

Effect of silymarin on oxidative damage in chicken liver cell membranes

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Recibido: 21/4/2022. Aprobado: 31/5/2022

ABSTRACT

In living beings, antioxidants are of vital importance for protection against oxidative damage caused by reactive oxygen species. Silymarin (SM), a plant-derived flavonoid present in the fruits and seeds of milk thistle *Silybum marianum* (L.) Gaertn., has a recognized hepatoprotective effect. In this work, the *in vitro* silymarin antioxidant effect on non-enzymatic peroxidation (NEP) in chicken liver mitochondria and microsomes was studied. Oxidative stress in the organelles was induced by subjecting the samples (1 mg of protein) to an ascorbate-Fe⁺⁺-dependent prooxidant system at 37 °C. Oxidative damage was quantified by chemiluminescence (CL) using a Packard 1900 TR liquid scintillation counter (Meriden CT, USA). CL expressed as cpm (counts per minute) was read every 10 minutes to establish the course of peroxidation as a function of time. Likewise, the total cpm value (sum of the readings) was used to compare the inhibitory effect of SM using different concentrations corresponding to 6.25; 12.5, and 25 µg of the active ingredient (silymarin phosphatide) per mg of mitochondrial and microsomal protein. Controls were run simultaneously without the addition of ascorbate. Peroxidation inhibition was dependent on the concentration of SM in the incubation mixture. The results show that a protective effect on induced oxidative damage was found for all concentrations tested.

Keywords: silymarin, liver, chicken, chemiluminescence.

Efecto de la silimarina sobre el daño oxidativo en membranas celulares de hígado de pollo

RESUMEN

En los seres vivos, los antioxidantes son de vital importancia para la protección contra el daño oxidativo causado por las especies reactivas del oxígeno. La silimarina (SM), un flavonoide de origen vegetal presente en los frutos y semillas del cardo mariano *Silybum marianum* (L.) Gaertn., tiene un reconocido efecto hepatoprotector. En este trabajo se estudió el efecto antioxidante *in vitro* de la silimarina sobre la peroxidación no enzimática (PNE) en mitocondrias y microsomas de hígado de pollo. El estrés oxidativo en los orgánulos se indujo sometiendo las muestras (1 mg de proteína) a un sistema prooxidante dependiente de ascorbato-Fe⁺⁺ a 37 °C. El daño oxidativo se cuantificó por quimioluminiscencia (QL) utilizando un contador de centelleo líquido Packard 1900

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TR (Meriden CT, EE. UU.). QL expresado como cpm (recuentos por minuto) se leyó cada 10 minutos para establecer el curso de la peroxidación en función del tiempo. Asimismo, se usó el valor total de cpm (suma de las lecturas) para comparar el efecto inhibitorio de SM mediante diferentes concentraciones correspondientes a 6,25; 12,5 y 25 µg del ingrediente activo (fosfátido de silymarina) por mg de proteína mitocondrial y microsomal. Los controles se realizaron simultáneamente sin la adición de ascorbato. La inhibición de la peroxidación dependía de la concentración de SM en la mezcla de incubación. Los resultados muestran que para todas las concentraciones probadas se encontró un efecto protector sobre el daño oxidativo inducido

Palabras clave: silymarina, hígado, pollo, quimioluminiscencia.

INTRODUCTION

Antioxidants are substances that in minimal concentrations delay or cancel the oxidation of different organic substrates. In living beings, they are of vital importance for the protection against oxidative damage produced by reactive oxygen species (ROS)—also known as free radicals—and other degenerative alterations (Maleki *et al.* 2019). ROS are highly reactive molecules with an unpaired electron in the outermost orbital, which are constantly produced during the course of many reactions essential for aerobic life, such as electron transfer reactions in the mitochondrial respiratory chain and also in certain cytoplasmic reactions (Zhao *et al.* 2020). From the pathophysiological point of view, ROS are involved in numerous diseases and tissue injuries such as those that occur at the pulmonary, cardiac, gastrointestinal, blood, ocular, cerebral, and hepatic levels and those associated with aging processes. The excessive production of reactive oxygen species, particularly hydroxyl radicals, can alter membrane lipids with the subsequent production of lipid peroxides. Free radical-induced lipid peroxidation is a phenomenon that occurs in different alterations such as ischemia-reperfusion of tissues, chronic inflammation, the toxicity of certain drugs, etc. To counteract the

