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Evaluation of the Effects of an Aqueous Extract of *Schinus terebinthifolius* Involving Wild Bacterial Culture of *Escherichia Coli*

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ABSTRACT

Stannous chloride ($SnCl_2$) is the most used reducing agent in the marking of radiopharmaceuticals, but is highly toxic to cells, including cultures of *Escherichia coli*, because the formation of free radicals. Some plant extracts have protective actions against the damaging effect of $SnCL_2$. We evaluate these damaging effects on wild culture of *E. coli* AB1157 (wild-type), and the effect of the agent together with an aqueous extract prepared from fresh leaves of *Schinus terebinthifolius* , already known as an extract help the healing process. Two experimental evaluations were conducted, evaluation of bacterial survival in liquid and solid medium. In the evaluation in liquid medium, aliquots of bacterial suspension was incubated with: (i) 0.9% NaCl, (ii) *S. terebinthifolius* extract, (iii) $SnCl_2$ + *S. terebinthifolius* extract, (iv) $SnCl_2$. After 60 min, aliquots of each culture was spred on petri dishes containing solid medium. In the evaluation of solid medium, plates containing solid medium prepared with bacterial culture for assessment of growth inhibition. Five discs of 6 mm were placed on each plate, and each was given samples of the following solutions: (i) 0.9% NaCl, (ii) *S. terebinthifolius* extract (iii) *S. terebinthifolius* extract + $SnCl_2$, (iv) $SnCl_2$, (v) Amoxicillin + Clavulanic acid. After overnight incubation of the samples of the evaluation in a liquid medium, growth of cultures of *E. coli* was observed in the plates that cultures were incubated with a solution of $SnCl_2$ with *S. terebinthifolius* extract. And after overnight incubation of samples of the evaluation in a solid medium, there was no inhibition zone where the disks were administered solution of $SnCl_2$ along with *S. terebinthifolius* extract. This suggests that the extract has no toxicity over the *E. coli* cultures and protects cells from the damaging action of $SnCl_2$.

INTRODUCTION

Stannous chloride ($SnCl_2$), has a great relevance in nuclear medicine, widely used as a reducing agent for technetium-99m (99mTc), widely used in the labeling of cells and molecules (Saha, 2004). Assessments of the use of $SnCl_2$ on colonies of microorganisms has been used frequently. One of these studies demonstrated the cytotoxic effects on cultures of *Escherichia coli(E. coli)* in liquid medium, reducing the survival fraction of cells, presumably by formation of excess free radicals in the medium, resulting in oxidative stress, thus causing damage to the cell [1-4].

Oxidative stress is caused by an imbalance between the mechanism of production of reactive oxygen species and the defense mechanisms of the biological system necessary to eliminate these reactive, and has been accepted as a factor involving various diseases, that may be chronic or acute and even involved in physiological aging [5].

Because of the wide use of tin in society, especially in nuclear medicine, it is evident the importance of studies involving this element in the assessment of biological effects.

Phytotherapy is the branch of medicine that uses natural products with known pharmacological effects, for therapeutic purposes. Where it is used as feedstock plant parts like leaves, stems, roots, flowers and seeds [6-7]. Many scientific studies have been developed and made in recent years, confirming the growing interest in the scientific community for this area, in order to better understand the biological effects associated with phytotherapy [8].

Schinus terebinthifolius, also known as Aroeira, corneíba, fruit-of-thrush, Cambuí, White Aroeira is a big tree, bark thin and scaly. It has compound leaves of leaflets lanceolate and pointed, numerous flowers arranged in pedicles, small white or greenish yellow. Its fruit is a red drupe and glossy, which resembles the smell of pepper. It is native to Peru and is also found in Europe, Asia and American countries, including Brazil [9]. The main parts of *S. terebinthifolius* reported in scientific studies are the bark, liber and leaves [10]. Examples of its use is the preparation of the ethanol extract of the bark of *S. terebinthifolius* for its use as an agent assisting in the healing of skin wounds, the healing of surgical wounds, and treatment of cervicitis and genital discharge [8, 11-13].

The objective of this study was to evaluate the biological effects of aqueous extract prepared from fresh leaves of *S. terebinthifolius* using experimental models with bacteria, assessing the survival of strains of wild *E. coli* in liquid and solid medium, using $SnCl_2$ as a damaging agent to the cells.

