Revista Argentina de Microbiología (2004) 36: 136-138

ISSN 0325-7541

Revista Argentina de Microbiología (2004) 36: 136-138

Virucidal activity presence in *Trichilia glabra* leaves

M. CELLA[1], D.A. RIVA[2]*, F.C. COULOMBIÉ[2], S.E. MERSICH[2]

[1] *CEFYBO. Serrano 669. 1414. Buenos Aires.* [2] *Lab. Virología. Dto. Química Biológica. FCEyN. UBA. Pab2 P4. Ciudad Universitaria. 1428. Buenos Aires. Argentina*
**Correspondence. E-mail: diegor@qb.fcen.uba.ar*

SUMMARY

Different immunomodulatory activities present in *Trichilia glabra* (TG) leaf extracts have already been described. Particularly, chloroform-methanol extracts were responsible for an *in-vivo* anti-inflammatory effect. The effect of such extracts on the infectivity of enveloped and naked viruses were investigated. Methanolic fraction extracts were active against herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV), while no activity against poliovirus type 3 was observed. VSV was slightly more affected than HSV-1: 2.8 $\log_{10}$ reduction in VSV titer against 2.4 $\log_{10}$ reduction in HSV-1 titer when 0.25 mg/ml F2 fraction was tested and a reduction of 2.7 $\log_{10}$ in VSV virus titer and of 1.5 $\log_{10}$ in HSV-1 virus titer was observed when 0.25 mg/ml F3 fraction was tested. Results obtained in this work suggest a potential pharmaceutical use of TG extract components.

Key words: Virucidal activity, methanolic extracts, *Trichilia glabra* leaves

RESUMEN

Presencia de actividad antiviral en hojas de *Trichilia glabra*. Previamente se han descripto distintas actividades inmunomoduladoras, presentes en extractos de hojas de *Trichilia glabra* (TG). En particular, se ha demostrado una actividad antiinflamatoria presente en extractos metanólicos. En este trabajo se investigó la actividad virucida de dichos extractos sobre virus envueltos y desnudos. Distintos extractos metanólicos han inactivado en forma moderada los virus herpes simplex tipo 1 (HSV-1) y el virus de la estomatitis vesicular (VSV), mientras no evidenciaron actividad sobre poliovirus tipo 3. VSV resultó algo mas afectado que HSV-1: se observó una reducción en el título viral de 2,8 $\log_{10}$ para VSV y de 2,4 $\log_{10}$ para HSV-1 cuando se uso una concentración de 0,25 mg/ml de la fracción F2 y una reducción de 2,7 $\log_{10}$ para VSV y de 1,5 $\log_{10}$ para HSV-1 cuando se usó una concentración de 0,25 mg/ml de la fracción F3. Los resultados obtenidos en este trabajo, sugieren un potencial uso farmacéutico de los componentes presentes en los extractos de TG.

Palabras clave: actividad virucida, extractos metanólicos, hojas de *Trichilia glabra*

The development of potential drugs from phytoche-mical preparations for the control of viral infections, is nowadays a cardinal goal. Meliaceae family have long been recognized for its medicinal properties (5). Aqueous or hydroalcoholic extracts obtained from leaves or roots of large trees of *Melia azedarach L.* or *Cedrela tubiflora* exert an antiviral and virucidal action against DNA and RNA viruses (1, 6). In previous reports, we described different immunomodulatory activities present in *Trichilia glabra* (TG) leaf extracts (3, 4). Particularly, methanolic extracts were responsible for an in-vivo anti-inflammatory effect. The present study was carried out to investigate the effect of such extracts on the infectivity of enveloped and naked viruses.

The preparation of TG fractions was done from dessicated leaves of TG, which had previously been collected in Buenos Aires in May 1999, and identified as already described (as Argentina BAA 2722) (4). In order to isolate the active principles, a first methanol extract (EM) was applied to a Silicagel 80 column and eluted with different proportions of chloroform-methanol (4). Fractions were named after the ($CHCl_3$:CH_3OH) mixture used for elution (100:0) F1, (95:5) F2, (90:10) F3. Percentage weights of the initial dried leaves were 19.5% EM, 1.9% F1, 0.3% F2, 0.2% F3.

