Abstract. This research aimed to conduct a systematic review and meta-analysis to compare the frequency of cell damage in crack users and nonusers, through Micronucleus (MN) test in buccal mucosa cells. A comprehensive search was carried out on MEDLINE via PubMeb, Web of Science, LILACS and the grey literature without restrictions. It was included case-control studies that report the frequency of micronuclei in the oral mucosa of adult crack users and nonusers. A review protocol was registered with PROSPERO (CRD42018115672), and conducted in accordance with the PRISMA guidelines for the report of this systematic review. Furthermore, study quality was evaluated using an adapted Newcastle-Ottawa Scale for cross-sectional studies. The original search yielded 27 references, after eligibility criteria only five articles were included. The number of micronuclei was higher in crack users compared to nonusers. Also, secondary outcomes: binucleated cells, nuclear buds, pyknosis, karyorrhexis and karyolysis had higher prevalence in crack users. Crack use is associated with genotoxic and mutagenic effects because there is a higher frequency of micronuclei in exfoliated cells of crack users. In addition, MN test proved to be a good biomarker to assess the mutagenic impact of crack use in oral epithelium.

Keywords: Illicit drugs; Crack cocaine; Micronucleus tests; Buccal mucosa cells.

Introduction
The last World Drug Report has estimated that approximately 1 of every 18 people aged 15–64 years used an illicit drug, corresponding to 5.6 per cent of the global population prevalence. There is a potential supply-driven expansion of drug markets at the highest levels ever recorded, expanding beyond their usual regions. At the same time, more new psychoactive substances are being synthesized and more are available than ever, with increasing reports of associated harm and fatalities (UNODC 2018).

World crack cocaine consumption has increased, spreading mainly after the 80’s (Balbino et al. 2011). Crack is a substance obtained from cocaine hydrochloride through conversion processes to make it suitable for smoking, is a cheap product, which can reach all social classes (UNODC 2018). Crack is a potent stimulant of the central nervous system with a high potential for addiction (Lima et al. 2016). This drug is
rapidly absorbed by mucous membranes when smoked, reaching cerebral circulation within 6 to 8 seconds. Another factor responsible for the intense crack action is the fact that the substance is more fat-soluble than the cocaine chloride, allowing quick passage to the central nervous system (Oliveira and Dinis-Oliveira 2018). Besides several psychotropic and neurotoxic effects, crack has high potential to generate damage to the user’s health (Lima et al. 2016). Crack is a major cause of lesions in the upper respiratory emphysema (de Freitas et al. 2014; Oliveira and Dinis-Oliveira 2018). Also, there is evidence that crack produces carcinogenic effects (Oliveira and Dinis-Oliveira 2018), cardiovascular disease, neurological problems, and gastrointestinal complications in adults (Butler et al. 2017; Antoniazzi et al. 2018). This drug appears to be able to induce inflammatory changes in the oral epithelium, increasing periodontal disease (Cury et al. 2017) and oral DNA damage such as an increase in keratinization, decrease in the area of nuclei, and increase in the number of nucleolar organizer regions (Antoniazzi et al. 2018). Micronucleus (MN) are extranuclear bodies composed of chromosomes or chromosomal fragments, which are separated from the daughter nuclei during nuclear division (mitosis) (das Graças et al. 2014). The presence of MN indicates DNA breaks, that is, clastogenicity or aneuploidy due to disturbances in the mitotic spindle caused by exposure to genotoxic agents (Thomas and Fenech 2011; das Graças et al. 2014). The MN test is considered a fast, inexpensive and non-invasive biomarker (Da Silva Pinto et al. 2017; da Silva Júnior et al. 2018; Aguiar Torres et al. 2019). It can be used to prevent and individuals monitoring at risk for cancer (Holland et al. 2008; Lorenzoni et al. 2017; Espitia-Pérez et al. 2018), especially when evaluated in oral exfoliated cells (Bonassi et al. 2011), in susceptible population such as chronic users of mutagenic substances as crackcocaine (Lima et al. 2016). The cytotoxicity of mutagenic substances has already been associated with MN in oral cells (Webber et al. 2016; de Geus et al. 2018). However, few studies have assessed the potential effects of crack induce genetic damage and there is no consensus about the effect of crack in oral mucosa and frequency of MN. Thus, due the high prevalence of crack cocaine use and its associated serious health outcomes, the purpose of the present study was to conduct a systematic review and meta-analysis to compare the frequency of cell damage in crack users and non-users, through MN test in buccal mucosa cells.

