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Research Article

Evaluation of Oral Administration of L-Citrulline on Lipid Peroxidation and Some Antioxidant Enzymes Activities on Hyperlipidemic Wistar Rats

S.V. Ajibade[1*], A. Jimoh[1], Y. Tanko[1], A. Mohammed[1], E. A. Alex[1], J. Zuberu[1], S. Sani[2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria and [2] School of pharmaceutical education and research, Hamdard University, India.

Keywords:
l-citrulline, Hyperlipidemia, poloxamer 407, oxidative stress, Antioxidants

ABSTRACT

Background: Hyperlipidemia is one of the most important modifiable risk factors for cardiovascular disease (CVD). It is characterized by an elevation of any or all lipid profiles and/or lipoproteins in the blood. Oxidative stress has been shown to play a role in hyperlipidemia, CVD, infectious diseases, cancer, diabetes, anemia and neurodegenerative pathology. Aim: The aim of the study was to evaluate the effect of oral administration of l-citrulline on lipid peroxidation and some antioxidant enzymes activities on hyperlipidemic wistar rats. **Methods**: The animals were made hyperlipidemic by intraperitoneal injection of poloxamer-407. Twenty-five (25) male Wistar rats weighing 100-120g, were grouped into five of 5 animals each. Animals in Group I (Normal Control) received distilled water only, while animals in Groups II, III, IV and V were given Poloxamer-407 intraperitoneally after every 48 hours and subsequently Groups III, IV and V were treated orally with Atorvastatin (10mg/kg), L-Citrulline (L-Citt) 400 mg and L-Citrulline 800 mg respectively for twenty-one (21) days. **Result**s: Malondialdehyde (MDA) concentration an index of lipid peroxidation, decreased in a dose dependent manner compared to the HLD only group (35.76±2.32, 35.02 ±2.55) ng/dl vs. (41.86±2.19) ng/dl. however, it was not significant($p<0.05$). Catalase was significantly ($p<0.05$) decreased from (33.47±3.13) ng/dl to (18.35 ± 2.19) ng/dl. Superoxide dismutase level increased from (40.96±3.08) ng/dl to (46.30±1.42, 45.14±1.39) ng/dl however, it was insignificant ($p<0.05$). Reduced glutathione peroxidase recorded no significance. **Conclusion**: Administration of L-citrulline fails to proffer significant decrease in lipid peroxidation and increase in antioxidant enzyme SOD.

INTRODUCTION

Hyperlipidemia is a disorder characterized by increase in lipoprotein or cholesterol blood levels. It is viewed as the primary mediator of a cascade of heart damaging events such as renal injury, stroke, atherosclerosis and metabolic syndrome (Xu *et al.,* 2007; Baby and Anuradha, 2013).

Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidemia in rats. P-407 is a biocompatible, non-ionic surfactant, considered non-toxic and safe during chronic administration for long term studies (Megalli *et al.,* 2005). Oxidative stress as a result of hyperlipidemia has been shown to damage the structural and functional integrity of the cell either by directly modifying cellular DNA, proteins, and membrane lipids or by initiating chain reactions that cause extensive oxidative damage to DNA, proteins, and membrane lipids (Zhao *et al.,* 2014). Oxidative stress also occurs as a result of decreased antioxidant defenses and has been related to the pathogenesis of atherosclerosis, and diabetes mellitus (Fisher-wellman *et al.,* 2009). Antioxidants are known to play important roles in biological systems by scavenging free radicals which may result in oxidative damage of biological molecules such as lipids, proteins and DNA.

*Address for correspondence:
Email: ajibadevictoria@gmail.com

L-Citrulline is a colorless, water soluble α-amino acid with an asymmetric carbon. It derives its name from watermelon *Citrullus lanatus* (Eberhardt *et al.,* 2014). It is a naturally occurring nonessential amino acid endogenous to most living systems (Davis *et al.,* 2011). L-Citrulline is the natural precursor of L-arginine, substrate for nitric oxide synthase (NOS) in the production of nitric oxide (NO) (Maritza *et al*., 2006). It plays an important role in the metabolism and regulation of NO (Jabłecka *et al.,* 2012; Suzuki *et al.,* 2016). The hallmark of most cardiovascular disorders is NO dysfunction. Hence, optimizing eNOS function and availability might be helpful in mitigating CVD, making L-Citrulline a possible alternative candidate for supplementation.

