

| Received | 2025/09/10 | تم استلام الورقة العلمية في |
|-----------|------------|-----------------------------|
| Accepted | 2025/10/10 | تم قبول الورقة العلمية في أ |
| Published | 2025/10/11 | تم نشر الورقة العلمية في |

Investigating the impact of cannabis on oral Lactobacillus and Candida albicans among addicts

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Abstract:

Cannabis, a plant rich in cannabinoid compounds such as tetrahydrocannabinol (THC) and cannabidiol (CBD), has attracted significant interest due to its medical applications psychoactive/addictive effects. This study aimed to investigate the association between cannabis use and oral health by examining the growth of Lactobacillus bacteria and Candida albicans among addicts. The study was conducted in Libya's Al-Jabal Al-Akhdar region with 60 participants divided into three groups: non-smokers (control group), tobacco smokers, and cannabis users. The results revealed a statistically significant relationship between cannabis use and decreased salivary flow rate along with lower salivary pH levels, creating a more acidic oral environment. Additionally, a marked increase in Lactobacillus bacterial growth and Candida albicans colonization was observed among cannabis users compared to the other two groups. These findings indicate adverse oral health effects that warrant specific public health attention.

Keywords: Cannabis, Oral health, Salivary flow rate, *Candida albicans*, *Lactobacillus* bacteria, Salivary pH Levels.



التحقق من تأثير القنب على لاكتوباسلس والكانديدا البيكانس في أفواه المدمنين

عبد الوهاب سليمان عبد الرحيم بوهنية و2نجاح سليمان عبد الله بوبكر

أقسم الأحياء الدقيقة، الأكاديمية الليبية للدارسات العليا، البيضاء، ليبيا ² قسم علم النبات، جامعة عمر المختار، البيضاء، ليبيا

الملخص:

القنب، وهو نبات غني بمركبات الكانابينويد مثل رباعي الهيدروكانابينول (CBD)، حظي باهتمام واسع نظرًا لتطبيقاته الطبية وتأثيراته النفسية وكانابيديول (CBD)، حظي باهتمام واسع نظرًا لتطبيقاته الطبية وتأثيراته النفسية والإدمانية. هدفت هذه الدراسة إلى بحث العلاقة بين استخدام القنب وصحة الفم من خلال فحص نمو بكتيريا لاكتوباسلس وفطر الكانديدا البيكانس لدى المدمنين. أُجريت الدراسة في منطقة الجبل الأخضر في ليبيا بمشاركة 60 متطوعًا تم تقسيمهم إلى ثلاث مجموعات: غير المدخنين (مجموعة ضابطة)، ومدخني التبغ، ومتعاطي القنب. أظهرت النتائج وجود علاقة ذات دلالة إحصائية بين تعاطي القنب وانخفاض معدل تدفق اللعاب وانخفاض مستوى الأس الهيدروجيني (pH) في اللعاب، مما يخلق بيئة فموية أكثر حموضة. كما لوحظ ارتفاع ملحوظ في نمو بكتيريا لاكتوباسلس وزيادة استعمار والكانديدا البيكانس لدى متعاطي القنب مقارنة بالمجموعتين الأخريين. وتشير هذه النتائج إلى أثار سلبية على صحة الفم تستدعى اهتمامًا خاصًا من الجهات الصحية العامة.

الكلمات الدالة: القنب، صحة الغم، معدل تدفق اللعاب، الكانديدا البيكانس، بكتيريا الاكتوباسلس، مستويات الأس الهيدروجيني للعاب.



http://www.doi.org/10.62341/aban1110

Introduction:

Cannabis addiction has been associated with significant and long-lasting health consequences. Studies consistently highlight a strong correlation between cannabis misuse and compromised oral health, particularly the onset of gum disease (Chaffee *et al.*, 2023).

Cannabis smoking and oral disease:

Cannabis smoke contains toxic chemicals in concentrations comparable to or exceeding those in tobacco and is identified as a potential respiratory tract carcinogen. Cannabis-related oral cancer commonly appears on the anterior floor of the mouth and the tongue (Keboa *et al.*, 2020). The carcinogenic effects of cannabis smoke are linked to harmful compounds such as aromatic hydrocarbons, benzopyrene, and nitrosamines, which are known to increase cancer risk (Howden and Naughton, 2011).

