RESEARCH ARTICLE

Editorial Process: Submission:09/15/2024 Acceptance:04/14/2025

Evaluation of Therapeutic Activity of Tualang Honey on Oral Cancer: Histopathological Study

May Al-Koshab[1], Rola Ali-Saeed[2], Aied Mohammed Alabsi[1,3]*, Siddig Ibrahim Abdelwahab[4], Meram Azzani[5], Muhamad Fareez Ismail[6], Maged A. Al-Garadi[7], Vetriselvan Subramaniyan[8]

Abstract

Objective: The use of natural products has been gaining interest, notably, in the area of cancer therapy. In this study, we determined the chemotherapeutic activity of Tualang Honey (TH) Malaysian wild local honey, on an animal model induced for oral cancer using 4NQO. **Methods:** A total of 28 male Sprague-Dawley (SD) rats were randomized into four groups (n=7 per group) as non-treated, treated (1000 mg/kg) 1000, treated 2000 mg/kg and control group. All groups' rats received 4NQO, followed by 10 weeks administration of TH at 1000 and 2000 mg/kg, respectively, for the treated groups. All rats from all experiments were sacrificed to evaluate the incidence of oral neoplasms with histopathological changes and histological evaluation of oral cancer metastasis. **Results:** TH significantly reduced the incidence of SCC from 100% to 28.6 % in the TH high dose group of 2000 mg/kg. furthermore, TH has the ability to inhibit the metastasis of oral cancer to adjacent organs. **Conclusion:** TH inhibited the proliferation of the cancer cells in an animal model of oral squamous cell carcinoma and therefore, has the potential to be developed as a chemotherapeutic agent.

Keywords: 4-nitroquinoline 1-oxide- Chemotherapeutic- Oral squamous cell carcinoma- Tualang Honey

Asian Pac J Cancer Prev, **26 (4)**, 1269-1275

Introduction

With 377,713 incident cases and 177,757 fatalities, oral cancer is considered as the 16th most common cancer worldwide [1]. Nearly two-thirds (65.8%) of all new cases occur in Asian countries, where roughly 60% of oral cancer cases are found [2]. Chewing betel quid, smoking tobacco, excessive alcohol consumption, and microbial infections are strongly linked to oral cancer [3-6]. Although alcohol itself does not cause cancer, it can be converted to the known carcinogen acetaldehyde by enzymatic activity in the human oral mucosa tissues or by oral cavity microbes [3]. Despite recent advancements in treatment, patients with oral cancer continue to have a poor prognosis, with a five-year survival rate for only 65% of OSCC patients [7].

In recent years, oral cancer has been successfully treated with therapeutic medicine. However, the toxicity of chemotherapeutic drugs often leads to serious side effects. As a result, there has been growing interest in using natural products and medicinal plants for cancer treatment. Honey, rich in antioxidants such as phenolic and flavonoid compounds, is one of these natural products that has been extensively studied [8, 9]. Natural substances can alter pathways linked to cancer and inhibit the growth of cancerous cells [10]. Phytochemicals found in plant products have also been shown to reduce the negative side effects of traditional treatments [11].

In Malaysia, there are many varieties of honey including the Tualang Honey that can be obtained from the Malaysian wild plants. When comparing Tualang Honey (TH) to other local honey varieties in Malaysia, including commercial honey, pineapple honey, and gelam honey, it was found that TH has a stronger antioxidant effect against various reactive oxygen species (ROS) [12]. TH has demonstrated antiproliferative and antitumor activity against various cancer cell types [13]. In breast cancer cell lines, TH's effect upregulated enzymes linked to double-strand DNA structure repair, preserving the integrity of the cells' DNA [14]. Additionally, TH was found to

*[1]Department of Oral & Craniofacial Sciences, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. [2]Department of Preclinical Sciences, Faculty of Dentistry, MAHSA University, Selangor, Malaysia. [3]School of Dentistry, Management and Science University, Shah Alam, Selangor, Malaysia. [4]Health Research Centre, Jazan University, Jazan, Saudi Arabia. [5]Department of Public Health Medicine, Faculty of Medicine, Universiti Teknologi MARA, Selangor, Malaysia. [6]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia. [7]Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. [8]Department of Medical Sciences, School of Medical and Life Sciences, Sunway University, Selangor, Malaysia. *For Correspondence: aied_absi@yahoo.com*

enhance tamoxifen-induced apoptotic cell death in breast cancer cell lines [14]. According to Kishore et al., the high concentration of phenolic compounds in TH contributes to its antioxidant and free-radical scavenging activity [12]. TH has also shown anti-proliferative and early apoptotic effects in human osteosarcoma, oral squamous cell carcinoma, cervical cancer, and human breast cancer cell lines [15, 16]. Another study revealed that TH exhibits chemopreventive activity in an animal model and may potentially be developed as a chemopreventive treatment for oral cancer [17].