According to the WHO, ischemic heart disease attributable to high cholesterol, is the leading cause of cardiovascular disease mortality rate globally. Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYs), or 2% of total DALYs. In 2008, the global prevalence of raised total cholesterol among adults (≥5.0 mmol/L) was 39% (37% for males and 40% for females (WHO, 2015).

Therefore, this study was designed to evaluate the effect of oral administration of l-citrulline on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase and Catalase) activities in hyperlipidemic wistar rats.

METHODS

Experimental Site

This study was carried out in the Physiology Laboratory of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Kaduna state, Nigeria.

Chemicals and Reagents

L-Citrulline pure powder (1.5g) of pharmaceutical grade was obtained from NOW FOODS, USA. Atorvastatin tablets 10 mg, Normal saline, distilled water used for this study were purchased from the Pharmacy of the Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria. All chemicals were commercially obtained and were of analytical grade.

Ethical Approval

The rats were handled in accordance with the principles guiding the use and handling of experimental animals of Ahmadu Bello University Zaria, Nigeria.

Experimental animals

(25) Male wistar rats weighing 100-120g were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University Zaria, Nigeria. The animals were kept and maintained under laboratory condition of ambient temperature, relative humidity and 12-hour light-dark cycle. They were fed on standard commercial feeds with water *ad libitum.* The animals were housed five animals per cage in five groups.

Induction of Experimental Hyperlipidemia

Hyperlipidemia was induced by single intraperitoneal injection of Poloxamer-407 at a dose of 500 mg/kg twice a week for 3 weeks (Woo *et al*., 2010). Poloxamer-407 (Lutrol F127; BASF, Ludwigshafen, Germany) was dissolved in fresh 0.13 g/ml cold normal saline and stored in a refrigerator overnight to facilitate its proper dissolution. P-407 increases serum lipoproteins via its actions at various levels in lipid metabolism, largely by inhibiting lipoprotein lipase, which facilitates the hydrolysis of triglycerides (TG).

Experimental Protocol and Treatment

A total of 25 male Wistar rats were used; they were randomly divided into five groups of five animals each as shown in Table 1.

Table1: Showing the Animal Grouping and Experimental Protocol

Groups	Treatment	Sample size(n)	Duration
I	Distilled water	5	21 days
II	P-407 only (500mg/kg)	5	48 hourly
III	P-407+ Atorvastatin(10mg/kg)	5	21 days
IV	P-407+ L-Citrulline (400mg/kg)	5	21 days
V	P-407+ L-Citrulline (800mg/kg)	5	21 days

L-Citrulline dose preparation and administration

The stock concentration was prepared by dissolving 5 g of L-Citrulline supplement in 10 ml of distilled water. The preparation was administered orally at 400 mg and 800 mg body weight of rats for twenty-one (21) days.

Sample Collection and Serum Preparation

At the end of the experiment (21 days) the animals were fasted overnight and anaesthetized using ketamine. Blood samples was collected from the apex of the rat's heart in all the groups via cardiac puncture technique using a 5ml syringe into plain specimen bottles and allowed to clot, and separated by centrifugation at 2,000

g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany).

Estimation of Biomarkers of Oxidative Stress and Lipid Peroxidation

Determination of serum superoxidase dismutase activity

Superoxide dismutase (SOD) was determined by the method described by Fridovich, (1989). The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2 forms the basis of this assay. 0.1ml of Serum was diluted in 0.9ml of distilled water to make 1:10 dilution of micro some. An aliquot mixture of 0.2ml of the diluted microsome was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm.

