

Potential Anti-Cancer Properties of Frankincense (*Boswellia Sarca*) Chewing Gum and its Role in Reduction of Tobacco Smoking Genotoxicity

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<https://dx.doi.org/10.13005/bpj/2602>

(Received: 05 February 2023; accepted: 16 March 2023)

Arabic frankincense is distinguished by its many medical and therapeutic benefits, as it treats many backward diseases that affect the human body. Smoking causes a long list of cancers, on top of which are lung cancer. Smoking cessation can prevent a third of cancer-related deaths. The study aimed to reveal the role of frankincense in reducing the genotoxicity of smoking on the buccal mucosa using buccal micronucleus cytome (BMCyt) assay. For this purpose, 50 smokers and 30 non-smokers participated; all of them were students of Wasit University. The study showed that smoking causes a significant increase in the level of nuclear abnormalities. Apoptosis showed the biggest change, with an about twenty-fold increase, followed by cytotoxicity (about four folds increase), and mutagenicity (about three-folds) as compared with control. A significant decrease in mutagenicity and cytotoxicity was observed ($P= 0.038$ and 0.051 , respectively) after 4 weeks of chewing frankincense gum by smokers while increase was observed with apoptosis ($P= 0.071$). We conclude from this study results that chewing gum exhibited pro-apoptotic and anti-proliferative activities against cancer-damaged cells.

Keywords: Apoptosis; Buccal Micronucleus Cytome (BMCyt) assay; Carcinogenesis; Frankincense; Genotoxicity; Micronucleus; Smoking.

Frankincense was a great traditional medicine in the Arab countries. It has been used to treat burns, bruising, digestive disorders, joint pain, and respiratory distress. It also exhibits antibacterial activity against a variety of microorganisms¹. In the family Burseraceae, the *Boswellia* genus of trees produce frankincense, an aromatic resin that hardens into irregular lumps of yellowish brown gum (Figure 1)¹. Alkaloids, polyphenols, penta- and tetracyclic terpenoids, tannins, saponins, essential oils, and other chemicals are found in high concentrations in frankincense resin^{2,3}.

Recent research has shown that frankincense extracts can inhibit the growth of tumors in animal cells and induce tumor apoptosis⁴. *Boswellia sacra* essential oil decreases tumor aggressiveness and encourages tumor-specific apoptosis in cultured human breast cancer cells⁵. Boswellic acids have also been shown to have anticancer potential due to their pro-apoptotic and anti-proliferative activities several of human cancer cell lines, including leukemia cells, meningioma cells, and hepatoma cells^{6,7}. In a naked mouse model of pancreatic cancer, *boswellia sacra* gum reduces

tumor growth. It possesses cytotoxicity and tumor cell growth arrest in multiple types of human cancer cell lines in cultures, simultaneously activating multiple pathways leading to cancer cell-specific death⁶. Additionally, it has been hypothesized that more than 50% of natural cancer therapies can trigger apoptosis⁸.

Smoking tobacco is recognized as a significant public health issue with global adverse effects. The effects of tobacco smoking on health were primarily limited to industrialized nations; nevertheless, the promotion and uptake of this habit in emerging countries are currently causing a massive rise in smoking-related illness and mortality on a global scale^{7,9}. There are an estimated more than 1 billion smokers worldwide, and smoking is thought to be the cause of about 3 million deaths each year, with this number increasing to 10 million in the next 30 to 40 years¹⁰. According to estimates, half a billion of those alive now will pass away from a disease linked to tobacco use¹¹.

Smoking-related genotoxic effects have been discovered in all eight organs that have been studied. Oral/nasal, pharynx/larynx, esophagus, lung, myeloid organs, pancreas, uterine cervix and bladder/ureter cancer are all brought on by smoking in people¹². Smokers' lung tumors display a high frequency and specific spectrum of KRAS and TP53 mutations, which point to the presence of PAHs (and possibly other substances) in the smoke^{12,13}. These findings are consistent with a concept of tobacco smoke carcinogenesis wherein the carcinogenic process is driven by mutations that accumulate in a field of tissue as a result of tobacco smoke's components leading to smoking.

Over the past 20 years, there has been significant research how cigarette smoke encourages these cancers and other health issues. One method concerns the tobacco smoke's mutagenic properties, which have been amply shown and discussed two decades ago^{14,15}. Recent reviews have gathered research on DNA and protein adducts-related smoking in human tissues¹⁵,¹⁶ adding to the cigarette smoke exposure-related chemical biomarkers¹⁷.

