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PROTECTIVE EFFECT OF AN AQUEOUS EXTRACT OF *Harpagophytum procumbens* UPON *Escherichia coli* STRAINS SUBMITTED TO THE LETHAL ACTION OF STANNOUS CHLORIDE.

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Abstract – Regardless of its lethal effects upon *Escherichia coli* (*E. coli*) cultures trough the production of free radicals (FR), stannous chloride ($SnCl_2$) remains to be the most used reducing agent on the production of technetium-99m radiopharmaceuticals, to obtain images on nuclear medicine. Moreover, authors have reported that vegetal extracts are able to protect *Escherichia coli* cultures against the cytotoxicity of this agent. *Harpagophytum procumbens*, also known as Devil's Claw, is a plant used in folk medicine, as an analgesic and anti-inflammatory in cases of joint and back pain, on the treatment of degenerative rheumatoid arthritis, osteoarthritis, kidney inflammation and heart diseases. The presence of this extract reduced the lesive effects of $SnCl_2$ upon *E. coli* AB1157 (proficient in DNA repair), BW9091 (deficient in the *xth*A gene) and BH110 (deficient in the *xth*A, *nfo* and *fpg* genes) cultures, and the deficient strains (BW9091 e BH110) were more sensible to this $SnCl_2$ action than the proficient one. The substances in the extract could be acting as: (i) chelator of the stannous ions, avoiding the generation of FR, (ii) FR scavenger, protecting the cells against the oxidation, and/or (iii) an oxidant compound acting upon the stannous ions, reducing the $SnCl_2$ cytotoxicity.

Key words: *Harpagophytum procumbens*, stannous ion, DNA repair, *Escherichia coli*, free radicals.

INTRODUCTION

In nuclear medicine, radiopharmaceuticals (radiobiocomplexes) labeled with technetium-99m (^{99m}Tc) are widely used as imaging agents (18). Red blood cells and plasma proteins labeled with ^{99m}Tc are also used in cardiovascular system images, detection and localization of gastrointestinal hemorrhages (34). This labeling technique is based on the reducing ability of stannous salts on 99mTc, as sodium pertechnetate, to a lower oxidation state (29,33). The most important stannous salt used for this purpose is stannous chloride ($SnCl_2$) (29). Furthermore, humans are widely exposed to stannous ion as a result of processing and packaging in food industry (5). Due to its several applications, the knowledge and the understanding of the biological effects of $SnCl_2$ become highly relevant (3).

Deoxyribonucleic acid (DNA) damages are related to cancer, among other diseases (14). Metal ions can strongly bind in nucleic acid preparations (12), and some transition metals, such as iron, copper, zinc, chromium and tin, are able to mediate Fenton or Fenton-like reactions that generate free radicals (FR) (16).

The genotoxic and/or mutagenic activities of stannous compounds are still poorly comprehended. Assays with *Bacillus subtilis* deficient in recombination repair showed an absence of those effects (21). It has also been demonstrated that the $SnCl_2$ would probably not be a carcinogenic compound, as demonstrated in experiments using wing primordial cells from *Drosophila melanogaster* (34). However, some reports have shown that $SnCl_2$ produced extensive DNA damage, detected in Chinese hamster ovary cells (26). Genotoxic potentiality of $SnCl_2$ was demonstrated in proficient (wild-type) and deficient *Escherichia coli* (*E. coli*) strains on DNA repair genes (3). Studies have revealed that $SnCl_2$ promotes strand breaks in plasmid DNA (11). Nevertheless, recent studies have demonstrated a reduction on the lethal effect of $SnCl_2$ on the survival of *E. coli* cultures by the presence of vegetal extracts (4,27,31).

The interest in phytotherapy has been increasing worldwide, due to the use of vegetal extracts for their pharmacological activities, and in nutrition, as dietary supplements. However, the amount of scientific information about their safe and effective use is still quite limited. In general, most of this information does not have scientific support and, moreover, the use as phytotherapic drugs is based only on traditional folk medicine (13).