effects mentioned above, antioxidants participate at different levels within the oxidative sequence through one of the following mechanisms: 1. decreasing the oxygen concentration by interception of singlet oxygen; 2. preventing the initiation step, scavenging hydroxyl radicals; 3. by decomposition of lipid peroxides, catalyzed by metal ions; 4. decomposing primary products to components without radical function; 5. preventing the breaking of the substrate chains by hydrogen abstraction (Zeb 2020). Antioxidants also act as hydrogen and/or electron donors to radicals formed from unsaturated lipids, thus stabilizing the active molecule (He *et al.* 2017). Natural antioxidants include phenolic and polyphenolic compounds, chelators, enzymes, and antioxidant vitamins. Vegetables in particular provide a rich source of natural antioxidants including vitamins C, E, carotenoids, and phenolic compounds. These substances have a similar basic molecular structure in which there is at least one aromatic ring and one hydroxyl group, such as phenolic acids, flavonoids and isoflavonoids, hydrolyzable tannins, lignans, coumarins, stilbenes, flavanones, and oligomeric proanthocyanidins. These molecules act by different mechanisms, conferring an effective defense system against free radical

attacks in plants (Džugan *et al.* 2018; Van Hung 2016). In turn, the presence of antioxidants of plant origin in food prevents, among other alterations, cardiovascular problems, cancer, cataracts, etc. (Li *et al.* 2015). Among the organs susceptible to oxidative damage, the liver constantly faces the effects of ROS. It represents the largest gland in the body and has a central place in the metabolism of proteins, fats, and carbohydrates, it actively participates in the neutralization of potentially toxic substances (it synthesizes, among other compounds, glutathione) (Trefts *et al.* 2017), vitamin storage (regulates vitamin E levels), etc. In the case of poultry production, for example, the great advances in the genetic selection of production animals (greater growth speed and feed efficiency) must have been associated with increases in hepatic metabolic activity (Lambrecht *et al.* 2020). Birds raised in intensive production systems are subject to stress, a situation that promotes the production of free radicals. Broilers suffering from pulmonary hypertension syndrome or ascitic syndrome result in significant economic losses in world poultry farming and the presence of oxidative damage or reduction in the antioxidant capacity associated with these and other diseases has been documented (Xu *et al.* 2021). That is why in intensive poultry farming substances with antioxidant capacity are used. The hepatoprotective effect of SM in poultry farming is poorly understood, although it may be used as a cheap, nontoxic, and safe feed additive to manage liver-related disorders and to replace synthetic drugs in poultry diets (Saeed *et al.* 2019). SM was initially found in the mountains of the Mediterranean, Asia, and North Africa regions; however, today, it is grown in many parts of the world. SM

is a complex flavonoid isolated from the fruits and seeds of milk thistle (*Silybum marianum*) that contains a mixture of six flavonolignans (silybin, isosilybin, silychristin, isosilychristin, silydianin, and silimonin) and other flavonoids (taxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodictyol, and chrysoeriol); among these, silybin is the principal active component (MacDonald-Ramos *et al.* 2021). Silibinin is used as an active ingredient in a wide spectrum of dietary supplements and has received much attention over the last decade as an herbal remedy (Rajnochová *et al.* 2016; Surai 2015). The objective of this work was to analyze the *in vitro* effect of SM on oxidative damage in mitochondrial and microsomal membranes of chicken liver. In order to know the benefits of plant antioxidants in broilers, different concentrations of SM (6.25, 12.5, and 25 µg/mL) obtained from a mother solution in sodium phosphate buffer (0.04 M, pH7.0) were used.

MATERIALS AND METHODS

Chemicals

SM was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum albumin (BSA) (Fraction V) was obtained from Wako Pure Chemical Industries Ltd, Japan. L (+) ascorbic acid, butylene hydroxytoluene (BHT), and phenylmethyl-sulfoxide fluoride (PMSF) were obtained from Merck Laboratories. All other reagents and chemicals were analytical grade from Sigma.

