BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Artículo Original / Original Article

Ascorbic supplementation attenuates juglone induced metabolic derangement

[La suplementación con ácido ascórbico atenúa el trastorno metabólico inducido por un derivado de juglona]

Fernando Ayavire[1], José Miguel Fonseca[1], Felipe Salas[1], Julio Benites[1], Chukwuemeka R. Nwokocha[2], Adrián Paredes[3], Fredi Cifuentes[4] & Javier Palacios[1]

[1]Departamento de Química y Farmacia, Facultad Ciencias de la Salud, Universidad Arturo Prat, Iquique, Chile
[2]Department of Basic Medical Sciences Physiology Section, Faculty of Medical Sciences, The University of the West Indies, Mona, Kingston, Jamaica
[3]Departamento de Química, Facultad de Ciencias Básicas, Universidad de Antofagasta, Antofagasta, Chile
[4]Laboratorio de Fisiología Experimental, Instituto Antofagasta, Universidad de Antofagasta, Antofagasta, Chile

Reviewed by:
Daniela Russo
University of Basilicata
Italy

Ricardo Diego de Albuquerque
Universidade de Vassouras
Brazil

Correspondence:
Javier **PALACIOS**
clpalaci@unap.cl

Section **Biological activity**

Received: 8 June 2020
Accepted: 31 August 2020
Accepted corrected: 13 September 2020
Published: 30 March 2021

Citation:
Ayavire F, Fonseca JM, Salas F, Benites J, Nwokocha CR, Paredes A, Cifuentes F, Palacios J. Ascorbic supplementation attenuates juglone induced metabolic derangement
Bol Latinoam Caribe Plant Med Aromat
20 (2): 195 - 202 (2021).
https://doi.org/10.37360/blacpma.21.20.2.15

Abstract: Juglone derivatives like 2-(4-hydroxyphenyl) amino-1,4-naphthoquinone (Q7) are used as antitumor agents, and act through reactive oxygen species (ROS) generation. Such may lead to abnormal lipid metabolism and ROS dysregulation. The objective of this study was to evaluate the effect of ascorbate on the metabolism of lipids and carbohydrates following Q7-induced oxidative stress. Male Wistar rats were administered Q7 (10 mg/Kg) and/or ascorbate (500 mg/Kg) orally for 20 days. Rats treated with Q7 showed an increase in serum triglycerides, VLDL cholesterol and lipid peroxidation levels. When Q7 treatment was followed up by ascorbate (500 mg/Kg) administration, we observed a reduction in serum triglycerides, VLDL cholesterol and lipid peroxidation. The oral administration of ascorbate reduced the Q7-induced increases in lipids, and postprandial glycemia. This could be associated with the redox activity of ascorbate that reduced the oxidative stress induced by Q7. We thus conclude that ascorbate modulates the Q7-induced increase of lipid and carbohydrate metabolism.

Keywords: Ascorbate; Naphthoquinone; Lipid; Glucose; Metabolism; Rat
.

Resumen: Los derivados de juglona, como 2-(4-hidroxifenil) amino-1,4-naftoquinona (Q7), son conocidos agentes antitumorales. Ellos generan especies reactivas de oxígeno (ROS), que podrían producir un desbalance de ROS y un metabolismo anormal de lípidos. El objetivo del estudio fue evaluar el efecto del ascorbato sobre el metabolismo de lípidos y carbohidratos en condición de estrés oxidativo inducido por Q7. A ratas Wistar macho, se les administró oralmente Q7 (10 mg/Kg) y/o ascorbato (500 mg/Kg) durante 20 días. Las ratas tratadas con Q7 mostraron un aumento de los triglicéridos en suero, del colesterol VLDL y de los niveles de peróxidación lipídica. Cuando el tratamiento con Q7 fue seguido de la administración de ascorbato (500 mg/Kg), observamos una disminución de los triglicéridos en suero, del colesterol VLDL y de la peroxidación lipídica. La administración oral de ascorbato redujo el aumento de lípidos inducido por Q7 y la glicemia postprandial. Esto podría estar asociado con la actividad redox del ascorbato, que reduce el estrés oxidativo inducido por Q7. Concluimos que el ascorbato modula el aumento del metabolismo de lípidos y carbohidratos inducido por Q7.

Palabras clave: Ascorbato; Naftoquinona; Lípido; Glucosa; Metabolismo; Rata.

INTRODUCTION

Walnut seeds are used by the traditional medicine in cancer treatment (Hayes *et al*., 2016), and juglone (5-hydroxy-1,4-napthoquinone) isolated from walnut is responsible for its antitumor effects (Catanzaro *et al*., 2018). Juglone derivatives possess potent anticancer activities because of their ability to generate oxide-reduction processes (Benites *et al*., 2010). These derivatives induce reactive oxygen species (ROS), such as superoxide anion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH) that can cause cell death (Tabrizi & Chiniforoshan, 2017).

