Journal of Agricultural Science; Vol. 9, No. 9; 2017
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Effect of the *Cymbopogon citratus* Infusion on the Activity of Acetylcholinesterase Enzyme and on the Redox Profile in Farmers' Erythrocytes

Natacha Cossettin Mori[1,2], Roberta Cattaneo Horn[1,2,3], Caroline Oliveira[1,2], Gabriela Tassotti Gelatti[1,2], Jonatas Zeni Klafke[2], Ana Caroline Tissiani[1], Viviane Cecília Nunes Deuschle[1], Cecília Gabriela Rubert Possenti[3] & Bruna Tomazetti Micheloti[4]

[1] Laboratório de Plantas Medicinais e Estresse Oxidativo, Grupo de Pesquisa em Atenção Integral a Saúde, Universidade de Cruz Alta, Brazil

[2] Programa de Pós-Graduação em Atenção Integral a Saúde, Universidade de Cruz Alta e Universidade do Noroeste do Rio Grande do Sul, Brazil

[3] Mestrado Profissional em Desenvolvimento Rural, Universidade de Cruz Alta, Brazil

[4] Programa de Pós-Graduação em Zootecnia, Universidade Federal de Santa Maria, Brazil

Correspondence: Roberta Cattaneo Horn, Laboratório de Estresse Oxidativo, Programa de Pós-Graduação em Atenção Integral a Saúde, Universidade de Cruz Alta e Universidade do Noroeste do Rio Grande do Sul, Campus Universitário Dr. Ulisses Guimarães, Rod. Municipal Jacob Della Méa, km 5.6, Parada Benito, 98020-290, Cruz Alta, RS, Brazil. E-mail: rcattaneo@unicruz.edu.br

Received: June 21, 2017 Accepted: July 25, 2017 Online Published: August 15, 2017

doi:10.5539/jas.v9n9p68 URL: https://doi.org/10.5539/jas.v9n9p68

Abstract

Agrochemicals were more prominent in 1960, marked due to the agricultural modernization process. As a result of this widespread use for food production, there was also an increase in cases of intoxication caused by these agents which made it necessary to search for alternative therapies for agricultural workers. Thus, considering that phytochemical characterization revealed the presence of antioxidants in *Cymbopogon citratus* extract, the objective of this study was to evaluate the effect of this plant infusion on the enzyme acetylcholinesterase activity (AChE) and on the redox response in farmers' erythrocytes. These erytrocytes were processed and subjected to treatment with the *Cymbopogon citratus* infusion (5, 10, 25 and 50 g/L). In these samples the following were determined: the AChE enzyme activity, the levels of thiobarbituric acid reactive substances (TBARS), protein carbonyls (CPs) and reduced glutathione (GSH). In general, it was discovered that the inhibition of AChE activity is negative regarding to the increase of protein carbonyl levels and positive regarding the GSH levels. In addition, *Cymbopogon citratus* infusions could not even reverse this inhibition or the high levels of TBARS and CPs. On the other hand, levels of GSH were increased by infusions demonstrating the increased antioxidant activity in rural workers' erythrocytes.

Keywords: farmworkers, pesticides, treatment, *Cymbopogon citratus*

1. Introduction

Agriculture has been practicing by the humanity for over ten thousand years, however the intensive pesticides use for the pests and diseases control on the tillage has existed since a little more than half a century. In Brazil, the agro-chemists started being more prominent in the 1960's, marked by the agricultural process modernization (Recena et al., 2006; Silva et al., 2015). Thus, there was also an increase in the cases of intoxication caused by the widespread use of pesticides for the food production these agents. According to the National System of toxic-pharmacological information (Fiocruz, 2015) 33.86% of the deaths by intoxication recorded in Brazil in 2012 were due to contact with pesticides.

The agrochemicals promote the acetilcolinesterase enzyme (AChE) inhibition, causing the acetylcholine accumulation in the synoptical cleft and consequent Acute cholinergic syndrome, what leads to the development of several symptoms, such as muscle stiffness, fatigue, tiredness, headache, among others (Bayrami et al., 2012; Cárdenas et al., 2005). Besides, several "*in vitro*" studies indicate that blood markers are negatively affected by

increased oxidative stress, considering that the AChE activity is also inhibited by the increase of the reactive species (Molochkina et al., 2005). According to Milatovic, Gupta, and Aschner (2006) the agricultural pesticides promote the oxidative phosphorylation inhibition in the central nervous system, debilitating the cells capacity to keep their energy levels, what possibly increases the quantity of reactive oxygen species and reactive nitrogen species in the organism, characterizing the oxidative stress condition. This mechanism has also been pointed as relevant to justify the adverse effects in the farmers' health who were exposed to the agricultural pesticides (Surajudeen et al., 2014). Despite of this, the biological AChE role present in the erythrocyte membrane is not well explained, and it is known that this enzyme is present in the red blood cells and it has several similar proprieties like in the purified shape obtained from the brain tissue (Sorensen, Gentinetta, & Brodbeck, 1982). Therefore, the AChE activity in erythrocytes can be considered an indicator of the central cholinergic state (Kaizer et al., 2008).

