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Evaluation of Cytological Parameters Induced by Aqueous Extracts of Seven Plants Used as Antihypertensive Agents in Argentine Folk Medicine

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SUMMARY. The aqueous extracts of seven species used in Northeastern Argentina folk medicine for treating hypertensive diseases were studied in order to evaluate their action on mitosis as indicative of presumable antimitotic and genotoxic actions, using the *Allium cepa* test. Results showed fall of Mitotic Index for all species but the antimitotic action was only statistically significant in *Aristolochia triangularis* and *Cayaponia bonariensis* extracts. Significant production of chromosome abnormalities (%) were recorded in *Solanum granuloso-leprosum, Urera baccifera* and *Cayaponia bonariensis.* Production of micronuclei was high but not significant in the latter two species.

RESUMEN. "Evaluación de Parámetros Citogenéticos inducidos por Extractos Acuosos de Siete Plantas usadas como Agentes Antihipertensivos en Medicina Popular Argentina". Han sido estudiados los extractos acouosos de siete especies utilizadas en el NE de Argentina para el tratamiento de la hipertensión, con el propósito de evaluar su acción sobre la mitosis como indicador de presunción de actividad antimitótica o genotóxica, usando el test de *Allium cepa.* Los resultados mostraron una disminución del índice mitótico en todas las especies estudiadas, pero la acción antimitótica fue estadísticamente significativa sólo en el caso de los extractos de *Aristolochia triangularis* y *de Cayaponia bonariensis.* Se registró una significativa producción (%) de anormalidades cromosómicas en *Solanum granuloso-leprosum, Urera baccifera* y *Cayaponia bonariensis.* La producción de micronúcleos fue alta pero no significativa en las últimas dos especies.

INTRODUCTION

Leaves of *Eugenia uniflora* L. *(Myrtaceae),* young leaves and flowers of *Solanum granuloso-leprosum* Dunal (= *S. verbascifolium* L. *var. auriculatum* (Ait.) O.K., *Solanaceae),* rhizomes of *Urera baccifera* (L.) Gaudich. and *Costus arabicus* L. *(Zingiberaceae: Costoideae),* aerial parts of *Cuphea calophylla* Cham. et Schlecht. subsp. *mesostemon* (Koehne) Lourteig *(Lythraceae),* roots of *Cayaponia bonariensis* (Miller) Martínez Crovetto *(Cucurbitaceae),* used as substituent of *U. baccifera,* and secondary growth stems of twings vines of *Aristolochia triangularis* Cham. *(Aristolochiaceae)* are species from which aqueous extracts of the aforementioned parts are used as antihypertensive agents in Northeastern Argentina folk medicine [1,2].

Different classes of constituents have been

KEY WORDS: Acetaminophen, *Allium cepa* Test, *Aristolochia triangularis, Cayaponia bonariensis,* Chromosome abnormalities, Clastogenic activity, C-Mitotic activity, *Costus arabicus, Cuphea calophylla* ssp. *mesostemon, Eugenia uniflora,* Genotoxic activity, *Solanum granuloso-leprosum, Urera baccifera.*

PALABRAS CLAVE: Acetaminofeno, Actividad clastogénica, Actividad C-Mitótica, Actividad genotóxica, Anormalidades cromosómicas, *Aristolochia triangularis, Cayaponia bonariensis, Costus arabicus, Cuphea calophylla* ssp. *mesostemon, Eugenia uniflora, Solanum granuloso-leprosum,* Test de *Allium cepa, Urera baccifera.*

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identified in some of these species: primary and secondary amines, triterpenoids, steroids, naphtoquinones, anthraquinones [3,4], essential oil [5], sesquiterpenes, lignans [6], alkaloids [7,8], and aristolochic acid [7] in *Aristolochia triangularis;* alkaloids and bitter principles [8] in *Cayaponia bonariensis;* phenolic compounds, flavonoids [9,10], leucoanthocyanidins, steroids and/or triterpenoids [9], essential oil with fatty acids, phenols, β-pinene, limonene, cineol, pulegone, camphor and sesquiterpenic compounds [11] in *Eugenia uniflora;* alkaloids [8,12], solasodine [8], and steroidal sapogenins [5,12] in *Solanum granuloso-leprosum*, and oxidases [5] in *Urera baccifera*.

Studies concerning toxicological and potential genotoxic activity of extracts of these species are very scarce, in spite of their wide use and trade in the country. In order to determine their potential antimitotic activity and genotoxicity actions, extracts prepared according ethnotherapeutic procedures and doses were analyzed using the *Allium cepa* test, a first-tier assay that have been proposed as a good indicator of these types of activities in complex substances, that may be used prior to perform mammalian tests.