Determination of Catalase Activity (CAT)

Catalase (CAT) activity was measured using Aebi's method (1974). Exactly 100µl of serum was added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM¯1 -cm¯1 was used to calculate the Catalase activity. **Unit:** One unit was the amount of Catalase that decompose 1µmol of H_2O_2 per min at pH 7.0

Assay of Glutathione Concentration (GSH)

Reduced glutathione (GSH) concentration measurement was done according to Ellman (1959) as described by Rajagopalan *et al.* (2004). It is based on the reaction of 5,5- dithiobis nitro benzoic acid (DNTB) and reduced Glutathione (GSH). To 150µl of serum homogenate (in phosphate - saline buffer pH 7.4), 1.5ml of 10% TCA was added and centrifuge at 1500g for 5 minutes. 1 ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.The quantity of GSH was obtained from the graph of the GSH standard curve.

Assessment of lipid peroxidation (Malondialdehyde Concentrations)

Malondialdehyde (MDA) concentrations is one of the many low molecular weight end-products of lipid hydroperoxide decomposition and is the most often measured as an index of lipid peroxidation. Lipid peroxidation is evident by formation of TBARS measured by the modified method of Niehaus and Samuelson (1968) and described by Akanji *et al.* (2009). 150µl of serum homogenate where treated with 2 ml of TBA-TCA-HCL reagent (1:1:1 ratio) and place in a water bath at 90°C for 60 minute, the mixture was cooled and centrifuged at 3000rpm for 5 minutes and the absorbance of the pink supernatant (TBA-Malonaldehyde complex was then measured at 535nm. Malonaldehyde formed was then calculated using the Molar extinction coefficient of 1.56 x 10^{-5} $cm^{-1}M^{-1}$

Statistical Analysis

Data collected was expressed as Mean ± SEM. It was analyzed by one-way analysis of variance, ANOVA, and *Tukey's post-hoc* test was used to compare the level of significance between the controls and treatment groups, using SPSS version 22.0. Values of $p < 0.05$ was considered significant

REULTS

Effect of L-Citrulline on Superoxide Dismutase Activity in Hyperlipidemia Induced Male Wistar Rats

Figure 1 Shows the effects of treatment on superoxide dismutase. Superoxide dismutase was lower in the HLD only group compared to the normal control (40.96±3.08 ng/dl vs 53.40±2.59 ng/dl) respectively. SOD in the 400 mg/dl L-Citt and 800 mg/dl L-Citt group was insignificantly higher compared to the HLD only group with a value of (46.30±1.42 ng/dl, 45.14±1.39 ng/dl vs 40.96±3.08 ng/dl respectively).

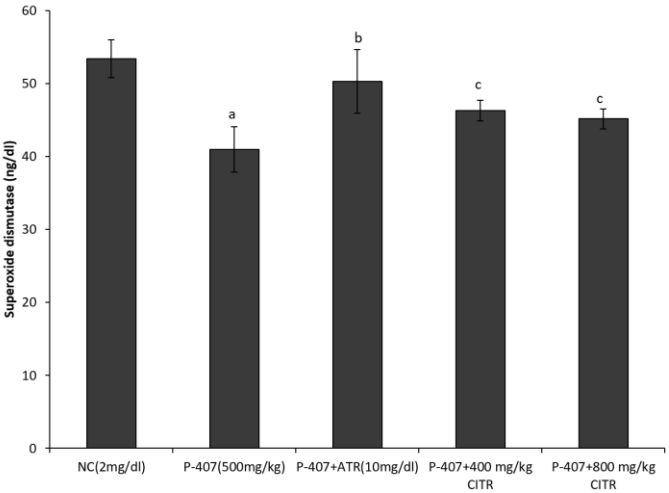

Fig. 1: Effect of L-Citrulline on superoxide dismutase activity in hyperlipidemia induced male Wistar rats. Values with different superscript are statistically significant ($p<0.05$). superscript [a,b,c] indicate statistical significant compared to control. HLD= hyperlipidemia, ATR= atorvastatin, L-CITT=L-Citrulline

Effect of L-Citrulline on Serum Catalase Activity Hyperlipidemia Induced Male Wistar Rats

Figure 2 shows the effects of treatment on catalase. Catalase increased in the HLD only group when compared with the normal control group (33.47±3.13 ng/dl vs 10.31±0.64 ng/dl) respectively. Catalase was significantly decreased in the 400 mg L-Citt as compared to the HLD only group (18.35 ± 2.19 ng/dl vs 33.47±3.13 ng/dl) respectively. Catalase was significantly increased ($p<0.05$) in the 800 mg L-Citt group as compared to the 400 mg L-Citt. Group (30.67±1.03 ng/dl vs 18.35 ± 2.19 ng/dl respectively).