Oxidative stress is linked to an imbalance between the production and the capacity of the cell to eliminate ROS. The generation of ROS could be responsible for insulin resistance, lipotoxicity, and obesity, leading to the induction of metabolic syndrome (Hurrle & Hsu, 2017). ROS enhances lipid peroxidation products and protein oxidation, thus damaging cellular components (Yilmazer, 2018). However, ROS are also important as signaling molecules in cell proliferation (Ray *et al*., 2012).

Cellular physiological processes acts to regulates the production of ROS, in order to reduce the adverse effects of naphthoquinone derivatives (Palacios *et al.*, 2016). For example, the superoxide dismutase enzyme (SOD) is an endogenous antioxidant that acts by suppressing or preventing the formation of ROS in cells. SOD converts free radicals of superoxide anions, which are harmful to cells, into hydrogen peroxide and molecular oxygen (Afonso *et al*., 2007; Reid *et al*., 2018).

It has been shown that the contribution of exogenous antioxidants contributes to human well-being (Yeung *et al*., 2019). They increase the antioxidant potential of the body to minimize the risk of health problems related to free radicals (Hill *et al*., 2018). In this study, we evaluated the joint administration of a naphthoquinone derivative with molecules showing reducing power (Palacios *et al*., 2018). Ascorbate (vitamin C) may decrease oxidative stress due to its reducing power, contributing one or two electrons (Pires *et al*., 2016). In accordance with previous studies (Hoffman *et al*., 2012), we postulated that the effects of ascorbate on oxidative stress induced by a naphthoquinone derivative would be; to modulate the metabolic regulations, through the decrease of triglycerides and glycemic levels, among others. In this study, we sought to evaluate *in vivo* the effect of ascorbate on the naphthoquinone derivative response [2- (4-hydroxyphenyl) amino-1,4-naphthoquinone; Q7] through the lipid profile (triglycerides, cholesterol types), glucose tolerance test, and determination of oxidative stress.

METHODS AND MATERIALS

Chemicals and reagents

The following drugs were used in this study: 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck, Darmstadt, Germany); menadione (Merck, Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; Merck, Darmstadt, Germany); malondialdehyde (Merck, Darmstadt, Germany); glucose (Winkler, Santiago, Chile); ascorbate (Asc) (Winkler, Santiago, Chile); butylated hydroxytoluene (Merck, Darmstadt, Germany); thiobarbituric acid (Merck, Darmstadt, Germany); trichloroacetic acid (Winkler, Santiago, Chile). Drugs were dissolved in distilled deionized water. For triglycerides, the Triglycerides GPO liquicolor kit (GPO-PAP method, Human, Magdeburg, Germany) was used, for total cholesterol, the Cholesterol liquicolor kit (CHOD-PAP method, Human, Magdeburg, Germany), and for HDL, the HDL-Cholesterol direct kit (Human, Magdeburg, Germany).

2-(4-hydroxyphenyl) amino-1,4-napththo-quinone (Q7, 265.07 g/mol) was synthesized under aerobic conditions by amination of 1,4-naphthoquinone with 4-hydroxyphenylamine, using $CeCl_3 \cdot 7H_2O$ as the Lewis acid catalyst as previously reported (Benites *et al*., 2010).

Animals

The study used male Wistar rats (6-8 weeks old; n = 20) weighing between 180 g and 200 g. The animals were provided by the Height Institute of the Arturo Prat University, Iquique, Chile. The healthy rats were randomly distributed in cages and maintained at a suitable temperature (22-24°C) and humidity (45 to 50%). All groups had *ad libitum* access to water and with standard rat chow (Champion, Santiago). They were divided into four groups of five rats each.

The first group (control) did not receive any treatment. The second group (Q7) received orally administered 2- (4-hydroxyphenyl) amino-1,4-naphthoquinone (10 mg/Kg, 20 days). The third group (Asc) received orally administered ascorbate (500 mg/Kg, 20 days) and the fourth group (Q7+Asc) received Q7 (10 mg/Kg) in combination with ascorbate (500 mg/Kg, 20 days). Q7 and ascorbate were administered orally by gavage, and peanut butter was used as vehicle. We selected the doses of ascorbate and Q7 according to a previous study

(Ourique *et al*., 2015). Q7 was synthesized by the laboratory of Medicinal Chemistry of the Faculty of Health Sciences, Arturo Prat University. The trials were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health of the United States (NIH, publication revised in 2013) and the Ethics Committee of Arturo Prat University (CEC-17).

