Journal of Animal and Veterinary Advances 3 (5): 335-338, 2004

Study of the Effect of an *Ageratum conyzoides* Linn. Extract on the Plasmid pUC 9.1 DNA

[1,2]Franklin P. S. Soares, [1,2] Fabrício M. Rapuano, [1,2]Roberta A. Framil, [1,2]Gláucio F. Diré, [1,2]Maria L. Gomes and [2,3]Mario Bernardo-Filho
[1]Universidade Estácio de Sá, Centro de Ciências da Saúde, Faculdade de Fisioterapia, Campus Resende, Rio de Janeiro, Brazil; [2]Universidade do Estado do Rio de Janeiro, Instituto de Biologia Alberto Alcantara Gomes, Departamento de Biofisica e Biometria, Av. 28 de setembro n 87, 20551-030, Rio de Janeiro, Brasil; [3]Instituto Nacional do Câncer, Coordenadoria de Pesquisa, Praça Cruz Vermelha, 23, Rio de Janeiro, RJ, Brasil, 20230-130

Abstract: Stannous (Sn), as fluoride or chloride, is frequently employed to label cells with Technetium-99m (^{99m}Tc) to be used as radiopharmaceuticals or radiotracers. 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 *Ageratum conysoides* is a plant with analgesic, antibacterial, anti-inflammatory, depurative, febrifuge, stimulant and vulnerary properties. In order to analyze the effects of the referred extract, in this study plasmid deoxyribonucleic acid (DNA) was exposed to the *A. conysoides* extract (aqueous extract) ($0.1 g.mL^{-1}$) in presence of stannous chloride ($SnCl_2$). Samples of the plasmid DNA were analyzed through agarose gel electrophoresis. Concerning to the results obtained it was noticed that the refereed extract were capable of damaging the DNA in the presence and in the absent of $SnCl_2$.

Key words: Plasmidial DNA, *Ageratum conysoides*, *Escherichia coli*, stannous chloride

Introduction

Stannous (Sn^{+2}), as fluoride or chloride, is frequently employed to label cell or molecules with Technetium-99m (^{99m}Tc) to be used as radiopharmaceuticals or radiotracers. Howerver, Sn^{+2} ions can be involved with several risks for human health. Stannous chloride ($SnCl_2$) can cause skin and mucosal irritation in humansand 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 and 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 and 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 $^{\cdot}OH$ as follows: $Fe^{2+} + H_2O_2 \rightarrow OH^- + {}^{\cdot}OH$. 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). $SnCl_2$ 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 developedand 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 and Sallé, 1996). *Ageratum conysoides* is known in Brazil as *Catinga de Bode*. It is a plant from the Central and Meridian

America. *A. conysoides* is a commonly used medicinal plant for a variety of indications due to its analgesic, antibacterial, anti-inflammatory maybe due to the presence of alkaloids with vase constrictor vase action, depurative, febrifuge, stimulant and vulnerary properties. Shirwaikar *et al.* (2003) have been demonstrated the gastroprotection effect of the referred extract in rats. It has been related that this plant has immunostimulant, antioxidantand more recently antimutagenic properties. The findings suggested that the significant gastroprotective activity could be mediated by its antioxidant activity, Ca^{2+} channel blocking and antiserotogenic properties. Jagetia *et al.* (2003) described that the radioprotection afforded by *A. conysoides* may be in part due to the scavenging of reactive oxygen species induced by ionizing radiation. 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 *A. conysoides* extract on the topology on gel electrophoretic of plasmid DNA submitted to $SnCl_2$.

Materials and Methods

Characterization of the *A. conysoides* Sample: A commercial dried powder of *A. conysoides* was obtained from the Laboratory Herbarium, Laboratório Botânico, Brazil, Lot 923661 (June, 2001 and validity June 2004). To prepare the solution, which was considered like 100% it was diluted, 10g of *A. conysoides* into 10mL of saline solution (NaCl 0.9%) obtained a solution 100% (0.1mg mL^{-1}).

The presence of toxic compounds was evaluated and we did not find them in the extract of *A. conysoides* 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 acethylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of *A. conysoides*. 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 *A. conysoides*)

Nucleic Acid Manipulations: Plasmids were diluted, dispensed into eppendorf tubes (200ng per tube) and incubated with 200μg.mL^{-1} of $SnCl_2$. To evaluate the influence of the extract of *Ageratum conyzoides* in the DNA breakage, a concentration on a par with 0.1g.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 (10μL) were mixed to 2μL 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 (0.5μg.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.

Results

In the Fig. 1 is shown the electrophoresis in agarose gel of. pUC. 9.1 plasmid treated with $SnCl_2$ and/or the extract of *A. conysoides*. Trough the analysis of the result it was noticed that the refereed extract was capable of inducing lesion in the plasmid deoxyribonucleic acid in the presence and in the absent of $SnCl_2$.

