Abstract

Lemongrass (Cymbopogon citratus) is a medicinal herb grown in almost all tropical countries, including Brazil. Your still therapeutic use is homemade, taking into account the ethnobotanical knowledge. Despite the long history with no reports of serious side effects, research has shown that they may have genotoxic potentialities. Therefore, this study aimed to examine the genotoxic effects of aqueous extract of lemongrass, prepared in 2%, 4% and 8% in the animal test system - Mus musculus L. The results showed that the bend the concentration of the aqueous extract of fresh leaves of lemon grass, bent also genotoxic effects on bone marrow Mus musculus L, inducing an increased frequency of micronucleus. This way, the consumption of lemon grass tea should be done with moderations and monitoring of health professionals.

Keyword: Cymbopogon citratus (A.D.) Stapf., Genotoxicity, micronucleus.
Genotoxic POTENTIAL OF AQUEOUS EXTRACT FRESH SHEETS DO GRASS - LEMON (Cymbopogon citratus(A.D.) Stapf). IN MICE IN MICRONUCLEUS TEST MEANS

EVALUATION OF THE EFFECTS OF DIET WITH COCONUT OIL ON THE METABOLISM OF CARBOHYDRATES AND LIPIDS IN WISTAR RATS

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SUMMARY

Lemongrass (Cymbopogon citratus) it is a medicinal herb grown in almost all tropical countries, including Brazil. Your still therapeutic use is homemade, taking into account the ethnobotanical knowledge. Despite the long history with no reports of serious side effects, research has shown that they may have genotoxic potentialities. Therefore, this study aimed to examine the genotoxic effects of aqueous extract of lemongrass, prepared in 2%, 4% and 8% in the animal test system - Mus musculus L. The results showed that the bend the concentration of the aqueous extract of fresh leaves of lemon grass, bent also genotoxic effects on bone marrow Mus musculus L, inducing an increased frequency of micronucleus. This way, the consumption of lemon grass tea should be done with moderations and monitoring of health professionals.

Key words: Cymbopogon citratus(A.D.) Stapf., Genotoxicity, micronucleus.

INTRODUCTION

The use of biological assays to monitor the plant isolated extracts of bioactivity has often been incorporated into the identification of potentially toxic substances. However, the cytotoxicity and genotoxicity of many plants considered medicinal, have not yet been verified with the necessary scientific rigor, as in the case of Cymbopogon citratus (AD.) Stapf. Which is an all ochthonous species of Indian
origin, but perfectly acclimatized and widespread throughout Brazil, where it is grown in gardens, home gardens and commercial way and sachets infusion envelopes.

*Cymbopogon citratus* (AD) Stapf., also known as lemon grass, can receive various names in Brazil, as lemongrass, cheap grass, grass cidrô, holy grass, cane balm, tea-road, among others.

The ethnomedicinal standpoint, lemon grass has been used as a carminative, sedative, sudorific, febrifuge, diuretic, antipyretic, antirheumatic, soothing and light spasmylic, analgesic, anticancer, antibacterial, antihelmintico, antifungal, insecticide and larvicide, which are assigned mainly to major compounds from the group of monoterpenes: 65-86% citral, which is formed by a mixture of geranial (citral α) and neral (β-citral), further containing citronellal (7-8%) (SACCHETTI et al., 2005; RAUBER, Guterres and SCHAPOVAL, 2005; Lemos et al., 2013).

Other aldehydes such as isovaleraldehyde decyl aldehyde and can also be found in addition to alcohols and ketones, such as, nerol, methyl heptenone, farnesol, and limonene (GUIMARÃES et al., 2008). In the pharmaceutical industry, the essential oil of lemongrass is widely used as a flavoring agent in soaps, colognes and deodorants, as well as obtaining citral, which is used as starting material for the synthesis of vitamin A and ianonas.

Although plant extracts and essential oil of lemon grass have been widely used several years in folk medicine and in the pharmaceutical industry, without toxicity reports (BRAZIL, 2006; Costa et al, 2011.; SANTOS NETO, and ALVES MARTINS, 2015; PEREIRA and PAULA, 2018), scientific research, also point since antimutagenic activities to the genotoxic potential (MACHRAOUI et al., 2018).

The controversy in relation to the potential toxic effects and/or protective healing may be due to the plasticity phenotypic what *Cymbopogon citratus* can be provided for different concentrations of metabolic compounds according to the environment in which it is planted crop management and/or compounds are extracted as fresh or dried leaves, essential oils, ethanol extracts, methanolic, decoctions or infusions in water.