Smoking cannabis is linked to oral premalignant lesions, such as leukoplakia and erythroplakia. These lesions have the potential to be precancerous and may heighten the risk of developing oral cancer (Hashibe *et al.*, 2000). Cannabis use has been linked to a heightened risk of oral cancers, especially on the anterior floor of the mouth and the tongue. Cannabis smoke contains carcinogens like aromatic hydrocarbons, benzopyrene, and nitrosamines, which are present in amounts 50% greater than that found in tobacco smoke. These harmful compounds can contribute to cancer development. Additionally, smoking cannabis is associated with the appearance of oral premalignant lesions, such as leukoplakia and erythroplakia. However, its role as a definitive risk factor for oral cancer remains uncertain and warrants additional research (Temilola *et al.*, 2017).

Relationship between cannabis use and dry mouth:

Cannabis was widely misused globally, leading to various social and health challenges across nations. Among the identified cannabis-associated oral effects is xerostomia, a condition stemming from reduced salivary flow. The subsequent dry mouth resembles that caused by cigarette smoking and tends to appear shortly after cannabis use in most individuals (Darling and Arendorf, 1993). The prevalence of cannabis use is increasing among adults. Studies suggest that cannabis consumption negatively impacts oral health, identifying it as a distinct risk factor for multiple dental problems. This underscores the importance of maintaining good oral hygiene and regular dental check-ups for cannabis users (Chaffee *et al.*, 2023).



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Cannabis use has long been linked to dry mouth and hyposalivation. For most users, these effects typically persist for one to six hours following consumption (Bellocchio *et al.*, 2021). This condition was regarded as a major risk factor for dental caries, as saliva plays a vital role in sustaining oral pH balance, suppressing bacterial growth, and facilitating the remineralization of teeth (Le *et al.*, 2022).

Effects of dry mouth on oral health:

Xerostomia has numerous negative effects on oral health (Kapourani *et al.*, 2022). Reduced salivary flow heightens the risk of dental caries because saliva plays a key role in neutralizing acids generated by oral bacteria (Rusu *et al.*, 2022). Dry mouth also contributes to a heightened risk of oral infections, including candidiasis (Torres *et al.*, 2002).

Saliva is vital for preserving oral health, and reduced salivary flow can result in the impairment of its critical functions, such as protecting against infections, aiding digestion, and maintaining a balanced oral environment, such as cleaning the mouth, digesting food particles and debris through salivary enzymes (Proctor *et al.*, 2021), and removing acids produced by oral microbiota as end metabolic products, which lowers the pH level (Dipalma *et al.*, 2021). This decline in pH fosters an environment suitable for acid-tolerant bacteria to thrive, ultimately becoming the predominant bacteria within the oral flora (Boisen *et al.*, 2021). The prevalence of acid-tolerant bacteria significantly elevates the risk of developing dental caries and periodontal diseases, as these bacteria thrive in the altered pH environment and contribute to the deterioration of oral health (Banas *et al.*, 2020).

Increased risk of fungal and bacterial infections:

The connection between cannabis uses and oral infections has become an area of increasing focus for researchers and dental professionals. Studies have demonstrated that cannabis use may contribute to various oral health issues, including infections and changes in the oral environment, particularly smoking, may contribute to a range of oral health problems, such as periodontal conditions and xerostomia, and an increased risk of oral infections such as candidiasis by *Candida albicans* (Keboa *et al.*, 2020).

Cannabis use can disrupt the oral environment, establishing conditions that promote the growth of harmful bacteria and fungi. This imbalance exacerbates oral health issues, potentially leading to infections and other complications (Shekarchizadeh *et al.*, 2013).



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This study aims to:

- 1-Detect the presence of *Lactobacillus* bacteria and *Candida albicans* in the oral cavities of cannabis users, smokers, and non-users.
- 2- Investigate potential oral infections, identify their causative agents, and examine their association with salivary flow rate and salivary pH levels among cannabis users.

Materials and Methods:

Study design: This study was conducted in Al-Jabal Al-Akhdar, Libya, between September 1, 2024, and April 1, 2025, following a prospective randomized study design. Cannabis users were selected through the snowball sampling method, ensuring gradual expansion of the participant pool (Parker et al., 2019). Additionally, smokers and non-users were recruited based on predefined criteria to establish a well-balanced sample. A total of 60 participants were enrolled, divided into three groups: 20 cannabis users, 20 tobacco smokers, and 20 non-users. Ethical approval was obtained from the Libyan National Committee for Biosafety and Bioethics. Inclusion criteria ensured sample homogeneity, with control group participants having no prior experience with smoking or cannabis use, while smokers and cannabis users were selected based on consumption frequency and duration. Exclusion criteria eliminated participants with hypertension, diabetes, hormonal disorders, vitamin D deficiency, alcohol intake, or hallucinogenic drug use to prevent confounding factors (Mayl et al., 2020).

