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Potential role of thymoquinone in management of Dental Caries and Gingival disease in the animal model

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Thymoquinone (TQ), the active principle of *Nigella sativa*, has various pharmacological properties. Aims: This study aimed to investigate TQ's potential protective effect on caries initiation and gingival inflammation by inhibiting plaque formation in rats' gingivitis model. Sixteen germ-free 21-day old Fischer rats were randomly assigned to four groups, G1 – negative control consumed regular diet without intervention; G2 – positive control consumed caries inducing food (CID); G3 – consumed CID and treated by TQ oral gel; G4 – consumed CID and treated by TQ aqueous solution. Experimental gingivitis and caries formation were done via feeding animals a sucrose-rich diet after oral challenge with *Streptococcus mutans*. Plaque index caries score, subgingival bacterial count, and bleeding on probing were measured. Indicator dyes were used to evaluate plaque score and caries fissures scores. Histological studies were conducted on mandibular arches to determine the degree of inflammation. The results revealed that rats treated with TQ as mouth gel or TQ aqueous solution showed less significant differences among all parameters. The histological study showed a minimum or absence of inflammation in gingival tissues. This study showed that treatment via TQ oral gel or TQ aqueous solution reduced caries initiation, plaque formation, and gingival inflammation. Therefore, it could be recommended for future toothpaste and mouthwash manufactures.

Keywords: Caries prévention; Thymoquinone; Plaque; Gingival inflammation; *Nigella sativa*

INTRODUCTION

Many factors contribute to the formation of dental caries, usually starting as small-demineralized areas, due to lactic acid produced by bacterial fermentation from dietary carbohydrates in the dental plaque (Beighton and Lynch, 1995). Dental caries is an irreversible microbial disease that progresses in calcified tissues and undergoes different stages. It has a

high incidence worldwide; 60% –90% of school-level kids are influenced, while older age is highly significant. In specific nations, the frequency is roughly 100% with various severity (Petersen et al. 2005). Dental caries thought to be the most transmissible youth infection, particularly in the United States, from age 5 to age 17years. Its predominance is multiple times higher than asthma (Kopycka-Kedzierawski et al. 2008). One research in Saudi Arabia revealed that 84% of healthy children and 92% of medically compromised kids suffer from dental caries (Brown, 2009). Dental decay has numerous etiological factors. The top risk factors causing dental caries are dental plaque, cariogenic food, financial variables, the liability of teeth to decalcification, and the period of introduction to the cariogenic bacteria factor.

The essential etiologic operator of caries, either coronal or radically, are the mutants *Streptococci*, especially *S. mutans* and *S. sobrinus* (Bradshaw and Lynch, 2013). The documented leading cause among pathogenesis is *S. mutans*, which produce extracellular polysaccharides, predominantly glucans (Yamashita et al. 1993), (Angius et al. 2015).

Dental plaque was characterized as a network of pathogens found over the tooth structure (Davey and O'Toole, 2000). Sucrose fermentation is probably caused by low pH that changes the balance of plaque microflora toward the cariogenic microorganisms (Marsh, 1992). Mechanical and synthetic strategies are typically used to manage dental plaque. However, mechanical techniques may not work correctly in medically compromised patients (Owens et al. 1997). In this manner, there is an enthusiasm for assessing an alternative way of preventing caries.

Nigella sativa (*N. Sativa*) is a yearly herbaceous plant fitting to Ranunculaceae. It is recognized under a few unique names; habbatu-Sawada (Arabic), dark caraway seeds (U.S.A.), shonaiz (Persian) (Khan, 1999). Few active elements have been identified from *N. Sativa*, including thymoquinone (TQ), thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alphahederin (Randhawa and Alghamdi, 2011). TQ (2-Isopropyl-5-methylbenzo-1,4-quinone) is the essence of the unstable oil of *N. Sativa*. Its effects in vitro and in vivo studies have been proven since it was processed during the 1960s. TQ has a powerful cancer prevention agent (Akhondian et al. 2011).

