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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION OF LYCOPENE'S PREVENTIVE EFFECT ON SODIUM NITRITE-INDUCED TONGUE CANCER IN ALBINO RATS

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ABSTRACT

Background: Sodium nitrite is a popular food ingredient used to preserve processed meat. It is still in use today even though it is classified as a potential human carcinogen. Lycopene is a carotenoid with potent anti-inflammatory, anti-cancer, and antioxidant qualities.

Aim: The purpose of this study was to determine whether giving sodium nitrite to albino rats may cause cancer. It also looked at the potential preventive impact of lycopene against this effect.

Material and Methods: Four groups of adult male albino rats were randomly assigned. Macroscopic inspection, histological analysis, immunohistochemical assay, PCR for Cadherin 1 gene expression, and statistical analysis were all used to determine the impact of lycopene on tongue cancer caused by sodium nitrite in albino rats.

Results: The results indicated that group II had put on weight, group IV had almost reached the same weight as the control group, and group III had lost weight considerably. Histopathologically, the oral mucosa of groups I and II was normal, while group III met the requirements for dysplastic criteria, and group IV showed less modification. In terms of immunohistochemical analysis, group III showed weak cellular adhesion while groups I, II, and IV displayed strong levels. Group III displayed a decrease in the expression of the cadherin 1 gene when compared to the other groups.

Conclusion: In albino rats, lycopene offered protection against tongue cancer induced by sodium nitrite.

KEYWORDS: Sodium Nitrite, Lycopene, Cancer, Albino Rats.

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INTRODUCTION

Food additives are compounds, either synthetic or naturally occurring, that are added to food to preserve its color, quality, and organoleptic qualities[1]. Since some of the components in food additives may be harmful to consumers' health, strict food safety regulations are required when utilizing them [2].

Sodium nitrite is a white to slightly yellow crystalline powder with the chemical formula $NaNO_2$. It is used as a preservative, color fixative, and antibacterial in fish and meats. The European Union restricts the marketing of sodium nitrite only when it is labeled "for food use" or in conjunction with salt (NaCl) [3].

Sodium nitrite and other digestive system constituents combine to form N-nitroso compounds (NOCs), which are thought to be potential human carcinogens and have been demonstrated to cause cancer in a number of animal species [4].

These compounds induce oxidative stress, inflammation, and carcinogenesis. These dangerous chemical byproducts can originate from $NaNO_2$ in an acidic stomach or at high temperatures while overcooking or scorching meat [5].

Sodium nitrite has the potential to be both a pro-oxidant and pro-carcinogen. It can increase hydrogen peroxide levels, lipid peroxidation, protein oxidation, DNA damage, and DNA-protein crosslinking in a dose-dependent manner [6].

Today, there is a worrisome rise in the consumption of $NaNO_2$ above the daily non-toxic recommended dosage, particularly in high-risk groups such as newborns and children [5].

Oxidative stress causes DNA damage and tissue harm, which is associated with the development of cancer. Antioxidants have been shown in several studies to prevent oxidative damage and reduce aberrant cell proliferation and therefore preventing cancer [7].

Lycopene is a tetraterpene molecule that is prevalent in tomatoes and tomato-based products. It is a member of the carotenoids. It is regarded widely as a strong antioxidant .Studies have shown that lycopene effectively reduces the risk of cancer recurrence, oxidative stress-related dysfunctions and inflammation issues [8].

Lycopene induces apoptosis and/or the arrest of cell cycle, so it has direct anticancer properties. However, the preventive role of lycopene against cancer is likely based on its excellent antioxidant property [9].

Lycopene possesses the ability to quench singlet oxygen and is a potent antioxidant, by decreasing the degree of lipid peroxidation and increasing the activity of glutathione-dependent enzymes, so it decreases the oxidative damage and has anticancer effect [7].

Lycopene may have anti-proliferative effects because it decreases the S phase and induces the G0 - G1 phase, which results in cell cycle arrest. Additionally, lycopene can prevent cancer by correcting the imbalance between apoptosis and cell growth and blocking p53-dependent apoptosis [7].

The anti-inflammatory effect of lycopene helps in preventing the growth and spread of cancer. Additionally, lycopene inhibits angiogenesis, cell invasion, and metastasis [9]. Lycopene has an anticancer effect on oral cancer by inhibiting cell proliferation, migration, invasion and apoptosis [10].

Angiogenesis, tumorigenicity, and the spread of cancer may all be associated with the insulin-like growth factor 1 (IGF1) pathway, which is a crucial regulatory signal pathway in the tumor microenvironment. It was found that the lethal effects of lycopene therapy on oral squamous cell carcinoma (OSCC) are related to its regulatory role on IGF1 pathway [11].

The symptoms of leukoplakia, oral lichen planus, and oral submucous fibrosis can all be successfully alleviated with lycopene [12].

The cadherins, a family of transmembrane glycoproteins, have drawn a lot of attention. E-cadherin, a member of the cadherin superfamily, is a calcium-regulated transmembrane glycoprotein that is necessary for epithelial cell-cell attachment [13].

MATERIAL AND METHODS

Material

The Ethical Declaration:

The study's experiments were all conducted in compliance with the Ethical Guidelines of the Research Ethics Committee of the Faculty of Dentistry, Minia University, Egypt, with approval number 775 on 25th July 2023.

Experimental Animals and Care

This investigation involved fifty adult male albino rats weighing an average of 200 gm. The animals were divided into four groups after being taken from Minia University's animal house.

Each group was housed individually in cages. They were housed in suitable conditions and fed a stock diet. The drinking water was changed daily. Room temperature was maintained at 22 ± 3 ºC, with a 12-hour light/dark cycle and a 50–60% relative humidity.

