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Effects of a Chayotte (*Sechium edule*) Extract (Macerated) on the Biochemistry of Blood of *Wistar* Rats and on the Action Against the Stannous Chloride Effect

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Abstract: The use of natural products as medicines has been growing in the entire world. There are concerns that these products may contain potentially toxic ingredients and contaminants such as heavy metals. The labeling of blood constituents with technetium-99m has been influenced by the presence of natural extracts. We evaluated the influence of a chayotte (*Sechium edule*) extract (100%v/v macerated) on the labeling of blood elements with ^{99m}Tc. The animals were treated with the extract during 15 days. Samples of blood were carried out with specific blood biochemistry kits. The present study analyzed the influence of chayotte in the survival of the strain of *Escherichia coli* AB1157 submitted to reactive oxygen species induced by stannous chloride. There was a reduction of the lethal effect induced by stannous chloride on the survival of the *E. coli* culture in the presence of chayotte. The results indicated a decrease in the level of glucose and globulin The effect of the extract could be explained by its metabolic transformation inducing the generation of oxidant metabolites. The culture of bacteria when was treated with stannous chloride and chayotte simultaneously, the extract could be reacting with stannous chloride ions, protecting them against the oxidation avoiding the generation of reactive oxygen species.

Key words: Chayotte, biochemistry, stannous chloride, *Escherichia coli*

INTRODUCTION

The use of herbal products is gaining popularity around the world, as they are considered to be effectual and to have few side-effects (Kam and Liew, 2002). Many drugs and vegetable extracts have been reported to affect the biodistribution of different compounds and pharmaceuticals (Capriles *et al*., 2002; Gomes *et al*., 2002; Moreno *et al*., 2002; Braga *et al*., 2000; Diré *et al*., 2001; Santos *et al*., 1995). The chayotte is a subtropical vegetable with potent diuretic action and very useful as food (Flores, 1989). It was related that chayotte is capable to reduce the diastolic pressure (Gordon, 2000).

Some pharmaceuticals may be used in the nuclear medicine as radiobiocomplexes to obtained a physiological image (Bernardo-Filho *et al*., 1994; Early and Sodee, 1995; Halliwell, 1994; Hesslewood and Leung, 1994; Srivastava and Straub, 1992). Reactive Oxygen Species (ROS) are generated during a variety of cellular events with beneficial as well as deleterious effects to the organism (Caldeira-de-Araújo *et al*., 1996; Halliwell, 1994). It was determined that *Escherichia coli* strains (*E. coli)* proficient in Deoxyribonucleic Acid (DNA) repair mechanisms were more resistant to Stannous Chloride ($SnCl_2$) treatment than deficient *E. coli* strains, suggesting that inactivation would be occurring at

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genetic level (Melo *et al*., 2001; Caldeira-de-Araújo *et al*., 1996; Halliwell, 1994). The present study evaluated the influence of a chayotte on the biochemistry of the blood of the animals treated with the referred vegetable extract during 15 days and also in a competent wild type of *E. coli* strain (AB1157).

MATERIALS AND METHODS

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 (Melo *et al*., 2001). In this method, brain acethylcholinestarase is utilized as an *in vitro* detector of organophosphorus and carbamate insecticides. Briefly, a preparation of acethylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayotte. 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).

The animals were treated during 15 days with chayotte extract. After that, samples of 4.0 mL of blood of each animal were withdrawn. Assays to evaluate the level of blood compounds were performed through of a biochemistry tests using specific kits. The level of glucose, uric acid and creatinine and total proteins was available by Dried Chemistry Method in a Vitros machine from Johnson, USA. The level of albumin and globulin was available by Bromocresol Green Method in a Mega machine from Merck, USA. The level of cholesterol and trigliceridics was utilized the Cholesterol oxidize Method in a Mega machine from Merck and the level from HDL was determined by the Direct Method without desproteinization in a Integra machine from Switzerland. The experiments were performed with the chayotte extract administrated to the animals. Whole blood was withdrawn from animals that received water or chayotte extract, as drinking water, for 15 days. The vegetable extract was prepared in the concentration of 0.1 g mL^{-1} and it was used the skin of the chayotte. For experimental which was used the bacteria colonies, it was obtained from a stock (in glycerol 50% v/v) a sample of the culture was grown on liquid LB medium 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 was taken from this culture and further incubated under the same conditions to exponential growth (10^8 cells mL^{-1}). The cells were collect by centrifugation, washed twice in 0.9% NaCl and resuspended in the same solution until they reached 10^8 cells mL^{-1}. Samples (1.5 mL) of these washed cultures (10^8 cells mL^{-1}) were incubated on the shaking water bath with stannous chloride, as $SnCl_2.2H_2O$ (24 μg mL^{-1}) or with 0.9 NaCl solution (0.5 mL), for different periods of time at 37°C. Both cultures were also treated with chayotte 250 μL (0.1 g mL^{-1}). At various periods of time after treatment, 0.1 mL aliquots were diluted with 0.9% saline and spread onto Petri dishes containing solidified LB medium (1.5% agar). Colonies were formed after overnight incubation at 37°C and the Survival Fraction (SF) was calculated by the division of the numbers of the viable cells obtained per mL, in each time of the treatment (N_t), by the number of viable cells obtained per mL at the zero time (N_o).

RESULTS

Figure 1 has shown the level of the blood compounds of *wistar* rats treated with chayotte extract and treated with water during 15 days. The analysis of the results indicates that there is a significant decrease ($p<0.05$) in the level of glucose (from 118.40±10.69 to 97.20±4.32) and globulin (from 3.52±0.13 to 3.02±0.19).

