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Original Research Article

Examination Of Antioxidants And Hepatic Enzymes Responses Of L-arginine On Aspartame-induced Oxidative Stress In Rats

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Abstract

This study aimed to examine the antioxidants and hepatic enzymes responses of L-arginine on aspartame-induced oxidative stress in 30 male Wistar rats. Aspartame (1000 mg Kg^{-1} of body weight) was administered by oral intubation daily for 21 days. Aspartame treatment significantly ($P < 0.05$) increased ferric reducing antioxidant power, total protein, thiobarbituric acid reacting substances, catalase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, but decreased superoxide dismutase. Aside from aspartate aminotransferase that was not reduced, L-arginine 20 mg $Kg^{-1}$was administered alone, and aspartame respectively administered with vitamin C 100 mg Kg^{-1}, L-arginine 20 mg Kg^{-1} and 40 mg Kg^{-1}, significantly ($P < 0.05$) decreased ferric reducing antioxidant power, total protein, thiobarbituric acid reacting substances, catalase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, but increased superoxide dismutase. These effects induced by aspartame (1000 mg Kg^{-1}) were mitigated by L-arginine irrespective of dose, and in a comparable pattern as a standard antioxidant, vitamin C. Thus, L-arginine significantly mitigated aspartame-induced oxidative stress and impaired hepatic enzymes in the rats. The responses were via probable up-regulated mechanisms in rats' serum antioxidants and hepatic enzymes responses.

Keywords : nitric oxide; vitamin c; oxidant risk; serum enzymes; superoxide dismutase; catalase

Introduction

L-arginine is a sole precursor of a conditional antioxidant, nitric oxide.[1] L-arginine is in common natural foods and in diets and drugs.[2] Aspartame, an artificial sweetener, ensures reduced sugar intake.[3]Aspartame is a methyl ester that comprises natural amino acids (L-aspartate and L-phenylalanine) with potential toxicity in animals upon hydrolysis to its constituent amino acids and methanol.[4,5] Aspartate and its inter-convertible products - asparagine, glutamine and glutamate are excitatory amino acids with hyper-excitability potential.[6] The oxidation of methanol results to the formation of formaldehyde, formic acid and formate that are cytotoxic.[5]

Aspartame metabolites increased significantly upon its consumption with an attendant spike in the generation of free radicals in rats.[7] Free radicals or oxidants account for tissue oxidative stress and underlies agent-related toxicity mechanisms. Free radicals are unstable atoms with unpaired valence electrons. They could easily damage vital organs by attacking and transforming cellular bio-molecules into other free radicals, including hydrogen peroxide, hydroxyl radical, and nitric oxide.[8] Disruption in the normal expression and function of biomarkers of physiological functions result in ill-health usually accompanied with oxidative stress and altered hepatic enzymes expression and functions. Oxidative stress is a state of excess pro-oxidant as against antioxidant that favours excess free radical generation in the body. It is the underlying basis for many diseases.[2] Excess free radicals (oxidants) leading to oxidative stress could be scavenged through well-developed multiple antioxidant defence systems.[9] Antioxidants transfer an electron to oxidants thereby inhibiting free radical production and

consequent oxidative stress-related cell damage. [10]

L-arginine with aspartame could be co-consumed in diets and drugs with unknown responses on the antioxidants and hepatic enzymes functions in animals. Aspartame generates free radicals through its metabolites, while L-arginine exerts antioxidant and oxidant effects through its metabolite, nitric oxide. It is necessary to study the possible effects of concomitant use of L-arginine and aspartame to establish whether arginine could aggravate, mitigate or fail to alter the potential oxidant and impaired hepatic enzymes effects of aspartame. Therefore, this study was aimed at examining the potential interactive effects and responses of arginine on aspartame-induced oxidant effects and impairment of the functions of the hepatic enzymes in rats' serum.

Methodology

Chemicals and Drug

Vitamin C (100 mg) tablet was procured from Emzor Pharmaceuticals, Lagos, Nigeria. L-arginine and aspartame were obtained from Sigma Chemical Company, St. Louis, MO. USA. Other chemicals were of certified analytical grade.