It can be very difficult to identify metastatic foci in distant organs or lymph nodes. Traditionally, the presence of metastasis is evaluated in euthanised mice after the completion of a study. For pathological assessment, most researchers resect the lungs and draining lymph node basins in OSCC [18]. The purpose of this study is to evaluate the chemotherapeutic activity of Tualang Honey (TH) and its anti-metastatic effect on oral cancer in an animal model induced by 4NQO, as there is currently limited information available on the antitumor activity of TH on oral cancer.

Materials and Methods

Ethical approval was obtained from Faculty of Medicine, Institutional Animal Care and Use Committee, University of Malaya (FOM-IACUC) prior to commencement of the study (Ethic reference no. 2016-171103/DENT/R/AMH). Information pertaining to animal treatment and preparation, and dose selection for the Tualang honey and other chemicals used in this study have been described in in our previous work [17, 19].

Animal treatment protocol

Drinking water containing 4NQO was freshly prepared twice a week in RO water and administered to the rats in light-shielded water bottles at a concentration of 20 ppm for 8 weeks [20]. The rats were randomly divided into four groups. Group 1 was given normal RO water (normal control), while Groups 2, 3, and 4 were given 4NQO solution as drinking water for 8 weeks. Starting from the 9th week, the groups were administered (vehicle (water), TH 1000 mg/kg, and TH 2000 mg/kg), respectively, in a volume of 10 ml/kg body weight daily for 10 weeks. Oral administration to the rats was carried out using gastric intubation by force-feeding with needle size 18 G (Harvard Apparatus, INC) [21], starting 1 week after the cessation of the 4NQO treatment (Figure 1). The rats were sacrificed by cervical dislocation, followed by the excision of the whole tongue.

Tumor size

The animals' tongues were examined during and after the experimental period, and the tumour volume was recorded for each group. To determine if the tumour size was affected by the administration of Tualang Honey (TH) and FD extract, the tumour size was measured using the formula ($\pi/6 \times$ width $\times$ length $\times$ height), as mentioned previously by Wali et al. [22] in 4NQO cancer-induced rats .

Histopathological evaluation of tongue

Histological evaluations were performed blindly with light microscopy by a qualified pathologist. The tongue tissue sections were assessed and graded as normal, hyperplasia, dysplasia or squamous cell carcinoma for each animal [23].

Histological evaluation of oral cancer metastasis

In this study, the cervical lymph nodes, kidneys, liver and lungs of all animals from each group were excised at the time of necropsy and submitted for histological evaluation. All specimens were fixed in 10% formalin, embedded in paraffin and the serial sections were cut and stained with hematoxylin. They were then examined in detail by a qualified pathologist to identify the foci for metastasis.

Statistical analysis

All assays were conducted in at least five separate experiments. Quantitative results were expressed as the mean+SD. Histological qualitative data were analyzed using the chi-square statistical test. For RT² PCR array, data were analysed using the GeneGlobe Data Analysis Center on QIAGEN's website (http://www.qiagen.com/

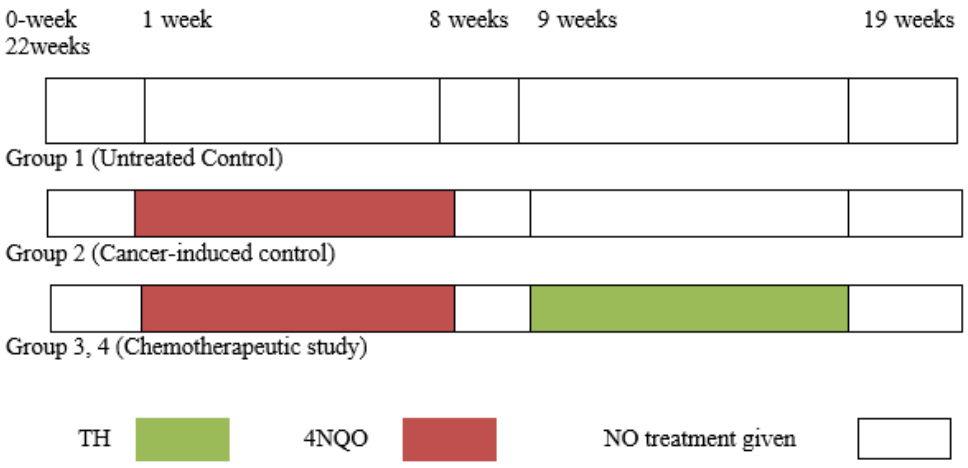

Figure 1. Experimental Protocol for Administration of 4NQO and Tualang Honey in Rats

my/shop/genes-and-pathways/data-analysis-center-overview-page/). Results were considered statistically significant at p-value < 0.05.

