

Full Length Research Paper

Effects of an aqueous extract of Three Ballerina on the survival of *Escherichia coli* AB1157 cultures and in the action of stannous chloride

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Accepted 1 April, 2011

The global interest in natural substances has increased. Three Ballerina (TB) is a natural formula that has medicinal properties. Scientific information about this formula is not found in the PubMed and this fact has stimulated investigations about its biological effects. An experimental model using a reducing agent, stannous chloride (SnCl_2), was carried out. Although, SnCl_2 is used in nuclear medicine to obtain technetium-99 m radiopharmaceuticals, it is capable to generate free radicals. The aim of this work was to evaluate the biological effects of an aqueous extract of TB on the survival of *Escherichia coli* (*E. coli*) AB1157 (wild type) cultures and to verify if this extract protect a *E. coli* culture against the action of SnCl_2 . *E. coli* AB1157 cultures (exponential growth phase) were incubated with TB extract (23.4 mg/ml) or 0.9% NaCl solution as a control. *E. coli* cultures were also incubated with SnCl_2 (25 $\mu\text{g/ml}$) + TB extract (23.4 mg/ml) or SnCl_2 (25 $\mu\text{g/ml}$) alone. Aliquots from these treatments were spread onto Petri dishes containing solidified LB medium. The colony-forming units (CFU) were counted after overnight and the survival fractions calculated. Data reveal that the TB did not alter the survival of the *E. coli* culture, however, it has protected the cells against the lethal effect of the SnCl_2 . In conclusion, the TB extract did not present cytotoxic effects, but it appears to have redox action.

Key words: *Escherichia coli*, three ballerina, stannous chloride, cytotoxic effect.

INTRODUCTION

The global interest in natural substances has increased over the world (Rotblatt and Ziment, 2002; Li et al., 2008; Monbaliu et al., 2010) and, there is a growing interest in the studies about various properties of medicinal and dietary plants (Bahramikia and Yazdanparast, 2008). The prevalence of obesity is growing worldwide and in the United States, it is estimated that this prevalence has reached almost 30% (Flegal et al., 2010). Due to the lack

and limitation of weight loss medicines, herbal teas and functional food ingredients have become important tools in improving obesity-related parameters. Tea has been consumed as a popular beverage worldwide for the last thousands of years because of its health benefits and pleasant aroma (Watson and Preedy, 2008). Green tea is one of the most extensively studied plants for the prevention of metabolic syndrome by stimulating fat oxidation and increasing energy expenditure (Dulloo et al., 1999). "3 Ballerina" Tea Dieters' drink is blended with the premium natural herbs. *Cassia angustifolia* and *Malva verticillate* are the vegetables of this formula. Following the instructions of the manufacturer (Truong Giang Corp.),

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“this special formula Dieters’ drink is all natural tea, soothing and relaxing especially delightful for those desiring to adjust weight, although this statement has not been evaluated by the Food and Drug Administration” (Soyuncu et al., 2008).

Different experimental models have been used to try to get scientific information about the natural products (Bahramikia and Yazdanparast, 2008; Li et al., 2008; Lima et al., 2002). Investigations to evaluate the cytotoxicity of medicinal plants (Raphael et al., 2009; Almeida et al., 2007) have been performed. The effects of extracts of *Baccharis genistellodes* (Scheila et al., 2002) in the action of a reducing agent, the stannous chloride (SnCl_2), have been assessed using *Escherichia coli* (*E. coli*) cultures.

SnCl_2 is used in nuclear medicine to obtain technetium-99m ($^{99\text{m}}\text{Tc}$) radiopharmaceuticals (Saha, 2010; Guedes et al., 2006). However, some deleterious effects of this substance have been described. In humans, it has been reported that it is highly irritant to the mucous membrane and skin, although it presents low systemic toxicity. In animals, it can produce stimulation or depression of the central nervous system. As for bacterial assays, SnCl_2 appears to be capable of inducing and/or producing injuries in deoxyribonucleic acid (DNA), being considered as a potential genotoxic agent (Soares et al., 2004; Guedes et al., 2006). These effects may be, at least in part, attributed to free radicals (FR), generated during SnCl_2 treatment (Bernardo-Filho et al., 1994; Dantas et al., 1999; Mattos et al., 1999).

As the scientific publications about “3 Ballerina” was not found in the PubMed databank, (TB), we have investigated the effects of an aqueous extract of TB on the survival of *E. coli* AB1157 (wild type) cultures and if this extract protect a *E. coli* culture against the action of SnCl_2 .

MATERIALS AND METHODS

Strategy in the PubMed

A search using the key words “Three Ballerina” or “3 Ballerina” was performed in the PubMed (www.ncbi.nlm.nih.gov/sites/entrez) on March 15th 2011.

