Original Article

Immunohistochemical Expression of Transforming Growth Factor-Beta (TGF-β) and Ki-67 in Filiform Papillae of Colchicine Treated Adult Albino Rats

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ABSTRACT

Introduction: Transforming growth factor-beta (TGF-β) is considered as a regulator of cells' growth and differentiation. Ki-67 is a broadly known nuclear protein accompanied by cell growth as well as division. Colchicine is a traditional mitotic inhibitor used in treating several diseases.

Objectives: To assess Colchicine administration outcome on the histology of filiform papillae with the TGF-β and Ki-67 immunohistochemical expression.

Design: Forty male albino rats (200-250gm) were employed in the study. Animals were assigned equally as two groups, Control Group: received no drug and Colchicine treated group: received Colchicine (3mg/Kg/day) for 7 days. After sacrifice, processing of tongue specimens was done, then the slides were stained by Hematoxylin and Eosin, anti-TGF-β and anti-Ki-67. Area% of positive TGF-β and number of Ki-67 positive cells were statistically analyzed.

Results: Histologically, Colchicine treated group showed shortened and narrowed simple conical filiform papillae with increased interpapillary areas. The epithelial covering revealed some dark hyperchromatic nuclei and vacuolations. Thickness of lamina propria and connective tissue (CT) cores were reduced with few fibroblasts, dilated and congested blood vessels. The papillae of Colchicine group showed wide distribution of strong intensity positive TGF-β immunoreactivity in both epithelial and CT cells, in addition to low intensity few positive Ki-67 basal and parabasal cells with rare positive CT cells. Statistical analysis showed highly significant increase in positive area% of TGF-β and highly significant decrease in Ki-67 positive epithelial as well as CT cells in this group when compared to the control.

Conclusions: Colchicine induced degenerative histological changes in filiform papillae, in addition to a highly significant increase of TGF-β and a highly significant decrease of Ki-67 immunohistochemical expression.

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Key Words: Colchicine; filiform papillae; Ki-67; TGF-β.

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INTRODUCTION

Transforming growth factor-beta (TGF-β) is a widely expressed cytokine from the superfamily of activins and bone morphogenetic proteins.[1] Its signaling provides useful means of controlling cell behavior. It has a role in affecting TGFβ-correlated cytokines as in proliferation and differentiation of cells, and tissue regeneration.[2]

The immunoreactivity of TGF-β was increased in hepatic parenchyma in Carbon tetrachloride induced liver fibrosis.[3] Intermediate and chronic cyclophosphamide-induced cystitis lead to high-intensity immunoreactivity of TGF-β1 in the urothelium as well as the detrusor smooth muscle.[4]

The Ki-67 protein is a noticeable marker of proliferation used in pathology. It was first identified as an antigen in proliferating cells' nuclei. In contrast to its diagnostic significance, the results of its functions were only published recently. It is required for heterochromatin organization[5]. The Ki-67 average level in proliferating cells is similar whatever the cell type is. Its level in a cell depends on the cell-cycle phase.[6] Ki-67 is degraded constantly in G0 as well as G1 and is constantly formed from S phase beginning till the end of mitosis.[7]

The use of many medications results in alterations in the expression of some markers. Some drugs caused decreased expression of Ki-67 in different tissues such as Cladribine, a cytostatic drug which leads to apoptosis,[8] and an immunosuppressant and antiproliferative drug Rapamycin.[9] In addition to Anastrozole which inhibits estrogen synthesis,[10] and recently the anti-hepatitis virus drug, Sofosbuvir.[11] Additionally, Colchicine was able to increase TGF-β1in the periodontal ligament fibroblasts that was dose-dependent except at the low dose of 0.1 ug/ml.[12]

