

**EFFECT OF AN EXTRACT OF *Solanum melongene* AND *Plantago major*  
ON MEMBRANE OF HUMAN ERYTHROCYTE**

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**Summary**

**Introduction:** The use of erythrocyte is an optimal biological model for the study of the activity on biological membranes. In our work we evaluated the photohemolytic activity of two medicinal plants: *Solanum melongene* and *Plantago major* by means of an in vitro test in red cells. **Materials and Methods:** Red cells from human blood containing heparine of healthy donors and the hydroalcoholics extracts of the studied plants were elaborated in the Laboratory of Medicine Plants of Villa Clara. The evaluation of photohemolysis in order to determine the percentage of hemolysis in every case was carried out according to protocol 81 of INVITTOX. Concentrations of 8, 9, 10, 11, 12 and 13 mg/mL in the case of *Solanum melongene* and 0,8, 1.0, 1.2, 1.4, 1.8 mg/mL for the *Plantago major* were evaluated. **Results:** In the case of *Solanum melongene* 7, 5 mg/mL as fifty hemolytic concentration (HC50) for the irradiated samples (IS) and 0,1 mg/mL for the unirradiated samples (UIS) was obtained. For the *Plantago major* it was estimated that HC50 for both the irradiated and the unirradiated samples is higher than 1.4 m/ml. A HC50 UIS/HC50 IS relation lower than 3 units for both cases was obtained. **Conclusions:** Both evaluated extracts are not photohemolytic. The extracts of *Solanum melangene* and *Plantago major* proved to have a higher HC50 compared to chlorpromazine, the positive control.

**Key words:** photohemolysis, erythrocytes, *Solanum melangene*, *Plantago major*.

## Introduction

The erythrocyte constitutes an ideal model for the study of activity on biological membranes. These cells have a relatively structural and functional simplicity as well as a lipid layer like the rest of the organism cells. Which allows us to infer that a damage in the membrane of the erythrocyte would correlate with a higher probability that other more complex cells with a higher quantity of organelles susceptible to the damage of their membranous system could respond in the same way or with greater intensity than the erythrocytes (1,2).

The damage to the cell membranes producing the disruption of the composition of the lipid layer up to the cell lysis can contribute to the development of many degenerative diseases such as neoplasias and cardiovascular diseases (3-8).

In the present work, we evaluated the photohemolytic activity of two medicinal plants: *Plantago major* and *Solanum melongena*, by means of an in vitro test in red cells. In photohemolysis test (also known as Photo RBC), we observed the hemolytic degree that a product in the presence of UV light can produce and so we evaluated the harmful or beneficial effects of light upon the action that the product under study exerts upon the cell membranes. The determination of the hemolytic concentration 50 (HC50) leads to a classification criterion of the phototoxic potential of the evaluated product (9-11).

The *Plantago major*, known as “llantén” in Cuba, grows in humid lands, meadows and boundaries. Among its active components there are acid and neutral mucilages, flavonoids, phenolic acids, hydroxycoumarins, lannins and others (12). Several reports are related to pharmaco-toxicological properties of this plant. Toxicological studies have revealed the slightly irritant behavior of an absorbent unguent in rabbit’s model with a single dose. This result does not invalidate the use of this formulation in the therapeutic practice (13). Pharmacological studies of this plant have shown hypolipemia (14), anti-inflammatory (15) and antifungal activity (16).

*Solanum melongene* which is known as eggplant in our country is a vegetable that is cultivated in the whole island. It has a low protein hydro carbonate, mineral and vitamin content, compared to other fruits and vegetables (17). Nevertheless, it contains a valuable quantity of antioxidants that justifies its medicinal properties (17, 18).

## Methods

Hydroalcoholic extracts of the plants under study were elaborated in the Laboratory of Medicinal Plants in Villa Clara. Then they were evaporated in a heating plate until

the alcoholic content was eliminated and the soft extract was obtained. Starting from the total solid of the soft extract the concentrations under study were evaluated.

The red cells blood from heparin human blood of healthy donors were isolated by means of centrifugation to 2,500 mm during 10 minutes and washed three times with solution of isotonic phosphate saline buffer (PBS) until traces of plasma and white cells were eliminated reducing spontaneous lysis in this way.

In order to determine the percentage of hemolysis in each case an evaluation of photohemolysis according to protocol 81 of INVITTOX was carried out.

In the case of *Solanum melongene* concentrations of 8, 9, 10, 11, 12 and 13 mg/mL were evaluated. For the study of the *Plantago major* the evaluated concentrations were 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mg/mL. It was taken into account that in both cases the values of HC50 was in the rank of the test concentrations.

Two series of identical plates were prepared. One of them was placed in the darkness (unirradiated samples UIS) and the other exposed to ultraviolet light during 60 minutes (irradiated samples IS). After this time the plate exposed to the light is put in the dark for 30 minutes. Later the content of the plates was transferred to tubes and centrifuged during 5 minutes (3500 rpm).

