Study of the Effects of Chayotte (*Sechium Edule*) Extracts on the Plasmid pUC. 9.1 DNA

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Abstract: Stannous chloride ($SnCl_2$) is employed as a reducing agent to obtain Technetium-99m-labelled radiopharmaceuticals in nuclear medicine kits, being inject endovenously in humans. Toxic effects of these kits were not studied, thus making it important to evaluate their impact in humans. The use of natural extracts as medicines is growing around the world. The chayotte (Sechium edule) is a subtropical vegetable with potent diuretic action. It is used in folk medicine due its hypotensor effect. In this study, plasmid deoxyribonucleic acid (DNA) was exposed to chayotte extracts (macerated and decoct) (0.1 $g.mL^{-1}$) in presence of stannous chloride ($SnCl_2$). Samples of the plasmid DNA were analyzed through agarose gel electrophoresis. The results show that the chayotte extracts were capable of damaging the DNA in the presence and in the absent of $SnCl_2$. [Nature and Science. 2004;2(3):48-54].

Key words: plasmidial DNA, chayotte, nuclear medicine, stannous chloride.

1 Introduction

Stannous chloride can cause skin and mucosal irritation in humans, and when this salt is injected into laboratory animals, it can produce stimulation and subsequent depression of the central nervous system (Gleason *et al*., 1969). It has been suggested that $SnCl_2$ is a powerful genotoxic (McLean *et al*., 1983; Oliver and Marzin, 1987), mutagenic (Singh, 1983; Triphaty *et al*., 1990) and carcinogenic (Ashby and Tennant, 1991) compound. In nuclear medicine, $SnCl_2$ has been employed in scintigraphic test as Technetium–99m (99mTc) reducing agent. Besides the use of $SnCl_2$ in nuclear medicine, this salt is also used in dentistry (dentifricies) (Hallas and Cooney, 1981; McLean *et al*., 1983; Rader, 1991; White, 1995; Budavery, 1996).

There are other sources of $SnCl_2$ to which human beings are exposed to such as from environmental contamination by biocide preparations containing organic compound dimethyl stannous chloride [$SnCl_2(CH_3)_2$] (Hallas and Cooney, 1981). It is hypothesized that the toxicity of $SnCl_2$ might be mediated by generation of reactive oxygen species (ROS) through the reaction: $Sn^{2+} + O_2 + 2H^+ \rightarrow Sn^{4+} + H_2O_2$. The generation hydrogen peroxide undergoes by Fenton reaction to generate $^{\bullet}OH$ as follows: $Fe^{2+} + H_2O_2 \rightarrow OH^- + {}^{\bullet}OH$ (4). It was also described that $SnCl_2$ mediates single strand breaks in plasmid DNA through ROS formation in a dose-dependent manner (Dantas *et al*., 1996). In addition, the mutagenic potentiality of $SnCl_2$ was identified by *supF* gene mapping (Cabral *et al*., 1998). It was also determined that *Escherichia coli* (*E. coli*) strains proficient in DNA repair mechanisms were more resistant to $SnCl_2$ treatment than deficient ones, suggesting that inactivation was due to DNA damage (Aherne and O'Brien, 1999). Biological effects of metals have been reported: (i) transition metals catalyze free radical production that can be related to aging processes and neurodegenerative diseases such as Alzheimer's diseases, Parkinson's disease, and others (Stohs and Bagchi, 1995); (ii) the association between human diseases and metal ions metabolism can be also demonstrated by Huntington's disease and amyotrophic lateral sclerosis; (iii)