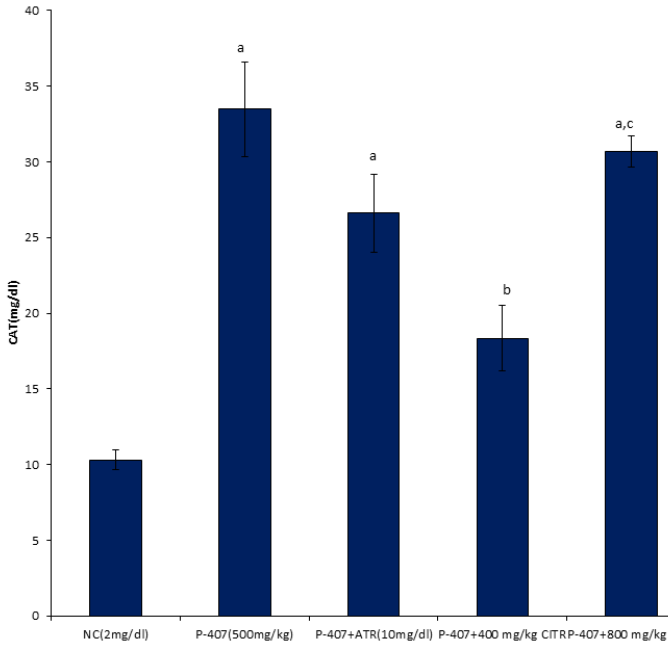

Fig. 2. Effect of L-Citrulline on catalase in hyperlipidemia induce male Wistar rats. Values with different superscript are statistically significant ($p<0.05$). Superscript ^{a,b,c} indicate statistical significan compared to control, HLD only and HLD+ 400 mg/kg L-Citt HLD= hyperlipidemia, ATR= atorvastatin, L-CITT=L-Citrulline

Effect of L-Citrulline on Reduced Glutathione Activity in Hyperlipidemia Induced Male Wistar Rats

Figure 3 shows the effects of treatment on reduced glutathione peroxidase. Reduced glutathione peroxidase was insignificantly lower in HLD only group as compared to the normal control group (15.40±0.65 ng/dl vs 18.24±1.49 ng/dl respectively). The 400 mg L-Citt and 800 mg L-Citt group showed no significant difference compared to the HLD only group (15.14±0.55 ng/dl, 14.36±0.48 ng/dl vs 15.4±0.65 ng/dl respectively).

Effects of L-Citrulline on Malondialdehyde In Hyperlipidemic male Wistar Rats

Figure 4 Shows the effects of treatment on Malondialdehyde. Malondialdehyde was significantly higher in HLD only, HLD+ATR, 400mg L-Citt and 800mg L-Citt group compared to the normal control group (41.86±2.19 ng/dl, 35.76±2.03 ng/dl, 35.76±2.32 ng/dl, 35.02 ±2.55 ng/dl vs 25.06±1.81 ng/dl). MDA levels in the 400mg L-Citt and 800mg L-Citt group showed a slight but insignificant decrease compared to the HLD only group (35.76±2.32 ng/dl, 35.02 ±2.55 ng/dl vs 41.86±2.19 ng/dl).