Following the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals, all experiments were carried out according to the research protocol approved by the Animal Care Committee of the National Study Center, Egypt.

The Used Chemicals

A. Sodium Nitrite

It is a white crystalline powder with a chemical formula of $NaNO_2$ that is highly dissolved in water. It was purchased from Merck KGaA (Sigma-Aldrich), Darmstadt, Germany.

B. Lycopene

It was obtained in the form of soft gel capsules: each capsule contains 40 mg of lycopene from natural tomato extract. It was dissolved in olive oil to increase its absorption. It was manufactured and purchased from Puritan's Pride Inc, NY, USA.

Experimental Design

Sample Size Calculations

The minimum sample size was calculated using G power 3.1 9.2 software. Based on a priori analysis for one way ANOVA with an effect size 0.45 according to data obtained from a pilot study. The required number of albino rats was 50 to provide 80% power at the level of 5% significance.

The fifty albino rats were randomly divided into four groups. Each group was composed of fifteen albino rats, except for the control group, which was composed only of five rats to avoid the unnecessary sacrifice of the rats, following the 3 Rs principle [14].

National and international regulations that govern animal experimentation in research are founded on the principle of 3 Rs: reduction, refinement, and replacement. Reduction refers to decreasing the number of animals required in order to obtain reliable data and significant results [15].

Group I (Control Group): Five albino rats did not receive any treatment.

Group II (Lycopene Group): Fifteen albino rats were given 10 mg/kg of lycopene.

Group III (Sodium Nitrite Group): Fifteen albino rats were given 30 mg/kg sodium nitrite dissolved in distilled water.

Group IV (Lycopene and Sodium Nitrite Group): Fifteen albino rats were given both lycopene and sodium nitrite at the same doses and duration as in groups II and III, respectively.

Both Lycopene and sodium nitrite were administrated orally by gastric tube, daily for 2

months. Albino rats were sacrificed under anesthesia following the concept of euthanasia at the end of the experiment.

Methods

1. Clinical Evaluation

A. Assessment of the Weight of the Albino Rats

A digital scale was used to weigh the albino rats both before and after the agents were administered.

B. Gross Morphology Assessment of the Albino Rat Tongues

The tongues were carefully inspected with the naked eye to look for any changes in size, consistency, color, shape, or any other abnormalities.

2. Biopsy Collection

The albino rats from each group were sacrificed after two months. Then specimens containing the lesions were dissected from each albino rat and each specimen was obtained and immediately cleaned in a 0.9% saline solution.

Each specimen was divided into two sections: the first was kept as a fresh specimen to be utilized for RNA extraction for PCR testing, and the second was immediately processed and embedded in paraffin for staining operations by immersing it in 10% formalin solution for 24 hours. Each block was divided into two sets of four micron-thick portions for histological and immunohistochemical assessment.

3. Microscopic Examination Using Hematoxylin and Eosin Stains

After obtaining biopsy specimens, they were immediately preserved in 10% neutral buffered formalin. The samples were thoroughly cleaned under running water, dehydrated with ascending concentrations of alcohol, and then placed in xylene to remove the remaining alcohol.

A melted soft paraffin wax was infiltrated into the tissues to replace the xylene then embedded in paraffin wax. Sections from paraffin blocks were cut to a thickness of 4-5 microns, mounted on glass slides, and stained with hematoxylin and eosin stain.

4. Immunohistochemical Procedure Using E-cadherin Immunomarker

The peroxidase-labelled streptavidin biotin technique was used for immunohistochemical staining. The technique started with deparaffinization of the sections, then rehydration using xylene and descending alcohol grades. Antigen retrieval was done in a microwave using a 10-mM citrate buffer (pH 6.0) at high and low power for 15 min and 10 min respectively.

To inhibit endogenous peroxidase activity, slices were covered with 4% hydrogen peroxide for 30 minutes. Furthermore, the slides were incubated with the primary anti-E-cadherin monoclonal antibody (Biogenex Life Sciences Private Limited, CA, USA, 6 ml, ready to use), then with secondary-linking antibody (biotinylated anti-immunoglobulins/ super-enhancer).

After that, they were treated with a prediluted secondary antibody (enzyme-conjugated streptavidin). The diaminobenzidine chromogen was then added, and Mayer's hematoxylin was used as a counterstain.

5. Image Analysis Procedures

The area fraction of E-cadherin immunoreactivity was calculated using Image J software (Image J, 1.54h, NIH, USA). The area fraction was measured for fifteen fields in groups II, III, and IV, and five fields in group I. using a 200x magnification light microscope and a monitored screen.

Brown regions exhibiting E-cadherin immunoreactivity were selected for measurement; the software then measured these regions after masking them with the red binary color.

6. Evaluation of the Cadherin 1 Gene by Real-Time Polymerase Chain Reaction

The human gene Cadherin 1 (CDH1) encodes e-cadherin, which is located on chromosome 16q22. In order to establish a correlation between the gene encoding the E-cadherin protein and its expression on the tongue's dorsal surface, the CDH1 gene was measured using a quantative real-time polymerase chain reaction (PCR) [16].

Total RNA was isolated from samples using TRI Reagent as a primer to determine the relative quantities of the CDH1 gene. Using real-time PCR, the exonic region of the E-cadherin gene primers were utilized to amplify the E-cadherin molecules found in the samples.

The used kit was GoTaq® 1-Step RT-qPCR system, whose reagents were purchased from PROMEGA life sciences (Madison, Wisconsin, USA).

