Original Article

The Effect of 5-Fluorouracil on the Tongue Mucosa of Adult Male Albino Rat and the Possible Protective Role of Melatonin: A Light and Scanning Electron Microscopic Study

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ABSTRACT

Background: 5-Fluorouracil (5-FU) is a common anti-tumor drug that is used in the management of different forms of malignancies. Mucosal inflammation of the oral cavity is the most common adverse effect of its administration. Melatonin has valuable activities like antioxidant and anti-inflammatory effects. Melatonin has protective effects on different organs against various side effects caused by anti-cancer treatments.

Aim of the work: To evaluate the protective effect of melatonin against 5-fluorouracil –induced changes in the tongue mucosa of rats using different histological techniques.

Materials and Methods: Forty adult male rats were randomly divided into four main groups: group I acted as control, group II was given melatonin at a dose 10 mg/kg/day, group III was given 5-FU at a dose 60 mg/kg on day 0 and 40 mg/kg on day 2, and group IV was given melatonin one hour before 5-FU administration. The specimens of the tongue were processed for light and scanning electron microscopic study. Immunohistochemical study was done by employing the nuclear marker for proliferation of the cells (Ki67) antibody.

Results: 5-FU induced structural changes in the tongue mucosa in the form of focal loss of lingual papillae, marked thinning and shortening of the filiform papillae, separation of the keratin layer from the underlying epithelium, vacuolated cytoplasm of the basal and suprabasal epithelial cells, and congestion of blood vessels with cellular infilteration in the lamina propria. A significant decrease in the papillae height, papillae width, ventral epithelial thickness, and percentage of Ki67 immunopositive cells were also detected. Scanning electron microscopy exhibited atrophy of the filiform papillae with desquamation of their epithelial covering. Deformed fungiform papillae with ill-defined taste pores were also revealed. On the other hand, these changes were less pronounced in rats received melatonin before 5-FU administration.

Conclusion: 5-FU induced significant structural changes in the tongue mucosa of albino rats. Melatonin attenuated these mucosal changes.

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Key Words: 5-Fluorouracil; ki67; melatonin; scanning electron microscopy; tongue mucosa.

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INTRODUCTION

5-Fluorouracil (5-FU) is a common anti-tumor drug that is used in the management of various forms of malignancies, including cancers of head, neck, colon, and breast[1]. However, many harmful effects may occur during the treatment period, such as mucositis, bone marrow suppression, dermatitis, gastrointestinal problems, and cardiomyopathy[2]. The most common adverse effect of 5-FU use is oral mucositis[3]. This condition is known as mucosal inflammation of the oral cavity induced by the disruption of the epithelial cells of the oral mucosa and inhibition of their growth due to treatment of cancer with either chemotherapy drugs or radiotherapy[4]. This mucosal inflammation usually results in pain, local and systemic infections, parenteral nutrition, longer hospital days, and may lead to stoppage of the treatment[5].

The tongue mucosa in rats is made up of an outermost keratinized stratified squamous epithelium below the lamina propria, which is formed of dense connective tissue. The mucosa of the dorsal surface shows various forms of papillae. The anterior part of the dorsal mucosa reveals filiform and fungiform papillae, while the posterior part shows few circumvallate papillae[6]. It is believed that filiform papillae serve as a reflection of the health condition, so dietary insufficiency or medication toxicity will cause changes to these papillae[3].

Melatonin is a hormone naturally secreted by the pineal gland. Previous researches revealed other sources of melatonin as blood lymphocytes, gastrointestinal tract, and retina[7]. Moreover, it is present in many plants as cereals, ginger, olives, tomatoes, walnuts, pineapple, and legumes[8]. Melatonin is mainly concerned with the circadian control of biological activities as sleep and aging. Moreover, it regulates the endocrine and immune functions[9]. It also attracted considerable attention due to its antioxidant and anti-inflammatory effects[10]. A previous study showed that melatonin had protective effects on gut mucosa[11]. Moreover, melatonin was reported to have protective effects on different organs against various side effects induced by anti-cancer treatments, either chemotherapy or radiotherapy[12,13].

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Multiple compounds were employed for treatment or inhibition of oral mucositis, but there is no effective procedure[14]. Therefore, new materials need to be identified that can prevent or ameliorate the mucosal injury induced by 5-FU. Based on the properties of melatonin mentioned before, the current research aimed to investigate the ameliorative effect of melatonin against changes in the tongue mucosa of rats caused by 5-fluorouracil.

MATERIALS AND METHODS

Animals

Forty adult male albino rats were employed in this research. Their average weight is (180 – 200) grams. The animals were acclimatized to the laboratory condition for 14 days. All animal work was approved by the local ethical committee of the Faculty of Medicine, Tanta University, Egypt (Approval number: 33940/7/20).

Drugs

5-Fluorouracil (Utoral) (250 mg/5 ml) ampoules were purchased from EIMC United Pharmaceuticals Company, Egypt. Melatonin was supplied by Sigma Aldrich Company in the form of powder that was dissolved in 1% ethanol in normal saline (100 mg melatonin was dissolved in 0.25 ml ethanol then diluted with normal saline to make a total volume of 25 ml. Each 1 ml contained 4 mg melatonin).

Study Design

The rats were randomly divided into four groups:

Group I (Control group): included ten rats that were further subdivided into two equal subgroups:

Subgroup (i) which received no treatment until the end of the experiment and Subgroup (ii) that received an intraperitoneal injection of 0.5 ml of 1% ethanol in normal saline, the diluting vehicle for melatonin, for 10 consecutive days.

Group II (Melatonin group): included ten rats that were administrated melatonin at a dose 10 mg/kg /day i.p for consecutive ten days[15].

Group III (5-FU group): included ten rats that were administrated 60 mg/kg i.p of 5-FU on day 0, and 40 mg/kg i.p on day 2[3].

Group IV (melatonin-5-FU group): included ten rats that were given melatonin one hour before 5-FU administration and continued once daily until anesthesia. Melatonin and 5-FU were administrated at a dose and duration as that of the previous groups.

Lastly, 24 h after the last injection of melatonin, all animals were anesthetized with sodium pentobarbital at a dose 30 mg/kg i.p[16]. The tongues were excised and cleaned. The anterior two-thirds of the tongues were prepared for light and scanning electron microscopic study.

Light microscopy

Sagittal tongue specimens from each animal in each group were fixed in 10% formal saline for 24 h, dehydrated in ascending series of ethyl alcohol and embedded in paraffin. 5 μm histological sections were cut and subjected to hematoxylin and eosin staining[17].

Immunohistochemistry

Briefly, sections of 5 μm were dewaxed and were put in the antigen retrieval solution to amplify the signal. After abolishing the activity of endogenous peroxidase with 3% hydrogen peroxide for 10 min and nonspecific protein binding, by 10% normal goat serum in PBS for 1 h at room temperature, the slides were then incubated for two hours with the diluted primary antibody against Ki67 (mouse monoclonal antibody, 1:20 dilution, SC-126, Santa Biotechnology, INC) which is a nuclear marker for proliferation of the cells. After washing the slides with phosphate-buffered saline (PBS), the corresponding biotinylated secondary antibody was added to the tongue sections followed by the streptavidin-biotin complex. The reactions were completed with Diaminobenzidine (DAB) as chromogen, counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted with DPX[18].

Light microscope examination was done at the histology department, Faculty of Medicine, Tanta University.

Scanning electron microscopy

After fixation of the tongue specimens in 4% phosphate-buffered glutaraldehyde (0.1 mol/L, pH 7.4), they were post-fixed in1% phosphate-buffered osmium tetroxide. Then the specimens were dehydrated in serial dilutions of ethanol and placed into amyl acetate. The samples were then dried with liquid CO2 and coated with gold particles[19]. The dorsal surfaces of the tongue samples were examined under a JEOL JSM-5200LV scanning electron microscope at the Electron Microscopic Unit, Faculty of Medicine, Tanta University, Egypt.

