GENOTOXIC EVALUATION OF THE EFFECT OF *Thuya occidentalys* TINCTURES

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ABSTRACT

Three tinctures samples from extracts of the popular medicinal plant *Thuya occidentalis* were tested *in vitro* through two short term tests for measuring the activity of genotoxic chemicals. Using the *Salmonella*/mammalian-microsome (Mutatest) assay and the SOS-chromotest (induction of β-galactosidase in *Escherichia coli*), none of the extract was effective in inducing mutagenesis or β-galactosidase synthesis (as an indicator of general and early sign of DNA damage), even with metabolization.

Key words: *Thuya occidentalis*, *Salmonella*/mammalian-microsome assay, SOS-chromotest, popular medicine.

RESUMO

Avaliação genotóxica do efeito de tinturas de *Thuya occidentalys*

Três amostras de tinturas de extratos da planta *Thuya occidentalis*, utilizada em medicina popular, foram testadas *in vitro* por meio de dois testes rápidos para medida da atividade genotóxica de químicos. Utilizando o teste de Ames (Mutateste) e o SOS-cromoteste (indução de β-galactosidase em *Escherichia coli*), verificou-se que nenhum dos extratos foi efetivo na indução de mutagênese ou na síntese de β-galactosidase (como o indicador de um sinal geral e precoce de lesão no DNA), mesmo com metabolização.

Palavras-chave: *Thuya occidentalis*, *teste de Ames*, SOS-cromoteste, medicina popular.

INTRODUCTION

The pharmacologic activity of plants and herbs in teas, syrups, cataplasm and tinctures is well established in popular medicine. However, some of them have deleterious effects, for example, some plants, taken as infusion by the population of developing countries have shown mutagenic, toxic and carcinogenic activities (Evans, 1991; Roeder, 1995). Among the plants used in Brazil as popular medicine, there is one, *Thuya occidentalis* which is recomended as expectorant, diuretic, anti-helmintic, stimulating and in rheumatism treatment. It acts in the renal ephitelium and has a toxic action in bladder muscles (Balbach, 1957; Van der Bergh, 1982). It is used in the treatment of cystitis and prostatic hypertrophy in senile and in the urinary incontinence in women. As tincture, it is popularly used as an abortive, as an agent for cauterization in papilomas treatment and wart candilomas (Dr. P. Maldonado, personal communication). In the present study, we used two reliable, standardized and accepted short-term tests developed by Ames *et al*. (1975) and Quillardet *et al*. (1982), testing three different samples of *Thuya occidentalis* tinctures.

MATERIAL AND METHODS

The *Salmonella typhimurium* and *Escherichia coli* strains used in this work, are described elsewhere (Ames *et al*., 1975; Felzenszwalb &

Alcantara Gomes, 1982; Valsa *et al*., 1990) and maintained according to the revised methods of Maron & Ames (1983) and Quillardet *et al*. (1982).

To evaluate the lethal effect of *Thuya occidentalis* samples, 2 ml of *E.coli* K12 AB1157 overnight cultures in Luria broth (LB) medium (Miller, 1972) were incubated with 50 µl of test solution (diluted 1:10) at 37ºC, for 25 min, with shaking. After that, 100 µl of properly diluted treated cultures were spread onto LB plates (LB medium solidified with 1.5% agar-agar). The colonies were counted after 24-36 h of incubation at 37ºC and the surviving fraction was calculated as mentioned elsewhere (Felzenszwalb & Alcantara Gomes, 1982).

In the Mutatest assays, the experimental procedure adopted was the pre-incubation method (Maron & Ames, 1983) slightly modified (Valsa *et al*., 1990).

In the SOS-chromotest assays, logarithmic-phase cells of *E.coli* PQ37 in LB medium were diluted 10 times in culture medium and 600 µl was incubated with 20 µl of different dilutions of *Thuya occidentalis* samples or controls (positive and negative) for 2 h at 37ºC. Then the β-galactosidase and alkaline-phosphatase activities were measured by colorimetric assay. The alkaline-phosphatase synthesis is constitutive and is used as representative of the action of the drug tested in general protein synthesis. The experiments were carried out in triplicate and the results presented are the average mean of two independent assays. The standard deviation did not exceed 20%.