Preparation of TB extract

As the commercial extract of Three Ballerina (dried powder), (Truong Giang Corp. South El Monte, CA 91733) has only small solubility, a solution with 2.34 g of Three Ballerina extract was prepared with 100 ml of a hot (ebullition) 0.9% NaCl solution (saline). The preparation was centrifuged (clinical centrifuge, 2000 rpm, 15 min) and the supernatant was isolated. Then, the obtained solution was considered 23.4 mg/ml. Saline was used in all the dilutions. All experiments were carried out during the period of validity of this product. The quality control of the preparation of this extract was controlled by the optical density of 0.93 ± 0.01 obtained at 490 nm that was used as a marker and the quality control of each preparation of TB in the all experiments.

Preparation of the *E. coli* culture to be used

The *E. coli* AB1157, a wild-type strain, proficient to repair damage in the DNA, was used in this work and its characteristics are reported in Howard-Flanders et al. (1964). From stock (in glycerol 50% v/v) a sample (50 μl) of the culture was grown on liquid LB medium (5 ml, Luria 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 (20 ml, liquid LB medium) under the same conditions, for 2 h to obtain an exponential growth phase (exponential growth culture with approximately 10^8 cells/ml). The cells were collected by centrifugation, washed with 10 ml of saline and suspended again in the same solution until they reached 10^8 cells/ml.

Effect of the TB extract on the survival of *E. coli* culture

Samples (0.8 ml) of the washed cultures (10^8 cells/ml) were incubated on the shaking water bath with (1) 0.2 ml of saline, or (2) 0.1 ml of saline and 0.1 ml of the TB extract 23.4 mg/ml up to 60 min, at 37°C. During the assay, at 0 and 60 min, aliquots (0.1 ml) were taken and diluted with saline and spread onto Petri dishes containing solidified LB medium (1.5% agar). Colony-forming units (CFU) formed after overnight incubation at 37°C were counted and the survival fraction was calculated as described previously (Dantas et al., 1996). 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 (No).

Effect of the TB extract on the action of the stannous chloride in *E. coli* culture

Samples (0.8 ml) of the washed cultures (10^8 cells/ml) were incubated on the shaking water bath with (1) 0.1 ml of SnCl_2 (25 $\mu\text{g/ml}$) and 0.1 ml of saline, or (2) 0.1 ml of the TB extract 23.4 mg/ml and 0.1 ml of saline, or (3) 0.2 ml of saline as control, on initial time and after 60 min, at 37°C. During the assay, at 0 and 60 min, aliquots (0.1 ml) were taken and diluted with saline and spread onto Petri dishes containing solidified LB medium (1.5% agar). CFU formed after overnight incubation at 37°C were counted and the survival fraction was calculated as described previously (Dantas et al., 1996). 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 (No).

RESULTS

The search that has been done in the PubMed has revealed that no items were found with the key words “Three Ballerina” or “3 Ballerina”.

Results shown in Figure 1 reveal that the TB was not capable to interfere on survival of the *E. coli* AB1157 culture up to 60 min with the treatment using a concentration of 23.4 mg/ml.

Figure 2 shows that the strong lethal effect of the stannous chloride has already described for different authors (Bernardo-Filho et al., 1994; Almeida et al., 2007). In addition, it is shown that the TB extract has a protective effect against the treatment with the SnCl_2 , abolishing the effect of the reducing agent in the concentration used in the assay.

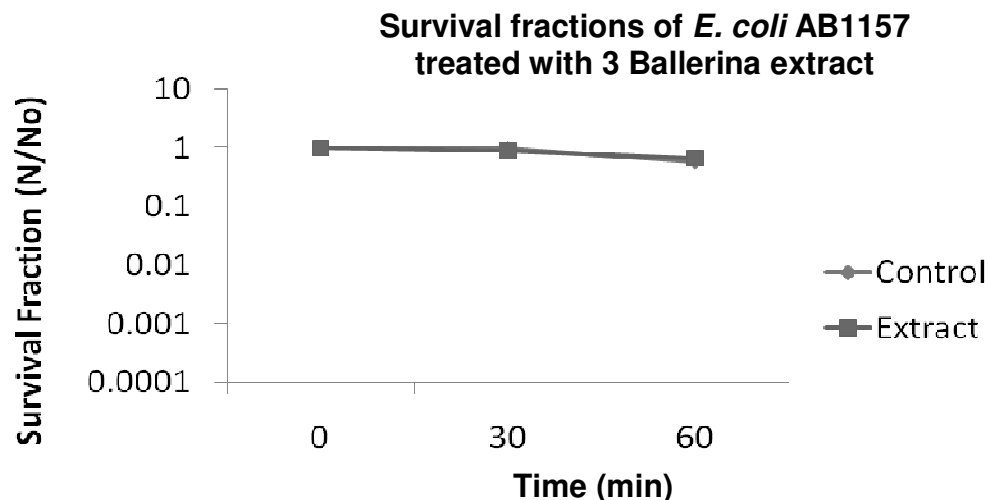


Figure 1. Absence of an effect of the TB extract on *E. coli* AB1157. Cells suspended in 0.9% NaCl were treated with TB extract. Survival fractions of *E. coli* AB1157 strain treated for different time of incubation in the presence or absence of the extract. The survival fractions were determined. ♦, 0.9% NaCl; ■, extract. N, number of viable cells obtained per ml in each time of the treatment; No, number of viable cells obtained per ml in zero time.

