In vitro anti-trypanosomal activity of elatol isolated from red seaweed *Laurencia dendroidea*

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SUMMARY

Chagas' disease is a debilitating but comparatively neglected illness that affects about 15 million people. There is an urgent need to develop new, more effective, and less-toxic compounds. In this study, we assessed the *in vitro* anti-trypanosomal activity of the sesquiterpene elatol from the Brazilian red seaweed *Laurencia dendroidea*. We used electron microscopy to evaluate the effect of elatol on the morphology and ultrastructure of the parasite. Elatol showed a dose-dependent effect against the epimastigote, trypomastigote, and amastigote forms, with IC_{50} values of 45·4, 1·38, and 1·01 μM, respectively. Observation of treated intracellular amastigotes by light microscopy demonstrated a total elimination of the infection at a dose of 3·0 μM. In addition, the compound did not affect the red blood cells, and the CC_{50} value for $LLCMK_2$ cells was 27·0 μM. Transmission and scanning electron micrographs showed aberrant-shaped cells and breaks in the plasma membrane, prominent swollen mitochondria, and extensive formation of cytoplasmic vacuoles in all the forms. This is the first report of the anti-trypanosomal effect of the sesquiterpene elatol.

Key words: *Trypanosoma cruzi*, *Laurencia dendroidea*, elatol, anti-trypanosomal activity, mitochondrion.

INTRODUCTION

American trypanosomiasis or Chagas' disease is a debilitating illness caused by *Trypanosoma cruzi*, and is highly prevalent in Latin America where it affects about 10–16 million people, causing the death of around 12 500 patients annually. Economic hardship and political problems have spurred migration from Chagas-endemic countries to developed countries (Schmunis, 2007; WHO, 2006). The infection is characterized by an acute phase that results in 2–8% mortality, in which the parasite circulates in the bloodstream as trypomastigotes and proliferates within the cytoplasm of a variety of cells as amastigotes. While in the chronic phase, most patients remain asymptomatic, and 30–40% of cases develop cardiac symptoms or digestive lesions (Prata, 2001; Dantas *et al*. 2006). Introduced in the 1960s and 1970s, nifurtimox and benznidazole are the currently accepted nitroderivatives for treatment of this disease. These compounds are active in the acute stage of Chagas' disease, but their efficacy during the chronic phase is still controversial. Moreover, the therapeutic dose is very close to the toxic dose and severe side-effects have been reported during their clinical use, including polyneuritis, lymphadenopathy, dermatitis, anorexia, allergic dermopathy, and depression of bone marrow. There is an urgent need to develop new compounds or novel strategies to make Chagas' disease chemotherapy more effective and less toxic (Coura and Castro, 2002; Urbina and Docampo, 2003; Urbina, 2009).

Marine algae have been used in traditional remedies in Asian countries including China, Japan, and Korea (Wang *et al*. 2009). Species of *Laurencia* (order Ceremiales, family Rhodomeleceae) have

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proved to be a rich source of halogenated secondary metabolites, predominantly sesquiterpenes, diterpenes, and C_{15} non-terpenoids (Iliopoulou *et al*. 2002: Kladi *et al*. 2008). The probable role of these halogenated metabolites is to defend the algae against marine herbivores (Vairapan *et al*. 2001; Iliopoulou *et al*. 2002; Salgado *et al*. 2008; Sudatti *et al*. 2008). Published reports describe important biological activities of seaweeds, including antibacterial, antifungal, anti-leishmanial, anti-trichomonal, antihelmintic, antimalarial, antioxidative, antiviral, antipyretic, analgesic, anti-inflammatory, and anticoagulant (Schaeffer and Krylov, 2000; Vairappan *et al*. 2001; Vairappan, 2003; Matsuhiro *et al*. 2005; Kang *et al*. 2008; Kladi *et al*. 2008; Moo-Puc *et al*. 2008; Freile-Pelegrin *et al*. 2008; Mayer *et al*. 2009; Wang *et al*. 2009).

In the present study, we assessed the *in vitro* anti-trypanosomal activity of the sesquiterpene elatol, the major constituent of the Brazilian red seaweed *Laurencia dendroidea* (Hudson) J. V. Lamouroux, against *Trypanosoma cruzi*. We used electron-microscopy techniques to evaluate the effect of elatol on the morphology and ultrastructure of the parasite.

MATERIALS AND METHODS

Algal material and obtention of elatol

Specimens of *Laurencia dendroidea*, a seaweed that occurs in the intertidal zone along almost the entire Brazilian coast, were collected at Cabo Frio Island, Rio de Janeiro state (22°59′S, 42°59′W). This seaweed was previously described as *L. obtusa*, but through molecular techniques, it was recently identified as *L. dendroidea* (Cassano, 2009). The specimens of *L. dendroidea* used in this study were identified by Dr Mute Toyota Fujii, and voucher specimens were deposited in the herbarium of the Instituto de Botânica, São Paulo state, Brazil (SP number: 399789).

