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BENEFICIAL EFFECT OF *BERBERIS BUXIFOLIA* LAM, *ZIZYPHUS MISTOL* GRISEB AND *PROSOPIS ALBA* EXTRACTS ON OXIDATIVE STRESS INDUCED BY CHLORAMPHENICOL

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Abstract The chemiluminescence of luminol, a measure of oxidative stress, increased immediately as a consequence of reactive oxygen species (ROS) stimulated by this antibiotic. The effect of Ch was dose dependent with maximum stimulus at 8 mg/ml (V_{max}); above this concentration the cells began to reduce the production of ROS. The oxidative injury of Ch was counteracted by water extracts of *Berberis buxifolia* lam, *Zizyphus mistol* Griseb and *Prosopis alba*, indigenous fruits from Argentina. The relatively light units (RLU) emitted decreased immediately as a consequence of a protective effect exerted by the extracts of these fruit extracts on blood cells. The three indigenous fruit extracts reduced to a different extent the oxidative injury caused by Ch. *B.buxifolia* lam exhibited the highest antioxidant capacity followed by *Z.mistol* Griseb. Water *e*xtracts of both fruit extracts were the most effective against the oxidative stress, while *P.alba* presented better antioxidant capacity in the ethanolic fraction obtained. Hexane extracts showed low protective action on blood cells, with little reduction of area under curve (AUC) of RLU plotted versus time. Leukocytes remained viable in blood samples incubated for 3h with Ch and water extracts of *B. buxifolia* lam or *Z. mistol* Griseb (97.1% and 92.5% viability by Trypan blue exclusion, respectively); whereas with Ch only the cells were stressed and viability decreased to 30%. The three fruit extracts protected the viability of leukocytes in parallel with the decrease of ROS. Erythrocytes were not lysed in the presence of Ch.

Key words: *Berberis buxifolia* lam, *Zizyphus mistol* Griseb, *Prosopis alba*, antibiotic, oxidative stress, chloramphenicol

Resumen ***Efecto benéfico de extractos de* Berberis buxifolia *Lam,* Zizyphus mistol *Griseb* y Prosopis alba *sobre el estrés oxidativo inducido por cloramfenicol.*** Se estudió el efecto antioxidante de tres extractos de frutas autóctonas, *Berberis buxifolia* lam (michay), *Zizyphus mistol* Griseb (mistol) and *Prosopis alba* (algarrobo). Las células sanguíneas humanas sufrieron estrés oxidativo por acción de cloramfenicol (Ch), con un aumento inmediato de especies reactivas del oxígeno (ERO), que fue determinado por quimioluminiscencia con luminol. La respuesta fue dependiente de la dosis, con un máximo a 8 mg/ml. Los extractos de frutas autóctonas de la Argentina fueron capaces de contrarrestar el estrés generado por el antibiótico. El michay y el mistol resultaron más efectivos en la fase acuosa, y el algarrobo fue más antioxidante en extractos etílicos, mientras que las fracciones obtenidas con hexano no fueron activas. La viabilidad de los leucocitos se mantuvo elevada con Ch en presencia de extractos, entre 92.5 y 97.1%, cayendo hasta un 30% con Ch solo. Tanto los eritrocitos como los leucocitos fueron protegidos del efecto estresante por la capacidad antioxidantes de los extractos de las tres frutas investigadas, lo que podría ser importante a considerar en la dieta de niños, y pacientes en general, sometidos a Ch u otras terapias causantes de estrés oxidativo.

Palabras clave: *Berberis buxifolia* lam, *Zizyphus mistol* Griseb, *Prosopis alba*, antibiótico, estrés oxidativo, cloramfenicol

Berberis buxifolia lam or "michay", a member of *Berberidaceae family,* is a native shrub of Patagonia (Argentina) that provides a traditional tint and has medicinal applications due to its anti-inflammatory, hepatic protector and hypotensor properties. It can be cropped and produced in a magnitude which allows commercialization. Besides, this fruit has great importance as nutrient for of "in vitro" propagation in the rooting stage for 1-2 months. The anti-inflammatory, hepatic protector and hypotensor effects could be associated with protection against oxidative damage. Moreover, it has been described that Berberry fruits have medicinal properties, with anticholinergic and antihistaminic activity, and also in hypertension[1-3].