Figure 2 has shown the curve of inactivation of the cultures of *E. coli* AB 1157 treated with stannous chloride and/or chayotte extract.

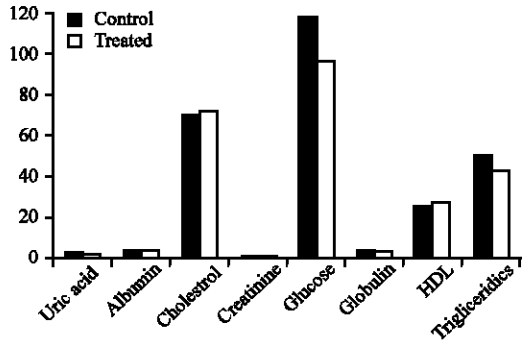

Fig. 1: Effect of chayotte extract on the biochemistry of blood. In these samples of blood (n = 10) were determined the concentrations of the blood compounds. The animals were treated during 15 days with chayotte extract. The animals of control group received water. The blood was withdrawn in the morning period after a break of 8 h on an empty stomach. The values of. globulin is expressed in g mL^{-1}, the one of HDL in UI and the other in mg mL^{-1}.

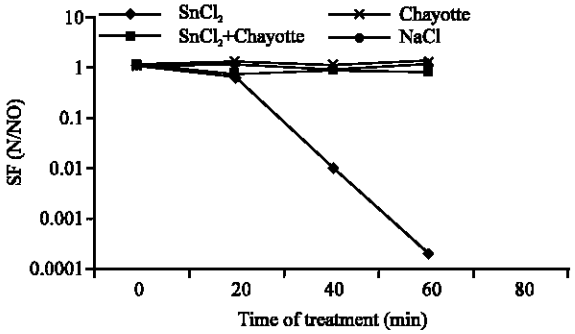

Fig. 2: Curve of inactivation of the cultures of *E. coli* AB 1157 treated with stannous chloride and/or chayotte extract. Cultures of wild bacteria (AB1157) were treated with NaCl (0.9%) as control, with $SnCl_2$ or chayotte extract or simultaneously ($SnCl_2$+chayotte extract), with chayotte extract and stannous chloride. Aliquots were obtained in each 20 min, diluted and spread onto petri dishes. After the counting of the colonies, the survival fractions were calculated.

The analysis of the results indicated a strong inactivation induced by $SnCl_2$ in the DNA repair of proficient *E. coli* cells. In addition, we can also observe the protective effect induced by chayotte extract against the inactivation produced by the treatment with the referred reducing agent. Moreover, the $SnCl_2$ toxic effect is abolished by the treatment in the presence of the chayotte extract.

DISCUSSION

The effect of chayotte extract probably, could be explained by the its metabolic transformation that could be capable to induce the generation of active metabolites with oxidant property. These active metabolites could act directly or indirectly, by the generation of reactive oxygen species. As reported for other plants, as *Solanum melongena* (Capriles *et al*., 2002), *Maytenus ilicifolia* (Oliveira *et al*., 2000), *Thuya occidentalis* (Oliveira *et al*., 1997), *Fucus vesiculosus* (Oliveira *et al*., 2003), *Paullinia cupana* (Oliveira *et al*., 2002), *Mentha crispa L.* (Santos-Filho *et al*., 2002) and *Nicotiana tabacum* (Vidal *et al*., 1998), chayotte extract could has an oxidant action as this effect could be supported by the results described by an *in vivo* and *in vitro* study which Diré *et al*. (2002) have observed that chayotte extracts (decoct and macerated) administrated to *Wistar* rats were able to alter the efficiency of radio labeling of blood constituents. The oxidative effect may induce an decrease on the level of glucose and globulin.

The possible oxidant action of the referred extract was observed in the experimental which was utilized *E. coli* (AB1157). Like *Brassica oleracea* (Lima *et al*., 2002), *Cymbopogon citratus*, *Maytenus ilicifolia and Baccharis genistelloides* (Melo *et al*., 2001) extracts, the cultures were treated in the presence of stannous chloride (lethal) and/or chayotte extract and the survival of the cultures due to the treatment with stannous chloride was increased by the presence of the referred extracts. Probably, the oxidant agents present in the extract of chayotte would oxidize the stannous ions, preventing that the same could act against the *E. coli* cultures. The same way, was showed by Reiniger *et al*. (1999), showing that the lethal effect of stannous chloride was abolished by boldine extract (*Peumus boldus*). As observed with Rutin (Bernardo *et al*., 2002) extract, a flavonoid isolated from *Ruta graveolens, C. citratus, M. ilicifolia* and *B. genistelloides* (Melo *et al*., 2001) and *F. vesiculosus* (Oliveira *et al*., 2003) extracts, the *Sechium edule* extract were not capable to induce important alteration in the survival of *E. coli* strain.

CONCLUSIONS

The present study suggest, that the oxidant effect of the extract had promoted the decreased of the levels of glucose and globulin fraction. The effect of chayotte extract, probably, could be explained by its metabolic transformation which could be capable to induce the generation of active metabolites with oxidant property. In addition, as we could observed in the experimental using the bacteria colonies of *E. coli* (AB1157), we speculated that the protective effect of chayotte extract against the lethal action of $SnCl_2$ might be explained by an oxidant action. This fact could oxidize the stannous ion and, consequently, avoiding the generation of active oxygen species which might induce lesions directly in the DNA molecule.

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