Antioxidant Responses of L-Arginine on Aspartame-Induced Oxidative Stress in Rats' Liver

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ABSTRACT

Background: *L-arginine a major precursor of a conditional antioxidant nitric oxide could be co-consumed with a widely used oxidant-prone artificial sweetener, aspartame with unknown outcomes on the antioxidant status in animals' liver.*

Objectives: *This study aimed to examine the possible liver antioxidant and histomorphologic responses of L-arginine on oxidative stress induced by aspartame assault.*

Methods: *Thus, aspartame, at a dose of 1000 mg Kg^{-1} of body weight was administered to male Wistar rats by oral intubation daily for 21 days.*

Results: *Aspartame treatment significantly ($P < 0.05$) increased ferric reducing antioxidant power, FRAP ($0.78 \pm 0.98 \mu g\ mL^{-1}$), total protein, TP ($2.42 \pm 0.19 g\ mL^{-1}$), thiobarbituric acid reacting substances, TBARS ($0.65 \pm 0.07\ mg\ mL^{-1}$), catalase, CAT ($6.61 \pm 1.81$ IU/L) and hepatic histo-congestion, but decreased superoxide dismutase, SOD (0.09 ± 0.01 IU/L). Aside ferric reducing antioxidant power and catalase that were not reduced, L-arginine 20 mg Kg^{-1} 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 total protein, thiobarbituric acid reacting substances and hepatic histo-congestion, but increased superoxide dismutase. Results revealed that these effects induced by aspartame at a dose of 1000 mg Kg^{-1} were significantly ($P < 0.05$) mitigated by L-arginine in a comparable pattern as standard antioxidant, vitamin C.*

Conclusion: *Thus, L-arginine mitigated aspartame-induced oxidative stress and histo-congestion in rats' liver via probable up-regulated mechanisms in rats' antioxidant responses.*

Keywords: Histo-integrity, Nitricoxide, Vitamin C, Oxidant risk, Histo-congestion

Introduction

L-arginine is a semi-essential basic amino acid. It is a sole-precursor of a conditional antioxidant, nitric oxide, which mediates animal health protection. [1] L-arginine is in natural foods, diets and drug. [2] L-arginine with artificial sweeteners may be co-consumed. Artificial sweeteners are increasingly used without considering their relative reactivity and health hazards. [3,4] Aspartame is notable artificial sweetener. It is a methyl ester that comprises natural amino acids (L-aspartate and L-phenylalanine). It could exert significant toxic manifestations in animals following hydrolysis to its constituent amino acids and methanol. [5,6] Aspartate is an excitatory

amino acid that could, in concert with its inter-convertible products - asparagine, glutamine and glutamate, result to hyper-excitability and adverse response. [7] Methanol on oxidation forms formaldehyde, formic acid and formate that are injurious to the cells. [6] Aspartame metabolites significantly increased following its consumption with a spike in generation of free radicals in rats. [8,9] Free radicals are oxidants. Free radical production underlies agent-related toxicity mechanism and oxidative stress by attacking and transforming cellular bio-molecules into other free radicals. [10] Oxidative stress is a state of excess pro-oxidant as against antioxidant that favors excess free radical generation in the body. It is the underlying basis for many diseases. [2,11]

The liver is notably prone to oxidative stress and related injury owing to its high metabolic rate as occasioned by its central role in xenobiotic metabolism. Well-developed multiple antioxidant defense systems protect the liver from oxidative stress due to free radicals attack. [12] Antioxidants transfer electron to oxidants thereby inhibiting free radical production and consequent oxidative stress-related cell damage. [13] L-arginine with aspartame could be co-consumed. Aspartame could generate free radicals through its metabolites, while L-arginine could exert 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 effects of aspartame. Therefore, this study was aimed at examining the potential interactive effects of arginine on aspartame-induced oxidant effects to elucidate the possible antioxidant and histomorphologic responses of L-arginine on hepatic oxidative stress induced by aspartame assault in rats.

MATERIALS AND METHODS

Chemicals and Drug

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

Animals and treatments

The animals used in this study were adult male Wistar rats obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. Thirty adult male rats with body weight range of 50 – 70 g were kept in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria 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}) 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}) alone where as 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.