The specimens of *L. dendroidea* were collected by hand during low tide and transported to the laboratory between sheets of moist paper in coolers. In the laboratory, this algal material was air-dried in the dark at room temperature, in order to avoid photolysis and thermal degradation. Dried seaweeds were submitted to exhaustive extraction in hexane or dichloromethane, and a crude extract was obtained. The solvent was eliminated in a rotary evaporator, and the elatol was isolated on pre-coated TLC plates, identified by TLC (thin-layer chromatography: Merck Al TLC 20 × 20 cm silica gel 60F254) and ^{1}H NMR (nuclear magnetic resonance). The spectra were measured on a Varian Unity Plus spectrometer, operating at 299·9 MHz for ^{1}H and 75·0 MHz for ^{13}C, as proposed by Da Gama *et al*. (2003), and compared with the literature (Sims *et al*. 1974; Konig and Wright, 1997).

Parasites and cells

Epimastigote forms of *T. cruzi, Y* strain were maintained at 28 °C by weekly transfers in liver infusion tryptose medium – LIT (Camargo, 1964), supplemented with 10% inactivated foetal bovine serum (FBS) (Gibco Invitrogen Corporation, NY, USA). Trypomastigote forms were obtained from the supernatant of a monolayer of infected $LLCMK_2$ cells in Dulbecco's modified Eagle's medium (DMEM, Gibco Invitrogen Corporation, New York, USA) in 5% CO_2 at 37 °C. $LLCMK_2$ (epithelial cells of monkey kidney – *Macaca mulatta*) were maintained in DMEM supplemented with 2 mM L-glutamine, 10% FBS, and 50 mg/l gentamicin, buffered with sodium bicarbonate.

Anti-proliferative activity of elatol on the epimastigote form

Epimastigote forms of *T. cruzi* (10^6 cells/ml) were cultured in LIT medium supplemented with 10% FBS, in the absence or presence of different concentrations (3·0 to 300·0 μM) of elatol (from stock solution in 1% dimethyl sulfoxide). Parasites were incubated at 28 °C for 96 h in 24-well microplates. After that, cell growth was determined by counting the parasites with a Neubauer haemocytometer (Improved Double Neubauer) and the results were expressed as the percentage of inhibition in relation to the control cultured in medium alone. The IC_{50} (concentration that inhibited 50% parasite growth) and IC_{90} (concentration that inhibited 90% parasite growth) were determined by logarithm regression analysis of the data obtained. Benznidazole (N-benzyl-2-nitro-1-imidazolacetamide) – Rochagan® (Roche Pharmaceuticals, Rio de Janeiro, Brazil) was used as the reference drug. Each experiment was conducted in duplicate and repeated at least 3 times.

Effect of elatol on the viability of the trypomastigote form

Trypomastigote forms (10^7 cells/ml) were re-suspended in DMEM medium supplemented with 20% FBS, containing 10% mouse blood, in the absence or presence of different concentrations (0·3 to 60·0 μM) of elatol. Parasites were incubated at 37 °C in a 5% CO_2 air mixture for 24 h in 96-well microplates. The IC_{50} (concentration which lysed 50% of the parasites) was calculated in accordance with the Pizzi-Brener method (Brener, 1962). Crystal violet was used as the reference drug. Each experiment was conducted in duplicate and repeated at least 3 times.

Activity of elatol on the intracellular amastigote forms

To assess the *in vitro* activity against intracellular *T. cruzi* amastigotes, $LLCMK_2$ cells were seeded at a

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concentration of $2{\cdot}5 \times 10^5$ cells/ml in 24-well microplates containing glass cover-slips and DMEM medium, and then maintained at 37 °C for 24 h to allow cell adhesion to the cover slips. Trypomastigotes were added to the wells at a concentration of 10 parasites per host cell and incubated for 24 h. Then, non-internalized trypomastigotes were washed and the infected $LLCMK_2$ cells were treated with different concentrations (1·5 to 12·0 μM) of elatol for 96 h at 37 °C with 5% CO_2 atmosphere, following fixation in methanol and Giemsa staining. The number of amastigotes was determined by counting at least 200 cells in duplicate cultures, and the results were expressed as the survival index. The survival index was obtained by multiplying the percentage of infected cells by the number of amastigotes per infected $LLCMK_2$ cell. Each experiment was conducted in duplicate and repeated at least 3 times.

Red blood cell lysis assay

The potential haemolytic effect of elatol was evaluated in this assay. A 4% suspension of fresh defibrinated human blood was prepared in sterile 5% glucose solution. Several concentrations (1·2 to 60·0 μM) of the elatol were added to individual test tubes and gently mixed, and the tubes were incubated at 37 °C. After 1 h of incubation, the visual reading was made, and after 2 h the samples were centrifuged at 250 ***g*** for 5 min. The absorbance of the supernatant was determined at 540 nm for estimation of haemolysis. The results were expressed as the percentage of haemolysis. Amphotericin B (Cristalia, São Paulo, Brazil) was used as the reference drug, Triton X-100 (Vetec, Rio de Janeiro, Brazil) was used as the positive control, and the cell suspension alone was used as the negative control. Each experiment was conducted in duplicate and repeated at least 3 times.