The oxidative stress occurs due to the existence of an unbalance between the oxidant and antioxidant compounds, in favor of the excessive generation of reactive species or a speed detriment during the removal of the reactive species. This process leads to the biomolecular oxidation with consequent loss of its biological functions and/or homeostatic unbalance, whose manifestation is the oxidative-potential damage against the cells and tissues (Barbosa et al., 2010). The reactive species promotes a rupture in the DNA chain, lipid peroxidation of the cellular membrane and protein degradation. In the biological systems these damages are reflected through performing a comet test and the micronucleus determination, determination of the substance levels reactive to the thiobarbituric acid (TBARS) and by the quantification of carbonyls protein levels, respectively (Vasconcelos et al., 2007).

The excess of reactive oxygen species in the organism is combated by antioxidants produced by the body or absorbed from the diet. The endogenous antioxidants divide themselves among the ones that act enzymatically, as for instance the glutathione peroxidase, catalase and superoxide dismutase, or non-enzymatically, for example the reduced glutathione (GSH), histidine peptides and proteins bound to iron (transferrin and ferritin). Moreover, it is noteworthy the antioxidant function of the compounds obtained from food, such as the alpha-tocopherol (vitamin-E), beta-carotene (pro-vitamin A), ascorbic acid (vitamin-C) and phenolic compounds (Barreiros, David, & David, 2006). According to Barbosa et al. (2010), despite of the effects of the dietetic antioxidants over the oxidative stress are still not conclusive, diet is a factor of great importance in the oxidative stress modulation.

Plants have important phenolic compounds resource, such as: flavonoids, phenolic acids, tannins and tocopherols (Shahidi & Janitha, 1992; Neves & Cunha, 2006). Alvis, Martines, and Arrazola (2012), and Soares et al. (2013) studies show the presence of these same phytochemical compounds in the *Cymbopogon citratus* leaves extract. However, it should be considered that certain plants exhibit potentially dangerous substances (Rodrigues et al., 2011), what makes it indispensable more bodies of research about the utility of the natural resources that surround us.

The *Cymbopogon citratus,* known popularly as lemongrass, is highlighted due its high level of citral, that besides therapeutic efficacy, seems to be a powerful captor of reactive oxygen species, promoting reduction of the same (Halabi & Sheikh, 2014). In addition, Soares et al. (2013) evaluated the total antioxidant capacity of the extract *Cymbopogon citratus* leaves with different solvents (water, methanol and ethanol) and they detected the elimination of reactive species activity in all of them.

Therefore, considering that phytochemical characterization of *Cymbopogom citratus* revealed the presence of antioxidant substances in this plant and that the contact with the agrochemicals promotes alterations in the AChE activities with possible generation of oxidative stress, it becomes relevant the evaluation of the effect of the same on the AChE and on the redox answer in farmers' erythrocytes, since the lemongrass can be considered a future therapeutic alternative for the workers exposed to the agrochemicals.

2. Method

2.1 Ethical Aspects

The present research paper was accepted by the Research Ethics Committee, from Cruz Alta University (UNICRUZ) under the protocol: 0071.0.417.000-11. The participants of this study were consulted about the participation viability at the research and they signed the Free Enlightened Consent Form (TCLE).

2.2 Population Studied

The samples used were from farmers who live in COREDE Alto Jacuí, RS, Brazil, who signed the TCLE, aged from 18 to 59 years old and that worked for at least 3 years with agriculture (inclusion criteria). To compose the control group healthy individuals were selected with sex and age similar to the farmers' group. All the volunteers

answered a questionnaire about occupational health with structured questions in order to use these data to select the research participants concerning the exclusion criteria, like: being non-smoker, not being alcoholic addict and not having chronic diseases.

Table 1 shows the participants' characteristics in this study.

Table 1. Profile of the participants

	Healthy individuals (Group S)	**Farmers**
Male (%)	90	96.67
Age (years old)	38±9	41±12
Sedentarism (%)	90±6	83.3±3
Exposure period (years)	-	21.9±12.2
Use of individual protection equipment (%)	-	40
Last application (Days)	-	58.9±10.4

Thus, 30 of the 40 farmers who accepted to participate in this research were randomly selected and divided in 5 groups:

Group 0 (basal): erythrocytes from farmers, without lemongrass treatment.

Group 5: erythrocytes from farmers, treated with 5 g/L lemongrass infusion.

Group 10: erythrocytes from farmers, treated with 10 g/L lemongrass infusion.

Group 25: erythrocytes from farmers, treated with 25 g/L lemongrass infusion.

Group 50: erythrocytes from farmers, treated with 50 g/L lemongrass infusion.

The control group (group S) was also composed by samples from 30 healthy individuals and they did not receive treatment with the *Cymbopogom citratus* infusion.

The participants' blood collection was performed by using vacutainers with ethylenediamine tetraacetic acid and the samples were centrifuged immediately on 3000 rpm during 10 minutes and the plasmas were removed. The erythrocytes were washed three times with cold isotonic saline solution and centrifuged again. After the final washing, the erythrocytes were resuspended in saline solution, and then diluted until they reached 5% hematocrit according to the technique described by Catalgol et al. (2007), with small adaptations. After the erythrocytes dilution, a treatment with the plant leaves infusion with concentrations (5-50 g/L) closer to those ones used by the population was carried out. So, the samples remained incubated for 1 hour in Bain Marie at 37 °C, after this period the samples were hemolyzed in vortex for 30 seconds and centrifuged on 3600 rpm for 15 seconds. The samples were stored at -20 °C for posterior analytical determinations.