Liver tissue collection and preparation

Liver samples of the rats sacrificed following mild anesthesia 24 h after 21 days treatment were excised individually. A part was fixed (for histological assessment) and the remainder homogenized (to obtain supernatant for biochemical evaluation). Ten (10) % liver tissue homogenate was obtained by separately grinding a 0.5 g of respective liver sample in 5 ml of phosphate buffer saline (p^{H} 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 homogenate FRAP, TP, TBARS, CAT and SOD. Liver tissues for histomorphologic examination were fixed in 10 % buffered formal-saline until prepared for according to the method described by Egbuonu and Ejike (2017). [2] Photomicrographs were taken using a Motic 9.0 megapixels microscope camera at × 400 magnifications. The study was carried out in accordance with the ethical guidelines of the National Research Council, USA. [14]

Determination of antioxidant status indicators in the rats' liver homogenate

The determination of superoxide dismutase (SOD) activity was by the method of Madesh and Balasubramanian [15] while catalase (CAT) activity was determined by the method described by Johansson and Borg. [16] The thiobarbituric acid reactive substance (TBARS) concentration was determined by the method of Wallin *et al*.

[17] while the ferric reducing antioxidant power (FRAP) was determined as described by Benzie and Strain. [18] The total protein (TP) concentration was determined with Randox kit based on the Biuret method of Weichselbaum. [19]

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. The Dunnett's test was used for the *post-hoc* multiple comparison of means. Differences in mean were considered significant at $p<0.05$ level of significance. The results were presented as mean ± standard error of mean (SEM).

RESULTS

FRAP and TP concentrations in the rats' liver homogenate

Table 1 shows a significant increase of FRAP and TP in aspartame-assaulted rats (group B) and in rats that were fed with L-arginine 20 mg Kg^{-1} and aspartame together (group E). A quantitative reduction of FRAP and TP 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).

Table 1. Response of L-arginine and Aspartame + L-arginine on FRAP ($\mu g\ mL^{-1}$) and TP ($g\ mL^{-1}$) concentrations in rats' liver homogenate

Group/Treatment (mg Kg^{-1})	FRAP ($\mu g\ mL^{-1}$)	TP ($g\ mL^{-1}$)
A: Distilled water	0.70 ± 0.81^{c}	2.26 ± 0.15^{e}
B: Aspartamealone	0.78 ± 0.98^{d}	2.42 ± 0.19^{f}
C: Aspartame + vitamin C	0.63 ± 0.50^{b}	0.89 ± 0.05^{b}
D: L-arginine alone (20)	0.68 ± 0.47^{b}	1.46 ± 0.103^{c}
E: Aspartame + L-arginine (20)	0.78 ± 0.46^{d}	1.90 ± 0.28^{d}
F: Aspartame + L-arginine (40)	0.47 ± 0.15^{a}	0.61 ± 0.05^{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 TBARS ($mg\ mL^{-1}$), CAT (IU/L) and SOD (IU/L) levels in rats' liver homogenate

Group/Treatment (mg Kg^{-1})	CAT activity (IU/L)	SOD activity (IU/L)	TBARS ($mg\ mL^{-1}$)
A: Distilled water	4.47 ± 0.26^{b}	0.11 ± 0.01^{c}	0.08 ± 0.15^{a}
B: Aspartame alone	6.61 ± 1.81^{d}	0.09 ± 0.01^{a}	0.65 ± 0.07^{e}
C: Aspartame + vitamin C	8.19 ± 0.38^{e}	0.30 ± 0.06^{f}	0.29 ± 0.04^{b}
D: L-arginine (20) alone	5.28 ± 0.73^{c}	0.20 ± 0.03^{d}	0.08 ± 0.08^{a}
E: Aspartame + L-arginine (20)	2.79 ± 0.38^{a}	0.10 ± 0.01^{b}	0.41 ± 0.07^{c}
F: Aspartame + L-arginine (40)	17.33 ± 3.44^{f}	0.24 ± 0.03^{e}	0.54 ± 0.06^{d}

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 $p<0.05$.