One of the best biomarkers that may be utilized to identify the genotoxicity of smoking is micronuclei (MN). Micronuclei have been used as markers for genotoxic exposure since

1937¹⁸. Micronucleus refers to the small nucleus that originates whenever a whole chromosome or chromosome fragments is not integrated into the main daughter nuclei during cell division. It indicates chromosomal instability and genotoxic events¹⁹. Besides the micronuclei, there are other nuclear abnormalities that were considered as additional biomarkers for genotoxic effects of numerous compounds, such as binucleated cells (BN), nuclear bud (NB), pyknosis (PN), karyorrhexis (KR), karyolysis (KL).^{19,20}

Therefore, we used the micronuclei test on buccal mucosal cells from young smokers to investigate the potential inhibitory effect of frankincense gum chewing on cytogenetic abnormalities development and risks of tumor growth.

MATERIAL AND METHODS

Frankincense chewing gum preparation

Frankincense chewing gum was obtained from herbal stores in one of the famous markets in the Wasit governorate in January 2022. The species of frankincense (*Boswellia sacra*) was identified by the professors who specialized in plant sciences at the Faculty of Science, Wasit University. Participants were provided with approximately 2±0.2 gm of gum and were asked to chew gum for more than three hours per day and during smoking times.

Study group

Fifty smokers who had smoked more than ten cigarettes a day for at least a year were included in this study. They were all male students at Wasit University in Iraq, between the ages of 20 and 25. As a control sample, 30 more students who did not smoke were chosen. The following were included as exclusion criteria: the presence of a visible lesion on the oral mucosa, chronic illnesses, a history of cancer or diabetes, and recent X-ray exposure or medical treatment. Following consent from the Student Committee of Wasit, Iraq, and the Scientific Council of the Science College, university of Wasit, this study was conducted in the pathological analysis department's labs in the college of science.

Sample collection

Both smokers and non-smokers' buccal exfoliated cells were collected on September 3 in

accordance with Thomas *et al* instructions with a few minor modifications²¹. Then, buccal exfoliated cells from all smokers were collected at 0 weeks and considered as control. Then the participants were asked to chew Frankincense gum daily for at least 3 hours. Buccal samples were taken weekly for four weeks and sent directly to the laboratory for examination. The sample collection was carried out in the period of 10 September - 25 October 2022. participants were asked to rinse their mouth twice with distal water before sample collection. The exfoliated buccal mucosa cells were scrapped gently using cylindrical cytological brush from both cheeks. The brush's head was then dipped into tubes containing 3 mL of phosphate buffered saline (PBS) (Sigma-Aldrich-USA), centrifuged for 5 minutes at 800 rpm, and the supernatant was removed. The pellet was re-suspended and dropped onto microscope slides, fixed in 3:1 methanol/acetic acid (Merck, Germany) for 15 min. Then, Coded slides were dyed for 30 minutes at room

temperature with Giemsa dye (Hercules, CA, USA), and examined under a light microscope (400x).

Buccal micronucleus cytome (BM Cyt) assay

A total of 1,000 cells per subjects from the control and exposed groups were examined following the method of Buajeeb *et al*²². Micronuclei were scored as a parameter of DNA damage (mutagenicity). Karyorrhexis (nuclear disintegration) and karyolysis (nuclear dissolution) were classified as apoptosis. For cytotoxicity, the following nuclear abnormalities were considered: binucleated cells, nuclear buds and pyknosis, the frequencies of nuclear abnormalities were reported in 1000 differentiated cells.

Statistical analysis

All of the biological examination results were entered into a database created in Microsoft Excel, and SPSS version 23 was used for statistical analysis. The data on genetic damage between the smokers and control groups were examined using

Table 1. Nuclear abnormalities (‰) in the buccal exfoliated cells of smokers and non-smokers

| Category | Sample size | mutagenicity (mean ‰) | Other nuclear alterations (mean ‰) | |
|----------------|-------------|------------------------|-------------------------------------|-------------|
| | | | Cytotoxicity | Apoptosis |
| Control | 30 | 6.96 ± 1.38 | 2.01 | 3.24 ± -1.7 |
| smokers | 50 | 19.47 ± 4.04 | 9.85± 6.46 | 67.55±13.18 |
| <i>P</i> value | | 0.032 | 0.026 | 0.007 |

