

*Full Length Research Paper*

# Experimental model to evaluate the effect of aqueous extract of 'Buzhong Yi Qi Wan' on the survival of *Escherichia coli* submitted to treatment with stannous ion

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'Buzhong Yi Qi Wan' is a combination of medicinal herbs widely used in Traditional Chinese Medicine to treat digestive and circulation disorders. It is an anti-inflammatory biocomplex with energizing and anti-cancer properties, and it should have an effect in natural killer cell (NK) activity, improving immunological capacity in elderly people. Substances present in the 'Buzhong Yi Qi Wan' extract could alter the cell membrane and plasma of blood constituents and have a hemolytic effect, causing the generation of free radicals that have oxidant properties, and could alter Nuclear Medicine procedures. Stannous chloride ( $\text{SnCl}_2$ ) is the reducing agent utilized with technetium-99 m (99mTc) in the labeling of blood constituents. The aim of this work was to evaluate the effect of aqueous extract of 'Buzhong Yi Qi Wan' on the survival of *Escherichia coli* submitted to the treatment with stannous ion, to understand this reducing agent action. The extracts of 'Buzhong Yi Qi Wan' were not capable of interfering in the survival of *E. coli*. Moreover, this extract protected the *E. coli* against the  $\text{SnCl}_2$  action; this fact can be related to the free radical-scavenging properties of the chemical compounds of the extract.

**Key words:** Stannous chloride, oxidant agent, 'Buzhong Yi Qi Wan', *Escherichia coli*.

## INTRODUCTION

In nuclear medicine, single photon emission computed tomography (SPECT) is a scintigraphy technology that allows for the measurement of physiologic processes and alterations related to various diseases. Herbs could alter this process (Fonseca et al., 2007). This nuclear medicine process with technetium-99 m (99mTc) requires a reducing agent, and stannous chloride ( $\text{SnCl}_2$ ) is widely used (Sampson, 1999; Saha, 2004). It was observed that  $\text{SnCl}_2$  can act directly on deoxyribonucleic acid (DNA) or indirectly, generating free radicals (FR) and leading to its genotoxic effects (Dantas et al., 1999; Nunes et al., 2006).

'Buzhong Yi Qi Wan' (Buzhong) is a medicinal plant with (biocomplex) anti-inflammatory, energizing and anti-cancer properties, and should have an effect on NK activity, improving immunological capacity in elderly people and endocrine function in stressed mice, an effect in chronic hepatitis B and in myasthenia gravis (Ji et al., 1989; Du et al., 1993; Tang and Wu, 1994; Kuroiva et al., 2004; Seki et al., 2005).

In Traditional Chinese Medicine, Buzhong has been widely used also as a middle Jiao tonic and chi stimulator (vital energy) to harmonize Xue (blood energy) and increase the physical vitality of the spleen and stomach (Zang-Fu) (Wang et al., 2002). Substances present in the Buzhong extract could alter the cell membrane and plasma of blood constituents and have a hemolytic effect,

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**Table 1.** Data of stannous chloride on the survival of *E. coli* AB1157 cultures.

Solution	Data (00 min)	Data (30 min)	Data (60 min)	Standard deviation	p-value
Control	1.000	1.0252	1.0126	0.55	p<0.05
SnCl <sub>2</sub>	1.000	0.0710	0.0355	0.01	p<0.05
SnCl <sub>2</sub> +BYQW	1.000	0.9412	0.4706	0.29	p<0.05
BYQW	1.000	1.0411	1.0823	0.04	p<0.05

Software Statistic 9.0 - StatSoft, USA; 2010; BYQW: Buzhong Yi Qi Wan; SnCl<sub>2</sub>: stannous chloride.

causing the generation of free radicals with oxidant properties, in procedures (SPECT) of Nuclear Medicine - study with blood of wistar rats experiences (Giani et al., 2007). The Nuclear Medicine aspect used 99 mTc and SnCl<sub>2</sub> on a SPECT-kit and drugs, medicinal plants, natural or synthetic products can alter this labeling process (Melo et al., 2001; Moreno et al., 2002; Abreu et al., 2006; Rebello et al., 2007; Almeida et al., 2007). Buzhong has this capacity. Human beings can use Buzhong and several effects about this natural product remain poorly understood. The aim of this work was to evaluate the effect of aqueous extract of Buzhong on the survival of *E. coli* submitted to the treatment with stannous ion, to understand this reducing agent action and the protective properties of Buzhong. The authors studied the effects of the SnCl<sub>2</sub> and *E. coli* AB1157, BW9091 and others authors too (Dantas et al., 1996; Assis et al., 1998; Reineger et al., 1999; Lima et al., 2002; Bernardo et al., 2002; Guedes et al., 2006) but with Buzhong we could find no data on databases.