Nigella sativa and its oils have been utilized for a considerable number of years both as food added substances, environmental medicines for some diseases, and against different sicknesses, including neurological and psychological illness, cardiovascular, malignancy, diabetes, hypertension, inflammatory conditions, asthma, bronchitis, migraine, skin inflammation, eczema, dizziness, flu, and infertility just as different infectious illnesses caused by viral, parasitic, and bacterial contaminations (Salem, 2005; Yimer et al. 2019). Moreover, N. Sativa was proven for its impact on bacteria adherence to epithelial tissue as it hinders the connection of microorganisms to the host tissues (Hull Vance et al. 2010). It potently affects gram-positive microscopic organisms (Chaieb et al. 2011; Kokoska et al. 2008). Also, *N. Sativa* and its active elements were proposed to inhibit the progress of antibiotic resistance of *Staphylococcus aureus* (Mouwakeh et al. 2019). Likewise, TQ exhibits decisive antimicrobial action against a few oral pathogens, which give a synergistic impact for specific antibiotics (Kouidhi et al. 2011; Vlachojannis et al. 2018). In this unique situation, it reported an anti-carcinogenic effect against *S. mutans*, which can provide a sound way to deal with the prevention of dental caries (Al-Attass et al. 2016; Jrah Harzallah et al. 2011). Numerous preventive measures are based on oral wellbeing experts via adjustment of the cariogenic condition that promotes the healing of enamel and dentine (Yon et al. 2019). In any case, not every person is experiencing a similar degree of caries progress (2000). Regardless of the perceptible development in prevention, caries is still complicated infections focusing on sound and comprehensive available preventive strategies (Blinkhorn and Davies, 1996). Public health procedures have focused on fluoride-containing dental items like toothpaste, mouthwashes, and topical fluoride application, and the utilization of water fluoridation (Bagramian et al. 2009). However, fluoride's positive impact in anticipation of dental decay risk doesn't eradicate dental decay, and numerous populations are not evident to the ideal measure of fluoride (Moynihan, 2005). Elimination of the plaque was thought to assume a crucial role in anticipating dental caries, gingival inflammation, and periodontitis (Loe et al. 1965). The present investigation aimed to assess the anti-inflammatory and antibacterial impacts of TQ against plaque and cariogenic microorganisms. In addition to evaluating TQ viability in preventing dental decay and improving gingival wellbeing.

We hypothesized that TQ would prevent caries initiation by suppressing bacterial adherents and reducing gingivitis by inhibiting plaque.

MATERIALS AND METHODS

A randomized animal study was done prospectively at Tufts University School of Dental Medicine, USA. The work was conducted after approval by Use Committee (IACUC) and Tufts Medical Center Institutional Animal Care, and it was complied with (Declaration of Helsinki for animal experiments).

2.1. Sample size calculation

Sixteen rodents (four for each group) were the sample size. This would convey an 80% power to locate a 1-unit difference in each outcome (caries score, plaque score, bacteria sample collection, and bleeding on probing) among the four groups, supposing a regular standard deviation of 0.75 units (nQuery Advisor, 7.0).

2.2. Cariogenic Bacteria

Streptococcus mutans microorganisms (ATCC® 25175TM) were bought from ATCC (Manassas, VA). Microorganisms were initiated as indicated by the producer instructions, which include rehydration of the microscopic organisms in disinfected saline (0.5 mL), resuspending in brain infused agar broth (5 mL), and incubation at 37 ºC overnight, after which the suspension was centrifuged for 10 minutes at 3000 rpm; the sediment pellets were resuspended again in sterile saline (1mL) (working suspension). A Microbiologics KWIK-STIK™ applicator was used to apply the bacterial suspension into the rodents' maxillary and mandibular teeth.

2.3. Thymoquinone preparation

Thymoquinone (TQ) was obtained by Sigma Aldrich (St. Louis, MO) and was prepared as a topical oral sticky gel and as a solution mixed in drinking water (TQ aqueous solution)

TQ topical gel:

The aseptic condition was adopted in the pharmaceutical lab at King Abdulaziz University, KSA. The oral mucoadhesive was prepared according to a previously reported method (Aslani et al. 2016). The basic formula includes Hydroxypropyl methylcellulose (HPMC) 4 gm: sorbitol 10 gm, sodium para-hydroxybenzoate 0.15 g distilled water to 100 gm. The pure gel (Vehicle) was supplemented to the TQ solution in propylene glycol (20 mg TQ in 1 mL) with appropriate mixing and geometric dilution. The weight of the oral gel was adjusted to 10 g (i.e., 0.2% w/w of TQ) and the pH (7.4) by sterile phosphate buffer. The formulation was subjected to pharmacopeia quality control testing, including microbial limits and stability (Partha et al. 2016).

Aqueous TQ solution:

Aqueous TQ solution was made as to the following: 20 mg of TQ was initially liquefied in two mL of propylene glycol, hybrid via vigorous vortexing approximately 20 sec, and then placed into 1L of distilled water.

2.4. Animals and treatments

Sixteen 21-days old Fisher male rats were obtained from Taconic (Hudson, NY) and kept in separate confine in standard conditions (temperature 72.5°F humidity 27%, and 12-hour light/dark cycles). The experiment lasted for five weeks (the complete animal grouping and treatment design were represented in table 1).