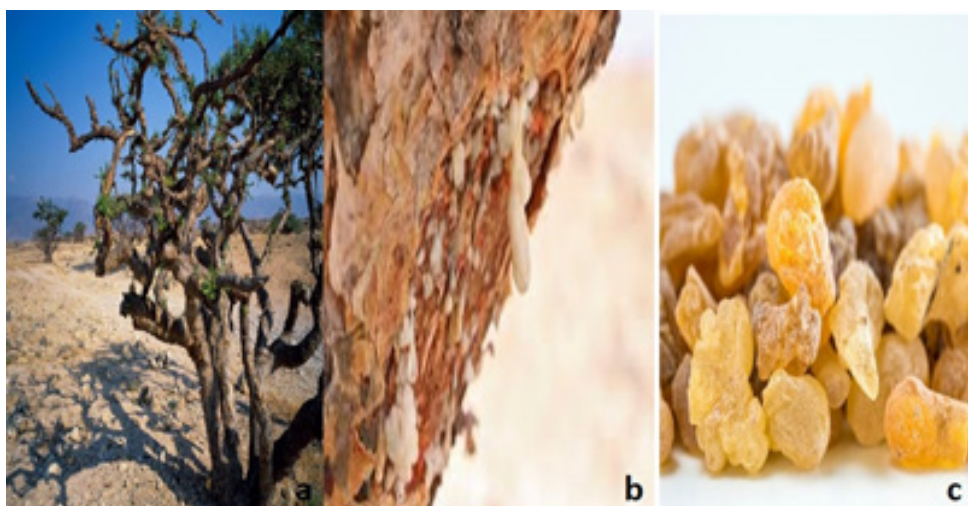


Fig.1. Frankincense (*boswellia sarca*): (a) slow-growing tree that reaches a height of 9 to 10 meters, (b) irregular lumps of yellowish brown gum that the tree's trunk exudes, and (c) frankincense chewing gum

a Poisson regression analysis. one-way analysis of variance (ANOVA) were applied to compare MN, BN, NB, KR, KL, and PN frequencies with chewing period. The frequencies of nuclear abnormalities per 1000 cells (%) was reported, with counts indicated as mean \pm standard deviation (SD). Statistical significance was defined as a *P* value 0.05.

RESULT AND DISCUSSION

Characteristic of participants

This study included 50 smokers and 30 nonsmokers who were 22.9 years old. All individuals were of male gender, to avoid the influence of sex on the results. Furthermore, due to Arab cultural and religious norms, there aren't