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Colchicine, found in autumn crocus plant, blocks or represses cell division by inhibiting mitotic spindles development during the division of nuclei. Under normal conditions, the cell uses its spindle fibers to arrange chromosomes then copy them, and divides into new daughter cells, where each one has a set of chromosomes[13]. Colchicine binds to microtubules ends and inhibits their polymerization. It is a famous anti-mitotic drug which inhibits mitotic cells from entering metaphase.[14]

Colchicine has been used in treating many diseases like acute gouty arthritis, Behcet's syndrome, familial Mediterranean fever, cirrhosis, psoriasis, necrotizing vasculitis, scleroderma, sarcoidosis and amyloidosis.[15] The frequent adverse effects of Colchicine are fatigue, headache, pharyngo-laryngeal pain, respiratory and thoracic mediastinal disorders, diarrhea, abdominal cramps and lactose intolerance. Other rare but toxic side effects include: nausea, vomiting, diarrhea, dyspnea, cardiac depression, shock, bone marrow depression, multiple organ failure, and renal damage.[16]

Thus, the objectives of the current study were to evaluate Colchicine administration influence on the histology of simple conical filiform papillae, in addition to TGF-β and Ki-67 immunohistochemical expression.

MATERIALS AND METHODS

The study was approved by "Research Ethics Committee" Faculty of Dentistry, Ain Shams University (FDASU-RecIR022315).

Animals

The sample size calculation depended on a previous research.[17] G. Power program 3.1.9.2 software was utilized with input parameters: effect size (0.925), power of 80% for Independent-samples t-test with 5% significant and 95% confidence interval. The sample size was calculated as 20 rats per group.

Forty Wistar male adult albino rats (200-250gm) were utilized in the research. Animals were kept in "Medical Research Center" animal house of Ain-Shams University and housed in sterile circumference with standard diet and tap water. Rats were maintained under good ventilation during the whole experimental period.

Rats were assigned into two groups (20 rats in each group):

Control Group: Rats were given no drug.

Colchicine treated group: Rats received 3mg/Kg/day Colchicine (Colchicine tablets, 500 mcg, El-Nasr Pharmaceutical Chemicals Co., Egypt). The tablets were ground into powder, dissolved in distilled water, then orally given by gastric tube for seven successive days.[17]

Specimen preparation

Rats were sacrificed by ketamine over dose and their tongues were dissected immediately. The tongue specimens were fixed in 10% phosphate buffered formalin for two days then processed. Slides with four µm sections were stained by Hematoxylin & Eosin (H&E),[18] TGF-β and Ki67 immunohistochemical stains.

TGF-β Immunohistochemical staining

Endogen peroxidase blockage was done by sections immersion for 5min in 30% hydrogen peroxide, absolute ethanol and phosphate buffered saline (PBS), washed 3 times in PBS (5min each). Heat mediated antigen retrieval with Tris/EDTA buffer was done. For background blockage, sections were placed in normal goat serum 1:30 diluted in PBS for an hour, incubated with primary antibody (Rabbit monoclonal Anti-TGF-β) at dilution 1:50 in PBS in a humid chamber overnight at 4°C. Application of a secondary antibody at dilution 1:100 in PBS (Goat Anti-Rabbit Immunoglobulin G Antibody, Peroxidase Conjugated) for an hour. Then, sections were dipped in PBS, hydrogen peroxide and diaminobenzidine for 5min, led to brown benzidine precipitates creation, localizing the expression of TGF-β.[19]

Ki-67 Immunohistochemical staining

Endogenous peroxidase blockage was performed using 3% hydrogen peroxide in distilled water for 5min. The antigen retrieval was done by heating in microwave, in 10mM citric acid for 2 times (4min each). Sections were incubated with normal goat serum for 30min at room temperature, then with a primary antibody at dilution 1:50 (Rabbit monoclonal Anti-Ki-67 antibody) at 4˚C overnight. Sections were washed with buffered saline, then 45min incubation in NovoLink Polymer detection system. Brown color was developed after sections incubation in 3-3-diaminobenzidine tetrahydrochloride (1mg/ml) in a Tris-buffered solution, with hydrogen peroxide, for 5min[9,20].