Discussion

The use of medicinal plants or natural products has increased in the last decades all over the world. Much effort has focused on the identification of phytochemicals in plants, which exert biological effects. The developing of models that permit evaluation of the biologic properties of natural products is worthwhile. The knowledge of these effects are expanding 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 and 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 and 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

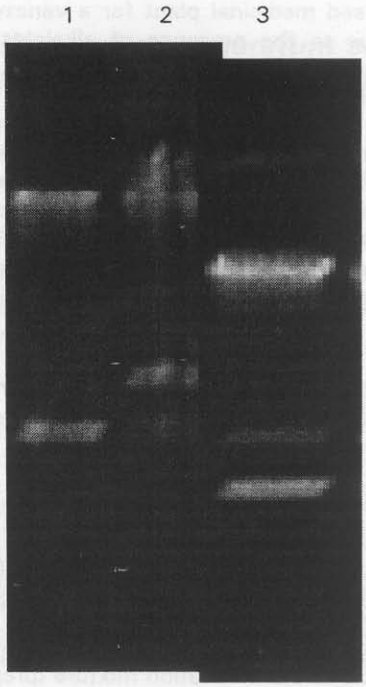

Fig.1: Electrophoresis in 0.8% agarose gel of pUC 9.1 plasmid treated with $SnCl_2$ and with an extract of Ageratum conyzoides
Lanes: 1, untreated control; 2, SnCl2 (200μg/ml); 3, extract (100%). I, supercoiled form; II, open circle form.

mediated by ROS (Caldeira-de-Araújo *et al.*, 1996; Dantas *et al.*, 1996; Felzenszwalb *et al.*, 1998; Dantas *et al.*, 1999 and Reiniger *et al.*, 1999).

The way observed in the study of the cauliflower extract (Lima *et al.*, 2002) the extract of *A. conysoides* 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 *A. conysoides* extract has not induced lesion in the DNA molecule. In this study the extract of *A. conysoides* has induced lesions in the DNA molecule in the presence and in the absent of $SnCl_2$. Reiniger *et al.* (1999) in the analysis of *Peumus boldus* extract have 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. The effect of *A. conysoides* extract may be due to its oxidant properties.

Conclusion

In conclusion, we may speculate that the extract of *A. conysoides* was capable of inducing damages in pUC 9.1 DNA probably due to its oxidant properties.

Acknowledgments

This research was supported by CNPq, CAPES, FAPERJ and UERJ.

References

Ashby, J. and R. W. Tennant, 1991. Definitive relationships among chemical and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutat. Res., 257: 229-306.

Aherne, S. A. and M. N. O'Brien, 1999. Protection by the flavonoids myricetin, quercetin and rutin against hydrogen peroxidase-induced DNA damage in Caco-2 Nd Hep G2 cells. Nutr. Cancer, 34:160-166.

Bernardo-Filho, M., M.C. Cunha, J.O. Valsa, A .C. Araujo, F.C.P. Silva and A.S. Fonseca, 1994b. Evaluation of potential genotoxicity of stannous chloride: inactivation, filamentation and lysogenic induction of *Escherichia coli*. Food. Chem. Toxicol., 32:477-479.

Bernardo, L.C., M.B.N. Oliveira, C.R. Silva, F.J.S. Dantas, J.C.P. Mattos, A. Caldeira-de-Araújo, R.S. Moura and M. Bernardo-Filho, 2002. Biological effects of rutin on the survival of Escherichia coli AB1157 and on the electrophoretic mobility of plasmid pUC 9.1 DNA. Cell. Mol. Biol., 48: 517-520.

Budavery, S., 1996. The Merck Index. Merck. pp. 1500-1501. White-house Station, NJ.

Cabral, R.E.C., A.C. leitão, C. Lage, A. Caldeira-de-Araújo, M. Bernardo-Filho, F.J.S.Dantas and J.B. Cabral-Neto, 1998. Mutational potentiality of stannous chloride: an important reducing agent in the Tc-99m radiopharmaceuticals. Mutat. Res., 408: 129-135.

Caldeira-de-Araújo, A., F.J.S. Dantas, M.O. Moraes, I. Felzenszwalb and M. Bernardo- Filho, 1996. Stannous chloride participate in the generation of reactive oxygen species. J. Braz. Assoc. Adv. Sci., 1996, 48: 109-113.

Capasso, R., A.A. Izzo, L. Pinto, T. Bifulco, C. Vitobello and M. Mascolo, 2000. Phytotherapy and quality of herbal medicines, Fitoterapia 71: S58-S65.

Cunha Bastos, V.L.F., J.F. Cunha Bastos and J.S. Lima, 1991. Brain acethylcholinesterase as an *in vitro* detector of organophosphorus and carbamate insectides in the water. Water Res., 7: 835-840.