Sample collection: Urine samples were successfully collected from the participants for detailed drug testing. This critical step ensured the confirmation of cannabis's active substance absence in the groups, these examinations were based on the protocols detailed in (Hadland and Levy, 2016). Salivary samples were collected following a standardized protocol (Navazesh and Kumar, 2008) to measure the salivary flow rate accurately. The pH levels were determined using strip-type tests, these examinations were conducted based on (Baliga *et al.*, 2013).

Two oral swabs were collected from each participant, the first oral swab was utilized to evaluate bacterial growth, while the second focused on fungal growth, the procedures followed the guidelines established in (Valinetz and Cangelosi, 2021).

Inclusion criteria: The inclusion and exclusion criteria for this study were meticulously designed in accordance with relevant



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guidelines to ensure the reliability and validity of the findings. These criteria were designed to ensure a homogeneous group of participants, thereby reducing variability within the sample and minimizing the influence of external factors on the study outcomes, The inclusion were conducted according to the methods referenced in (Keung *et al.*, 2020).

Control group:

- Individuals who have never smoked or used cannabis.
- Serve as a comparison group to measure differences between groups.
- Subjects must match the experimental groups in age, gender, and demographics.

Smokers group:

- Individuals who regularly smoke tobacco.
- Used to study the impact of smoking on the studied variables.
- Selected based on criteria such as the amount and duration of smoking.

Cannabis users group:

- Individuals who regularly use cannabis and have a history of its use.
- Used to study the effects of cannabis use on the studied variables.
- Selected based on criteria such as the amount and duration of cannabis use.

Exclusion criteria:

- Suffer from hypertension (high blood pressure)
- Diabetes.
- Hormonal imbalances.
- Deficiency in vitamin D.
- Significant health issues.
- Consume alcohol.
- Use hallucinogenic drugs.
- Engage in vigorous exercise.

Evaluation before participating:

- Informed consent was secured from all participants, ensuring confidentiality and the anonymity of their identities to researchers and anyone involved in the study. The privacy and confidentiality of the participant is maintained by classifying the data in an anonymous, non-referenced manner.
- Participants had option to be operated out of the study at any time without compromising their right.



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- All participants underwent a set of questions, which incorporated the established inclusion and exclusion criteria to ensure appropriate selection for the study. (Keung *et al.*, 2020).
- Detailed past medical and surgical history.

Complete drug test:

Drug testing was performed to detect the active ingredient in cannabis (THC), This test provides detection of more than one active drug substance in a urine sample, including the following: methamphetamine, cocaine, tramadol, barbiturates, benzodiazepine, Tetrahydrocannabinol and Amphetamine. This testing was necessary to accurately identify participants who used cannabis and ensure the validity of the study results (Owen *et al.*, 2012).

Collection of urine samples:

Urine samples were successfully collected from the participants for detailed drug testing. This critical step ensured the confirmation of cannabis's active substance absence in the smoker and control groups, while validating its presence among cannabis users. Participants were supplied with urine collection containers and instructed to provide morning samples, which had to be submitted to the laboratory within two hours for accurate results. This procedure ensured the accuracy and validity of group classification based on cannabis use.

Collection of saliva and measurement of saliva flow rate:

Saliva collection tubes were distributed to the participants as the next step in the process. These tubes were used to obtain saliva samples required for laboratory analysis

Measurement of saliva pH level:

The pH levels were determined using strip-type tests, to assess the acidity or alkalinity of the oral environment.

Statistical Analysis:

Statistical analysis data from the control group, smoker group, and cannabis users group included saliva flow rate, saliva pH, *Lactobacillus* bacterial growth, and *Candida albicans* growth. The data was further analyzed using Minitab (version 20), with a significance threshold set at P < 0.05. The ANOVA test was employed to compare the mean salivary flow rates and pH levels among the three groups: control, cannabis users, and smokers. Post hoc comparisons using Tukey's HSD test further highlighted significant differences between groups. Additionally, the Chi-Square test was utilized to compare the proportions of Lactobacillus bacterial growth and *Candida albicans* fungal growth across the



groups. Furthermore, correlation coefficients were calculated to assess the relationships between cannabis use and various salivary parameters (such as salivary flow rates and pH levels), as well as the prevalence of *Candida albicans* and *Lactobacillus*. These analyses provided valuable insights into the significant associations and trends observed among the studied variables.