TGF-β and Ki-67 immunostained sections were then counterstained by Hematoxylin, dehydrated in ethanol, followed by clearing with xylene.

Histomorphometric analysis

From each specimen, three sections for each immunohistochemical staining were assessed with selection of five equal representative fields/section of simple conical filiform papillae. Each selected field, with an area 0.6 mm^2, was captured at a magnification (x200). Histomorphometric analysis was done by image J software. In TGF-β immunostained sections, the areas of interest were only selected. 8-bit monochrome type conversion and color threshold for the images were done, finally area% of TGF-β positive reaction was measured. While for Ki-67 immunostained sections, images brightness adjustment was performed to inspect only the positive cells. Then, binary and watershed for each image were selected, dividing adjacent cells that identified as one. At last, the number of Ki-67 positive epithelial and connective tissue (CT) cells were measured automatically.

Statistical Analysis

Obtained results of area% of TGF-β positive reaction and number of Ki-67 positive epithelial and CT cells that expressed as cells/mm^2 were analyzed by SPSS. Data were given as mean ± standard deviation and ranges. Data were checked for normality by tests of Kolmogorov-Smirnov and Shapiro-Wilk. Independent-samples t-test of significance was performed to compare between two means. 95% confidence interval and 5% margin of error accepted. *P-value* <0.001 (highly significant), *P-value* <0.05 (significant) and *P-value* >0.05 (insignificant).

RESULTS

H&E results

Simple conical filiform papillae were seen at the anterior part of anterior two thirds of rat's tongue. In control group of this study, the papillae appeared as conical pointed projections that were regular in shape, size and orientation. Papillae were covered with stratified squamous epithelium of normal cell layers and regular orthokeratin. Thin lamina propria and CT cores, which were projected deeply into the epithelium, revealed well-formed dense fibrous CT with numerous spindle-shaped fibroblasts and collagen fiber bundles (Figures 1a,b). In Colchicine treated group, the papillae appeared shortened and narrowed with increasing in the interpapillary areas in some regions. The epithelial covering revealed basal and parabasal cell layers with dark hyperchromatic nuclei, numerous intracellular vacuolations, and covered by separated and torn orthokeratin. The thickness of lamina propria and CT cores was noticeably reduced, in addition to a decreased number of fibroblasts as well as dilated and congested blood vessels in some regions (Figures 1c,d,e).

TGF-β immunohistochemical results

Control group showed low intensity few positive TGF-β immunoreactivity in the epithelial and CT cells (Figure 2a). Meanwhile, the other group revealed wide distribution of strong intensity positive immunoreactivity in both epithelial and CT cells (Figure 2b).

Ki-67 immunohistochemical results

Control group demonstrated markedly detected high intensity positive Ki-67 immunoreactions in nearly all basal, some parabasal and few prickle cells with some positive CT cells (Figure 2c). However, the other group showed low intensity few positive basal and parabasal cells with rare positive CT cells (Figure 2d).

Statistical results

Area% of TGF-β

Colchicine group presented a statistically high significant increase in positive area% of TGF-β than control (Table 1, Figure 3).

Number of Ki-67 positive cells

Colchicine group revealed a statistically high significant decrease in Ki-67 positive epithelial and CT cells number than control group (Table 1, Figure 3).

