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**Original Article**

# The Role of Plant Extracts in the Perspective of Oral Squamous Cell Carcinoma Treatment

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## ABSTRACT

Oral squamous cell carcinoma remains a pressing issue due to high incidence, late diagnosis, and limited effectiveness of standard treatments with severe toxic effects. This study compared the antitumor activity and safety of *Curcuma longa*, *Camellia sinensis*, and *Nigella sativa* extracts in a rat model of DMBA-induced oral cancer. The experiment involved 78 adult male Wistar rats. Acute toxicity was assessed at 5000 mg/kg. Tumors were induced by applying 0.5% DMBA to the buccal mucosa three times weekly for 16 weeks. Animals received saline, plant extracts (500 mg/kg each), or cisplatin (2 mg/kg) for 8 weeks. Tumor measurements were performed weekly. Histological, immunohistochemical (Ki-67, caspase-3), and biochemical analyses were conducted. All extracts showed antitumor activity without toxicity. Black cumin extract demonstrated the highest efficacy, reducing tumor incidence to 70% (vs. 100% in controls) and decreasing tumor volume 2.8-fold (650 vs. 1850 mm³), achieving 64.9% growth inhibition. Immunohistochemistry revealed decreased Ki-67 to 31.4% (vs. 68.3%) and increased caspase-3 to 34.2% (vs. 8.2%), approaching cisplatin values (28.7% and 38.5%). Turmeric and green tea extracts showed moderate effects with 80% incidence and 47.0-52.4% inhibition. Cisplatin caused significant hepato- and nephrotoxicity, while plant extracts maintained normal parameters. All extracts suppress proliferation and induce apoptosis. Black cumin extract showed cisplatin-comparable efficacy without toxicity, supporting its further investigation for oral cancer management.

**Keywords:** Oral squamous cell carcinoma, Curcuma longa, Camellia sinensis, Nigella sativa, DMBA-induced carcinogenesis, Plant extracts

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## Introduction

Oral squamous cell carcinoma remains one of the most significant challenges in modern oncology [1, 2]. It ranks as the sixth most common cancer worldwide, with over 400,000 new cases diagnosed annually [3, 4]. In the Russian Federation, malignant neoplasms of the oral cavity account for 2-4% of all cancer cases. Approximately 13,500 new patients are identified each year, and the incidence of this pathology increases by an average of 2-3% annually [5]. The tongue is the most frequently affected anatomical site, representing

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65% of all cases, followed by the mucosa of the floor of the mouth at 11% and the buccal mucosa at 13% [6, 7].

Epidemiological studies show a clear male predominance among affected individuals, with a male-to-female ratio reaching 4-5:1. The peak incidence occurs in patients over 50-60 years of age, although recent decades have seen a trend toward younger patients [8, 9]. A particularly concerning fact is that despite the visible location of these tumors and their accessibility for examination, a significant proportion of patients are diagnosed at advanced stages. This substantially worsens the prognosis and reduces survival rates [10, 11].

Tobacco smoking and alcohol abuse are traditionally considered the main risk factors for developing oral squamous cell carcinoma. Up to 92% of patients with this pathology are smokers. The risk of developing the disease in heavy smokers is 13 times higher than in non-smokers. Combining smoking with excessive alcohol consumption increases cancer risk by 38 times in men and 100 times in women [12-14]. Chronic mechanical trauma to the oral mucosa also plays an important role. Such trauma may result from poorly fitting dentures, sharp edges of fillings, or broken teeth [15, 16]. Human papillomavirus type 16, transmitted through oral contact, also contributes to the etiology of the disease, particularly in younger patients [17, 18].

Current approaches to treating oral squamous cell carcinoma include surgery, radiation therapy, chemotherapy, and their combinations. For early stages, organ-preserving methods such as brachytherapy are preferred as they help avoid functional and cosmetic defects. For locally advanced disease, combined treatment with postoperative radiation or chemoradiation therapy remains the standard [19-22]. However, despite improvements in treatment methods, therapeutic effectiveness remains limited, especially in advanced disease. Studies show that excessively intensifying chemoradiation therapy inevitably increases the frequency and severity of adverse reactions and complications [23, 24]. This limits the potential of this approach to improve patient survival. Even with modern treatment protocols, 5-year survival does not exceed 54-55%. The toxic effects of chemotherapeutic agents, including cisplatin, remain a serious clinical problem manifesting as hepatotoxicity, nephrotoxicity, and hematological disorders [25, 26].