Cytotoxicity assay

A suspension of $LLCMK_2$ cells was seeded at a concentration of $2{\cdot}5 \times 10^5$ cells/ml in a 96-well microplate containing DMEM medium supplemented with 10% FBS, and then maintained at 37 °C in 5% CO_2 air mixture for 24 h until confluence was achieved. Thereafter, the cells were treated with different concentrations (0·3 to 300·0 μM) of elatol for 96 h under the same conditions as above. Control wells without elatol were included, and Benznidazole was used as the reference drug. Subsequently, the sulforhodamine B colourimetric assay was carried out. Absorbance was read in a 96-well plate reader (BIO-TEK Power Wave XS) at 530 nm. The 50% Cytotoxicity Concentration (CC_{50}) was extrapolated by linear regression analysis. The cytotoxicity of elatol on $LLCMK_2$ cells was also compared with the activity against trypomastigote and intracellular amastigote forms of *T. cruzi*, by using the selective index (SI) (ratio: CC_{50} $LLCMK_2$ cells/IC_{50} protozoa). All experiments were performed in duplicate. The means were determined from at least 3 experiments.

Electron microscopy

Epimastigote, trypomastigote, and intracellular amastigote forms of *T. cruzi* were treated with elatol and then processed for electron microscopy. Parasite cells were harvested and washed twice with PBS, and fixed with 2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer at 4 °C. For transmission electron microscopy (TEM), cells were post-fixed in a solution containing 1% OsO_4, 0·8% potassium ferrocyanide, and 10 mM $CaCl_2$ in 0·1 M cacodylate buffer, dehydrated in an increasing acetone gradient, and embedded in Epon® resin. Next, ultrathin sections were stained with uranyl acetate and lead citrate, and images were obtained on a Zeiss 900 TEM. For scanning electron microscopy (SEM), epimastigote and trypomastigote forms of *T. cruzi* were fixed as before. Next, small drops of the sample were placed on a specimen support with poly-L-lysine. The samples were dehydrated in graded ethanol, critical-point dried in CO_2, coated with gold, and observed on a Shimadzu SS-550 SEM.

Statistical analyses

Statistical analysis was performed with the program GraphPad Prism 4 (GraphPad Software, San Diego, California, USA). Student's *t*-test was applied, and a *P* value less than 0·05 was regarded as significant. All experiments were performed in duplicate. The means and standard deviations were determined from at least 3 experiments.

RESULTS

Chemical composition of L. dendroidea

The physical and spectroscopic properties of the compound elatol are shown in Table 1, and were identical with previously reported data (König and Wright, 1997). Elatol (Fig. 1) is a colourless oil, and has been described in several species of the genus: *Laurencia elata* (Sims *et al*. 1974), *Laurencia obtusa* (Hay *et al*. 1987), and *Laurencia rigida* (König and Wright, 1997). The oil plays several ecological (Hay *et al*. 1987, 1988; de Nys *et al*. 1996; König and Wright, 1997; Steinberg *et al*. 1998; Da Gama *et al*. 2003) and pharmacological roles (Vairappana *et al*. 2001; Bansemira *et al*. 2004).

Anti-trypanosomal activity of elatol

Elatol was initially tested *in vitro* against epimastigote forms of *T. cruzi* Y strain. Figure 2 A shows that elatol had a dose-dependent activity after 96 h of

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Table 1. ^{1}H NMR ($CDCl_3$, 299·9 MHz) and ^{13}C NMR ($CDCl_3$, 75·0 MHz) data for elatol

	Present study	König and Wright (1997)	Present study	König and Wright (1997)
H#C	d_H (nH, *m*, *J*)[a]	d_H (nH, *m*, *J*)[a]	Dc ^{13}C-APT	Δc ^{13}C
1	2·08 (1 H, *br s*)	2·08 (1 H, *br d*, 17·5)	38·6 (CH_2)	38·4
	n.o	2·19 (1 H, *br d*, 17·5)		
2	—	—	128·0 (C)	127·9
3	—	—	124·1 (C)	124·0
4	1·85 (1 H, *m*)	1·82 (1 H, *m*)	29·3 (CH_2)	29·2
	1·98 (1 H, *d*, 3·0)	1·96 (1 H, *m*)		
5	1·63 (1 H, *m*)	1·62 (1 H, *m*)	25·6 (CH_2)	25·4
	1·81 (1 H, *m*)	1·80 (1 H, *m*)		
6	—	—	49·1 (C)	49·0
7	—	—	140·7 (C)	140·6
8	2·50 (1 H, *dd*, 3·0, 14·7)	2·49 (1 H, *dd*, 2·8, 14·4)	38·0 (CH_2)	37·8
	2·36 (1 H, *dm*, 15·0)	2·19 (1 H, *dm*, 14·4)		
9	4·15 (1 H, *dd*, 3·0, 6·6)	4·14 (1 H, *m*)	72·1 (CH)	72·0
10	4·61 (1 H, *d*, 2·7)	4·61 (1H, *d*, 2·9)	70·8 (CH)	70·7
11	—	—	43·1 (C)	43·0
12	1·07 (3 H, *s*)	1·06 (3 H, *s*)	20·7 (CH_3)	20·6
13	1·08 (3 H, *s*)	1·07 (3 H, *s*)	24·2 (CH_3)	24·1
14	4·80 (1 H, *br s*)	4·79 (1 H, *br s*)	115·8 (CH_2)	115·7
	5·13 (1 H, *br s*)	5·12 (1 H, *br s*)		
15	1·71 (3 H, *br s*)	1·70 (3 H, *br s*)	19·4 (CH_3)	19·3
OH	n.o.	2·19 (1 H; *br s*)		

[a] nH, Number of hydrogens; m, multiplicity; *J* (Hz), coupling constant; n.o., not observed.

