



Influence of an aqueous extract of *Hypericum perforatum* (Hypericin) on the survival of *Escherichia coli* AB1157 and on the electrophoretic mobility of pBSK plasmid DNA

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RESUMO: “Influência de um extrato aquoso de *Hypericum perforatum* (Hipericina) na sobrevivência de *Escherichia coli* AB1157 e na mobilidade eletroforética de DNA plasmidial pBSK”. Hiperico (*Hypericum perforatum* or St John’s worth) tem sido usado como uma planta medicinal para tratar a depressão. Hipericina é o principal componente do hiperico. O cloreto estano (SnCl₂) é o agente redutor mais utilizado em medicina nuclear. O objetivo desse trabalho foi verificar o efeito de um extrato de hiperico na sobrevivência de *Escherichia coli* AB1157 e na topologia do DNA plasmidial. Culturas de *E. coli* AB1157, em fase exponencial, foram incubadas com SnCl₂ na presença ou ausência de hipericina. Aliquotas foram espalhadas em placas de Petri contendo meio sólido, as unidades formadoras de colônias foram contadas após incubação e as frações de sobrevivência calculadas. DNA plasmidial foi incubado com SnCl₂ na presença ou ausência de hipericina durante 40 minutos, eletroforese em gel de agarose a 0,8% foi realizada, o gel foi corado com brometo de etídio e as formas (bandas) topológicas do plasmídeo visualizadas. Os resultados revelaram que o extrato de hiperico não foi capaz de alterar a sobrevivência da cultura de *E. coli* e a topologia do DNA plasmidial, mas protegeu as bactérias contra a ação do SnCl₂. Os resultados sugerem ausência de efeitos citotóxicos e genotóxicos do extrato aquoso do hiperico e um efeito protetor nas células de *E. coli* contra a ação do SnCl₂.

Unitermos: *Hypericum perforatum*, Hypericaceae, *Escherichia coli*, DNA plasmidial, eletroforese, cloreto estano.

ABSTRACT: Hiperico (*Hypericum perforatum* or St John’s worth) has been widely used as an herbal medicine to treat depression. Hypericin is the main chemical compound of hiperico. Stannous chloride (SnCl₂) is the most used reducing agent in nuclear medicine. The aim of this work was to verify the effect of a hiperico extract on the survival of *Escherichia coli* AB1157 and on the plasmid DNA topology. Exponentially *E. coli* AB1157 cultures were incubated with SnCl₂ in the presence or absence of hiperico. Aliquots were spread onto Petri dishes containing solidified rich medium, the colonies units were counted after overnight and the survival fraction was calculated. Plasmid DNA samples were incubated with SnCl₂ in presence or absence of hiperico extract during 40 minutes, 0.8% agarose gel electrophoresis was performed, the gel was stained with ethidium bromide and the plasmid topological forms (bands) were visualized. The results revealed that hiperico extract is neither capable of altering the survival of *E. coli* cells nor the plasmid DNA topology but it may have protected these cells against the SnCl₂ action. The data suggest absence of cytotoxic and genotoxic effects of the aqueous hiperico extract and a protective effect on *E. coli* cells against the action of SnCl₂.

Keywords: *Hypericum perforatum*, Hypericaceae, *Escherichia coli*, DNA, electrophoresis, *Hypericum perforatum*, stannous chloride.

INTRODUCTION

The use of natural products, as medicinal plants, is very frequent worldwide. Hiperico (*Hypericum*

perforatum or St John’s worth) has been widely used as an herbal medicine (Carvalho et al., 2008; Cordeiro et al., 2005). Hypericin is the main chemical compound of the hiperico (McGarry et al., 2007). Authors have

reported several pharmacological actions of this chemical compound (hypericin) (Wurglics and Schubert-Zsilavecz, 2006). Several studies have demonstrated the efficacy of St John's wort as an antidepressant agent in human beings, besides of its testing in animal behavioral models of depression (Hunt et al., 2001; Werneke et al., 2004; Linde et al., 2005; McGarry et al., 2007) and dysthymia (Rotblatt and Ziment, 2002). This natural product has been also used in folk medicine to treat hemorrhoids and bruises, as well as to induce vasodilatation (Hunt et al., 2001; Siepmann et al., 2004; Jaric et al., 2007). St John's wort reduces the effects of several drugs including immunosuppressants, oral contraceptives, oral anticoagulants and HIV protease inhibitors (Mannel, 2004; Busti et al., 2004; Bobrov et al., 2007). Pharmacodynamic effects on neuronal excitability also were reported (Neagoe et al., 2004). Recently, an anti-inflammatory action was demonstrated in an experimental model (Sosa et al., 2007).

Free radicals (an unpaired electron in their outer valence shell) are highly reactive species of molecules that could be produced by some oxidative cellular mechanisms (Infanger et al., 2006). If these molecules are produced in excess, they can induce tissue damage. There are internal mechanisms in the living organisms constituted by enzymes and other molecules to protect the cells of these free radicals (Hsieh et al., 2005; Kinoshita et al., 2005; Bao and Lou, 2006; Marcus et al., 2006). In addition to these defense mechanisms, certain medicinal compounds, including vitamins and other nutrition products could inhibit the production of free radicals (Infanger et al., 2006; Barbosa-Filho et al., 2008).