MATERIAL AND METHODS

The studied materials were collected in their natural habitats, obtained from cultivation and/or acquired in popular markets at Posadas, Province of Misiones, Argentina. Voucher specimens were deposited in the Herbarium, Department of Pharmacy, FCEQyN-UNaM.

Aqueous extracts were obtained according ethnotherapeutic procedures (decoctions), as consigned in Table 1.

Species	Part of the plant used	Decoction concentration
Aristolochia triangularis	secondary growth stems of twings vines	25 g/l
Cayaponia bonariensis	roots	75 g/l
Costus arabicus	rhizomes	90 g/l
Cuphea calophylla ssp. *mesostemon*	aerial parts	3 g/l
Eugenia uniflora	leaves	3 g/l
Solanum granuloso-leprosum	young leaves and flowers	5 g/l
Urera baccifera	rhizomes	75 g/l

Table 1. Composition of the aqueous extracts of the seven plants studied.

Genotoxic activity was analyzed using the *Allium cepa* test [13-15]. Mitotic Index (MI), Prophase (PI), Metaphase (MeI), Anaphase (AI), and Telophase (TI) Indexes were the cytological parameters studied, as well as chromosome abnormalities (AN%) and production of micronuclei (MN) and binucleate cells (BN) at Interphase. The obtained values were statistically analyzed by means of the Student´s t-Test.

Roots of *Allium cepa* (onion) bulbs were exposed for 48 h to the extracts, then the tips were separated, fixed for 12-20 h in ethyl alcohol-acetic acid (3:1), stained with lacto-propionic orcein and mounted for examination. At least samples of five onion bulbs were analyzed for each extract and respective controls; the amount of counted cells is indicated in Table 2 for each case. Tap water was used as negative control and 300 mg/l of paracetamol (acetaminophen) was used as positive clastogenic standard [13,16].

RESULTS

Cytogenetic parameters as well as cytogenetic abnormalities induced by aqueous extracts of the studied species are reported in Tables 2 and 3, and Figures 1-2.

CONCLUSIONS

The aqueous extracts of all the assayed species induce a fall in the MI in relation to controls (Table 2), but only for *Aristolochia triangularis* and *Cayaponia bonariensis* extracts the values are statistically significant (antimitotic ac-

Species	Counted cells	MI	PI	MeI	AI	TI
Eugenia uniflora	5895	5.27	53.70	18.60	13.50	14.10
Solanum granuloso-leprosum (leaf)	5914	4.31	44.96	27.61	15.85	11.56
Solanum granuloso-leprosum (flowers)	5455	5.39	40.25	25.57	22.01	12.15
Urera baccifera	13027	1.96	60.15	14.45	6.25	19.14
Cuphea calophylla	5954	6.19	61.65	14.36	8.94	15.02
Costus arabicus	9608	4.37	48.03	20.82	12.89	18.27
Aristolochia triangularis	3207	0.62*	90	5.00	0.00	5.00
Cayaponia bonariensis	11468	2.29*	62.35	9.88	9.50	18.25
Paracetamol (Acetaminophen)	5593	5.27	70.50	14.57	6.10	8.81
Water	23536	7.32	52.46	17.97	12.40	17.16

Table 2. Cytogenetic parameters in the *Allium cepa* test for the studied species. MI: Mitotic Index; PI, MeI, AI, TI: Prophase, Metaphase, Anaphase and Telophase Indexes. * p≤ 0.05.

Species	MN	BN	AN%	Abnormalitie type
Eugenia uniflora	-	-	1.05	DMe
Solanum granuloso-leprosum (leaf)	1	-	7.84*	DMe; ABr; FA; AnCr
Solanum granuloso-leprosum (flowers)	1	-	3.40	FMe; MeBr
Urera baccifera	6	9	15.62**	StMe; C-Mit; DMe; A/DA; ABr; DT
Cuphea calophylla	-	-	0.00	-
Costus arabicus	-	-	8.81	ABr; A/DA; PolA; StMe; RCr
Aristolochia triangularis	-	16	5.00	C-Mit
Cayaponia bonariensis	15	2	23.73*	C-Mit; RCr; DMe; A/DA; ABr; FA; StA; DT; TBr
Paracetamol (Acetaminophen)	53**	-	11.27	FMe; A,T; DMe
Water	-	2	2.90	-

Table 3. Cytogenetic abnormalities in the *Allium cepa* test for the species studied. MN: production of miproduction at interphase; BN: binucleate cells at interphase; AN%: percentage of chromosome abnormalities; F: fragments; P: Prophase; Me: Metaphase; A: Anaphase; T: Telophase; DMe: dispersed metaphasic chromosomes; Br: bridges; RCr: ring or anular chromosomes at metaphase; A/DA: Arrested anaphase or disorganized chromosomes at anaphase; PolA: poliploid anaphase; St: sticky chromosomes; C-Mit: condensend or c-mitotic chromosomes. * $p \leq 0.05$ ** $p \leq 0.01$.