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Another indigenous plant that seems to have antioxidant components in adequate quantity for medicinal application is *Zizyphus mistol* Griseb or "mistol" found in the central areas of Argentina, used by the native population as hepatic and stomachal medicine. Although the antioxidant capacity of this plant was not investigated, antitumor action was observed[4,5].

Finally, *Prosopis alba* or "algarrobo" has a fruit used as medicine against asthma and ocular affections, since it contains iron and calcium . Furthermore, the sensorial evaluation by a panel consulted in a consumer acceptability test indicated pleasant flavour[6,7]. Even though this native species is known from ancient times, more investigation is necessary to unravel its medicinal properties.

Nowadays, the studies of medicinal plants indicate that certain pathologies, as well as aging, are associated with alterations in the delicate equilibrium between oxidants and antioxidants, which is normally maintained by a whole battery of reducing enzymes, vitamins and others tissue components. The accumulation of reactive oxygen species (ROS), such as anion superoxide ($O_2^{\cdot-}$), hydroxyl ($^{\cdot}OH$) and peroxyl ($ROO^{\cdot}$) radicals is harmful to cell viability, and causes oxidative stress. Although ROS are constantly produced by organisms, they can be incremented by exogenous agents like chloramphenicol (Ch) leading to oxidation of lipids, DNA and proteins. Oxidant agents or pathologic situations can increment ROS in diverse cells, such as leukocytes that represent an important source of ROS during the inflammatory process in infiltrated tissues. In addition, erythrocytes can also generate ROS in detectable amounts[8]. For this reason, whole blood was used to determine the effect of substances able to cause oxidative stress. The use of leukocytes, erythrocytes together with the plasma makes the assay more complete than those performed only with leukocytes, since "in vivo" the oxidant agents act in the presence of all these components. Furthermore, the evaluation of oxidative stress with whole blood can result convenient because small samples of blood are enough to verify the response to oxidative agents in hospitalized patients with severe pathologies, including neonates or oncologic patients.

Moreover, agents like chloramphenicol (Ch) have limited use as consequence of its toxicity. This antibiotic is hematoxic to humans and can lead to reversible anemia, aplastic anemia, and leukemia, effects that can be related to the oxidative alterations it evokes. Ch causes damage of blood forming organs with a progressive decline in the number of pluripotential stem cells and granulocytic progenitor cells, causing residual marrow damage; although it has been suggested that this antibiotic resulted hemotoxic only in susceptible individuals[9].

The preceding observations stimulated the investigation of the detoxifying effect of natural substances on oxidized metabolites originated by Ch; in particular, the aim of this work was to study by chemiluminiscence (CL) the effects of *B.buxifolia*, *Z.mistol* and *P.alba* on ROS produced by Ch in whole human blood.

Material and Methods

Heparinized blood of ten healthy volunteers from our laboratory was obtained according to the protocol of our University Human Ethics Committee. Routine biochemical analyses were performed on the blood samples, which included the full blood count, peripheral blood smears, erythrocyte sedimentation rate, glucose, cholesterol and triglyceride levels. The capability of Ch to generate ROS was examined in venous whole blood using 3-aminophthalhydrazide 5-amino-2.3-dihydro-1,4-phthalazinedione (luminol) sensitized CL. The following reagents mixture (RM) was prepared: 5 ml of 0.067 % luminol in Hanks'balanced salt solution (HBSS), 0.2 ml of 5% glucose, 1 ml of Ringer lactate solution and 3.6 ml distilled water. The production of ROS in 10ìl of whole blood was measured by adding 600 ìl of RM, 0.1 ml RM with our without 1 mg/ml fruit extracts, and 10 ìl of HBSS with or without 0.8 mg/ml Ch in.

Normal or spontaneous production of ROS was determined in samples without antibiotic after 3 h of extraction, compared to samples treated for 3 h with Ch 0; .1 ml of 1 mM phorbol myristate acetate was employed as positive control of ROS generation. Light emission results were expressed as relative light unities (RLU/second) at different times with subtraction of the background. The maximum value (V_{max}) of RLU/second and the area under the curve at 60 min (AUC), in the curves of RLU/second versus time, were calculated by computer origin program. AUC was expressed in relative unities of area (RUA). The viability of leukocytes was estimated by trypan blue dye exclusion.