Use of silymarin preparation

A stock solution was prepared with 10 mg of SM dissolved in 10 ml of distilled

water. Different dilutions of the SM (6.25, 12.5, and 25 $\mu\text{g/ml}$) obtained from the stock solution in sodium phosphate buffer (0.04 M, pH 7.0) were used.

Animals and preparation of mitochondria and microsomes

32-day-old Cobb chicks donated by Balnearios Patagónicos, Trelew, Argentina, were used for the study. Immediately after the arrival (one day of life) of the chicks at the experimental house, they were weighed (71.38 g). The birds were kept in boxes of 2 m², which were maintained with balanced feed and water *ad libitum*. The animals were sacrificed by cervical dislocation; the livers were removed, cut into small pieces, and washed repeatedly with a 0.15 M NaCl solution. The homogenate was prepared by adding 3 ml per gram of tissue of solution A (0.25 M sucrose; 10 mM Tris HCl buffer pH 7.4, 0.1 mM PMSF) in a potter Elvehjem homogenizer. The homogenate was centrifuged at 1,000 x g, the pellets were discarded, and the resulting supernatant was centrifuged at 20,000 x g for 10 min to obtain mitochondria. After centrifugation, 5 ml of the resulting supernatant was passed through a Sepharose 4 B column (1.6cm x 12 cm) equilibrated and diluted with 10 mM Tris-HCl buffer pH7.4 0.01% Na N₃. The microsomal fraction appeared in the void volume (12-20 ml) and the cytosol was discarded. All operations were performed at 4 °C and under dim light. The quality of this microsomal preparation is compositionally similar with respect to concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation (Tangen *et al.* 1973). Mitochondria were obtained by the method described by Schneider and Hogeboom (1958).

The CICUAL (Institutional Committee for the Care and Use of Laboratory Animals) of the Faculty of Veterinary Sciences, UNLP, approved all the housing and experimentation procedures

Peroxidation of chicken liver mitochondria and microsomes

Chemiluminescence and peroxidation were initiated by adding ascorbate-Fe⁺⁺ to mitochondria and microsomes (1 mg protein) (ascorbate-Fe⁺⁺ group) (Wright *et al.* 1979). To carry out the experiments, three groups were established: 1. control group (only organelles), 2. ascorbate-Fe⁺⁺ group (organelles + inducer), and 3. SM group (organelles + inducer + SM). SM added mitochondria and microsomes (6.25, 12.5, and 25 $\mu\text{g-SM}$ group) were incubated at 37 °C with 0.01 M phosphate buffer pH 7.4, 0.4 mM ascorbate, final volume 2 ml. The phosphate buffer is contaminated with enough iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15 μM) for peroxidation (Tadolini and Hakim 1996). Simultaneously, preparations were made with mitochondria and microsomes, which lacked ascorbate (control group). Light emission from the membranes was determined over a period of 120 min, chemiluminescence was recorded as cpm every 10 min, and the sum of the total chemiluminescence was used to calculate cpm/mg protein. Chemiluminescence was measured as counts per minute on a Packard 1900 TR liquid scintillation analyzer with chemiluminescence software.

Protein determination

Proteins were determined by the method of Lowry *et al.* (1951), using BSA as a standard.

Statistical analysis

Data were expressed as means \pm SD of thirty independent determinations. The statistical analysis used was the Student's t-test. The 0.05 level was selected as the point of least statistical significance. The statistical criterion of significance was selected at different p-values and was indicated in each case.

RESULTS

Light emission from chicken liver mitochondria during peroxidation

Incubation of chicken liver mitochondria in the presence of ascorbate-Fe⁺⁺ resulted in peroxidation of the membranes as shown by light emission (chemiluminescence) when the control and ascorbate-Fe⁺⁺ groups were compared. The values were 298.704 ± 9.72 in the control group, while 1617.68 ± 69.37 in the ascorbate-Fe⁺⁺