2.5. Measurements of the outcomes

2.5.1. Subgingival plaque collection and processing.

Rodents were anesthetized utilizing isoflurane. The entire sample was taken and handled by a similar examiner to normalize the testing strategy. The subgingival plaque samples were gathered from the buccal site of each trial tooth (first molar from every quadrant, upper right central incisor, and lower left central incisor) utilizing the paper point strategy (Tanner and Goodson, 1986). Delicate insertion of disinfected paper points (ISO #40 tighten 0.02 mm/mm) into gingival sulcus till tissue obstruction and held for 20 seconds. Paper points were pulled out of the sulcus cautiously without contacting the surrounding tissues. Then immediately placed inside disinfected vial has brain heart infusion for microbiological assessment. Samples were prepared immediately after collection. The bacterial testing was completed at the baseline and the following five weeks. The vial was blended by vortexing for 20 seconds. Next, a 2 mL aliquot was inoculated onto blood agar plates, including a pancreatic digest of casein 14.5 g/L, papaic digest of soybean meal five g/L, growth factors 1.5 g/L, agar 14 g/L, sodium chloride five g/L, and defibrinated sheep blood 5% (Fisher Scientific, Waltham, MA). Following incubation, the number of colony-forming units per 100 mL

(CFU/100 mL) was obtained. The whole plates were incubated in a typical incubator at a temperature of 7°C for 24h.

2.5.2. Bleeding on probing (BOP).

BOP was estimated from each trial tooth, from the four surfaces (mesial, distal, buccal, and lingual), utilizing a sterile probe with a 0.45 mm tip measurement (PCPUNC15; Hu Friedy, Chicago, IL). This method was performed in the gingival sulcus by using probe insertion till tissue resistance, keeping it in place around 20 sec. At that point, grade the score as (1) for bleeding surface and (0) for no bleeding. Afterward, the percentage of bleeding on propping was determined by summing the number of bleeding surfaces together, dividing the total by the number of surfaces assessed, and multiplying the score by 100.

2.5.3. Caries detection.

Rodents were sacrificed, and the maxilla was isolated, cleaned to evacuate every connected muscle and food fragment, and then fixed in 10% neutral buffered formalin for one day. Maxillary surfaces were colored with a caries indicator (SableTM SeekR and SeekR, Ultradent Products, Inc, South Jordan, Utah) placed for 10 sec. The maxilla surfaces were washed with regular water, dried, and explored via stereomicroscope (Olympus, SZ16) at 60X power, set by an advanced camera. Two independent investigators did the caries assessment for statistical analysis.

2.5.4. Plaque detection.

The maxillary teeth were utilized to assess the plaque using a plaque disclosing color (Reveal, Henry Schein Inc. Melville, NY). O'Leary Plaque Control Record evaluated the percentage of the plaque (Morita et al. 1987). The plaque stain was placed over the entire maxillary surfaces for 10 sec. At that point, washed, dried, and evaluated via a stereomicroscope (Olympus, SZ16) at 60X power, outfitted with an electronic camera.

Each tooth's occlusal surface was evaluated separately and divided into four surfaces. Each surface scored as (1) for the appearance of plaque or (0) for the nonappearance of plaque. The plaque index was determined by dividing the number containing plaque of all teeth over the total number of accessible teeth surfaces and then multiplying the outcome by 100. Two independent investigators statistically analyzed the plaque assessment with the mean.

2.6. Histological Studies.

The mandible arches were processed to identify any features of inflammation and dental decay histologically. Arch from each rodent was separated, then cleaned and fixed in neutral buffered formalin 10% for one day. Mandibular arches were decalcified before paraffin embedding via inserting the arches in the Cal-Rite Fixation/Decalcifying Solution (Thermo Scientific/Richard-Allan Scientific, Kalamazoo, MI). At that point, ammonium hydroxide was utilized to test the arches for decalcification. After decalcification, the samples were dried out in alcohol solutions, routinely processed, and implanted in paraffin wax. Almost 6-10 um thick sections were prepared to utilize a microtome then stained with hematoxylin and eosin (Fukuzato et al. 2009).

2.7. Data Presentation and Statistical Analyses.

SAS software package version 9.2 (SAS Institute, Caryhowed, NC) was used for statistical analysis Shapiro-Wilk test**.** Showed that the data were not normal; therefore, we presented the data as medians ± IQR (interquartile range Kruskal-Wallis tests (non-parametric test) to test the differences between the groups for the TQ effects. The variables are caries rating, plaque index, bleeding on propping, and microbiological cultures. Mann-Whitney U test (non-parametric test for pair-wise comparison) was used due to the not normally distributed data and the small sample size; p values < 0.05 were reflected as significant.

RESULTS

Effect of TQ on body weight.

The animals' weight was measured for the whole study duration every 3-4 days. It was noticed that treatment with TQ did not significantly affect animal weight among the entire various groups, neither at the baseline nor the end of the investigation. As shown in (Figure 1)

Effect of TQ on the bacterial count.

The bacterial collection was achieved at baseline and by the end of the study. After exposing the rats to high sucrose diet for five weeks, more bacterial colonies were detected in the negative control and positive control group. While the groups treated with TQ oral gel or aqueous solution has a significantly lower bacterial count (Figure 2, Tables 2, 3).

Effect of TQ on BOP.

At baseline, bleeding on probing was not noticed in any examined rats (data not presented). Subsequently, after five weeks of feeding the animals with a high sucrose meal, bleeding was recognized in the positive and negative control groups (Figures 3A and B). Conversely, in rats treated with TQ in both oral gel or an aqueous solution, bleeding with gingival inflammation was absent (Figure 3C and D). Bleeding was significantly higher in the positive control group than in the negative control group (Figure 3E). After TQ treatment, bleeding in the oral gel and aqueous solution groups was significantly lower than the negative and positive control groups (Figure 3, and Tables 4, 5).