Animals and Treatments

The animals used in this study were adult male Wistar rats. They (thirty adult male rats with a body weight range of 50 – 70 g) were obtained from competent rats' breeder. They were kept in the animal house of the institution (south-east, Nigeria) where the study was carried out for 2 weeks to acclimatize. Then, the animals were randomly assigned to six groups of five rats each. Group A rats, control, were given distilled water (1 mL Kg^{-1}). Group B rats were fed aspartame (1000 mg Kg^{-1} bwt) alone, whereas group C rats were fed aspartame (1000 mg Kg^{-1}) with dietary water-soluble antioxidant standard, vitamin C (100 mg Kg^{-1}). Group D rats were given L-arginine (20 mg Kg^{-1}bwt) alone whereas groups E and F rats were fed aspartame (1000 mg Kg^{-1}bwt) with L-arginine (20 mg Kg^{-1}) and (40 mg Kg^{-1}), respectively. Treatment was by daily oral intubation for 21 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.

Blood Collection and Preparation

Blood samples of the rats sacrificed following mild anaesthesia 24 h after 21 days of treatment were collected individually with sterile capillary tubes into properly labeled plain polystyrene centrifuge tubes by ocular puncture technique. The blood samples thus collected were allowed to clot. Then, the serum was removed by centrifugation at 3000 rpm for 5 minutes, collected individually and stored in a deep freezer for determination of the serum ferric reducing antioxidant power (FRAP), total protein (TP), thiobarbituric acid reacting substances (TBARS), catalase (CAT), superoxide dismutase (SOD) alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The study was carried out in accordance with the institution's standard for humane handling of laboratory animals based on the ethical guidelines of the National Research Council, USA.[11]

Determination of Antioxidant Status Indicators in the Rats' Serum

The determination of superoxide dismutase (SOD) activity was according to the method of Madesh and Balasubramanian [12] while catalase (CAT) activity was determined according to the method described by Johansson and Borg.[13] The thiobarbituric acid reactive substance (TBARS) concentration was determined by the method of Wallin et al.[14] while the ferric reducing antioxidant power (FRAP) was determined following the description by Benzie and Strain.[15] The total protein (TP) concentration was determined with the Randox kit according to the Biuret method of Weichselbaum.[16]

Determination of Liver Enzymes Activities in the Rats' Serum

The serum ALT and AST activities were determined with Randox kits according to the method of Reitman and Frankel.[17] The serum ALP activity was determined with Randox kit according to the method of Kochmar and Moss.[18]

Statistical Analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA). The statistical package for social sciences (SPSS) for windows version 16.0 was used. Dunnett's test was employed to carry out the *posthoc* multiple comparisons of means. Differences in mean were considered significant at $p<0.05$ level of significance. The results obtained were presented as mean ± standard error of the mean (SEM).

Results

FRAP and TP Concentrations in the Rats' Serum

Table 1 shows a significant increase of FRAP concentration in aspartame-assaulted rats (group B) compared to control. A quantitative reduction was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone.

The study recorded a significant increase of TP concentration in aspartame-assaulted rats (group B) compared to control while a quantitative reduction in TP concentration was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone (Table 1).

CAT and SOD activities in the Rats' Serum

The CAT enzyme was elevated in the serum of aspartame-exposed rats (group B) compared to control. But, a quantitative reduction was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone (Table 2).

The study recorded a significant decrease of SOD activity in aspartame-assaulted rats (group B) compared to control while a quantitative elevation in SOD activity was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone (Table 2).

TBARS Concentration in the Rats' Serum

The results presented in Table 2 show a significant increase in the rats' serum TBARS concentration in aspartame-assaulted rats (group B) compared to control. A quantitative reduction was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone.

ALT, AST and ALP activities in the Rats' Serum

The serum activities of ALT, AST and ALP enzymes were elevated in aspartame-fed rats (group B) compared to control. A quantitative reduction of serum activities of ALT and ALP was observed in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D), vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) compared to aspartame-fed alone. But, aside from rats that were fed L-arginine 20 mg Kg^{-1} alone (group D), a quantitative increase of serum activity of AST was observed in rats that were fed with vitamin C 100 mg Kg^{-1} and aspartame together (group C), and L-arginine either at 20 mg Kg^{-1} or 40 mg Kg^{-1} and aspartame together (groups E and F) on comparison with aspartame-assaulted rats and control (Table 3).