Morphometric study

Leica Qwin 500 image analyzer computer system was used to measure the mean length and width of the filiform and fungiform lingual papillae and the mean ventral epithelial thickness in H&E-stained sections at a magnification of × 400. The mean number percentage of Ki67 positive cells, in DAB-stained sections at a magnification of × 400 was also measured. Ten non-overlapping randomly selected fields in the slides from each rat in all groups were measured.

Statistical analysis

The morphometric data were compared by using one-way ANOVA and Tukey-kramer post-test. The values were expressed as mean ± SD. The probability (*P*) value less than 0.05 indicated a significant difference[20].

RESULTS

Only one animal died in the 5-FU group. Both subgroups of the control group showed no statistical difference in the histological results, so they were presented as the control group. As regards the histological, immunohistochemical results in both groups I (control group) & II (melatonin group), they showed a non-significant statistical difference.

Histological results

H&E-stained sections

Examination of tongue sections of the control group demonstrated the normal histological structure of the rat tongue. The dorsal surface revealed numerous papillae and was covered by a mucous membrane composed of keratinized stratified squamous epithelium with underlying connective tissue of the lamina propria and lingual skeletal muscles that run in different directions (Figure 1). The anterior two-thirds of the dorsal surface of the tongue had two types of papillae; filiform papillae were numerous conical in shape with pointed tips and covered by keratinized stratified squamous epithelium (Figure 2). The fungiform papillae were few and scattered in-between the filiform ones. They had a single taste bud on its upper surface and were covered by keratinized stratified squamous epithelium (Figure 3). The ventral surface of the tongue was covered by a smooth mucus membrane composed of keratinized stratified squamous epithelium with underlying connective tissue and lingual muscles (Figure 4).

Examination of tongue sections of the 5-FU group revealed numerous changes in both dorsal and ventral surfaces. The dorsal surface showed focal loss of lingual papillae with shallow epithelial ridges (Figure 5), marked thinning and shortening of the filiform papillae (Figure 6), keratin separation from the underlying epithelium (Figure 7), vacuolated cytoplasm of the basal and suprabasal epithelial cells (Figure 8), and congested blood vessels with cellular infilteration in the lamina propria (Figure 9). As regards the fungiform papillae, they were disfigured (Figure 10). The ventral surface showed focal thinning of the epithelium with loss of the keratin layer (Figure 11). Vacuolated cytoplasm of the basal epithelial cells (Figure 12) was also detected. Moreover, the lamina propria showed dilated congested blood vessels (Figure 13).

Examination of tongue sections of the melatonin-5-FU group revealed almost restoration of the structure of the tongue mucosa. The dorsal surface was covered by keratinized stratified squamous epithelium and exhibiting numerous papillae (Figure 14). Most of the dorsum was covered by normal filiform papillae with tapering ends while few areas showed short papillae with blunt ends (Figure 15). The fungiform papillae appeared normal as that of the control group (Figure 16). The ventral surface that appeared normal and was covered by keratinized stratified squamous epithelium with underlying connective tissue (Figure 17). The morphometric analysis revealed a significant decrement in the mean length and width of both the filiform and fungiform papillae of group III (198.95±3.083 μm, 98.671±1.538 μm, 96.143±3.159 μm, 127.314±3.856 μm, respectively) in comparison with the control group (336.786±8.235 μm, 119.557±2.077 μm, 206.129±3.406 μm, 149.314±0.908 μm, respectively). Also, group IV revealed a significant decrement in the mean length of the filiform papillae (327.914±4.931 μm) in comparison with the control group. As regards the mean ventral epithelial thickness of group III it revealed a significant decrement (19.114±2.737 μm) in comparison with the control group (42.657±3.793 μm) (Table 1).

Immunohistochemical results

Examination of Ki67-immunostained tongue sections of the control group exhibited numerous Ki67 positive cells in the basal and suprabasal epithelial cells of the dorsum of the tongue (Figure 18). The ventral surface showed numerous Ki67 positive cells in the basal and suprabasal epithelial cells (Figure 19). While sections from the 5-FU group showed only some Ki67 positive cells in the basal epithelial cells in both dorsal and ventral surfaces (Figures 20,21). As regards the melatonin-5-FU group, it revealed many Ki67 positive cells in the basal and suprabasal epithelial cells of both surfaces (Figures 22,23). The statistical analysis of the mean number percentage of Ki67 immunopositive cells of group III revealed a significant decrement in the mean number percentage of Ki67 positive cell of both dorsal and ventral surfaces (36.400±5.094, 17.229±0.335, respectively) when compared with the control group (64.429±4.041, 29.357±0.257, respectively) (Table 1).

Scanning electron microscopic results

Examination of the tongue from the control group displayed the dorsal surface covered with numerous filiform papillae with scattered fungiform ones in-between (Figure 24). The filiform papillae were elongated conical in shape with tapering ends ad had one direction (Figure 25) and had intact covering (Figure 26). The fungiform papillae had broad surfaces and taste pores on their upper surfaces (Figure 27). While the 5-FU group revealed marked shortening and thinning of the filiform papillae with loss papillae in some areas (Figure 28). Moreover, the filiform papillae showed desquamation of their covering epithelium (Figure 29). Also, deformed fungiform papillae with ill-defined taste pores appeared among disfigured filiform ones (Figure 30). As regards the melatonin-5-FU group, it revealed partial preservation of the tongue papillae. Some areas showed almost normal filiform papillae (Figure 31). While other areas revealed short filiform papillae with blunt ends and intact covering (Figures 32,33). The fungiform papillae were normal and showed the characteristic taste pores on their upper flat surface (Figure 34).

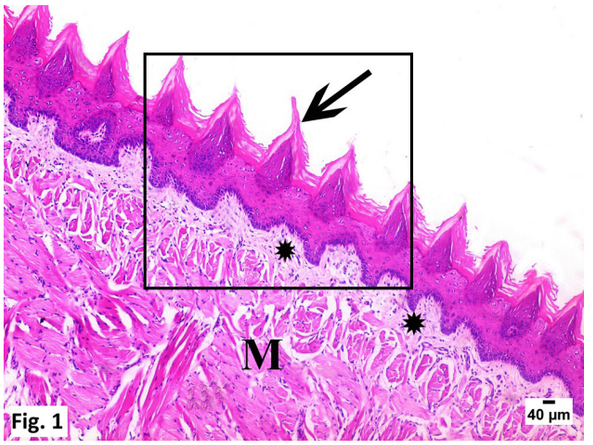

Fig. 1: The dorsal surface of the tongue has many papillae and is covered by a mucous membrane composed of keratinized stratified squamous epithelium (arrow) with underlying connective tissue of the lamina propria (asterisks) and lingual skeletal muscles that run in different directions (M). (Control group, H&E × 100, scale bar = 40 µm).

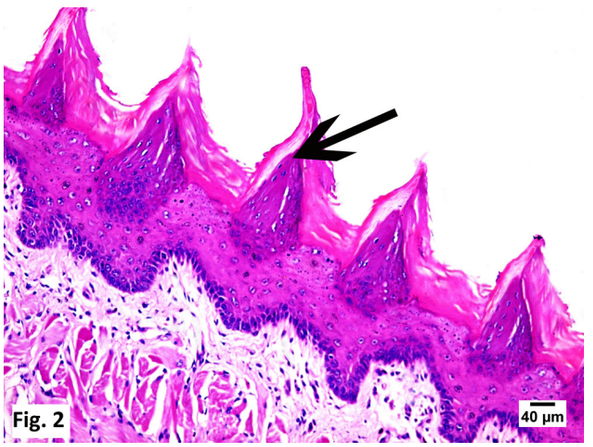

Fig. 2: A higher magnification of the previous figure revealing numerous regularly arranged filiform papillae which are conical in shape with pointed tips covered by keratinized stratified squamous epithelium (arrow). (Control group, H&E × 200, scale bar = 40 µm).