Methods
Protocol and registration
This study protocol were registered at the PROSPERO (international prospective register of systematic reviews) database (CRD42018115672), and conducted in accordance with the PRISMA guidelines for the report of tis systematic review (Moher et al. 2009).

Search strategy
The controlled vocabulary (mesh terms) and free keyword in the search strategy was defined based on the following PECO question: 1. Population (P): adults 2. Exposure (E): crack users 3. Comparison (C): non-crack users 4. The outcome (O): frequency of micronuclei 5. Study design (S): cross-sectional studies
To identify trials to be included for this review, we searched on the electronic databases MEDLINE via PubMed Web of Science, Latin-American and Caribbean Literature on Health Sciences (LILACS), and the grey literature (google-scholar). No restrictions to publication date or languages were performed. The search terms used were: "crack" OR "crack cocaine" and “micronucleus” OR “micronuclei”.

Eligibility criteria
To be included, studies must have been published as an original survey reporting the frequency of micronuclei in users and non-users of crack in adult patients of any group. The primary outcome of the study was the frequency of micronuclei. It was included only cross-sectional studies in humans. Non-controlled clinical trials, editorial letters, pilot studies, historical reviews, in vitro studies, and descriptive studies, such as case reports and case series, were excluded.

Study selection and data collection
process and data extraction
Initially, the articles were selected by title and abstracts. Full-text articles were obtained when the title and abstract had insufficient information to make a clear decision. Subsequently, two reviewers individually classified those that met the inclusion criteria with approximately 95% agreement. Differences were resolved by discussion and consultation with a third researcher when it was needed. Two
researchers completed data extraction for all studies, one review author checked text entries, and one independent quality control person checked numeric outcome data. To find additional relevant articles, the reference lists of all of the retrieved studies were examined.

**Quality Assessment**
Study quality was evaluated using an adapted Newcastle-Ottawa Scale (NOS) (Wells et al. 2012) for cross-sectional studies, which assesses the design and quality of nonrandomized studies and further facilitates the task of incorporating quality assessments into the interpretation of meta-analysis results. The evaluation of each article was given by a star rating from three perspectives: selection (4 criteria), comparability (1 criterion) and exposure (3 criteria). After the sum of the stars received by each article, it is possible to classify them in unsatisfactory (1-4 stars), intermediate (5 stars), good (6-7 stars) and very good quality (8-9 stars) studies. Only studies classified as good or very good quality were included in the meta-analysis. It is important to mention that higher quality studies have lower risk of bias, and so on. The NOS was presented in the supplementary material.

**Statistical Analysis**
When authors evaluated the micronuclei frequency in different regions in the oral cavity or when more than one group of crack users was added, we combined these data to make a single entry in the meta-analysis. In the meta-analysis to compute the overall effect size, it was used fixed effects model due to the number of studies (less than 10 studies). Between-study heterogeneity was examined using Cochrane’s Q test and I-squared. Publication bias was assessed by visual inspection of funnel plots. Formal statistical assessment of funnel plot asymmetry was done with Egger's regression asymmetry test. The meta-analysis was performed using the software MetaXL 5.3 and the statistical analyses were done by using Stata, version 10. Values of < 0.05 were considered statistically significant.

**Results**
The flowchart of selected studies was exhibited in Figure 1. The original search yielded 27 references. Full-text reports on 8 studies were retrieved for complete review, no articles were excluded after quality assessment as well as no article were included from the reference list. A total of five articles were included in the review.

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**Figure 1.** Flow of information through the different phases of a systematic review
The characteristics of the selected studies are shown in Table 1. Majority of the studies included in this review used Feulgen staining procedure (Almeida et al. 2012; das Graças et al. 2014; Webber et al. 2015), one study used Giemsa (Antoniazzi et al. 2018) and one Feulgen-Rossenbeck (Lima et al. 2016). All studies scored 1000 cells and tested buccal mucosa cells. The smallest sample size per group was 10 (Almeida et al. 2012) and the highest was 54 (Antoniazzi et al. 2018). The mean age of participants ranged from 20.2 to 40.9 for nonusers of crack and 15.5 to 39.6 for crack users (Almeida et al. 2012; das Graças et al. 2014; Webber et al. 2015; Lima et al. 2016; Antoniazzi et al. 2018). The mean time of crack use ranged from more than 1 year to 6.4 years (Almeida et al. 2012; Lima et al. 2016; Antoniazzi et al. 2018), and two studies did not report this information (das Graças et al. 2014; Webber et al. 2015). Only, one study did not present significant statistical difference between the number of micronuclei between nonusers of crack compared to users of crack (Webber et al. 2015). To obtain the samples, four studies smeared only the mucosa of the cheek (Almeida et al. 2012; das Graças et al. 2014; Lima et al. 2016; Antoniazzi et al. 2018) and one smeared the floor of the mouth (Webber et al. 2015). All studies analyzed other genotoxic changes in addition to the main outcome (micronuclei).