In order to evaluate the endpoint the amount of hemoglobin released in the supernatant in 540 nm vs. a blank of erythrocytes in PBS was determined. Total hemolysis incubating the erythrocytes in distilled water was obtained.

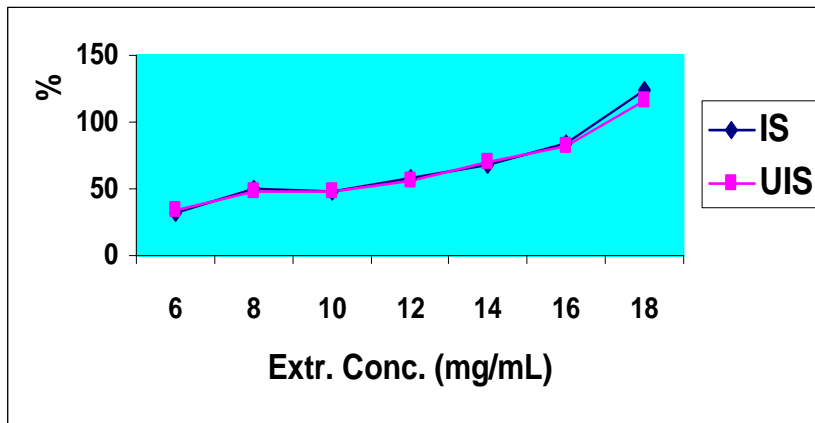
Finally the hemolysis percentage of each sample was calculated taking the total hydrolysis as a reference and then determining the HC50 of the evaluated product using the program SPSS version 11.5.1 of 2002.

The experimental procedures were made as fast as possible to minimize the kinetic effects due to the fact that hemolysis and the formation of methemoglobin are not stopped at the end of the irradiation.

## Results

In case of the *Solanum melongene* 7.5 mg/mL as HC50 for the irradiated samples and 8.1 mg/mL for the unirradiated was obtained by using a linear adjustment. In the *Plantago major* study a non linear adjustment was applied and it was estimated that in both irradiated and unirradiated samples the HC50 was higher than 1.4 mg/mL. In both cases the HC50 was very high in comparison with chlorpromazine, the positive control, which values of HC50 was below 0,3 mg/mL (Figure 1).

A)



B)

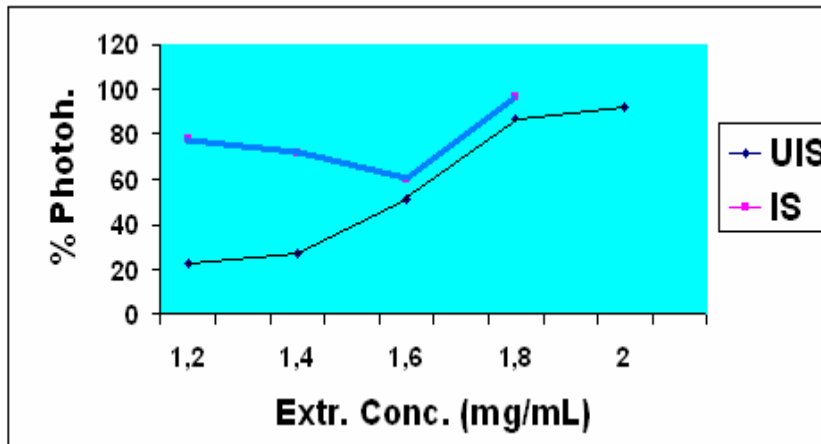


Figure 1. Results of Photohemolysis test. A) Hydroalcoholic extract of *Solanum melongene*. B) Hydroalcoholic extract of *Plantago major*.

Legend: % Photoh (Photohemolysis) vs Extr.Conc. (concentrations of extract) UIS (unirradiated samples), IS (irradiated samples).

### Discussion

Both plants demonstrated hemolytic activity in erythrocytes membranes humans. Nevertheless, the results indicate that any case can be classified as photohemolytic agent since the relation HC50 UIS/HC50 IS does not reach 3 units, which is the criterion followed to recognize a photosensitized agent (1,19).

*Solanum melongene* showed a very low hemolytic effect. This can be justified by the antioxidant properties that it has in relation to *Plantago major*, which could be a protective factor against the hemolysis caused by the light UV.

The high value of the HC50 obtained in the case of *Solanum melongene* is very encouraging about its therapeutic potentials, which justifies later studies in multiple biological models in which antioxidant properties, capillary fragility, disrupt in the permeability of membranes, among others biological activities could be evaluated.

### Conclusions

The extracts of *Solanum melongene* and *Plantago major* did not show photohemolysis activity because the relation of HC50 UIS/HC50 IS did not reach 3 units.

The high photoprotective potential of *Solanum melongene* can be estimated by the high HC50 that was obtained.

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