Effect of TQ on caries development.

The negative control group samples and, to a lesser extent, the TQ oral gel group exhibit early caries (Figure 4C). The positive control group samples revealed modifications in teeth morphology with cavitation and the occurrence of widespread tooth caries (Figure 4B). Conversely, the TQ aqueous solution group was free from dental decay (Figure 3D). Commercially caries detector dyes were used for further confirmation of the presence of caries. As shown in the illustrative microscopic photos in (Figure 5A-D). Dental decay was noticed negatively and more in positive control groups; however, both TQ-treated groups were free of caries. Two separate examiners' averaged data showed that caries scores were significantly higher in samples from the positive control group than from the negative control group. Caries scores in the TQ oral gel and aqueous solution group were lower significantly than the negative and the positive control (Figure 5C, D, and Tables 4, 5).

Effect of TQ on plaque score.

The index was evaluated based on the O'Leary method using a commercially available dye (O'Leary et al. 1972). As revealed in the representative photographs in (Figure 6A-D), a considerable amount of plaque was spotted in arches from both the negative and positive control groups. In contrast, arches from both TQ treated groups with oral gel or aqueous solution had minimal and no noticeable plaque, respectively.

Figure 6 demonstrates an averaged data gathered by two separates examiners. The plaque score in the positive control group was significantly higher compared to the negative control group. However, the plaque score in the group treated with oral gel and an aqueous solution significantly lower plaque score values in matching the negative and positive control groups (Figure 6C, D and Tables 4, 5).

Table 1: Experimental design

<table>
<tr><td>Group 1: (n=4)</td><td>Negative control fed a normal standard diet, no treatment, or bacterial challenge.</td></tr>
<tr><td rowspan="3">Group 2, 3&4
fed a high sucrose diet for five weeks (Harlan laboratories, Indianapolis, IN), S. mutans was applied to the rats’ maxillary and mandibular teeth using a Microbiologics KWIK-STIK™ applicator</td><td>Group 2: (n=4) (positive control, no treatment</td></tr>
<tr><td>Group 3: (n=4) (test TQ in oral gel) TQ Oral gel was applied daily over the teeth and gum</td></tr>
<tr><td>Group 4: (n=4) (test TQ in drinking water, divided equally to 4 separate plastic water bottles equipped with sipper pipes. Each rodent was contained in separated confine, and the measure of water used was recorded day by day</td></tr>
<tr><td colspan="2">The water was changed at regular intervals (every two days)
The measure of used diet besides the rodents ' weight was evaluated every four days.</td></tr>
</table>

Table 2: Evaluation of bacterial counts in subgingival plaque samples from the buccal site (Kruskal-Wallis test)

Bacterial count	Negative control		Positive control		TQ in gel		TQ in water		p-value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
At baseline	1.0	0.0-2.8	0.0	0.0-0.8	0.5	0.0-5.5	3.0	0.8-4.5	0.358
At study end	12.5	7.0-21.0	30.0	11.0-43.8	1.0	0.0-4.3	0.5	0.0-1.0	0.008
Difference	11.5	4.8-20.5	29.5	11.0-43.5	0.0	-1.5-0.8	-2.0	-4.3--0.5	0.006

The data are presented as median (interquartile range) and was analyzed using the Kruskal-Wallis test.

Table 3: Pair-wise evaluations of bacterial counts (Mann-Whitney U test)

Bacterial Count	Negative Control	Positive Control	TQ in Oral Gel	TQ in Water
At baseline	1.0 (0.0-2.8)	0.0 (0.0-0.8) [a] p=0.48	0.5 (0.0-5.5) [a] p=1.00 [b] p=0.48	3.0 (0.8-4.5) [a] P=0.34 [b] P=0.11
At study end	12.5 (7.0-21.0)	30.0 (11.0-43.8) [a] p=0.34	1.0 (0.0-4.3) [a] p=0.03 [b] p=0.03	0.5 (0.0-1.00) [a] p=0.03 [b] p=0.03
Difference	11.5 (4.8-20.5)	29.5 (11.0-43.5) [a] p=0.34	0.0 (-1.5-0.8) [a] p=0.03 [b] p=0.03	-2.0 (-4.3- -0.5) [a] p=0.03 [b] p=0.03

The data are presented as median (interquartile range) and were analyzed using the Mann-Whitney U test. [a] Denotes p values compared to the negative control group, [b] denotes p values compared to the positive control group.

Table 4: Evaluation of plaque, caries, and BOP (Kruskal-Wallis tests)

Variable	Negative control		Positive control		TQ in Oral-gel		TQ in water		p-value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Caries Score	10.0	7.8-13.8	18.5	17.3-20.5	5.0	0.8-7.0	0.5	0.0-2.5	0.005
Plaque Score	12.5	11.3-13.8	21.5	21.0-23.5	6.0	4.0-8.8	4.0	3.3-4.8	0.004
BOP	31.1	21.3-35.9	40.7	37.6-48.4	6.3	0.0-21.9	3.1	0.0-6.3	0.006

The data are presented as median (interquartile range) and were analyzed using the Kruskal-Wallis test.