7. Statistical Analysis

The statistical software IBM® SPSS® (ver. 27. SPSS Inc., IBM Corporation, Armonk, NY, USA) was used for data entry and analysis.

The mean and standard deviation were used to present the quantitative data. The significant differences between the various groups were compared using the One-way Analysis of Variance (ANOVA) test and the Bonferroni post hoc test. ***P-***value ≤ 0.05 was considered as the statistically significant level.

RESULTS

1. Clinical Evaluation

A. Assessment of the Weight of the Albino Rats

Following a two-month study period, the albino rats in groups I and II experienced weight gain, however group III exhibited a noteworthy loss in weight relative to the other groups. Group IV's albino rats were nearly identical in weight to group I's.

B. Gross Morphology Assessment of Albino Rat Tongues

As can be seen in figures 1(A) and (B), respectively, the dissected tongue specimens from groups I and II had normal morphological gross appearances. In contrast, Group III showed conspicuous ulcerations, inflammation, and congestion, as seen in figure 1(C).

Figure 1 (D) showed that while group IV's tongues were substantially better than group III's, the anterior portion of the tongues still showed some slight inflammation but no ulcerations.

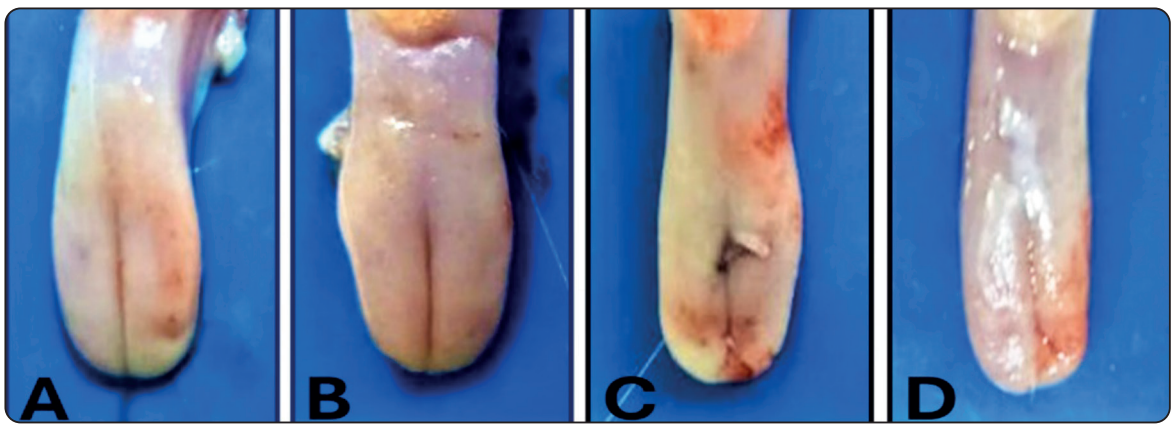

Fig. (1) A macroscopic analysis of the tongues' dorsal surfaces exhibiting: (A) Group I's tongue normal appearance, (B) Group II's tongue was identical to that of group I, (C) Group III demonstrated inflammation in addition to ulcerations on the dorsal surface of the tongue, and (D) The tongue's appearance improved in group IV.

2. Microscopic Examination Using Hematoxylin and Eosin Stains

The tongues of the control group were covered in many papillae, the most common of which were filiform papillae, which had conical and pointy points. As seen in figure 1 (A), the connective tissue papillae and epithelial rete pegs were regular.

Figure 2 (B) showed that group II's tongues resembled group I's, while group III's tongues revealed dysplastic and atrophic changes, as seen in figure 1 (C), including small, atrophied filiform papillae, and decreased epithelial layer thickness. The keratin layer was thin and fragmented, and nests of epithelial cells had penetrated the connective tissue.

Pleomorphism, hyperchromatism, an elevated nuclear to cytoplasmic ratio, and large nucleoli were examples of abnormal mitotic patterns that were seen in figure 3 (A, and B). There was also a disruption of the basal cell layer and a decrease in cellular adhesion. Red blood cells (RBCs) and dilated blood arteries were also observed. Figure 1 (D) illustrated the minor atrophic changes in the epithelial tissue and the marginal reduction in the keratin layer in group IV.

3. Immunohistochemical Analysis Using E-cadherin

Brown membranous staining was used to detect E-cadherin (E-cad) immunoreactivity in different groups. Group I displayed a dark brown membranous staining of the basal and suprabasal layers of the epithelium, except for the superficial layer, as shown in figure 4 (A).

As depicted in figure 4 (B), group II had the same appearance as group I. Up to half of the

