated diagnostic ratios of oxidative stress in MSG-challenged rats after oral exposure for 14 days using acceptable methods. Malondialdehyde (MDA) concentration (µmol/ml) was highest ($p < 0.05$) in rats exposed to therapeutic dose of AML, TAML + MSG (0.57 ± 0.16) followed by MSG only (0.56 ± 0.07) and least ($p < 0.05$) in therapeutic dose of AML, OAML, only (0.33 ± 0.03) compared to control (0.30 ± 0.08). Glutathione peroxidase (GP_X) activity (IU/L) was least ($p < 0.05$) in OAML + MSG (3.84 ± 0.36) followed by MSG only (3.98 ± 0.42) and highest ($p < 0.05$) in TAML only (5.85 ± 0.26) compared to control (5.93 ± 0.23). Similar variations in magnesium (Mg) concentration (mmol/l) and the calculated diagnostic ratios (MDA:GPx, MDA:Mg and GPx:Mg) revealed marked alteration ($p < 0.05$) of these parameters in MSG only, OAML + MSG and TAML + MSG treated rats compared to control and others. The results demonstrated that amlodipine, particularly at therapeutic dose, did not compromise the antioxidant integrity and did not lower the significant adverse effects on the oxidative parameters elicited by monosodium glutamate, in the rats' serum. This suggests probable negative interactive effects of amlodipine and monosodium glutamate which warrants caution in their combined use in rats.

Keywords: Amlodipine, antioxidant integrity, glutathione peroxidase, magnesium, malondialdehyde, monosodium glutamate

INTRODUCTION

The 8th Joint National Committee recommended calcium channel blockers as a first-line anti-hypertensive treatment for all patients regardless of age or race [1-2]. The fundamental goal of amlodipine-based treatment was the prevention of the important endpoints of hypertension,

including heart attack, stroke and heart failure [3]. The mechanism of action of amlodipine is through the blockage of calcium entrance and reduction in the contraction of the arteries to achieve vasodilation and reduction in blood pressure [4].

Strictly, amlodipine is not an over the counter drug, but it could be procured on request from pharmaceutical outlets. Thus, with self-purchase cum self-medication tendency, possibility of its abuse exists with significant adversity in animals [5]. Monosodium glutamate (MSG) is a sodium salt of glutamic acid commonly used for food flavouring [6-8]. MSG is used in canned food [9-11] and contained in condiment with varied brand names but without MSG content indication on the labels could be bought and consumed without restriction hence stands a high chance of inadvertent abuse with inherent MSG-intoxication [5]. In other studies, monosodium glutamate elicited adverse effects [5]. MSG adversity could induce toxic influences associated with oxidative stress [12-14]. Oxidative stress presents in many diseases [15-19]. The possibility of co-intake of amlodipine with monosodium glutamate flavour enhanced foods, at intoxicating dose exists.

The objectives of this study involved the determination of changes in some seric indicators of oxidative stress; malondialdehyde concentration, magnesium concentration and glutathione peroxidase activity in the rats after concurrent intake of amlodipine with intoxicating dose of MSG. The possible interactive effects of MSG with amlodipine (AML) on the antioxidant response apparatus may be significant in the serum indicators of oxidative stress, including glutathione peroxidase activity, malondialdehyde and magnesium concentration [20-22]. These warranted this study aimed to assess the effect of amlodipine on some serum indicators of oxidative stress in MSG-challenged Wistar rats. Altered glutathione peroxidase activity, malondialdehyde and magnesium concentration in the serum are common indicators of oxidative stress status in animals as could be supported by outcome of their combination ratios.

MATERIALS AND METHODS

Sample collection and preparation

Monosodium glutamate (99%) with the brand name Ajinomoto, marketed by West Africa seasoning company limited and Amlodipine (10 mg) marketed by Elbe Pharmacy Nigeria Limited, were procured from a reliable source (Blessed Pharmacy) in Umuahia, Abia State, Nigeria. Other chemicals for the serum assay were kits by Randox Limited and were of analytical grade. The standards were used without further purification. Thirty-six (36) male

Wistar rats each weighing 80-160 g were obtained from a commercial breeder, O'give Farms Aba, Abia State, Nigeria. They were maintained in stainless steel cages under laboratory conditions and allowed to acclimatize for 7 days before commencing the experiment.

Ethical adherence and experimental design

The study adhered to ethical guidelines on animal use [23]. The rats were allotted into six (6) groups of six (6) rats each. They were exposed freely to rat feed and potable tap water according to the following protocols: Group 1 (Control) rats were fed normal rat feed and water only. Group 2 (therapeutic dose of AML, TAML) rats were exposed to TAML (0.33 mg/kg body weight, bwt). Group 3 (overdose of AML, OAML) rats were exposed to OAML (1.65 mg/kg bwt). Group 4 (MSG group) rats were exposed to high MSG (8000 mg/kg bwt). Group 5 (TAML + MSG) rats were exposed to MSG (8000 mg/kg) co-treated with TAML (0.33 mg/kg) bwt. Group 6 (OAML + MSG) rats were exposed to MSG (8000 mg/kg) co-treated with OAML (1.65 mg/kg bwt).