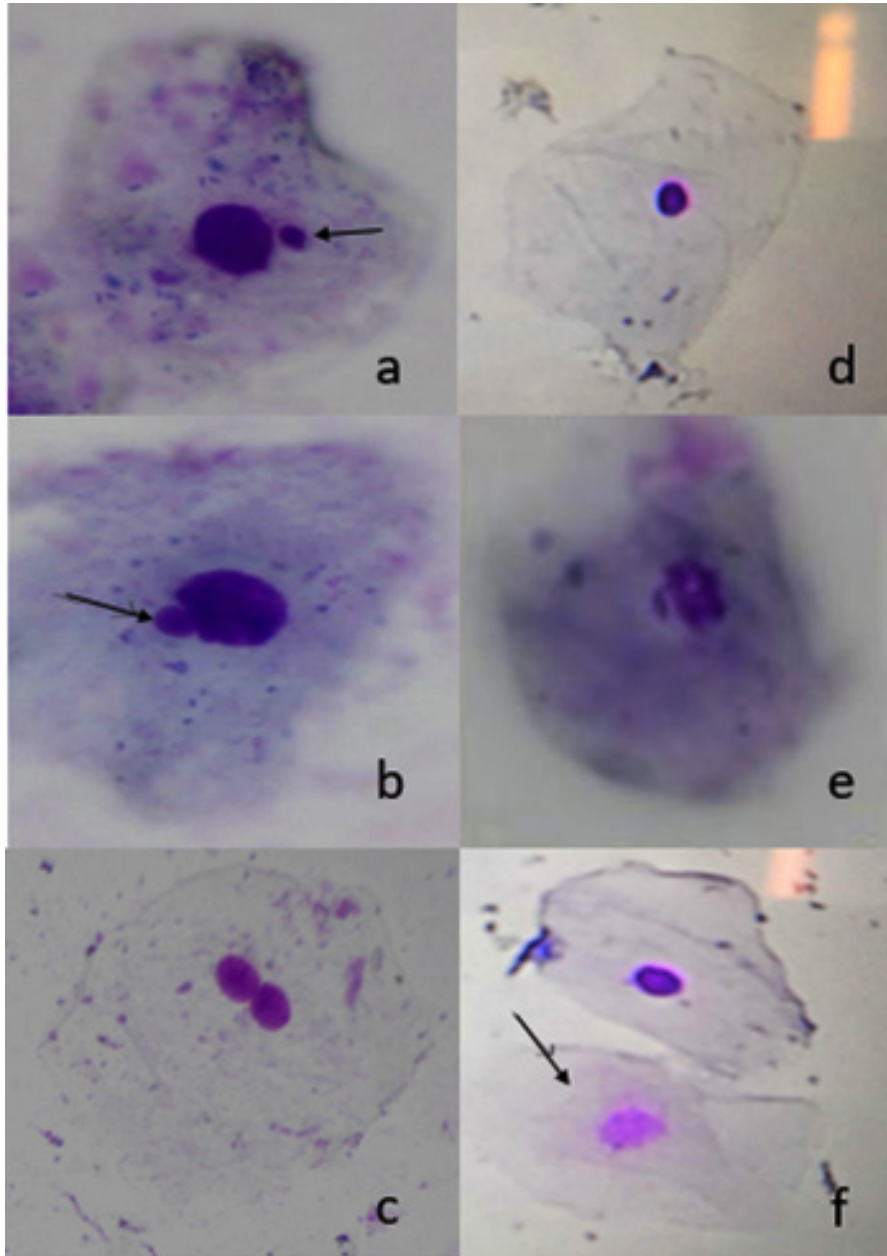


Fig. 2. Nuclear anomalies in oral mucosa cells: (a) cell with a micronucleus (arrow), (b) cell with a nuclear bud (arrow), (c) binucleated cell, (d) pyknosis, (e) karyorrhectic, and (f) karyolytic cell (arrow)

many female smokers in the study area who are of the target age.

Genotoxic effect of smoking

To identify the genotoxic effect of cigarette smoking and cancer development, the frequencies of nuclear anomalies in smokers buccal exfoliated cells were compared with frequencies among non-smokers students. Figure 2 shown the representative photographs of micronucleated cells and other nuclear abnormalities.

Only 0.0 to 0.9% of cells in the general population had micronuclei. Any variation in this range occurs as a result of an genotoxic effect that leads to chromosomal abnormalities²³. In this study, the results indicated significant increase in the mutagenicity (number of micronucleated cells), Cytotoxicity (binucleated, nuclear buds and pynkosis) and Apoptosis (karyolrrhetic and karyolitic cells) in smokers as compared to non-smokers as shown in table 1. The most significant was observed for apoptosis, where smokers had an about 20-fold higher rate than non-smokers, Cytotoxicity (4 folds increase), and mutagenicity (3-folds increase). It is a clear indication of the genotoxic effect of cigarette smoking. Alkaline smoke produced by cigarettes contains free radicals

which react with other combustion byproducts or living cells and cause DNA damage²⁴. Genotoxicity assay appeared to be a promising biomarker for assessing the impact of carcinogens in smoke. It has been observed that the number of chromosome breaks increases when exposed to carcinogenic substances, and it has been shown that these breaks are a precursor to the cancer development^{24, 25}. The present results indicated that apoptosis eliminates the cells with genetic damage and act as a surveillance mechanism²⁶. Thus, increased percentage frequencies of karyolysis may indicate of necrotic cells and genotoxic insult, as these occur in the pre-keratinization process²⁷.