2.3 Reagents and Samples

Sodium carbonate, Folin-Ciocalteau, gallic acid, aluminum chloride, vanillin solution, methanol, ethanol, hydrochloric acid, catechin, acethylthiocholine, 5,5-dithio-bis(2-nitrobenzoic acid), malondialdehyde, 2-thiobarbituric acid, trichloroacetic acid, 2,4-dinitrofenilhidrazina and sodium dodecyl sulfate were obtained from Sigma Chemical (St. Louis, MO). Distilled water (grade 3) was used in analytical determinations.

The *Cymbopogom citratus* was prepared in order to compare the phytochemical concentrations present in the same with the infusion concentrations. For this, the methodology described by Simões et al. (2010) was followed, which recommends the use of water and ethanol (70:30) as extractor solvents. The plant material (*Cymbopogon citratus* leaves) was submitted to daily manual agitation during 14 days, filtered and concentrated in rotary evaporator. This extract was lyophilized to remove water, obtaining thus, the crude hydroethanolic extract.

The *Cymbopogom citratus* leaves were from the UNICRUZ vegetable garden, Rio Grande do Sul, Brazil. The infusion preparation consisted of pouring boiling water on the plant and then putting the lid on covering the recipient for 10 min. According to the Brazilian pharmacopoeia (2012), this method is indicated for parts of the plants which do not have such a hard consistency as the leaves, flowers and fruits, or that have volatile active substances.

2.4 Analytical Determinations

The composition of *Cymbopogom citratus* infusion was evaluated and all determinations were performed in triplicate. Thus, the total polyphenols determination was performed by Folin-Ciocalteau method, described by Chandra and Mejia (2004), with alterations. For this, the sample was diluted in a 0.150 mg/mL concentration, plus 20% Sodium carbonate solution and 2 N Folin-Ciocalteau reagent. The solution was incubated for 10 minutes and the absorbances measured at 730 nm. The total polyphenols content was expressed as gallic acid based on the gallic acid calibration curve.

The total flavonoids contents were determined according to the method described by Woisky and Salatino (1998). The sample was diluted in a concentration of 1mg/mL plus aluminum chloride and methanol. The absorbances were measured at 420 nm.

The condensate tannins was determined by the method described by Morrison et al. (1995) with some changes. The sample was diluted in a concentration of 25 mg/mL in ethanol. Afterwards, a vanillin solution (10 g/L in ethanol) and hydrochloric acid (0,08 M) were added. The absorbances were measured at 500 nm. The total tannins content was expressed as catechin based in the catechin calibration curve.

The analysis of the AChE enzyme activity was performed following the methodology described by Ellman et al. (1961) which mixed potassium phosphate tampon (100 mM (pH 7.5)), 5,5-dithio-bis(2-nitrobenzoic acid) (10 mM) and substrate acetylthiocholine (1 mM). The absorbances were measured at 412 nm at a rate of 20 s during 2 min. The results were expressed in UI/mL.

The lipid peroxidation was determined according to Stocks and Dormandy (1971) protocols. Afterwards, it was added to the reactive mixture which has 28% (v/v) trichloroacetic acid; thiobarbituric acid (0.1 mol/L) followed by heating at 95 ºC for 15 minutes. The absorbances were measured at 532 nm. The results were expressed in nmol TBARS/g Hb.

The analyses of CPs levels were performed by means of a modification Levine et al. (1990) method, where trichloroacetic acid (10% (v/v)), hydrochloric acid (2 N), 2,4-dinitrofenilhidrazina (10 mM) and sodium dodecyl sulfate (3% (m/v)) were used as the reaction mixture. The absorbances were measured at 370 nm. The results were expressed in nmol carbonyl/mg CP.

The GSH levels were determined through the technique described by Ellman (1959) adapted for erythrocytes, where a potassium phosphate buffer (1 M, pH 7.4) and acid 5,5'-ditiobis-(2-nitrobenzoic) (10 mM) are used. The procedure was performed in ice bath and the absorbance measures were performed at 412 nm. The results were expressed in μmol GSH/mL.

2.5 Statistical Methods

Lemongrass extracts were obtained and characterized in triplicate and the results expressed by average ± standard deviation. The results obtained were submitted to the t-student test for parametric data considering the significantly different rates with a $p < 0.001$.

The analytical determinations of all the samples were performed in triplicate and their results were expressed by ± SEM (pattern mistake). The variables distribution was tested using the Kolmogorov-Smirnov test. The data obtained by all the studied groups, for the same parameter, were submitted to the one-way variance analysis followed by Tukey-Kramer test for the parametric data, or KrusKal-Wallis followed by Dunn Multiple Comparison or Mann Whitney test for the non-parametric data. The AChE, CPs and GSH results were transformed for the parametric analysis, where the Y = log (Y) and the TBARS results were analyzed by non-parametric tests. Significantly different rates with a $p < 0.05$ were considered.