neurotoxic properties of aluminum and its etiopathogenetic role in Alzheimer'a disease are still controversially argued and need further elucidation (Gutteridge *et al*., 1984); (iv) abnormal copper metabolism detected in the brain tissue of Wilson's and Menke's patients remains to be fully explained (Gutteridge *et al*., 1985); (v) substantial levels of zinc are found in the hippocampus and its brain deficiency can be the cause of several pathological events (Bettger and O'Dell, 1981), however; (vi) magnesium is successfully used for the treatment of migraine (Stohs and Bagchi, 1995). In addition, the molecular mechanisms underlying neurotoxicity associated with mercury, tin and manganese, also need further investigation. Tin is a heavy metal which has long been regarded as a contaminant of the environment (Wood, 1974). One of its inorganic salts, stannous chloride ($SnCl_2$), has been widely used in daily human life, to conserve soft drinks, in food manufacturing, as a result of processing and packaging. Studies on the biological effects of $SnCl_2$ revealed that it can generate reactive oxygen species (ROS) and breaks in deoxyribonucleic acid (DNA) (Caldeira-de-Araújo *et al.,* 1996) and induces lethality in *E. coli*, whose damage recovery depends on RecA-mediated repair (Bernardo-Filho *et al.,*1994b). Medicinal plants are mainly complex products with several components with different chemical and pharmacological characteristics (Moro and Basile, 2000). In addition, many of these products are also sold as dietary supplement, but, scientific information about their safe and effective use is hard to find because limited toxicological data are available on herbal remedies and support of rigorous clinical studies is lacking (Capasso *et al*., 2000). The use of natural products as medicines has been growing in the entire world. Because of this fact, many studies with natural products are being developed, and new drugs for treatments of diseases are being discovered. In the literature, the medicinal action mechanism of several plants has been described and different compounds, with various properties, have been isolated from the crude extracts (Leite *et al.,* 1986; Sallé, 1996). *Sechium edule* (chayotte), a subtropical vegetable with potent diuretic action, is a cucurbitaceus species which is used as food or as medication in popular medicine. It was reported a case of severe hypokalemia pregnancy and that a chayotte preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable stopped (Jensen and Lai, 1986). Gordon *et al*. (2000) described the hypotensor effect of the chayotte. Diré *et al.* (2002) have shown that the extracts of chayotte (macerated and infusion) were capable of altering the labeling of blood elements with technetium-99m (99mTc) in an *in vivo* study. In other research Diré *et al.* (2001) have demonstrated that a chayotte extract (macerated) was able to alter the biodistribution of 99mTc as sodium pertechnetate ($NaTcO_4$) as well as the shape of red blood cells through a qualitative analysis. The effect of stannous ion has been abolished by extracts of some medicinal plants (Reiniger *et al*., 1999; Melo *et al*, 2001; Lima *et al*., 2002, Silva *et al*., 2002). Bernardo *et al*. (2002) described that the rutin, a compound isolated from *Ruta graveolens*, was not capable of damaging DNA, protecting DNA from the $SnCl_2$ redox action and inactivating the *Escherichia coli* (*E. coli* AB1157) culture.

ROS are generated during a variety of cellular events with beneficial as well as deleterious effects to the organism (Halliwell, 1994). Some plant extracts may increase the effects of the deleterious actions of ROS (Lima *et al*., 2001). In the present study, we have evaluated the influence of a chayotte extracts on the topology on gel electrophoretic of plasmid DNA submitted to $SnCl_2$.

2 Material and Methods

2.1 Characterization of the chayotte sample

Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of the skin of chayotte were mixtured with 500 ml of water in an electric extractor. This preparation was filtered and this extract was considered 100%.

The presence of toxic compounds was evaluated and we did not find them in the extracts of chayotte used in our experiments. The method to verify the presence of these toxic products is based on inhibition of acethylcholinesterase in the presence of the pesticides (Cunha Bastos *et al*., 1991). In this method, brain acethylcholinestarase is utilized as an *in vitro* detector of organophosphorus and carbamate insecticides. Briefly, a preparation of acethylcholines-terase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of cauliflower. Enzyme

assay was performed by a potentiometric method based on the formation of acetic acid in the incubation mixture (preparation of acethylcholinesterase and extract of chayotte).