Antioxidant capacity of ascorbate: Inhibition of DPPH radical

This method is based on the ability to reduce antioxidant compounds that can trap free radicals or contribute a hydrogen atom. The synthetic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a way to replace the function of a natural radical in the organism. For an aliquot of 190 µL of 0.075 mM DPPH solution, 10 µL of each compound to be tested was added in increasing concentrations (0 to 1 mM) of Q7, menadione, ascorbate, and Trolox, and the solution was incubated at room temperature for 30 minutes in the dark. The absorbance was then measured at a wavelength of 517 nm. As a positive control, Trolox was used, and the results were expressed as percent inhibition of the DPPH radical using the formula:

$$\% \text{ Inhibition} = [(A_{blank} - A_{sample})/ A_{blank}] \times 100$$

where A_{blank} relates to the absorbance of blank and A_{sample} is the sample's absorbance after the reaction.

Determination of the lipid peroxidation products in rat

The thiobarbituric acid reactive substances (TBARS) in serum of rats were measured (Cifuentes *et al*., 2009). Sample (100 µL) was used and 200 µL of trichloroacetic acid (Sigma) was added. Butylhydroxytoluene (3 µL) was added and centrifuged at 4000 r.p.m (Centrifuge 5417R, Eppendorf, Ontario, Canada) for 20 min. 140 µL of the supernatant was extracted and 140 µL of thiobarbituric acid was added. The sample was incubated in a water bath (100°C) for 1 h and then exposed to ice for 10 min. The solution (280 µL) was cooled, and a mixture of butanol/pyridine (15: 1) was added to extract the malondialdehyde. Finally, 150 µL of the final solution was used and the absorbance was measured at 532 nm in the spectrophotometer (Infinite M200 Pro, Tecan, Männedorf, Switzerland).

Lipid profile in rat serum

To extract blood from the rat tails, they were anesthetized with a mixture of xylazine (5 mg/Kg) plus ketamine (42 mg/Kg). As for an anticoagulant, sodium heparin (100 IU/mL) was used in 1 mL of blood and subsequently centrifuged at 2500 r.p.m. (Centrifuge 5417R, Eppendorf, Ontario, Canada). The samples were kept refrigerated (-20°C) until they were analyzed. The levels of total cholesterol, lipoproteins (LDL, HDL, VLDL), and circulating triglycerides were determined in rats treated with Q7 and in the rats treated with the combination of Q7 and ascorbate. The analyses were conducted with a Hitachi 704 Chemistry analyzer (Roche-Hitachi, Basel, Switzerland). For triglycerides, the Triglycerides GPO liquicolor kit (GPO-PAP method, Human, Magdeburg, Germany) was used, for total cholesterol, the Cholesterol liquicolor kit (CHOD-PAP method, Human, Magdeburg, Germany), and for HDL, the HDL-Cholesterol direct kit (Human, Magdeburg, Germany).

Oral glucose tolerance test (OGTT)

After 20 days of treatment, the four groups of rats were left fasting for 12 hours to determine their glycaemia index. Each rat was administered glucose orally at a concentration of 2 g/Kg per body weight through an esophageal cannula. Finally, the glycaemia was determined in a blood sampled from the tail of the rat through the Accu-Chek Active Glucose meter (Sidney, Australia). To calculate the area under the curve (AUC), we used GraphPad Prim Version 6.0 Software (GraphPad Software, CA, USA).

Statistical analysis

Values are expressed as the mean ± standard error of the mean; *n* represents the number of animals studied. For the statistical analysis of the groups, a one-way or two-way ANOVA was used, as appropriate, followed by a Bonferroni *post-hoc* test. A value $p<0.05$ is considered statistically significant. We used GraphPad Prim Version 6.0 Software (GraphPad Software, CA, USA).

RESULTS AND DISCUSSION

Chemical Composition

Figure No. 1A shows the chemical structure of 2-[(4-hydroxyphenyl) amino]-1,4-naphthoquinone (Q7)**.** The structure of Q7 was established on the basis of their spectral properties (IR, ^{1}H NMR and ^{13}C NMR) and micro analytical data according to a previous study (Benites *et al*., 2010).