Given these limitations of standard therapy, recent years have seen growing interest in alternative and adjuvant agents based on natural compounds. Such compounds offer antitumor activity with minimal side effects. Plant polyphenols, flavonoids, and other phytochemicals attract researchers' attention due to their ability to modulate key signaling pathways involved in carcinogenesis, including proliferation, apoptosis, angiogenesis, and metastasis [27, 28]. Among the most studied natural agents are curcumin from *Curcuma longa*, epigallocatechin gallate from green tea, and thymoquinone from *Nigella sativa*. Each of these compounds has unique mechanisms of action and has shown promising results in preclinical studies. Curcumin, the main polyphenolic component of *Curcuma longa*, is known for its ability to inhibit nuclear factor kappa B and the STAT signaling pathway. Both play key roles in inflammation and carcinogenesis [29, 30]. Green tea polyphenols, primarily epigallocatechin gallate, exhibit antioxidant properties and can influence epigenetic regulation by modulating DNA methyltransferase activity [31, 32]. Thymoquinone from *Nigella sativa* attracts particular attention due to its ability to induce apoptosis and autophagy in tumor cells through activation of p53-dependent signaling pathways. It also exhibits selective cytotoxicity, causing minimal damage to normal cells [33, 34]. Most current studies emphasize the need for comparative evaluation of different plant extracts under identical experimental conditions. This approach helps identify the most promising candidates for further clinical investigation.



This study aimed to compare the antitumor activity of *Curcuma longa*, *Camellia sinensis*, and *Nigella sativa* extracts in a rat model of dimethylbenzanthracene-induced oral squamous cell carcinoma. We analyzed their effects on tumor incidence, growth dynamics, proliferative activity, and apoptosis. We also evaluated the safety profile of these extracts in comparison with the reference drug cisplatin.

## Materials and Methods

*Preparation of plant extracts*

Three types of plant material were used in this study: rhizomes of turmeric (*Curcuma longa*), leaves of green tea (*Camellia sinensis*), and seeds of black cumin (*Nigella sativa*). Dried and ground raw materials of each plant were subjected to extraction with 70% ethanol at a 1:10 ratio at 40°C for 72 hours. The obtained extracts were filtered through a paper filter. Ethanol was evaporated on a rotary evaporator under reduced pressure. Residual water was removed by freeze-drying to obtain a dry extract [35, 36]. The yield of extractive substances was 12.4% for turmeric, 18.7% for green tea, and 9.3% for black cumin. The finished extracts were standardized by the content of the main active compounds. Turmeric extract contained at least 95% curcuminoids calculated as

curcumin. Green tea extract contained at least 80% epigallocatechin-3-gallate. Black cumin extract contained at least 5% thymoquinone. The extracts were stored in a light-protected place at 4°C until use. Immediately before administration to animals, extract samples were dissolved in physiological saline to achieve the required concentration. The administration volume did not exceed 1 ml per 100 g of animal body weight.

*Animals and housing conditions*

The experiment was performed on 78 adult male Wistar rats weighing 200-220 g. The animals were obtained from a laboratory animal nursery. They were housed in the vivarium of Dagestan State Medical University under standard light conditions (12-hour light/dark cycle), temperature of 22±2°C, and relative air humidity of 55±10%. Rats had free access to granulated feed and purified water [37]. The distribution of animals into experimental groups and the study design are shown in **Figure 1**. All animal procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The study was approved by the local ethics committee of the university.