Preliminary tests of concentrated tinctures showed a toxic effect for both *S. typhimurium* and *E. coli* strains (data not shown). All experiments were then performed with dilutions of the concentrated tinctures in deionised and destiled water.

Ethanolic extracts of *Thuya occidentalis* were kindly supplied by Dr. Ribeiro de Miranda (Arte de Curar – Homeopatia, Três Rios, RJ), and we analysed three different tinctures samples, called sample 1, 2 and 3.

RESULTS AND DISCUSSION

The absorption spectra of the three solutions were constructed between 300 and 600 nm, using Incibras Spectrophotometer MF200 UVVIS and showed to be the same with a maximum of absorption in 350 nm (data not shown). The determination of the absorption spectra was important as there are some preparations of *Thuya* tinctures that are composed of mixtures, instead of *Thuya occidentalis* alone (Dr. R. de Miranda, personal communication).

There is a strong correlation between the ability of chemicals to be genotoxic to bacteria and their mutagenic and tumour initiating properties in mammals (Purchase, 1982; Venitt *et al*., 1984). The capacity of a chemical to induce bacterial death, mutagenesis or SOS system expression is widely used to detect potential carcinogenic effects (Quillardet & Hofnung, 1988).

Although an inactivation effect was observed for the three tested samples (Table 1), their mutagenic capacity, assayed by the Ames method, was not higher than the background level (revertant mutants present in culture medium without treatment) even with metabolic activation (+S9) (S9 mix: Maltox™), as shown in Table 2. Difference in survival is in agreement with the absorption of the three samples, where sample 1 showed to be less concentrated (data not shown). The results obtained with SOS-chromotest confirm the absence of SOS functions induction effect (Table 3) and the toxicity of concentrated solutions, measured by alkaline-phosphatase activities.

TABLE 1

Survival fractions of *Escherichia coli* K12 AB1157 cells treated with ethanolic extracts of *Thuya occidentalis*.

***Thuya occidentalis* (dilution 1/10)**	**Survival fraction (number of viable cells after 25 min treatment/number of viable cells at time zero)**
Culture medium	1.3
Sample 1	2.2×10^{-1}
Sample 2	2.2×10^{-2}
Sample 3	2.1×10^{-2}

TABLE 2

Salmonella*/mammalian-microsome assay with *Thuya occidentalis.

Compound	Dilution	Volume (µl)	Revertant colonies per plate									
			TA97		TA98		TA100		TA1535		TA102	
			–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
Culture medium	–	50	108	219	33	58	161	198	24	19	327	412
SAMPLE 1	1/10	50	101	216	18	48	159	174	13	15	287	372
		100	87	206	25	48	202	187	22	16	302	272
	1/100	100	94	180	21	43	183	184	13	16	281	392
	1/1000	50	75	212	25	50	192	220	17	17	287	352
		100	85	220	27	45	208	197	16	19	339	388
SAMPLE 2	1/10	50	90	215	39	53	163	159	17	18	324	371
		100	79	193	34	46	197	208	23	20	320	429
	1/100	100	95	217	36	54	146	187	16	20	329	412
	1/1000	50	91	217	32	48	159	171	24	18	295	437
		100	89	199	34	46	155	187	17	20	323	411
SAMPLE 3	1/10	50	117	208	27	49	164	167	19	21	293	387
		100	115	202	34	52	184	177	22	19	308	415
	1/100	100	121	203	33	53	169	157	26	16	308	377
	1/1000	50	117	199	31	56	163	179	26	19	264	416
		100	110	197	31	55	164	166	28	20	289	408
4NQO (0,5 µg)	–	50	537	–	231	–	5,052	–	104	–	591	–
2-AF (10 µg)	–	50	–	1,776	–	4,060	–	3,508	–	101	–	648

Observation: non-diluted samples were tested as described in Maron & Ames (1983) with the spot-test and showed to be toxic (data not shown). Positive controls: 4-nitroquinoline-N-oxide and 2-aminofluorene (Sigma Chemicals).