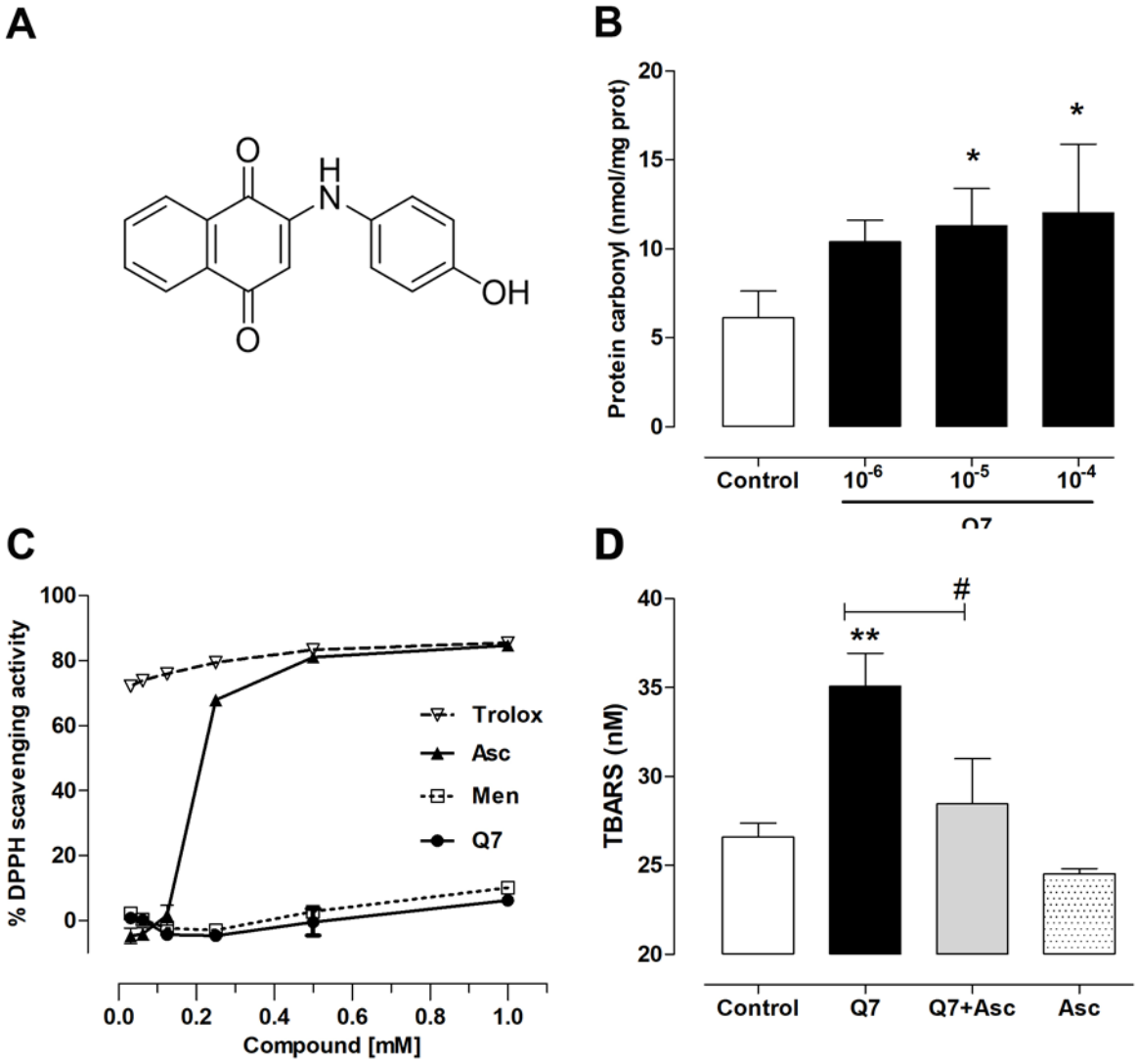

Figure No. 1

Chemical structure of 2-[(4-hydroxyphenyl) amino]-1,4-naphthoquinone (Q7; 265.07 g/mol) (A). Bovine serum albumin (10 mg/mL) was exposed to different concentrations of Q7 (10^{-6} to 10^{-4} M) for 60 min at 37°C (B). Comparison of the trapping capacity of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH). They are compared in increasing concentrations (0-1 mM) of different compounds: trolox (positive control), ascorbate (Asc), menadione (negative control), and Q7 (C). Oral administration of ascorbate (500 mg/Kg) for 20 days in Q7+Asc group decreased Q7 (10 mg/Kg Q7)-induced lipid peroxidation (D). The data represent the mean ± standard error of the mean (SEM). *p<0.05, **p<0.01 versus control; #p<0.05 versus Q7

Q7 induces oxidative stress and role of ascorbate

The cytotoxic effect of naphthoquinone derivatives involves the generation of reactive oxygen species (ROS) through quinone reduction in a redox-cycling reaction (O'Brien, 1991; Bolton *et al*., 2000; Shang *et al*., 2012; Tabrizi *et al*., 2017). The NADPH-cytochrome P450 reductase enzyme catalyzes the reduction of an electron from Q7 to the semiquinone (•Q7) which becomes very reactive. This semiquinone •Q7 is reoxidized to the quinone in the presence of molecular oxygen, while oxygen is reduced to superoxide radical ($^{\bullet}O_2^-$) (Benites *et al*., 2018).