Fresh fruits of *B. buxifolia, Z. mistol and P. alba* were selected, 20 g of each were washed with distilled water and then crushed in a mortar. Four samples of 4 g of each fruit were separated and treated with 50 ml of the following solvents: 80% ethanol, acetone, hexane or water. The extracts were sifted by Whatmann filter and then they were concentrated by vacuum in a rotator evaporator at 57 °C. The extracts were maintained in dark recipients at -20 °C; they were diluted to 1 mg/ml in RM to perform the CL assay.

Statistical comparisons were made using ANOVA analysis. t-test analyse was performed in independent treatments. Instat Statistical Software was applied in ANOVA for two factors, time and extract type, in paired samples. Triplicate assays were performed to estimate means and standard deviation (SD). Differences were considered significant when $p< 0.05$.

Results

Blood samples that exhibited normal full blood count, peripheral smears, erythrocyte sedimentation rate, glucose, cholesterol and triglycerides, were applied to this study. Ch increased ROS in all samples (n = 10). In absence of antibiotic the spontaneous production of ROS did not exceed 0.035 RLU, while with Ch there was a significant increase ($p < 0.05$) and ROS remained elevated for the 100 min assayed by CL. This pattern was similar to that evoked by phorbol mirystate acetate (Fig. 1). The V_{max} obtained with the antibiotic was 17 fold the V_{max} obtained in controls without Ch.

The incubation of blood cells with *B. buxifolia* lam extracts exerted an antioxidant effect (Fig. 2). The extracts of this berberry diminished significantly the V_{max} of ROS in the following order: water = alcohol > acetone > hexane. The samples obtained from *Z. mistol* Griseb exhibited reductions of ROS in all the fractions, being water the most effective solvent to extract the antioxidant components of this fruit (Fig. 3) while hexane was the less effective. The extracts from *P. alba* were able to reduce in different magnitude the oxidative stress caused by Ch in blood cells. The alcoholic extraction exerted the strongest inhibition (Fig. 4); while the water-extracted fraction decreased ROS production more than acetone and hexane extracts. V_{max} did not differ significantly in assays with acetone and hexane.

The comparison of AUC in the curve RLU vs. time indicated that *B. buxifolia* lam was able to decrease the

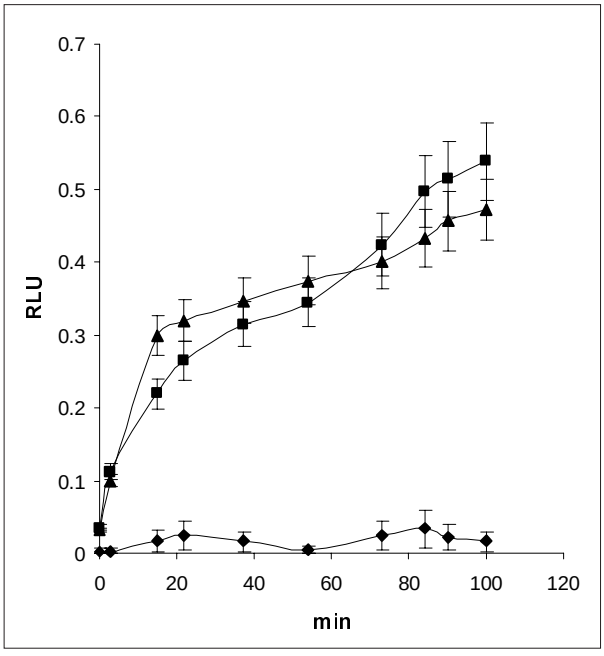


Fig. 1.– Production of ROS by blood cells spontaneously (♦) expressed in relative light unities (RLU); with the stimulant of ROS phorbol mirystate acetate (▲) and with Ch (■).