## MATERIAL AND METHODS

### Extracts preparation

A commercial 'Buzhong Yi Qi Wan' (Buzhong) was used in the assays (Gansu Medicines and Health Products Import and Export Corporation, valid November/2008). As indicated by this manufacturer, compact herbs of 'Buzhong Yi Qi Wan' (no flavors, no conservatives) - *Radix astragalus* (27.8%), *Radix codonopsis* (8.3%), *Radix glycyrrhizae* (14%), *Rhizoma atractylodis macrocephalae* (8.3%), *Radix angelicae sinensis* (8.3%), *Rhizoma cimicifugae* (8.3%), *Radix bupleuri* (8.3%), *Pericarpium citri reticulatae* (8.3%), *Rhizoma zingiberis recens* (2.8%) and *Fructus jujubae* (5.6%) - were used to prepare this solution (with macerated pills). In the preparation of the extract, 128 mg of the material was put in a tube with 10 ml of saline solution (NaCl 0.9%) that was gently shaken in a vortex (3 min). This suspension was centrifuged in a clinical centrifuge (Bio. Eng. Industry, type 1001) at 1500 rpm (5 min) and the supernatant was considered to be 12.8 mg/ml (100%).

### Bacteria inactivation

Cells from *E. coli* AB1157 cultures in the exponential growth phase (1-2 x 10<sup>8</sup> cells/mL) show the effect of the Buzhong extract (12.8 mg/ml) on the inactivation induced by stannous chloride in the *E. coli* AB1157 strain. These extracts were capable of protecting the

cells of *E. coli* against the lesive action of SnCl<sub>2</sub>. Moreover, they were not capable of interfering with the survival of the culture. Samples (1mL) of these cultures were incubated on a water bath shaker with: (a) SnCl<sub>2</sub> (25 µg/mL), (b) SnCl<sub>2</sub> (25 µg/mL) + extract (25 mg/mL), (c) extract (25 mg/mL), (d) 0.9%NaCl. At 30 and 60 min intervals, aliquots were withdrawn, diluted and spread onto glass Petri dishes with solid LB medium (1.5% agar). Colonies formed after overnight incubation (37°C), and the survival fractions (N/N0) were calculated. Experiments were carried out in triplicate with Buzhong extract, and the results presented are the average mean of three independent assays. Standard deviations were calculated (p<0.05 and 10%<β<20%).