Table 5: Pair-wise evaluations of plaque, caries, and BOP (Mann-Whitney U test)

Variable	Negative Control	Positive Control	TQ in Oral Gel	TQ in Water
Caries Score	10.0 (7.8-13.8)	18.5 (17.3-20.5) [a] p=0.02	0.5 (0.8-7.0) [a] p=0.04 [b] p=0.02	0.5 (0.0-2.5) [a] P=0.02 [b] P=0.02

Plaque Score	12.5 (11.3-13.8)	21.5 (21.0-23.5) [a] p=0.02	6.0 (4.0-8.8) [a] p=0.02 [b] p=0.02	4.0 (3.3-4.8) [a] p=0.02 [b] p=0.02
BOP	31.1 (21.3-35.-9)	40.7 (37.6-48.4) [a] p=0.02	6.3 (0.0-21.9) [a] p=0.04 [b] p=0.02	3.1 (0.0-6.3) [a] p=0.03 [b] p=0.03

The data are presented as median (interquartile range) and were analyzed using the Mann-Whitney U test. [a] Denotes p values compared to the negative control group, [b] denotes p values compared to the positive control group.

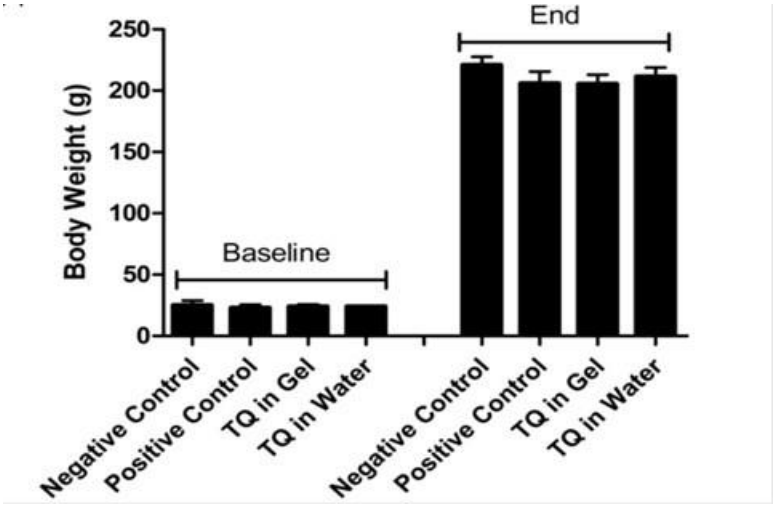

Figure 1: Effect of TQ on body weight

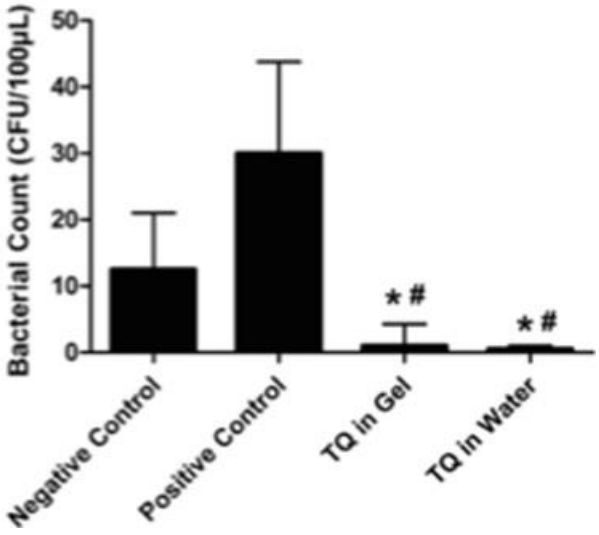

Figure 2: Effect of TQ on the bacterial count.