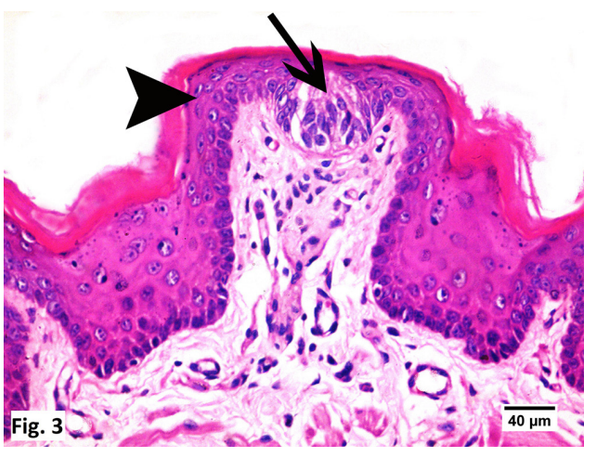

Fig. 3: A fungiform papilla with a single taste bud on its upper surface (arrow) and is covered by keratinized stratified squamous epithelium (arrowhead). (Control group, H&E × 400, scale bar = 40 µm).

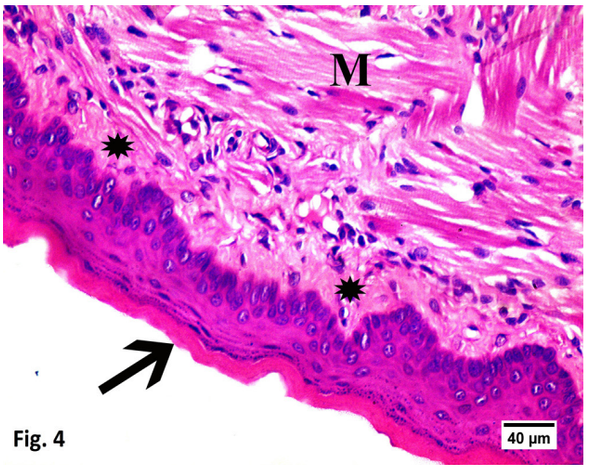

Fig. 4: The smooth ventral surface of the tongue is lined by keratinized stratified squamous epithelium (arrow) with underlying connective tissue (asterisks) and lingual muscles (M). (Control group, H&E × 400, scale bar = 40 µm).

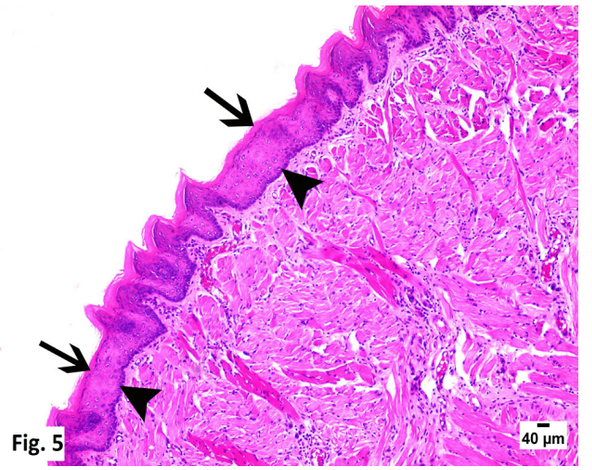

Fig. 5: Focal loss of lingual papillae (arrows) with shallow epithelial ridges (arrowheads). (5-FU group, H&E × 100, scale bar = 40 µm).

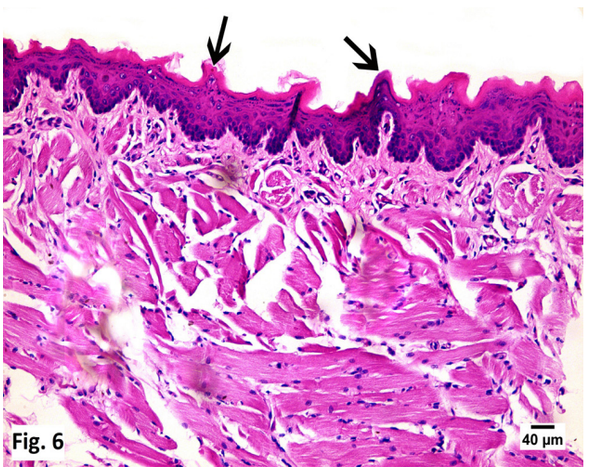

Fig. 6: Marked shortening and thinning of the filiform papillae (arrows). (5-FU group, H&E × 200, scale bar = 40 µm).

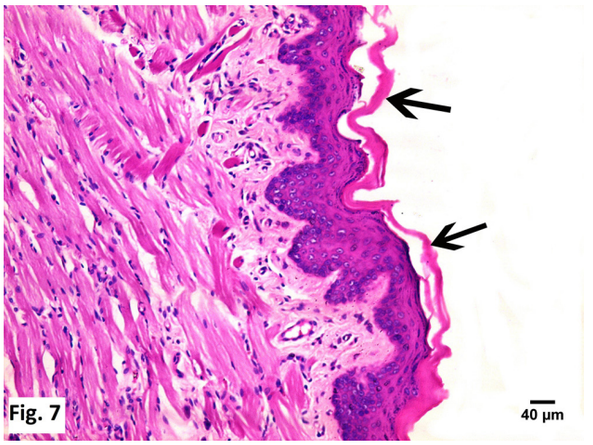

Fig. 7: Separation of the keratin layer from the underlying epithelium of the dorsal surface (arrows). (5-FU group, H&E × 200, scale bar = 40 µm).

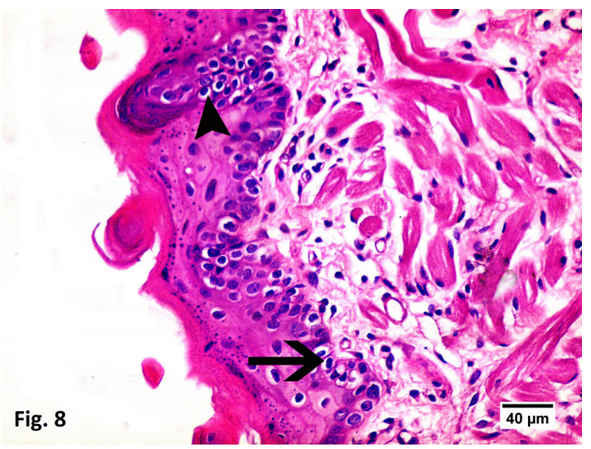

Fig. 8: Vacuolated cytoplasm of the basal (arrow) and suprabasal epithelial cells (arrowhead) of the dorsal surface of the tongue. (5-FU group, H&E × 400, scale bar = 40 µm).

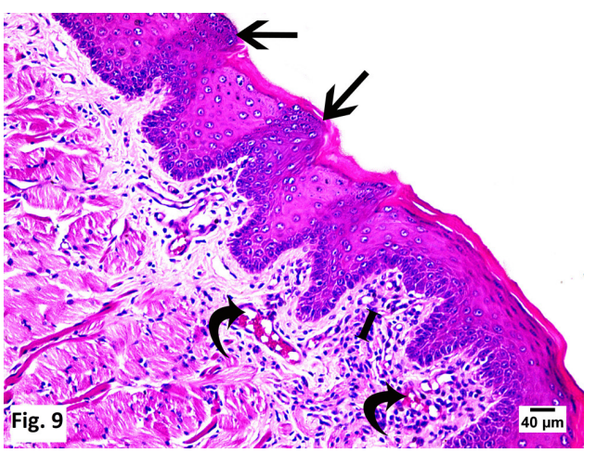

Fig. 9: Congested blood vessels (curved arrows) and cellular infilteration (I) in the connective tissue of the lamina propria of the dorsal surface. Notice, the presence of short filiform papillae (arrows). (5-FU group, H&E × 200, scale bar = 40 µm).

