

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

<https://doi.org/10.36673/AJPCR.2020.v08.i04.A19>



THE ROLE OF ORGANIC COMPOUND (ASCORBIC ACID) ON ANTIOXIDANT ENZYMES IN GROWN-UP RABBITS

Hameda T. Algalbati^{*1} and Fayrouz. A. Khaled¹

^{1*}Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University, Al -Bayda-Libya.

ABSTRACT

Vitamin C or ascorbic acid (AA) is a naturally occurring organic compound with antioxidant properties, found in both animals and plants. It capacities as a redox buffer which can decrease, and in this manner neutralize, receptive oxygen species. It may be a cofactor for proteins included in controlling photosynthesis, hormone biosynthesis, and recovering other cancer prevention agents; which too controls cell division and development, is included in flag transduction, and has parts in a few physiological forms, such as safe incitement, amalgamation of collagen, hormones, neurotransmitters, and press retention, has too parts in detoxifying the body of overwhelming metals. The objective of this study was to determine the impact of ascorbic acid (AA, 40mg/kg BW) on antioxidant enzymes in male rabbits every other day for 12 weeks. Results showed that treatment with Ascorbic acid significantly ($p < 0.05$) increased (GSH), (GPx), (GST), (CAT) and (SOD) activities in plasma as compared with control group on the other hand treatment with ascorbic acid alone significantly ($p < 0.05$) decreased TBARS concentration in plasma as compared with control group.

KEYWORDS

Ascorbic acid, Antioxidant enzymes, TBARS and Rabbits.

Author for Correspondence:

Hameda T. Algalbati,
Department of Chemistry,
Faculty of Science, Omar Al-Mukhtar University,
Al -Bayda-Libya.

Email: idress.hamad@omu.edu.ly

INTRODUCTION

Vitamin C (ascorbic acid) is a hydrophilic antioxidant found in green vegetables such as spinach, tomatoes, and fruits like lemons and strawberries¹. Vitamin C (l-ascorbic acid) may be a water-soluble micronutrient required for numerous natural capacities. It is vital for typical development and improvement, and is an essential protein cofactor for a few proteins within the post-translational hydroxylation of collagen, biosynthesis of carnitine, change of the

neurotransmitter dopamine to norepinephrine, peptide amidation, and in tyrosine digestion system. It is additionally an antioxidant that makes a difference security against disease and press assimilation. A few creature species have misplaced the capacity for l-ascorbate union, for that reason, they are subordinate upon count calories to guarantee satisfactory levels of vitamin C for digestion system and oxidative assurance. The high l-ascorbate contents found in plants make them the primary source of vitamin C intake for humans². Vitamin C is one of the strong diminishing operators and forager of free radicals in organic frameworks, working as a forager of oxidizing free radicals and destructive oxygen-derived species, such as hydroxyl radical, hydrogen peroxide (H₂O₂), and singlet oxygen².

Vitamin C acts as a prooxidant, contributing to the formation of hydroxyl radicals that may lead to lipid, DNA, or protein oxidation³. There are distinctive instruments to lighten oxidative stretch and repair harmed macromolecules. Enzymatic and nonenzymatic cancer prevention agents have imperative parts in rummaging free radicals and receptive oxygen species (ROS). The antioxidant chemicals, catalase (CAT), superoxide dismutase (Grass), glutathione reductase (GR), glutathione peroxidase (GSHpx) and, in plants, ascorbate peroxidase (AA-px) and the nonenzymatic cancer prevention agents, counting glutathione (GSH) and ascorbate (ASC), have been shown to be significantly affected by oxidative stress³. Antioxidant compounds can anticipate the uncontrolled arrangement of free radicals or repress their response with organic destinations; too, the annihilation of most free radicals depends on the oxidation of endogenous antioxidants mainly by scavenging and reducing molecules³. Vitamin C is thought to be an imperative water dissolvable antioxidant which is detailed to neutralize ROS and decrease the oxidative stress⁴. Ascorbic acid is additionally known as a diminishing operator and a radical forager. As a radical forager AsCH₂ gives successful security of films and proteins against oxidation by receptive oxygen species (ROS):

superoxide (O₂•-), hydrogen peroxide (H₂O₂), hydroxyl radicals (•OH), peroxy radicals (•OOH), and singlet oxygen 1O₂, especially at high concentrations⁵.

L-ascorbic acid could be a dibasic corrosive with an enediol bunch built into a five membered heterocyclic lactone ring. The chemical and physical properties of ascorbic acid are related to its structure⁶. The structure of dehydroascorbic corrosive, the primary oxidation item of ascorbic acid (Plot No.2), has been analyzed by x-ray crystallography to be a dimer. Electrochemical thinks about have demonstrated that ascorbic acid and dehydroascorbic acid frame a reversible redox couple.