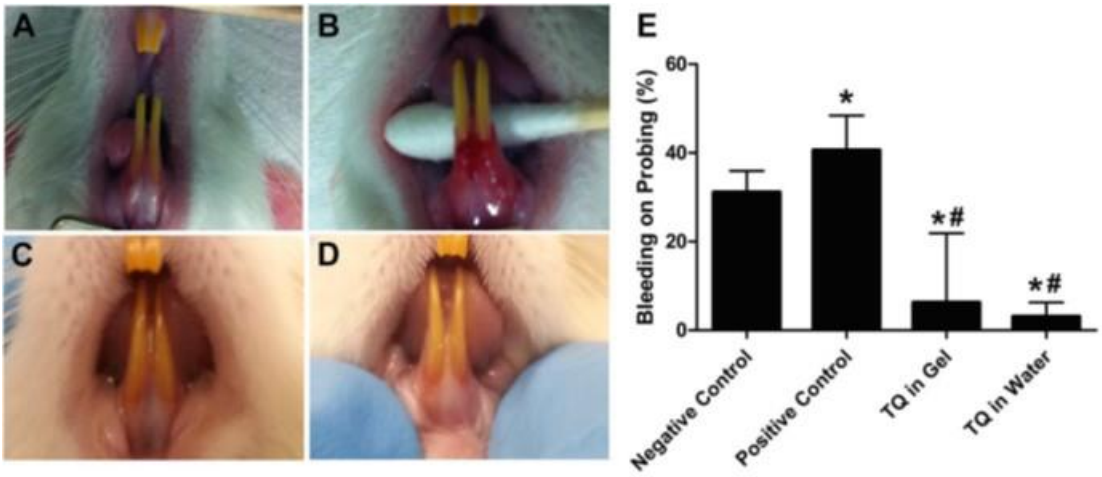

Figure 3: Effect of TQ on BOP

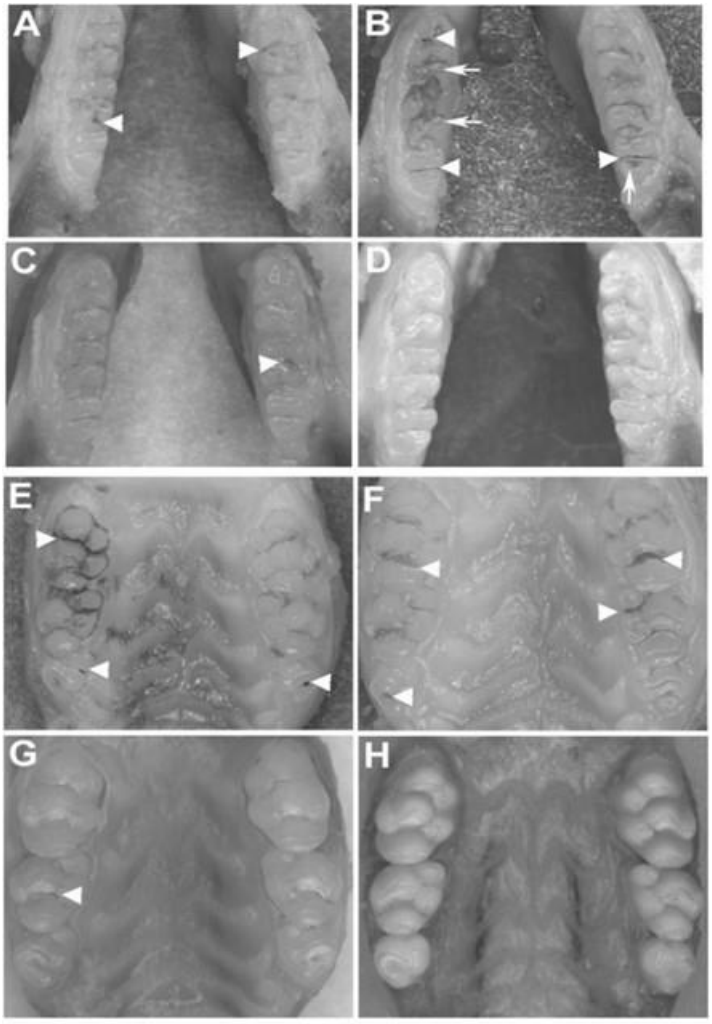

Figure 4: Effect of TQ on caries development.

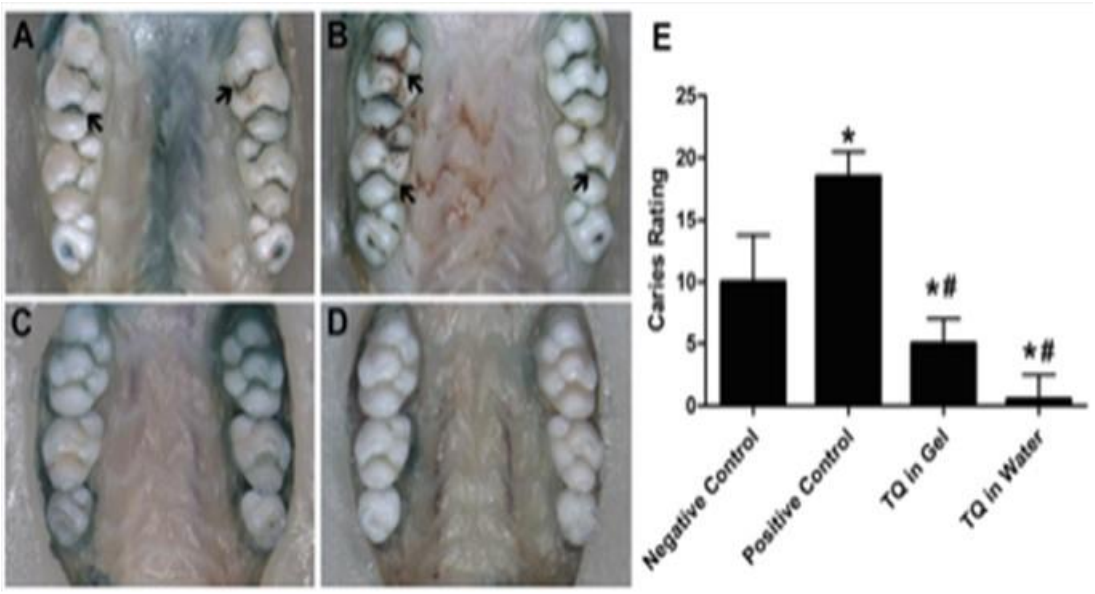

Figure 5: Effect of TQ on caries development.