MATERIALS AND METHODS

Reagent and extract preparation

For the preparation of the solution of $SnCl_2$, salt tin was weighed, dissolved in 0.9% NaCl solution and used immediately. And the concentration used (25µg/mL) was the same used in other studies [1, 14,15].

The aqueous extract processed from fresh leaves of *S. terbinthifolius* was prepared according to the Brazilian Pharmacopoeia (1ª edition), with some modifications. Every 2 L of mineral water were used 800 g of fresh leaves. The extract was stored in amber bottles, identified and kept in freezer (Blood Plasma Freezer - Indrel. Model: GPS 10-D) until its lyophilization. The extract was lyophilized (Lyophilized Liotop. Model: L202 - Liobras) and stored at - 28°C freezer (Blood Plasma Freezer - Indrel. Model: GPS 10-D).

The aqueous extract of *S. terebinthifolius* was obtained from the dilution of 100 mg of lyophilized in 10 ml of 0.9% NaCl solution, thereby obtaining an extract concentration of 10 mg/mL. The extract was homogenized in vortex (vortex tubes AP56 Mod Phoenix - Bivolt) for 1 min.

Bacterial strain

The strain used in the experiments was Escherichia coli (*E. coli*) AB1157, wild-type strain proficient for DNA damage repair [16].

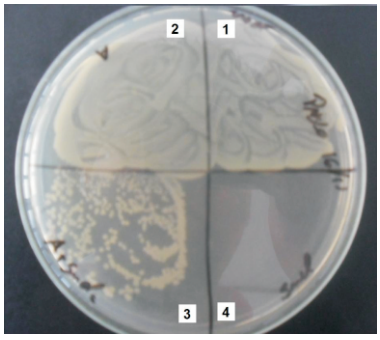

Figure 1: Plate on LB solid medium seeded with culture of *E. coli* in exponential phase, incubated with different experimental solutions (1 - 0.9% NaCl, **2** – *S. terebinthifolius* extract (10 mg / mL), **3** – *S. terebinthifolius* extract (10 mg / mL) + SnCl2 (25 mg / mL) **4** - $SnCl_2$ (25 mg / mL))

Growth media and cunture conditions

The culture of *E. coli* was grown in overnight in bath shaker at 37°C in liquid LB medium, So they could reach the stationary phase of growth. The starting aliquot (0.2 mL) was taken from this culture, inoculated in fresh the same medium, and incubated once again, for 2 hours, up to reach the exponetial phase (1-2 x 10^8 cells/mL) [1].

Survival of bacteria in liquid medium

Culture of *E. coli* in exponential growth phase was centrifuged, washed and resuspended in 0.9% NaCl. Cultures were incubated in bath shaker at 37°C with: (1) 0.9% NaCl (600 µL), (2) *S. terebinthifolius* extract (100 µL of extract 10 mg/mL + 500 µL of 0.9% NaCl); (3) $SnCl_2$ + extract (500 µL of $SnCl_2$ 25µg/mL + 100 µL of *S. terebinthifolius* extract 10 mg/mL); (4) $SnCl_2$ (500 µL de $SnCl_2$ 25µg/mL + 100 µL of 0.9% NaCl). After 60 min, aliquots were withdrawn, diluted and spread on glass Petri dishes with solid LB (Luria Broth) medium plus 1.5% agar noble.

Bacterial survival in solid medium

For this evaluation two different aqueous extracts of *S. terebinthifolius* were prepared from the dilution of 300 mg and 400 mg of lyophilized in 10 ml at 0.9% NaCl solution, thereby obtaining an extract concentration of 30 mg/mL and a 40 mg/mL, respectively. The extract was homogenized in vortex (vortex tubes AP56 Mod Phoenix - Bivolt) for 1 min.

Aliquot (0.1mL) from suspension culture of *E. coli* in exponential phase was added to LB medium (3 ml) plus 1.5% Noble agar, maintained at 45°C, and administered on solid LB medium. After 10 min, were added to each plate five discs of filter paper 6 mm in diameter. On the discs were given to 5µ: (i) 0.9% NaCl, as a negative control; (ii) *S. terebinthifolius* extract; (iii) *S. terebinthifolius* extract + $SnCl_2$ (25 µg/mL); (iv) $SnCl_2$ (25 µg/mL); (v) Amoxicillin + Clavulanic acid (50 µg/mL + 12,5 µg/mL), as a positive control. After 10 minutes of inoculation, the plates were incubated overnight for 24 hours at 37ºC.