We confirmed that Q7 causes oxidative stress in proteins. Albumin carbonylation was significantly increased in the presence of Q7 in comparison to the control (6.1 ± 0.7 nmol/mg protein Control versus 11.2 ± 1.0 nmol/mg protein with 10^{-5} M Q7; $p<0.05$; **Figure No. 1B**). On the other hand, ascorbate had a significant radical scavenging capacity generated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), while Q7, as expected, had a zero scavenging capacity (**Figure No. 1C**).

The findings above were confirmed by the lipid peroxidation (TBARS) assay (**Figure No. 1D**). Oral treatment with only Q7 (10 mg/Kg) produced a significant elevation in the production of TBARS (35 ± 2 nM in Q7 group) in the serum of the rats when compared to the control group (27 ± 1 nM in control group; $p<0.01$), an effect prevented by the additional treatment with ascorbate (500 mg/Kg) (28 ± 3 nM in Q7+Asc group). We thus conclude that ascorbate is able to revert some of the Q7-induced effects.

Effect of ascorbate on lipid metabolism in rats chronically treated with Q7

To determine whether the naphthoquinone derivative Q7 induces an alteration of lipid metabolism, the lipid profile was determined in the rats. The level of triglycerides and VLDL cholesterol increased significantly ($p<0.05$) in the rats treated with Q7 in comparison to the control group (**Table No. 1**). Q7 slightly increased LDL and HDL cholesterol relative to the control, but it was not significant.

In agreement with this result, several authors reported that high doses of menadione (2-methyl-1,4-naphthoquinone) increases the serum triglycerides, but not the total cholesterol in rats (Alarcon *et al*., 1995; Azharuddin *et al*., 2007). Whereas naphtha-quinone derivatives induce ROS that can cause cell damage (Tabrizi *et al*., 2017), the elevated levels of triglycerides and cholesterol in the blood could be a consequence of the peroxidation of unsaturated membrane lipids mediated by ROS production induced by Q7 (Palacios *et al*., 2016; Anwar *et al*., 2019).

Oral administration of ascorbate reduced the increase in triglycerides and VLDL cholesterol in the rats treated with Q7 (Q7+Asc, **Table No. 1**). This finding partly coincides with a hypolipidemic effect of ascorbate in rats fed a high-fat diet (Santillo *et al*., 1995). However, in another study of rats fed a balanced diet, no hypolipidemic effect of ascorbate was observed, or the effect was slower than in hyperlipidemic rats (Hayashi *et al*., 1976). A meta-analysis of 13 trials demonstrated that ascorbate supplementation decreases serum triglycerides and LDL cholesterol (McRae, 2008). Ascorbate enhances the carnitina transport and, consequently, the fatty acid β-oxidation in the liver, leading to a decrease of the plasma triglyceride concentration (Otsuka *et al*., 1999; Lee *et al*., 2019). Also, ascorbate supplementation can regulate the cholesterol homeostasis, such as LDL cholesterol, decreasing the hepatic 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) activity, and then, the endogenous cholesterol biosynthesis (Deng *et al*., 2019). Our results confirmed the protective role of ascorbate over lipid metabolism.

The administration of ascorbate to the rats treated with Q7 (Q7+Asc) significantly increased HDL cholesterol (**Table No. 1**). To explain this result, we assume that the increase in HDL lipoproteins leads to a better redistribution of lipid levels mediated by reverse cholesterol transport, which means that HDL lipoprotein removes unesterified cholesterol from cell membranes of tissues and transfers back to the liver for excretion through the bile (McRae, 2008).

Effect of Juglone derivatives (Q7) on carbohydrate metabolism

It is also possible that treatment with Q7 alters glycaemia through the increase of ROS (Palacios *et al*., 2016; Palacios *et al*., 2018). To determine this, we performed the oral glucose tolerance test (OGTT) on the rats. As shown Figure No. 2B, the postprandial glycemia decreased significantly in Q7+Asc (10,481 ± 115 mg/dL x min; $p<0.01$) compared to Control (11,602 ± 115 mg/dL x min). Interestingly, the significant decrease of the postprandial glycemia caused by ascorbate in rats treated with Q7 occurred at 15 min after administration of the glucose (183 ± 1 mg/dL Control versus 120 ± 11 mg/dL, $p<0.001$;

Figure No. 2A). This could be explained by the reduction of the intestinal absorption of glucose, an inhibition of the gluconeogenesis in the liver or an increase in the glucose uptake by the tissues.