group, showing a significance value of $p < 0.0005$. After incubation of mitochondria in an ascorbate-Fe⁺⁺ system at 37 °C for 120 min in the presence of increasing amounts of SM (6.25, 12.5, and 25 μ g of SM) per mg of protein, the values of cpm caused by light emission were lower (concentration-dependent) in the SM group than in the ascorbate-Fe⁺⁺ group. The values were 1190.79 ± 104.19 with the addition of 6.25 μ g SM/mg protein; 705.41 ± 152.44 with the addition of 12.5 μ g of SM/mg protein and finally 244.9 ± 29.05 cpm with the addition of 25 μ g of SM/mg protein. The significance values were $p < 0.005$ with the 6.25 μ g SM/mg protein dose, while a significance value of $p < 0.0005$ was obtained for the 12.5 and 25 μ g SM/mg protein doses. Figure 1 shows the light emission of chicken liver mitochondria obtained from the control groups, ascorbate-Fe⁺⁺ and the SM group.

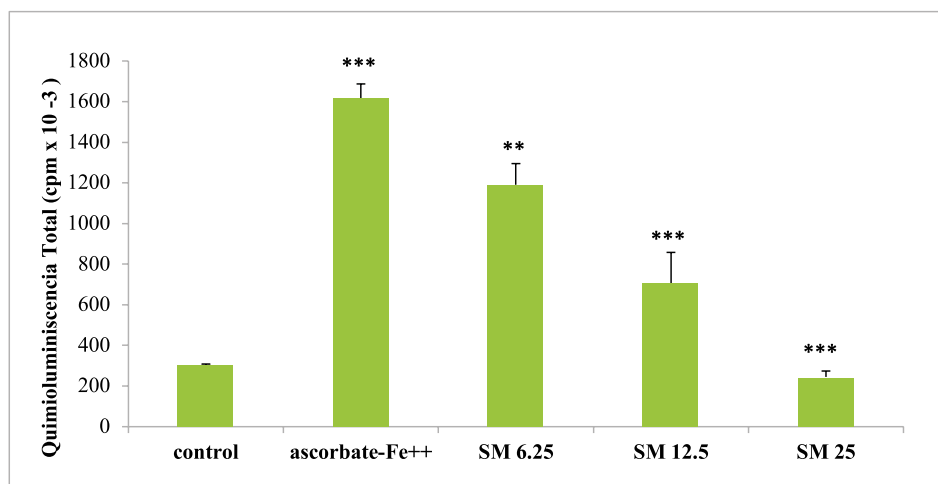


FIGURE 1. Light emission of chicken liver mitochondria during peroxidation with different concentrations of SM.

The results are expressed as the mean \pm S.D. of thirty independent experiments. The light emission values show highly significant (** $p < 0.005$) and highly significant (***) $p < 0.0005$) statistical differences between the compared groups.

Source: own elaboration.

Light emission from chicken liver microsomes during peroxidation

Incubation of chicken liver microsomes in the presence of ascorbate-Fe⁺⁺ resulted in peroxidation of the membranes as evidenced by light emission (chemiluminescence) when the control and ascorbate-Fe⁺⁺ groups were compared. The values were 347.58 ± 29.18 in the control group while in the ascorbate-Fe⁺⁺ group, it was 1440.56 ± 13.53, showing a significance value of $p < 0.0005$. After incubation of mitochondria in an ascorbate-Fe⁺⁺ system at 37 °C for 120 min in the presence of increasing amounts of SM (6.25, 12.5, and 25 µg of SM) per mg of protein, the values of cpm caused by light emission were lower (concentration-dependent) in the SM group than in the ascorbate-Fe⁺⁺ group.

The values were 1034.81 ± 56.13 with the addition of 6.25 µg SM/mg protein; 810.59 ± 20.96 with the addition of 12.5 µg SM/mg protein and finally 377.83 ± 41.91 cpm with the addition of 25 µg SM/mg protein. The significance values were $p < 0.05$ with the 6.25 dose, while for the 12.5 and 25 µg SM/mg protein doses a highly significant value corresponding to $p < 0.0005$ was obtained. Figure 2 shows the light emission of chicken liver microsomes obtained from the control groups, ascorbate-Fe⁺⁺ and the SM group.