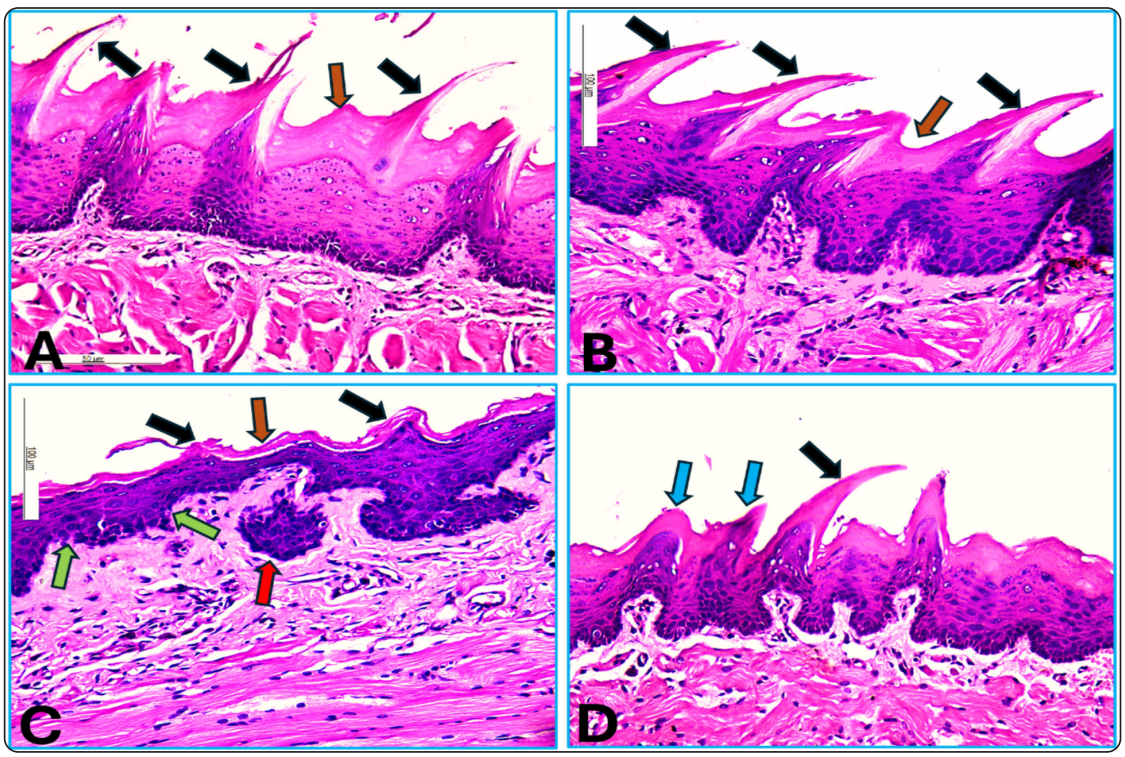

Fig. (2) A photomicrograph of sections stained with hematoxylin and eosin displaying: (A, and B) Groups I and II had conical-shaped papillae (black arrows) and were covered with a thick layer of keratin (brown arrow), (C) Group III had short atrophied papillae (black arrows), a thin keratin layer (brown arrow), invasion of masses of epithelial cells with signs of cellular atypia (red arrow), and breakdown of basal cell adhesion (green arrows), and (D) Group IV exhibited pointy points papillae (black arrow), and papillae were shorter with blunted tips (blue arrows) (200x magnification).

thickness of the epithelial cell membrane in Group III was stained a moderate shade of brown. Figure 4 (C) illustrated the decreased E-cad membranous staining intensity observed in basilar hyperplasia and invading cell nests. Like group I, Group IV's basal and suprabasal layers exhibited significant membranous brown staining, as seen in Figure 4 (D).

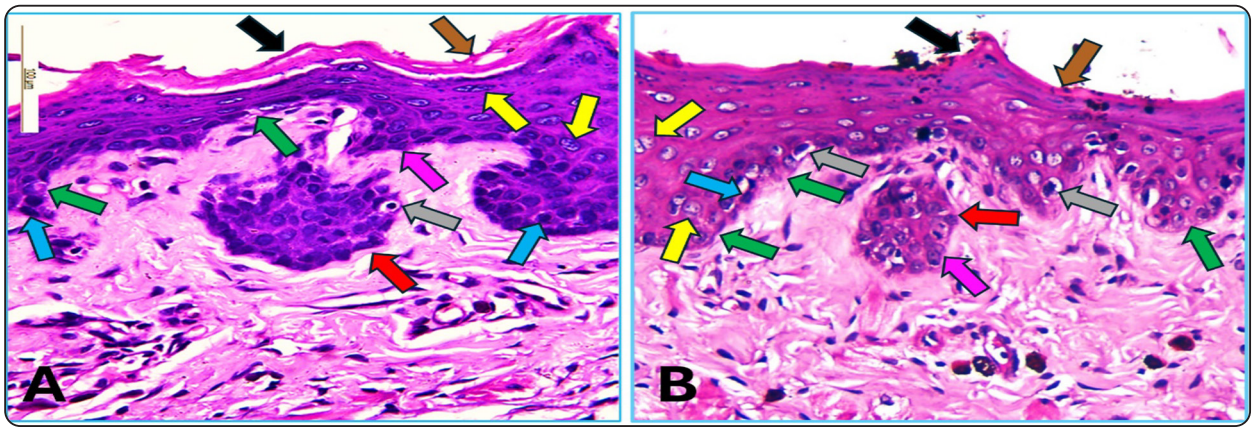

Fig. (3) A photomicrograph of the hematoxylin and eosin stained sections showing: (A, and B) Atrophied filiform papillae (black arrows) covered by thin and detached keratin layer (brown arrows), epithelial cell nest invading the C.T (red arrows), disruption and loss of adhesion of the basal cell layer (green arrows), prominent nucleoli (grey arrows), hyperchromatic nuclei (blue arrows), nuclear pleomorphism (pink arrows), abnormal mitotic figure (yellow arrows) (400x magnification).