3. Results and Discussion

In Table 2 the levels of phytochemicals antioxidants present in the extract (E) and in the (I) lemongrass infusion in the 50 g/L concentration. These results demonstrate that both the extract and the infusion of this plant contain compounds with antioxidant activity, however, a significant difference was verified between the concentration of these components, when both preparations were compared, highlighting the minor quantification for the tested infusion. These results confirm, corroborating with Cheel et al. (2005), that found the main ERs eliminator both in the infusion and in the decoction of the *Cymbopogom citratus* extract, but with important differences in the relative proportions in each preparation.

Table 2. Quantification of total polyphenols, tannins and flavonoids of the extract and *Cymbopogom citratus* infusion

Sample	Quantity (mg/g)		
	Polyphenols Total	**Flavonoids**	**Tannins**
Cymbopogom citratus extract (E)	59.36±0.28	28.3±0,006	0.613±0.006
Cymbopogom citratus 50 g/L Infusion (I)	22.98±0.8 *	12.4±0.32 *	0.0 *

Note. The results were expressed by the average±pattern deviation; *indicates significantly different results, considering $p < 0.01$.

Although the presence of polyphenols and tannins is evidenced in the lemongrass, it is possible to point out the quantity of flavonoids both in the extract and in the infusion of this plant, fact which according to Balakrishnan, Paramasivam, and Arulkumar (2014), indicates an important antioxidant lemongrass activity, due to the fact that, these authors report that the flavonoids have strong activities of reactive oxygen species elimination, and it is effective regarding the cell protection against the oxidative damages.

However, it is useful to consider that according to Simões et al. (2010) and Sousa et al. (2007), the total polyphenols contribute in the initiation period, just like in the oxidative process propagation and the tannins seem to intercept with the reactive oxygen forming more stable radicals.

Figure 1 demonstrates the results concerning the AChE enzyme activity, where it is possible to notice that in the farmers' erythrocytes without the plant treatment (group 0), this enzyme activity is found significantly reduced (0.53±0.15 UI/mL), when compared to the control group (3.78±0.22 UI/mL), indicating that these rural workers are intoxicated with pesticides that inhibit the AChE activity.

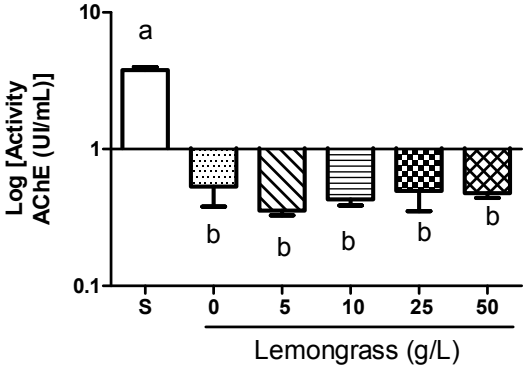

Figure 1. AChE enzyme activity in erythrocytes treated with lemongrass infusion at different concentrations: without lemongrass infusion (Group S and basal), 5 g/L (group 5), 10 g/L (group 10), 25 g/L (group 25) and 50 g/L (group 50). Different letters means significant differences ($p < 0.05$)

This inhibition of the AChE activity causes the acetylcholine accumulation in the synaptic cleft of the nervous terminals, what can cause several undesirable physiological effects, such as: back pain, and intense headache (Murussi et al., 2014), symptoms that are often reported by workers who are exposed to pesticides.

With the purpose of verifying if the *Cymbopogom citratus* would revert the inhibition of the AChE enzyme activity, farmers' erythrocytes were treated with different concentrations of lemongrass infusion. No significant alterations were observed after these treatments, suggesting that this plant does not have action on the AChE activity. Similarly, Adaramoye and Azeez (2014) did not observe relevant differences between AChE and rats treated with the *Cymbopogom citratus* extract and the control group (rats not treated with the plant).

The erythrocyte membrane is a direct target to the lipid peroxidation and protein carbonylation in oxidative stress conditions. Figure 2 shows that the TBARS and CPs levels analyzed in the farmers' erythrocytes were higher than the quantity of these oxidative markers in the control group, showing an occurrence of oxidative stress in these individuals that were chronically exposed to the tillage chemical products, what increases the risk of development of some pathologies, like: atherosclerosis, diabetes, neurodegenerative diseases and cancer (Cruzat et al., 2007; Deresz et al., 2007; Duarte et al., 2007, 2010; Choi et al., 2014).