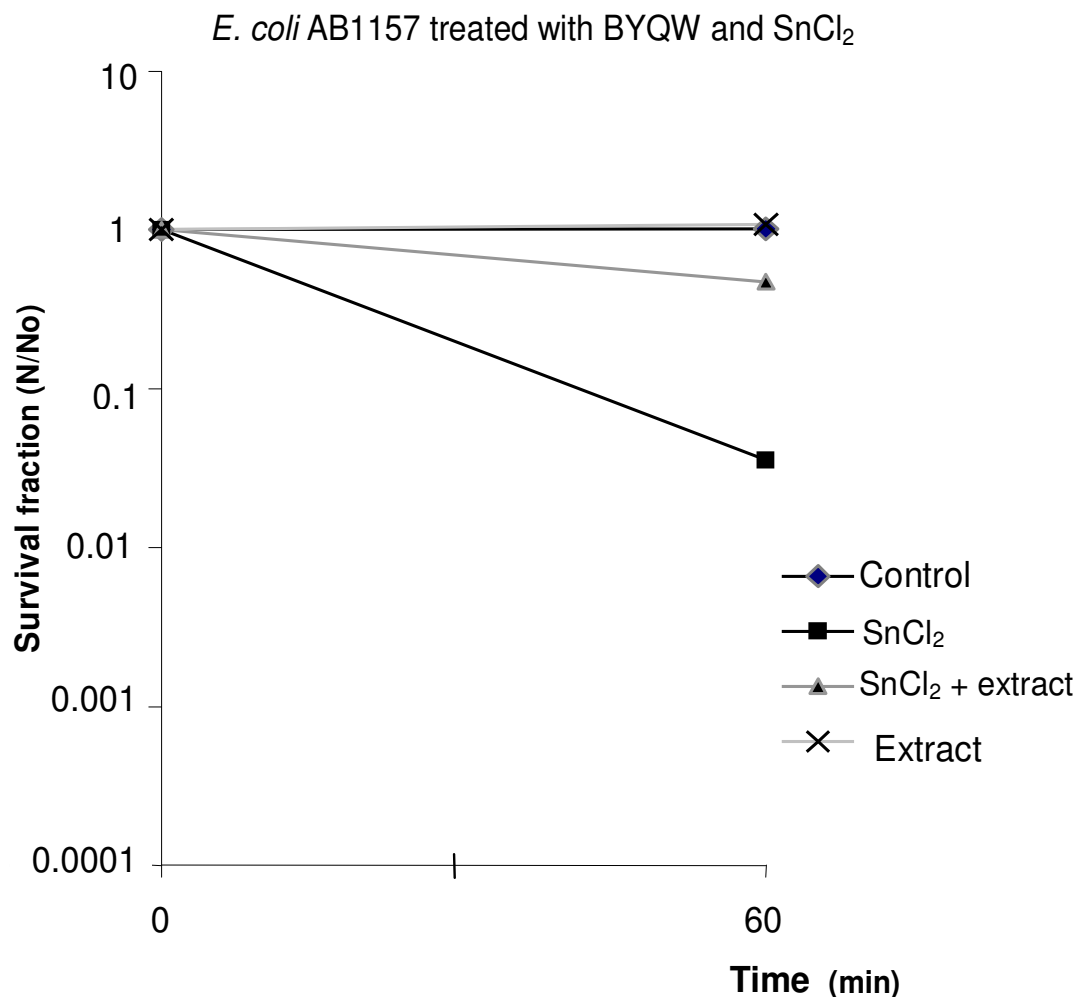
## RESULTS

The data (Table 1 and Figure 1) show the effect of the Buzhong extracts on the inactivation induced by SnCl<sub>2</sub> in the *E. coli* AB1157 strain. These extracts were capable of protecting the cells of *E. coli* against the lethal effect of SnCl<sub>2</sub>; moreover, they were not capable of interfering with the survival of the culture.

## DISCUSSION

Medicinal plant extracts can generate metabolites capable of promoting morphological and/or physiological modifications to structures, tissues or pH values. Stannous chloride (SnCl<sub>2</sub>) has been widely used in nuclear medicine as a reducing agent of pharmaceutical products radiolabelled with technetium-99 m (99 mTc). Some authors have reported that vegetal extracts are able to protect *E. coli* cultures against the cytotoxicity of SnCl<sub>2</sub> (Lima et al., 2002; Bernardo et al., 2002; Guedes et al., 2006). Cytotoxic and genotoxic effects are related to SnCl<sub>2</sub> and mediated by medicinal plants (Assis et al., 1998; Nunes et al., 2006). These extracts protected *E. coli* cells against SnCl<sub>2</sub>-induced lesions. Moreover, these extracts did not interfere with the survival of this strain. These results have already been reported in *E. coli* AB1157 treated with SnCl<sub>2</sub> and plant extract; chelated stannous ions, protecting the *E. coli* against oxidation and avoiding the generation of free radicals (FR) (Nunes et al., 2006).

Our results with yeast support of the earlier research papers on the genotoxic effects for SnCl<sub>2</sub> in the bacteria and indicate that the biological effect of this reducing



**Figure 1.** Effect of aqueous extracts and of stannous chloride on the survival of *E. coli* AB1157 cultures.

agent may be related to the generation of reactive oxygen species (ROS); we conclude that experimental models with SnCl<sub>2</sub> and *E. coli* permit the study of drug interactions and of the biological activities of vegetal extracts. Moreover, these findings could be worthwhile to try to understand some alters in the nuclear medicine imaging (Giani et al., 2007; Lima et al., 2002; Bernardo et al., 2002; Guedes et al., 2006). However, the substances in various herbs that form the basis for medicinal drugs, as in Buzhong extract, could oxidize these ions sufficiently to protect the *E. coli* culture against the lethal effect of the stannous ion. Others effects of Buzhong and others herbs is the vital energy increase to respond to several diseases, which should have an effect on the natural killer cell (NK) activity, thereby improving, to some degree, the immunological capacity (Kuroiva et al., 2004). In Traditional Chinese Medicine Buzhong extract could be associated, at least in part, with its property of “xue-harmonized energy” (blood-harmonized energy) and increased energy of the “Jiao” middle (Giani et al., 2007),

protecting the structures of SnCl<sub>2</sub> lethal aggression in the labeling of blood constituents treated of 99 mTc. In the *E. coli* colonies in the presence of SnCl<sub>2</sub>, the extract of Buzhong interfered in certain cultivate colonies (Figure1).

This Buzhong protective action toward *E. coli* survival could be associated with action in the plasma membrane and/or related to the generation of FR that have oxidant properties that chelated the stannous ions. The extracts of Buzhong Yi Qi Wan were capable of continuing the existence of *E. coli*. This aqueous extract (12.8 mg/ml - NaCl 0, 9%) protected the *E. coli* against the SnCl<sub>2</sub> action and can be related to the free radical-scavenging properties of the chemical compounds of the extract.

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