Table 1. Summary of the studies selected for this systematic review.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n participants/group</th>
<th>Age (years)+</th>
<th>Time of Crack use (years)</th>
<th>Micro nuclei Means± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al. 2012</td>
<td>10 NC</td>
<td>20.2 ± 1.0 NC</td>
<td>3.3 ± 1.4</td>
<td>0.1 ± 0.3 NC</td>
</tr>
<tr>
<td></td>
<td>10 C</td>
<td>15.5 ± 1.4 NC</td>
<td>-</td>
<td>4.3 ± 4.1 C*</td>
</tr>
<tr>
<td>das Graças et al. 2014</td>
<td>30 NC</td>
<td>31.4 ± 9.3 NC</td>
<td>-</td>
<td>0.7 ± 0.2 NC</td>
</tr>
<tr>
<td></td>
<td>30 C</td>
<td>33.6 ± 11.6 C</td>
<td>-</td>
<td>1.3 ± 1.4 C*</td>
</tr>
<tr>
<td>Weber et al. 2015</td>
<td>34 NC</td>
<td>40.9 ± 11.0 NC</td>
<td>-</td>
<td>0.2 ± 0.4 NC</td>
</tr>
<tr>
<td></td>
<td>26 C</td>
<td>39.6 ± 12.8 C</td>
<td>-</td>
<td>0.4 ± 0.6 C</td>
</tr>
<tr>
<td>Lima et al. 2016</td>
<td>15 NC</td>
<td>30.2 ± 10.65</td>
<td>6.4</td>
<td>0.6 ± 1.6 NC</td>
</tr>
<tr>
<td></td>
<td>15 C</td>
<td></td>
<td>-</td>
<td>2.9 ± 3.5 C*</td>
</tr>
<tr>
<td>Antoniazzi et al. 2018</td>
<td>54 NC</td>
<td>22.5 (18.0 - 31.0) NC</td>
<td>&gt; 1.0</td>
<td>3.8 ± 7.6 NC</td>
</tr>
<tr>
<td></td>
<td>54 C</td>
<td>25 (18.0 - 32.2) C</td>
<td></td>
<td>17.3 ± 21.8 C*</td>
</tr>
</tbody>
</table>

NC non-users of crack; C users of crack; *p ≤ 0.05. All studies report year in mean± SD (standard deviation) expect + median (25th–75th percentile).