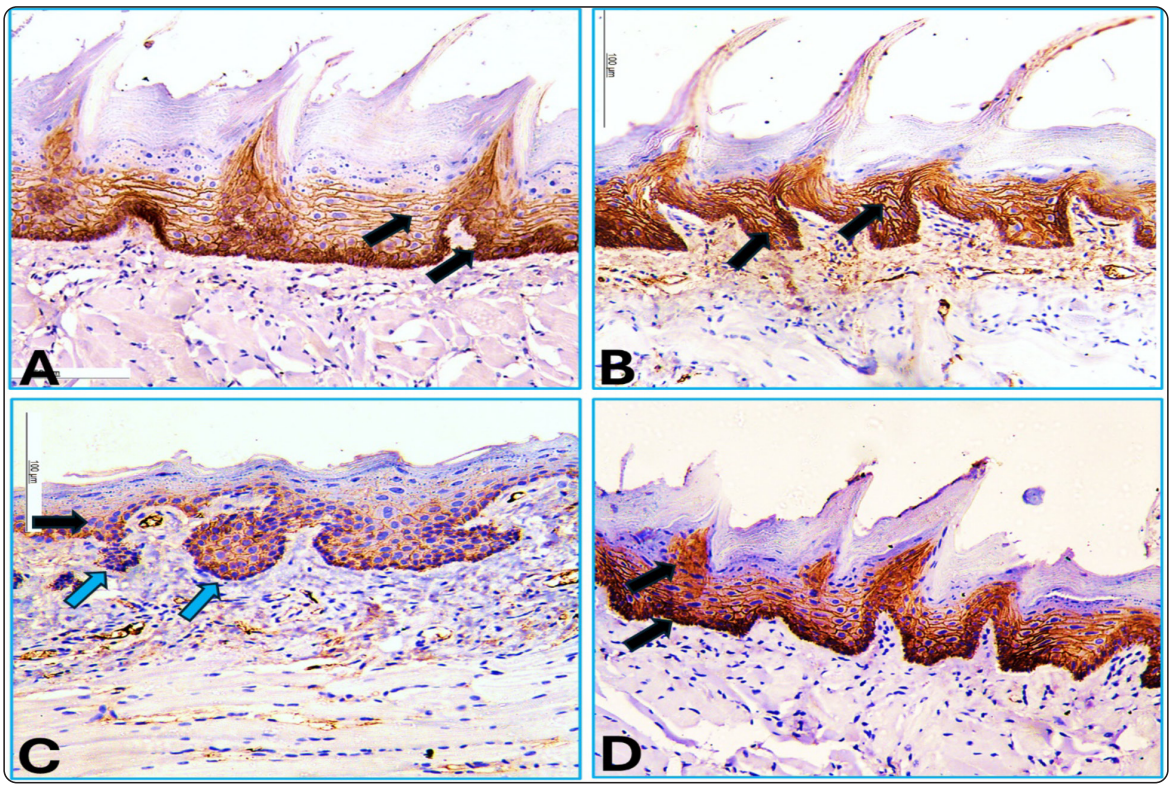

Fig. (4) A photomicrograph of E-cadherin expression showing: (A, and B) Groups I and II had high membranous immunostaining in the basal and suprabasal epithelium layers (black arrows), (C) Group III displayed weak immunostaining (black arrow) and the nests of epithelial cell invade the connective tissue (blue arrow), and (D) Group IV exhibited strong immunostaining of the lower two-thirds of the epithelium (black arrows) (200x magnification).

4. Evaluation of the Cadherin 1 Gene by Real-Time Polymerase Chain Reaction

RT-PCR investigation was made on 3 tongue samples from each group and indicated that the levels of Cadherin 1 (CDH1) gene expression in groups I and II were almost equal. Table 1 showed that, in contrast to the previous groups, Group III's number was lower and Group IV's number was higher.

5. Statistical Analysis

In comparison to group I, groups II and III a statistically significant difference with ***P-***values less than 0.05 in both groups (***P-***values were 0.001, 0.001 respectively). Group IV showed an insignificant difference (***P-***value was 1) when compared with group I, as shown in tables 2 and 3.

TABLE (1) Polymerase chain reaction in real time, quantitative (qRT-PCR) in the various categories.

Sample	GAPDH	DNTT	ΔCT	ΔΔCT	2^ΔΔCT	Fold Change
Group I	16.8	29.6	12.8	0.38	0.00995033	1.01
Group II	17.5	29.54	12.04	-0.38	0.19062036	1.21
Group III	16.3	22.5	6.2	-6.22	-0.8675006	0.42
Group IV	16.1	23.2	7.1	-5.32	-0.0943107	0.91

TABLE (2) An analysis of variance test conducted on the average E-CAD ± standard deviation for multiple groups.

Groups	Number	E-cad		F	*P*-value
		Mean±SD	95% CI		
Control G.	5	186.25±13.4shown6	178.79-193.71	70.049	**<0.001**
Lycopene G.	15	217.53±10.53	211.70-223.36		
Sodium Nitrite G.	15	147.34±9.08	142.32-152.37		
Combination G.	15	192.03±18.60	181.73-202.33		

TABLE (3) Bonferroni post hoc test for comparing the various groups.

Groups		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control G.	Lycopene G.	-31.281*	4.901	0.001*	-44.68	-17.87
	Sodium Nitrite G.	38.902*	4.901	0.001*	25.49	52.31
	Combination G.	-5.778	4.901	1.00	-19.18	7.62
Lycopene G.	Sodium Nitrite G.	70.183*	4.901	0.001*	56.77	83.59
	Combination G.	25.503*	4.901	0.001*	12.09	38.91
Sodium Nitrite G.	Combination G.	-44.680*	4.901	0.001*	-58.08	-31.27

(*) The mean difference is significant at the 0.05 level.

DISCUSSION

Oral cancer was regarded as a global health concern with over 300,000 newly diagnosed cases and 177,000 deaths globally, particularly in less developed nations without access to diagnostic or therapeutic facilities. Oral cancer ranked 20th in cancer-related deaths and 21st in newly diagnosed cases in Egypt [17].

Among cancers of the oral cavity, tongue cancer was the most prevalent and aggressive type with approximately 354,864 cases annually in incidence and 177,384 cases in mortality [18].