The relatively simple C. citratus and cultivation, because it is a very hardy species to soil and climate variations. Santos et al. (2009) observed that clay or sand-clay soils are those that offer the best conditions for its development. The ideal climatic conditions for its development are hot and humid climate, with full sun exposure and evenly distributed rainfall. Also, it was reported by the same authors as the plant obtained at periods of the year with higher temperature and rainfall rate resulted in greater yield of essential oil compared with other times of the year (Lucena, 2015).

The composition and yield of the essential oil of lemon grass, may depend on factors that affect the plant, such as genetic structures, soil and climatic conditions, agricultural practices and extraction methods (Martinazzo et al, 2007; PINTO et al, 2014; SILVA, RECK and FONSCENCA, 2016).

Detection of potentially cytotoxic and genotoxic substances and their likely effects on organisms, is important in the sense of the impact that they can bring to people, animals, plants and humans. The Unified Health System (SUS) describes in his publications (Table 7) the program herbal medicines Central (PPPM/CEME) *C. citratus* which has no toxic effect in preclinical toxicology studies realized in the year 1985.
A current review of technical reports supported by PPPM/CEME, which gave rise to the herbal medicine program in the SUS in Brazil, it is necessary to continue, especially those in more advanced stages of research and development (R & D), as the *C. citratus*, incorporating the cytogenetic techniques, non-existent at the time the projects were contracted by PPPM / CEME.

to present scientific controversies regarding their toxicity and it is a medicinal plant widely used in Brazil, searched in this study had checked genotoxic potential of different concentrations of the aqueous extract of fresh leaves of *Cymbopogon citratus* (AD) Stapf., of frequency by means of micronucleus (MCN) in bone marrow cells of mice treated *in vivo* as a bioindicator species as the animal test system.

**MATERIAL AND METHODS**

**Collection of botanical material**

Fresh leaves of *Cymbopogon citratus* (DC) Stapf. was collected at the site of medicinal plants of the Center for Biological Studies, in the University Center of Caratinga (UNECD), at 19 ° 47' 23" S, 42 ° 08' 21" W, in august, in the morning shaded conditions. Immediately after collection, the leaves were placed in sterile plastic bags and brought to the Pharmaceutical Botany Laboratory in UNEC for the preparation of the aqueous extract.

**Preparation of the aqueous extract of fresh of grass lemon leaves**

The aqueous extract was prepared according to ethnobotanical recommendations, 20g of fresh leaves in 1L of boiled water, amounting to 2% extract, after reaching room temperature. Subsequently, using the same methodology, the extracts to 4% and 8% were prepared seeking to determine the dose-response relationship of infusions.

**Test system Animals (*Mus musculus* L.)**

Mice were used in the Swiss strain, adults with an average weight of 28g. The animals spent 6 days adjustment period, with water and food *ad libitum* commercial environment with a photoperiod of 12h light and 12h dark, average temperature of 23°C. Groups of 6 male mice were treated with aqueous extracts of fennel, orally, in acute treatment, the three experimental concentrations (2%, 4% and 8%). For the negative control (NC) was used distilled water and the positive control (PC) was used cyclophosphamide, 24h before euthanasia. The genotoxicity was evaluated by counting all micronucleated erythrocytes (CCM's) bone marrow smear of each femur in each treatment, comprising three blades per replicate (Abreu et al., 2019).

All solutions (extracts, CN and CP) were administered by gavage a single dose of 50mg/kg, introducing special needle through the mouth into the stomach of the animal, and the like via the accidental or intentional ingestion of the test substance.

**Statistical analysis**

The experimental design for the evaluation test system was randomized (DIC) using variance analysis method one way ANOVA and subsequent Tukey post test for the comparison of averages, at 5% probability.
RESULTS AND DISCUSSION

After cytogenetic analysis showed a significant difference (p < 0.01) between the average MCN’s of all treatments tested (Table 1 and Figure 1), the CN group the smallest average and the most CP group, as expected. For, as it exponential or concentrations of the extract (2%, 4% and 8%), it was found also apparent exponential increase in CCM’s frequency, 94%, 66% and 39%, respectively, to the concentration (8%) compared to the CN, which is equivalent to the concentration of 0%.