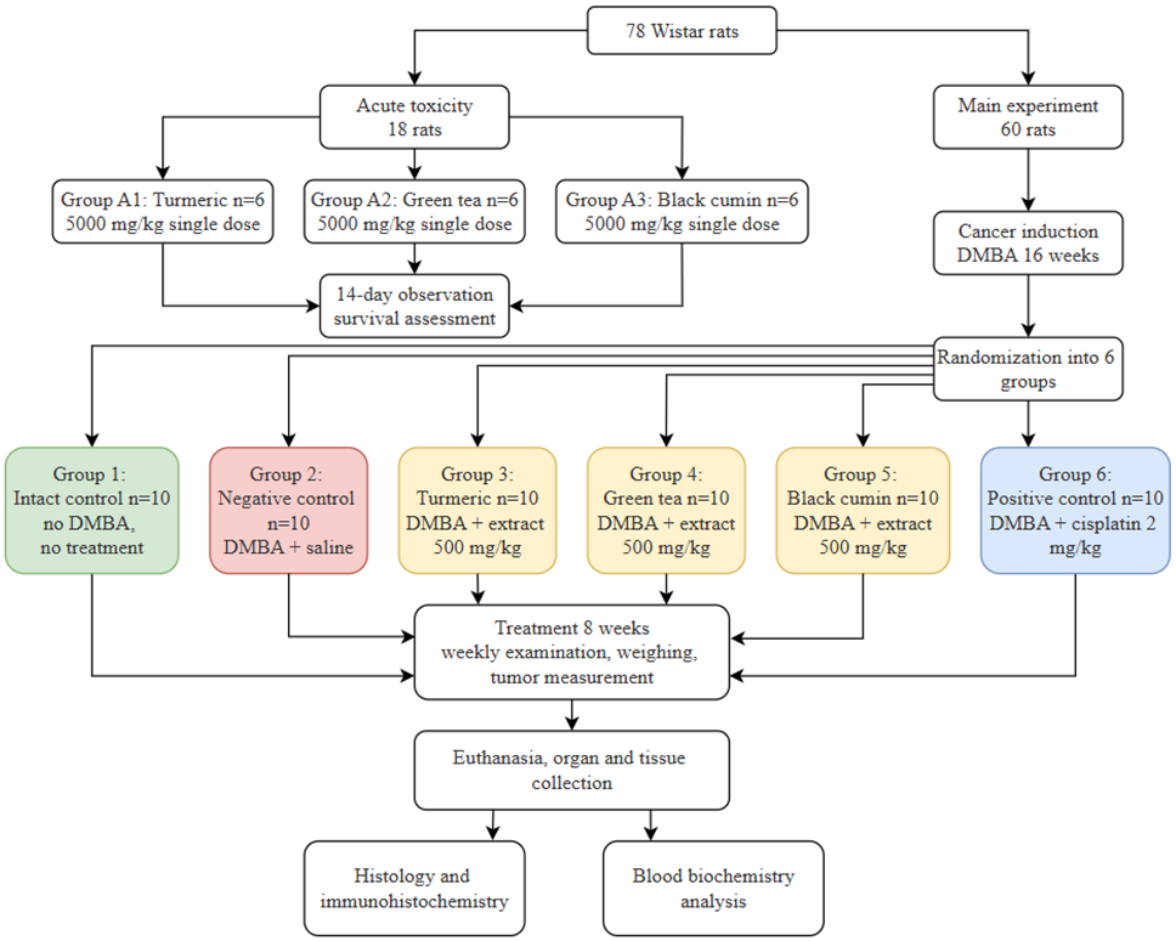



**Figure 1.** Experimental design for studying the antitumor activity of plant extracts in a rat model of oral cance

*Acute toxicity study*

Acute toxicity was assessed using 18 rats divided into 3 groups of 6 animals each. The extracts were administered intragastrically via a gavage tube as a single dose of 5000 mg/kg. This dose follows OECD guidelines for acute toxicity testing (Guideline 423) and allows classification of the studied substances into hazard categories [38, 39]. Animals were observed for 14 days. General condition, behavioral responses, food and water consumption, body weight, and mortality were recorded. Lethality was assessed daily.

*Induction of buccal mucosa squamous cell carcinoma*

Tumors were induced by applying 0.5% solution of 7,12-dimethylbenzanthracene (DMBA, Sigma-Aldrich, USA) dissolved in acetone. The carcinogen concentration was selected based on numerous previous studies confirming that 0.5% DMBA applied three times weekly for 16 weeks produces squamous

cell carcinoma of the buccal mucosa in 90-100% of animals with minimal mortality [40, 41]. The carcinogen was applied to the right buccal mucosa using a brush at a volume of 0.1 ml per application three times weekly for 16 weeks. The left cheek served as an intact control. Animals were weighed weekly. The buccal mucosa was examined, and the timing of tumor appearance and tumor growth dynamics were recorded.

*Experimental design and treatment protocol*

After completing the 16-week tumor induction period, 60 animals were randomized into 6 groups as shown in **Figure 1**. Group 1 (intact control, n=10) consisted of animals not exposed to the carcinogen and served to determine normal histological and biochemical parameters. Group 2 (negative control, placebo group, n=10) included tumor-bearing animals that received intragastric physiological saline in an equivalent volume to control the natural course of the tumor process. Groups 3, 4, and 5 (experimental groups, n=10 each) included tumor-bearing animals that received turmeric, green tea, and black cumin extracts, respectively. The dose of 500 mg/kg for plant extracts was based on acute toxicity data showing no toxic effects at this dose and on literature data regarding the therapeutic efficacy of similar doses in rodent cancer models. Group 6 (positive control, reference group, n=10) included tumor-bearing animals that received the reference drug cisplatin at 2 mg/kg intraperitoneally once weekly. Cisplatin was chosen as the comparator because it is a standard chemotherapeutic agent for head and neck squamous cell carcinoma in clinical practice. The dose of 2 mg/kg administered intraperitoneally once weekly corresponds to the average therapeutic dose for rodents and allows comparative evaluation of plant extract efficacy. Treatment continued for 8 weeks. Tumor sizes were measured weekly using an electronic caliper. Tumor volume was calculated using the formula $V = (a \times b^2)/2$, where a is the largest diameter and b is the smallest diameter. Antitumor efficacy was evaluated by tumor incidence in each group, mean tumor volume, and tumor growth inhibition rate.