Tobacco, alcohol, betel quid, and chemicals like food additives were crucial contributors particularly in Africa and the Middle East [17].

Atypical mitotic figures, increased nucleus: cytoplasm ratio, increased number and size of nucleoli, nuclear hyperchromatism, and nuclear pleomorphism were among the criteria of epithelial dysplasia observed in tongue squamous cell carcinoma[19].

Several therapeutic techniques had been developed for TSCC treatment, including chemotherapy, radiation therapy, and surgery. Recently, the use of natural compounds as antioxidants had been used to avoid the drawbacks of the other treatment options [20].

Humans used sodium nitrite ($NaNO_2$) mostly as a food preservative in processed and cured meats. Dietary nitrite sources can be hazardous, and epidemiological research has related nitrite levels in food to cancer [21]. Human overexposure mostly happened because of contaminated food or water [22].

It initially had effect on the gastrointestinal tract before entering the bloodstream and reaching multiple other organs, where it continues to cause toxicity and oxidative stress [22].

The correlation between $NaNO_2$ intake and harmful human effects was illustrated by ***Crowe, Elliott et al. 2019***, who investigated the relationship between processed meat consumption, that contained nitrite, and the colorectal cancer [23].

Through the production of reactive oxygen species (ROS), oxidative stress had been shown to be a significant mediator of nitrite-induced oxidative damage; antioxidant therapy could reduce or even reverse this action [22].

Antioxidants had become more popular as chemoprotective agents in recent times. Lycopene, a carotenoid found in tomatoes and watermelon, was a highly potent antioxidant. Lycopene exhibited anticancer properties against a variety of cancer types, such as breast, lung, and prostate cancer. Additionally, increasing the consumption of tomato products might reduce the incidence of oral cancer [10].

Lycopene had protective and curative roles, by providing a therapeutic nonsurgical aid in treating oral diseases such as lichen planus, OSCC, leukoplakia, and oral submucous fibrosis [24].

Albino rats had been used as animal models because of their many benefits, including small size, economical maintenance requirements, short lifespans, and easy access to a huge genetic resource pool [25].

In the current study, the impact of lycopene on tongue cancer caused by sodium nitrite in albino rats was evaluated using macroscopic inspection, histological analysis of the hematoxylin and eosin-stained sections, the epithelial cadherin immunostained sections, real-time PCR analysis for the Cadherin 1 gene expression, and statistical analysis.

First, after two months, group III's albino rats' body weight was macroscopically inspected and shown to have dropped in comparison to groups I and II, whereas group IV's body weight increased to a level nearly comparable to the control group. Group III's cancer-related tongue ulcerations contributed to their lower body weight, which made feeding more challenging.

This result was in line with the findings of ***Helal, El-Sayed et al. (2017)***, who reported that rats given 0.1 mg/kg of NaNO2 for a month had considerable decrease in body weight, which might be related to the reduction of food utilization or due to increased catabolic processes in the body [26].

The body weight of group II was higher than that of control I. This was demonstrated by ***Mezbani, Kavan et al. 2019***, who reported that 42 days of conventional grower diet supplemented with 100 mg/kg of lycopene had increased the growth performance of broiler chickens. They linked the enhanced dietary intake and the antioxidant impact of lycopene to the improvement in performance [27].

Macroscopically, the tongues' dorsal surfaces showed that both the control and lycopene groups' appearances were normal. Along with noticeable congestion and inflammation, group III also showed ulcerations on the dorsal surface of the anterior two thirds of the tongue. Group IV had improved tongue appearance; there was some irritation but with no ulceration.

Second, histological examination of hematoxylin and eosin-stained sections of tongue specimens in rats treated with $NaNO_2$ revealed dysplastic changes occurred in the epithelium and ended with the invasion of epithelial cell nests into the underlying connective tissue, in comparison with the group I and II. Group IV showed only some histological changes but without any invasion.

This epithelial invasion indicated cancer formation and its mechanism was illustrated *by* ***Clark and Vignjevic 2015***, who suggested that the stromal and tumor cells, which were separated by an intact basement membrane, were interacting with one another, causing activation of the stroma, so the randomly arranged collagen bundles became denser and formed a track perpendicular to the BM, which facilitated the passage of tumor cells from the BM into the stroma, which leaded to cancer formation[28].

After 2 months of treating the rats in group III with sodium nitrite, the signs of epithelial atrophy and dysplasia were observed, which came in co-ordinance with ***Moubarak, Essawy et al. 2020***, which included nuclear pleomorphism, nuclear hyperchromatism, increased nuclear to cytoplasmic ratio, abnormal mitotic figures, basilar hyperplasia and loss of cellular adhesion between the cells and finally invasion of the epithelial cells into the underlying connective tissue [29].

Atrophy of the filiform papillae in rats treated with $NaNO_2$ was also observed at the end of the study. They also showed detached keratin with decreased thickness of the epithelial layer. Edema, spacing, vacuolation and fibrosis in the connective tissue were also observed in the $NaNO_2$ group. This edema caused an increased infiltration of the inflammatory cells, resulting in inflammation and fibrosis.

This chronic inflammation was the main cause of cancer, this theory was supported by ***Aita and Mohammed 2014*****,** who treated rats with 30 mg/kg of $NaNO_2$ for 2 months [30], and by ***AlThanoon and Taha 2022***, in which pulmonary edema resulted from giving 120 mg/kg of NaNO2 to the rats [31].