2.2 Nucleic acid manipulations

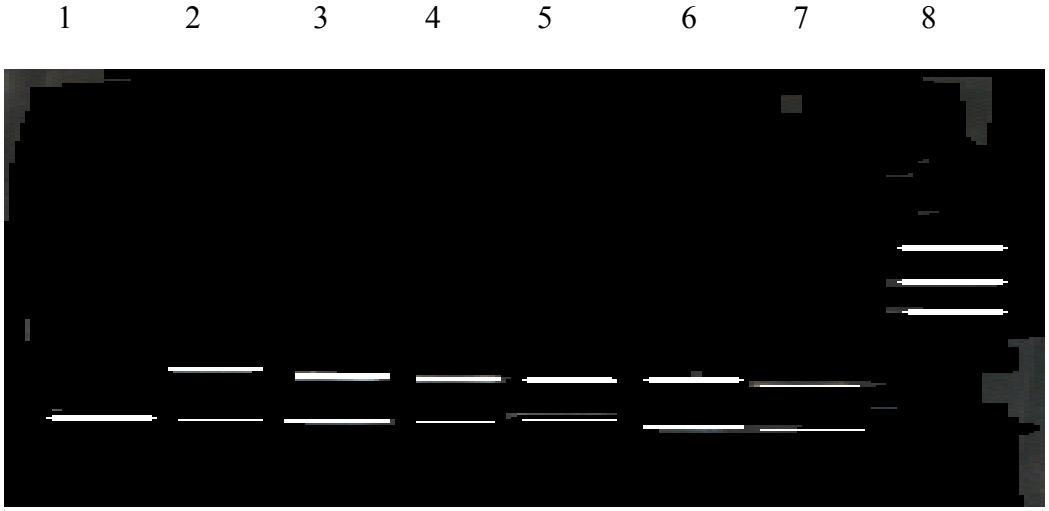
Plasmids were diluted, dispensed into eppendorf tubes (200 ng/tube) and incubated with 200 μg.mL^{-1} of $SnCl_2$. To evaluate the influence of the extract of the chayotte in DNA breakage, a concentration on a par with 0.1 g.mL^{-1} was used. In all cases, reaction mixtures were incubated at 37°C for 40 min. The analysis of the single breaks (SSB) formation was performed using 0.8% agarose gel electrophoresis in order to separate the conformations of plasmid DNA: form I supercoiled native conformation and form II open circle resulting from SSB. Aliquots from each sample (10 μL) were mixed to 2 μL of 6x concentrated loading buffer (0.25% xylene cyanol FF; 0.25% bromofenol blue; 30% glycerol), and applied in a horizontal gel electrophoresis chamber in Tris acetate-EDTA buffer at pH 8.0. After electrophoresis, the gel was stained with ethidium bromide (0.5 μg.mL^{-1}) and the DNA bands were visualized by fluorescence in an ultraviolet (UV) transiluminator system. Permanent records were performed using a polaroid MP-4^{+}system.

3 Results

Figure 1 shows electrophoresis of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of macerated extract, and the electrophoretic mobility of plasmidial DNA in various experimental conditions (macerated extract) in agarose gel.

Figure 2 showselectrophoresis pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of decoct extract (heated till 100°C) and electrophoretic mobility of plasmidial DNA in various experimental conditions (decoct extract) in agarose gel.

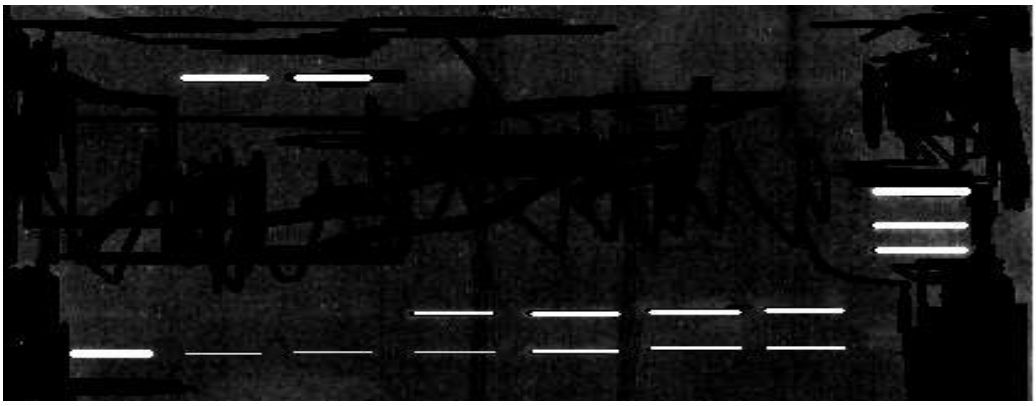
Figure 3 shows electrophoresis of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of decoct extract (heated till 50°C). and electrophoretic mobility of plasmidial DNA in various experimental conditions (heated extract) in agarose gel.