Results:

All urine samples from the control and smoker groups tested negative for the listed drugs, indicating that these participants were not exposed to any of the substances assessed. This underscores the importance of a control group as a baseline for comparison. In contrast, all urine samples from the cannabis user group tested positive for Tetrahydrocannabinol (THC), the active compound in cannabis, and negative for the other drugs listed, confirming cannabis use exclusively in this group, Salivary flow rate measurements revealed notable differences across the study groups. The volume of each saliva sample was measured using a precision balance, allowing for accurate assessment of salivary flow rates. A one-way ANOVA revealed a statistically significant difference among the groups (P < 0.001). Post hoc analysis using Tukey's HSD test showed that the control group exhibited significantly higher salivary flow rates (0.98 \pm 0.11 g/min) compared to smokers (0.70 ± 0.13 g/min; p < 0.001) and cannabis users (0.52 ± 0.12 g/min; p < 0.001). Additionally, smokers had significantly higher flow rates than cannabis users (p < 0.001), indicating a graded reduction in salivary flow with cannabis users showing the most pronounced impairment (**Table 1**). Additionally, smokers demonstrated significantly higher flow rates than cannabis users (p < 0.001). These results suggest a graded reduction in salivary flow, with cannabis users showing the most pronounced impairment.

Table :(1). Statistical analysis of saliva flow rate among groups

| Comparison | Mean Difference (g/min) | P-value | Significance (α=0.05) |
|-------------------------------|----------------------------|---------|-----------------------|
| Control vs. Smokers | 0.28 | < 0.001 | Yes |
| Control vs. Cannabis users | 0.46 | < 0.001 | Yes |
| Smokers vs. Cannabis users | 0.18 | < 0.001 | Yes |



The pH level of each saliva sample was measured utilizing pH test strips. This provided insightful data on the saliva pH levels across the studied groups, contributing to a thorough evaluation of how factors like cannabis use and smoking influence the variables under investigation. The effect of cannabis on salivary pH levels, One-way analysis of variance was conducted to evaluate the differences in salivary flow rates among three groups: control, cannabis users, and smokers group. There was significant difference between groups as indicated by P-value = < 0.001, which is well below the predetermined significance level (P < 0.05). This finding suggests that there are meaningful variations in salivary pH levels between the groups, Post hoc comparisons using Tukey's Honestly Significant Difference test further delineated these differences: the control group exhibited significantly higher pH levels (6.85 \pm 0.6) compared to both smokers (6.15 \pm 0.81; p = 0.011) and cannabis users (5.95 \pm 0.69; p < 0.001). However, no significant difference was observed between smokers and cannabis users (p = 0.112). These results demonstrate a progressive acidification of saliva, with cannabis users showing the most pronounced pH reduction (**Table** 2).

Table :(2). Statistical analysis of saliva pH among groups

| 2 400 20 1 (2)1 5 144 25 27 242 442 442 | J | 1 | |
|---|-------------------------|---------|-----------------------|
| Comparison | Mean Difference (g/min) | P-value | Significance (α=0.05) |
| Control vs. Smokers | 0.60 | 0.011 | Yes |
| Control vs. Cannabis users | 1.00 | <0.001 | Yes |
| Smokers vs. Cannabis users | 0.40 | 0.112 | Yes |

The Chi-Square test was used to compare the of *Lactobacilli* growth between the groups (**Table 3**). There was significant difference between groups as indicated by P-value = 0.017, which is well below the predetermined significance level (P < 0.05), *Lactobacilli* positive growth is observed in 5% of the control group, 15% of the smokers group, and 35% of the cannabis users group. Upon closer examination, the cannabis users group demonstrated a notably higher proportion of positive *Lactobacilli* growth compared to the other groups. These findings indicate a significant correlation between cannabis use and an increased likelihood of *Lactobacilli* growth, The Chi-Square test was used to compare *Candida* albicans



growth between the groups (**Table 4**). There was significant difference between groups as indicated by P-value = 0.001, which is well below the predetermined significance level (P < 0.05). Candida albicans.