TABLE 1 - Effect of aqueous extracts from fresh leaves of Cymbopogon citratus (AD.) Stapf. of the total and average number of erythrocytes per repetition in MCN’s and treatment.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>CN</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>13</td>
<td>22</td>
<td>34</td>
<td>68</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>9</td>
<td>19</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>11</td>
<td>21</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>9</td>
<td>25</td>
<td>28</td>
<td>64</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>12</td>
<td>18</td>
<td>51</td>
<td>56</td>
</tr>
</tbody>
</table>

SAW 1 16 18 32 68

Averages 2.17 (a) 11.67 (b) 20.50 (c) 33.83 (d) 63.83 (e)

*Means followed by the same letter are not significantly different by Tukey test (p < 0.01)

At Figure 1 can be seen that average frequency of MCN’s was directly proportional to the increase of the concentration of the aqueous extract of fresh leaves of lemon grass, with a linear relationship between the variables. This result demonstrates that the higher the concentration of the extract, the effect Greater and more genotoxic than frequencies of MCN’s.

![Graph showing the relationship between concentration of the extract and average frequency of MCN's](image)

FIGURE 1 - Effect of aqueous extracts from fresh leaves of Cymbopogon citratus (AD) Stapf. on the frequency MCN’s average erythrocyte per treatment.
By bending the concentration of the aqueous extract of fresh leaves of lemon grass, bent also genotoxic effects on bone marrow of mice, inducing a higher frequency of micronuclei (FIGURE 2), which may be the result of chromosomal damage or damage of the mitotic apparatus of erythrocyte hematopoiesis.

![FIGURE 2 - Micronuclei (red arrows) in mouse erythrocytes (Mus musculus L.) gives Swiss strain exposed to aqueous extracts of fresh leaves Cymbopogon citratus(AD.) Stapf. Orally, in a single dose. Total x400.](image)

Seize to be simple, cheap and fast, the micronucleus test is able to detect efficiently genetic changes resulting from chromosomal injury and/or damage to the mitotic apparatus.

The induction of micronucleus is an excellent parameter irreversible DNA loss and a good test for preclinical studies, since it is already envisaged scientifically that there is a positive correlation between the increased frequency of micronucleus and the appearance of tumors in rodents and being humans. Thus, this finding serves as a warning to the indiscriminate use of homemade herbal teas, used by the general population, to find that it comes from a natural product, it does not generate harmful effects to long-term body, only immediate benefits over the reduction of symptoms.

The aqueous extract of fresh leaves of lemon grass, orally administered in mice in acute treatment acted as a xenobiotic, because with only 24 hours after its administration caused genetic alterations in bone marrow.

Like any xenobiotic, the products of their biotransformation can be potentially toxic; and thus they must be regarded, until otherwise proven. From a toxicological point of view, should be considered a medicinal plant not only has immediate effect and easily correlated with their intake, but also the effects that settle over time and asymptotically, such as carcinogenic, hepatotoxic and nephrotoxic (SIMÕES et al., 2002).

Medicinal plants are able to alleviate or cure diseases and have tradition of use as a drug in a population or community. However, to use them it is imperative knowledge of the collection process, stabilization and drying, may be full, erased, crushed or pulverized and, especially, how to prepare it (BRAZIL, 2006).

Furlan et al. (2010), showed that the content of essential oil of Cymbopogon citratus, collected in different regions of the State of Sao Paulo, was higher in areas with higher monthly rainfall index. Furthermore, it was found that citral content was higher in the low sunlight period (18:30 - 21:30). These results suggest that the pattern of a single gene can control the accumulation of these constituents and have epigenetic effects.
Hanaa et al. (2012) found essential oil concentration difference of the dried leaves of lemon grass three different methods of drying in the sun for 36h, 48h by shade drying oven at 45°C for 7h. After analysis in capillary GC instruments and GC/mass, there were significant differences in the essential oil content in the different methods used. Drying in the oven showed the highest percentage of oil (2.45%) compared to the shade drying methods (2.12%) and sun drying (2.10%). The authors also compare the tenor of different components of the essential oil of fresh leaves vs dry, checking that the concentrations of the main components, geranial (α-citral), neral (β-citral) and myrcene were higher in dry leaves.

Several pharmacological activities of the essential oil extracts and/or isolated from lemongrass compounds have been described in a contradictory manner in the scientific literature.

The antimutagenic activity lemongrass ethanolic extract was verified in human lymphocytes when they were exposed to mitomycin C; in micronucleus formation in mice when they were exposed to cyclophosphamide, the retardation of tumor growth and reduction of the number of transplanted in mice metastases with cells fibrous (MEEVATEE et al., 1993.; SUAEYUN et al, 1997). The effects of ethanol extract of lemongrass also been observed in experiments of chemical carcinogenesis by reducing the GST-P positive foci in the liver of F344 rats initiated by DEN (PUATANACHOKCHAI et al., 2002) and in the incidence and/or multiplicity of foci of aberrant crypts in F344 rats, the extract is administered for the phases initiation and promotion of carcinogenesis in colon by AOM (SUAEYUN et al., 1997).