Stannous chloride (SnCl_2) is the most used reducing agent in nuclear medicine to label cellular and molecular structures with biological interest with technetium-99m to be used as radiobiocomplexes. Some authors have performed studies about the cytotoxic/genotoxic potentials of SnCl_2 (Pungartnik et al., 2005; Fernandes et al., 2005; Almeida et al., 2007; Presta et al., 2007; Aquino et al., 2007; Paoli et al., 2008). Using bacterial cultures and plasmid DNA has been suggested that stannous chloride appears to induce damages in the deoxyribonucleic acid (DNA) by oxidative mechanisms related to free radicals generation (Dantas et al., 2002; El-Demerdash et al., 2005; Dantas et al., 1996). Data from assays with *Escherichia coli* (*E. coli*) deficient in DNA repair mechanisms suggested that this chemical agent could induce different lesions in DNA (El-Demerdash et al., 2005; Almeida et al., 2007). Thus, the aim of this work was to verify the effect of the hiperico aqueous extract on the survival of *E. coli* AB1157 and on the electrophoretic mobility of pBSK plasmid DNA.

MATERIAL AND METHODS

Preparation of the extract

The extract of hiperico was prepared with 5g of a purified dust of *Hypericum perforatum* (*Herbarium Laboratório Botânico LTDA*, Brazil, lot 954661) in 100 ml of 0.9% NaCl (saline). The quality controls of this hiperico preparation performed by the *Herbarium Laboratório Botânico LTDA* revealed that the major chemical compound is hypericin (95%). The preparation was homogenized in a vortex mixer and filtered through paper (quality filter paper) and 1ml was considered to contain 50 mg of the hypericin extract and regarded as 100% of the filtered solution. The quality control of this extract was evaluated with the analysis of the absorption spectra of a hypericin recorded in the range from 400 up to 700 nm with intervals of 20 nm using a spectrophotometer (Analyser 800M, *Analyser Comércio e Indústria LTDA*, São Paulo, Brazil), using a cuvette of 1 cm path length. All spectrophotometric measurements were performed on the same spectrophotometer and the value of the absorbance (0.414 ± 0.016) at 580 nm was used as marker and the quality control of each prepared hypericin in all experiments.

Bacteria inactivation

The *E. coli* AB1157, a wild-type strain, proficient to repair damage in the DNA, was used in this work. From stock (in glycerol 50% v/v) a sample (50 μl) of the culture was grown on liquid LB medium (5 ml, Lúria and Burrous, 1957) at 37 °C overnight on a shaking water bath (reciprocal water bath shaker, model R76, New Brunswick, USA) up to the stationary growth phase. A sample (200 μl) was taken from this culture and further incubated (20ml; liquid LB medium) under the same conditions to exponential growth (10^8 cells/ml). The cells were collected by centrifugation, washed twice in 10 ml of saline and suspended again in the same solution until they reached 10^8 cells/ml. Samples (1.0 ml) of these washed cultures (10^8 cells/ml) were incubated on the shaking water bath with (i) 0.5 ml of SnCl_2 (75 $\mu\text{g/ml}$) and 0.1 ml of saline, or (ii) 0.1 ml of hiperico extract (50 mg/ml) and 0.5 ml of saline, or (iii) 0.1 ml of hiperico extract (50 mg/ml) and 0.5 ml of SnCl_2 (75 $\mu\text{g/ml}$), or (iv) 0.6 ml of saline as a control, on initial time and after 60 min, at 37 °C. During the assay, at 0 and 60 min, aliquots (100 μl) were diluted with saline and spread onto Petri dishes containing solidified LB medium (1.5% agar). Colonies units formed after overnight incubation at 37 °C were determined. The survival fraction was calculated dividing the number of viable cells obtained per ml in each time of the treatment (N) by the number of viable cells obtained per ml in zero time (N0).

Analysis of DNA mobility alterations

Preparation of plasmids was performed using alkaline method, described by Sambrook et al (1989).

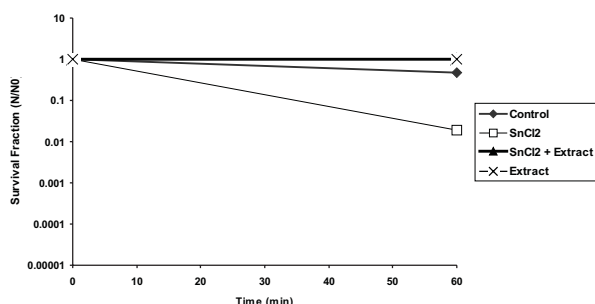


Figure 1. Effect of the hiperico extract on the inactivation induced by stannous chloride on *E. coli* AB1157. Exponential growth of *E. coli* suspended in saline and treated with stannous chloride for different incubation times (min) related with the presence or absence of the extract and with the extract alone.