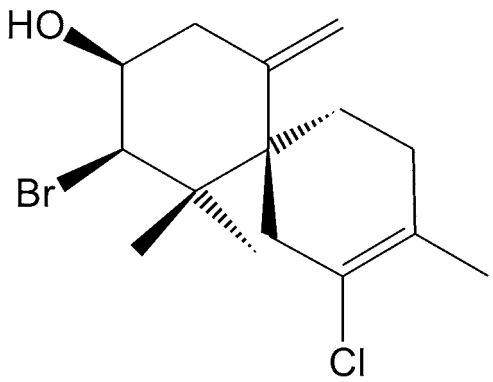

Fig. 1. Chemical structure of elatol, the major secondary metabolite isolated from *Laurencia dendroidea*.

treatment, exhibiting an IC_{50} of $45·4 \pm 1·9\ \mu M$ and IC_{90} of $138·8 \pm 3·0\ \mu M$. At $300·0\ \mu M$ of the compound (the highest concentration tested), the parasites were completely arrested. The IC_{50} of the reference drug benznidazole was $7·8 \pm 1·4\ \mu M$. Figure 3A shows the activity of elatol on trypomastigote forms of the parasite. After 24 h of treatment, the effective concentration (IC_{50}) was $1·38 \pm 0·15\ \mu M$. The IC_{50} of the reference drug crystal violet was $12·8 \pm 2·6\ \mu M$. Elatol showed better activity against trypomastigotes than did the reference drug. We also investigated the activity of elatol against intracellular amastigotes during 96 h of incubation (Fig. 4A). The elatol showed a strong effect against intracellular amastigotes, with an IC_{50} and IC_{90} of $1·01 \pm 0·65\ \mu M$ and $3·0 \pm 1·8\ \mu M$, respectively. The survival indices were calculated as 427·5 for $1·5\ \mu M$, 186·5 for $3·0\ \mu M$, 98·0 for $6·0\ \mu M$, and 35·5 for $12·0\ \mu M$, while the control was determined as 1137·7. The EC_{50} value for benznidazole, the reference drug, was $24·3 \pm 1·4\ \mu M$. Therefore, elatol showed better activity against intracellular amastigotes than did the reference drug. All results were significant at $P \leqslant 0·05$ as compared to the control group, by Student's *t*-test. Direct observation of treated parasites by light microscopy showed a dose-dependent effect of elatol on intracellular amastigotes until the infection was completely eliminated (Fig. 4 B, C, D).

Cytotoxicity and haemolytic assay

In Table 2, the cytotoxicity of elatol from *L. dendroidea* on $LLCMK_2$ cells was compared with the activity against trypomastigote and intracellular amastigote forms of *T. cruzi*. Elatol caused no cytotoxic effect against the cell line after 96 h of treatment with concentrations up to $27·0 \pm 0·51\ \mu M$. The compound was more selective (about 20·0 times) for trypomastigotes than for $LLCMK_2$ cells. The results for intracellular amastigote forms showed that elatol is 26·7 times more selective against the parasites than the mammalian cells. In the haemolytic assay, we evaluated the toxicity of elatol to human red blood cells (Fig. 5). The treatment with elatol did not affect red blood cell integrity at concentrations that inhibit the growth of forms of *T. cruzi*. At $60·0 \pm 0·44\ \mu M$ (the highest concentration tested), elatol caused only 21% haemolysis. In contrast, cells treated with