Results

Effect of TH on body weight

The mean body weight is shown in Figure 2. Although the rats showed signs of weight loss during the study, the weight loss was only significant ($p<0.05$) from week 17 until the end of the study period. It was observed that, at the end of the study (22 weeks), a statistically significant difference in the mean body weight was seen between the normal untreated group and the cancer-induced group using 4NQO (Table 1) ($p<0.05$). No significant differences were found between the 4NQO control group and any of the groups in the chemotherapeutic study (Table 1).

Effect of FD extract and TH on tumor size

It was observed that most of the lesions were located in the posterior third of the tongue, except for one rat in the 4NQO control group, where the lesions were localised on both the anterior and posterior third of the tongue (Figure 3). There was no significant difference between the TH treated groups compared to the cancer control group (Table 2).

Incidence of OSCC and pre-cancerous lesions of rat's tongue induced for oral cancer using 4NQO and treated with TH

The diagnosis of each tongue sample was based on the final histopathological changes. For each rat specimen diagnosed as having OSCC, the presence of hyperplasia and dysplasia lesions could still be detected in some parts of the same tongue.

In the 4NQO (induced cancer) groups, the incidence of OSCC was 85.7%, while in rats treated with TH at 1000 mg/kg and 2000 mg/kg, the decrease in the incidence of OSCC was statistically significant at 42.9 % and 28.6 % respectively. The presence of preneoplastic lesions such as dysplasia and hyperplasia was also observed in the present study. Dysplasia was diagnosed in 14.6% of the animals in the 4NQO (cancer) group, while in rats treated with TH of 1000 and 2000 mg/kg, 57.1% and 28.6% of the lesions were diagnosed as dysplasia, respectively. The incidence of hyperplasia in the TH-treated groups was 14.3% and 57.1% for 1000 mg/kg and 2000 mg/kg, respectively (Table 3).

Histological observation of rat's tongue induced for oral

Table 1. The Effects of Tualang Honey on the Body Weights of Rats during 4NQO-Induced Oral Carcinogenesis after 22 Weeks. (A post hoc Dunnett test)

Rats Group sN=7	Body weight (g)
Normal group (control)	*551±17
Oral cancer induced rats by administration of 4NQO (Untreated group)	395±00
Oral cancer induced rats by administration of 4NQO treated with TH(1000 mg/kg)	421±67
Oral cancer induced rats by administration of 4NQO treated with TH(2000 mg/kg)	396±67

*The mean difference is significant at the 0.05 level compared to Oral cancer induced rats by administration of 4NQO (Untreated group)