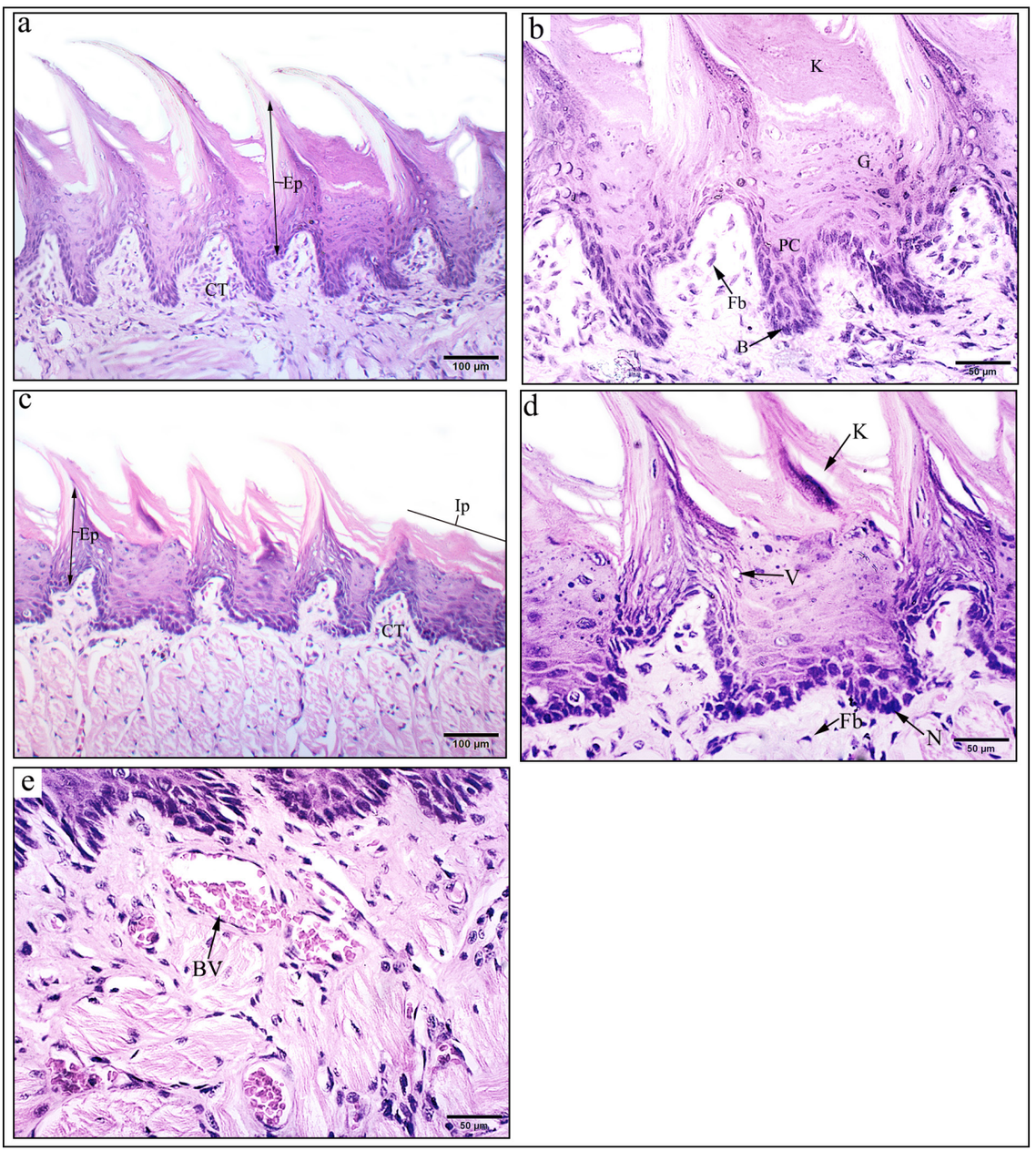

Fig. 1. Photomicrographs of simple conical filiform papillae showing; control group: (a)- Regular conical projections with normal orthokeratinized stratified squamous epithelium (Ep) and well-formed dense fibrous CT. (b)- Normal epithelial covering with basal (B), prickle (PC), granular (G) and keratin (K) cell layers. Normal CT with numerous fibroblasts (Fb) and collagen fiber bundles. Colchicine group: (c)- Shortened and narrowed papillae covered by orthokeratinized stratified squamous epithelium (Ep) with increased interpapillary (Ip) areas in some regions and apparently narrowed CT. (d)- Epithelial covering with dark hyperchromatic nuclei (N) in basal and parabasal cells, numerous intracellular vacuolations (V), separated and torn orthokeratin layer (K). CT with decreased number of fibroblasts (Fb) (e)- Some regions showed dilated and congested blood vessels (BV) (a & c; H&E, x200). (b, d & e; H&E, x400).

