PHARMACOTOXICOLOGICAL STUDIES OF *Gracylaria cylindrica* ALGAE

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Summary

Introduction: marine products, specialy algae have a high demand for the cosmetic and pharmaceutical industries. The *Gracilarya cylindrica* alga has big potentialities due to its properties and exploitation possibilities. Materials and Methods: Experimental Paharmacology studies concerning photoprotective effect and the influence of the conditions of extractions in such effect were carried out, Toxicological studies were Acute Toxicity Class (ATC), an alternative accepted method and Dermic and Oftalmological Irritation. For the photoprotective effect it was obtained the absorbance spectrum of each one of the extracts prepared (12) from 600 to 200 ηm using a spectrophotometer. The UV area maxims evidence was analyzed. The ATC method used 2 groups of 3 Wistar rats, sex ratio 1:1, whiche were administered with single 2000 and 5000 mg/kg BW doses. They were observed during the next 14 days, body weight was measured on days 1, 7 and 14 and anatomopathological studies were also done. Dermal and Eye irritations tests were applied to dry and to the aequous extracts using New Zeland rabbits, assigning the values according to the accepted Draize scale. Results: The extract does not has absorption maxims in the UV region and the extractions conditions studied had no influence in the absorption characteristics. ATC study did not show toxicity symptoms neither signs, only an animal died at the dose of 5000 mg/kg BW. Dermal and eye irritations studies di not show any lession related to the products applied. Conclusions: the studied extract does not show photoprotective effect, toxicologicaly speaking the product is classified as NON TOXIC, and as NON PRIMARY DERMAL IRRITANT nor EYE IRRITANT for both types of extracts.

Keywords: *Gracilaria cylindrica*, photoprotection, acute toxicity, dermal irritancy, ocular irritancy.

Introduction

The products of marine origin, specially the seaweed, are used widely, as much at international level as in our country, in the cosmetics industry, where they have an ample demand. At the moment they have begun to be introduced in the medical and pharmaceutical industries, due to the great pharmacological potential that they have (1-3). The photoprotective effect of diverse marine organisms has been evaluated, because this protection includes substances that absorb ultraviolet (UV) radiations (1,4). Cuba, being an island surrounded by the sea, has a privileged position for the operation of this resource. The objectives of the present work are: to evaluate the chemical photoprotective effect of the watery extract of *Gracilaria cylindrica* algae and to determine the influence of the conditions of extraction in the photoprotective effect, to evaluate the toxic potential of the dry extract after a single dose exposure by oral route and to determine the dermal and ocular irritancy of dry and watery extracts.

Materials

Chemical Photoprotective Effect: (2): the extracts to be evaluated were obtained in an installation on laboratory scale, following this procedure: distilled water was added and it was warmed up to the working temperature. The mass of the algae was added and the mixture, with the use of a mechanical agitator during the time of operation, was shaken. The mixture was then filtered and centrifuged to 1 000 rpm during 5 minutes and the supernantant constituted the extract to evaluate, which was packaged in a suitable container, conserving it in refrigeration. Algae mass to use, temperature and extraction time were fixed according to the operations conditions (Temperature in °C, ratio algae/water in g/ml, time in minutes, algae particle size in millimeters). There were obtained 12 extracts which were analyzed in 3 series of 4 extracts each one, variying only one of the three factors mentioned above.

The samples for spectrophotometry had to be diluted. In order to determine the optimal dilution solutions of 0,01 g and 0,1 g of extract were prepared in 10 mililiter of distilled water. For the evaluation of the activity as chemical photoprotective (capacity to absorb the radiations) of the watery extracts, the absorbance spectrum of each one of them was obtained from 600 to 200 ηm, using a sweeping spectrophotometer. The appearance of maximums of absorption was analyzed, fundamentally in UV zone, to choose the optimal conditions of extraction and to classify the extracts according to the zone where they offer protection.

Acute Toxic Classification (ATC) (5): Wistar rats, coming from the National Center for Laboratory Animal Production, weighing between 190 and 230 grams, of both sexes were used and maintained in standard experimental environmental conditions, 6 animals distributed by sex in 2 groups of 3 animals were used.

The test substance was prepared suspending the dry, sifted and molinated algae, (particle size of 0,1 mm) in a watery vehicle with carboximetilcelulosa to 2%. 4 hours from retiring the food, the test substance by oral route with an intragastric cannula was administered in a unique dose of 2 000 mg/Kg BW using an administration volume of 15 ml/Kg, beginning by the females. On the following day males were administered following the same procedure. The animals were systematicaly observed during the first 24 hours postadministration and daily until experiment termination (14 days). Later all animals were sacrificed under anesthesia, to carry out anatomopathological examinations of organs and weaves. Body Weight (BW) was controlled at the beginning, seventh day and at the end of the experiment. The final classification of the product took place according to the criteria established by the OECD. Later a level of 5000 mg/Kg BW was administered following the same above mentioned procedure by means of a grater concentration solution in order to keep the 15 ml/kg administration volume, divided in two administrations, with an interval of 4 hours between them.

Dermal and Ocular Irritancy: for these studies two extracts were evaluated: dry (raw material) and watery.
Dermal irritancy (6-8):
The study was carried out in three healthy, adults, males rabbits for each extract, New Zealand line, coming from the National Center for Laboratory Animal Production, with a body weight between 2 and 3 kg. Approximately 24 hours before the application of the substance, the skin of the dorsal region in both sides of the dorsal thorn was carefully shaved and only intact skin ones were chosen for the experiment.