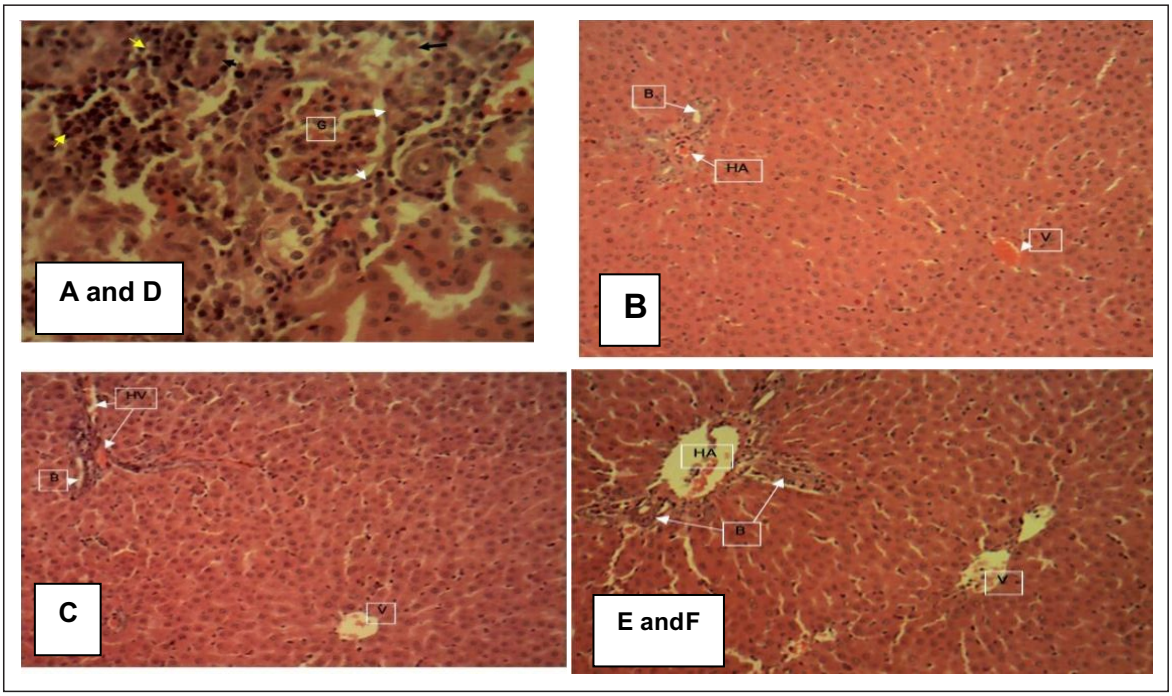

Figure 1. Response of L-arginine and Aspartame + L-arginine on hepatic histomorphology of rats (H & E ×400).

Notes: A = Rats' Group A, Distilled water; B = Rats' Group B, Aspartame alone; C = Rats' Group C, Aspartame + vitamin C; D = Rats' Group D, L-arginine alone at 20 mg Kg^{-1}; E = Rats' Group E, Aspartame + L-arginine at 20 mg Kg^{-1} and F = Rats' Group F, Aspartame + L-arginine at 40 mg Kg^{-1}.
G = central vein (normal).
V = central vein (congested).
Black and white arrowheads =cords of hepatocytes.
Yellow arrowheads = epithelia cells.
HA = portal hepatic artery.
B = bile duct.

TBARS concentration, CAT and SOD activities in the rats' liver homogenate

The results presented also in Table 2 show a significant increase in liver homogenate TBARS concentration in aspartame-assaulted rats (group B). 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 CAT enzyme was elevated in the liver homogenate of aspartame-exposed rats (group B). This was further quantitatively potentiated (over 2-fold) in rats co-administered with L-arginine 40 mg Kg^{-1} and aspartame (group F) on comparison with aspartame- assaulted rats and others (Table 2).

The SOD enzyme diminished in aspartame-exposed rats (group B). A quantitative elevation 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).