Comparative percentage inhibition of light emission in chicken liver mitochondria and microsomes

After incubation of mitochondria and microsomes in an ascorbate-Fe⁺⁺ system

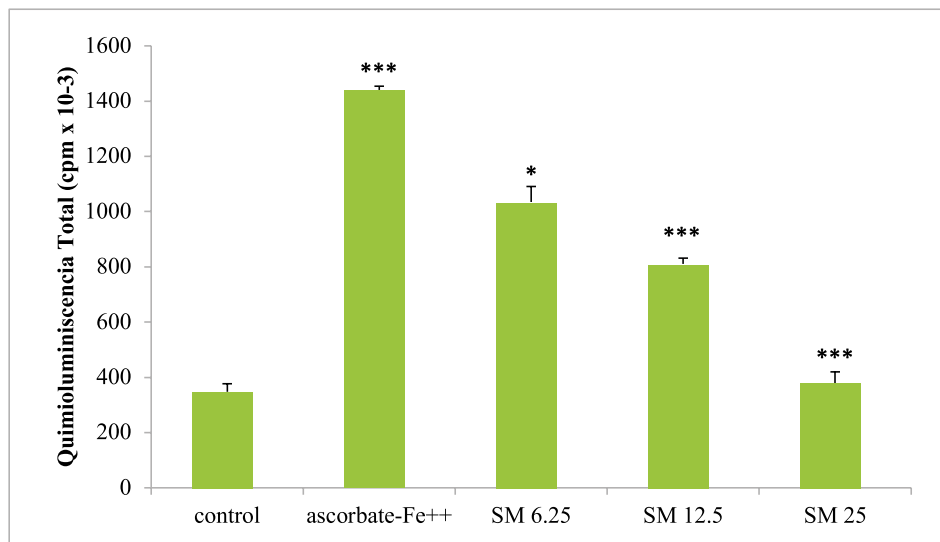


FIGURE 2. Light emission of chicken liver microsomes during peroxidation with different concentrations of SM.

The results are expressed as the mean ± S.D. of thirty independent experiments. The light emission values show statistically significant (* $p < 0.05$) and highly significant (***) $p < 0.0005$) differences between the compared groups. Source: own elaboration.

(120 min at 37°C), it was observed that the percentage cpm/mg of protein from light emission (chemiluminescence) was lower in liver mitochondria and microsomes from chicken with the addition of SM. Thus, the highest percentage of inhibition of peroxidation observed with the addition of SM was 84.86% in mitochondria and 73.77% in microsomes for the dose of 25µg of SM/mg of protein (figure 3).

DISCUSSION

During cellular respiration, univalent reduction of oxygen occurs (Oddone *et al.* 2019) and therefore most of the ROS formed under physiological conditions are different forms of oxygen radicals: superoxide anion, hydroxyls, and singlet oxygen. The balance between the generation of free radicals and their neutralization is controlled by complex mechanisms that include: a. the maintenance of the spatial separation between the sites of radical

production and the biomolecules vulnerable to them, b. the enzymatic mechanisms of radical trapping: superoxide dismutase, catalase, and glutathione peroxidase, and c. the presence of substances with antioxidant properties: vitamin C, vitamin E, and glutathione (Parcheta *et al.* 2021). If the protective mechanisms are altered and the rate of ROS generation exceeds the antioxidant capacity, the effects produced by these reactive molecules can have great pathophysiological significance (Di Meo and Venditti 2020) on different cellular components: proteins, nucleic acids, and lipids having studied with greater emphasis the attack of ROS caused on lipids, an effect that leads to lipoperoxidation: the oxidation of the α -methylene bonds of unsaturated fatty acids that results in the formation of lipoperoxides and hydroperoxides, and finally the fragmentation of the lipid molecule (Zhong *et al.* 2019). Due to the fact that biomembranes are rich in polyunsaturated fatty acids, these