TABLE 3

SOS-Chromotest with *Thuya occidentalis* ethanolic tinctures with or without metabolic activation.

T. occidentalis ethanolic tinctures (amount 20 µl)	Dilutions	Phosphatase units		β-GAL units		R $\frac{\text{β-GAL units}}{\text{Phosp. units}}$		Induction factor $I = \frac{R(c)}{R(o)}$	
		–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
SAMPLE 1	2.5×10^{-1}	179	5.4	32.6	18.7	0.14	2.3	0.87	1
	5×10^{-1}	183	4	27.3	15	0.11	2.5	0.68	1
	1×10^{-1}	167	3.7	30.3	20	0.14	3.6	0.87	1.5
	5×10^{-2}	180	3.6	28.6	19.4	0.12	3.5	0.75	1.5
	1×10^{-2}	182	4.4	32	20	0.14	3.1	0.87	1.3
	5×10^{-3}	179	4	31	19.2	0.13	3.2	0.81	1.3
	1×10^{-3}	172	3.6	30.3	21.6	0.14	4	0.87	1.7
SAMPLE 2	2.5×10^{-1}	112	4.8	19	26.8	0.20	3.9	0.95	1.6
	5×10^{-1}	122	5.4	13	21.3	0.10	2.7	0.47	1.1
	2×10^{-1}	110	6	20	26.5	0.20	3.1	0.95	1.3
	5×10^{-2}	112	5	29	27.3	0.26	3.8	1.23	1.6
	1×10^{-2}	110	4.1	18	28.7	0.17	4.9	0.80	2.1
	5×10^{-3}	115	5	13	27	0.12	3.8	0.57	1.6
	2×10^{-3}	120	3.2	15	26.4	0.13	5.8	0.60	2.5
SAMPLE 3	2.5×10^{-1}	83	6.6	19	11	0.22	1.25	1.04	0.5
	5×10^{-1}	84	4.4	20	10	0.25	1.73	1.19	0.7

TABLE 3 (*Continued*)

T. occidentalis **ethanolic tinctures (amount 20 µl)**	**Dilutions**	**Phosphatase units**		**β-GAL units**		**R β-GAL units / Phosp. units**		**Induction factor** $I = \frac{R(c)}{R(o)}$	
		–S9	**+S9**	**–S9**	**+S9**	**–S9**	**+S9**	**–S9**	**+S9**
	1 x 10^{-1}	73	6.2	22	9.1	0.3	1.10	1.42	0.4
	5 x 10^{-2}	72	6.6	17	9.3	0.24	1.00	1.14	0.4
	1 x 10^{-2}	72	6.5	19	8.5	0.25	0.98	1.19	0.4
	5 x 10^{-3}	73	7.2	19	9.3	0.26	0.96	1.20	0.4
	2 x 10^{-3}	72	8.0	16	0.3	0.22	0.87	1.04	0.3
4NQO (20 mg)		89	–	160	–	1.8	–	8.5	–
AFB1 (5 mg)		–	5.8	–	175	–	20	–	8.6
DMSO		179	6.3	32.6	19.2	0.14	2	0.75	0.8
Ethanol 95%		162	4.5	18	9.8	0.08	1.4	0.5	0.6

Observations: (a) non-diluted samples were toxic; (b) it was used 20 µl of samples (1, 2 and 3) and controls (negative and positive) for incubation with 0.6 ml of tester strain (*E. coli* PQ 37); DMSO (dimethyl sulfoxide – Merck); AFB1 – Aflatoxin B1 (Sigma Chemical).

Presence of the activating mixtures has a slight inhibitory effect on protein synthesis which is reduced by a a factor of about three in the two hours incubation period. In addition, the presence of phosphastes inhibits competitively the activity of alkaline phosphatase so that the ratio of b galactosidase to alkaline phosphatase activities is increased by a factor of 13 in the presence of S9 mix (Dr. P. Quillardet, personal communication).

As natural products from flora and fauna are frequently used as medicaments (Gomes *et al*., 1996), we call attention to the control of such compounds since they may present genetic toxicity and moreover it is very important to check their cancer inducing potentiality, as measured by the capacity of activating SOS functions, in the form they are consumed by population.

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