The ascorbic acid atom comprises of two topsy-turvy carbon particles, C-4 and C-5⁵. Hence, in expansion to L-ascorbic acid itself, there are three other stereoisomers: D-ascorbic acid, D-isoascorbic acid, and L-isoascorbic acid and L-isoascorbic acid. These three isomers have very little or no antiscorbutic activity⁶.

MATERIAL AND METHODS

In this study Vitamin C Solution for oral administration (250mg/ml) was supplied from chemistry department, faculty of science Omar Al-Mokhtar University, El-Beida-Libya. The dosage of AA was 40mg/kg BW each other day⁸. Male New Zealand White rabbits (age of 6 months and initial weight of 2662 ± 72g) were used. Animals were individually housed in cages and weighed weekly throughout 3-months experimental period. A total of 10 mature male rabbits were randomly divided into two equal group. Group 1 served as control, while groups 2 was given AA (40mg/kg body weight). The doses of AA was calculated according to the animal's body weight on the week before dosing. The proper doses of AA for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oesopharyngeal region. The other part of heparted blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 860xg for 20 min, and was stored at -20°C until

used for analyses. The Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to⁹. Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide¹⁰. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to¹¹. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method¹².

Statistical analysis

Data were analyzed as a completely randomized design¹³ using the general linear model procedure of¹⁴. Means were statistically compared using least significant difference (LSD) test at 0.05 significant level¹³. The following model was used: $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$ where Y_{ijk} = experimental observation; μ = overall mean; α_i = treatment effect; β_j = week effect; $\alpha\beta_{ij}$ = interaction effect of treatment and week; ϵ_{ijk} = random error.

RESULTS AND DISCUSSION

The activities of glutathione (GSH), glutathione peroxidase (GPx), glutathione S transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were measured in plasma of male rabbits treated with ascorbic acid. Data in Table No.1 indicated that treatment with Ascorbic acid significantly ($p < 0.05$) increased (GSH), (GPx), (GST), (CAT) and (SOD) activities in plasma as compared with control group. Thiobarbituric acid reactive substances (TBARS) are produced by lipid peroxidation (LPO) and are considered as indicators of oxidative stress. LPO was assessed by measuring the concentrations of thiobarbituric acid-reactive substances TBARS in plasma of male rabbits treated with ascorbic acid. Data in Table No.1 indicated that treatment with ascorbic acid alone significantly ($p < 0.05$) decreased TBARS concentration in plasma as compared with control group.

Discussion

Antioxidants are known to reduce oxidative-radical induced reactions. Vitamin C is a basic dietary supplement required as a co-factor for numerous proteins, and people are among the few creatures

that need the capacity to orchestrate the compound from glucose. The reduced form of the vitamin, ascorbic acid, is an especially effective antioxidant owing to its high electron-donating power and ready conversion back to the active reduced form. There's great intrigued within the clinical parts of Vitamin C because of prove that oxidative harm may be a root cause of, or at slightest related with, numerous infections. Populace thinks about appear that people with tall immaterial of Vitamin C have lower chance of a number of inveterate maladies, counting heart illness, cancer, eye infections, and neurodegenerative conditions. The prove that ascorbic acid acts as an imperative antioxidant in numerous body tissues is persuading¹⁵. Ascorbic acid is one of the critical antioxidants. Consequently, It avoids spread of chain responses which activated by free radicals. It is also has the capability to prevention of lipid peroxidation and decreasing of oxidative harm, which hurtful for the layer and damage spermatozoa DNA¹⁶.

Vitamin C is normally happening free radical forager, as such, its nearness helps different other components in diminishing various troublesome free radical forms from taking put, counting lipid peroxidation¹⁷. The decrease in the levels of TBARS, an increase in (GSH), (GPx), (GST), (CAT) and (SOD) activities in plasma of rabbits treated with ascorbic acid (Table No.1) are in understanding with the finding of¹⁸. Also¹⁹ reported that Vitamin C (500mg ascorbate per day) supplementation in nonsmokers reduced lipid peroxidation. Additionally, AA can protect biomembranes against peroxidative damage. Two major properties of Vitamin C make it an perfect antioxidant. To begin with is the moo one-electron decrease possibilities of both ascorbate and its one-electron oxidation item, the ascorbyl radical. These moo decrease possibilities empower ascorbate and the ascorbyl radical to respond with and diminish fundamentally all physiologically pertinent radicals and oxidants. The moment major property that produces Vitamin C such a successful antioxidant is the soundness and moo reactivity of the ascorbyl radical shaped when ascorbate scavenges a