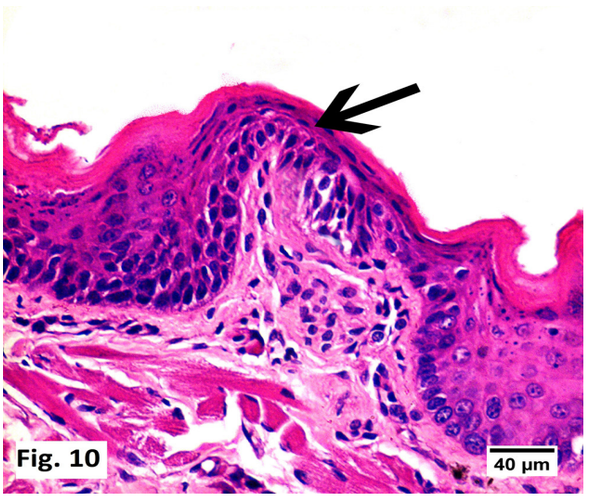

Fig. 10: A disfigured fungiform papilla (arrow). (5-FU group, H&E × 400, scale bar = 40 µm).

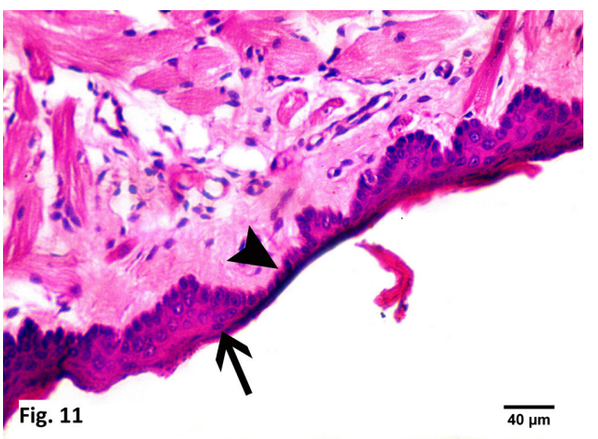

Fig. 11: Focal thinning of the epithelium (arrowhead) and loss of the keratin layer (arrow) from the ventral surface of the tongue. (5-FU group, H&E × 400, scale bar = 40 µm).

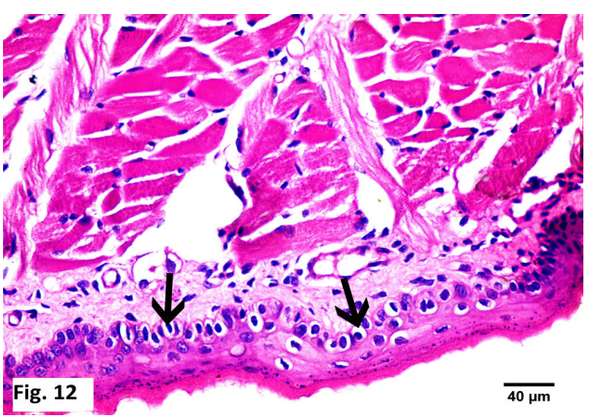

Fig. 12: Vacuolated cytoplasm of the basal epithelial cells (arrows) of the ventral surface of the tongue. (5-FU group, H&E × 400, scale bar = 40 µm).

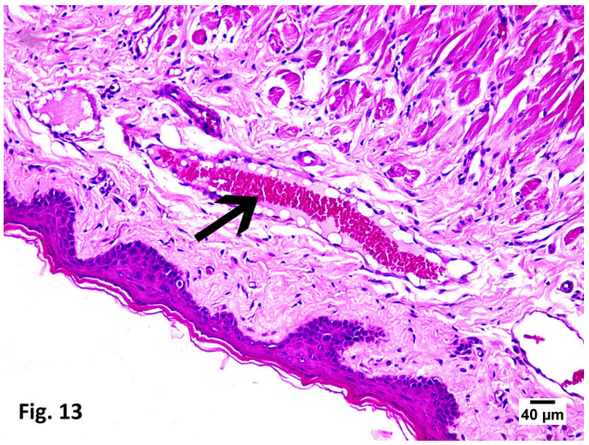

Fig. 13: A dilated congested blood vessel in the lamina propria (arrow) of the ventral surface of the tongue. (5-FU group, H&E × 400, scale bar = 40 µm).

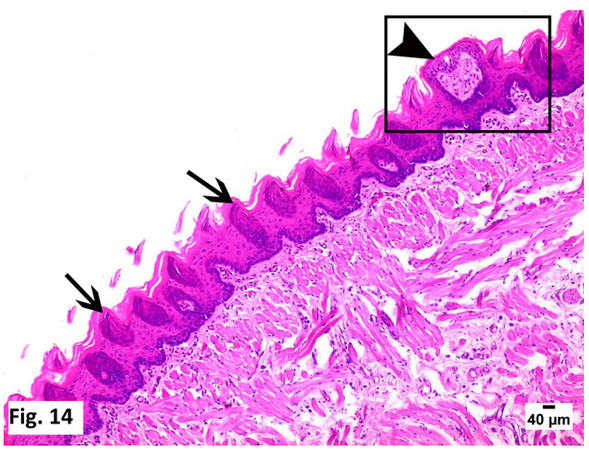

Fig. 14: The dorsal surface of the tongue is covered by keratinized stratified squamous epithelium and exhibiting numerous filiform papillae (arrows) and a fungiform one (arrowhead). (Melatonin-5-FU group, H&E × 100, scale bar = 40 µm).

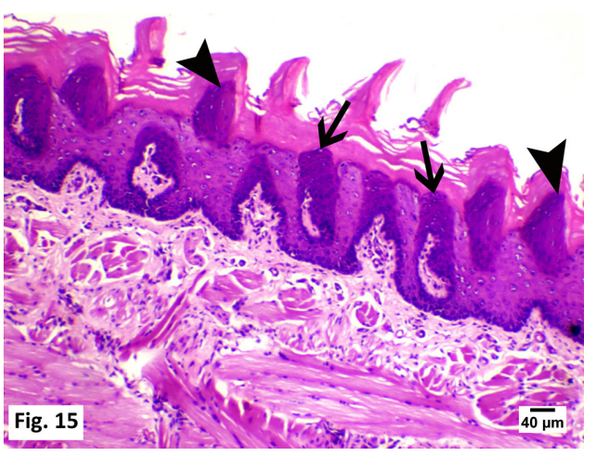

Fig. 15: The dorsal surface of the tongue revealing numerous normal filiform papillae with tapering ends (arrowheads). Notice a focal area with short papillae with blunt ends (arrows). (Melatonin-5-FU group, H&E × 200, scale bar = 40 µm).

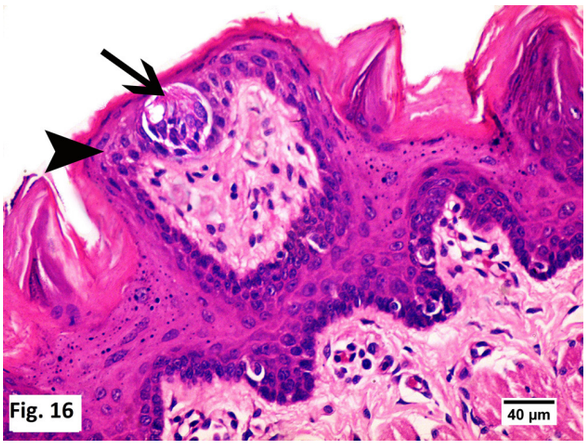

Fig. 16: A higher magnification of figure 14 showing a fungiform papilla covered by stratified squamous epithelium keratinized (arrowhead) and having a single taste bud on its top (arrow). (Melatonin-5-FU group, H&E × 400, scale bar = 40 µm).