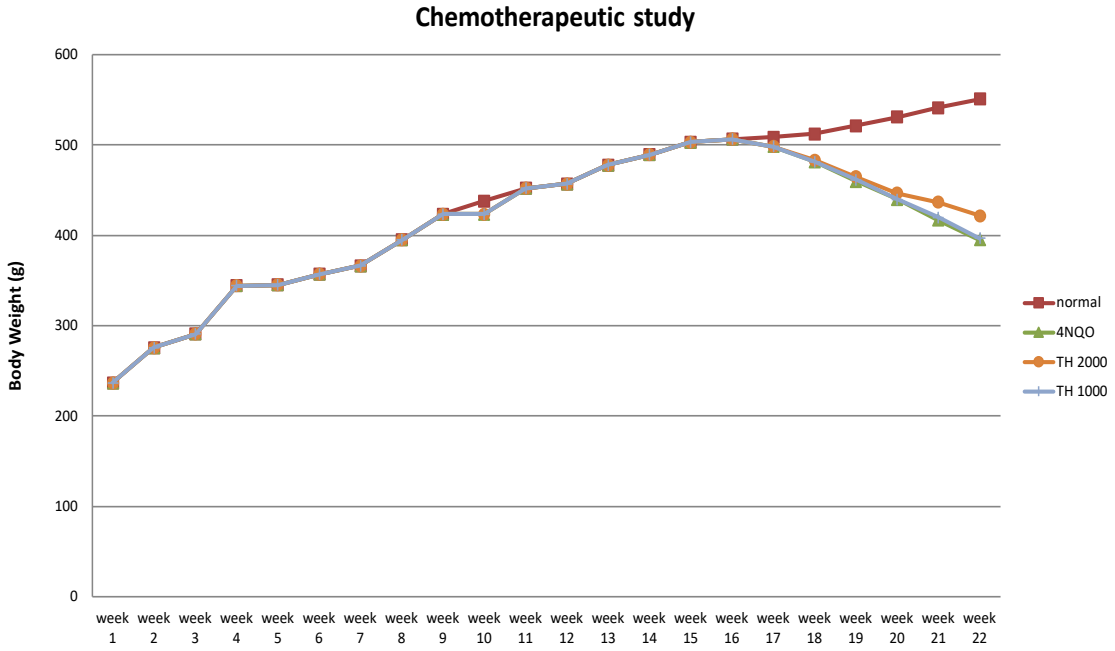

Figure 2. Mean Body Weights of Rats during 4NQO-Induced Oral Carcinogenesis in a Chemotherapeutic Study.

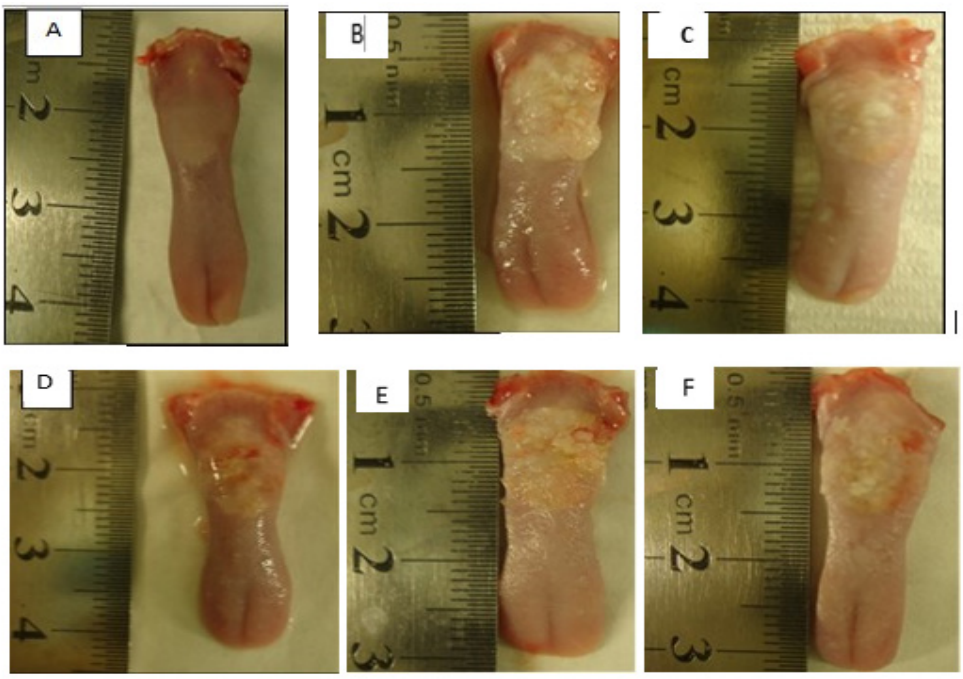

Figure 3. Macroscopic Photos of Rats Tongue Obtained from an Animal Model Study for Oral Carcinogenesis. (A) Normal rat tongue. (B) rat tongue induced for oral cancer using 4NQO (control cancer group). Rats treated with TH 2000 mg/kg (C) and FD 500 mg/kg (D) in a chemo-preventive study. Rats treated with TH 2000 mg/kg (E) and FD 500 mg/kg (F) in a chemotherapeutic study.

Table 2. Effect of FD Extract and TH on Tongue Tumour Size in 4NQO-Induced Oral Cancer Rats (Chemotherapeutic Study)

Groups	Tongue tumor volume (mm^3) (Mean± SD)	P value
Cancer control group	87.03±4.73	--
TH 1000 mg/kg	63.22±13.6	0.062
TH 2000 mg/kg	76.28±29.2	0.166

*p value less than 0.05, (p< 0.05) significant (A post hoc Dunnets test)

cancer using 4NQO and treated with TH

Histological evaluations were performed blindly with light microscopy by a qualified pathologist. The tongue tissue sections were assessed and graded as normal, hyperplasia, dysplasia or squamous cell carcinoma for each animal.

The rat's tongue obtained from the normal untreated group (Group 1) exhibited histological features of normal oral mucosa with keratinized stratified squamous

Table 3. Effect of TH on the incidence of pre-neoplastic and neoplasm in rats administered with 4NQO.