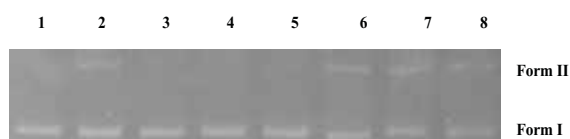


Figure 2. Agarose gel electrophoresis of pBSK Plasmid DNA (100 ng) treated with different concentrations of hiperico, alone or associated with stannous chloride (100 µg/ml). Lane 1: control; Lane 2: stannous chloride (100 µg/ml); Lane 3: hiperico (50 mg/ml); Lane 4: hiperico (5 mg/ml); Lane 5: hiperico (0.5 mg/ml); Lane 6: hiperico (50 mg/ml) and stannous chloride (100 µg/ml); Lane 7: hiperico (5 mg/ml) and stannous chloride (100 µg/ml); Lane 8: hiperico (0.5 mg/ml) and stannous chloride (100 µg/ml); Form I: DNA supercoil (white band); Form II: open circular and/or linear.

Experiments were done with plasmid DNA (200 ng) exposed to hiperico extract (50 mg/ml). Aliquots were also incubated with 200 µg/ml of SnCl₂ in order to evaluate the influence of hiperico extract in the DNA lesions induced by stannous ion. The experiments were carried out at room temperature for 40 min. Aliquots of each sample (10 µl) were mixed to 2 µl of 6x concentrated loading buffer (0.25% xylene cyanol FF, 0.25% bromophenol blue, 30% glycerol in water), applied in a horizontal gel electrophoresis chamber in Tris-acetate-EDTA buffer at pH 8.0 and the electrophoresis were performed. Following, the gel was stained with ethidium bromide (0.5 µg/ml), the DNA bands were visualized by fluorescence in an ultraviolet transilluminator system and the gel was recorded using a Polaroid system.

RESULTS

Figure 1 shows the survival fractions of *E. coli* AB1157 cultures treated with SnCl₂ in the presence or absence of hiperico extract during 60 minutes. The data suggest no alteration on the survival fraction of *E. coli* incubated with hiperico extract at the used concentration (50 mg/ml). The results reveal inactivation of *E. coli* cells induced by SnCl₂. The data also show a protective

effect induced by the extract of hiperico against the inactivation produced by the treatment with the SnCl₂.

The electrophoretic profile of pBSK plasmid DNA in different experimental conditions is shown in figure 2. In lane 1, the plasmid DNA alone is found mostly as a supercoil form (form I). In lane 2 is shown the efficient cleavage of the plasmid DNA by SnCl₂ evidenced by formation of open circular (form II). Lanes 3 to 5 show the electrophoretic profile of plasmid DNA incubated with hiperico at different concentrations (50, 5, 0.5 mg/ml) suggesting no modifications in plasmid topology when compared with control (lane 1). In lanes 6 to 8 is shown that hiperico extract was not capable of protect the plasmid DNA against the stannous chloride action.

DISCUSSION

Free radicals (FR) have been related to the primary destructive intermediates molecules in a wide range of environmental conditions, as well as these species are involved in various biological phenomena, as mutagenesis, apoptosis and aging (Ozben, 2007).

Although cytotoxic and genotoxic effects of SnCl₂ have been demonstrated in different experimental models and these appeared to be mediated by free radicals (El-Demerdash et al., 2005; Almeida et al., 2007). Moreno et al. (2004) related that an extract of *Ginkgo biloba* protected the plasmid DNA from the lesions induced by SnCl₂.

The results herein obtained revealed absence of cytotoxic effect of hiperico extract on *E. coli* wild type, in the concentration tested (Figure 1). The data also show that the aqueous hiperico extract protected these bacterial cells against the lethal action of SnCl₂. This result may well be explained by: (i) its antioxidant properties; (ii) its free radicals scavenger action; or (iii) its metal ions chelating action related to this hiperico extract.

Some authors have reported an antioxidant action to the hiperico extract (Breyer et al., 2007; Gioti et al., 2007). Hunt et al (2001) have found that hiperico could be a potent inhibitor of the superoxide radical in a cell-free, as well as in the human vascular system. In addition, hiperico extract, standardized to both hypericin and hyperforin, appears to have significant free radical scavenging properties in cell-free and human vascular systems (Hunt et al., 2001; Wahlman et al., 2003). Our results indicated no protective action of the aqueous hiperico extract in plasmid DNA treated with SnCl₂ (Figure 2). Moreover, hiperico was not also capable to induce modifications in the DNA mobility in agarose gel.

In conclusion, our experimental data suggest absence of cytotoxic and genotoxic effects of the aqueous hiperico extract and a protective effect on *E. coli* cells against the lethal action of SnCl₂. However, additional

studies should be performed to try to elucidate the action mechanisms involved in the effects of hiperico extract obtained in this work.

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