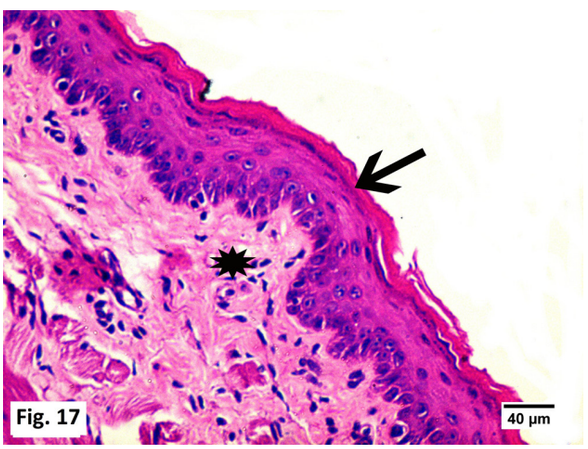

Fig. 17: The ventral surface of the tongue appears normal and is covered by keratinized stratified squamous epithelium (arrow) with underlying connective tissue (asterisk). (Melatonin-5-FU group, H&E × 400, scale bar = 40 µm).

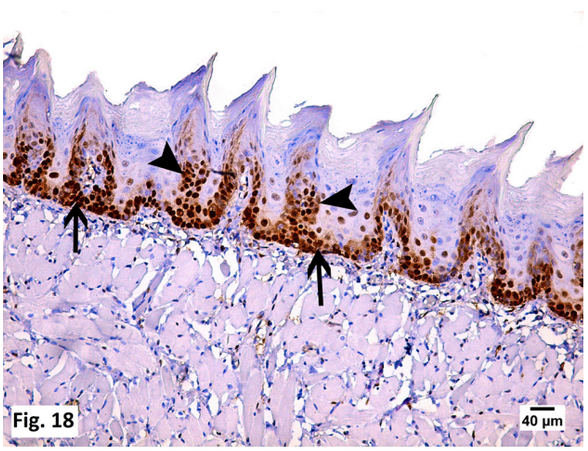

Fig. 18: The dorsal surface of the tongue exhibiting numerous Ki67 positive cells in the basal (arrows) and suprabasal (arrowheads) epithelial cells. (Control group, Ki67 immunostaining × 200, scale bar = 40 µm).

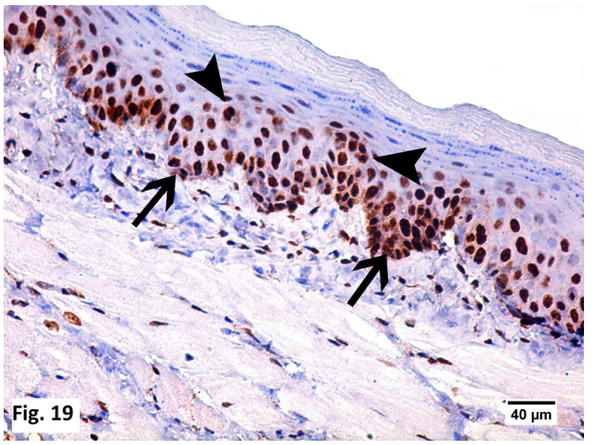

Fig. 19: The ventral surface of the tongue exhibiting numerous Ki67 positive cells in the basal (arrows) and suprabasal (arrowheads) epithelial cells. (Control group, Ki67 immunostaining × 400, scale bar = 40 µm).

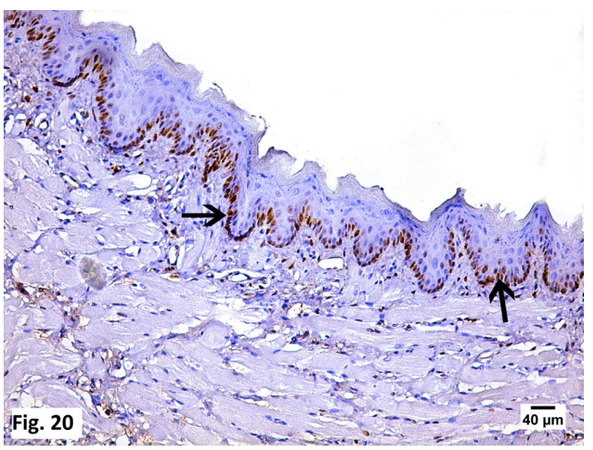

Fig. 20: The dorsal surface of the tongue revealing some Ki67 positive cells in the basal epithelial cells (arrows). (5-FU group, Ki67 immunostaining × 200, scale bar = 40 µm).

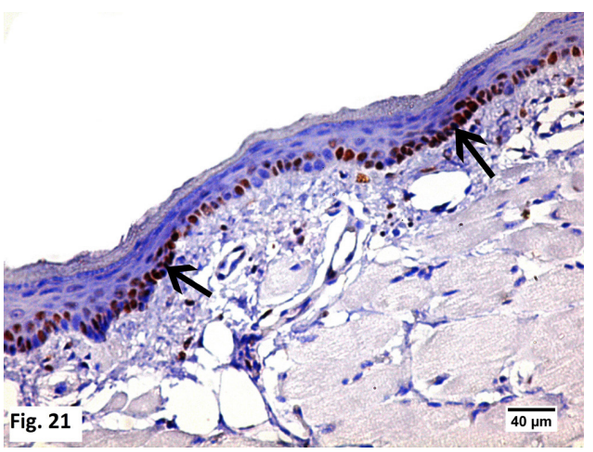

Fig. 21: The ventral surface of the tongue exhibiting some Ki67 positive cells in the basal epithelial cells (arrows). (5-FU group, Ki67 immunostaining × 400, scale bar = 40 µm).

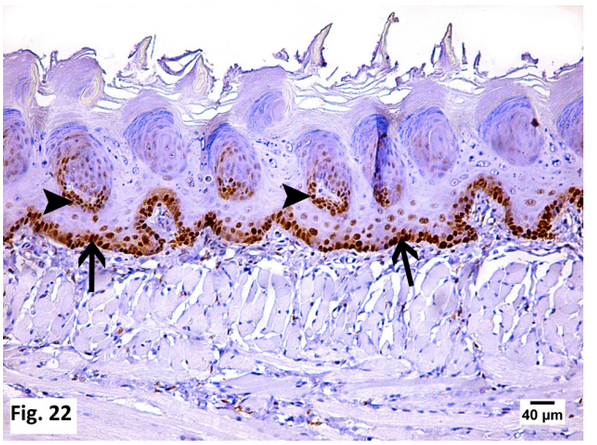

Fig. 22: The dorsal surface of the tongue has many Ki67 positive cells in the basal (arrows) and suprabasal (arrowheads) epithelial cells. (Melatonin-5-FU group, Ki67 immunostaining × 200, scale bar = 40 µm).