tion). In spite of the record of micronuclei (indicator for clastogenic action) in *Solanum granuloso-leprosum*, *Urera baccifera* and *Cayaponia bonariensis*, only the concentration assayed for acetaminophen was statistically significant. Binucleate cells in asynchronic division was observed in *Solanum granuloso-leprosum*. Chromosome abnormalities (Table 3) during division (AN%) were significant in *Solanum granuloso-leprosum* (leaves) and *Urera baccifera* and highly significant in *Cayaponia bonariensis.*

Is expected that the present results contribute to characterize the toxicity and bioactivity profile of extracts of analyzed species, and could be subsequently used in pharmacological and toxicological research.

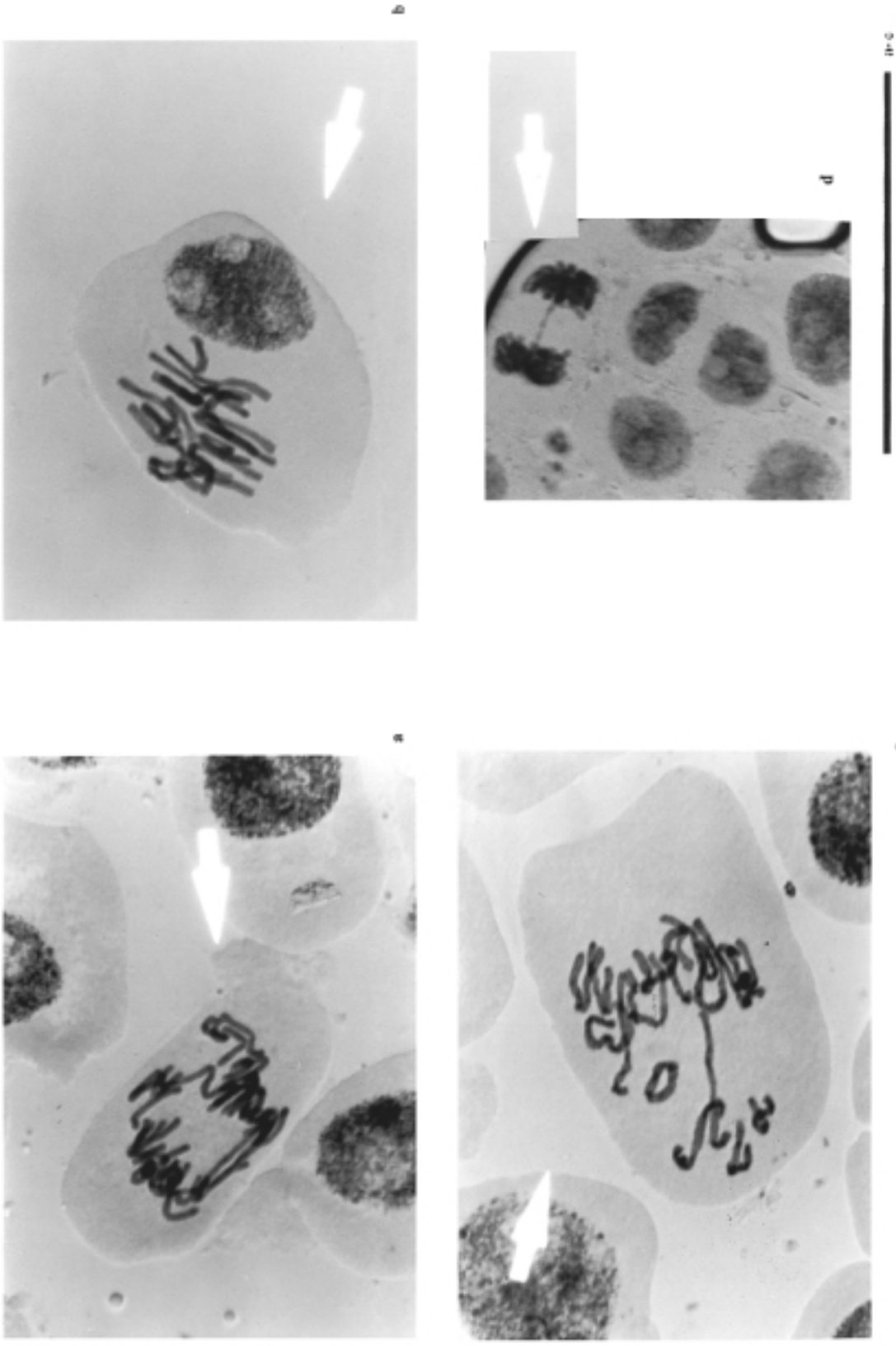

Figure 1. Cytogenetic abnormalities produced by *Solanum granuloso-leprosum* extract. a: disorganized anaphase with bridges; b: binucleate cell in asynchronic division; c: abnormal anaphase with inequal distribution and arrest of chromosomes and an "annular" chromosome (typical malformation of *c*-mitosis); d: bridge at latter anaphase. Bar: 400 µm.