Table :(3). Lactobacilli growth among groups.

| Table :(3): Laciobactiti growth among groups. | | | | |
|---|----------------------|----------------|---------|-----------------------|
| Groups | Positive cases/Total | Percentage (%) | P-value | Significance (α=0.05) |
| Control | 1/20 | 5% | | |
| Smokers | 3/20 | 15% | 0.017 | Yes |
| Cannabis users | 7/20 | 35% | | |

Table :(4). Candida albicans growth among groups

| Groups | Positive cases/Total | Percentage (%) | P-value | Significance (α=0.05) |
|--------------------|----------------------|----------------|---------|-----------------------|
| Control Smokers | 0/20 0/20 | 0% 0% | | |
| Cannabis users | 6/20 | 30% | 0.001 | Yes |

Positive growth is observed in 30% of the cannabis user group, while no positive growth cases were recorded in either the control group or the smoker group. Upon closer examination, the cannabis users group demonstrated a notably higher proportion of positive *Candida albicans* growth compared to the other groups. These findings indicate a strong correlation between cannabis use and an increased likelihood of *Candida albicans* growth, The correlation coefficient was employed to determine the relationships between various variables (**Table 5**). This statistical method facilitated the measurement of the strength and direction of associations between cannabis consumption and factors such as *Candida albicans* prevalence, *Lactobacilli* presence, and various salivary parameters, including pH levels and flow rate. The findings revealed a strong positive correlation between cannabis

consumption and an increase in *Candida albicans* prevalence (0.63), as well as a moderate positive correlation with *Lactobacilli* presence (0.41). Furthermore, reduced salivary acidity was significantly associated with an increase in *Candida albicans* (-0.52) and *Lactobacilli* (-0.31). Additionally, decreased salivary secretion rates were linked to increased *Candida albicans* (-0.47). Interestingly, despite their respective associations with cannabis consumption, no



direct correlation was observed between *Lactobacilli* and *Candida* albicans.

Table :(5). Correlation analysis of cannabis use, salivary parameters,

and microbial growth.

| and inicional growth. | | | |
|----------------------------------|-----------------------------|---------|-------------------------------|
| Parameter Pair | Correlation coefficient (r) | P-value | Interpretation |
| Cannabis use vs C. albicans | 0.63* | <0.001 | Strong positive association |
| Cannabis use vs Lactobacillus | 0.41* | 0.002 | Moderate positive association |
| Saliva pH vs C. albicans | -0.52* | <0.001 | Moderate inverse correlation |
| Saliva flow vs C. albicans | -0.47* | 0.001 | Moderate inverse correlation |
| Lactobacillus vs C. albicans | 0.12 | 0.38 | Non-significant association |
| Saliva pH vs Lactobacillus | -0.31* | 0.02 | Weak inverse correlation |

Discussion:

Salivary flow rates are a vital indicator of oral health in this study demonstrated that, cannabis users exhibited significantly lower salivary flow rates compared to both the control group and smokers' group. These findings correspond to (Prestifilippo et al., 2006) observed that, cannabis use reduces saliva secretion by impacting cannabinoid receptors (CBs) located in salivary glands. Similarly, (Grotenhermen, 2003) found that, cannabis users had lower salivary flow rates compared to non-users. Furthermore, Evidence from this research shows that, observed that, the effects of cannabis on saliva flow rate and pH were analyzed in comparison to a smoker group and a control group. The findings revealed a distinct relationship between reduced saliva flow rate and decreased pH values within the cannabis user group. The data demonstrated that samples with lower saliva flow rates tended to have more acidic pH values. These results are consistent with the observations of (Bechir et al., 2021) reported that, a decrease in salivary flow rate is associated with a decline in pH levels. In this study identified that, the percentage of Lactobacillus growth in the cannabis users' group was significantly higher compared to the other groups, *Lactobacillus* growth can be an indicator of changes in the oral environment These results are agreement with the study by (Habib et al., 2021) which demonstrated that, cannabis use can enhance Lactobacillus growth



http://www.doi.org/10.62341/aban1110

in the mouth. In this investigation illustrated a correlation between low pH and *C. albicans* overgrowth among cannabis users, Samples that show *C. albicans* overgrowth have relatively low pH values. respectively, which in agreement with the study of (Davis, 2003) concluded that,

C. albicans exhibits remarkable adaptability and growth in acidic environments, contributing to its ability to colonize various tissues in the human body.

Conclusion:

This study confirms that, cannabis use significantly impairs oral health by reducing salivary flow and increasing acidity, fostering dry mouth and dental decay. The low pH environment promotes overgrowth of *Lactobacillus* and *Candida albicans*, disrupting microbial balance. Acidic conditions enhance *C. albicans* pathogenicity and allow *Lactobacillus* to further acidify the oral cavity, collectively raising the risk of caries and periodontal disease

Recommendations:

- We recommend that oral healthcare providers take appropriate measures when treating cannabis users
- We recommend doing studies with large number of participants.
- . We recommend doing studies with long period of follow up.

Ethics:

Ethical approval for this study was granted by the National Committee for Biosafety and Biological Ethics (Approval No: NBC: 004.H.25.3).

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