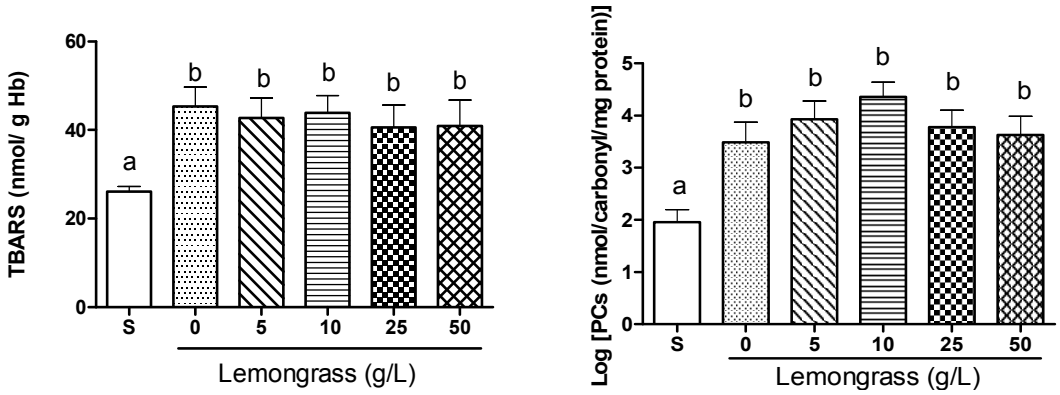

Figure 2. TBARS and CPs levels in erythrocytes treated with different concentrations of lemongrass infusion: without lemongrass infusion (Group S and basal), 5 g/L (group 5), 10 g/L (group 10), 25 g/L (group 25) and 50 g/L (group 50). Different letters mean significant differences ($p < 0.05$)

These results can be still associated with the incapability of lemongrass infusion to revert the AChE enzyme inhibition, due to the fact that the reduction of this enzyme activity can induce the reactive oxygen species production and consequently to promote damages to the macromolecules (Milatovic, Gupta, & Aschner, 2006; Adaramoye & Azeez, 2014).

GSH plays a key role in the xenobiotic elimination and in the cells defense against the oxidative stress, once that it helps the glutathione S-transferases and the glutathione peroxidase in the reduction of oxidant species (Huber, Almeida, & Fatima, 2008). Thus, considering the importance of this antioxidant agent for the defenses against the reactive oxygen species in this study the evaluation of the GSH levels in all the groups was also done (Figure 3). This way, it was possible to observe that the GSH levels were lower in the basal group (farmers) comparing to the control group, what suggests that the organism increased the consumption of this non-protein tiol as a response to the increase of the TBARS and CPs levels found in the erythrocytes from these same rural workers (Figure 2), probably in order to neutralize the reactive oxygen species that operate damaging lipid, protein and DNA (Barbosa et al., 2010). Besides, after the samples treatment with lemongrass infusion the GSH levels were significantly decreased when compared to the healthy group and the group without treatment with the plant, indicating that these plant infusions (5-50 g/L) are able to stimulate the antioxidant action.

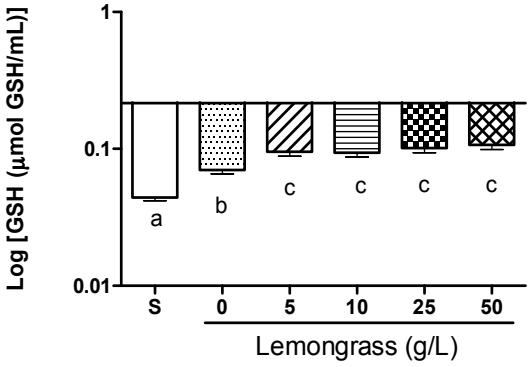

Figure 3. GSH levels in erythrocytes treated with different concentrations of lemongrass infusion: without lemongrass infusion (Group S and basal), 5 g/L (group 5), 10 g/L (group 10), 25 g/L (group 25) and 50 g/L (group 50). Different letters mean significant differences ($p < 0.05$)

Figures 4A and 4B exhibit the correlation between the lipid peroxidation and protein carbonylation before the erythrocytes treatment with lemongrass infusion, showing that the enzyme activity and the oxidative markers levels were inversely proportional, confirming what Milatovic, Gupta, and Aschner (2006) describe in their studies; the hyper cholinergic stimulation promoted by the AChE inhibition damages the oxidative

phosphorylation, increasing hence, the reactive oxygen species production which in turn, promotes the cell components destruction.

It should also be noted that this discovery corroborates with Jha and Rizvi's (2009) studies that observed correlation between the antioxidant capability and the AChE activity.

Moreover, in Figure 4C it is observed that the lower the AChE activity in the farmers' erythrocytes, the lower the GSH levels were on the same, what indicates a possible consumption of this endogenous antioxidant agent on the attempt to revert the damages promoted by the TBARS and CPs levels elevation generated by the AChE activity inhibition.