Table No. 1
Effect ascorbate on lipid metabolism in rats chronically treated with Q7
Values of triglycerides, very-low-density lipoprotein cholesterol (VLDL), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) were measurement in serum of rats

	Control	Q7	Q7+Asc	Asc
Triglycerides, mg/dL	92 ± 6	132 ± 15*	93 ± 15	68 ± 4$^{\#\#}$
VLDL Cholesterol, mg/dL	18 ± 1	27 ± 3*	19 ± 3	13 ± 1$^{\#\#}$
LDL Cholesterol, mg/dL	19 ± 2	26 ± 3	12 ± 6	35 ± 14
HDL Cholesterol, mg/dL	22 ± 1	24 ± 2	38 ± 4***	20 ± 1
Increased body weight (g)	17 ± 1	25 ± 3*	18 ± 1	17 ± 1

The rats treated with Q7 (10 mg/Kg) were orally administered with ascorbate (Asc; 500 mg/Kg) for 20 days. Values are the mean ± SEM of 5 experiments in mg/dL. Statistically significant differences: **p*<0.05, ****p*<0.001 versus control; $^{\#}$*p*<0.05 and $^{\#\#}$*p*<0.01 vs. Q7

On the other hand, postprandial glycemia was increased after administration of ascorbate as shown by the area under the curve (13,125 ± 247 mg/dL x min with Asc; $p<0.001$). It is likely that ascorbate competes with glucose for transportation into the tissues, because both chemical structures are similar. Ascorbate is transformed into dehydroascorbic acid (DHA) in the body, and most cell types are capable of recycling DHA to ascorbate (Lykkesfeldt & Tveden-Nyborg, 2019). Intriguing, DHA presents a higher affinity for GLUT1, GLUT3 and GLUT-4 transporters than glucose (Rivas *et al*., 2008). In fact, DHA is rapidly transport by GLUT 1-4 and 8 and reduced to ascorbic acid in different tissues (Lykkesfeldt & Tveden-Nyborg, 2019).

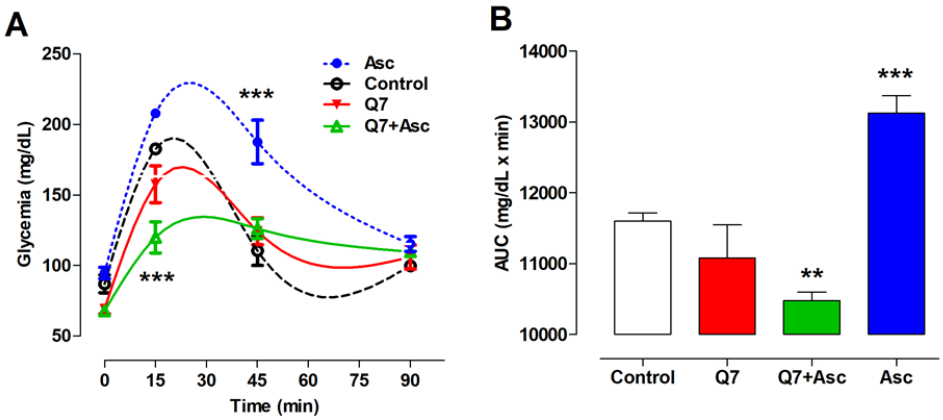

Figure No. 2
Oral administration of Q7 decreases glycaemia in rats. Glycaemia determination (mg/dL) in different groups of rats (A). The control group (black), the group treated with Q7 (10 mg/Kg Q7) (red), the group treated with both Q7 and ascorbate (500 mg/Kg) (green) and group treated with ascorbate (Asc) (blue). Area under the curve (AUC) of plasma glucose levels in the different groups under treatment (B). The data represent the mean ± SEM. ***p*<0.01 and ****p*<0.001 vs. Control; n = 5

CONCLUSION

The oral administration of ascorbate reduced the Q7-induced increases in lipids. This could be associated with the antioxidant activity of ascorbate that reduced the oxidative stress induced by Q7. Although Q7 did not alter the glycemia in rats, ascorbate plus Q7 reduced it. Furthermore, a synergistic effect between ascorbate and the naptotquinone derivative seems to take place, but further experiments are necessary to throw more light on this. These results suggest an *in vivo* protective effect of ascorbate (taken orally) in animals treated with a naphthoquinone derivative, which induces oxidative stress. They may be of interest for future research in different pathologies such as inflammatory disorders, cardiovascular diseases, and cancer.

ACKNOWLEDGMENTS

Financial support was provided by Vicerrectoría de Investigación, Innovación y Postgrado Universidad Arturo Prat (VRIIP0047-19 and VRIIP0179-19), FONDECYT 1200610 to JP and the Network for Extreme Environments Research project (NEXER, Project ANT1756, Universidad de Antofagasta, Chile). These sources of funding are gratefully acknowledged.