The secondary outcomes are present in table 2. Three studies evaluated binucleated cells (Almeida et al. 2012; Webber et al. 2015; Lima et al. 2016), four evaluated nuclear buds (Almeida et al. 2012; Webber et al. 2015; Lima et al. 2016; Antoniazzi et al. 2018), three analyzed pyknosis (Almeida et al. 2012; das Graças et al. 2014; Lima et al. 2016), five evaluated karyorrhexis (Almeida et al. 2012; das Graças et al. 2014; Webber et al. 2015; Lima et al. 2016; Antoniazzi et al. 2018) and three analyzed karyolysis (das Graças et al. 2014; Lima et al. 2016; Antoniazzi et al. 2018). In all cases, except for Webber et al. (2015), significant differences were observed between groups. More significant DNA damage was found in the crack users groups (Almeida et al. 2012; das Graças et al. 2014; Lima et al. 2016; Antoniazzi et al. 2018). The quality assessment of selected studies was presented in Table 3. In summary, four studies were considered of very good quality (Webber et al. 2015; das Graças et al. 2014; Lima et al. 2016; Antoniazzi et al. 2018) and one of good quality (Almeida et al. 2012), having low risk of bias, according to the quality assessment components of the Newcastle-Ottawa Scale (Wells 2012). Therefore, all the studies met the best requirement features for inclusion in the meta-analysis of the frequency of micronuclei in crack users.
Table 2. Data from secondary outcomes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Binucleation</th>
<th>Nuclear Buds (Broken eggs)</th>
<th>Pyknosis</th>
<th>Karyorrhexis</th>
<th>Karyolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al. 2012</td>
<td>10.2 ± 9.6 NC</td>
<td>0.5 ± 1.2 NC</td>
<td>0.2 ± 0.6 NC</td>
<td>34.4 ± 24.1 NC</td>
<td>-</td>
</tr>
<tr>
<td>das Graças et al. 2014</td>
<td>-</td>
<td>-</td>
<td>91 ± 48 NC</td>
<td>10 ± 9 NC</td>
<td>19 ± 17 NC</td>
</tr>
<tr>
<td>Weber et al. 2015</td>
<td>1.2 ± 0.2 NC</td>
<td>0.7 ± 1.6 NC</td>
<td>-</td>
<td>1.7 ± 2.9 NC</td>
<td>-</td>
</tr>
<tr>
<td>Lima et al. 2016</td>
<td>9.3 ± 6.8 NC</td>
<td>5.0 ± 5.8 NC</td>
<td>10.8 ± 6.8 NC</td>
<td>24.9 ± 24.0 NC</td>
<td>8.3 ± 9.3 NC</td>
</tr>
<tr>
<td>Antoniazzi et al. 2018</td>
<td>-</td>
<td>0.02 ± 0.1 NC</td>
<td>-</td>
<td>10.11 ± 24.04 NC</td>
<td>9.46 ± 12.9 NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NC nonusers of crack; C users of crack;* p ≤ 0.05.
All studies report year in mean± SD (standard deviation)

Table 3. Assessment of the quality of case control studies included in the meta-analysis using the Newcastle-Ottawa Scale.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Selection 1 2 3 4</th>
<th>Comparability</th>
<th>Exposure 1 2</th>
<th>Total</th>
<th>Final Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al. 2012</td>
<td>* * *</td>
<td>*</td>
<td>* * *</td>
<td>7/9</td>
<td>Good quality</td>
</tr>
<tr>
<td>das Graças et al. 2014</td>
<td>* * *</td>
<td>* *</td>
<td>* * *</td>
<td>9/9</td>
<td>Very good quality</td>
</tr>
<tr>
<td>Weber et al. 2015</td>
<td>* * *</td>
<td>* *</td>
<td>* * *</td>
<td>9/9</td>
<td>Very good quality</td>
</tr>
<tr>
<td>Lima et al. 2016</td>
<td>* * *</td>
<td>* *</td>
<td>* * *</td>
<td>9/9</td>
<td>Very good quality</td>
</tr>
<tr>
<td>Antoniazzi et al. 2018</td>
<td>* * *</td>
<td>* *</td>
<td>* * *</td>
<td>9/9</td>
<td>Very good quality</td>
</tr>
</tbody>
</table>