Since it has been shown that anti-inflammatory and virucide effects can be ascribed to different chemical constituents of a natural extract (11) and we have already demonstrated that F2 and F3 display immunomodulatories activities, only EM, F2 and F3 biological activities were studied in this report. Each fraction was evaporated and resuspended in a mixture of 0.87M dimethyl sulfoxide and 0.7M ethanol. Sterile extracts were stored at –20 °C until use. Concentration was calculated as weight of dried material per volume of final solution. The cellular toxicity of the different fractions against Vero cells was determined by the MTT

test (8), carried out on confluent monolayers in the presence of MEM 1.5% of bovine serum over a period of two hours. The dose that diminished 50% viability of cell cultures treated with each fraction was calculated from a dose-response curve, by regression analysis in comparison with untreated cultures (Cell cytotoxicity 50 or CC_{50}). EM, F1, F2 and F3 fractions exhibited a CC_{50} higher than 5 mg/ml.

The virucidal effect of the fractions against herpes simplex virus type 1 strain F, vesicular stomatitis virus, (Indiana serotype, San Juan strain) and polio-virus type 3 was assayed. Virus stocks were prepared in Vero cells, and titration was performed by plaque formation in Vero cells (6). Each virus sample, containing approximately 10^6 PFU, was mixed either with or without the fractions F2, F3 or EM, for 1 h at 25 °C. Then, samples were chilled and diluted to determine residual infectivity by plaque formation. Virucidal activity was calculated as the ratio of TG-treated virus titer and MEM-treated virus titer. Dose-response curves were done with each fraction and CI_{50} was defined as the concentration required to reduce virus titer with respect to MEM-treated virus titer by 50% (6). No difference was observed between titers obtained after incubation with MEM or with MEM plus DMSO and/or ethanol, solvents used to solubilize F2 and F3 fraction. In kinetic studies the pre-incubation period varied between 5 and 60 min. Positive controls with chlorine were carried out by incubating different dilutions of chlorine diluted in PBS with the viral stocks. Reduction of 40% VSV titer incubated with 10 mg/ml chlorine was equivalent to incubation with 0.22 mg/ml F3, 0.19 mg/ml F2 or 0.42 mg/ml EM. On the other hand, 33% HSV titer reduction was observed when incubation with 10mg/ml chlorine, 0.27 mg/ml F2, 0.35 mg/ml F3 or 0.5 mg/ml EM was done.

F2, F3 and EM fractions determined a reduction in HSV-1, VSV and polio plaque forming units. Titers were inversely related to the concentrations of the different *T. glabra* fractions in a dose-dependent manner. CI_{50} values for each fraction are summarized in Table 1. The virucidal effect of *T. glabra* leaf fractions F2 and F3, against VSV and HSV was different according to the concentration assayed. While values in Table 1 indicate that HSV is more sensitive than VSV, at high concentrations (>0.2 mg/ml) results are opposite. The influence of time of incubation on the virucidal activity of TG fractions is shown in Fig. 1. VSV was slightly more affected than HSV-1: 2.8 $\log_{10}$ reduction in VSV titer against 2.4 $\log_{10}$ reduction in HSV-1 titer when 0.25 mg/ml F2 fraction was tested. A reduction of 2.7 $\log_{10}$ in VSV titer and of 1.5 $\log_{10}$ in HSV titer was observed when 0.25 mg/ml F3 fraction was tested. In turn, when 0.25 mg/ml EM fraction was incubated with VSV or HSV-1, HSV-1 titer reduction was higher than VSV titer reduction, indicating that EM fraction contained different proportions of the components present in F2 and F3 fractions. Interestingly, only 1 log reduction in Polio virus titer was obtained when incubated with any of the fractions. On the other hand, TG fractions were not active as antiviral agents and had no effect on viral replication following pretreatment of Vero cells (data not shown).

In the present study, fractions F2, F3 and EM obtained from leaves of TG showed a moderate virucidal effect against HSV-1 and VSV and a slight reduction in Polio virus titer. This effect could be due to a direct interaction with both enveloped virions, since it has been proposed that plant-derived substances exhibit extracellular virucidal activities either by binding to the proteins and/or by denaturing them. In fact water crude extracts from leaves of *Cedrela tobiflora* exerted a virucidal effect against HSV, pseudorabies virus and VSV by an unknown mechanism (6).