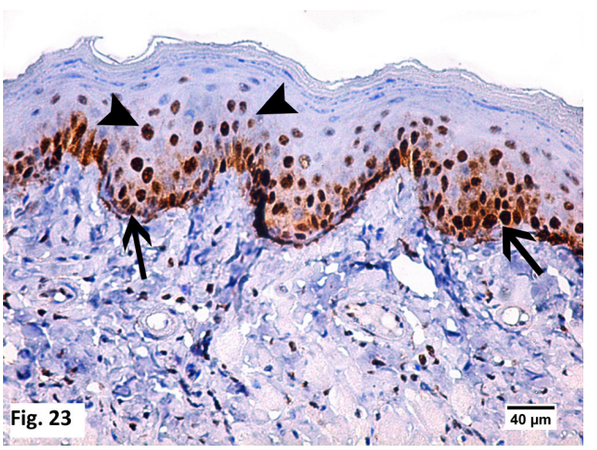

Fig. 23: The ventral surface of the tongue has many Ki67 positive cells in the basal (arrows) and suprabasal (arrowheads) epithelial cells. (Melatonin-5-FU group, Ki67 immunostaining × 400, scale bar = 40 µm).

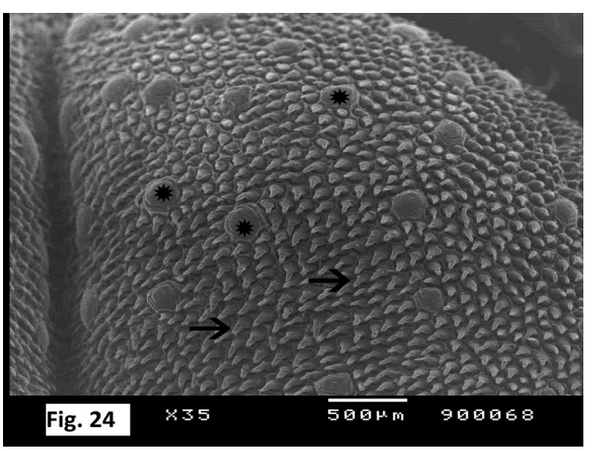

Fig. 24: The dorsal surface of the tongue is covered with numerous filiform papillae (arrows) with scattered fungiform ones in-between (asterisks). (Control group, SEM × 35, scale bar = 500 µm).

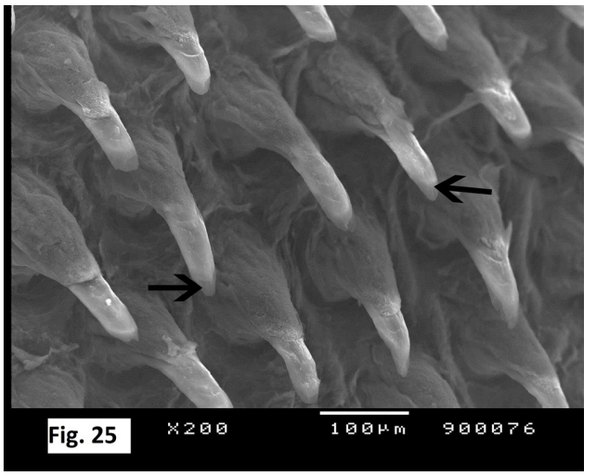

Fig. 25: Unidirectional filiform papillae which are elongated conical in shape with tapering ends (arrows). (Control group, SEM × 200, scale bar = 100 µm).

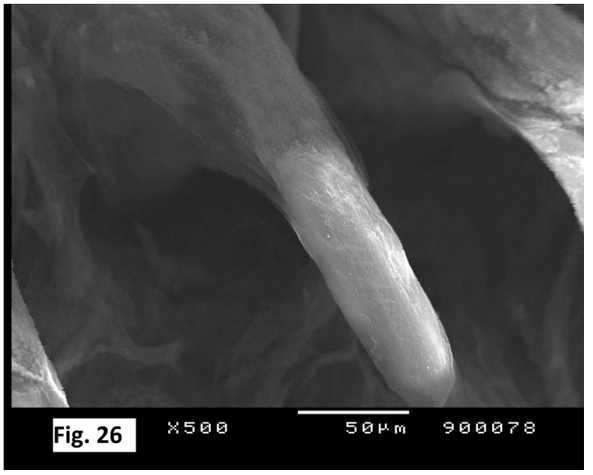

Fig. 26: A filiform papilla with an intact epithelial covering. (Control group, SEM × 500, scale bar = 50 µm).

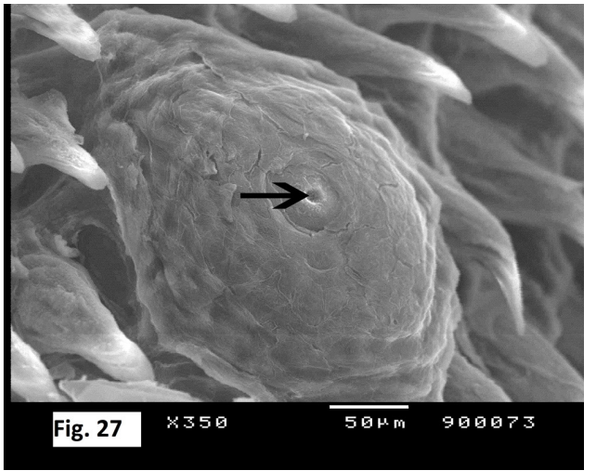

Fig. 27: A fungiform papilla with a broad surface and has a taste pore on its upper surface (arrow). (Control group, SEM × 350, scale bar = 50 µm).

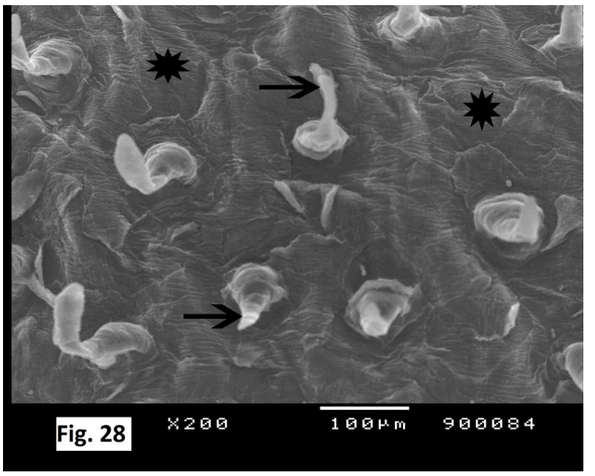

Fig. 28: Marked shortening and thinning of the filiform papillae (arrows) with areas of papillae loss (asterisks). (5-FU group, SEM × 200, scale bar = 100 µm).

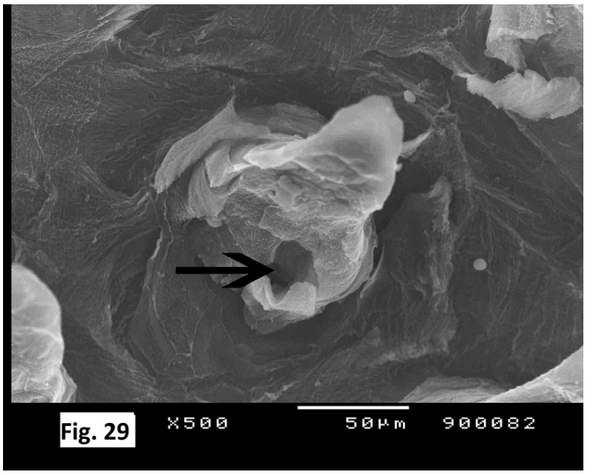

Fig. 29: A filiform papilla with desquamation of its covering epithelium (arrow). (5-FU group, SEM × 500, scale bar = 50 µm).