Histomorphologic outcome of the rats' liver

The histomorphologic changes in the rats' liver were shown in Figure 1. A marked congestion of the central vein (V) and portal artery (HA) was observed in aspartame-assaulted rats (group B). The central vein (V) and portal artery (HA) werenot congested with blood in the control rats (group A) and in rats that were fed with L-arginine 20 mg Kg^{-1} alone (group D). There was mild blood congestion of the central vein (V) and portal artery (HA) in rats that were fed with aspartame + vitamin C (group C). There was sparse blood congestion of the central vein (V) and portal artery (HA) and clear bile duct (B) in rats that were fed

with aspartame + L-arginine at 20 mg Kg^{-1}(group E) or aspartame + L-arginine at 40 mg Kg^{-1} (group F).

DISCUSSION

This study examined the possible antioxidant responses of L-arginine on liver oxidative stress and histo-congestion induced by aspartame assault in rats. The outcome of this study revealing that aspartame treatment increased ferric reducing antioxidant power (FRAP), total protein (TP), thiobarbituric acid reactive substance (TBARS) and catalase (CAT), but decreased superoxide dismutase (SOD) in the rats' liver indicated induction of oxidative stress sequel to compromised antioxidant response mechanisms in the rats liver. This is consistent with previous reports that associated induction of oxidative stress or compromised antioxidant response mechanisms in animals with increased bio-indicators of antioxidant status reported herein. [20,21] Aspartame could compromise these bio-indicators of rats' antioxidant defense mechanisms leading to oxidative stress. [22] Metabolites of aspartame notably methanol triggered free radicals hyper-production in previous study. [8]

Simultaneous increase in CAT and TBARS as adaptive response to oxidative stress has been documented. [23] The increased CAT activity in aspartame-assaulted rats herein was consistent with earlier report. [8] Proteins play primary role in antioxidant defenses. They are important general scavengers of reactive oxygen species (ROS) generated under oxidative stress conditions. They are targets for ROS attack but during such attack, functional groups of proteins interact with ROS to generate inactive adducts. [23] The increased protein concentration in aspartame-assaulted rats herein aligned with the work of Gul *et al*. [24] indicating compromised protein metabolism-related bio-functions. Diminution in FRAP suggested free radical scavenging potential and *vice versa*.[2] Diminution in SOD as in this study indicated oxidative stress and consequence of key participation of SOD enzyme in antioxidant response against superoxide radicals as an important component of first line of antioxidant response defense mechanism [25]. The diminution could result from down-regulation in the synthesis and increased utilization in converting superoxide radicals generated following aspartame assault in the rats to molecular oxygen and hydrogen peroxide. [26,27] As shown in the present study, increased lipid per-oxidation product, MDA and decreased SOD indicated oxidative stress status. [28]

In the present study, administering L-arginine alone decreased FRAP, TP, TBARS and CAT but increased SOD in the rats' liver. This indicates L-arginine-related benefits on the rats' antioxidant response system with possible potential to mitigate oxidative stress in the rats' liver. The observation and suggestion thereto conformed to previous study outcomes. [20,29,28] The outcome of these bio-indicators following administration of aspartame with either low or high L-arginine compared favorably with that following administration of aspartame with vitamin C and was opposite and significant on comparison with that following administration of aspartame alone. The present outcome demonstrates significant and dose-dependent mitigation response of L-arginine against oxidative stress induced in the rats' liver following aspartame assault. The present outcome is expected. Vitamin C is a standard antioxidant known to mitigate oxidative stress. [30] Previously, L-arginine mitigated oxidative stress. [20,21] Herein, L-arginine exerted potential benefit on FRAP, TP, TBARS and CAT expression in the rats' liver. L-arginine administered with aspartame significantly down-regulated the contribution of the expression of these bio-pointers on aspartame-induced hepatic oxidative stress in the rats. On comparison to administration of aspartame alone, outcome on these antioxidant bio-pointers 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 overriding potential of L-arginine at a higher dose (40 mg Kg^{-1}) to enhance the mitigation of hepatic oxidative stress contributed by compromised expression and functions of these bio-pointers in the rats.