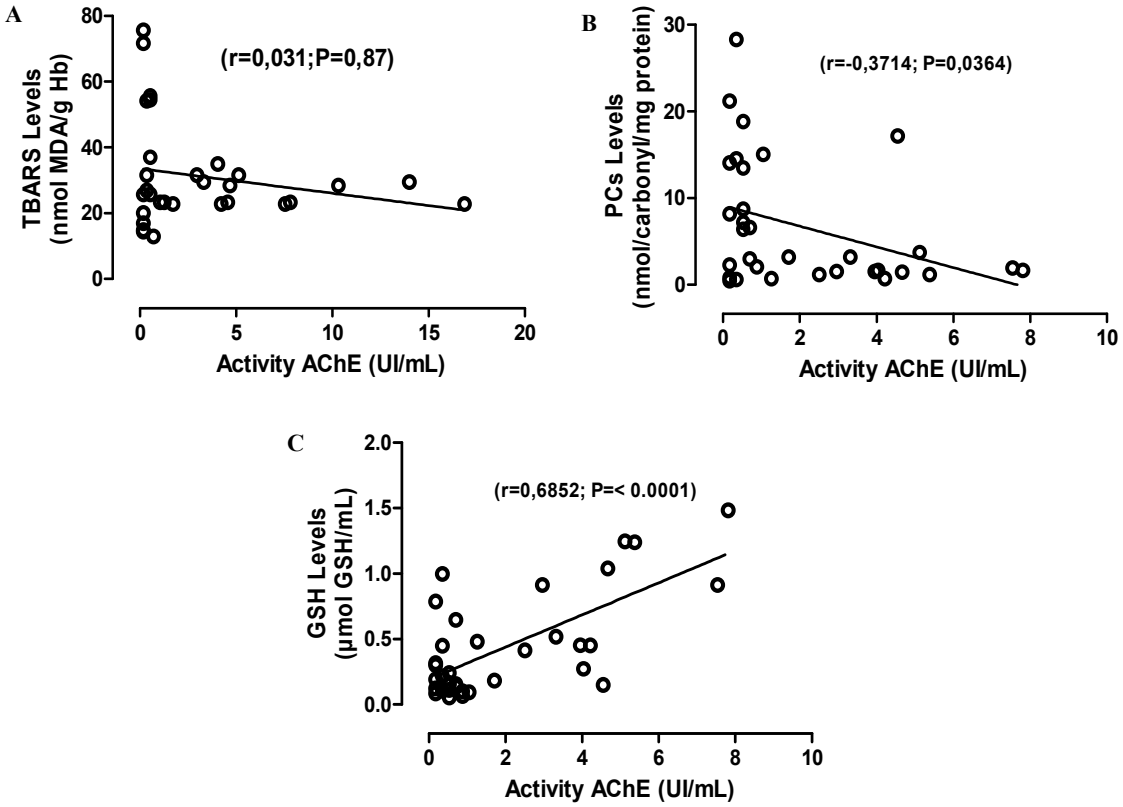

Figure 4. Correlation between AChE enzyme activity and TBARS (A), CPs (B) and GSH (C) levels in healthy individuals' erythrocytes (group S) and erythrocytes without lemongrass infusion treatment (group 0)

Generally speaking, the decrease of AChE activity caused by the pesticides use seems to be correlated with the oxidative stress occurrence, once that it presents negative correlation with the TBARS and CPs levels and positive correlation with the antioxidant capability verified through the GSH levels evaluation (Milatovic, Gupta, & Aschner, 2006; Adaramoye & Azeez, 2014).

Therefore, it was observed the presence of polyphenols, flavonoids and tannins in the lemongrass infusions (5-50 g/L), but these concentrations were inferior to the ones that are present in the extract of this plant. Probably because of this low quantity of antioxidant phytochemical, the lemongrass infusions (5-50 g/L) did no trevert the AChE activity inhibition verified in the farmers who were exposed to the pesticides and thus, could not revert the oxidative markers levels at the 1-hour exposure performed in this study. On the other hand, the *Cymbopogom citratus* infusions (5-50 g/L) increased the antioxidant capability in the erythrocytes by elevating the GSH consumption, what decreased the reactive oxygen species generation and increased the reactive oxygen species neutralization (Barbosa et al., 2010; Oliveira et al., 2013).

The lemongrass infusions tested in this study (5, 10, 25 and 50 g/L) were not able either to reverse the AChE enzyme inhibition observed in the farmers' erythrocytes, or to decrease the TBARS and CPs levels, however, they increased the GSH levels, proving hence that the *Cymbopogom citratus* increased the antioxidant capacity

in the rural workers' erythrocytes. Moreover, according to this study, the hypothesis is confirmed that the AChE activity inhibition is accompanied by the increase of the oxidative markers levels and of the consumption of the most important antioxidant endogenous.

References

Adaramoye, A. O., & Azeez, F. A. (2014). Evaluation of antioxidant and acetylcholinesterase-inhibitory properties of methanol extracts of *Nauclealatifolia*, *Cymbopogon citratus* and *Cocos nucifera*: An *in vitro* study. *British Journal of Medicine and Medical Research, 4*, 2156-2170. https://doi.org/10.9734/BJMMR/2014/7206

Agência Nacional de Vigilância Sanitária. (2011). *Brazilian pharmacopoeia*. São Paulo, SP: Agência Nacional de Vigilância Sanitária.

Alvis, A., Martínez, W., & Arrazola, G. (2012). Obtención de extractos hidro-alcohólicos de limoncillo (*Cymbopogon citratus*) como antioxidante natural. *Información Tecnológica, 23*, 3-10. https://doi.org/10.4067/S0718-07642012000200002

Balakrishnan, B., Paramasivam, S., & Arulkumar, A. (2014). Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. *Asian Pacific Journal of Tropical Disease, 4*, 134-139. https://doi.org/10.1016/S2222-1808(14)60428-X

Barbosa, K. B. F., Costa, N. M. B., Alfenas, R. C. G., De Paula, S. O., Minim, V. P. R., & Bressan, J. (2010). Estresse oxidativo: Conceito, implicações e fatores modulatórios. *Revista de Nutrição Campinas, 23*, 629-643. https://doi.org/10.1590/S1415-52732010000400013