*Assessment of systemic effects*

Throughout the treatment period, the general condition of animals, motor activity, coat condition, and food and water consumption were recorded. Body weight dynamics were assessed weekly. To evaluate the systemic effects of the extracts, blood was collected from all animals at the end of treatment for biochemical analysis of liver function (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin) and kidney function (creatinine, urea). The obtained values were compared with those of the intact control group to identify potential toxicity.

*Euthanasia and tissue collection*

After completing the 8-week treatment course, all animals were euthanized by diethyl ether overdose in a closed chamber [42]. Following deep anesthesia and respiratory arrest, the abdominal and thoracic cavities were opened. For histological examination, fragments of the buccal mucosa with tumor and adjacent unchanged tissue, regional lymph nodes, and internal organs (liver, kidneys, spleen, lungs, heart) were collected. Tissue samples were fixed in 10% buffered formalin solution (pH 7.4) for 24-48 hours at room temperature. The fixative volume exceeded tissue volume by at least 15 times. After fixation, samples were dehydrated in graded alcohols and embedded in paraffin using standard techniques. For the lungs, formalin instillation through the trachea was performed before fixation to expand the tissue. Sections of 4-5 μm thickness were prepared on a rotary microtome.

*Histological and immunohistochemical examination*

The obtained sections were stained with hematoxylin and eosin for light microscopy. Malignancy of neoplasms was confirmed based on the presence of cellular atypia, invasive growth, pathological mitoses, and keratinization. The degree of epithelial dysplasia and tumor differentiation was assessed semi-quantitatively. Immunohistochemical examination was additionally performed with antibodies to Ki-67 (proliferation marker) and caspase-3 (apoptosis marker) following a standard protocol with antigen retrieval. The immunohistochemical reaction was assessed by the percentage of positively stained nuclei (Ki-67) or cells (caspase-3) in at least 10 fields of view at ×400 magnification. Microscopic examination was performed using a light microscope at ×100, ×200, and ×400 magnifications.

*Statistical analysis*

Statistical data processing was performed using Statistica 10.0 software package. Normality of distribution was assessed by the Shapiro-Wilk test. One-way analysis of variance followed by Tukey's post hoc test was used for multiple comparisons. Differences in frequency indicators were assessed using Fisher's exact test. Data are presented as the arithmetic mean ± standard error of the mean. Differences were considered statistically significant at $p<0.05$.

**Results and Discussion**

*Acute toxicity of plant extracts*

Following a single intragastric administration of the extracts at a dose of 5000 mg/kg, no animal deaths were recorded in any of the three groups during the 14-day observation period. Rats receiving turmeric, green tea, and black cumin extracts maintained normal motor activity, appetite, and water consumption. Their appearance, coat condition, and mucous membranes did not differ from those of intact animals. Body weight dynamics in the extract-treated groups showed no statistically significant differences from physiological norms. Based on these data, the studied extracts can be classified as low-toxicity substances (hazard class V according to the OECD classification). The dose of 500 mg/kg selected for the main experiment is safe for long-term administration.

*Effects of plant extracts on tumor incidence and volume*

In the negative control group (Group 2) receiving only physiological saline, tumor incidence reached 100%, with all 10 animals developing multiple neoplasms of the buccal mucosa. Experimental groups receiving plant extracts showed reduced tumor incidence. The best effect was recorded in the group receiving black cumin extract (Group 5), where tumors developed in 7 out of 10 rats (70%). In the turmeric (Group 3) and green tea (Group 4) groups, tumors were detected in 8 out of 10 animals (80%). In the positive control group (cisplatin, Group 6), tumors developed in 6 out of 10 rats (60%), confirming the adequacy of the selected model and the validity of experimental conditions. No signs of tumor growth were detected in the intact group (Group 1).

Weekly tumor measurements allowed assessment of the studied extracts' effects on tumor progression rates. By the end of week 8 of treatment, the mean tumor volume in the negative control group reached 1850±210 mm³. In the turmeric group, this value was 880±95 mm³. In the green tea group, it was 980±105 mm³. In the black cumin group, it was 650±70 mm³. In the cisplatin group, it was 470±55 mm³. Tumor volume dynamics and final values are presented in **Table 1** and **Figure 2**.