Outcome of the rats' liver histomorphologic assessment as presented based on Figure 1 supported the serum and homogenate chemistry results obtained in this study. Confirmation of clinical chemical results from assessed organ histology had been suggested. [2] Herein, L-arginine irrespective of dose demonstrated potential benefit on the restoration of the rats' liver congested with blood following aspartame assault. This is consistent with earlier study outcomes demonstrating arginine benefits. [31,32]. Thus, L-arginine significantly mitigated aspartame-induced blood congestion in the rats'

liver and could offer potential benefit in restoration of rats' liver histology damaged following aspartame assault.

CONCLUSION

Results obtained and discussed herein revealed that these effects induced by aspartame at a dose of 1000 mg Kg_{-1} were mitigated by L-arginine irrespective of dose, and in a comparable pattern as standard dietary antioxidant, vitamin C. Thus, L-arginine mitigated aspartame-induced oxidative stress and hepatotoxicity in rats *via* probable up-regulated mechanisms in rats' antioxidant and enzymatic responses.

Acknowledgements

The authors acknowledge the academic staff, laboratory technologists and histopathologist in the relevant departments and colleges of Michael Okpara University of Agriculture Umudike for the important input and guidance while this study lasted.

Declaration of interest statement (Disclosure statement)

The authors declare that they have no interests to report.

References

1. El-Mesallamy, H.O., Hamid, S.G.A. and Gad, M.Z. (2008). Oxidative stress and asymmetric dimethylarginine are associated with cardiovascular complications in hemodialysis patients: improvements by L-arginine intake. Kidney and Blood Pressure Research, 31: 189-195.
2. Egbuonu, A.C.C. and Ejike, G.E. (2017). Effect of pulverized *Mangiferaindica* (mango) seed kernel on monosodium glutamate-intoxicated rats' serum antioxidant capacity, brain function and histology. EC Pharmacology and Toxicology, 4: 228-243.
3. Toews, I., Lohner,S., Daniela, K., Sommer, H. and Meerpohl, J.J. (2019). Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. British Medical Journal, 364 k4718: 1-13.
4. Fagherazzi, G., Vilier, A., SaesSartorelli, D., Lajous, M., Balkau, B. and Clavel-Chapelon, F. (2013). Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes. American Journal of Clinical Nutrition, 97: 517-523.
5. Chattopadhyay, S., Raychaudhuri, U. and Chakraborty, R. (2014). Artificial sweeteners - a review. Journal of Food Science and Technology, 51: 611–621.
6. Yagasaki, M. and Hashimoto, S.(2008). Synthesis and application of dipeptides; current status and perspectives. Applied Microbiology and Biotechn\ology, 81: 13-22.
7. Soffritti, M., Belpoggi, F., Degli-Esposti, D. and Lambertini, L .(2005). Aspartame induces lymphomas and leukaemias in rats. European Journal of O\ncology, 10: 107-116.
8. Ashok, I., Sheeladevi, R. and Wankhar, D. (2015). Acute effect of aspartame- induced oxidative stress in Wistar albino rat brain. The Journal of Biomedical Research, 29: 390-396.
9. Prokić, M.D., Paunović, M. G., Matić, M.M., Djordjević, N.Z., Ognjanović, B.I., Štajn, A.S. and Saičić, Z.S. (2014). Prooxidative effects of aspartame on antioxidant defense status in erythrocytes of rats. Journal of Biosciences, 39: 859-866.
10. Phaniendra, A., Jestadi, D.B. and Peryasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. Indian Journal of Clinical Biochemistry, 30: 11-26.
11 Sharifi-Rad, M., Kumar, N.V.A., Zucca, P., Varoni, E.M. et al. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases.Frontier in Physiology, 11(694): 1-21.
12. Poljšak, B. and Fink, R.(2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. Oxidative Medicine and Cellular Longevity, Article ID 671539: 1-22.
13. Valko, M, Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160: 1-40.
14. NRC (National Research Council, USA). (2011). Guide for the Care and Use of Laboratory Animals. 8th Edition, Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington DC, USA: National Research Council, National Academies Press.
15. Madesh, M. and Balasubramanian, K.A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics, 35: 184-188.