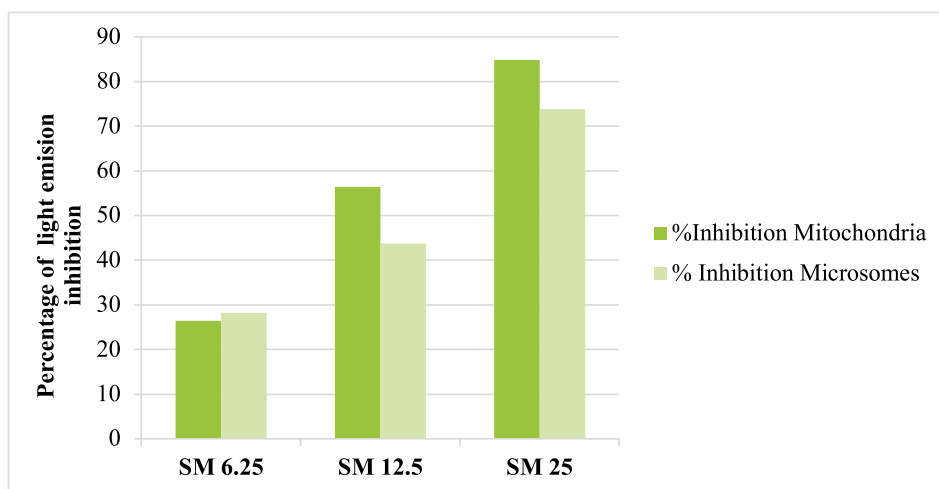


FIGURE 3. Percentage of inhibition of light emission in chicken liver mitochondria and microsomes by SM.

Source: own elaboration.

reactions lead to the disintegration of the membrane structures and ultimately irreversible cell damage (Lawrence 2021). ROS have also been associated with cellular aging: the theory of progressive membrane aging emphasizes the damage produced to membrane fatty acids in the following aspects: a. membrane fatty acids are found in the primary site of production of ROS (mitochondrial membrane) and are so close to them that no antioxidant defense system is capable of preventing their peroxidation; b. lipid peroxidation constitutes an autocatalytic chain reaction that, once started, will continue until it is eventually stopped by antioxidant mechanisms; c. many of the products of lipid peroxidation are themselves very reactive molecules and are therefore highly damaging agents on other molecules (Su *et al.* 2019). The aim of our study was to evaluate the protective *in vitro* effect of SM on chicken liver mitochondria and microsomes against peroxidation. *In vitro* peroxidation studies are useful to elucidate the possible mechanism of peroxide formation *in vivo* (Rauchová *et al.* 1993). Silymarin is used in humans in different liver disorders, particularly in chronic liver diseases, cirrhosis, and hepatocarcinoma, due to its antioxidant, anti-inflammatory, and antifibrotic power (Federico *et al.* 2017). Although considerable research has already been done to characterize changes in the structure, composition, and physical properties of membranes subjected to oxidation (Detaille *et al.* 2019; Gaschler and Stockwell 2017), it is important to know how biological compounds with properties antioxidants such as SM contribute to the protection of specialized membranes against the harmful effects produced by reactive oxygen species and other free

radicals in other species, such as broilers in this case (Armanini *et al.* 2021). In the present study, we have determined that both chicken liver mitochondria and microsomes with the addition of SM were protected against peroxidation compared to similar membranes obtained from the ascorbate-Fe⁺⁺ group, as shown by the results obtained by chemiluminescence. Our results are in agreement with previous work carried out with SM in rat kidney mitochondria and microsomes (Marmunti *et al.* 2016). Likewise, with the addition of the maximum dose of SM tested 25 µg/mg of protein, a higher percentage of peroxidation inhibition was observed in mitochondria than in microsomes in chicken liver. On the other hand, Saeed (2019) considers that the hepatoprotective effect of silymarin in poultry farming is poorly understood, but according to other authors it could provide a safe and cheap feed additive to promote growth performance and support liver function.

CONCLUSION

Our results are consistent with the hypothesis that SM can act as an antioxidant in cell membranes, showing greater protection in chicken liver mitochondria. However, further studies are needed to better evaluate these observations, in order to find an effective antioxidant dose in poultry.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This work was supported by the Secretariat of Science and Technology, National University of La Plata (Project 11/V276).