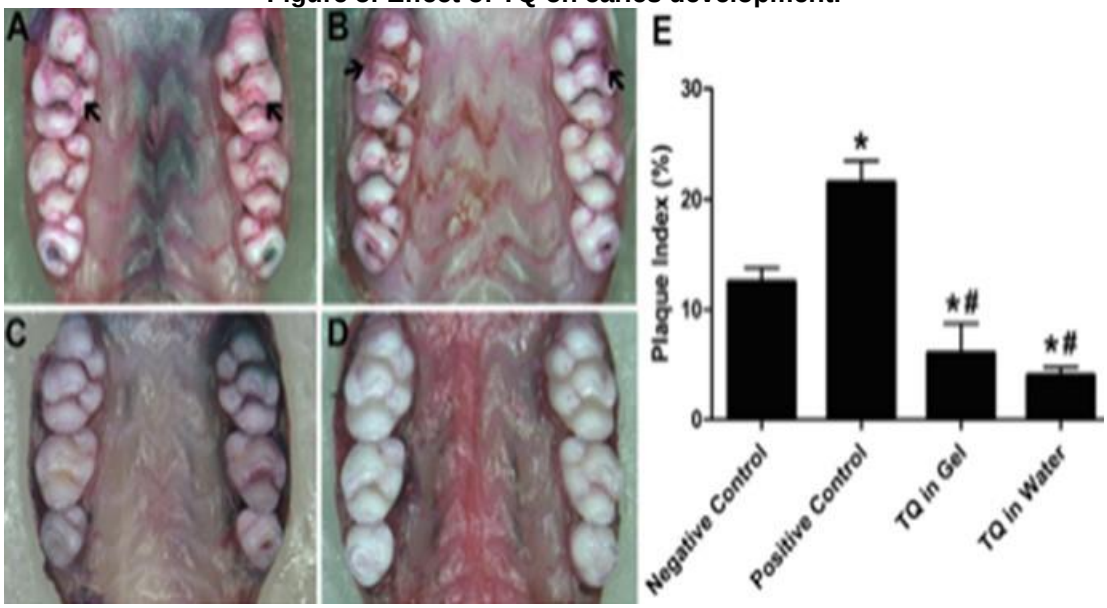

Figure 6 Effect of TQ on plaque score.

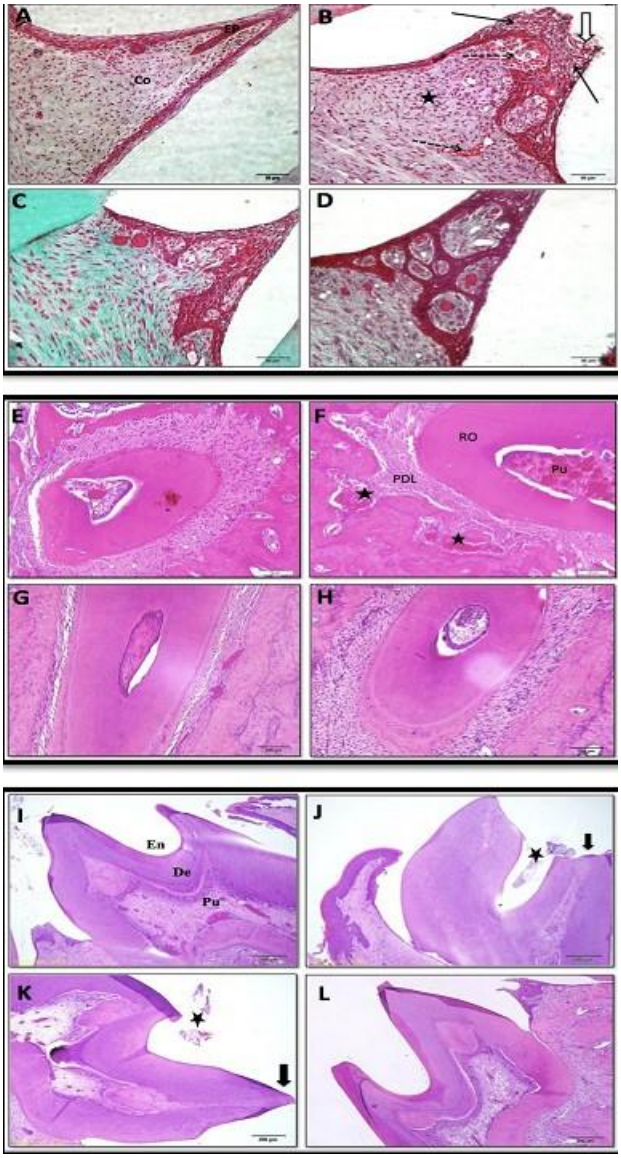

Figure 7: Histological findings

Histological findings.