It is not generally possible to correlate the bioactivity of plant extracts with specific constituents (13). Our results are similar to those already described on hypericin, a naphthodiantrone isolated from the herb *Hypericum triquetrifolium* Turra, that inactivated enveloped viruses if incubated with purified virions for 1 h at 37 °C, but it was ineffective as antiviral agent (12). It was recently demonstrated that this virucidal activity could be due to hypericin binding to the low-density lipoproteins (7). Besides, virucidal activities present in plant extracts against herpes virus have been ascribed to different chemical compounds (9, 10, 11). Flavones and chalcones are two type of major constituents, consistent with solubility and biological properties observed in this study for EM, F2 and F3 fractions, that might be present in TG extracts (10, 11). However non-polar residues are in a high proportion in F2 fraction. In summary, we have described a virucidal activity against enveloped viruses, present in organic extracts from TG leaves, whose mechanism of action remains to be determined. Since we have already demonstrated that hot extraction with methanol recovers small molecules (2), studies on the chemical nature of the bioactive components, charac-terized in this report, is an important step to be done in the context of a potential pharmaceutical use.

Acknowledgements: These studies were supported by University of Buenos Aires (UBA-X-004) grant. We thank Dr. F. Benecia for useful discussions.

REFERENCES

1. Alche JF, Ferek GA, Meo M, Coto CE, Maier MS (2003) An antiviral meliacarpin from leaves of *Melia azedarach L.* Z. Naturforsch. 58: 215-219.
2. Benencia F, Coulombié FC (1998) Immunomodulatory activities of *Trichilia glabra* leaf aqueous extracts. Fitoter Res. 12: 161-171.
3. Benencia F, Courrèges MC, Coulombié FC (1999) *Trichilia glabra*: Effect on the phagocytic and respiratory burst response of peritoneal macrophages. Immunopharmacol. 41: 45-53.
4. Benencia F, Courrèges MC, Coulombié FC (2000) Anti-inflammatory activities of *Trichilia glabra* aqueous leaf extract. J. Ethnopharmacol. 71: 293-300.
5. Bray DH, Warhurst DC, Connolly JD, O'Neill MJL (1990) Plants as sources of antimalarial drugs. Part 7. Activity of some species of Meliaceae plants and their constituent limonoids. Phytother. Res. 4: 29-35.
6. Cordoba MA, Coto C, Damonte EB (1991) Virucidal activity in aqueous extracts obtained from *Cedrela tubiflora* leaves. Phytother. Res. 5: 250-253.
7. Delaey EM, Obermueller R, Zupko I, De Vos D, Falk H, de Witte PA (2001) In vitro study of the photocytotoxicity of some hypericin analogs on different cell lines. Photochem. Photobiol. 74: 164-71.
8. Denizot F, Lang R (1984) Rapid colorimetric assay for cell growth and survival. Modifications of the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods. 89: 271-277.
9. Goncalves JS, Leitao SG, Delle Monache F, Miranda MS, Santos MM, Romanos MV, et al. (2001) In vitro antiviral effect of flavonoid-rich extracts of *Vitex polygama* (Verbenaceae) against acyclovir-resistant herpes simplex virus type 1. Phytomedicine. 8: 477-480.
10. Hayashi K, Hayashi T, Otsuka H, Takeda Y (1997) Antiviral activity of 5,6,7- trimethoxyflavone and its potentiation of the antiherpes activity of acyclovir. J. Antimicrob. Chemother. 39: 821-824.
11. Phrutivorapongkul A, Lipipun V, Ruangrungsi N, Kirtikara K, Nishikawa K, Maruyama S, et al. (2003) Studies on the chemical constituents of stem bark of *Millettia leucantha*: isolation of new chalcones with cytotoxic, anti-herpes simplex virus and anti-inflammatory activities. Chem Pharm Bull (Tokyo). 51: 187-190.
12. Tang J, Colacino JM, Larsen SH, Spitzer W (1990) Virucidal activity of Hypericin against enveloped and non-enveloped DNA and RNA viruses. Antiviral Res. 13: 313-326.
13. Wagner H, Bladt S (1994) Pharmaceutical quality of hypericum extracts. J. Geriatr. Psychiatry. Neurol. 7: 65-68.

Recibido: 18/02/04 – Aceptado: 12/07/04