The standardized mean difference of the frequency of micronuclei between groups was -0.72, with a 95% confidence interval of -0.96 to -0.48 (p < 0.001). Based on these studies, a significant statistical difference between the groups could be identified (Figure 2). Data were homogeneous (chi-square test, p < 0.52; I² = 0%), which means that all studies included in the analysis share a common effect size.
Discussion
Mutagenic and genotoxic effects of crack has been a topic of interest to scientific community and public health, due to drug abuse increases deleterious effects on genetic material, such as genotoxic and carcinogenic products resulting from pyrolysis of substances present in crack smoke (Nakahara and Ishigami 1991). Crack use was associated with a range of health outcomes in humans (Butler et al. 2017). Beyond that, according to Yujra et al. (2015) in their study evaluating genomic damage in multiple organs of mice following acute exposure to crack cocaine, this substance induced DNA damage in peripheral blood and brain cells by single cell gel (comet) assay data. The results of our systematic review and meta-analysis point that there is a higher frequency of MN in oral mucosa cells in crack users when compared to non-users, statistically significant, demonstrating the potentially mutagenicity of this drug (Almeida et al. 2012; das Graças et al. 2014; Webber et al. 2015; Lima et al. 2016; Antoniazzi et al. 2018). Moreover, the detection of an elevated frequency of micronuclei in some tissues and/or organs indicates increased risk of cancer (Butler et al. 2017). It is important to highlight that literature report differences in MN assay methodology, which might influence the results found in this study. Some protocols Feulgen (Almeida et al. 2012; das Graças et al. 2014; Webber et al. 2015; Lima et al. 2016) and others used Giemsa (Antoniazzi et al. 2018) as staining method. A review proposed by Nersesyan et al. (2006) indicated that nuclear anomalies, like the MN, might be misinterpreted with non-specific DNA dyes, such as Giemsa, and lead to false positives. However, in the results found in our review, the staining method used by the different authors showed no significant difference. Similar results were observed in the review developed by De Geus et al. (2018) with cigarette smokers. Even though this review included databases that have article repositories from all around the world, all selected articles were developed in Brazil. This is probably due to Brazil being considered the world’s largest market for crack cocaine (Laranjeira et al. 2014; UNODC 2018). The results of this systematic review corroborate to the data from the Brazilian National Survey on the use of Crack, since it reports that the majority of crack users are young adults - with an average age of 30.28 years and that approximately 1/3 of them is concentrated in the age group of 18 to 24 years. Also, it was observed high mean time of crack use contradicting the commonly reported that crack users would have survival less than 3 years of consumption (Bastos and Bertoni 2014). Furthermore, of the five selected studies, four were conducted with crack users who were in chemical dependency treatment programs and one used an interview with users who lived on the street. Beyond MN assay, evaluated studies have addressed cytotoxicity characteristics that were usually related to anomalies that reflect acute cell death (Nersesyan et al. 2013). Among these changes are: binucleation (cells that contain two nuclei); nuclear buds (MN-like bodies attached to the nucleus by a thin nucleoplasmic connection); pycnosis (cells containing dark, dense small nuclei without noticeable chromatin); karyorrhexis (cells that exhibit particle chromatin condensation showing nucleus fragmentation into intact small round or oval bodies); and karyolysis (absence of nucleus in cells) (Lima et al. 2016). Although these parameters are approached associated with MN, only the results of pycnosis, karyorrhexis and karyolysis showed statistical difference between users and nonusers in the studies. This may indicate that crack consumption leads to inflammatory changes and these parameters may serve as bioindicators that precede carcinogenesis (Nersesyan et al. 2013; Lima et al. 2016). However, it is important to note that the presence of MN in the oral mucosa cells of crack users may also be related to the products generated during pyrolysis and also the exposure of the mucosa to high temperatures, as pointed out by De Geus et al. (2018) in their systematic review on MN in cigarette smokers. The present review highlights the very low heterogeneity degree between studies, which provide results that are considered more consistent. The homogeneity of the results may be due all studies evaluated MN in the same cell type. In addition, all studies were from Brazil, which may be related with the high prevalence of crack addiction in the country. According to Abdalla et al. (2014) 370,000 Brazilians regularly used crack and similar for at least six months in 2012, corresponding to 0.8% of the country’s capitals population. In Brazil, approximately 2 million of people (aged 14 years and older) smoked cocaine (crack) at least once in their lifetime (1.5% among adults, 0.8% among adolescents) and one in one hundred adults used crack in the past year (Abdalla
et al. 2014). As observed, there is a gap in the literature on this thematic elsewhere in world. Thus, there is the possibility that many drug users susceptible to DNA damage may have different outcomes than those of the studies included in this review and, therefore, require further research in different populations.

**Conclusion**

Summary, the micronuclei frequency was higher in users of crack compared to non-users, as well as, secondary outcomes such as binucleated cells, nuclear buds, pyknosis, karyorrhexis and karyolysis. Therefore, there is a negative impact of crack use in oral mucosa exfoliated cells and MN assay proved to be a good biomarker to assess the mutagenic impact of crack use in oral epithelium.

**Authors Disclosures**

**Contributors**

Key search parameters, including search strategy, screening and data extraction were designed by FMRSJ, MS, RAT, TBS and CLFF. Screening, data extraction, and summarizing was performed primarily by FMRSJ, MS and RAT with validation performed by two independent reviewers. Statistical analyses were performed by FMRSJ and CLF with input from RAT. Newcastle-Ottawa quality assessment scale adapted for cross-sectional studies was performed by RAT and validated by FMRSJ. Data interpretation, manuscript drafting and revising was conducted jointly by all authors, led by FMRSJ. The corresponding author (FMRSJ) had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

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