16. Johansson, L.H. and Borg, L.A. (1988). A spectrophotometric method for determination of catalase activity in small sample tissues. Analytical Biochemistry, 174: 331-336.
17. Wallin, B., Rosengren, B., Shertzer, H.G. and Camejo, G. (1993). Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of antioxidants. Analytical Biochemistry, 208: 10-15.
18. Benzie, I.F.F. and Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299: 15-27.
19. Weichselbaum T.E. (1946). An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American Journal of Clinical Pathology, 10: 40-49.
20. Qiu, Y., Yang, X., Wang, L., Gao, K. and Jiang, Z. (2019). L-arginine inhibited inflammatory response and oxidative stress induced by lipopolysaccharide *via* arginase-1 signaling in IPEC-J2 cells. International Journal of Molecular Sciences, 20: 1800-1813.
21. Zhang, H., Peng, A., Yu, Y., Guo, S., Wang, M.Z. and Wang, H. R. (2019). L-arginine protects ovine intestinal epithelial cells from lipopolysaccharides-induced apoptosis through alleviating oxidative stress. Journal of Agricultural and Food Chemistry,67: 1683-1690.
22. Egbuonu, A.C.C. and Elendu, D.S. (2021). Alterations in brain histomorphology and some homogenate antioxidant bio-pointers in L-arginine co-exposed aspartame-assaulted rats. Animal Research International, 18: 4116-4124.
23. Ilaria, M., Fabio, A. and Ilaria, P. (2017). Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxidative Medicine and Cellular Longevity, Article ID 6501046: 1-32.
24. Gul, S.S., Hamilton, A.R.I., Munoz, A.R., Phupitakphol, T. et al. (2017). Inhibition of the gut enzyme intestinal alkaline phosphatase may explain how aspartame promotes glucose intolerance and obesity in mice. Applied Physiology, Nutrition, and Metabolism, 42: 77-83.
25. Mao, C., Yuan, J., Yue-Bin, L., Gao, X. et al. (2019). Associations between superoxide dismutase, malondialdehyde and all-cause mortality in older adults: a community-based cohort study. BMC Geriatrics, 19(104): 1-9.
26. Ighodaro, O.M. and Akinloye, O.A.(2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54: 287-293.
27. Egbuonu, A.C.C., Opara, C.I., Atasie, O.C. and Mbah, U.O. (2017). Vitamins composition and antioxidant properties in normal and monosodium glutamate-compromised rats' serum of avocado pear (*Pearsea americana*) seed. Open Access Journal of Chemistry, 1:19-24.
28. Mandal, A., Das, S., Roy, S., Ghosh, A.K. et al. (2016). Deprivation of L-arginine induces oxidative stress mediated apoptosis in Leishmania donovani promastigotes: Contribution of the polyamine pathway. PLoS Neglected Tropical Diseases, 10: 1-26.
29. Liang, M., Wang, Z., Li, H., Cai, L. et al. (2018). L arginine induces antioxidant response to prevent oxidative stress via stimulation of glutathione synthesis and activation of Nrf2 pathway. Food and Chemical Toxicology, 115: 315-328.
30. Ljiljana, M.P., Nebojsa, R.M., Dijana, M., Boban, B., Mirjana, M. and Brankica, P. (2015). Influence of vitamin C supplementation on oxidative stress and neutrophil inflammatory response in acute and regular exercise. Oxidative Medicine and Cellular Longevity, Article ID 295497: 1-7.
31. Ozsoy, Y., Ozsoy, M., Coskun, T., Namlı, K., Var, A. and Ozyurt, B. (2011). The effects of L-arginine on liver damage in experimental acute cholestasis an immunohistochemical study. HPB Surgery, Article ID 306069:1-5.
32. Zhang, H., Zhang, Y., Liu, X., Elsabagh, M., Yu, Y., Peng, A., Sifa, D. and Wang, H. (2021). L-Arginine inhibits hydrogen peroxide-induced oxidative damage and inflammatory response by regulating antioxidant capacity in ovine intestinal epithelial cells, Italian Journal of Animal Science, 20(1), 1620-1632.