Dantas, F.J.S., M.O. Moraes, J.C.P. De Mattos, R.J.A.C. Bezerra, E.F. Carvalho, M. Bernardo-Filho and A. Caldeira-de-Araújo, 1999. Stannous chloride mediates single strand breaks in plasmid DNA through reactive oxygen species formation. Toxicol. Lett., 110: 129-136.

Dantas, F.J.S., M.O. Moraes, E.F. Carvalho, J. O. Valsa, M. Bernardo-Filho and A. Caldeira-de-Araújo, 1996. Lethality induced by stannous chloride on Escherichia coli AB1157: participation of reactive oxygen species. Food Chem. Toxicol., 34: 959-962.

Felzenszwalb, I., J.C.P. De Mattos, M. Bernardo-Filho and A. Caldeira-de-Araújo, 1998. Shark cartilage-containing preparation: protection against reactive oxygen species. Food Chem. Toxicol., 36: 1079-1084.

Gleason, M.N., R.E. Gosselin, C.H. Hodge and R.P. Smith, 1969. Clinical toxicology of Commercial Products (Acue Poisoning), Williams and Wilkins, Baltimore.

Hallas, L.E. and J.J. Cooney, 1981. Tin and tin-resistante microorganisms in Chesapeake Bay. Appl. Environ. Microb., 41: 466-471.

Halliwell, B., 1994. Free radicals and antioxidants: a personal view. Nutr. Rev., 52: 256-258.

Hladik III, W.B., G.B. Saha and K.T. Study, 1987. Essentials of Nuclear Medicine Science. Williams and Wilkins, Baltimore, London.

Jagetia, G.C., A. Shirwaikar, S.K. Rao and P.M. Bhilegaonkar, 2003. Evaluation of the radioprotective effect of Ageratum conyzoides Linn. extract in mice exposed to different doses of gamma radiation. J. Pharm Pharmacol., 8: 1151-1158.

Leite, J.R., M. de L. Seabra, E. Maluf, K. Assolant, D. Suchecki, S. Tufik, S. Klepacz, H.M. Calil and E.A. Carlini, 1986. Pharmacology of. lemongrass (*Cymbopogon citratus* Stapf). Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. J. Ethnopharmacol. 17: 75-83.

Lima, E.A.C., G. Diré, D.M.M. Mattos, M.N. Oliveira, J.C.P. Mattos, F.J.S. Dantas, A. Caldeira-de-Araújo and M. Bernardo-Filho, 2001. Effect of the leaf extract from cauliflower (Brassica oleracea L.Var.Botrytis) on the biodistribution of the radiopharmaceutical sodium pertechnetate in mice and on the electrophoretic mobility of plasmid pUC 9.1 DNA. J. Labelled Cpd. Radiopharm., 44: 642-644.

Lima, E.A.C., G. Diré, D.M.M. Mattos, R.S. Freitas, M.L. Gomes, M.B.N. Oliveira, M.V.C. Faria, R.L. Jales and M. Bernardo-Filho, 2002. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99m and on the survival of Escherichia coli AB1157 submitted to the treatment with stannous chloride. Food. Chem. Toxicol., 40: 919-923.

McLean, J.R.N., D.H. Blakey, G.R. Douglas and J.R. Kaplan, 1983. The effect of stannous and stannic (tin) chloride on DNA in Chinese hamster ovary cells. Mutat. Res., 119: 195-201.

Moro, C.O. and G. Basile, 2000. Obesity and medicinal plants, Fitoterapia, 71: S73-S82.

Oliver, P. and D. Marzin, 1987. Study of genotoxic potential of 48 inorganic derivates with the SOS chromotest. Mut.. Res., 189: 263-269.

Rader, J.I., 1991. Anti-nutritive effects of dietary tin. Adv. Exp. Med. Biol., 289: 509-524.

Reiniger, I.W., C.R. Silva, I. Felzenszwalb, J.C.P. Mattos, J.F. Oliveira, F.J.S. Dantas, A.A.C. Bezerra and M. Bernardo-Filho, 1999. Boldine action against the stannous chloride effect. J. Ethnopharmacol., 68: 345-348.

Sallé J. L.O., 1996. Totum Em Fitoterapia. Robe Editorial, São Paulo, p.237.

Singh, I., 1983. Introduction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisae*. Mutat. Res., 117: 149-152.

Shirwaikar, A., P.M. Bhilegaonkar, S. Malini and J.S. Kumar, 2003. The gastroprotective activity of the ethanol extract of Ageratum conyzoides. J Ethnopharmacol., 1:117-121.

Triphaty, N.K., F.E. Wurgler and H. Frei, 1990. Genetic toxicity of six carcinogens and six non carcinogens in the *Drosophila* wing spot test. Mut. Res., 242:169-180.

White, D.J., 1995. Return to stannous fluoride dentifrices. J. Clin. Dentol., 6:29-36.