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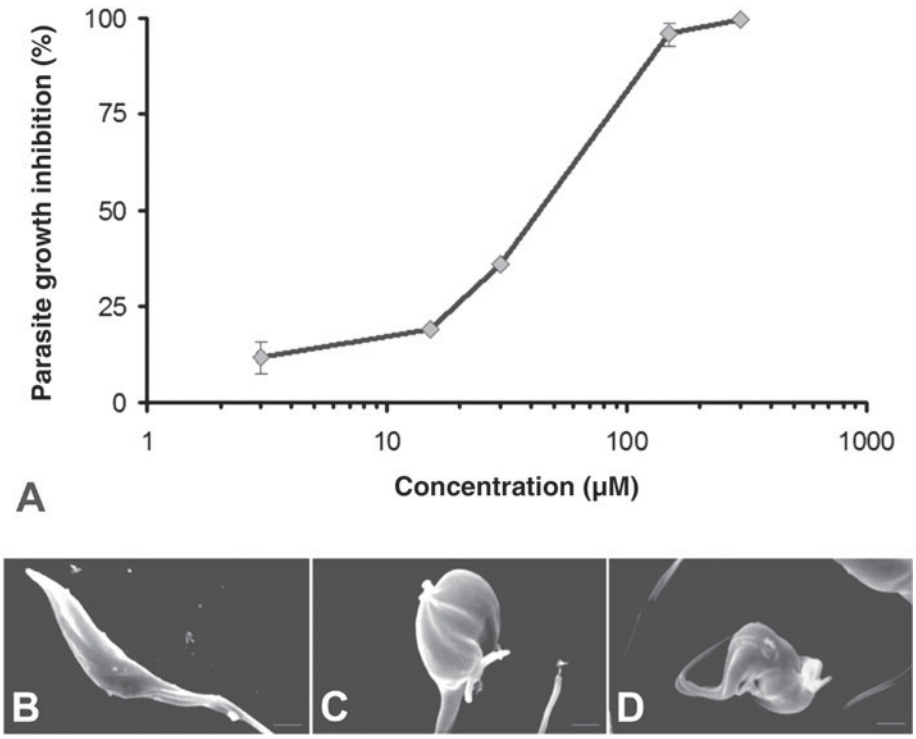

Fig. 2. Effects of elatol against the epimastigote form of *Trypanosoma cruzi Y* strain. (A) Activity of the compound on growth of *Trypanosoma cruzi*. The protozoa were cultured for 96 h in the presence of concentrations of 3·0, 15·0, 30·0, 150·0, and 300·0 μM, and an untreated control. Each experiment was conducted in duplicate and repeated at least 3 times. The results were analysed as percentages of growth inhibition in relation to untreated parasites. Bars represent standard errors. All results were significant at $P \leqslant 0{\cdot}05$ as compared to the control group, by Student's *t*-test; morphological alterations were observed by S.E.M. (B) Control; (C) Parasites treated with IC_{50} and (D) Parasites treated with IC_{90} for 96 h. Scale bars = 1 μM.

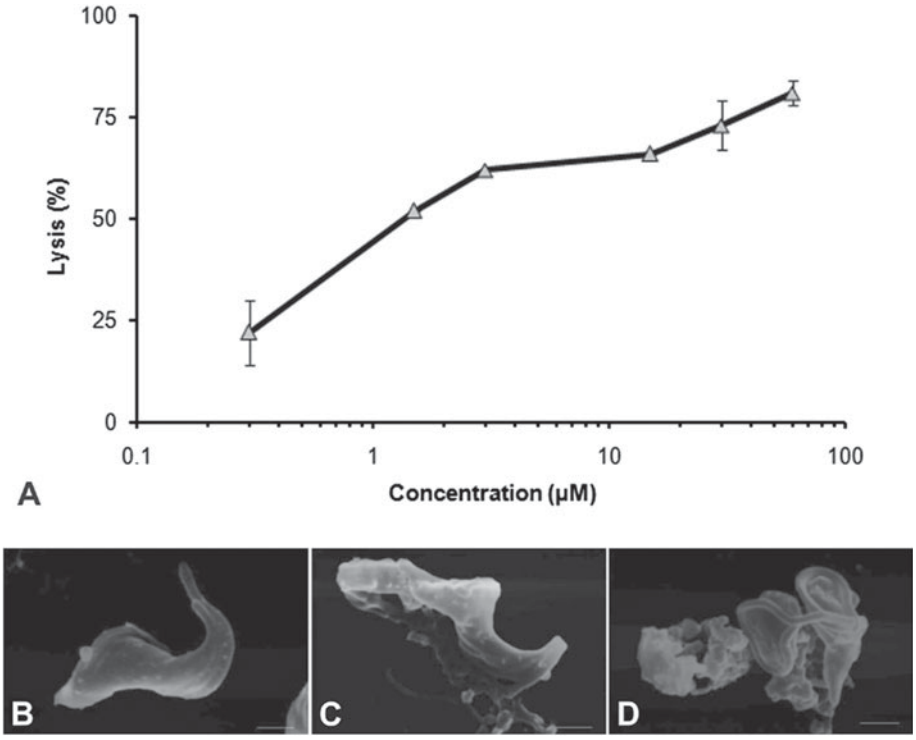

Fig. 3. Activity of elatol on trypomastigotes, the infective form of *Trypanosoma cruzi*. (A) The parasites (10^7 cells/ml) were re-suspended in the absence or presence of different concentrations of the compound (0·3, 1·5, 3·0, 15·0, 30·0, and 60·0 μM). Each experiment was conducted in duplicate and repeated at least 3 times. All results were significant at $P \leqslant 0{\cdot}05$ as compared to the control group, by Student's *t*-test; morphological alterations were observed by S.E.M. (B) Control, (C) parasites treated with IC_{50} and (D) parasites treated with IC_{90} for 96 h. Scale bars = 1 μM.