Kouame et al. (2015) and Machraoui et al (2018), citing several authors, extensive reviews on Ethnopharmacology, Phytochemistry, toxicological and pharmacological activities of essential oil compounds and the majority of lemon grass, described several studies that indicate toxicity Cymbopogon citratus. As DL50s in shrimp larvae (Artemia salina L.) and mice were respectively 9.83 g / ml and 460 ug / ml (Lizard, et al, 2001). In J774G8 macrophages, cytotoxic concentration 50 (CC50) of the essential oil and its main component (citral) were respectively 25 mg / mL and 50 mg / mL (SANTIN et al., 2009) . The acute toxicity was observed between 2000 mg / kg and 3000 mg / kg if symptoms of numbness, and nose bleeding eyelid Wistar rats (FANDOMON et al., 2008). Histological examination revealed atrophy of the stomach lining end necrosis of hepatocytes, and the E3500 LD50 foi 3250 mg / Kg, respectively in rats and young adults (Bidinotto, et al., 2011).

The toxicity was attributed to Cymbopogon citratus and β-myrcene, citral compounds that would embryofetotoxic not doses greater than 125 mg / kg E1200 mg / kg, respectively (Nogueira et al., 1995). In humans, limonene is toxic single dose of 100 mg / kg, effects as nausea, vomiting and diarrhea with no liver dysfunction, renal and pancreatic (Crowell et al., 1994; Vigushin et al., 1998). respectively (Nogueira et al., 1995).

Already the hepatotoxic activity, nephrotoxic and cytotoxicity of aqueous extract lemon grass was verified in mice by Guerra et al. (2000); Negrelle and Gomes (2007) .The genotoxic, cytotoxic and / or cell cycle of the aqueous leaf extract of lemon grass effects were observed in plant test system (AKINBORO Bakare, 2007; Sousa, and VICCINI SILVA, 2008; azeez et al 2016 FAGUNDES et al, 2017), or by allelopathic studies (Souza et al., 2005.; walls and GAYOZO, 2018).

Villaverde et al. (2013) reported that the essential oil of lemon grass was able to produce significant antimitotic effect on the human melanoma SKMEL 147, shown by the results obtained in the proliferation
curve, cell viability, MTT reduction, LDH and cell morphology as observed no effect cytotoxic to normal human keratinocytes HaCaT, at the same concentrations used to SK MEL-147 cells.

Recently, Souza et al. (2019) found exposure citral, major phytochemical component *Cymbopogon citratus* in cultured human cells in different experiments. Blue Cytotoxicity assays and trypan MTT demonstrated toxicity in HepG2 cells, which contrasted with the lack of toxicity in leukocytes. The micronucleus test showed no clastogenic effects aneugenic or in both cells. Since the test comet assay, it was found significant in both Hep, G2 genotoxic effects as in leukocytes.

From the above, Kitchen Apron that the great controversy of experimental results in lemongrass toxicity is related to the test used, the type of bio-indicator cell and the use of essential oils or extracts that may present mechanisms of different pharmacological actions. It is known that essential oils are soluble and low stability, especially in the presence of air, light, heat, moisture and metals. As it is observed, the variables that can influence the stability and reliability of the active ingredients of many essential oils. Moreover, the composition and yield of the essential oil of lemon grass, can depend on factors affecting the plant such as genetic structures, soil and climatic conditions, agricultural practices and methods extraction. Furthermore Kumar et al. (2008) reported the importance of hydrophobicity of essential oils and their constituents, by interact with the lipid layer of cell membranes, causing changes in their structures and becoming less selective.

The population, mostly, do not intake of oils and essences nor ethanolic extracts of lemongrass, when they want to use their medicinal properties; but infusions and / or decoctions of fresh leaves, which are equivalent to the aqueous extracts. Therefore, the present study we tried to use methodologies used in ethnobotany place to be to have a scientific parameter alert the communities about potentially toxic pharmacological properties of extracts of aquaosos *Cymbopogon citratus*.

**CONCLUSIONS**

- *Cymbopogon citratus* In vivo model used experimental in has genotoxic potential in bone marrow cells of mice.
- The higher the concentration of the aqueous extract *Cymbopogon citratus*. The greater the genotoxic effect in relation to micronucleus frequencies in bone marrow of mice erythrocytes.
- The tea consumption *Cymbopogon citratus* should be done with moderations and monitoring of health professionals, it may be harmful to health.

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