0,5 mililiter of the watery extract of the alga were directly applied on the skin keeping it in contact with it during 4 hours by means of gauze dressings of 6 cm^2, fixed with nonirritating sticking plaster (two applications by animal), later the surface of the skin was washed with warmed water to eliminate the residual substance and the application areas were observed at 1, 24, 48 and 72 hours later to the retirement of the dressings with the objective to determine the degree of erithem and oedema, assigning the corresponding score according to the scale proposed by Draize, taking the neighboring zones as control to the application sites. With the collected data the Index of Primary Irritancy, for the final classification of products, was calculated.

Ocular irritancy (9-11):
Three healthy, adults, males New Zealand line rabbits for each extract were used, New Zealand line, coming also from the National Center for Laboratory Animal Production, with a body weight between 2 and 3 kg. 24 hours before the beginning of the experiment both eyes of each animal were examined and those with no damage were selected for the experiment. It was instilled 0,1 milliliter of the extract in the conjunctival coat of the right eye of each animal, maintaining the eyelids united during 5 seconds. The left eye was taken as control.

One hour after the application the eyes were washed with saline solution and the later readings were made at 24, 48 and 72 hours to graduate the damages in cornea, iris and conjunctive, according to the Draize accepted scale. In order to determine the corneal damages the eye was dyed with sodic Fluorescein 2% and observed with the aid of an ultraviolet light lamp. With the obtained results the Ocular Irritancy Index was determined to classify the products.

Results

In the case of the evaluation of the chemical photoprotective effect when evaluating the spectra obtained with the solutions prepared from the dilutions of 0.01g/10ml and 0.1g/10ml, both displayed optimal characteristics for the spectrophotometric evaluation and the dilution effect did not influence significantly in the results. Therefore, it was decided to use the most diluted for the study, considering the great viscosity of the extracts. None of the extracts showed absorption maximums in visible zones of UV. The behavior is similar for all the extracts, independently of the conditions of its obtaintion.

In the ATC study (5), in the level of 2000 mg/Kg BW, the experiment finished with a 100% of survival. Neither signs nor clinical symptoms of toxicity after the administration were observed and the animals throughout the experiment showed a normal behavior. The body weight as a toxicity index did not show alterations, because the animals gained weight within the rank established as normal for the growing curve of the species and line. The macrocospic anatomopathological analysis did not reveal alterations in the studied organs and weaves. In the test with the level of 5000 mg/kg BW only an animal died. The rest of the animals showed a both normal behavior and weight, not being observed signs nor clinical symptoms of toxicity. In the case of the dermal irritancy (8) of the watery extract it did not appear oedema nor erithem nowhere, finally Dermal Irritancy Index took a a value of “0”, reason why the product is evaluated as non dermal irritating agent. There were not either affectations in any of the parameters evaluated in the eyes of each animal in the ocualr irritancy study and the Ocualr Irritancy Index was “0” evaluating the product as non ocular irritant (9). For the dry extract dermal irritancy appeared neither oedema nor erithem either in any of the application during the course of the experiment, taking also a value of “0” the Index of Primary Irritancy and classifiying the substance as non dermal irritant (8). A slight damage in conjuctive in two animals to the first time of observation for the dry extract was appraised, as well as little secretions to this same time in the three animals. All these findings disappeared for the next observation. The calculation of the Ocualr Irritancy Index threw a value of 0.42, reason why the product is evaluated as non ocular irritant (9).

Discussion

The study of the photoprotective effect of a compound begins generally by the evaluation of its capacity UV radiations absorption, due to the simplicity and accessibility of the technique (12,13). The results obtained in the study indicate that the watery extract of the algae does not have the ability to act like chemical photoprotective agent, under none of the studied conditions of extraction. In literature other techniques are reported to evaluate different photoprotective mechanisms (14-16). There are studies where extracts of Cuban marine seaweed have been evaluated that indicate that the photoprotective effect of this compunds sholud be by antioxidant and colagen fiber repair mechanisms. We considered that the algae should be evaluated by other techniques before reaching definitive conclusions in this respect.

To the present time, to give a criterion of acute toxicity it was necessary to use a great number of animals, nevertheless, in the last years have arisen alternative methods whichs objectives are to replace, to reduce and to refine the number of animals (17), for this reason and cosidering the type of substance to evaluate, the study started for the dose of 2000 mg/kg BW. The generated experimental evidences allow locating the LD50 of this product over 2000 mg/Kg BW, which corresponds with the class "Not classified", meaning non toxic. However, it was decided to prove, with informative aims (screenig), the effects produced by a 5000 mg/Kg BW single dose since the application of this product in later studies is anticipated which can imply the use of high doses, which also allows to corroborate the results obtained with the first level of studied dose, that is in correspondence with the awaited ones for a product of this nature.

The studies of dermal and ocular iritancy did not demonstrate damage to such structures and those produced in ocular irritancy in the conjunctive are adjudicables to the physical effect of the dry extract (raw material) being deposited in the eye which is corroborated when disappearing such damages in the next reading, despite of its expression in mathematical value still they locate it in the category of non ocular irritant.

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