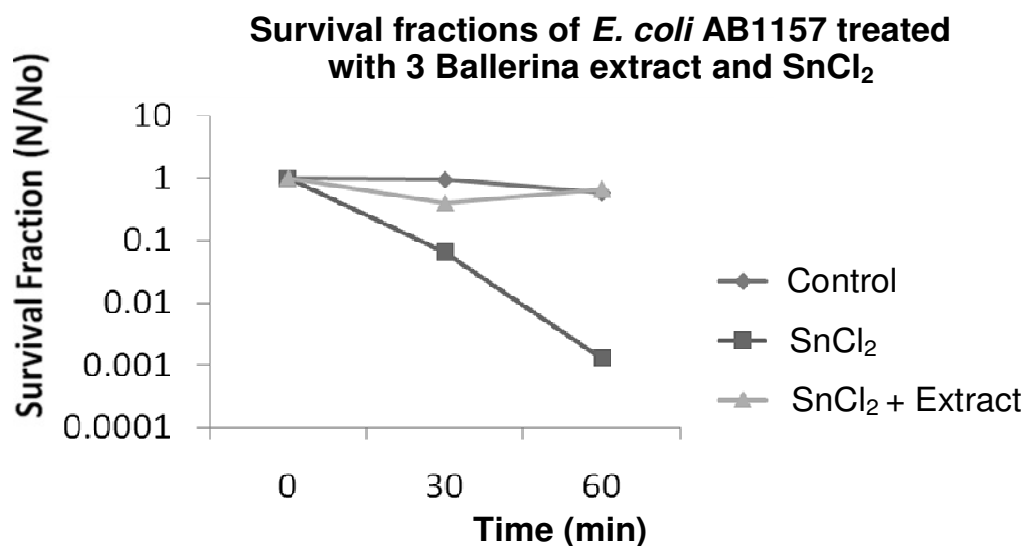


Figure 2. Effect of the extract from TB on the inactivation induced by SnCl₂ on *E. coli* AB1157. Cells suspended in 0.9% NaCl were treated with SnCl. Survival fractions of *E. coli* AB1157 strain treated with SnCl₂ for different time of incubation in the presence or absence of the extract and with the extract alone. The survival fractions were determined. ♦, 0.9% NaCl; ■, SnCl₂; ▲, SnCl₂ + extract. N, number of viable cells obtained per ml in each time of the treatment; No, number of viable cells obtained per ml in zero time.

DISCUSSION

Due to the use of the medicinal plants, it is increasing around the world, and given the limited scientific information about the effect of many of them, it is highly relevant to try to assess biological properties of extract of

natural products. Concerning to the Three Ballerina, there are no publications in the PubMed. This fact stimulates scientific investigations about this formula.

The extract of Three Ballerina was not capable in interfering in the survival of an *E. coli* culture AB1157 (Figure 1), demonstrating an absence of cytotoxic effect

in the conditions used in the experiments. This fact has already been demonstrated to another extracts, as cauliflower (Lima et al., 2002).

Although the stannous chloride has been used in the nuclear medicine (Saha, 2010), important deleterious effects associated to this reducing agent has been reported (Melo et al., 2001; Guedes et al., 2006; Souza et al., 2009). Another important finding of our work was demonstrated that the extract of Three Ballerina abolished the lethal effect of the stannous chloride (Figure 2). As the action of this reducing agent is related with the generation of free radicals, we can suggest that the TB extract has redox properties. The protective effect in *E. coli* AB1157 induced by the extract of TB against the inactivation produced by the treatment with SnCl_2 was also observed with *Peumus boldus* (Reiniger et al., 1999) and with *Cymbopogon citratus*, *Maytenus ilicifolia* and *Baccharis genistelloides* (Melo et al., 2001).

In conclusion, the findings in this work show that the chemical products present in extracts of TB, in the concentration used, are not toxic to the *E. coli* AB1157 culture. Moreover, as the TB extract has probably redox properties, it can prevent the generation of free radicals or act as a scavenger. Additional studies should be performed to try to elucidate the action mechanisms involved in the effects of TB extract obtained in this work.

ACKNOWLEDGEMENTS

The authors are grateful to the following CNPq, CAPES and UERJ for supporting this work.

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