Harpagophytum procumbens (*H. procumbens*), commonly known as Devil's Claw, is a prostrate, perennial herb, indigenous to Southern Africa. The powdered tubers can be used either directly in a tea infusion or as pills produced by pharmaceutical companies (20). Vita Hervas Brazil (www.vitalab.com.br) is a manufacturer that distributes Devil's Claw capsules, and it suggests that a patient can take up to 6 pills per day, each pill containing 400mg of dry extract. Its tuberous secondary roots are used in the folk medicine of several countries as an analgesic and anti-inflammatory, in cases of joint and back pain (9,30), on the treatment of degenerative rheumatoid arthritis, osteoarthritis, kidney inflammation (22) and heart diseases (32). The anti-inflammatory properties of Devil's Claw are due to its capability to inhibit the production of the iNOS enzyme (inducible nitric oxide synthase), due to the presence of iridoid glycosides, such as the harpagoside (22).

Therefore, the purpose of the present study was to verify: (i) the effect of $SnCl_2$ in different *E. coli* strains, proficient or deficient in DNA repair genes; (ii) the biological effects of an aqueous extract of *H. procumbens* in different *E. coli* strains, and (iii) the biological effects of this extract on the damage caused by $SnCl_2$ in different *E. coli* strains.

MATERIALS AND METHODS

Reagents and extract preparation

Stannous chloride was purchased from Sigma Chemical Co., USA, used in the concentration of 25µg/ml, as previously used in various experimental models in basic research (2,3,7) Commercial dried powder of *H. procumbens* was obtained from Vita Hervas capsules, (Brazil; lot: 02, validity: July 2004 to July 2006) prepared through the addition of 0.9% NaCl (10ml) to 400mg of the powder, which is the amount of extract found in one pill (20). Then, it was centrifuged (1500 rpm, 5 min) and the supernatant phase was isolated, which is the resulting aqueous extract, a light-brown powdery crude solution (25). Once the exact solubility of the powder extract in the aqueous solution is not known, the supernatant phase was considered as 40mg/ml. This same procedure has already been described in aqueous extractions of other plants (1,19). The experiments were carried out in the period of time from November 2005 until May 2006.

Bacterial strains

The bacterial strains used in this work are listed on Table 1.

Table 1. *E. coli* bacterial strains used on the experiments and their main characteristics.

Strains	Genetic markers	References
E. coli AB1157	wild type	(17)
E. coli BW9091	*xth*A	(35)
E. coli BH110	*fpg nfo xth*A	(6)

Bacterial survival and treatments

Cells from *E. coli* AB1157, BW9091 and BH110 cultures in exponential growth phase (1-2 x 10^8 cells/ml) were collected by centrifugation, washed and ressuspended in 0.9% NaCl solution. Samples (1ml) of these cultures were incubated in water bath shaker with: (a) $SnCl_2$ (25µg/ml), (b) $SnCl_2$ (25µg/ml) + extract (40mg/ml), (c) extract (40mg/ml), (d) 0.9% NaCl (control). After 60 min, aliquots were withdrawn, diluted and spread onto glass Petri dishes with solid LB (Luria Broth) medium (1.5% agar). Colonies were formed after overnight incubation (37ºC) and the survival fractions (SF) calculated. Experiments were repeated in 3 separated sequences, and 2 samples of each treatment were used in each sequence.

RESULTS

Results shown in figure 1 indicate that the aqueous extract of *H. procumbens* did not present cytotoxicity over the *E. coli* AB1157 (proficient in DNA repair), BW9091 (deficient in the *xth*A gene) and BH110 (deficient in the *xth*A, *nfo* and *fpg* genes) strains. Although these strains have different capabilities of DNA damage repairing, the survival fractions of any of these cultures were not modified by the presence of the studied extract when compared to the control (NaCl 0.9%).

