Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. II, 67-70, 1991

GENOTOXICITY OF PLANT EXTRACTS

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*Aqueous extracts of seven species used in Brazilian popular medicine (*Achyrocline satureoides, Iodina rhombifolia, Desmodium incanum, Baccharis anomala, Tibouchina asperior, Luehea divaricata, Maytenus ilicifolia*) were screened to the presence of mutagenic activity in the Ames test (Salmonella/microsome). Positive results were obtained for* A. satureoides, B. anomala *and* L. divaricata *with microsomal activation. As shown elsewhere (Vargas et al., 1990) the metabolites of* A. satureoides *extract also show the capacity to induce prophage and/or SOS response in microscreen phage induction assay and SOS spot chromotest.*

The positive results were related to the presence of flavonoids and tannins in the aqueous extracts.

Key words: Ames test – flavonoids – tannins – plant extracts – *Achyrocline satureoides* – *Luehea divaricata* – *Baccharis anomala*

The effect of environmental factors and their role in human mutagenesis/carcinogenesis has been significantly emphasized in the last decade. Epidemiological research has shown significant variations in cancer incidence both between and within countries (Brown, 1980; Ames, 1983).

The human diet contains a great variety of natural mutagens and carcinogens and this is an area deserving careful investigation. In nature plants synthesize toxic chemicals in large amounts, apparently as a primary defense against the horders of bacterial, fungal, insect and other animal predators (Ames, 1983).

Preparations of medicinal plants are widely used in human therapy although little information on their potential risk to health is available.

Aqueous extracts of plants widely used in medicinal practice in Brazil were investigated for the presence of genotoxic activity in microorganisms.

MATERIAL AND METHODS

Samples – The plants were collected from unpolluted areas and botanically classified. The dried plant material (Table I) was used to prepare the extracts in boiling water at a concentration of 50% weight/volume. Samples were sterilized using Sartorius filter with 0,22 μm pore size.

Strains – The *Salmonella typhimurium* strains TA98, TA100 and TA102 were kindly provided by B. M. Ames, University of California, Berkeley, CA, USA.

Salmonella/microsome assay – Mutagenicity was analyzed by the preincubation procedure (Maron & Ames, 1983). Mutation tests were performed on samples of plant extracts in the presence or absence of S9 mix (20 μl of 59 fraction prepared from Sprague-Dawley rat livers pretreated with 3-methylcholanthrene containing 16,3 mg/ml protein per 500 μl of S9 mix). The screening test was performed using TA100 and TA98 strains. In infusions of *A. satureoides, B. anomala* and *L. divaricata* the TA102 strain was also used.

RESULTS

Table II shows the results of mutagenic activity for seven plant extracts screened in the Ames test using TA100 (detects base-pair substitution mutagens) and TA98 (detects frameshift mutagens) strains with and without metabolization.

This work was supported by CNPq – FAPERGS – FINEP – PIMUT.

+ Research fellow CNPq.

TABLE I

Medicinal plants screened for mutagenicity in Ames test

Sample	Popular name	Plant part	Therapeutical use (Alice et al., 1985)
Achyrocline satureoides Lam D. C.	marcela	flowers	antispasmodic analgesic anti-inflammatory
Iodina rhombifolia Arn.	cancorosa	leaves	analgesic antiseptic cicatrizant
Desmodium incanum D. C.	amor do campo	leaves stems	diuretic anti-inflammatory
Baccharis anomala D. C.	uva do mato	leaves stems	diuretic
Tibouchina asperior (CHAM.) Cogn.	douradinha	leaves stems	diuretic
Luehea divaricata Mart.	açoita cavalo	leaves stems	diuretic anti-inflammatory
Maytenus ilicifolia Mart.	espinheira santa	leaves	cholagogue choleretic

TABLE II

Analysis of mutagenic activity of plant extracts in Ames test utilizing TA98 and TA100 strains of *Salmonella typhimurium* with metabolization