Barreiros, A. L. B. S., David, J. M., & David, J. P. (2006). Estresse oxidativo: Relacão entre geracão de espécies reativas e defesa do organismo. *Química Nova, 38*, 113-123. https://doi.org/10.1590/S0100-40422006000100021

Bayrami, M., Hashemi, T., Malekirad, A. A., Ashayeri, H., Faraji, F., & Abdollahi, M. (2012). Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. *Toxicology and Industrial Health, 28*, 90-96. https://doi.org/10.1177/0748233711407243

Cárdenas, O., Silva, E., Morales, L., & Ortiz, J. (2005). Epidemiological study of organophosphate and carbamate pesticide exposure in 7 separated zones in Colombia, 1998-2001. *Biomédica, 25*, 170-180. https://doi.org/10.7705/biomedica.v25i2.1339

Catalgol, B. K., Ozden, S., & Alpertunga, B. (2007). Effect of molondialdehyde on trichlorfon and antixidant system in human eryhrocytes. *In Vitro Toxicology, 21*, 1538-1544. https://doi.org/10.1016/j.tiv.2007.06.002

Chandra, S., & Meija, E. G. (2004). Polyphenolic compounds, antioxidant capacity and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to Mate (*Ilex paraguaiensis*) and Green teas (*Camellia sinensis*). *Journal of Agricultural and Food Chemistry, 52*, 3583-3589. https://doi.org/10.1021/jf0352632

Cheel, J., Theodoluz, C., Rodrigues, J., & Schmeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from Lemongrass (*Cymbopogon citratus* (DC.) Stapf.). *Journal of Agricultural and Food Chemistry, 53*(7), 2511-2517. https://doi.org/10.1021/jf0479766

Choi, S. I., Yoo, S., Lim, J. Y., & Hwang, S. W. (2014). Are sensory TRP channels biological alarms for lipid peroxidation? *International Journal of Molecular Sciences, 15*, 16430-16457. https://doi.org/10.3390/ijms150916430

Cruzat, V. F., Rogero, M. M., Borges, M. C., & Tirapegui, J. (2007). Current aspects about oxidative stress, physical exercise and supplementation. *Brazilian Journal of Sporting Medicine, 13*(5), 336-342.

Deresz, L. F., Lazzarotto, A. R., Manfroi, W. C., Gaya, A., Sprinz, E., De Oliveira, A. R., et al. (2007). Oxidative stress and physical exercise in HIV positive individuals. *Brazilian Journal of Sporting Medicine, 13*(4), 275-279.

Duarte, M. M., Loro, V. L., Rock, J. B., Leal, D. B., Well, A. F., Dorneles, A., ... Schetinger, M. R. (2007). Enzymes hydrolyze adenine nucleotides que of patients with hypercholesterolemia and inflammatory process. *FEBS Journal, 274*(11), 2707-2714. https://doi.org/10.1111/j.1742-4658.2007.05805.x

Duarte, M. M., Moresco, R. N., Duarte, T., Santi, B. M. D., Da Cruz, I. B., Schetinger, M. R., & Loro, V. L. (2010). Oxidative stress in hypercholesterolemia and its association with Ala16Val superoxide dismutase

gene polymorphism. *Clinical Biochemistry, 43*(13-14), 1118-1123. https://doi.org/10.1016/j.clinbiochem.2010.07.002

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82*(1), 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*, 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

FIOCRUZ/CICT/SINITOX. (2015). *Annual Intoxication and Poisoning Statistics*. Oswaldo Cruz Fundation/Scientific and Technological Information Center/National System of Toxic-Pharmacologic Information, Brazil. Retrieved Mar 15, 2015, from http://www.fiocruz.br/sinitox

Halabi, M. F., & Sheikh, B. Y. (2014). Antiproliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. *BioMed Research International, 2014*, 1-8. https://doi.org/10.1155/2014/906239

Huber, P. C., Almeida, W. P., & De Fátima, A. (2008). Glutationa e Enzimas Relacionadas: Papel biológico e importância em processos patológicos. *Química Nova, 31*, 1170-1179. https://doi.org/10.1590/S0100-40422008000500046

Jha, R., & Rizvi, S. I. (2009). Age-dependent decline in erythrocyte acetylcholinesterase activity: correlation with oxidative stress. *Biomedical Papers, 153*, 195-198. https://doi.org/10.5507/bp.2009.032

Kaizer, R. R., Correa, M. C., Gris, L. R. S., Da Rosa, C. S., Bohrer, D., Morsch, V. M., & Schetinger, M. R. C. (2008). Effect of long-term exposure to aluminum on the acetylcholinesterase activity in the central nervous system and erythrocytes. *Neurochemical Research, 33*, 2294-2301. https://doi.org/10.1007/s11064-008-9725-6

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., … Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology, 186*, 464-478. https://doi.org/10.1016/0076-6879(90)86141-H

Lionetto, M. G., Caricato, R., Calisi, A., Giordano, M. E., & Schettino, T. (2013). Acetylcholinesterase as a biomarker in environmental and occupational medicine: New insights and future perspectives. *BioMed Research International, 2013*, 1-8. https://doi.org/10.1155/2013/321213

Milatovic, D., Gupta, R. C., & Aschner, M. (2006). Anticholinesterase toxicity and oxidative stress. *The Scientific World Journal, 6*, 295-310. https://doi.org/10.1100/tsw.2006.38

Molochkina, E. M., Zorina, O. M., Fatkullina, L. D., Goloschapov, A. N., & Burlakova, E. B. (2005). H_2O_2 modifies membrane structure and activity of acetylcholinesterase. *Chemico-Biological Interactions, 157*, 401-404. https://doi.org/10.1016/j.cbi.2005.10.075

Moreira, R. C. T., Costa, L. C. B., Costa, R. C. S., & Rocha, E. A. (2002). Abordagem etnobotânica acerca do uso de plantas medicinais na Vila Cachoeira, Ilhéus, Bahia, Brasil. *Acta Farmacêutica Bonaerense, 21*, 205-2011.