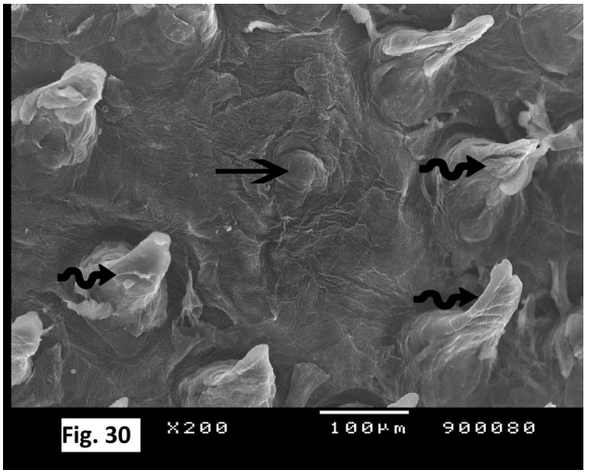

Fig. 30: A deformed fungiform papilla with ill-defined taste pore (arrow). Notice disfigured filiform papillae (wavy arrows). (5-FU group, SEM × 200, scale bar = 100 µm)

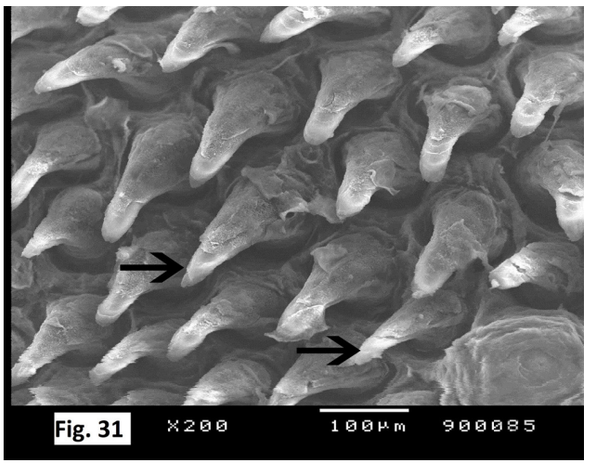

Fig. 31: Conical filiform papillae with tapering ends (arrows). (Melatonin-5-FU group, SEM × 200, scale bar = 100 µm).

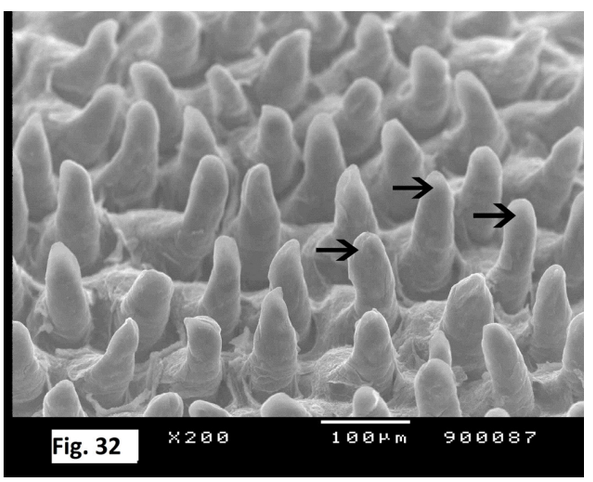

Fig. 32: Apparent shortening of the filiform papillae with blunt ends (arrows). (Melatonin-5-FU group, SEM × 200, scale bar = 100 µm).

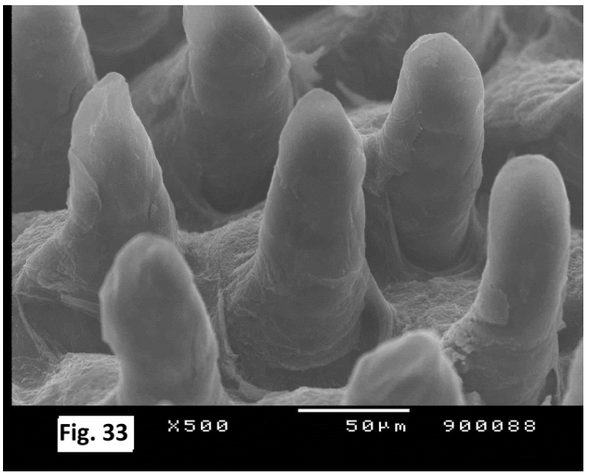

Fig. 33: The filiform papillae with intact epithelial covering. (Melatonin-5-FU group, SEM × 500, scale bar = 50 µm).

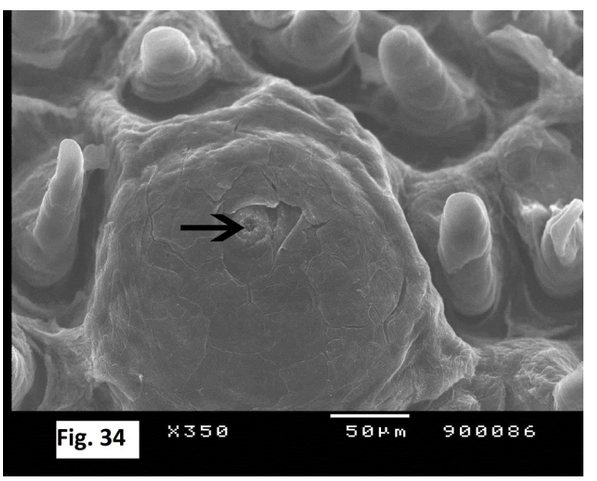

Fig. 34: A fungiform papilla with a characteristic taste pore on its upper flat surface (arrow). (Melatonin-5-FU group, SEM × 350, scale bar = 50 µm).

Table 1: Morphometrical and statistical analysis of different studied groups

	Control group (Group I) Mean± SD	Melatonin group (Group II) Mean± SD	5-FU group (Group III) Mean± SD	Melatonin-5-FU group (Group IV) Mean± SD
Filiform papillae Mean length (µm)	336.786±8.235	333.600±3.093	198.957±3.083*	327.914±4.931*
Filiform papillae Mean width (µm)	119.557±2.077	118.914±2.902	98.671±1.538*	117.071±2.210
Fungiform papillae Mean length (µm)	206.129±3.406	203.086±3.724	96.143±3.159*	201.029±3.948
Fungiform papillae Mean width (µm)	149.314±0.908	147.686±2.594	127.314±3.856*	145.486±2.581
Ventral epithelial thickness (µm	42.657±3.793	44.443±3.908	19.114±2.737*	40.371±3.617
Mean number percentage of Ki67 positive cell of dorsal surface	64.429±4.041	61.829±6.701	36.400±5.094*	60.371±2.657
Mean number percentage of Ki67 positive cell of ventral surface	29.357±0.257	29.971±0.972	17.229±0.33*	28.571±0.528

* Significant versus control

DISCUSSION

Chemotherapy and radiation therapy are considered the first line of treatment of different types of malignant tumors, but they have harmful effects on patients[21]. Cancer treatments usually affect proliferating cells either neoplastic or healthy[22]. The mucosa of the oral cavity is vulnerable to damage by chemotherapy due to the high proliferative activity of its cells, as well as the presence of different microflora within the oral cavity[23]. Inflammation of the oral mucosa is the usual side effect of cancer treatment[24]. It occurs in 20-40% of cases administrating the usual dose and 80% of persons received a high dose protocol as well as in all cases having radiotherapy for treatment of cancers in the head and neck[25,26]. In our research, we described the 5-FU-induced oral mucosal injury in a rat model that represented the human histopathological reactions of the oral mucosa to 5-FU. Moreover, we investigated the role of melatonin in alleviating this mucosal injury. The dorsal surface of the tongue exhibited numerous filiform papillae and they are vulnerable to changes including loss as well as they undergo atrophy more easily and rapidly than other papillae[27]. Moreover, the filiform papillae have high metabolic activity, so they are usually affected by enzymatic disturbance or drug toxicity resulting in their atrophy[6].