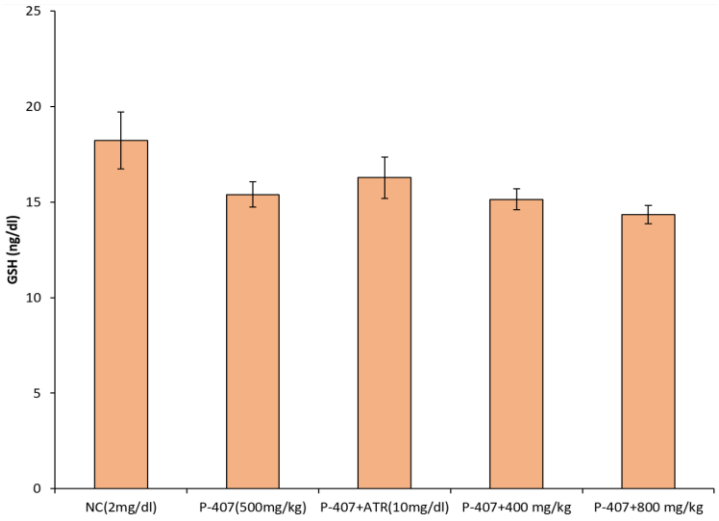

Fig. 3: Effect of L-Citrulline on reduced glutathione in hyperlipidemia induced male Wistar rats. Values with different superscript are statistically significant ($p<0.05$). HLD= hyperlipidemia, ATR= atorvastatin, L-CITT=L-Citrulline

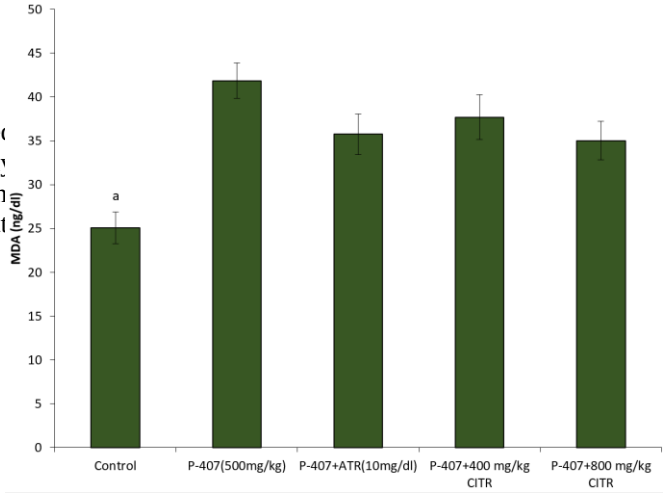

Fig. 4: Effect of L-Citrulline on Malondialdehyde (MDA) in hyperlipidemia induced male Wistar rats. Values with different superscript are statistically significant ($p<0.05$). Superscript a indicate statistically significant compared to control. HLD= hyperlipidemia, ATR= atorvastatin, L-CITT=L-Citrulline

DISCUSSION

This study investigated the antioxidant activity of L-Citrulline in hyperlipidemic Wistar rats. The experimental model used was Poloxamer-407 (P-407) to

induce hyperlipidemic in rat model. Oxidative stress occurs when the effect of reactive oxygen (ROS) and reactive nitrogen (RNS) species overcomes the physiological antioxidant defense mechanisms (Luciano and Omidreza, 2014). The health-related applications of oral L-citrulline supplementation are largely predicated on the capacity for L-citrulline to increase L-arginine availability for NO production (Luciano and Omidreza, 2014).

Superoxide dismutase level (SOD) is known to offers the first line of antioxidant defense. From the result obtained in our study, (SOD) was lowered in p-407 only group, compared to the control and treatment groups which could probably be due to the increase in the ROS level of the p-407 only group and the used-up of the SOD in doing the oxidation to prevent ROS formation. L-Citrulline antioxidant effect has been thought to work by probably decreasing reactive oxygen species (ROS), thus preventing (ROS) mediated damage and also increasing NO bioavailability. However, increase in SOD in the L-Citrulline treated groups could be as a result of ROS reacting with NO at a higher rate than SOD to form peroxynitrite, thereby improving NO bioavailability and SOD activity concomitantly. That is l-citrulline upregulating SOD to do the oxidation. Previous research reported that Dietary antioxidant could initiate antioxidant defense to combat excessive ROS production. This was done either by direct scavenging of hydroxyl radicals to form water or inhibition of hydroxyl radical production (Coles, 2007). This agrees with the work of Victoria *et al.*, (2020) who worked on dietary l-citrulline supplementation in hens and recorded an increase in enzymatic activity of serum SOD in heat stressed hen. Santos and Jennifer, (2016), also demonstrated an increased in serum SOD level in endothelial cells arterial dysfunction.