Morrison, M., Asiedu, E. A., Stuchbury, T., & Powell, A. A. (1995). Determination of lignin and tannin contents of cowpea seeds coats. *Annals of Botany, 76*, 287-90. https://doi.org/10.1006/anbo.1995.1097

Murussi, C., Horn, R. C., Santi, A., Clasen, B. E., Reis, G., Souza, D., … Loro, L. L. (2014). Changes in oxidative markers, endogenous antioxidants and activity of the enzyme acetylcholinesterase in farmers exposed to agricultural pesticides—A pilot study. *Ciência Rural, 44*(7), 1186-1193. https://doi.org/10.1590/0103-8478cr20130516

Neves, J. M., & Cunha, S. (2006). Plantas medicinais. *Revista Ciência e Saúde, 3*, 50-57.

Oliveira, J. S., Sostisso, Q. C. B., Junqueira, C. R., Librelotto, C. S., Possenti, C. G. R., & Horn, R. C. (2013). Níveis indesejáveis de colesterol total no organismo humano e a ocorrência de estresse oxidativo. *Revista Biomotriz, 7*(2), 95-107.

Recena, M. C. P., Caldas, E. D., Pires, D. X., & Pontes, E. R. (2006). Pesticides exposure in Culturama, Brazil: Knowledge, attitudes, and practices. *Environmental Research, 102*(2), 230-236. https://doi.org/10.1016/j.envres.2006.01.007

Rodrigues, H. G., Meireles, C. G., Lima, J. T. S., Toledo, G. P., Cardoso, J. L., & Gomes, S. L. (2011). Efeito embriotóxico, teratogênico e abortivo de plantas medicinais. *Revista Brasileira de Plantas Medicinais, 13*, 359-66. https://doi.org/10.1590/S1516-05722011000300016

Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*(1), 67-103. https://doi.org/10.1080/10408399209527581

Silva, F. M. de, Coelho, D. C., de Lima Ferreira, P. M., Lacerda de Sousa, E. M., de Bezerra Azevedo, P., de Almeida, I. P., ... Borges Maracajá, P. (2015). Os riscos no uso indiscriminado de agrotóxicos: uma visão bibliográfica. *Informativo Técnico do Semiárido, 9*, 77-84.

Simões, C. M., Schenkel, E. P., Gosmann, G. J., Mello, C.P., Mentz, L. A., & Petrovick, P. R. (2010). *Farmacognosia: Da planta ao medicamento*. Porto Alegre, RS: UFGRS/UFSC.

Soares, M. O., Alves, R. C., Pires, P. C., Oliveira, M. B. P., & Vinha, A. F. (2013). Angolan *Cymbopogon citratus* used for therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts. *Food and Chemical Toxicology, 60*, 413-418. https://doi.org/10.1016/j.fct.2013.07.064

Sorensen, K., Gentinetta, R., & Brodbeck, U. (1982). An amphiphile-dependent form of human brain caudate nucleus acetylcholinesterase: Purification and properties. *Journal of Neurochemistry, 39*(4), 1050-1060. https://doi.org/10.1111/j.1471-4159.1982.tb11496.x

Sousa, C. M. M., Silva, H. R., Vieira Jr, G. M., Ayres, M. C. C., Costa, C. L. S., Araújo, D. S., … Chaves, M. H. (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova, 30*, 351-355. https://doi.org/10.1590/S0100-40422007000200021

Stocks, J., & Dormandy, T. L. (1971). The autoxidation of human red cell lipids induced by hydrogen peroxide. *British Journal of Haematology, 20*(1), 95-111. https://doi.org/10.1111/j.1365-2141.1971.tb00790.x

Surajudeen, Y. A., Sheu, R. K., Ayokulehin, K. M., & Olatunbosun, A. G. (2014). Oxidative stress indices in Nigerian pesticide applicators and farmers occupationally exposed to organophosphate pesticides. *International Journal of Applied and Basic Medical Research, 4*, S37. https://doi.org/10.4103/2229-516X.140730

Vasconcelos, S. M. L., Goulart, M. O. F., Moura, J. B. D. F., Manfredini, V., Benfato, M. D. S., & Kubota, L. T. (2007). Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: Principais métodos analíticos para sua determinação. *Química Nova, 30*, 1323-1338. https://doi.org/10.1590/S0100-40422007000500046

Woisky R. G., & Salatino, A. (1998). Analysis of própolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research, 37*(2), 99-105. https://doi.org/10.1080/00218839.1998.11100961