REFERENCES

- Armanini EH, Boiogo MM, De Oliveira Cécere BG, Oliveira PV, Teixeira CJS, Strapazzon JV, Bottari NB, Silva AD, Fracasso M, Vendruscolo RG, Wagner R, Da Gloria EM, Horn VW, Mendes RE, Baldissera MD, Vedovatto M, Da Silva AS. 2021. Protective effects of silymarin in broiler feed contaminated by mycotoxins: growth performance, meat antioxidant status, and fatty acid profiles. *Trop Anim Health Prod.* 53(4):442. <https://doi.org/10.1007/s11250-021-02873-2>
- Detalle D, Pasdois P, Sémont A, Dos Santos P, Dioliz P. 2019. An old medicine as a new drug to prevent mitochondrial complex I from producing oxygen radicals. *PLoS One.* 14(5):e0216385. eCollection 2019. <https://doi.org/10.1371/journal.pone.0216385>
- Di Meo S, Venditti P. 2020. Evolution of the knowledge of free radicals and other oxidants. *Oxid Med Cell Longev.* 2020:9829176. eCollection 2020. <https://doi.org/10.1155/2020/9829176>
- Dżugan M, Tomczyk M, Sowa P, Grabek-Lejko D. 2018. Antioxidant activity as biomarker of honey variety. *Molecules.* 23(8):2069. <https://doi.org/10.3390/molecules23082069>
- Federico A, Dallio M, Loguerco C. 2017. Silymarin/Silybin and Chronic Liver Disease: A Marriage of Many Years. *Molecules.* 22(2):191. <https://doi.org/10.3390/molecules22020191>
- Gaschler MM, Stockwell BR. 2017. Lipid peroxidation in cell death. *Biochem Biophys Res Commun.* 482(3):419-425. <https://doi.org/10.1016/j.bbrc.2016.10.086>
- He L, He T, Farrar S, Ji L, Liu T, Ma X. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol Biochem.* 44(2):532-553. <https://doi.org/10.1159/000485089>
- Lambrecht J, Van Grunsven LA, Tacke F. 2020. Current and emerging pharmacotherapeutic interventions for the treatment of liver fibrosis. *Expert Opin Pharmacother.* 21(13):1637-1650. <https://doi.org/10.1080/14656566.2020.1774553>
- Lawrence GD. 2021. Perspective: The Saturated fat-unsaturated oil dilemma: relations of dietary fatty acids and serum cholesterol, atherosclerosis, inflammation, cancer, and all-cause mortality. *Adv Nutr.* 12(3):647-656. <https://doi.org/10.1093/advances/nmab013>
- Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. 2015. The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci.* 16(11):26087-124. <https://doi.org/10.3390/ijms161125942>
- Lowry OH, Rosebrough NJ, Farr AL. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 193(1):265-75. PMID: 14907713
- MacDonald-Ramos K, Michán L, Martínez-Ibarra A, Cerbón M. 2021. Silymarin is an ally against insulin resistance. A review. *Ann Hepatol.* 23:100255. <https://doi.org/10.1016/j.aohep.2020.08.072>
- Maleki SJ, Crespo JF, Cabanillas B. 2019. Anti-inflammatory effects of flavonoids. *Food Chem.* 299:125124. <https://doi.org/10.1016/j.foodchem.2019.125124>
- Marmunti M, Gavazza M, Zeinsteger PA, Palacios A. 2016. Antioxidant Effect of Silymarin During Non-Enzymatic Peroxidation of Rat Kidney Microsomes and Mitochondria. *Biochem Mol Biol Lett.* 2(1):102. Disponible en: <https://www.tsijournals.com/articles/antioxidant-effect-of-silymarin-during-nonenzymatic-peroxidation-of-rat-kidney-microsomes-and-mitochondria.pdf>
- Oddone N, Pederzoli F, Duskey JT, De Benedictis CA, Grabrucker AM, Forni F, Angela Vandelli M, Ruozzi B, Tosi G. 2019. ROS responsive smart polymeric conjugate synthesis characterization and proof of concept study. *Int J Pharm.* 570:118655. <https://doi.org/10.1016/j.ijpharm.2019.118655>
- Parcheta M, Świsłocka R, Orzechowska S, Akimowicz M, Choińska R, Lewandowski W. 2021. Recent Developments in Effective Antioxidants: The Structure and Antioxidant Properties. *Materials (Basel).* 14(8):1984. <https://doi.org/10.3390/ma14081984>
- Rajnochová Svobodová A, Zálešák B, Biedermann D, Ulrichová J, Vostálová J. 2016. Phototoxic potential of silymarin and its bioactive components. *J Photochem Photobiol B.* 156:61-8. <https://doi.org/10.1016/j.jphotobiol.2016.01.011>
- Rauchová H, Kalous M, Drahotka Z, Koudelová J, Mourek J. 1993. Lipid peroxidation in isolated membranes of cerebral cortex, heart, and kidney. *Physiol Res.* 42(5):323-7. PMID: 8130178.