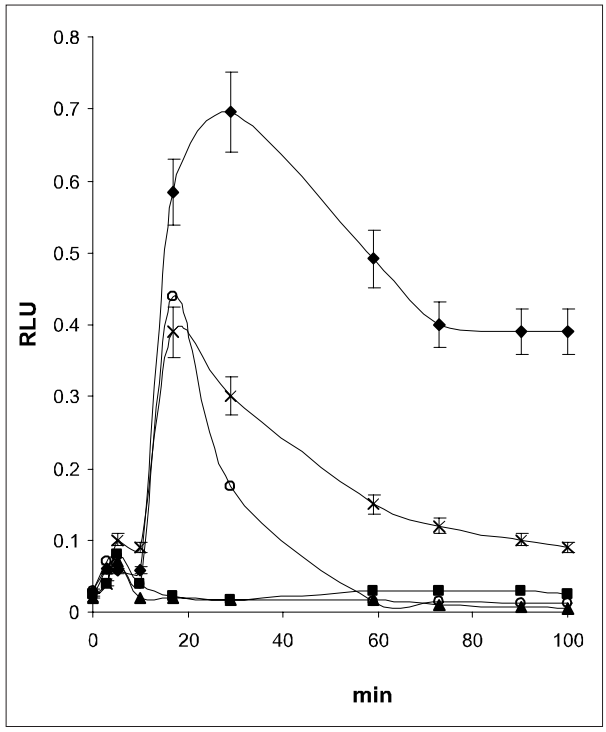


Fig. 2.– Stimuli of ROS by Ch expressed in relative light unities (RLU) (♦) and antioxidant effect of *B.buxifolia* extracted with water (▲), alcohol (■), acetone (X) and hexane (○). ANOVA: effect of extracts and time, significant differences ($p<0.05$) between the treatment with Ch and each solvent treatment.

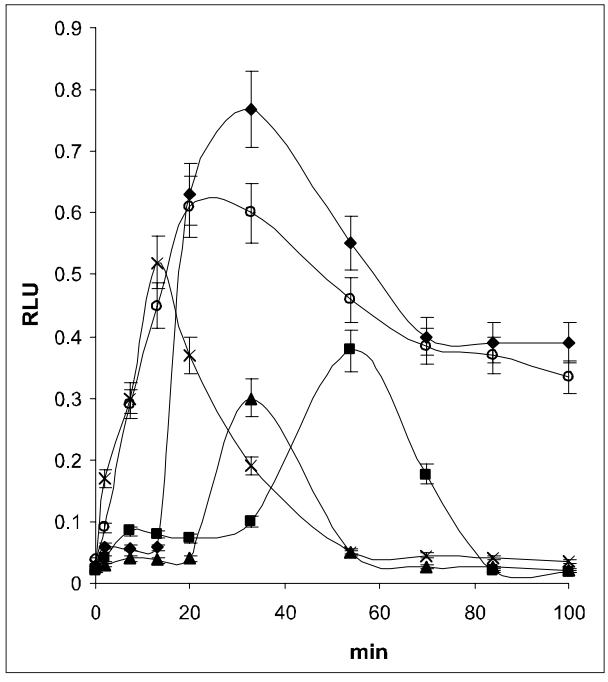


Fig. 3.– Stimuli of ROS by Ch expressed in relative light unities (RLU) (♦) and antioxidant effect of *Z.mistol* extracted with water (▲), alcohol (■), acetone (X) and hexane (○). ANOVA: effect of extracts and time, significant differences ($p<0.05$) between the treatment with Ch and treatments with water, alcohol or hexane.