Vascular changes were observed in the form of vasodilation of blood vessels (BVs) and congestion with RBCs. The same finding was reported by ***AlThanoon and Taha 2022*** [31] and also illustrated by ***Aita and Mohammed 2014*** who stated that there was a vasodilation in BVs present around bile ducts in rats treated with 30 mg/kg of $NaNO_2$ for 2 months via nitricoxide (NO) stimulation of the cyclic guanosine monophosphate (cGMP) signaling pathway in vascular smooth muscle cells [30].

The results of this study demonstrated that when lycopene mixed with sodium nitrite in group IV, the carcinogenic effect of the nitrite was reduced, and they exhibited only inflammation without ulcer formation. It also improved the growth performance of albino rats in group II when given alone.

Lycopene had been linked to preventing oral cancer, a finding proved by ***Shahi and Ahmadian 2018***. They illustrated that the administration of tomato and tomato paste had the potential to significantly lower the incidence of buccal pouch carcinogenesis by activating antioxidant enzymes, replenishing glutathione pools, and attenuating lipid peroxidation in hamsters [32]

This study's findings were consistent with those of the previous study made by ***Bahey and Elswaidy 2021***, who proved that a 10 mg/kg of lycopene co-administrated with a carcinogenic drug had prevented its damaging effect on the jejunal mucosa when given to the rats for 4 weeks [33].

Third, the expression of E-cadherin immunomarker revealed a significant decrease in the brown membranous pigmentation in group III, this finding proved that cell adhesion decreased in tumor cells, which was also proved by ***Xu, Lin et al. 2012***, who explained that the sodium nitrite improved the epithelial-mesenchymal transition of hepatoma cells by the generation of oxygen species in rats treated with graded doses of $NaNO_2$ (10, 20 and 30 mg/kg) [34].

This study also revealed that the E-cadherin brown staining increased in the lycopene group, which was also supported by ***Wang, Lu et al. 2020***, who approved that lycopene had anticancer effect on oral cancer by inhibiting the epithelial-mesenchymal transition and deactivating the PI3K/AKT/m-TOR signaling pathway through increasing the levels of E- cadherin, which in turn induced the apoptosis of oral cancer cells [10].

Fourth, PCR analysis of group III's Cadherin 1 (CDH1) gene expression showed a considerable decrease (0.42) in comparison to group I (1.01), but group IV (0.91) was still very close to group I. Group II demonstrated higher levels of CDH1 gene expression (1.21).

These outcomes were in consistent with ***El-Rouby 2011***, who proved that lycopene treatment dramatically reduced 4-NQO-induced tongue carcinogenesis, decreased PCNA-positive nuclei, and increased E-cadherin and β-catenin immunoexpression, when compared to the carcinogen group [35].

At last, statistical analysis demonstrated that there was a statistically significant difference between the sodium nitrite group and the control group (***P-*** value was 0.001). While group IV showed insignificant change in comparison to control group (***P-*** value was 1.00). Group II showed a significant change when compared to control one (***P-*** value was 0.01).

CONCLUSIONS

Sodium nitrite had a carcinogenic effect on the tongues of the albino rats. Lycopene significantly reduced the risk of tongue cancer when combined with sodium nitrite. Lycopene exhibited strong antioxidant and anti-carcinogenic characteristics.

Conflict of Interest

The authors have no financial conflicts of interest.

REFERENCES

1. Etwaroo DH, Goburdhun D, Ruggoo A. Classes of food additives in food products sold in Mauritius. British Food Journal. 2019;121(5):1125-39.
2. Martins FC, Sentanin MA, De Souza D. Analytical methods in food additives determination: Compounds with functional applications. Food chemistry. 2019;272:732-50.
3. Abdel-Reheim ES, Abdel-Hafeez H, Mahmoud BM, Abd-Allah EN. Effect of food additives (monosodium glutamate and sodium nitrite) on some biochemical parameters in albino rats. International Journal of bioassays. 2014;3(08):3260-73.
4. Chazelas E, Pierre F, Druesne-Pecollo N, Esseddik Y, Szabo de Edelenyi F, Agaesse C, et al. Nitrites and nitrates from food additives and natural sources and cancer risk: results from the NutriNet-Santé cohort. International journal of epidemiology. 2022;51(4):1106-19.
5. Elsherbini AM, Maysarah NM, El-Sherbiny M, Al-Gayyar MM, Elsherbiny NM. Glycyrrhizic acid ameliorates sodium nitrite-induced lung and salivary gland toxicity: Impact

on oxidative stress, inflammation and fibrosis. Human & Experimental Toxicology. 2021;40(4):707-21.