Discussion

The outcome of this study revealed that aspartame treatment increased FRAP, TP, TBARS

and CAT, but decreased SOD in the rats' serum. This indicated induction of oxidative stress due to compromised antioxidant response mechanisms in the rats. This is consistent with previous reports.[7,19,20] Simultaneous increase in CAT and TBARS reported herein was a consequence of oxidative stress.[21] The increased protein concentration in aspartame-assaulted rats herein is consistent with previous study results [22] indicating increased protein metabolism as a probable consequence of increased interactions between functional groups of proteins and ROS to generate inactive adducts.[21] Aspartame-related diminution in SOD as in this study could be a consequence of active participation of SOD enzyme in antioxidant response against superoxide radicals-related oxidative stress.[2] This probably involved increased utilization of SOD in converting superoxide radicals to molecular oxygen and hydrogen peroxide as a first-line antioxidant defence in the rats.[23,24] As shown in the present study, increased lipid peroxidation product, MDA and decreased SOD indicated oxidative stress status in the previous study[25]. Conversely, administering L-arginine alone decreased FRAP, TP, TBARS and CAT but increased SOD in the rats' serum; which is consistent with previous study outcomes.[25,26] This indicates L-arginine-related benefits on the rats' antioxidant response system and the potential to mitigate oxidative stress in the rats. The outcome of these bio-indicators following administration of aspartame with either low or high L-arginine compared favourably with that following administration of aspartame with vitamin C and was essentially opposite and significant on comparison with that following administration of aspartame alone. This expectedly demonstrates a significant mitigation response of L-arginine against oxidative stress-induced in the rats following aspartame assault. Vitamin C is a standard antioxidant known to mitigate oxidative stress.[27] Previously, L-arginine mitigated oxidative stress. [21]

The outcome of this study further reveals that aspartame treatment significantly increased serum ALT, AST, and ALP enzyme activities; which confirms successful induction of oxidative stress and impaired hepatic enzymes functions in the aspartame-fed rats. This is consistent with previous study outcomes.[28,29,30] The impaired hepatic enzymes reported herein (and apparent hepatotoxicity) is an expected consequence of successful induction of oxidative stress in the rats. The liver is a major organ for xenobiotics metabolism, and impaired hepatic enzymes leading to hepatic damage as a consequence of oxidative stress was suggested in an earlier study.[31] In contrast, administering L-arginine alone decreased ALT, AST and ALP in the rats' serum. This indicates L-arginine-related benefits in the rats' liver enzyme expression and function with possible potential to mitigate impaired hepatic enzymes functions in the rats. These support the outcome of the present study; and were consistent with the previous report.[32] The outcome of these bio-indicators following administration of aspartame with either low or high L-arginine compares with that following administration of aspartame with vitamin C; but was opposite, significant and dose-dependent on comparison with that following administration of aspartame alone. This demonstrates the definite potential of L-arginine to mitigate compromised liver enzymes functions in oxidative-stressed rats following aspartame assault. In comparison to administration of aspartame alone, the outcome on these liver enzymes following administration of L-arginine 40 mg Kg^{-1} with aspartame was marked, significant, consistent and out-compared that observed following administration of the standard dietary antioxidant, vitamin C 100 mg $Kg^{-1}$with aspartame. This could be a pointer to the overriding potential of L-arginine at a higher dose (40 mg $Kg^{-}1$) to enhance the mitigation of oxidative stress-related hepatic dysfunction contributed by compromised liver enzymes expression and functions in the rats. The observation and suggestion thereto show a similar trend to that in this study outcome on the antioxidants response in rats. This confirms the significant potential of L-arginine to mitigate oxidative stress and impaired hepatic enzymes in rats following intake of assault dose of aspartame. On the whole, the results demonstrate that L-arginine could significantly mitigate aspartame-induced oxidative stress and impair hepatic enzymes by decreasing FRAP, TP, TBARS, CAT, ALT, AST, and ALP while increasing SOD levels in the rats.

