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Toxicological studies on alcoholic extracts of Loranthaceae from northwestern Argentine

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INTRODUCTION

The use of medicinal plants for the treatment of a variety of health affections has been increased during the last decades. Scientific publications attribute multiple biological activities to plant extracts. However, not always the potential toxic effects of the evaluated species are taken into account. Under certain conditions, some bioactive substances show non desirable genotoxic effects. Genotoxicity is the toxicity manifested in the cell genetic material, and includes direct and indirect effects on DNA, which can derive in mutations and/or carcinogenesis. The present work studies the genotoxicity of plant extracts prepared from 2 species of the Loranthaceae family. Cytotoxicity on eucariotic cells was evaluated by the *in vivo* lethality test with larvae of *Artemia salina*.

METHODOLOGY

Tinctures were prepared from leaves of *Psittacanthus cuneifolius* (Ruiz & Pav.) Blume and *Tripodanthus acutifolius* (Ruiz & Pav.) Van Tieghem. The extractive forms were chosen according to popular use. The contents of phenolic compounds (Singleton *et al*., 1999) and the amount of extracted material (EM) were determined according to *Pharmacopeia Argentina, 6th edition.* The genotoxic activity was evaluated by the 'rec-assay' (Takigami *et al.*, 2002) with strains of *Bacillus subtilis*, which uses the differential growth of strains of *B. subtilis* H17 [rec (+)] and M45 [rec (-)] as genotoxicity index. Polyplates (of 96 wells) were used with the addition of 30 microL of suspension of each bacterial culture (10^5 UFC/mL), prepared in Muller Hinton (MH) broth, 60 microL of serial dilutions of plant extracts (or reference drug), and 10 microL of buffer phosphate (0.1 M, pH 7.4). Growth controls contain sterile distilled water instead of plant extract (or reference drug). The polyplates were stirred at 37°C for 1 hour (interaction period), then 100 microL MH broth were added to each well. The initial optical density was read (OD_o) at 595 nm. The polyplates were incubated then at 37°C for 7 h (rec (+) strains) and 8 h (rec (-) strains), time in which reached the exponential growth phase, the final optical density (OD_f) being determined. For each well the difference of readings was calculated (ΔOD); The 100% growth accounts for ΔOD of the controls. The % inhibition (100 - % growth) was graphed for each tested extract dose. Curves were linealized with the probit mathematical transformation, the area between both curves (S-probit), which represents the genotoxicity, was calculated by integration. Tests were carried out in triplicate for each strain. $K_2Cr_2O_7$ (known genotoxic agent), kanamycin (KM) and dimethylsulfoxide (DMSO) (non-genotoxic agents) were used for comparative purposes. The *in vivo* citotoxicity was evaluated by the lethality asssay with *Artemia salina* larvae (Meyer *et al.*, 1982). This assay consists on placing 10 larvae of *A. salina* (nauplii) in a medium with 5 mL marine water and the addition of serial dilutions of plant extracts. After 16 h incubation (at 2000 Lux) survived nauplii were counted, and the lethal dose 50 values (LD_{50}) were calculated by probit analysis ('Finney test' programm), corresponding to the extract concentration able to produce the death of 50% of the nauplii.

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RESULTS AND DISCUSSION

The genotoxic activity of the tested substances was expressed in terms of R50 (Relationship among LD50) and as a function of the"S-probit." The R50 criterion is an approximate index of evaluation. As higher this value is, higher will be the genotoxicity. S-probit constitutes a quantitative index, its values were compared with reported data (Matsui, 1980), which allowed to classify substances in different genotoxicity categories:

Higher than 0.593: Strong genotoxic response (++)
Between 0.200 and 0.592: Genotoxic response (+)
Between -0.123 and 0.199: Non-genotoxic response (-)
Lower than - 0.123: Inverse effect (r)

The 2 tested tinctures showed strong genotoxicity (S-probit > 0.593), the same as $K_2Cr_2O_7$, an agent of known genotoxic activity. Kanamycin is a non-genotoxic antimicrobial agent. The reverse effect that DMSO shows, can be understood like an independent toxicity of the *rec* gene.

Table 1. Results obtained with the rec-assay for extracts and reference drugs

Substance	R50	S-probit	Evaluation
K2Cr2O7	87.4	2.95	(++)
DMSO	0.73	- 0.302	(r)
KM	1	0.137	(-)
T. acut.	7.14	1.37	(++)
P. cun.	3.57	0.771	(++)

The citotoxicity assay afforded LD50 values of 112.2 and 112.1 microg of EM/mL for *T. acutifolius* and *P. cuneifolius*, respectively, lower than those observed with $K_2Cr_2O_7$ (cytotoxic reference).

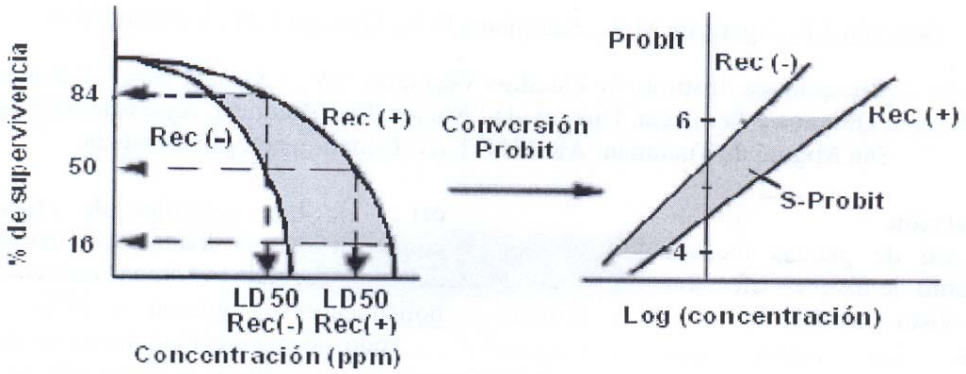

Figure 1. Graphic representation of the genotoxicity analysis.

CONCLUSIONS

The results of our assays indicate the necessity to include toxicity tests in experimental analyses, since a good biological activity can also be accompanied by a great toxicity. The 'rec-assay' is a powerful biochemical tool for the evaluation of genotoxicity, owing to its simple methodology, reproducible results, and generated in a short time of analysis. The test with *A. saline* is an assay that evaluates toxicity on an eucariont organism in a quick and not very expensive way.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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