Amlodipine overdose was calculated as therapeutic dose equivalent for 70 kg man × 5. The exposure was *per* oral and daily for 14 days [24]. The therapeutic concentration was prepared by dissolving adult dose (1 tablet) of amlodipine in 15ml of distilled water corresponding to the weight of the rat according to the instruction provided by the manufacturer with slight modification as described in recent report [20]. Intoxication of rats with MSG was achieved at 8000 mg/kg bwt and by daily exposure for 14 days as in earlier study [20]. After 14 days, the rats were sacrificed after overnight fast by cervical dislocation. Their blood samples were aseptically collected by cardiac puncture into plain polystyrene tubes, allowed to clot and centrifuged at 3000 rpm for 5 minutes. The serum obtained was stored in a refrigerator and used to determine the biochemical parameters.

Determination of oxidative stress parameters

Glutathione peroxidase activity in the serum was determined by the method of Paglia *et al.* [25]. Magnesium concentration was determined by the methods of Henry [26] and Faulkner [27]. Malondialdehyde concentration was determined based on thiobarbituric acid reaction described by Wallin *et al.* [28].

Calculation of diagnostic ratios and relative changes

Diagnostic ratios were calculated as simple combination ratios from the parameters determined in this study. Relative change to either control or the MSG group was calculated based on Cemaluk relative change formula [20].

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 error of mean (SEM). Difference was accepted as significant at $p < 0.05$ and by relative change of up to and above ten percent (10.00%) as calculated with Cemaluk relative change formula [20].

RESULTS AND DISCUSSION

It is known that the commonly used anti-hypertensive, amlodipine is prone to interaction and may interact with a common food flavour enhancer, monosodium glutamate to interfere with the antioxidant balance. This study investigated the effect of amlodipine on some serum indicators and associated diagnostic ratios of oxidative stress in monosodium glutamate-challenged rats. Results in Table 1 showed that malondialdehyde (MDA) concentration (µmol/ml) was highest ($p < 0.05$) in rats exposed to TAML + MSG (0.57 ± 0.16) followed by MSG only (0.56 ± 0.07) and least ($p < 0.05$) in TAML only (0.33 ± 0.03) compared to control (0.30 ± 0.08).

Table 1: Effect of amlodipine on some serum malondialdehyde (MDA) concentration (µmol/ml) in monosodium glutamate-challenged Wistar rats

Groups	MDA (µmol/ml)	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	0.30 ± 0.08	0	-4.67
2: TAML	0.33 ± 0.03	10.63	-4.11
3: OAML	0.34 ± 0.03	14.55	-3.90
4: MSG	0.56 ± 0.07	87.73	0
5: TAML+ MSG	0.57 ± 0.16	90.51	0.15
6: OAML+ MSG	0.49 ± 0.05	64.26	-1.25

Values are mean ± SEM for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p<0.05$ and relative change of up to and above 10.00 % [19]

These results indicated marked induction of oxidative stress by MSG in the rats probably through a spike in activities leading to increased lipid peroxidation of polyunsaturated fatty acids and generation of reactive oxygen species. The generation of reactive aldehyde,

malondialdehyde from lipid peroxidation of polyunsaturated fatty acids and reactive oxygen species serves as a notable biomarker to measure the level of oxidative stress [29-30].

Results in Table 2 showed GPX activity (IU/L) was least ($p < 0.05$) in OAML + MSG (3.84 ± 0.36) followed by MSG (3.98 ± 0.42) and highest ($p < 0.05$) in TAML (5.85 ± 0.26) compared to control (5.93 ± 0.23).

Table 2: Effect of amlodipine on some serum glutathione peroxidase (GP_X) activity (IU/L) in monosodium glutamate-challenged Wistar rats

Groups	GPx (IU/L)	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	5.93 ± 0.23	0	0.49
2: TAML	5.85 ± 0.26	-1.32	0.47
3: OAML	4.77 ± 0.39	-19.52	0.20
4: MSG	3.98 ± 0.42	-32.80	0
5: TAML+ MSG	5.82 ± 0.26	-1.83	0.46
6: OAML+ MSG	3.84 ± 0.36	-35.21	-0.04

Values are mean ± SEM for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p < 0.05$ and relative change of up to and above 10.00 % [19]

The significant depletion of GP_X activity in OAML + MSG and MSG only groups could be reflecting consequence of increased level of participation in protecting the rats from the respective treatment-induced oxidative-stress damage. The main biological role of GP_X (EC 1.11.1.9) as the others in the enzyme family with peroxidase activity is to protect the organism from oxidative damage [31].Results in Table 3 showed variations in magnesium (Mg) concentration (mmol/l) which revealed marked alteration ($p < 0.05$) of Mg in MSG only and in OAML + MSG treated rats compared to control and others.