Group	No. of animal	Hyperplasia	Dysplasia	OSCC
4NQO (group 2)	7 (100%)	0 (0.0%)	1 (14.3%)	6 (85.7%)
TH1000 (group 3)	7 (100)	0 (0.0)	4 (57.1)	3 (42.9)
TH 2000 (group 4)	7 (100)	0 (0.0)	5 (71.4)	2 (28.6)

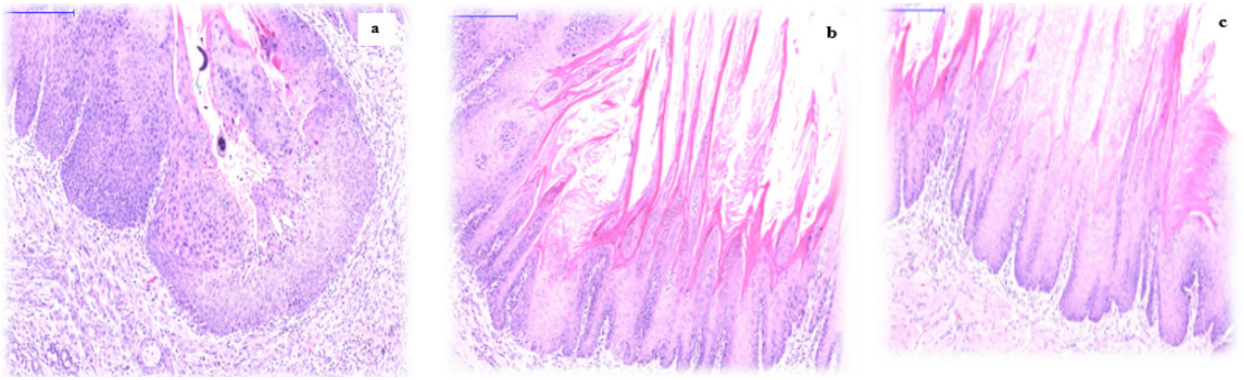

Figure 4. Histopathological Analysis of Rat's Tongue Obtained from a Chemotherapeutic Study. Rat's tongue following cancer induction with 4NQO (a), and treated with FD extract 500 mg/kg (b), and TH 2000 mg/kg (c) (scale bar, 200 µm).

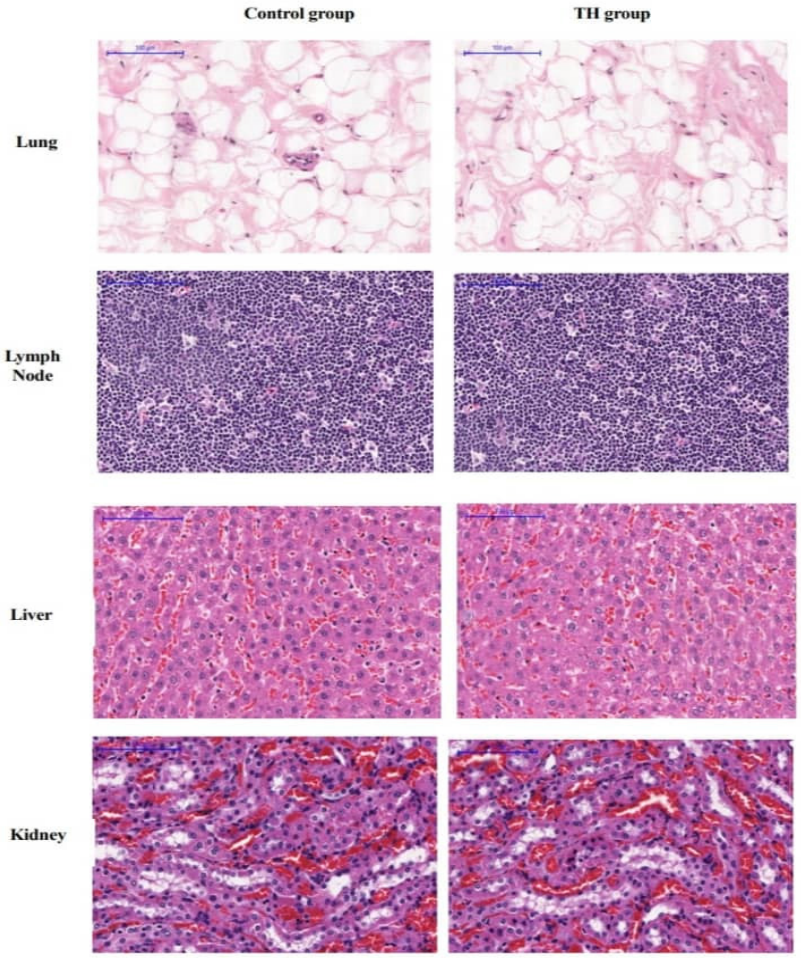

Figure 5. Photomicrograph of H&E Slides Obtained from Sections of Vital Organs (Lung, Lymph Nodes, Liver and Kidney) of Rats from the 4NQO Control Group (group 2) and TH (group 4) treated groups. (Scale bar, 100 μm)

epithelium. The architecture of the epithelium was normal with the presence of papillae, connective tissue and skeletal muscle beneath the mucosa (Figure 4).