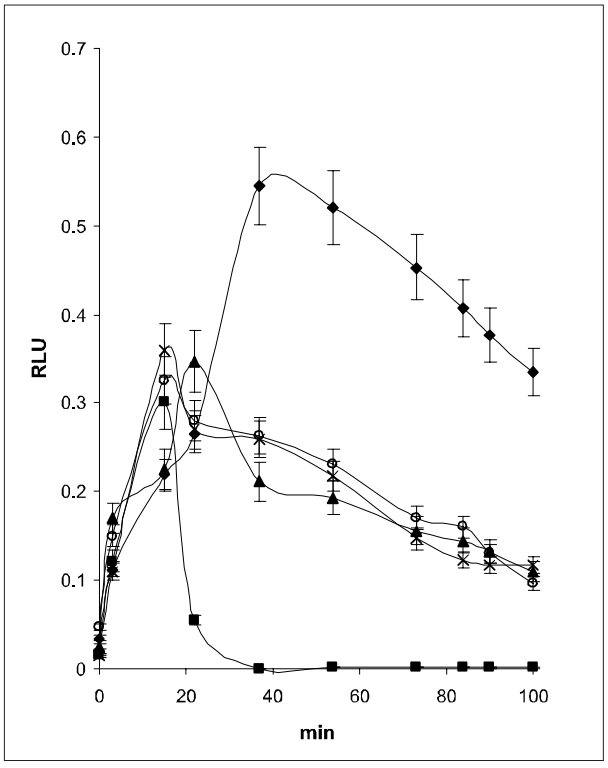
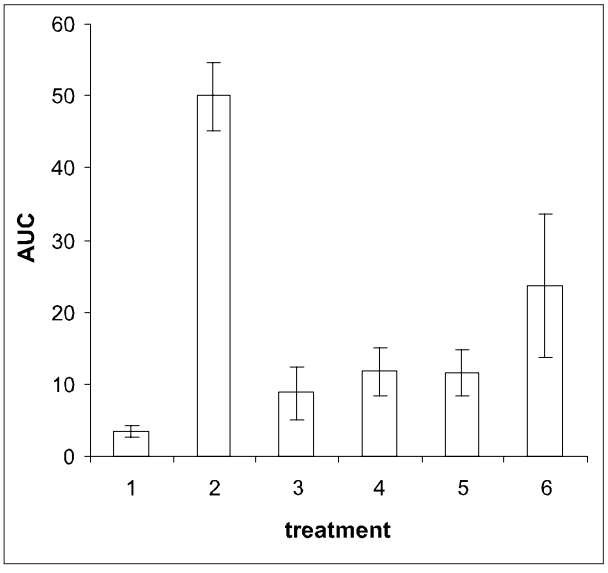
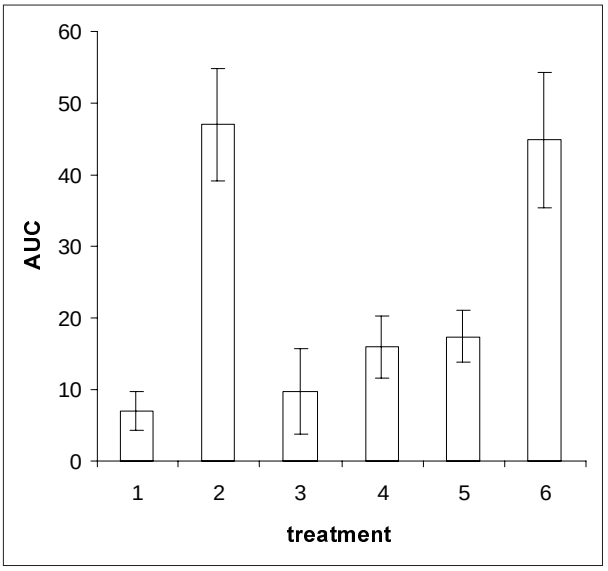


Fig. 4.– Stimuli of ROS by Ch expressed in relative light unities (RLU) (♦) and antioxidant effect of *P.alba* lam extracted with water (▲), alcohol (■), acetone (X) and hexane (○). ANOVA: effect of extracts and time, significant differences ($p<0.05$) between the treatment with Ch and each solvent treatment.



*p < 0.05 with respect to control (1)
**p < 0.05 with respect to treatment with Ch (2)

Fig. 5.– Area under de curve (AUC) of RLU vs time in blood cells. (1) control , (2) treated with Ch; (3) water extract of *B.buxifolia* lam, (4) alcoholic extract of *B.buxifolia* lam, (5) acetone extract of *B.buxifolia* lam or (6) hexane extract of *B.buxifolia* lam.



*p < 0.05 with respect to control (1)
**p < 0.05 with respect to treatment with Ch (2)

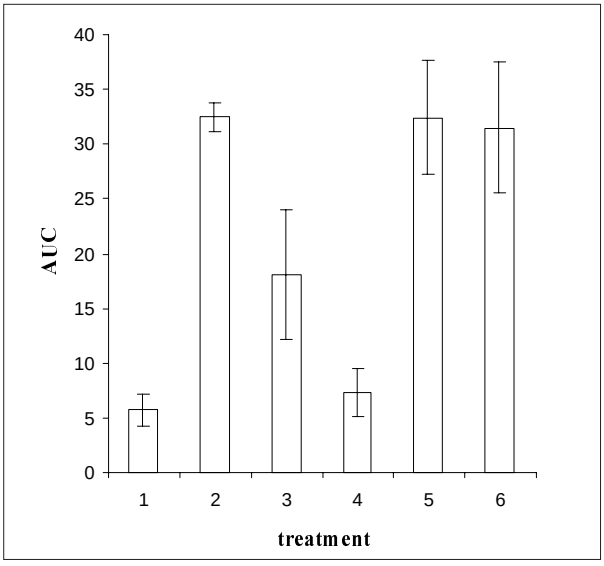
Fig. 6.– Area under de curve (AUC) of RLU vs time in: (1) control blood cells, (2) treated with Ch, (3) *Z.mistol* extracted with water, (4) *Z.mistol* extracted with alcohol, (5) *Z.mistol* extracted with acetone or (6) *Z.mistol* extracted with hexane.