receptive oxygen or nitrogen species²⁰. Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics²¹. Also, oxidative damage to biomolecules, such as lipids, DNA, and proteins, has been implicated in many chronic diseases, in particular, cardiovascular disease, cancer, and cataract²². There's prove that vitamin C restrains oxidative DNA harm in confined and refined cells uncovered to responsive oxygen species and UV/visible light²². Vitamin C has two major properties that make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate and its one-electron oxidation product, the ascorbyl radical that react with and reduce basically all physiologically relevant radicals and oxidants. The moment major property that produces vitamin C such a compelling antioxidant is the soundness and moo reactivity of the ascorbyl radical shaped when ascorbate scavenges a receptive oxygen or nitrogen species²⁰.

Table No.1: Plasma TBARS, glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) activities of male rabbits treated with ascorbic acid

S.No	Parameters	Animal Groups	
		Control	Ascorbic acid
1	TBARS (nmol/ml)	0.30 ± 0.016 ^b	0.23 ± 0.011 ^c
2	GSH(U/ml)	0.188 ± 0.06 ^b	0.90 ± 0.011 ^b
3	GPx(U/ml)	12.5 ± 0.24 ^b	15.0 ± 0.55 ^a
4	GST(μmol/hr/ mg protein)	1.08 ± 0.04 ^b	1.131± 0.036 ^a
5	Catalase(μmol H ₂ O ₂ consumed/min./ml)	50± 3.1 ^b	59 ± 4.99 ^a
6	SOD(U/mg protein)	1.165 ± 0.020 ^b	1.31 ± 0.0219 ^a

^{abcd} Within row, means with different superscript letters differ significantly (p < 0.05).

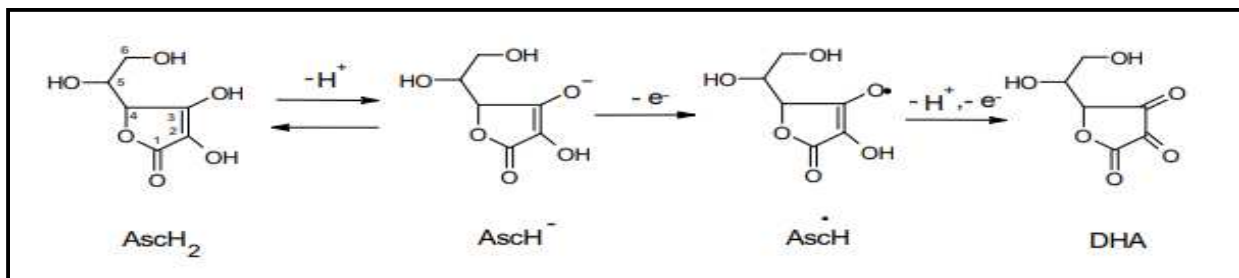


Figure No.1: The structure of ascorbic acid and the main products of redox reactions. Ascorbic acid—AsCH₂; ascorbate anion—AsCH⁻; ascorbyl radical—AsCH[•]; dehydroascorbic acid—DHA⁵

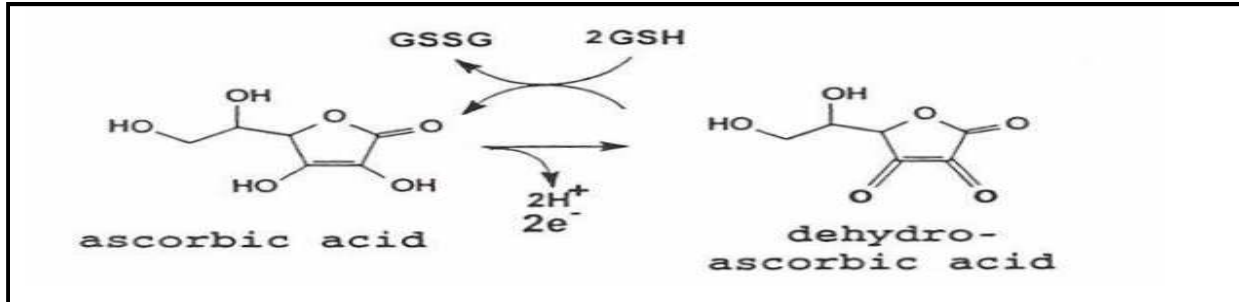


Figure No.2: Ascorbic acid and dehydroascorbic acid. Ascorbic acid is the reduced form of vitamin C. The oxidized form, dehydroascorbic acid, can be reduced back to ascorbic acid by glutathione (GSH)⁷