The detection of OSCC was made through the presence of the submucosal invasion of the epithelial tumour cells, as indicated by certain features such as islands, nests and sheets, and the discontinuation of the basement membrane. Altered nucleus characteristics, such as cytoplasmic ratio and the presence of keratin pearls in the connective tissue, were shown in the epithelial tumour cells, together with cellular and nuclear pleomorphism and hyperchromatic nuclei.

Histopathological changes, such as hyperplasia and dysplasia, were observed in rats that were subjected to drinking water which contained 20 ppm of 4NQO. Hyperplasia, with a clearly-defined basement membrane, was also seen in some samples in groups that were treated with TH extract (Groups 3 and 4). Dysplastic histological changes include hyperplasia of the stratum spinosum, basal cell hyperplasia, superficial mitosis, increased number and size of nucleoli, with the level of atypia presence in the lower, middle or upper third of the epithelium stratification. Irregular epithelial stratification was also evident at the base of the epithelium of rats' tongues treated with TH (Groups 3 and 4) (Figure 4).

Histological findings of oral cancer metastasis

Histological findings indicated that the cancer have not metastasized from the oral cavity. All histological features of the lymph nodes and vital organs (lung, liver and kidney) were found to be normal in both the treated (G4) and the cancer control (G2) groups (Figure 5).

Discussion

In this study, an in vivo experiment was performed to determine the chemotherapeutic activities of Tualang Honey (TH) on an animal model induced for oral cancer using 4NQO. Unlike other carcinogenic substances, such as 7,12-dimethylbenz(a)anthracene, which can cause necrosis, tissue sloughing, and lesions that are cytologically and morphologically different from human lesions, 4NQO-induced lesions typically do not produce nonspecific inflammatory changes. This is why many researchers have used similar animal models [24].

According to this study, losing body weight could be a helpful indicator of how OSCC is progressing clinically [25]. Animals given 4NQO for cancer induction may experience significant weight loss due to oral cancer, decreased appetite, difficulty eating, and elevated metabolic rate [26]. Cancer anorexia/cachexia syndrome, a wasting syndrome that occurs in patients

with advanced cancer, is characterized by weight loss and a poor prognosis [27]. In this study, the application of 4NQO resulted in a notable decrease in body weight among the cancer-induced animals, consistent with earlier reports [21, 25].

Weight loss in this study was only significant ($p<0.05$) following week 17 of 4NQO administration and continued until the study's conclusion. These findings are consistent with earlier research, which showed significant body weight decreases in rats only after 16 [19] and 14 weeks of 4NQO application [28].

In a chemotherapeutic study evaluating the effects of doxorubicin plus methotrexate in rats induced for cancer using 4NQO, Khiavi et al. [29] reported that body weight measurements during the 4NQO exposure period were non-significant. Additionally, it was noted that body weights in the chemotherapeutic and 4NQO control groups did not differ significantly. Rats treated with Tualang Honey (TH) in the chemotherapeutic study did not show significant differences in tumor volume compared to the cancer control group.

In this study, none of the animals in the untreated control group developed visible tongue epithelial lesions or experienced histopathological changes. However, after 8 weeks of administering 4NQO in drinking water at 20 ppm, precancerous and cancerous lesions on the tongue epithelium appeared by the end of the experiment. This is consistent with previous reports using 4NQO in animal models of oral carcinogenesis. After 8 weeks of administering 4NQO at 20-30 ppm, the incidence of developing SCC and dysplasia at 28 weeks was 100% and 21.4%, respectively [30].

In a previous study, Ohne et al. [31] induced oral cancer in rats with 10 ppm of 4NQO for 28 weeks and found that none of the sacrificed rats' lymph nodes showed signs of metastasis. Similarly, in the current study, no metastases were found in any of the vital organs (lung, lymph node, liver, or kidney) of rats induced for oral cancer with 4NQO. However, these results should be interpreted with caution, as it is unclear whether higher doses of 4NQO (greater than 20 ppm) and a longer experimental period could cause metastatic spread.