stress with all the extracts but principally with the water, alcohol and acetone fractions (Fig. 5) ($p < 0.05$) and *Z. mistol* Griseb decreased the AUC mainly with water (Fig. 6). *P. alba* counteracted the oxidative stress and presented the following decreasing order of antioxidant capacity in the extracts: alcohol > water > acetone > hexane, being significant ($p < 0.05$) with alcoholic and water fractions (Fig. 7).

The antioxidant capacity was investigated in relation with the viability of leukocytes, the counts of viable cells showed correlation with the inhibition of ROS. Ch decreased to 30% the number of live leukocytes in 3 h, while the water extracts of *B. buxifolia* lam*, Z. mistol* Griseb and *P. alba* plus Ch presented 97.1%, 92.5% and 82.4% of viable cells after 3 hours of contact; simultaneously, Ch-induced ROS generation decreased to 80.4%, 80% and 44.6% in presence of these extracts respectively. This indicated that inhibition of ROS generation was related to improved cell viability. The number of cells controlled by counts in Neubauer chamber indicated that the extracts obtained with different solvents and suspended in HBSS did not affect cell viability (data not shown).

Discussion

The injury provoked by diverse substances frequently takes place through the oxidation of macromolecules



*p < 0.05 with respect to control (1)
**p < 0.05 with respect to treatment with Ch (2)

Fig. 7.– Area under de curve (AUC) of RLU vs time in (1) control or spontaneous production of ROS by blood cells, (2) incubated with Ch, (3) water extract of *P.alba*, (4) alcoholic extract of *P. alba* l, (5) acetone extract of *P. alba* or (6) hexane extract of *P. alba*.

necessary for a normal function of different organs. The oxidative stress generated by new drugs and certain toxic drugs can be studied by their induction of ROS. Previous investigations proved that oxidative stress is one important factor that contributes to unfavourable toxic alterations in human, with mitochondrial changes and modifications in redox processes of the respiratory chain[10, 11].

Ch was included in this study in relation to its negative side effects, among them blood dyscrasias, allergic skin and eye reactions, or the irreversible aplastic anemia the most severe damage of this chemotherapeutic agent. Fortunately, most of the consequences suffered by normal individuals are reversible but represent serious problems to be controlled, such as sideroblastic anemia, in which red blood cells experiment hemolysis by metabolic disruption associated with alterations of mitochondrial function. These oxidative changes imply modifications of haemoglobin synthesis and hepatic oxidation[12, 13].

The susceptibility to stress caused by oxidant substances varies among humans; there are numerous pathologic situations in which effective antioxidant defence is loss, with diverse deleterious effects that increase with age. Previously, it was observed in our laboratory that fruit extracts can attenuate the noxious effects of oxidative stress[14], and in the present work we observed that blood samples of healthy individuals can be protected from the induced toxicity by three native fruits of our country. The evaluation of oxidative stress with whole blood constitutes a rapid and sensitive assay to detect generation of ROS, an important promoter of oxidation in diverse macromolecules. Certainly, the application of fresh blood cells, without the necessity of separation of plasma, presents several advantages such as the elimination of cell manipulation, rapid determination and the inclusion of all the blood cells that can be exposed to oxidative injury during treatment with Ch. The CL assay performed to determine the effects of Ch could be adequate to detect susceptible individuals.

The results obtained indicate that all the indigenous fruits investigated were effective against oxidative stress. The three plants studied were effective against the oxidative stress provoked by Ch; therefore, the co-administration of Ch with these fruits or their pharmacologic derivates could represent a dietary regimen or a medicinal treatment that may contribute to the detoxification of oxidized metabolites. The present investigation gives sustentation to the medicinal use in hepatic and gastrointestinal alterations, hypertension, asthma and inflammatory process, events associated to oxidative stress. Furthermore, the beneficial antioxidant capacity observed might counteract the toxicity of other drugs with clinical implications.

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Conflict of interests: None.