Potential anti-cancer activity of frankincense gum

Smokers were asked to start chewing gum for four weeks for three hours a day, preferably during the smoking period, after measuring the micronucleus and other nuclear abnormalities in their buccal mucosa (0 week). This was done to investigate the role of frankincense gum in treating and reducing cancer risks. The study showed a noticeable decline in the number of micronuclei and in the rate of Cytotoxicity with *P* value 0.038 and 0.051, respectively. While apoptosis showed

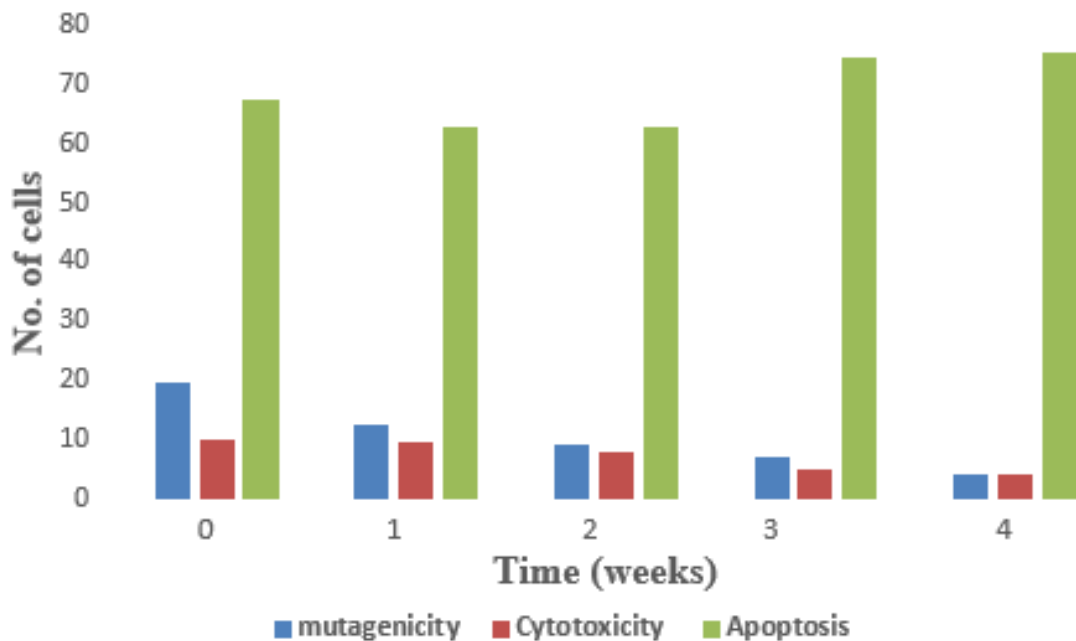


Fig. 3. Nuclear anomalies (%) in the buccal exfoliated cells of smokers per week from the start of chewing gum

slightly increased with insignificant difference ($P=0.071$) (Figure 3).

We found that frankincense has intense growth suppression activity and can induce apoptosis, this observation is in agreement with an earlier article that frankincense is help to treat cultured human colon, breast, bladder, and pancreatic tumor cells^{1,28}. Traditional Chinese medicine has included resins from frankincense gum as a component of anticancer medications. Many elements of frankincense essential oil's complex chemical composition may cooperate to produce a strong anti-cancer effect.

The pro-apoptotic and anti-proliferative and effects of frankincense essential oil may work together as anti-cancer. If PI3K/Akt activation and cell cycle progression are reduced while growth arrest genes are simultaneously expressed more highly, frankincense essential oil-induced growth inhibition may result¹. Based on caspase-3, -8, and -9 cleavages and activation, as well as poly (ADP-ribose) polymerase (PARP), in many human tumor cell, frankincense essential oil causes apoptosis^{5,28}.

CONCLUSION

We examined the frequency of nuclear abnormalities in the exfoliated buccal cells of 50 smokers as a biomarker of DNA damage and cytotoxic effects. According to the study, smoking significantly raises the levels of apoptosis, cytotoxicity, and mutagenicity when compared to a control group (20-folds, 4 folds and 3-folds increase, respectively). Frankincense has a broad range of pharmacological potential. It showed a noticeable decline in the number of micronuclei and in the rate of cytotoxicity with P value 0.038 and 0.051, respectively. Given that frankincense has anti-cancer properties via inducing apoptosis in cancer-damaged cells, apoptosis showed a slight increase with the insignificant difference ($P=0.071$). All ages can benefit from using this herbal medication to improve their oral hygiene. To administer frankincense essential oil for anti-cancer therapy as effectively as possible, pharmacokinetics and pharmacodynamic investigations are also necessary.

Conflict of Interest

The author declare that there is no conflict of interests regarding the publication of this paper.

Funding Sources

The authors received no financial support for the study, authorship, and publication of this article.

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