Ankita *et al.* (2019), describes catalase as a key regulator of ROS. it decomposes hydrogen peroxide to insignificant products such as water and oxygen. Serum Catalase level was increased significantly in the P-407 group only. An increase in serum catalase activity in the P-407 group could be due to an upregulation of the inherent antioxidant enzymes to abate the effect of oxidative stress. Moldogazieva *et al*. (2018), in their findings revealed that appropriate intracellular levels of ROS play a crucial role in physiological redox signaling via activation and regulation of endogenous defenses by protecting cells from oxidative stress damage. 800 mg/kg l-Citrulline had an increased catalase level compared to 400 mg /kg of l-Citrulline. This might probably be a synergetic act of upregulating the antioxidant system through the NO pathway. Antioxidants can act either as direct scavengers of superoxide or as blockers of the primary superoxide formation. Poor aqueous solubility and low stability limits the therapeutic potentials of antioxidants, which limit antioxidant bioavailability (Tan *et al*., 2018).

Serum MDA concentrations level is often considered as an index of free radical generation which increases in conditions of oxidative stress (Kehrer, 1993). Disorders of lipid metabolism such as hyperlipidemia, results in modification of cellular reaction that produces ROS. Oxidative stress results from over production of ROS, failure of host antioxidant defense or both (Fostermann, 2006). Increase in serum MDA concentrations demonstrated lipid peroxidation in P-407 group, compared to the normal control group. L-citrulline treated groups had a non-significant decline in serum MDA concentrations, demonstrating its possible ameliorative effect on lipid peroxidation and also stabilization of the cell membrane. This implies that oxidative stress was countered probably due to the up-regulation of the NO pathway. Elevations in reactive oxygen species (ROS), especially superoxide ($O2^-$), can reduce the bioavailability of NO through the generation of peroxynitrite ($ONOO^-$). Atorvastatin a standard drug is known to decreased lipid peroxidation; however, it was non-significant in our study. Several studies (Jones *et al*., 2003; Jukema *et al.,* 2004), recorded that low dose atorvastatin was less efficacious in reducing lipid peroxidation. Hence, this could be likened to the dosage of the atorvastatin used in the study. Reduced glutathione had no significant difference across the group; however, it was reduced in the P-407group.

CONCLUSION

P-407 induced hyperlipidemia and causes oxidative damage by lowering the antioxidant enzyme activity. L-Citrulline alone is not enough to ameliorate some conditions associated with oxidative stress in hyperlipidemia. We suggest that more studies should be carried out on the long-term effect of l-citrulline and its action with other drugs or supplements.

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REFERENCES

Aebi, H. (1974). Isolation and Characterization of Normal and Variant Erythrocyte Catalase and Their Subunits. *European Journal of Biochemistry* (48)1: 137-145

Akanji, M.A. Adeyemi, O.S. Oguntoye, S.O. and Sulyman, F. (2009) Psidium Guajava Extract Reduces Trypanosomosis Associated lipid peroxidation and raises glutathione concentrations in infected animals. *EXCLI Journal* 8: 148-154

Ankita, N., Liang-Jun, Y., Chandan, K.J., and Nilanjana, D. (2019) Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative medicine and cellular longevity*

Bahram, P., Vahideh E., Maryam R., and Abolfazl, G. (2009) *Journal of Medicinal Plants Research*. 3(10): 815-821

Bahri, S., Zerrouk, N., and Aussel, C. (2012) Citrulline: from metabolism to therapeutic use. *Nutrition.* 29(3):479-84,

Baron, R. (2005) Lipid Abnormalities. Current Medical Diagnosis and Treatment 44th ed. The McGraw-Hill Company. Pp.1202-13.