**Table 1.** Tumor incidence and neoplasm volume at week 8 of treatment

| Group | Group characteristics | Tumor incidence, % | Mean tumor volume, mm³ (M±m) | Growth inhibition, % |
|---|---|---|---|---|
| **Group 1** | Intact control | 0 | - | - |
| **Group 2** | Negative control (placebo) | 100 | 1850 ± 210 | - |
| **Group 3** | Turmeric extract | 80 | 880 ± 95* | 52.4 |
| **Group 4** | Green tea extract | 80 | 980 ± 105* | 47.0 |
| **Group 5** | Black cumin extract | 70 | 650 ± 70* | 64.9 |
| **Group 6** | Positive control (cisplatin) | 60 | 470 ± 55* | 74.6 |

*Note: differences from Group 2 are statistically significant at $p<0.05$



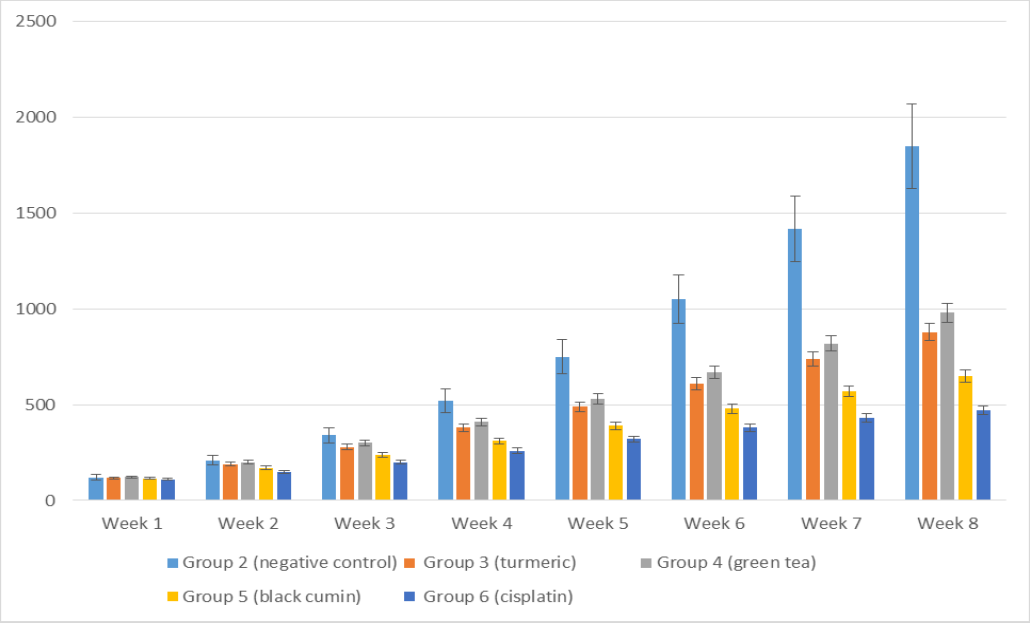



**Figure 2.** Dynamics of tumor volume changes during 8 weeks of treatment

The graph based on the obtained data shows steady tumor growth in the negative control group and a significant slowdown of tumor growth in all experimental groups. Among the plant extracts, Group 5 (black cumin) showed the best result, with its curve most closely approaching that of Group 6 (cisplatin).

*Assessment of systemic effects on the organism*

Throughout the experiment, animals in all groups except those receiving cisplatin maintained satisfactory general condition, normal motor activity, and appetite. In the positive control group (cisplatin), 4 out of 10 rats showed reduced food consumption and slight body weight loss by the end of week 3 of treatment. This represents an expected side effect of standard chemotherapy. Body weight dynamics are presented in **Figure 3**.