Table 3: Effect of amlodipine on some serum magnesium (Mg) concentration (mmol/l) in monosodium glutamate-challenged Wistar rats

Groups	Mg (mmol/l)	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	11.68 ± 0.52	0	-0.30
2: TAML	11.32 ± 0.43	-3.07	-3.36
3: OAML	10.15 ± 0.39	-13.03	-13.29
4: MSG	11.71 ± 0.76	0.30	0
5: TAML+ MSG	11.73 ± 0.52	0.45	0.15
6: OAML+ MSG	10.80 ± 0.19	-7.48	-7.75

Values are mean ± SEM for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p < 0.05$ and relative change of up to and above 10.00 % [19]

This agrees with the results of this study on MDA and GP_X levels that reflected induction of oxidative stress in these rat groups and consequence of increased attempt to protect the rats from oxidative-stressed damage. Magnesium, which is essential to the basic nucleic acid biochemistry of all cells, is significantly altered in the serum due to oxidative stress which is strongly associated with decreased GPx activity but increased MDA concentration as observed in this study [21-33]. Results in Table 4-6 showed similar variations in the calculated diagnostic ratios (MDA:GPx, MDA:Mg and GPx:Mg). These revealed marked alteration ($p < 0.05$) of the diagnostic ratios in MSG only, OAML + MSG and TAML +MSG treated rats compared to control and others. These agree with the results of the present study that indicated induction and consequence of oxidative stress in these treatment (MSG only, OAML + MSG and TAML + MSG) groups.

Table 4: Effect of amlodipine on some serum MDA: GPX ratio in monosodium glutamate-challenged Wistar rats

Groups	MDA:GPx	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	50.33	0	-64.21
2: TAML	56.43	12.11	-59.87
3: OAML	71.64	42.33	-49.06
4: MSG	140.62	179.38	0
5: TAML+ MSG	97.68	94.07	-30.53
6: OAML+ MSG	127.60	153.53	-9.25

Values are mean for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p < 0.05$ and relative change of up to and above 10.00 % [19]

Table 5: Effect of amlodipine on some serum MDA:Mg ratio in monosodium glutamate-challenged Wistar rats

Groups	MDA:Mg	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	25.55	0	-46.57
2: TAML	29.16	14.13	-39.02
3: OAML	33.65	31.72	-29.63
4: MSG	47.82	87.17	0
5: TAML+ MSG	48.46	89.65	1.33
6: OAML+ MSG	45.36	77.54	-5.15

Values are mean for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p < 0.05$ and relative change of up to and above 10.00 % [19]

Table 6: Effect of amlodipine on some serum GP_X:Mg ratio in monosodium glutamate-challenged Wistar rats

Groups	GPx:Mg	Change relative to the Control (%)	Change relative to MSG group (%)
1: Control	0.51	0	49.25
2: TAML	0.52	1.81	51.96
3: OAML	0.47	-7.45	38.14
4: MSG	0.34	-33.00	0
5: TAML+ MSG	0.50	-2.27	45.87
6: OAML+ MSG	0.36	-29.97	4.53

Values are mean for sample size (n) = 6. + denotes higher by; - denotes lower by. Difference considered statistically significant at $p < 0.05$ and relative change of up to and above 10.00 % [19]

Generally, increased serum MDA concentration indicates increased lipid peroxidation and generation of oxidative stress [28-34]. The probably increased lipid peroxidation led to the increased generation of MDA as recorded in MSG only, OAML + MSG and TAML + MSG treatment groups and attempts made to combat the oxidative stress damage led to a significant reduction in the level of GP_X and Mg in these rat groups. These positions were seemingly confirmed by the consistently marked and significant alteration of the calculated combined ratios of the parameters in these rat groups. Combination ratios of bioindicators could be a supportive diagnostic tool in confirming the outcome of the parent parameters [5].

CONCLUSION

The study was aimed at assessing the effect of amlodipine on some serum indicators of oxidative stress; MDA, Mg concentration and GPx activity in MSG-challenged Wistar rats. The results demonstrated that amlodipine, particularly at therapeutic dose, did not compromise the antioxidant integrity, and did not lower the significant adverse effects on the above listed oxidative parameters elicited by monosodium glutamate, in the rats' serum. This validates previous studies which established that MSG led induction of oxidative stress. It further suggests probable negative interactive effects of amlodipine and monosodium glutamate which warrants caution in their combined use in rats. The underlying mechanism of interaction between amlodipine and MSG to elicit these negative effects calls for further study.

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