REFERENCES

Afonso V, Champy R, Mitrovic D, Collin P, Lomri A. 2007. Reactive oxygen species and superoxide dismutases: Role in joint diseases. **Joint Bone Spine** 74: 324 - 329. **https://doi.org/10.1016/j.jbspin.2007.02.002**

Alarcon OM, Reinosa FA, Silva LTM, Padron FA. 1995. Changes of serum lipids in vitamin K-3 (menadione) treated rats. **Arch Latinoam Nut** 45: 286 - 289.

Anwar F, Khan R, Sachan R, Kazmi I, Rawat A, Sabih A, Singh R, Afzal M, Ahmad A, Al-Orab AS, Al-Abbasi FA, Bhatt PC, Kumar V. 2019. Therapeutic role of calcium and vitamin K3 in chemically induced hepatocarcinogenesis - new tools for cancer treatment. **Arch Physiol Biochem** 125: 270 - 275. **https://doi.org/10.1080/13813455.2018.1455708**

Azharuddin MK, O'Reilly DS, Gray A, Talwar D. 2007. HPLC method for plasma vitamin K1: effect of plasma triglyceride and acute-phase response on circulating concentrations. **Clin Chem** 53: 1706 - 1713. **https://doi.org/10.1373/clinchem.2007.086280**

Benites J, Valderrama JA, Bettega K, Pedrosa RC, Buc Calderon P, Verrax J. 2010. Biological evaluation of donor-acceptor aminonaphthoquinones as antitumor agents. **Eur J Med Chem** 45: 6052 - 6057. **https://doi.org/10.1016/j.ejmech.2010.10.006**

Benites J, Toledo H, Salas F, Guerrero A, Rios D, Valderrama JA, Buc Calderon P. 2018. *In vitro* inhibition of Helicobacter pylori growth by redox cycling phenylaminojuglones. **Oxid Med Cell Long** 8. **https://doi.org/10.1155/2018/1618051**

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. 2000. Role of quinones in toxicology. **Chem Res Toxicol** 13: 135 - 160.

Catanzaro E, Greco G, Potenza L, Calcabrini C, Fimognari C. 2018. Natural products to fight cancer: A focus on. **Toxins** 10: 1 - 38. **https://doi.org/10.3390/toxins10110469**

Cifuentes F, Bravo J, Norambuena M, Stegen S, Ayavire A, Palacios J. 2009. Chronic exposure to arsenic in tap water reduces acetylcholine-induced relaxation in the aorta and increases oxidative stress in female rats. **Int J Toxicol** 28: 534 - 541. **https://doi.org/10.1177/1091581809345924**

Deng JM, Zhang XD, Zhang JW, Bi BL, Wang HZ, Zhang L, Mi HF. 2019. Effects of dietary ascorbic acid levels on cholesterol metabolism in rainbow trout (Oncorhynchus mykiss). **Aquacult Nut** 25: 1345 - 1353. **https://doi.org/10.1111/anu.12955**

Hayashi E, Yamada J, Kunitomo M, Terada M, Tomita T, Kinoshita T. 1976. Fundamental studies on physiological and pharmacological actions of L-ascorbate 2-sulfate. I. On the hypolipidemic effects. **J Nutr Sci Vitaminol** 22: 201 - 208.

Hayes D, Angove MJ, Tucci J, Dennis C. 2016. Walnuts (*Juglans regia*) chemical composition and research in human health. **Crit Rev Food Sci Nutr** 56: 1231 - 1241. **https://doi.org/10.1080/10408398.2012.760516**

Hill A, Wendt S, Benstoem C, Neubauer C, Meybohm P, Langlois P, Adhikari NKJ, Heyland DK, Stoppe C. 2018. Vitamin C to improve organ dysfunction in cardiac surgery patients-review and pragmatic approach. **Nutrients** 10 (8). **https://doi.org/10.3390/nu10080974**

Hoffman RP, Dye AS, Bauer JA. 2012. Ascorbic acid blocks hyperglycemic impairment of endothelial function in

adolescents with type 1 diabetes. **Pediatric Diabetes** 13: 607 - 610. **https://doi.org/10.1111/j.1399-5448.2012.00882.x**

Hurrle S, Hsu WH. 2017. The etiology of oxidative stress in insulin resistance. **Biomed J** 40: 257 - 262. **https://doi.org/10.1016/j.bj.2017.06.007**

Lee H, Ahn J, Shin SS, Yoon M. 2019. Ascorbic acid inhibits visceral obesity and nonalcoholic fatty liver disease by activating peroxisome proliferator-activated receptor α in high-fat-diet-fed C57BL/6J mice. **Int J Obes** 43: 1620 - 1630. **https://doi.org/10.1038/s41366-018-0212-0**

Lykkesfeldt J, Tveden-Nyborg P. 2019. The pharmacokinetics of vitamin C. **Nutrients** 11 (10). **https://doi.org/10.3390/nu11102412**

McRae MP. 2008. Vitamin C supplementation lowers serum low-density lipoprotein cholesterol and triglycerides: a meta-analysis of 13 randomized controlled trials. **J Chiropract Med** 7: 48 - 58. **https://doi.org/10.1016/j.jcme.2008.01.002**

O'Brien PJ. 1991. Molecular mechanisms of quinone cytotoxicity. **Chem Biol Interact** 80: 1 - 41.