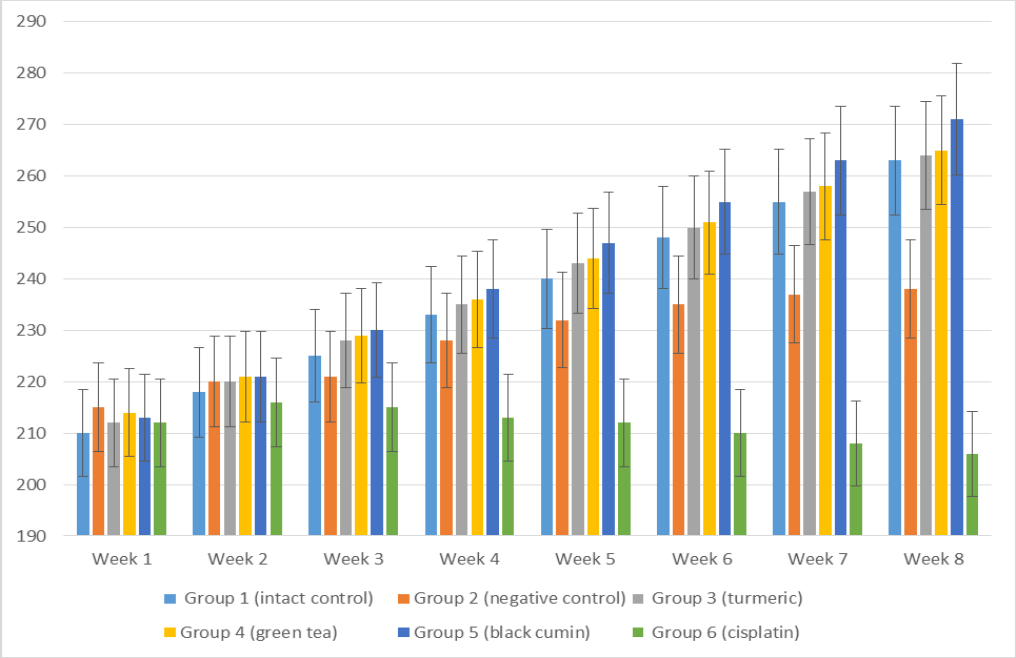



**Figure 3.** Body weight dynamics of animals during the experiment (M±m), grams

The data show that animals receiving plant extracts, particularly black cumin, demonstrated stable body weight gain comparable to the intact control group. In contrast, the cisplatin group showed progressive body weight loss starting from week 3 of treatment, indicating systemic toxicity of this drug.

*Blood biochemical parameters*

At the end of the experiment, blood biochemical analysis was performed to assess the functional state of the liver and kidneys. The results are presented in **Table 2**.

**Table 2.** Blood biochemical parameters at the end of the experiment (M±m)

| Parameter | Group 1 (intact) | Group 2 (negative) | Group 3 (turmeric) | Group 4 (green tea) | Group 5 (black cumin) | Group 6 (cisplatin) |
|---|---|---|---|---|---|---|
| **ALT, U/L** | 42.3 ± 4.1 | 45.6 ± 5.2 | 44.1 ± 4.8 | 43.8 ± 5.0 | 43.2 ± 4.5 | 78.4 ± 8.9* |
| **AST, U/L** | 86.5 ± 7.3 | 90.2 ± 8.1 | 88.7 ± 7.6 | 87.9 ± 7.4 | 87.1 ± 6.9 | 152.3 ± 14.2* |
| **Alkaline phosphatase, U/L** | 156.2 ± 14.5 | 168.4 ± 15.8 | 160.3 ± 14.2 | 162.7 ± 15.1 | 158.9 ± 13.8 | 245.6 ± 22.1* |
| **Total bilirubin, µmol/L** | 5.2 ± 0.6 | 5.8 ± 0.7 | 5.4 ± 0.6 | 5.5 ± 0.6 | 5.3 ± 0.5 | 9.7 ± 1.1* |
| **Creatinine, µmol/L** | 44.6 ± 4.2 | 46.3 ± 4.8 | 45.2 ± 4.3 | 44.9 ± 4.1 | 44.1 ± 3.9 | 98.5 ± 9.3* |
| **Urea, mmol/L** | 5.8 ± 0.5 | 6.1 ± 0.6 | 5.9 ± 0.5 | 6.0 ± 0.6 | 5.7 ± 0.5 | 12.4 ± 1.3* |

*Note: differences from Group 1 are statistically significant at $p<0.05$

Analysis of biochemical parameters revealed no statistically significant differences between groups receiving plant extracts and the intact control. All values remained within physiological norms. In the cisplatin group, significant increases in liver enzymes (ALT, AST) and renal function markers (creatinine,

urea) were observed, indicating the development of hepato- and nephrotoxicity characteristic of this chemotherapeutic agent.

*Histological and immunohistochemical examination*

Microscopic examination of buccal mucosa tissues in the intact control group revealed a normal structure of stratified squamous epithelium with no signs of dysplasia, inflammation, or tumor growth. In the negative control (placebo) group, all animals showed invasive tumors with pronounced cellular atypia, numerous pathological mitoses, foci of keratinization, and infiltration of underlying tissues, consistent with squamous cell carcinoma. In groups receiving plant extracts, the histological picture was more favorable. The least degree of atypia and the fewest pathological mitoses were observed in the black cumin group, in which some animals had no tumors, and the remaining neoplasms showed areas of necrosis and fibrosis, indicating tumor tissue regression.

For objective assessment of proliferative activity and apoptosis, immunohistochemical staining with antibodies to Ki-67 and caspase-3 was performed. The results are presented in **Table 3**.