The Histological study performed on the mandibular arches supported what has been detected in maxillary samples. Figure 7, panels A-D demonstrated representative photomicrographs of mandibular sections showing TQ's outcome on the gingiva. The positive control group exhibited thickened overlying epithelium, swollen interdental papilla along with congested vessels, and bacterial attachment (Figure 7B). These modifications were not present in TQ-treated groups (Figure 7C, D). Panel E-H showed sections of the periodontal ligaments. The positive control group revealed several widened congested vessels; however, those alterations were fewer in TQ-treated groups Figure 7G, H). Dental caries was also noticed histologically (panels I-L) as loss of cusp tips and presence of food fragments and cavitation (Figure 7J). In contrast, particularly in the aqueous solution group, TQ treated groups, the tooth morphology was almost similar to that from the negative control (Figure 7K, L, and I).

DISCUSSION

The caries rate was assessed by utilizing caries recognizing color, a generally acknowledged strategy for bench tests. Our outcomes exhibited that the TQ groups' caries

rates were significantly lower than the positive and negative control groups. This result is consistent with the in vitro study, which found that TQ has an antimicrobial role in cariogenic bacteria (Forouzanfar et al. 2014; Jrah Harzallah et al. 2011). Moreover, this result affirmed similar outcomes concerning *N. Sativa* extract, which exhibits the largest inhibit zone against the two cariogenic bacteria (*S. mutans and S. mitis)* (Najah, 2012). Likewise, we examined TQ's effect to counteract the breaking down impacts of dental plaque on gingival integrity and wellbeing by evaluating the microbiological and periodontal parameters. As a gel or blended in drinking water, we exhibited that TQ administration fundamentally diminished plaque accumulation. These phenomena can be clarified due to the known biofilm restraint and antimicrobial potencies of TQ. These outcomes are steady, with an investigation covered the antibacterial potential and antibiofilm action (Goel and Mishra, 2018). TQ indicated significant bactericidal activity against numerous human microorganisms, particularly gram-positive cocci. TQ was proven to prevent cell adhesion to glass surfaces (Chaieb et al. 2011).

Moreover, our outcomes showed a considerable diminishing in bacterial inclusion in the TQ treated groups, while the opposite was found in the negative and positive control groups. This finding is upheld by in vitro investigations, which showed that TQ possesses a particular antibacterial activity against oral microscopic organisms. Consequently, the authors recommended that TQ be utilized as an environmental component with resistance changing action (Goel and Mishra, 2018; Kouidhi et al. 2011). Our finding likewise demonstrated a critical decrease in BOP, clinically, and histologically with progress in improving gingival conditions. TQ-treated rodents showed no BOP and negligible indications of inflammation. These outcomes follow detailed studies exhibiting TQ affirmative action against alveolar bone loss and periodontal aggravation, and it additionally advances wound recovery. The authors credited these outcomes to TQ because of its antimicrobial, anti-inflammatory, and antioxidant properties (Nourbar et al. 2019; Ozdemir et al. 2012). The findings from the current study affirmed the direct stable relationship between the utilization of dietary sugars and the presentation of caries (Michalek et al. 1977). The plaque, caries, and bleeding scores introduced statistically significant higher patterns in the positive group, fed with a high sucrose meal compared with negative control rodents fed a standard eating diet. These outcomes are in concurrence with those expressed in a crossover report, which lasts for three weeks, then analyzed the effect of repeated sugar consumption on gingival inflammation. It was portrayed that repetitive sugar utilization brought about gingival inflammation when assessed by gingival BOP (Sidi and Ashley, 1984).

There are restrictions on the current investigation. Initially, the examination was performed on rodents, and to apply it to the human circumstance requires further research. Although the embryonic development in rodents is firmly corresponded to that of humans and demonstrated to be utilized to test the reasons for human infections and productivity of healings with prescient validity, rodents' reports don't reliably anticipate individual results. Next, rodent molars are smaller than human molars, even though they are comparative in anatomic configuration, making it hard to lead comprehensive periodontal parameters evaluation (Xu and Wei, 2006). Besides, the molar teeth were hard to access because of the constrained mouth opening.

CONCLUSION

The current study showed that Thymoquinone administration either in oral gel or blended in drinking water decreased caries incident, plaque accumulation, and gingival infection in a rodent gingival inflammation model. Therefore, it could be advised for future clinical application in the dental field.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HA, AA, CL, YH, SA, DZ. Performed the experiments: HA, CK, HM. Analyzed the data: HA, DZ. Wrote the paper: HA, DZ

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