Behradmanesh, S., and Nasri, P. (2012) Serum cholesterol and LDL-C in association with level of diastolic blood pressure in type 2 diabetic patients. *Journal of Renal Inj* Prev1:23–26

Block, T.A., Aarsvold, J.N. and Matthews, L., (1995) Nonthermally mediated muscle injury and necrosis in electrical trauma. *Journal of Burn Care Rehabilitation*.; (16): 581–588.

Castilla-Guerra, L., Fernández-Moreno, M., and Alvarez-Suero, J. (2009). Secondary stroke prevention in the elderly: new evidence in hypertension and hyperlipidemia. *European Journal of Internal Medicine*; 20:586-90.

Daniel, J.C., David, A.W., and Raphael, C.L. (2004) Surfactant Poloxamer 188-related decreases in inflammation and tissue damage after experimental brain injury in rats. ; 101:16-20.

Di Angelantonio, E., Gao, P., and Pennells, L. (2012*)* Emerging risk factors collaboration. Lipid-related markers and cardiovascular disease prediction. *Journal of American Medical Association; 307:2499–506.*

Ellefson, R.D., Caraway, W.T., and Dryer, R.L. (1982): Lipids and lipoproteins. *Fundamentals of clinical chemistry. 2*: Pp.474.

Ellman, G.L. (1959) Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, (82), 70-77

Fridovich, I. (1989) Superoxide Dismutases. An adaptation to a paramagnetic gas. *The Journal Of Biological Chemistry*, (264):7761-7764.

Gilbert, P., Jones M.V., and Allison D.G. (1998) The use of poloxamer hydrogels for the assessment of biofilm susceptibility towards biocide treatments. *Journal of Applied Microbiol*.;85: Pp985-90.

Ginsberg, H.N., (1998). Lipoprotein physiology. *Journal of Endocrinology and Metabolism* (27), Pp503–519

Gotto, A.M., and Moon, J. (2010). Pitavastatin for the treatment of primary hyperlipidemia and mixed dyslipidemia. *Expert Review of Cardiovascular Therapy*; 8: pp1079-90

Hamdy, D.A., and Brocks, D.R. (2009). Experimental hyperlipidemia causes an increase in the electrocardiographic changes associated with amiodarone. *Journal of cardiovascular pharmacology*.;53:1-8

Harikumar, K., Abdul-Althaf, S., Kishore-kumar, B., Ramunaik, M., and Suvarna, C.H. (2013) *international journal of novel trends in pharmaceutical sciences* (3) 4-10

Harper, H.A., Rodwell, V., and Mayers, P. (1981): Metabolism of plasma proteins: review of physiological chemistry. 18th Ed., Lange Med. Publs., 23; P.348.

Havel, R.J. (1982): Familial dysbetalipoproteins: New aspects of pathogenesis and diagnosis. *Medical Clinics of North America.;* 66:441

Hitesh, R.P., Rakesh, P. and Patel, M.M. (2009) *International Journal of Pharmacological Technology Research*. (1)2, pp 299-303

Hurst, J.W., Schlant, R.C., Rackley, C.E., Sonnenblick, E.H., and Wenger, N.K. (1990): Biochemistry of lipid regulation; in "The heart, arteries and veins," *McGraw-Hill information Services Company (Ed.)*; 7th Ed., Chapter 7, pp.106-111,

Jin, K., Simpkins, J. W., Ji, X., Leis, M., and Stambler, I. (2015). The critical need to promote research of aging and aging-related diseases to improve health and longevity of the elderly population. *Aging Disease* 6:1

Jones, P.H., Davidson, M.H., Stein, E.A., Bays, H.E., McKenney, J.M., Miller, E., Cain, V.A., and Blasetto, J.W. (2003). Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses. Am J Cardiol., 92: 152-160

Jukema, J., Liem, A., Dunselman, P., van der Sloot, J., Lok, D. and Zwinderman, A. (2004). LDL-C/HDL-C ratio in patients with coronary artery disease and low HDL-C: the radar study. Atheroscler Suppl., 5: 125-10

Kirsten Elizabeth Coles (2007). An investigation into the antioxidant capacity of L-arginine and L-Citrulline in relation to their vascular protective properties.