**Table 3.** Expression of proliferation and apoptosis markers in tumor tissue (M±m)

| Group | Ki-67, % positive nuclei | Caspase-3, % positive cells |
|---|---|---|
| **Group 2 (negative control)** | 68.3 ± 5.2 | 8.2 ± 1.1 |
| **Group 3 (turmeric)** | 42.1 ± 3.8* | 21.4 ± 2.3* |
| **Group 4 (green tea)** | 45.6 ± 4.1* | 19.7 ± 2.0* |
| **Group 5 (black cumin)** | 31.4 ± 2.9* | 34.2 ± 3.1* |
| **Group 6 (cisplatin)** | 28.7 ± 2.6* | 38.5 ± 3.5* |

*Note: differences from Group 2 are statistically significant at $p<0.05$

The obtained data clearly demonstrate that the negative control group showed high proliferation and low apoptosis. All experimental groups showed decreased Ki-67 and increased caspase-3 expression. The most balanced ratio of proliferation suppression to apoptosis enhancement was observed in Group 5 (black cumin), approaching the values of Group 6 (cisplatin). These findings indicate that the antitumor effect of the studied extracts is achieved through both suppression of tumor cell proliferation and activation of apoptotic mechanisms.

*Correlation analysis*

Correlation analysis between tumor volume and immunohistochemical parameters was performed. A strong positive correlation was found between tumor volume and Ki-67 expression ($r=0.82$, $p<0.05$). A strong negative correlation was observed between tumor volume and caspase-3 expression ($r = -0.79$, $p < 0.05$). These findings confirm the pathogenetic significance of the studied mechanisms in tumor development and progression.

This study provided a comparative evaluation of the antitumor activity of three plant extracts (turmeric (*Curcuma longa*), green tea (*Camellia sinensis*), and black cumin (*Nigella sativa*)) in a rat model of 7,12-dimethylbenzanthracene-induced oral squamous cell carcinoma. The obtained results indicate that all studied extracts possess significant antitumor activity, although the magnitude of the effect varied considerably among the plants.

Black cumin extract demonstrated the highest efficacy. In animals receiving this extract, tumor incidence decreased to 70% compared to 100% in the negative control group. Mean tumor volume decreased 2.8-fold, reaching 650 mm³ versus 1850 mm³ in controls. The tumor growth inhibition rate reached 64.9%, approaching that of cisplatin (74.6%), the reference drug. Importantly, unlike cisplatin, black cumin extract did not cause toxic effects. Animals maintained normal body weight, appetite, and motor activity, while biochemical parameters of liver and kidney function did not differ from intact controls.

These findings align with numerous studies confirming the high antitumor potential of thymoquinone, the main bioactive component of *Nigella sativa*. The scientific literature emphasizes thymoquinone's unique ability to induce apoptosis and autophagy in cancer cells through activation of p53-dependent signaling pathways while exhibiting selective cytotoxicity and causing minimal damage to normal cells [43-46]. Our immunohistochemical data fully support this mechanism. The black cumin group showed the most significant reduction in Ki-67 proliferation marker expression (to 31.4% vs. 68.3% in controls) and the greatest increase in caspase-3 apoptosis marker expression (to 34.2% vs. 8.2% in controls) among all plant extracts. The strong negative correlation between tumor volume and caspase-3 levels further confirms that apoptosis activation represents the key mechanism underlying the antitumor effect.

Turmeric and green tea extracts also exhibited moderate antitumor activity, reducing tumor incidence to 80% and decreasing tumor volume by 2.1-fold and 1.9-fold, respectively. The tumor growth inhibition rates were 52.4% for turmeric and 47.0% for green tea.

Immunohistochemical analysis showed significant reductions in proliferation and increases in apoptosis in both groups, although these changes were less pronounced than in the black cumin group. These results are consistent with the current understanding of these plants' mechanisms of action. Curcumin, the active component of turmeric, has been shown to modulate signaling pathways involved in inflammation and carcinogenesis [47-49]. Green tea polyphenols, particularly epigallocatechin-3-gallate, have shown the capacity to inhibit angiogenesis and influence epigenetic regulation [50-52].

All three plant extracts demonstrated a favorable safety profile. Unlike cisplatin, which caused characteristic chemotherapy side effects including body weight loss, elevated liver enzymes, and increased renal function markers, none of the plant extracts produced statistically significant deviations in biochemical parameters from physiological norms. Moreover, the black cumin group showed greater body weight gain than intact controls, potentially indicating an additional health-promoting effect. This observation aligns with the literature, which suggests that Nigella sativa possesses immunomodulatory and antioxidant properties that contribute to improved overall health [53, 54].