Otsuka M, Matsuzawa M, Ha TY, Arakawa N. 1999. Contribution of a high dose of L-ascorbic acid to carnitine synthesis in guinea pigs fed high-fat diets. **J Nutr Sci Vitaminol** 45: 163 - 171. **https://doi.org/10.3177/jnsv.45.163**

Ourique F, Kviecinski MR, Felipe KB, Correia JFG, Farias MS, Castro L, Grinevicius VMAS, Valderrama J, Rios D, Benites J, Buc Calderon P, Pedrosa RC. 2015. DNA damage and inhibition of akt pathway in MCF-7 cells and Ehrlich tumor in mice treated with 1,4-Naphthoquinones in combination with ascorbate. **Oxid Med Cell Longev** 10. **https://doi.org/10.1155/2015/495305**

Palacios J, Cifuentes F, Valderrama JA, Benites J, Ríos D, González C, Chiong M, Cartes-Saavedra B, Lafourcade C, Wyneken U, González P, Owen GI, Pardo F, Sobrevia L, Buc Calderon P. 2016. Modulatory effect of 2-(4-Hydroxyphenyl) amino-1,4-naphthoquinone on endothelial vasodilation in rat aorta. **Oxid Med Cell Longev** 2016: 3939540. **https://doi.org/10.1155/2016/3939540**

Palacios J, Fonseca JM, Ayavire F, Salas F, Ortiz M, Sandoval JM, Benites J, Nwokocha CR, Zavala E, Paredes A, Barría I, Vega JL, Cifuentes F. 2018. Ascorbate attenuates oxidative stress and increased blood pressure induced by 2-(4-Hydroxyphenyl) amino-1,4-naphthoquinone in rats. **Oxid Med Cell Longev** 11. **https://doi.org/10.1155/2018/8989676**

Pires AS, Marques CR, Encarnação JC, Abrantes AM, Mamede AC, Laranjo M, Gonçalves AC, Sarmento-Ribeiro AB, Botelho MF. 2016. Ascorbic acid and colon cancer: an oxidative stimulus to cell death depending on cell profile. **Eur J Cell Biol** 95: 208 - 218. **https://doi.org/10.1016/j.ejcb.2016.04.001**

Ray PD, Huang BW, Tsuji Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. **Cell Signal** 24: 981 - 990. **https://doi.org/10.1016/j.cellsig.2012.01.008**

Reid M, Spence J, Nwokocha M, Palacios J, Nwokocha CR. 2018. Chapter 2 - **The role of NADP(H) oxidase inhibition and its implications in cardiovascular disease management using natural plant products**. In: Studies in natural products chemistry. Elsevier, Amsterdam, The Netherlands.

Rivas CI, Zuniga FA, Salas-Burgos A, Mardones L, Ormazabal V, Vera JC. 2008. Vitamin C transporters. **J Physiol Biochem** 64: 357 - 375. **https://doi.org/10.1007/bf03174092**

Santillo M, Mondola P, Santangelo F, Gioielli A, Iossa S, Basilisco A, De Mercato R. 1995. Changes in apoprotein distribution between lipoprotein classes of hypercholesterolemic rats treated with ascorbate. **Int J Biochem Cell Biol** 27: 257 - 262.

Shang Y, Chen C, Li Y, Zhao J, Zhu T. 2012. Hydroxyl radical generation mechanism during the redox cycling process of 1,4-naphthoquinone. **Environ Sci Technol** 46: 2935 - 2942. **https://doi.org/10.1021/es203032v**

Tabrizi L, Chiniforoshan H. 2017. Designing new iridium(iii) arene complexes of naphthoquinone derivatives as anticancer agents: a structure-activity relationship study. **Dalton Trans** 46: 2339 - 2349. **https://doi.org/10.1039/c6dt04339a**

Yeung AWK, Tzvetkov NT, El-Tawil OS, Bungau SG, Abdel-Daim MM, Atanasov AG. 2019. Antioxidants: scientific literature landscape analysis. **Oxid Med Cell Longev** 11. **https://doi.org/10.1155/2019/8278454**

Yilmazer A. 2018. Cancer cell lines involving cancer stem cell populations respond to oxidative stress. **Biotechnol Rep** 17: 24 - 30. **https://doi.org/10.1016/j.btre.2017.11.004**