Conclusion

The effects induced by aspartame (1000 mg Kg^{-1}) were mitigated by L-arginine irrespective of dose, and in a comparable pattern as a standard antioxidant, vitamin C. Thus, L-arginine significantly mitigated aspartame-induced oxidative stress and impaired hepatic enzymes in the rats. The responses were *via* probable up-regulated mechanisms in rats' serum antioxidants and hepatic enzymes responses.

Limitations of the Study

This was only conducted using male Wistar rats.

Conflicts of interest/Competing interests: None

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Table 1. Response of L-arginine and Aspartame + L-arginine on FRAP and TP Concentrations in Rats' Serum

Group/Treatment (mg Kg^{-1})	FRAP (µg mL^{-1})	TP (g mL^{-1})
A: Distilled water	0.16 ± 0.09^{d}	6.47 ± 0.21^{d}
B: Aspartamealone	0.17 ± 0.05^{e}	7.01 ± 0.12^{f}
C: Aspartame + vitamin C	0.12 ± 0.03^{a}	6.56 ± 0.40^{e}
D: L-arginine alone (20)	0.13 ± 0.04^{b}	6.46 ± 0.25^{c}
E: Aspartame + L-arginine (20)	0.13 ± 0.11^{b}	5.75 ± 0.30^{b}
F: Aspartame + L-arginine (40)	0.15 ± 0.12^{c}	5.15 ± 0.57^{a}

Notes: The results are mean ± SEM for five rats in each group.
^{a,b,c,d,e,f} Means on a column with different superscript letters (arranged from ^{a} = least to ^{f} = highest) are significantly different at $p < 0.05$.

Table 2. Response of L-arginine and Aspartame + L-arginine on CAT and SOD Enzyme Activities and TBARS Concentration in Rats' Serum

Group/Treatment (mg Kg^{-1})	CAT activity (IU/L)	SOD activity (IU/L)	TBARS (mg mL^{-1})
A: Distilled water	0.22 ± 0.09^{d}	0.79 ± 0.02^{c}	0.02 ± 0.06^{a}
B: Aspartame alone	0.29 ± 0.04^{e}	0.68 ± 0.07^{a}	0.07 ± 0.14^{c}
C: Aspartame + vitamin C	0.17 ± 0.03^{b}	0.77 ± 0.06^{b}	0.02 ± 0.0^{a}
D: L-arginine (20) alone	0.13 ± 0.02^{a}	0.91 ± 0.02^{d}	0.03 ± 0.04^{b}
E: Aspartame + L-arginine (20)	0.19 ± 0.05^{c}	1.20 ± 0.06^{f}	0.03 ± 0.01^{b}
F: Aspartame + L-arginine (40)	0.22 ± 0.06^{d}	1.06 ± 0.16^{e}	0.03 ± 0.07^{b}

Notes: The results are mean ± SEM for five rats in each group.
^{a,b,c,d,e,f} Means on a column with different superscript letters (arranged from ^{a} = least to ^{f} = highest) are significantly different at $p < 0.05$.

Table 3. Response of L-arginine and Aspartame + L-arginine on ALT, AST and ALP Enzyme Activities in Rats' Serum

Group/Treatment (mg Kg^{-1})	Serum ALT activity (IU/L)	Serum AST activity (IU/L)	Serum ALP activity (IU/L)
A: Distilled water	24.98 ± 1.23^{c}	53.16 ± 2.62^{b}	40.54 ± 1.50^{a}
B: Aspartame alone	27.58 ± 1.58^{d}	54.96 ± 1.93^{c}	79.85 ± 3.99^{f}
C: Aspartame + vitamin C	20.62 ± 4.13^{a}	55.70 ± 3.46^{d}	58.96 ± 2.25^{c}
D: L-arginine (20) alone	24.02 ± 2.51^{c}	43.94 ± 4.35^{a}	48.47 ± 5.09^{b}
E: Aspartame +L-arginine (20)	24.18 ± 3.16^{c}	56.56 ± 3.74^{e}	65.33 ± 4.85^{d}
F: Aspartame + L-arginine (40)	22.54 ± 2.15^{b}	58.42 ± 3.43^{f}	68.20 ± 4.57^{e}

Notes: The results are mean ± SEM for five rats in each group.
^{a,b,c,d,e,f} Means on a column with different superscript letters (arranged from ^{a} = least to ^{f} = highest) are significantly different at $p<0.05$.

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