Column 1: (Control: DNA + water); Column 2: (Chayotte100%); Column 3: ($SnCl_2$ 200μg.mL^{-1});
Column 4: ($SnCl_2$- 200μg.mL^{-1} + Chayotte 100%); Column 5: (oxidized Chayotte-10min);
Column 6: (oxidized $SnCl_2$- 10min); Column 7 (oxidized Chayotte+ $SnCl_2$- 10min) ;
Column 8 (marker λ hind III). Photos of the gels were scanned.

Figure 1. Electrophoresis of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of macerated extract and electrophoretic mobility of plasmidial DNA in various experimental conditions (macerated extract) in agarose gel

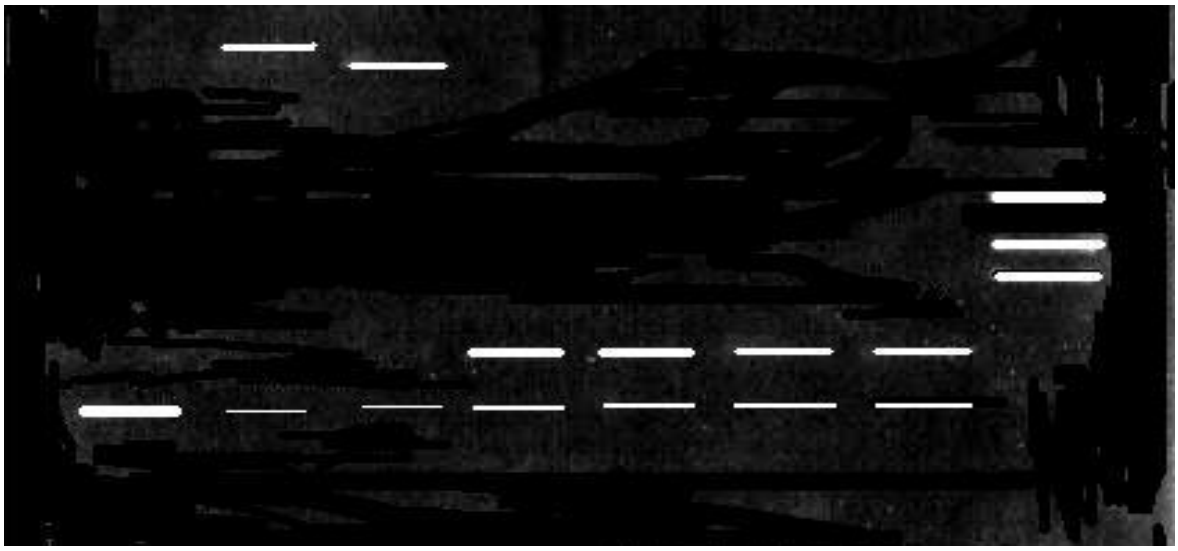
1 2 3 4 5 6 7 8



Column 1: (Control: DNA + water); Column 2: (Chayotte100%); Column 3: ($SnCl_2$ 200μg.mL^{-1});
Column 4: ($SnCl_2$- 200μg.mL^{-1} + Chayotte 100%); Column 5: (oxidized Chayotte-10min);
Column 6: (oxidized $SnCl_2$- 10min); Column 7 (oxidized Chayotte+ $SnCl_2$- 10min) ;
Column 8 (marker λ hind III). Photos of the gels were scanned.

