ANTIOXIDANT EFFECT OF LIGMED-A ON HUMANE ERITHROCYTES *IN VITRO*

García L[1], Abajo C[2], del Campo J[2], Mitjans M[2], Marrero E[1] and Vinardell M P[2]

[1]Chemical-Pharmacological Group, National Center of Animal and Plant Health, San José de las Lajas, La Habana (Cuba), e-mail: lgarcia@censa.edu.cu
[2]Dept. Physiology, Faculty of de Pharmacy, University of Barcelona (Spain)

Summary

Ligmed-A is prepared from sugarcane and is commercially available in as an antidiarrheal drug. This compound, which is composed of about 90% lignin, an insoluble polyphenolic constituent of plants and a component of dietary fiber, has been manufactured in Cuba since the 1990s. It shows a similar eficacy to other drugs used to treat diarrhea and is effective 2 or 4 days after administration. We investigated the antioxidant activity of an aqueous solution of Ligmed-A by studying its protective effect on the hemolysis induced by an initiator of radicals such as 2, 2'-Azobis(2-amidinopropane) dihydrochloride (AAPH). The antioxidant capacity was expressed as the IC50 (concentration inducing 50% of inhibition of hemolysis induced by AAPH). Ligmed-A was very active as antioxidant with a IC50 of 106,63 μg/ml. This property of Ligmed-A increase its benefits on animal health.

Key words: antioxidant, lignin, sugar cane

Introduction

Lignin is a natural phenolic polymer. It is one of the most abundant natural polymers, composing up to one-third of the material found in plant cell walls. Lignin serves to affect water transport, protect trees against chemical and biological attack, and provide structural integrity. Lignins are derived from abundant and renewable resources such as trees, plants, and agricultural crops. They are present in a large variety of foods, and are particularly abundant in cereal brands. These compounds are non-toxic and extremely versatile in performance, qualities that have made them increasingly important in many industrial applications and lignin uses have expanded into hundreds of applications (1-3).

Ligmed-A is prepared from sugarcane and is commercially available in as an antidiarrheal drug. This compound, which is composed of about 90% lignin, an insoluble polyphenolic constituent of plants and a component of dietary fiber, has been manufactured in Cuba since the 1990s. It shows a similar eficacy to other drugs used to treat diarrhea and is effective 2 or 4 days after administration (4). This product has been demonstrated to not affect intestinal enzyme activity and intestinal morphometry (5).

Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are lignins and lignans that are widely distributed within the plant kingdom (6).

Lignins have been demonstrated as antioxidants, acting as free radical scavengers. Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade and it is of particular interest in relation to human health. In addition to traditional application strategies, antioxidant is a potential application of lignin. Lignin is a free radical scavenger, and stabilizes the reactions induced by oxygen and its radical species (7,8).

Material and methods

Chemicals: The following reagents were obtained from Sigma Chemical Co. (St Louis, MO): sodium chloride, sodium phosphate dibasic, chlorpromazine, 2, 2'-Azobis(2-amidinopropane) dihydrochloride (AAPH).

Antioxidant effect: Blood samples were obtained from healthy donors by venipuncture (Blood Bank, Hospital Clinic, Barcelona, Spain). Citrated blood was centrifuged at 1000 *g* for 10 min and washed three times with phosphate-buffered saline (PBS). Supernatant and buffy coat were carefully removed by aspiration after each wash. Washed erythrocytes were finally suspended in buffered saline.

We determined the hemolysis of erythrocytes mediated by AAPH (a peroxyl radical initiator) using a modification of a method described elsewhere (9). 25 µl aliquots of erythrocyte suspension were incubated in the presence of 100 mM AAPH to induce hemolysis at 37ºC for 2.5 hours. Different concentrations of Ligmed-A ranging from 20 to 250 µg/ml were added to assay antihemolytic effect. The IC_{50} (inhibitory concentration 50) of the hemolysis induced by AAPH was determined. Hemolysis was monitored by spectrophotometry at 540 nm.

Results and Discussion

In this study, we tested the efficacy of an aqueous solution of Ligmed-A as an inhibitor of AAPH-induced erythrocyte hemolysis. AAPH, a water-soluble free radical generator, was used to stimulate the *in vivo* conditions of oxidative stress and peroxyl radicals were generated by thermal decomposition of an azo compound in oxygen. The advantages of this method are that the AAPH decomposes thermally to generate radicals without biotransformation or enzymes and the rate of radical generation is easily controlled by adjusting the concentration of initiator.

The antioxidant effect of Ligmed-A on the hemolysis induced by AAPH is shown in figure 1, which represents the percentage of hemolysis inhibition at several concentrations. The inhibitory effect is concentration dependant for all the lignins

studied, being the greatest inhibitory effect at the highest concentration studied. The IC_{50} was 106.63 μg/ml.

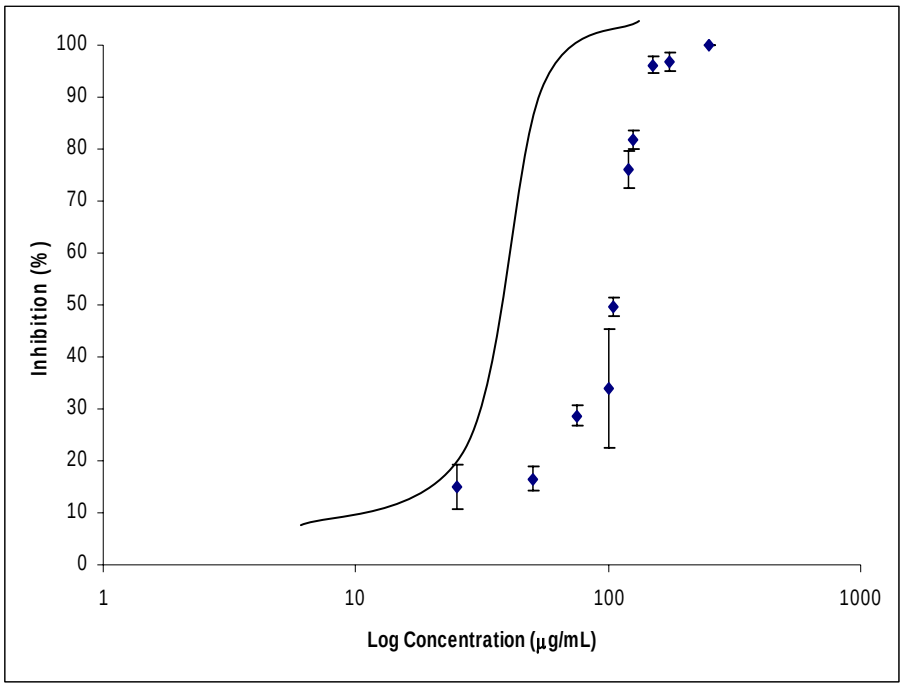

Figure 1. Inhibition of AAPH induced hemolysis by different concentrations of Ligmed-A. Mean values ± SEM of at least 4 independent experiments

Due to the molecular complexity of lignin, it has been difficult to assign these biological activities to specific structural components, compared to the activities of chemically defined tannins and flavonoids (10,11).

As a major component in dietary fiber, lignin can inhibit the activity of enzymes related to the generation of superoxide anion radicals and obstruct the growth and viability of cancer cells (12). In a recent paper it has been demonstrated that, in general, the lignins prepared at elevated temperature, longer reaction time, increased catalyst, and diluted ethanol showed high antioxidant activity (13).

Further studies are necessary to be conducted to understand the relationship between chemical structure of the lignins present in Ligmed-A and its antioxidant activities.

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