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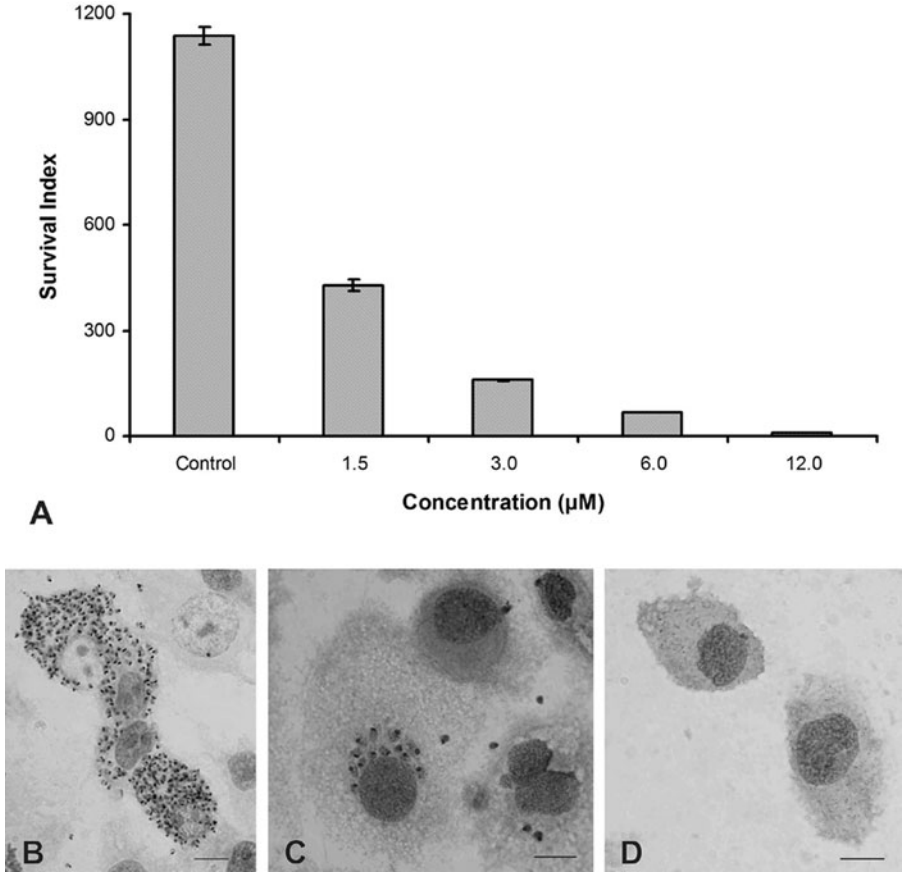

Fig. 4. Effect of elatol on the *Trypanosoma cruzi* – $LLCMK_2$ cell interaction. (A) $LLCMK_2$ cells were infected with the trypomastigote form and then treated with elatol. The survival indices were determined by the equation: number of infected cells × mean of amastigotes per cell. The data represent the mean values from 3 independent experiments. All results were significant at $P \leqslant 0{\cdot}05$ as compared to the control group, by Student's *t*-test. (B) Light microscopy of *T. cruzi*-infected $LLCMK_2$ cell line after 5 days, untreated. The cells were Giemsa-stained and examined under a light microscope at 40x magnification. Cells with intracellular amastigotes (arrow-heads). (C) Cells treated with 1·0 μM of elatol; (D) cells treated with 3·0 μM of elatol. Scale bars = 20 μM.

Table 2. Comparison of values of CC_{50} for $LLCMK_2$ cells with IC_{50} trypomastigote and intracellular amastigote forms of *T. cruzi*, and their respective selectivity indices (SI)

	IC_{50} (μM)	SI*
$LLCMK_2$ cells	27·0 ± 0·51	
Trypomastigote	1·38 ± 0·15	20·0
Intracellular amastigote	1·01 ± 0·65	26·7

* SI, CC_{50} $LLCMK_2$ cells/EC_{50} of *T. cruzi* forms.

Amphotericin B (AMPB) showed 75% haemolysis with this concentration. We also observed that the red blood cell control with or without 1·0% DMSO did not show haemolysis, whereas the Triton X-100 positive control showed 100% haemolysis.

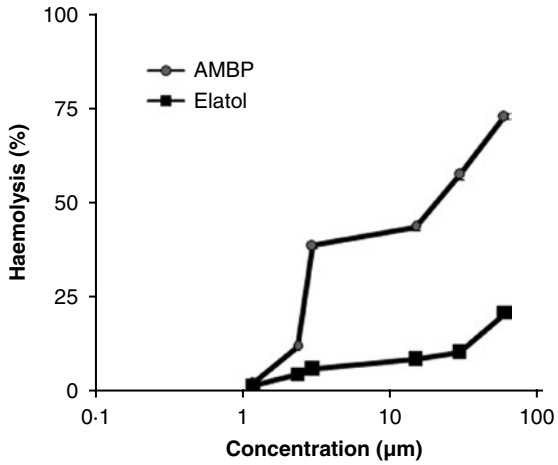

Fig. 5. Haemolytic properties of elatol obtained from *Laurencia dendroidea*. Amphotericin B (AMPB) was included in the assay as a reference drug. The data represent the mean values from 3 independent experiments. All the assays were carried out in duplicate.