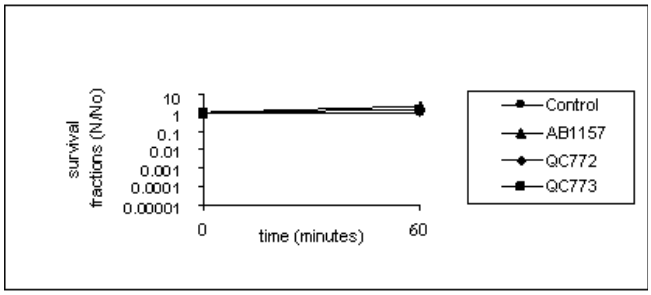

Figure 1. Survival fractions of the used strains treated with an aqueous extract of *H. procumbens* (40g/ml). a) • 0.9% NaCl; b) ▲ AB1157; c) ♦ BW9091; d) ■ BH110. Values are means of three isolated experiments. Standard deviations did not exceed 15%.

Figure 2 shows that $SnCl_2$ possesses an effect upon the survival of *E. coli* AB1157 cultures (proficient in DNA repair), as already described. Moreover, an important action in the presence of the extract is found, since it was able to protect this strain against the effects of the reducing agent in matter, once the survival fractions of the cultures treated simultaneously with the extract and $SnCl_2$ are at the same level of the control (NaCl 0.9%) and the extract alone.

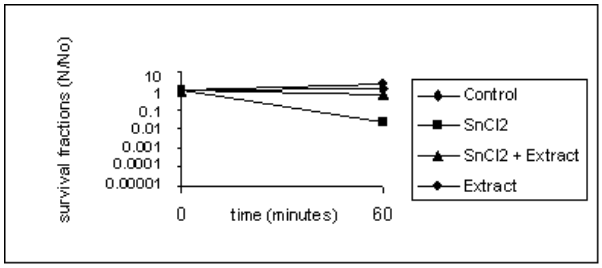

Figure 2. Survival fractions of *E. coli* AB1157 strain treated with $SnCl_2$ in the presence or absence of the extract. a) ♦ 0.9% NaCl; b) ■ $SnCl_2$ (25µg/ml); c) ▲ $SnCl_2$ (25µg/ml) + *H. procumbens* extract (40g/ml); d) • *H. procumbens* extract (40mg/ml). Values are means of three isolated experiments. Standard deviations did not exceed 15%.

Figure 3 shows that $SnCl_2$ possesses an effect upon the survival of BW9091 (deficient in the *xth*A gene), as already described. Moreover, an important action in the presence of the extract is found, since it was able to protect this strain against the effects of the reducing agent in matter, once the survival fractions of the cultures treated simultaneously with the extract and $SnCl_2$ are at the same level of the control (NaCl 0.9%) and the extract alone.

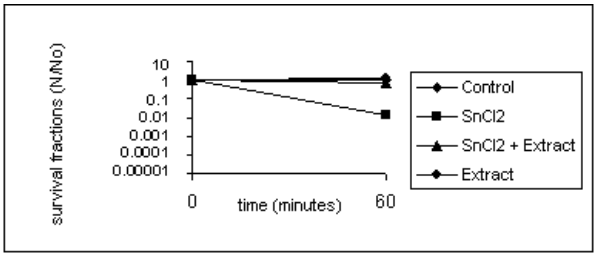

Figure 3. Survival fractions of *E. coli* BW9091 strain treated with $SnCl_2$ in the presence or absence of the extract. a) ♦ 0.9% NaCl; b) ■ $SnCl_2$ (25µg/ml); c) ▲ $SnCl_2$ (25µg/ml) + *H. procumbens* extract (40mg/ml); d) • *H. procumbens* extract (40mg/ml). Values are means of three isolated experiments. Standard deviations did not exceed 15%.

Furthermore, figure 4 shows that $SnCl_2$ possesses an effect upon the survival of BH110 (deficient in the *xth*A, *nfo* and *fpg* genes), as already described. Moreover, an important action in the presence of the extract is found, since it was able to protect this strain against the effects of the reducing agent in matter, once the survival fractions of the cultures treated simultaneously with the extract and $SnCl_2$ are at the same level of the control (NaCl 0.9%) and the extract alone.