Our research revealed that 5-FU caused structural changes in the tongue mucosa as manifested by light and scanning electron microscopic studies. These changes were in the form of focal loss of lingual papillae, marked thinning and shortening of the filiform papillae, separation of the keratin layer from the underlying epithelium, vacuolated cytoplasm of the basal and suprabasal epithelial cells, and congested blood vessels with cellular infilteration in the lamina propria. Moreover, the 5 -FU decreased the proliferation of tongue mucosa manifested by the significant decrement in the Ki67 positive cells in the 5-FU group in comparison with the control group. Results of the present research coincide with previous researches that demonstrated the same changes in the mucosa of the tongue of hamsters and mice induced by 5-FU[1,28], methotrexate[29], and irinotecan in rats[30].

The oral mucosal injury caused by chemotherapy including 5 -FU is thought to be due to the production of oxidative stress and generation of reactive oxygen species (ROS), that activate different cellular signals that lead to mucosal damage[31]. Moreover, chemotherapy activated the proinflammatory cytokines. These cytokines enhance damage and apoptosis of particularly basal epithelial cells and submucosal ones[32]. As well as the strong cytotoxic impact of anti-tumor drugs on the basal epithelial cells, connective tissue, and the oral microflora[33]. Such injury of the oral mucosa creates a site of entry of viruses, bacteria, and fungi with subsequent increase risk of infection[28].

The present work demonstrated atrophic changes in the tongue papillae in the 5-FU group accompanied with a considerable decrement in Ki67 positive cells. This may be due to the inhibition of epithelial reproduction by 5-FU. As it incorporates into DNA and RNA, preventing their functions and arresting the cells in S phase[34]. Thus, inhibition of DNA synthesis and damage of DNA caused by ROS impair the progenitor cell's metabolism, causing inhibition of mitosis and increasing apoptosis[3]. The present study showed dilated congested blood vessels accompanied with cellular infiltration, which are considered signs of inflammation. This could be explained by the generation of ROS and the release of proinflammatory cytokines, which directly or indirectly increased the vascular permeability[35].

Our work demonstrated that pre-treatment with melatonin attenuated the structural damages in the tongue mucosa caused by 5-FU. This was due to the antioxidant effect of melatonin, which stimulates the production and activation of endogenous antioxidant enzymes, including glutathione peroxidase, catalase, and superoxide dismutase, thus it is considered a powerful free radical scavenger[36,37]. Thus, melatonin is efficient in protecting against damage induced oxidative stress. It can cross the plasma membrane and reach different organelles, including mitochondria and protect them against damage[38,39].

Our study revealed that melatonin minimized the inflammation in the mucosa of the tongue. This result coincides with the result of Tahan *et al*.[40] who indicated the anti-inflammatory property of melatonin on colon inflammation in rats. This effect is attributed to the prevention of pro-inflammatory cytokines release which enhances adherence of leukocytes to the endothelial cells. Therefore, melatonin inhibits migration of the cells and inflammation, which cause tissue damage[41]. An increase in the Ki67 positive cells was demonstrated in rats pre-treated with melatonin. This result is in line with Mohseni *et al*.[42] who described the anti-apoptotic effect of melatonin on blood lymphocytes of rats and attributed this to the inhibition of apoptotic proteins and promoting the anti-apoptotic ones as well repairing the damaged parts in the DNA within the cell[43,44]. In the same line, Khan *et al*.[45] demonstrated that melatonin administration before a high dose of irradiation resulted in 100% survival and maintenance gastrointestinal system in mice through regulation of pro-apoptotic genes and elevation of anti-apoptotic ones in the organs as well as it protected against damage of proliferative progenitor stem cells and improved their ability to regenerate the damaged tissue.

Melatonin was reported to be synthesized in the mucosa of the oral cavity and is essential in the physiology of the oral mucosa[46]. Moreover, it has a valuable role in inhibiting the diseases of the oral cavity by attenuating the tissue injury caused by free radicals and by increasing the immune responses[47]. Accumulated data suggested that melatonin decreased the harmful effects of chemotherapy on different organs. Melatonin protected the heart tissue against Adriamycin and doxorubicin-induced damage in rats[48,49]. It also protected against cisplatin and cyclophosphamide-induced seminiferous tubules injury in rats[50]. In addition, it was reported that melatonin decreased the damage of

small intestine caused by methotrexate administration in rats through preventing oxidative stress, suggesting that oral administration of melatonin can ameliorate the methotrexate-induced intestinal damage and may be effective in improving enteritis caused by methotrexate in the human[11].

CONCLUSION

We can conclude that 5-FU has a harmful effect on the mucosa of the oral cavity causing histopathological changes. Importantly, melatonin might be useful in protecting against these changes. Therefore, it is recommended for patients administrating anti-tumor drugs to employ melatonin to decrease oral complications. This is an experimental study and its clinical application needs further investigations and more research to guide its optimal use.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير ٥-فلورويوراسيل على الغشاء المخاطي للسان في ذكورالجرذان البيضاء البالغة والدور الوقائي المحتمل للميلاتونين: دراسة بالمجهر الضوئي والمجهر الإلكتروني الماسح

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المقدمة: ٥-فلورويوراسيل هو دواء شائع مضاد للأورام يستخدم في علاج أشكال مختلفة من الأورام الخبيثة. وكان التهاب الغشاء المخاطي المبطن للفم هو التأثير الضار الأكثر شيوعًا لإستخدامه. الميلاتونين له أنشطة قيمة كمضاد للأكسدة ومضاد للالتهاب. الميلاتونين له تأثيرات وقائية على الأعضاء المختلفة ضد الآثار الجانبية المختلفة التي تسببها العلاجات المضادة للسرطان.

الهدف من البحث: تقييم التأثير الوقائي للميلاتونين ضد التغيرات المحدثة ب ٥-فلورويوراسيل في الغشاء المخاطي للسان الجرذان باستخدام مختلف التقنيات النسيجية.

مواد وطرق البحث: تم تقسيم أربعين جرذا من الذكور البالغين بشكل عشوائي إلى أربع مجموعات: المجموعة الأولى عملت كمجموعة ضابطة ، المجموعة الثانية أعطيت الميلاتونين بجرعة ١٠ مجم / كجم / يوم ، المجموعة الثالثة أعطيت ٥-فلورويوراسيل بجرعة ٦٠ مجم / كجم في اليوم ٠ و ٤٠ مجم / كجم في اليوم الثاني ، وتم إعطاء المجموعة الرابعة الميلاتونين قبل ساعة واحدة من تناول ٥-فلورويوراسيل. تم تجهيز عينات من اللسان للدراسة بالمجهر الضوئى والالكترونى الماسح. وقد أجريت دراسة هستوكيميائية مناعية باستخدام الأجسام المضادة للدلالة النووية لتكاثر الخلايا Ki.

النتائج: تسبب ٥-فلورويوراسيل في حدوث تغييرات في الغشاء المخاطي للسان في شكل فقدان بؤري للحليمات اللسانية ، وقصرملحوظ للحليمات الخيطية ، وفصل طبقة الكيراتين عن الخلايا الطلائية، ووجود تجاويف في سيتوبلازم الخلايا الطلائية، واحتقان الأوعية الدموية مع وجود تسلل خلوي. كما كان هناك انخفاض ذو دلالة احصائية في ارتفاع الحليمات ، وعرض الحليمات ، وسمك الخلايا الطلائية، ونسبة الخلايا المناعية Ki٦٧. كما أظهر الفحص بالمجهرالإلكتروني الماسح ضمورًا في الحليمات الخيطية مع تقشير في غلافها الظهاري. كما تم الكشف عن وجود حليمات فطرية مشوهة ذات مسام غير واضحة. من ناحية أخرى ، كانت هذه التغييرات أقل وضوحًا في الفئران التي تلقت الميلاتونين قبل إعطاء ٥-فلورويوراسيل.

الاستنتاج: ٥-فلورويوراسيل تسبب في حدوث تغييرات تركيبية في الغشاء المخاطي للسان الجرذان البيضاء. والميلاتونين قد خفف من هذه التغيرات.