References

1. Arena ME, Martinez Pastur G, Vater G. In vitro propagation of *Berberis buxifolia* Lam. *Biocell* 2000; 24: 73-80.
2. Fatehi-Hassanabad Z, Jafarzadeh M, Tarhini A, Fatehi M. The antihypertensive and vasodilator effects of aqueous extract from *Berberis vulgaris* fruit on hypertensive rats. *Phytother Res* 2005; 19: 222-5.
3. Hwang JM, Wang CJ, Chou FP, et al. Inhibitory effect of berberine on tert-butyl hydroperoxide-induced oxidative damage in rat liver. *Arch Toxicol* 2002; 76: 664-70.
4. Muñoz SE, Silva RA, Lamarque A, Guzmán CA, Eynard AR. Protective capability of dietary *Zizyphus mistol* seed oil, rich in 18:3, n-3, on the development of two murine mammary gland adenocarcinomas with high or low metastatic potential. *Prost Leuk Essen Fat Ac* 1995, 53: 135-8.
5. Munoz SE, Piegari M, Guzman CA, Eynard AR. Differential effects of dietary Oenothera, Zizyphus mistol, and corn oils, and essential fatty acid deficiency on the progression of a murine mammary gland adenocarcinoma - a powerful anticarcinogen from animal fat sources *Nutrition* 1999; 15: 208-12.
6. Bernardi C, Drago S, Sabbag N, Sanchez H, Freyre M. Formulation and sensory evaluation of *Prosopis alba*

(Algarrobo) pulp cookies with increased iron and calcium dialyzabilities *Plant Foods Hum Nut* 2006; 61: 39-44.
7. Felker P, Maurico Ewens M, Ochoa H. Environmental influences on grafting success of *Prosopis ruscifolia* (vinal) onto *Prosopis alba* (algarrobo blanco) *J Ar Environ* 2000; 46: 433-9.
8. Kondo M, Itoh S, Kusaka T, Imai T, Isobe K, Onishi S. The ability of neonatal and maternal erythrocytes to produce reactive oxygen species in response to oxidative stress. *Early Hum Develop* 2002; 66: 81-8.
9. Chen J. Animal models for acquired bone marrow failure syndromes. *Clin Med Res* 2005; 3: 102-8.
10. Matsuhashi T, Liu X, Nishizawa Y, Usukura J, Wozniak M, Wakabayashi T. Mechanism of the formation of mega mitochondria in the mouse liver induced by chloramphenicol. *Toxicol Let* 1996; 86: 47-54.
11. Ambekar CS, Lee JS, Cheung BM, Chan LC, Liang R, Kumana CR. Chloramphenicol succinate, a competitive substrate and inhibitor of succinate dehydrogenase: possible reason for its toxicity. *Toxico. In Vitro* 2004; 18: 441-7.
12. Farombi EO, Adaramoye OA, Emerole GO. Influence of chloramphenicol on rat hepatic microsomal components and biomarkers of oxidative stress: protective role of antioxidants. *Pharmacol Toxicol* 2002; 9: 129-34.
13. Li CH, Tzeng SL, Cheng YW, Kang JJ. Chloramphenicol-induced mitochondrial stress increases p21 expression and prevents cell apoptosis through a p21-dependent pathway. *J Biol Chem* 2005; 280: 26193-9.
14. Eraso AJ, Albesa I. *Eriobotrya japonica* counteracts reactive oxygen species and nitric oxide stimulated by Chloramphenicol. *Am J Chin Med* 2007; 5: 875-85.

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THE SCIENTIFIC MIND*****

A mind nimble and versatile enough to match the resemblances of things which is the chief point, and at some time steady enough to fix and discern their subtle differences; endowed by nature with the desire to seek, patience to doubt, fondness to meditate, slowness to assert, readiness to reconsider, carefulness to set in order, and neither affecting what is new nor admiring what is old, and hating every kind of impostor.

Francis Bacon

LA MENTE CIENTÍFICA

Una mente ágil y versátil como para captar la analogía de las cosas, que es el punto más importante, y al mismo tiempo suficientemente estable como para precisar y distinguir sus sutiles diferencias; dotada por la naturaleza con el deseo de investigar, paciencia para dudar, inclinación para meditar, prudencia para afirmar, disposición para reconsiderar, exactitud para ordenar, y que ni se deja seducir por lo nuevo ni fascinar por lo viejo, y aborrece toda clase de impostura.

Francis Bacon

* En los espacios que quedan libres aparecerán aforismos, frases o conceptos históricamente interesantes y muchas veces de validez actual.