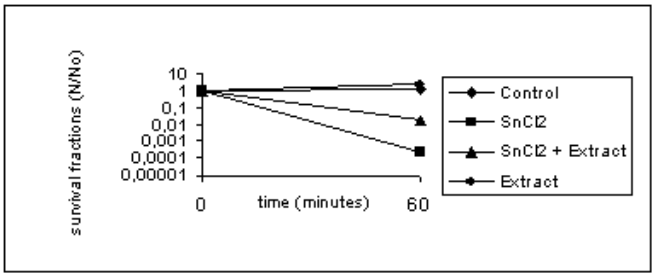

Figure 4. Survival fractions of *E. coli* BH110 strain treated with $SnCl_2$ in the presence or absence of the extract. a) ♦ 0.9% NaCl; b) ■ $SnCl_2$ (25µg/ml); c) ▲ $SnCl_2$ (25µg/ml) + *H. procumbens* extract (40mg/ml); d) • *H. procumbens* extract (40mg/ml). Values are means of three isolated experiments. Standard deviations did not exceed 15%.

DISCUSSION

Stannous chloride is the main ^{99m}Tc reducing agent to obtain radiobiocomplexes used in nuclear medicine examinations (29,33), in spite of the fact that the genotoxic and/or mutagenic activities of stannous compounds are still poorly comprehended (3,27).

Some enzymes involved on base excision repair (BER), which consists on several enzymatic reactions to remove DNA lesions, have already been well studied in *E. coli* cultures, such as exonuclease III, endonuclease IV and formamidopyrimidine-DNA-glycosylase, encoded by the *xth*A, *nfo* and *fpg* genes, respectively. Base related damage can cause lesions with mutagenic properties, contributing to possible carcinogenesis in superior organisms (28). $SnCl_2$ mediated the lethal effects observed in all three *E. coli* cultures used (Figures 2, 3 and 4). The three strains present the same phenotype; however, BW9091 (lacks the *xthA* gene) and BH110 (lacks the *xthA*, *fpg* and *nfo* genes) possess mutations in specific genes, and its enzymatic products are directly associated with the repair of DNA lesions. Therefore, the deficient strains (BW9091 and BH110) were more sensitive to the $SnCl_2$ lethal effects than the wild-type strain (AB1157). This result could be justified due to the deficiency on BER mechanism, since the *E.coli* strains used have been widely used to try to investigate the influence of physical and chemical agents in

different steps of the DNA lesion repair (15). Authors have reported that the genotoxic potential of stannous salts depends on the genome of the strains used, since the bacterial cultures with lack of only one DNA repair gene are more resistant than the ones with lack of two or three genes (3,8,10). Therefore, our findings would indicate the importance of BER on the repair of the damages mediated by $SnCl_2$. Galhardo et al. have also hypothesized the influences of the product of these same genes involved with BER on the effects caused by hydrogen peroxide, a FR generator such as $SnCl_2$ (15).

Authors have described the presence of antioxidant properties in vegetal extracts (23,24). An important antioxidant effect related to the *H. procumbens* aqueous extracts has been described, and it seems that it does not only involve the presence of the harpagoside, but its association with the other extract components (22). Figures 2, 3 and 4 also show the effect of the *H. procumbens* aqueous extracts on the inactivation induced by $SnCl_2$ in the *E. coli* strains. This extract was capable to protect the *E. coli* cells against the lesive action of $SnCl_2$. Moreover, it did not present cytotoxicity over these cultures, as shown in figure 1.

Hence, our results showed a protective effect of the referred extract upon the $SnCl_2$ cytotoxicity upon *E. coli* cultures. According to this finding, we may suggest that the substances in the aqueous extract could be acting as: (i) metal ion chelator of the stannous ions, avoiding the generation of FR, (ii) FR scavenger, protecting the cells against the oxidation, and/or (iii) an oxidant compound that could act upon the stannous ions, reducing the $SnCl_2$ lethal effect. Further investigation should take place, in order to elucidate the exact protective mechanism of action of these substances upon the $SnCl_2$ lethality.

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