Kishor, J.S., Kathivarin, M.K., Rahul, S., and Chamanal, J. (2007) The biology and chemistry of

hyperlipidemia. Bioorganic and Medicinal Chemistry, 15, pp 4674-4699

Luciano, S. and Omidreza, F. (2014). Pharmacological Applications of Antioxidants. *Current Drug Targets.* (15)13

Michelle, A., Hopkins, J., McLaughlin, C.W., Johnson, S. Warner, M.Q., LaHart, D., and Wright, J.D. (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall

Moldogazieva, N. T., Mokhosoev, I. M., Feldman, N. B., and Lutsenko, S. K. (2018). ROS and RNS signalling: adaptive redox switches through oxidative/nitrosative protein modifications. *Free Redic*. Res. 52, 507–543.

Niehaus, W. G. and Samuelsson, B. (1968) Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*. 6(1):126-30

Onwe, P.E., Folawiyo, M.A., Anyigor –Ogah, C.S., Umahi, G., Okorocha, A.E., and Afoke, A.O. (2015) IOSR *Journal of Dental and Medical Sciences*

Ortolani, E.L., and Marcondes, M.C. (1995) Treatment of ammonia intoxication in rats through the use of amino acids from the urea cycle. *Veterinary & Human Toxicology*. 37(3):217-20

Rajagopalan, R., Kode, A., Penumatha, S.V., kallikat, N.R. and Venugopal, P.M. (2004). Comparative of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacology and Pharmaceutical Sciences*, 83: 2747-2752

Rifai, N., Bachorik, P.S., and Albers, J.J. (1999). Lipids, lipoproteins, and apolipoproteins. Tietz Textbook of Clinical Chemistry. WB Saunders, Philadelphia, Pennsylvania, Pp. 809–861.

Shepherd, J., and Krauss, R. (1991). Pathophysiology of triglyceride-rich particles: Metabolism of triglyceride rich particles. *American Journal of Cardiology*.; 68:5A-7A.

Sureda, A., and Pons, A. (2013). Arginine and Citrulline supplementation in sports and Exercise: Ergogenic Nutrients. *Medical Sport Science.* 59: 18-28

Tan, B.L., Norhaizan, M.E., Liew, W-P-P., and Sulaiman, R.H. (2018). Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front. *Pharmacology*. 9:1162

Tania, B. (2007). Targetable biodegradable nanoparticles for delivery of chemotherapeutic and imaging agents to ovarian cancer. The University of Texas At Austin. Biomedical engineering. *ProQuest:* Pp 130

Upasana B, and Pravin T. A (2015). Comparative Study for Investigation into Beneficial Effects of Ketoconazole and Ketoconazole + Cholestyramine Combination in Hyperlipidemia and The Complications Associated with It. *Adv. Biores*. 6 [4]: 81-87.

Voight, B.F., Peluso, G.M., and Orho-Melander, M. (2012). Plasma *HDL* cholesterol and risk of myocardial infarction: a Mendelian randomization study. *Lancet; 380: 572-580.*

Weber, C., and Noels, H. (2011) Atherosclerosis: Current pathogenesis and therapeutic options. *National Medicine*.;17: 1410-1422

Woo, J., Jae, H.R. and Han, J.O. (2010). The Influence of Sam-Chil-Geun (Panax Notoginseng) on the serum lipid levels and inflammations of rats with hyperlipidemia induced by poloxamer-407. *Yonsei Medical Journal*, 51(4): 504-510.

World Health Organization. Global Health Observatory data. Cholesterol. http://www.who.int/gho/ncd/risk_factors/cholesterol_prevalence/en/ (accessed 14 Feb 2015).

Yusuf, S., Hawken, S., and Ounpuu, S. (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case control study. *Lancet; 364:937–952.*