6. Ansari FA, Ali SN, Arif H, Khan AA, Mahmood R. Acute oral dose of sodium nitrite induces redox imbalance, DNA damage, metabolic and histological changes in rat intestine. PLoS One. 2017;12(4):e0175196.
7. Kim MJ, Kim H. Anticancer effect of lycopene in gastric carcinogenesis. Journal of cancer prevention. 2015;20(2):92.
8. Imran M, Ghorat F, Ul-Haq I, Ur-Rehman H, Aslam F, Heydari M, et al. Lycopene as a natural antioxidant used to prevent human health disorders. Antioxidants. 2020;9(8):706.
9. Ono M, Takeshima M, Nakano S. Mechanism of the anticancer effect of lycopene (tetraterpenoids). The Enzymes. 2015;37:139-66.
10. Wang R, Lu X, Yu R. Lycopene inhibits epithelial–mesenchymal transition and promotes apoptosis in oral cancer via PI3K/AKT/m-TOR signal pathway. Drug design, development and therapy. 2020:2461-71.
11. Tao A, Wang X, Li C. Effect of Lycopene on oral squamous cell carcinoma cell growth by inhibiting IGF1 pathway. Cancer Management and Research. 2021:723-32.
12. Al-Maweri SA, Halboub E, Al-Qadhi G, Al-Wesabi M, Al-Sharani HM, Parveen S, et al. Efficacy of lycopene for management of oral potentially malignant disorders: A systematic review and meta-analysis. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2023;135(1):79-95.
13. Jain A, Gupta A. Immunohistochemical Evaluation of E-Cadherin in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma. Journal of Pharmacy and Bioallied Sciences. 2024;16(Suppl 2):S1838-S42.
14. Saliba I, Bachy-Razzouk M, Bensidhoum M, Hoc T, Potier E, Vialle R, et al. Analysis of a Chronic Lateral Ankle Instability Model in the Rat: Conclusions and Suggestions for Future Research. Life. 2024;14(7):829.
15. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, et al. Ethical considerations regarding animal experimentation. Journal of preventive medicine and hygiene. 2022;63(2 Suppl 3):E255.
16. Bashir A, Khan AS, Ahmed S, Shafiq F, Younas HM. Immunohistochemical Expression of E-Cadherin in Normal Oral Mucosa, Precancerous Lesions and Oral Squamous Cell Carcinoma. International Journal of Pathology. 2021:135-42.
17. Sakr Y, Hamdy O, Eldeghedi M, Abdelaziz R, Med Sidi El Moctar E, Alharazin M, et al. Shifting Epidemiology Trends in Tongue Cancer: A Retrospective Cohort Study. Cancers. 2023;15(23):5680.
18. Doghish AS, El-Husseiny AA, Khidr EG, Elrebehy MA, Elballal MS, Abdel-Reheim MA, et al. Decoding the role of miRNAs in oral cancer pathogenesis: A focus on signaling pathways. Pathology-Research and Practice. 2023:154949.
19. Odell E, Kujan O, Warnakulasuriya S, Sloan P. Oral epithelial dysplasia: recognition, grading and clinical significance. Oral diseases. 2021;27(8):1947-76.
20. Mortazavi H, Safi Y, Baharvand M, Rahmani S. Diagnostic features of common oral ulcerative lesions: an updated decision tree. International journal of dentistry. 2016;2016.
21. Al-Gayyar MM, Hassan HM, Alyoussef A, Abbas A, Darweish MM, El-Hawwary AA. Nigella sativa oil attenuates chronic nephrotoxicity induced by oral sodium nitrite: Effects on tissue fibrosis and apoptosis. Redox Report. 2016;21(2):50-60.
22. Soliman MM, Aldhahrani A, Metwally MM. Hepatoprotective effect of Thymus vulgaris extract on sodium nitrite-induced changes in oxidative stress, antioxidant and inflammatory marker expression. Scientific Reports. 2021;11(1):5747.
23. Crowe W, Elliott CT, Green BD. A review of the in vivo evidence investigating the role of nitrite exposure from processed meat consumption in the development of colorectal cancer. Nutrients. 2019;11(11):2673.
24. Gupta S, Jawanda MK, Arora V, Mehta N, Yadav V. Role of lycopene in preventing oral diseases as a nonsurgical aid of treatment. International journal of preventive medicine. 2015;6(1):70.
25. Hegazy A, Abdel-Azeem A, Zeidan H, Ibrahim K, Sayed EE. Hypolipidemic and hepatoprotective activities of rosemary and thyme in gentamicin-treated rats. Human & experimental toxicology. 2018;37(4):420-30.
26. Helal EG, El-Sayed RA, El-Gamal MS. Assessment of the physiological changes induced by sodium nitrite, annatto or mono sodium glutamate in male albino rats. The Egyptian Journal of Hospital Medicine. 2017;67(1):330-5.
27. Mezbani A, Kavan BP, Kiani A, Masouri B. Effect of dietary lycopene supplementation on growth performance, blood parameters and antioxidant enzymes status in broiler chickens. Livestock Research for Rural Development. 2019;31(1):12.

28. Clark AG, Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. Current opinion in cell biology. 2015;36:13-22.

29. Moubarak HS, Essawy TA, Mohammed SS. Carcinogenic effect of potassium bromate on tongue of adult male albino rats. Journal of Radiation Research and Applied Sciences. 2020;13(1):121-31.

30. Aita NAA, Mohammed FF. Effect of marjoram oil on the clinicopathological, cytogenetic and histopathological alterations induced by sodium nitrite toxicity in rats. Global Vet. 2014;12:606-16.

31. AlThanoon SA, Taha AM. Histopathological changes on the pregnant rat's lung induced by sodium nitrite and monosodium glutamate. Iraqi Journal of Veterinary Sciences. 2022;36(2):419-24.

32. Shahi S, Ahmadian E. The protective role of Lycopene in the treatment of oral disease. Journal of advanced chemical and pharmaceutical materials (JACPM). 2018;1(2):48-51.

33. Bahey N, Elswaidy N. Lycopene reduces potassium bromate-induced structural alterations in the jejunal mucosa of adult rats. Egyptian Journal of Histology. 2021;44(4): 1036-49.

34. Xu H-R, Lin B, Wang S-Q, Zhang Z, Lu X-X, Liu Y, et al. Sodium nitrite improves epithelial-mesenchymal transition of hepatoma cells in mice bearing H22. Yao xue xue bao= Acta Pharmaceutica Sinica. 2012;47(11):1470-6.

35. El-Rouby DH. Histological and immunohistochemical evaluation of the chemopreventive role of lycopene in tongue carcinogenesis induced by 4-nitroquinoline-1-oxide. Archives of oral Biology. 2011;56(7):664-71.