Effect of elatol on the morphology and ultrastructure of T. cruzi

Figure 2 C and D and Fig. 3 C and D show morphological alterations in epimastigotes and trypomastigotes, respectively, treated with concentrations corresponding to the IC_{50} and IC_{90} value of elatol for each form. Untreated control epimastigotes

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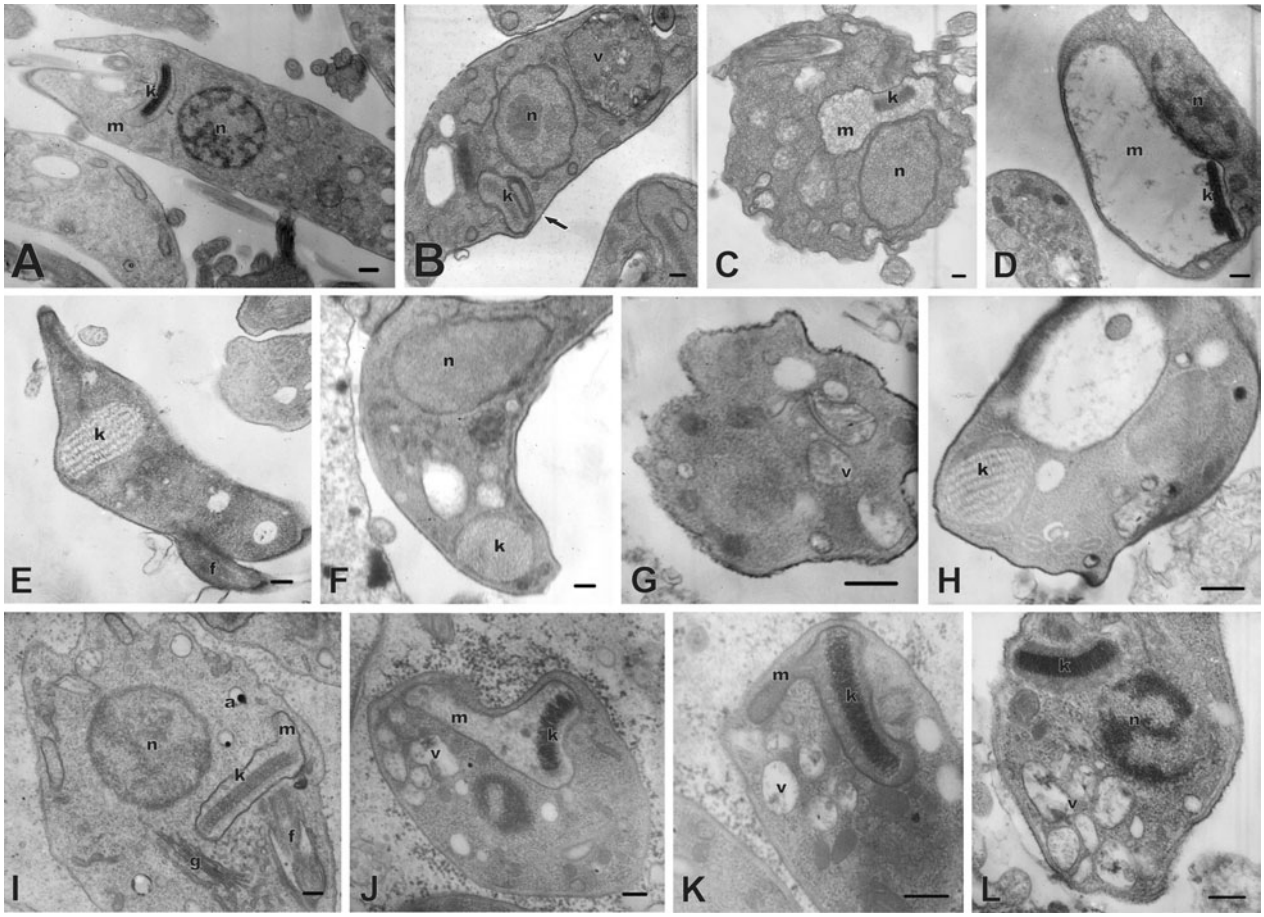

Fig. 6. Elatol caused marked ultrastructural changes in *Trypanosoma cruzi* epimastigote (A–D), trypomastigote (E–H), and intracellular amastigote forms (I–L); TEM images of (A) epimastigote control, kinetoplast (k), mitochondrion (m), and nucleus (n); (B–D) cell treated with IC_{50}; (E) trypomastigote control, kinetoplast (k) and flagellum (f); (F–H) cells treated with IC_{50}; (I) amastigote control, kinetoplast (k), mitochondrion (m), nucleus (n), Golgi complex (g), acidocalcisome (a), and flagellum; (J–L) cells treated with IC_{50}. Scale bar = 1 μM.

(Fig. 2 B) and trypomastigotes (Fig. 3 B) showed the typical elongated shape. However, when the parasites were treated with elatol, we observed notable morphological changes, such as the appearance of aberrant-shaped cells. Ultrastructural changes in the 3 forms of *T. cruzi* treated with elatol are illustrated in Fig. 6. Untreated epimastigotes, trypomastigotes, and intracellular amastigotes showed no plasma membrane alterations and organelles with normal morphology (Fig. 6 A, E and I). Similar ultrastructural alterations were observed in all forms of the parasite treated with elatol. The most prominent effects observed in treated parasites were swollen mitochondria (Fig. 6 B-D, J-L), and extensive formation of cytoplasmic vacuoles in all the treated cells.