CONCLUSION

Ascorbic acid (AA) reduced the levels of TBARS and Antioxidant Enzymes in plasma of rabbits. Thus, ascorbic acid can act as a solid, productive, and cheap antioxidant specialist.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University, Al -Bayda-Libya for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

BIBLIOGRAPHY

1. Padayatty S J, Sun H, Wang Y, Riordan H D, Hewitt S M, Katz A, Levine M. Vitamin C pharmacokinetics: Implications for oral and intravenous use, *Annals of Internal Medicine*, 140(7), 2004, 533-537.
2. Hacisevki A. An overview of ascorbic acid biochemistry, *Journal of Faculty of Pharmacy*, 38(3), 2009, 233-255.
3. Rouhier N, Lemaire S D, Jacquot J P. The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation, *Annu. Rev. Plant Biol.*, 59, 2008, 143-166.
4. Verma R S, Mehta A, Srivastava N. *In vivo* chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins, *Pestic Bioche and Physi*, 88(2), 2007, 191-196.
5. Rahman K. Studies on free radicals, antioxidants and co-factors, *Clinical Interventions in Aging*, 2(2), 2007, 219-236.
6. Aguirre R, May J M. Inflammation in the vascular bed: Importance of Vitamin C, *Pharmacology and Therapeutics*, 119(1), 2008, 96-103.
7. Mehlhorn R J. Ascorbate-and dehydroascorbic acid-mediated reduction of free radicals in the human erythrocyte, *Journal of Biological Chemistry*, 266(5), 1991, 2724-2731.
8. Khan P K, Sinha S P. Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb), *Mutagenesis*, 11(1), 1996, 33-36.
9. Habig W H, Pabst M J, Jakoby W B. Glutathione S-transferases the first enzymatic step in mercapturic acid formation, *Journal of Biological Chemistry*, 249(22), 1974, 7130-7139.
10. Luck H. Method of enzymatic analysis, *Verlag Chemic, Academic Press, New York*, 2nd Edition, 1974, 885.
11. Mishra H P, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biolog. Chem.*, 247(10), 1972, 3170-3175.
12. Tappel A L, Zalkin H. Inhibition of lipid peroxidation in mitochondria by Vitamin E, *Archives of Biochemistry and Biophysics*, 80(2), 1959, 333-336.
13. Steel R G D, Torrie J H. Principles and

- procedures of statistics, *Principles and Procedures of Statistics*, 1960.
14. SAS. User's guide: Statistics, *SAS Institute Inc, Cary, NC*, 1985, 956.
 15. Jacob R A, Sotoudeh G. Vitamin C function and status in chronic disease, *Nutri in Clini Car*, 5(2), 2002, 66-74.
 16. Sara M. Elgazwi and Fayrouz A. Khaled. The impact of ascorbic acid on semen overhaul in male rabbits, *Asi Jour of Resea in Biolo and Pharmaceu Scie*, 8(1), 2020, 1-6.
 17. Knight J A, Blaylock R C, Searles D A. The effect of vitamins C and E on lipid peroxidation in stored erythrocytes, *Ann. Clin. Lab. Sci*, 23(1), 1993, 51-56.
 18. Yousef M I, Salem M H, Kamel K I, Hassan G A, El-Nouty F D. Influence of ascorbic acid supplementation on the haematological and clinical biochemistry parameters of male rabbits exposed to aflatoxin B1, *J. Environ. Sci. Health*, B38(2), 2003a, 193-209.
 19. Huang H Y, Appel L J, Croft K D, Miller III E R, Mori T A, Puddey I B. Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial, *The Ameri Jour of Clin Nut*, 76(3), 2002, 549-555.
 20. Tsao C S. An overview of ascorbic acid chemistry and biochemistry, *Vitamin C in health and disease*, *Marcel Dekker Inc, New York*, 1997, 25-58.
 21. Anane R, Creppy E E. Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase+ catalase and Vitamins E and C, *Hum and Experi Toxi*, 20(9), 2001, 477-481.
 22. Carr A, Frei B. Does Vitamin C act as a pro-oxidant under physiological conditions? *The FASEB Journal*, 13(9), 1999, 1007-1024.

Please cite this article in press as: Hameda T. Algalbati and Fayrouz A. Khaled. The role of organic compound (ascorbic acid) on antioxidant enzymes in grown-up rabbits, *Asian Journal of Phytomedicine and Clinical Research*, 8(4), 2020, 169-174.