Samples		TA98 Mutag.[c]	TA98 R/pl[d]	TA100 Mutag.	TA100 R/pl	Main constituents (Alice et al. 1985)
Water[a]		–	44 ± 3[e]	–	149 ± 5	
Aflatoxin[b]		+	402 ± 2	+	439 ± 2	
A. satureoides	(f)	+	174 ± 3	+	310 ± 3	flavonoids
I. rhombifolia	(l)	–	37 ± 1	–	200 ± 2	flavonoids, alkaloids
D. incanum	(l)	–	76 ± 1	–	147 ± 5	flavonoids, tannins,
	(s)	–	40 ± 2	–	160 ± 3	saponins
B. anomala	(l)	+	186 ± 8	–	130 ± 5	tannins,
	(s)	–	41 ± 1	–	158 ± 2	saponins
T. asperior	(l)	–	45 ± 4	–	145 ± 4	flavonoids, tannins
	(s)	–	26 ± 7	–	136 ± 7	triterpenes, saponins
L. divaricata	(l)	+	141 ± 5	–	145 ± 5	flavonoids, tannins, saponins
	(s)	–	31 ± 3	–	134 ± 3	
M. ilicifolia	(l)	–	48 ± 2	–	139 ± 2	flavonoids, tannins, saponins, alkaloids

a: corresponds to the negative control; *b:* positive control (0,5 μg/plate); *c:* + mutagenic; – non mutagenic; *d:* R/pl = revertants His^+/plate; *e:* S. D.
Samples of plant extracts correspond to 100 mg of dried material/plate.

TABLE III

Analysis of mutagenic activity of plant extracts in Ames test utilizing TA102 strain of *Salmonella typhimurium* with metabolization

Samples	TA102 Mutagenic[c]	TA102 R/pl[d]	Main constituents (Alice et al. 1985)
Water[a]	–	385 ± 30[e]	
2-amino-antraceno[b]	+	950 ± 15	
A. satureoides	+	1295 ± 10	flavonoids
B. anomala	–	426 ± 15	tannins, saponins
L. divaricata	–	387 ± 20	flavonoids, tannins, saponins

For definition, see footnote to Table II.

The positive extracts were tested in TA102 strain, which detects a variety of oxidative mutagens, active forms of oxygen and alkylating agents, and which has an intact excision-repair pathway (Levin et al., 1982). Positive results were obtained for *A. satureoides* extracts (Table III).

DISCUSSION

The positive results obtained in genotoxic assays for *A. satureoides*, *B. anomala* and *L. divaricata* were correlated to the presence of tannins and flavones with certain hydroxylation patterns (5,7 hydroxyl substitution) such as quercetin and kaempferol in these extracts. (Macgregor & Wilson, 1988; Vargas et al., 1989, 1990). High intakes of tannins and related anthocyanins through plant materials are correlated to esophageal cancer (Morton, 1980). However some tannins have antioxidative characteristics and therefore, must work in some way as antimutagenic and anticarcinogenic agents (Kada et al., 1985).

In all assays, significant genotoxic effect was seen in the presence of microsomal activation only. Flavonols occur mainly in a pro-mutagenic form but the mutagenic activity is recovered by microsomal hydrolysis or by glycosidases. (Göggelmann & Schimmer, 1986; Ravanel et al., 1987).

The *A. satureoides* aqueous extract contains four polyphenols: quercetin and 3-methoxy-quercetin flavonols, luteolin flavone and caffeic-acid (Simões, 1984). The results in *A. satureoides* extracts are indicative of the mutagenic activity of quercetin, 3-methoxy-quercetin and caffeic acid mutagenic action (Ames, 1983; Mac Gregor, 1986; Ravanel et al., 1987; Vargas et al., 1990). The mutagenic acitivity is more intense in TA98 than TA100 strains. This is to be expected in accordance with the flavonol-like quercetin in *S. typhimurium* (MacGregor & Wilson, 1988).

The positive response obtained in TA102 strain for *A. satureoides* extract can be either due to by oxidative reactions of caffeic acid and quercetin originating quinones and generating hydrogen peroxide, or by alkyiating action of quercetin metabolization products (quinone – methides) in the bacterial DNA bases. (Ames, 1983; Ravanel et al., 1987; Vargas et al., 1990). The response obtained for this strain in *L. divaricata* and *B. anomala* aqueous extracts where tannins are present (Kada et al., 1985) was negative.

The *A. satureoides* extract was also analyzed in SOS spot chromotest (Quillardet & Hofnung, 1985) and Microscreen phage-induction assay (Rossman et al., 1984). These assays (Vargas et al., 1990) confirm the presence of genotoxicity of the *A. satureoides* extracts showing its capacity to induce prophage and/or SOS response. Positive SOS response in PQ230 strain which detects alkylating agents reinforces the hypothesis of the same action form as detected in the Ames test (Hofnung & Quillardet, 1986).

These studies show that high priority should be given to the evaluation of the potential risks to human health represented by the indiscriminate use of extracts of these plants.

ACKNOWLEDGEMENTS

To C. B. Alice for the plants selection and classification, and to Dr Eloy Julius Garcia for assistance in preparing the English version of the manuscript.

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