The experiment was conducted with two concentrations of extract, at 30 mg/mL and 40 mg/mL.

RESULTS

The results of the evaluation of bacterial survival in liquid medium is shown in Figure 1, indicating the damaging effect of $SnCl_2$ on cultures of *E. coli* in liquid medium and reduced of cell death in culture treated with *S. terebinthifolius* aqueous extract.

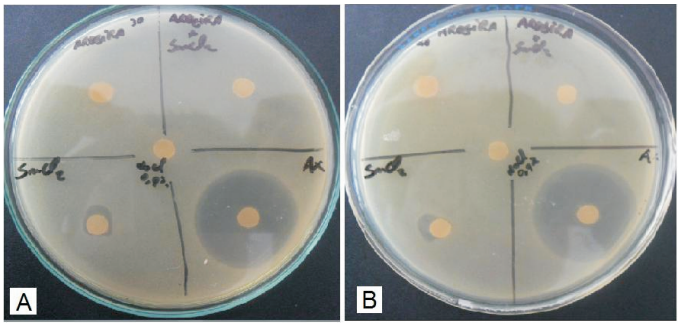

Figure 2: Analysis on solid medium about the effects of *S. terebinthifolius* aqueous extract in concentrations of 30 and 40 mg/mL on culture of *E. coli*. The first image (A) corresponds to analysis with the extract at 30 mg / mL and the second image (B) corresponds to analysis with the extract at 40 mg/mL.

You can view the results of the analysis of bacterial survival in solid medium in Figure 2. This figure enables the visualization of inhibition zone around the disk containing $SnCl_2$, again indicating its damaging effects, and the absence of inhibition zone on the disk where it was inoculated $SnCl_2$ solution, plus the *S. terebinthifolius* aqueous extract. It suggesting that the extract at a concentration of 30 mg / mL protects the cell from the damaging power of $SnCl_2$.

DISCUSSION

As shown in Figure 1, the first quadrant corresponding to bacterial growth in 0,9% NaCl solution was seen considerable growth of *E. coli*, also observed in the second quadrant, corresponding the bacterial growth in *S. terebinthifolius* extract (10 mg / mL). Through analysis of these plates may suggest that the aqueous extract of *S. terebinthifolius* has no damaging effect on bacterial growth in liquid medium.

The four quadrant corresponding to bacterial growth in a solution of $SnCl_2$ (25 mg / mL), where was not obtained any colony-forming unit. Based on this data, it is suggested that the dose of $SnCl_2$ is lethal to the bacterial culture of *E. coli* wild-type.

In the third quadrant, corresponding to bacterial growth in *S. terebinthifolius* Extract (10 mg / mL) in conjunction with a solution of $SnCl_2$ (25 mg / mL), bacterial growth was observed. Therefore, it can be suggested that the *S. terebinthifolius* extract carries a protective effect against the damaging effect of $SnCl_2$ in the culture of *E. coli*.

In the plates of the analysis of bacterial survival on solid medium, the results showed no inhibition zones around the disks related to the 0.9% NaCl solution, as a negative control for cell death. The samples showed massive inhibition zones around the disks for the antibiotic, as a positive control for cell death. On discs corresponding the solution of $SnCl_2$ (25 mg / mL), found little inhibition zones around the disks, suggesting that the damaging effect of stannous chloride is also observed in solid medium. However, the disks were inoculated *S. terebinthifolius* extracts concentrations of 30 and 40 mg / mL, demonstrated the absence of inhibition zones around the disk corresponding to the *S. terebinthifolius* extract, plus solution of $SnCl_2$ (25 mg / mL), and can thus suggest that the extract in these concentrations has a protective effect on bacterial cultures against injuring action of $SnCl_2$ on solid medium.

CONCLUSION

Before the results, we suggest that the aqueous extract processed from the infusion of fresh leaves of *S. terebinthifolius* at a concentration of 10 mg / mL, has a protective action against the harmful effects of $SnCl_2$ in liquid medium.

Additionally, based on analysis of the results is permissive to speculation that the extract studied at concentrations of 30 and 40 mg / mL has a protective action against the harmful effects of $SnCl_2$ in tests on solid medium.

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