In conclusion, Tualang Honey (TH) demonstrated a significant ability to inhibit the proliferation of cancer cells in an animal model of oral squamous cell carcinoma. This study highlights TH's potential as a viable chemotherapeutic agent. The results suggest that TH's rich composition of antioxidants, such as phenolic and flavonoid compounds, may play a crucial role in its antitumor activity. Furthermore, the reduced incidence of OSCC in TH-treated groups underscores its effectiveness and potential to minimize cancer progression. Given its natural origin and lower side-effect profile compared to conventional chemotherapy, TH represents a promising avenue for future cancer treatment development. Continued research and clinical trials will be essential to fully establish its therapeutic benefits and practical applications in oncology.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

The authors would like to express their appreciation to University of Malaya for providing facilities and materials for this study. This research was supported and funded by FRGS Grant (Fp 030-2015 A) from the Ministry of Education Malaysia and a University of Malaya Postgraduate Research Grant (PG 253-2015 B). The ethical approval was obtained from Faculty of Medicine, Institutional Animal Care and Use Committee, University of Malaya (FOM-IACUC) prior to commencement of the study (Ethic reference no. 2016-171103/DENT/R/AMH). The research project was conducted under the supervision of: Ass Professor Aied M Alabsi, PhD, it was a part of an approved PhD student thesis. Ass Professor Aied M Alabsi, PhD conceived the study, May Al-koshab carried out the experiments and performed the statistical analysis, all authors drafted and reviewed the manuscript. All authors have read and approved the final manuscript. There is no conflict of interest.

References

1. International agency for research on cancer. Lip oral cavity factsheet. Globocan; 2020. Available from: Https://www.Uicc.Org/who-we-work/-un-partners/international-agency-research-cancer.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90. https://doi.org/10.3322/caac.20107.
3. Bakri MM, Cannon RD, Holmes AR, Rich AM. Detection of candida albicans adh1 and adh2 mrnas in human archival oral biopsy samples. J Oral Pathol Med. 2014;43(9):704-10. https://doi.org/10.1111/jop.12193.
4. Scully C, Bagan J. Oral squamous cell carcinoma: Overview of current understanding of aetiopathogenesis and clinical implications. Oral Dis. 2009;15(6):388-99. https://doi.org/10.1111/j.1601-0825.2009.01563.x.
5. Kerishnan J, Mah M-K, Fawzi N, Ramanathan A, Lim GS, Abdul Aziz A, et al. The association between epstein-barr virus (ebv) past infection with the risk of oral squamous cell carcinoma (oscc). Sains Malays. 2019;48:1969-74. https://doi.org/10.17576/jsm-2019-4809-18.
6. Saraswat N, Pillay R, Everett B, George A. Knowledge, attitudes and practices of south asian immigrants in developed countries regarding oral cancer: An integrative review. BMC Cancer. 2020;20(1):477. https://doi.org/10.1186/s12885-020-06944-9.
7. Shah JP, Gil Z. Current concepts in management of oral cancer--surgery. Oral Oncol. 2009;45(4-5):394-401. https://doi.org/10.1016/j.oraloncology.2008.05.017.
8. Kamarulzaidi MA, Yusoff Z, Mohamed AM, Adli D. Tualang honey consumption enhanced hippocampal pyramidal count and spatial memory performance of adult male rats. 2016;45:215-20.
9. Othman NH. Honey and cancer: Sustainable inverse relationship particularly for developing nations-a review. Evid Based Complement Alternat Med. 2012;2012:410406. https://doi.org/10.1155/2012/410406.
10. Mitra S, Dash R. Natural products for the management and prevention of breast cancer. Evid Based Complement Alternat Med. 2018;2018:8324696. https://doi.