- Disponibile en: http://www.biomed.cas.cz/physiolres/pdf/42/42_323.pdf
- Saeed M, Babazadeh D, Arif M, Arain MA, Bhutto ZA, Shar AH, Kakar MU, Manzoor R, Chao S. 2019. Silymarin: a potent hepatoprotective agent in poultry industry. *Worlds Poultry Sci J.* 73:483-492. <https://doi.org/10.1017/S0043933917000538>
- Schneider WC, Hogeboom GH. 1951. Cytochemical studies of mammalian tissues; the isolation of cell components by differential centrifugation: a review. *Cancer Res.* 11(1):1-22. PMID: 14792549. Disponible en: <https://aacrjournals.org/cancerres/article/11/1/1/472487/Cytochemical-Studies-of-Mammalian-Tissues-The>
- Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY. 2019. Reactive oxygen species induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid Med Cell Longev.* 2019:5080843. <https://doi.org/10.1155/2019/5080843>
- Surai PF. 2015. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. *antioxidants.* Basel. 4(1):204-47. <https://doi.org/10.3390/antiox4010204>
- Tadolini B, Hakim G. 1996. The mechanism of iron (III) stimulation of lipid peroxidation. *Free Radic Res.* 25(3):221-7. <https://doi.org/10.3109/10715769609149047>
- Tangen O, Jonsson J, Orrenius S. 1973. Isolation of rat liver microsomes by gel filtration. *Anal Biochem.* 54:597-603. [https://doi.org/10.1016/0003-2697\(73\)90392-8](https://doi.org/10.1016/0003-2697(73)90392-8)
- Trefts E, Gannon M, Wasserman DH. 2017. The liver. *Curr Biol.* 27(21):R1147-R1151. <https://doi.org/10.1016/j.cub.2017.09.019>
- Van Hung P. 2016. Phenolic compounds of cereals and their Antioxidant capacity. *Crit Rev Food Sci Nutr.* 56(1):25-35. <https://doi.org/10.1080/10408398.2012.708909>
- Wright JR, Rumbaugh RC, Colby HD. 1979. The relationship between chemiluminescence and lipid peroxidation in rat hepatic microsomes. *Arch Biochem Biophys.* 192(2):344-51. [https://doi.org/10.1016/0003-9861\(79\)90102-4](https://doi.org/10.1016/0003-9861(79)90102-4)
- Xu T, Yue K, Zhang C, Tong X, Lin L, Cao Q, Huang S. 2021. Probiotics treatment of leg diseases in broiler chickens: a review. *Probiotics Antimicrob Proteins.* <https://doi.org/10.1007/s12602-021-09869-2>
- Zeb AJ. 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *J Food Biochem.* 44(9):e13394. <https://doi.org/10.1111/jfbc.13394>
- Zhao H, Huang J, Li Y, Lv X, Zhou H, Wang H, Xu Y, Wang C, Wang J, Liu Z. 2020. ROS-scavenging hydrogel to promote healing of bacteria-infected diabetic wounds. *Biomaterials.* 258:120286. <https://doi.org/10.1016/j.biomaterials.2020.120286>
- Zhong S, Li L, Shen X, Li Q, Xu W, Wang X, Tao Y, Yin H. 2019. An update on lipid oxidation and inflammation in cardiovascular diseases. *Free Radic Biol Med.* 144:266-78. <https://doi.org/10.1016/j.freeradbiomed.2019.03.036>

Forma de citación del artículo:

Gavazza MB, Marmunti ME, Palacios A. 2023. Effect of silymarin on oxidative damage in chicken liver cell membranes. *Rev Med Vet Zoot.* 70(1):20-29. <https://doi.org/10.15446/rfmvz.v70n1.102246>