The comparison of plant extract efficacy with the reference drug cisplatin deserves particular attention. Although none of the extracts surpassed cisplatin in antitumor activity, their complete absence of toxicity makes them promising candidates for long-term prevention or supportive therapy. Furthermore, given the well-known problem of cisplatin resistance in head and neck squamous cell carcinoma, combining low doses of this chemotherapeutic agent with plant extracts may represent a strategy to reduce toxicity and overcome drug resistance. Our data on the different yet complementary mechanisms of action of the studied extracts provide a theoretical basis for investigating their combinations in future studies.

The selected DMBA-induced oral cancer model in rats proved fully adequate. Tumor incidence in the negative control group reached 100%, and the histological picture corresponded to squamous cell carcinoma with signs of invasive growth and cellular atypia. This confirms the model's validity and the correctness of the obtained results. The model's reproducibility aligns with long-term research data confirming the effectiveness of 0.5% DMBA solution for inducing squamous cell carcinoma of the buccal mucosa in rodents.

This study has several limitations that should be considered when interpreting the results. The chemically induced carcinogenesis model, although standard for preclinical studies, does not fully replicate spontaneous human cancer, which develops through complex interactions of genetic, epigenetic, and environmental factors. The extracts were administered intragastrically, and we did not assess their bioavailability or actual concentration of active components in buccal mucosa tissue. For curcumin, for example, low bioavailability is well known, and using more bioavailable forms might enhance the observed effect. The 8-week treatment duration does not allow conclusions about long-term outcomes or potential relapses after therapy discontinuation.

Future research directions include studying the combined use of the extracts and low-dose cisplatin to evaluate possible synergism and reduce standard chemotherapy toxicity. Pharmacokinetic studies are needed to determine optimal doses and administration regimens, ensuring maximum concentration of active components in target tissues. Given the favorable safety profile, particularly of black cumin, investigating these extracts as chemopreventive agents in patients with precancerous oral mucosal conditions represents a promising avenue.

## Conclusion



This study provided a comparative evaluation of the antitumor activity of three plant extracts (turmeric (*Curcuma longa*), green tea (*Camellia sinensis*), and black cumin (*Nigella sativa*)) in a rat model of 7,12-dimethylbenzanthracene-induced oral squamous cell carcinoma.

All studied plant extracts demonstrated significant antitumor activity. Extract administration resulted in reduced tumor incidence, decreased mean tumor volume, suppressed tumor cell proliferative activity, and enhanced apoptosis. When ranked by antitumor effect magnitude, black cumin extract showed the highest activity, while turmeric and green tea extracts exhibited moderate effects.

In animals receiving black cumin extract, tumor incidence decreased to 70%, tumor volume decreased 2.8-fold, and the tumor growth inhibition rate reached 64.9%, approaching the values of the reference drug cisplatin. Immunohistochemical analysis confirmed that the mechanism of action involves proliferation suppression and apoptosis induction: the black cumin group showed decreased Ki-67 expression to 31.4% and increased caspase-3 expression to 34.2%, comparable to those of cisplatin.

A critically important finding is the confirmation of a high safety profile for all studied plant extracts. Unlike cisplatin, which caused characteristic toxic effects

including body weight loss and elevated hepatic and renal markers, none of the plant extracts produced statistically significant deviations in biochemical parameters from physiological norms. Animals receiving plant extracts maintained normal motor activity and appetite, and showed stable body weight gain throughout the experiment.

The identified differences in the mechanisms of action of the studied extracts provide a theoretical foundation for investigating their combinations in future research. The practical significance of this work lies in justifying the promise of further investigation of plant extracts, particularly black cumin, as potential agents for oral squamous cell carcinoma prevention and treatment. The combination of proven antitumor efficacy with the absence of toxicity makes these extracts especially attractive for long-term use in patients with precancerous oral mucosal conditions and as adjuvant agents during standard chemotherapy [55-58].

Future studies should focus on investigating the pharmacokinetics of active components, evaluating the effectiveness of combined extract use with each other and with low-dose cisplatin, and examining long-term therapy outcomes and the potential of these extracts for preventing relapse after surgical treatment and radiation therapy [59-64].

Thus, the study achieved its aim. The obtained results contribute to understanding the antitumor properties of the studied plants and justify the feasibility of their further preclinical and clinical investigation as agents for oral squamous cell carcinoma prevention and treatment.

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**Conflict of Interest:** None

**Financial Support:** None

**Ethics Statement:** All animals were housed under standard vivarium conditions with a 12/12-hour light/dark cycle and had free access to water and food. All procedures were approved by the Institutional Ethical Committee and fully complied with international guidelines for the humane treatment of laboratory animals, including the ARRIVE principles and Directive 2010/63/EU.

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