Figure 2. Electrophoresis of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of decoct extract (heated till 100°C) and electrophoretic mobility of plasmidial DNA in various experimental conditions (decoct extract) in agarose gel

1 2 3 4 5 6 7 8



Column 1: (Control: DNA + water); Column 2: (Chayotte100%); Column 3: ($SnCl_2$ 200μg.mL^{-1});
Column 4: ($SnCl_2$- 200μg.mL^{-1} + Chayotte 100%); Column 5: (oxidized Chayotte-10min);
Column 6: (oxidized $SnCl_2$- 10min); Column 7 (oxidized Chayotte+ $SnCl_2$- 10min);
Column 8: (marker λ hind III). Photos of the gels were scanned.

Figure 3. Electrophoresis of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of decoct extract (heated till 50°C). and electrophoretic mobility of plasmidial DNA in various experimental conditions (heated extract) in agarose gel

4 Discussion

Much effort has focused on the identification of phytochemicals in plants, which exert biological effects. The knowledge of these effects are worthwhile and can help to prevent possible undesirable actions of crude extracts and/or purified substances isolated from various plants. Moreover, many times the results reported in the literature are controversies. This fact could be explained by (i) various experimental conditions and models, (ii) the characteristic and the concentration of the used material (crude extract, isolated fraction, purified substance or heated extract), and (iii) the specific condition of the growth of the studied plant. Many biological effects have been associated with the flavonoids and other antioxidant molecules (Webster *et al*., 1996; Aherne *et al*., 1999). Reactive oxygen species (ROS) have been implicated as the primary destructive intermediates in a wide range of environmental conditions as well as in an increasing number of humans disorders (mutagenesis, apoptosis, aging) (Hladik *et al*., 1987). $SnCl_2$ has been used as a reducing agent (Bernardo-Filho *et al*., 1994; Caldeira-de-Araujo *et al*., 1996) in medical procedures.

Cytotoxic and genotoxic $SnCl_2$-induced damage were demonstrated in *E. coli* and the effects appeared to be mediated by ROS (Caldeira-de-Araújo *et al.,* 1996; Dantas *et al.,* 1996; Felzenszwalb *et al*., 1998; Dantas *et al*., 1999; Reiniger *et al*., 1999).

The treatment of the *E. coli* strains AB1157 with $SnCl_2$ in presence of *Peumus boldus* (Reiniger *et al*., 1999), *Cymbopogon citratus*, *Maytenus ilicifolia*, *Baccharis genistelloides* (Melo *et al*., 2001), Rutin (Bernardo *et al*., 2002) and with *Brassica oleracea* L. var *botrytis* (Lima *et al.*, 2002) induced protection of cells against the citotoxic-$SnCl_2$ effects. It could probably be due to oxidant properties of these extracts. However, the intensity of the protective effect against the $SnCl_2$ effects was dependent on the considered extract. Like observed in the study of the cauliflower extract (Lima *et al*., 2002) the extract of chayotte was capable of inducing lesion of break type in the plasmid pUC 9.1 DNA (Lima *et al.,* 2001). In comparison with the Bernardo *et al*., 2002, study, it was verified that the rutin different of the chayotte extracts have not induced lesion in the DNA molecule. In this study the extracts of chayotte (macerated and decoct) in all concentrations tested have induced lesions in the DNA molecule. In the analysis of *Peumus boldus* extract was noticed that it has reduced or abolished the effect of $SnCl_2$ although the lesive effect of boldine was observed when the highest concentration of this substance was used in the presence of the reducing agent despite boldine alone has not been capable of inducing alterations in the DNA. Silva *et al.,* 2002, working with different cultures of *E. coli* strains also have shown a reduction of the lethal effect induced by $SnCl_2$ on the survival of the cultures in the presence of *C. citratus*, *B. genistelloides*, *M. ilicifolia* and *P. boldus*. In the present work it was verified that the heated extracts have induced more breaks in the DNA molecule in comparison with the crude one.

5 Conclusion

In general we can speculate that the extracts of chayotte were capable of inducing damages in pUC. 9.1 DNA molecules and considering the heated extracts it was observed that these extracts have induce more breaks than the macerated one.

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