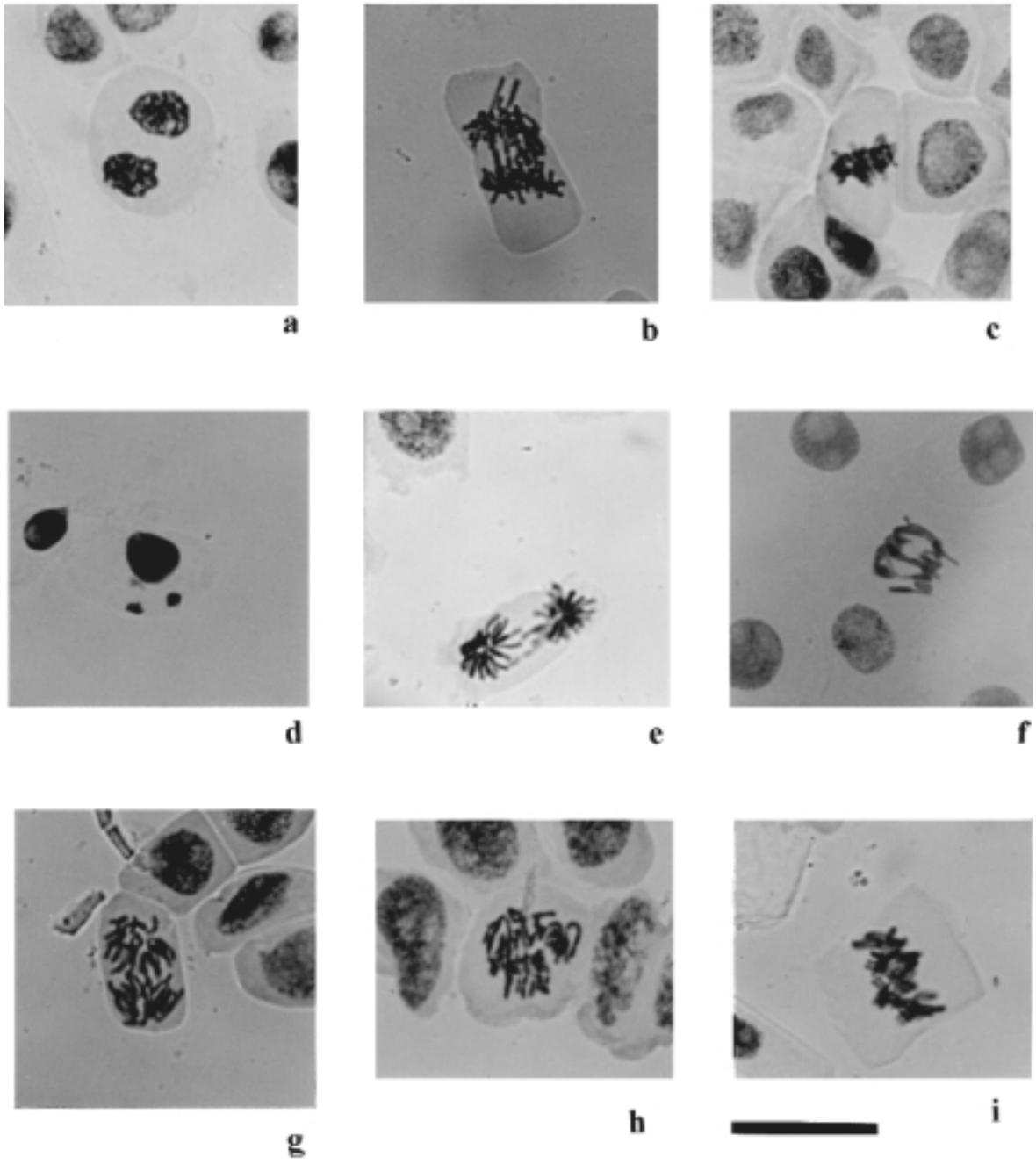

Figure 2. Cytogenetic abnormalities produced by *Urera baccifera* extract. a: prophase binucleated cell; b and e: brigdes at anaphase; c: sticky metaphase; d: interphase bi-micronucleated cell; f: sticky anaphase with bridges; g and h: dispersed anaphases; I: *c*-metaphase. Bar: 400 µm.

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