DISCUSSION

The undesirable side-effects associated with classical trypanocidal drugs, as well as the development of resistance, are encouraging research for alternative synthetic (Tonin *et al*. 2009; Valdez *et al*. 2009) or natural (Luize *et al*. 2005, 2006; Izumi *et al*. 2008; Moreira *et al*. 2009) compounds that are effective for the treatment of Chagas' disease. In this investigation, we demonstrated that the sesquiterpene elatol, the major constituent of the Brazilian red seaweed *L. dendroidea* (Hudson) J. V. Lamouroux, showed important activity against epimastigote, trypomastigote, and amastigote forms of *T. cruzi*. Our data showed that elatol had a dose-dependent activity against the epimastigote form after 96 h of treatment, exhibiting an IC_{50} of $45{\cdot}4 \pm 1{\cdot}9$ μM. Moreover, elatol was effective in killing trypomastigotes with a concentration of $1{\cdot}38 \pm 0{\cdot}15$ μM (IC_{50}), and intracellular amastigotes with an IC_{50} value of $1{\cdot}01 \pm 0{\cdot}65$ μM. The effect of the reference drug for the trypomastigote form, crystal violet, showed an IC_{50} of $12{\cdot}8 \pm 2{\cdot}6$ μM. In addition, the IC_{50} value of the reference drug used for intracellular amastigotes, benznidazole, was $24{\cdot}3$ μM. Therefore, elatol showed better activity against trypomastigote and intracellular amastigote forms than did the reference drug. These results are especially interesting because trypomastigotes and intracellular amastigotes are the forms that are present in the vertebrate host, and

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pose a challenge for treatment of Chagas' disease. A previous report described significant inhibitory action of 2 other marine algae, *Fucus evanescens* and *Pelvetis babingtonii*, on the infection rate and the amastigote growth of *T. cruzi* in HeLa cells, with a weak inhibitory effect on epimastigotes (Nara *et al.* 2005).

The search for bioactive compounds originating from the sea is recent. The red alga *Laurencia microcladia* has been reported to have properties against *Plasmodium falciparum* (Mendiola-Martínez *et al.* 2005). In addition, the sesquiterpenes ((8*R*)-8-bromo-10-epi-beta-snyderol) and aromatic compounds (*p*-hydroxybenzaldehyde and *p*-methoxybenzyl) isolated from *Laurencia* sp. show antimalarial activity (Wright *et al.* 1996; Topcu *et al.* 2003). Studies with members of the Phaeophyta demonstrated anti-trichomonal activity and activity against *Trypanosoma brucei rhodesiense* and *Leishmania donovani* (Orhan *et al.* 2006).

An important criterion in the search for compounds active against *T. cruzi* with therapeutic potential is that they are not toxic to the mammalian host cells. Elatol showed promising parasite inhibition at dosages that did not show cytotoxicity to mammalian $LLCMK_2$ cells, and this resulted in a good selective index against the forms of the parasite that are present in the vertebrate host. Additionally, elatol showed lower haemolytic activity.

Observation by SEM of elatol-treated epimastigotes revealed swelling of the parasite body and shortening of the flagellum, when compared to control cells. Elatol-treated trypomastigotes showed distortion in the cell body and loss of integrity of the membrane. Several other studies have also demonstrated ultrastructural alterations in *T. cruzi* treated with synthetic or natural compounds (Salas *et al.* 2008; Valdez *et al.* 2009). Transmission electron microscopy indicated that epimastigotes treated with elatol showed intensely swollen mitochondria and the matrix became less electron dense, containing myelin-like figures, and damage to the plasma membrane also occurred. The treatment of trypomastigotes and intracellular amastigotes caused mitochondrial swelling and the formation of small vesicles within organelles, especially in the mitochondrion. Mitochondria of trypanosomatid parasites exhibit unique structural and functional features which are remarkably different from mammalian mitochondria, making this organelle an exceptionally attractive chemotherapeutic target (Menna-Barreto *et al.* 2009). In fact, through the years, several trypanocidal compounds have been designed that target parasite mitochondrial function. Mitochondrial disorganization and dysfunction have been described after treatment with different drugs for *T. cruzi* (Van-Hellemond *et al.* 2005; Luize *et al.* 2006; Menezes *et al.* 2006; Menna-Barreto *et al.* 2007, 2009).

This is the first report of the *in vitro* antitrypanosomal effect of the sesquiterpene elatol. Although the mode of action likely includes a specific metabolic pathway of the parasites, it still remains to be elucidated, which will be the subject of our further studies as well as *in vivo* studies. Molecular identification and characterization of enzymes and metabolic pathways that are essential and distinct in *T. cruzi* show the greatest potential as primary targets for screening bio-resources *in vitro*, in the search for a new generation of chemotherapies.

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