org/10.1155/2018/8324696.
11. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, et al. Major phytochemicals: Recent advances in health benefits and extraction method. Molecules. 2023;28(2). https://doi.org/10.3390/molecules28020887.
12. Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res. 2011;31(4):322-5. https://doi.org/10.1016/j.nutres.2011.03.001.
13. Khalid A, Tan J, Yoke Keong FY. Malaysian tualang honey and its potential anti-cancer properties: A review. Sains Malaysiana. 2018;47:2705-11. https://doi.org/10.17576/jsm-2018-4711-14.
14. Yaacob NS, Nengsih A, Norazmi MN. Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid Based Complement Alternat Med. 2013;2013:989841. https://doi.org/10.1155/2013/989841.
15. Ghashm AA, Othman NH, Khattak MN, Ismail NM, Saini R. Antiproliferative effect of tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. BMC Complement Altern Med. 2010;10:49. https://doi.org/10.1186/1472-6882-10-49.
16. Fauzi AN, Norazmi MN, Yaacob NS. Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol. 2011;49(4):871-8. https://doi.org/10.1016/j.fct.2010.12.010.
17. Al-Koshab M, Alabsi AM, Bakri MM, Naicker MS, Seyedan A. Chemopreventive activity of tualang honey against oral squamous cell carcinoma-in vivo. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020;129(5):484-92. https://doi.org/10.1016/j.oooo.2020.01.009.
18. Milas z, myers j, caulin c. Animal models of oral cancer metastasis. In oral cancer metastasis. Springer new york. 2010. P. 135-161. https://doi.org/10.1007/978-1-4419-0775-2_7.
19. Al-koshab M, Alabsi A, Bakri M, Ali R, Naicker M. Antitumor activity of ficus deltoidea extract on oral cancer: An in vivo study. J Oncol. 2020;2020:1-10. https://doi.org/10.1155/2020/5490468.
20. Kanojia D, Vaidya MM. 4-nitroquinoline-1-oxide induced experimental oral carcinogenesis. Oral Oncol. 2006;42(7):655-67. https://doi.org/10.1016/j.oraloncology.2005.10.013.
21. Al-Afifi N, Alabsi A, Kaid F, Bakri M, Ramanathan A. Prevention of oral carcinogenesis in rats by dracaena cinnabari resin extracts. Clin Oral Investig. 2019;23(5):2287-301. https://doi.org/10.1007/s00784-018-2685-6.
22. Wali RK, Kunte DP, De La Cruz M, Tiwari AK, Brasky J, Weber CR, et al. Topical polyethylene glycol as a novel chemopreventive agent for oral cancer via targeting of epidermal growth factor response. PLoS One. 2012;7(6):e38047. https://doi.org/10.1371/journal.pone.0038047.
23. Al-Afifi NA, Alabsi AM, Shaghayegh G, Ramanathan A, Ali R, Alkoshab M, et al. The in vitro and in vivo antitumor effects of dracaena cinnabari resin extract on oral cancer. Arch Oral Biol. 2019;104:77-89. https://doi.org/10.1016/j.archoralbio.2019.05.030.
24. Hasina R, Martin LE, Kasza K, Jones CL, Jalil A, Lingen MW. Abt-510 is an effective chemopreventive agent in the mouse 4-nitroquinoline 1-oxide model of oral carcinogenesis. Cancer Prev Res (Phila). 2009;2(4):385-93. https://doi.org/10.1158/1940-6207.Capr-08-0211.
25. McCormick DL, Horn TL, Johnson WD, Peng X, Lubet RA, Steele VE. Suppression of rat oral carcinogenesis by agonists of peroxisome proliferator activated receptor γ. PLoS One. 2015;10(10):e0141849. https://doi.org/10.1371/journal.pone.0141849.
26. Thandavamoorthy P, Balan R, Subramaniyan J, Krishnan G, Rajendran R, Mani G, et al. Alleviative role of rutin against 4-nitroquinoline-1-oxide (4-nqo) provoked oral squamous cell carcinoma in experimental animal model. J Pharm Res. 2014;8(7):899-906.
27. Fearon KC, Preston T. Body composition in cancer cachexia. Infusionstherapie. 1990;17 Suppl 3:63-6. https://doi.org/10.1159/000222558.
28. Dayan D, Hirshberg A, Kaplan I, Rotem N, Bodner L. Experimental tongue cancer in desalivated rats. Oral Oncol. 1997;33(2):105-9. https://doi.org/10.1016/s0964-1955(96)00048-6.
29. Moradzadeh Khiavi M, Rostami A, Hamishekar H, Mesgari Abassi M, Aghbali A, Salehi R, et al. Therapeutic efficacy of orally delivered doxorubicin nanoparticles in rat tongue cancer induced by 4-nitroquinoline 1-oxide. Adv Pharm Bull. 2015;5(2):209-16. https://doi.org/10.15171/apb.2015.029.
30. Yamamoto K, Kitayama W, Denda A, Morisaki A, Kuniyasu H, Inoue M, et al. Suppressive effects of a selective cyclooxygenase-2 inhibitor, etodolac, on 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. Exp Toxicol Pathol. 2004;56(3):145-51. https://doi.org/10.1016/j.etp.2004.07.001.
31. Ohne M, Satoh T, Yamada S, Takai H. Experimental tongue carcinoma of rats induced by oral administration of 4-nitroquinoline 1-oxide (4nqo) in drinking water. Oral Surg Oral Med Oral Pathol. 1985;59(6):600-7. https://doi.org/10.1016/0030-4220(85)90189-6.