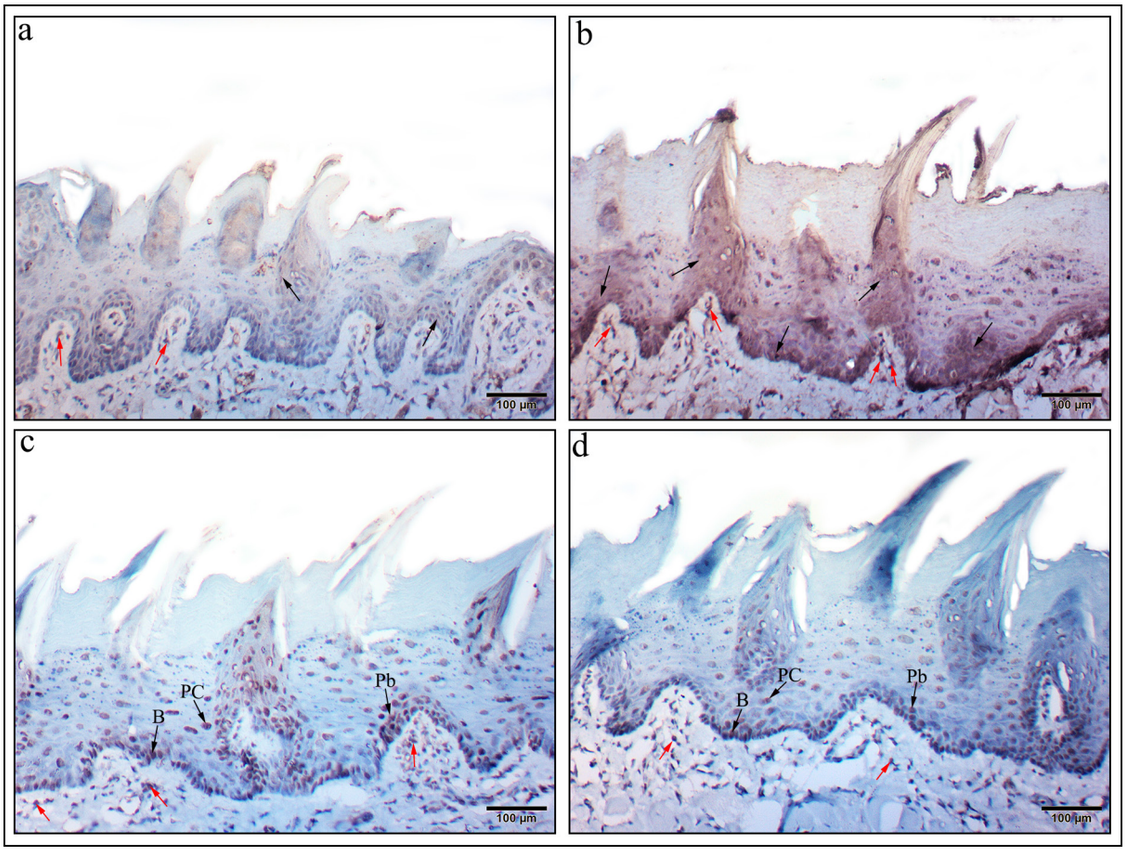

Fig. 2. (a)- Control group: Low intensity few positive TGF-β immunoreactivity in the epithelial (black arrows) and CT (red arrows) cells (b)- Colchicine group: Wide distribution of strong intensity positive immunoreactivity in epithelial (black arrows) and CT (red arrows) cells (a & b; Anti-TGF-β antibody, x200). (c)- Control group: High intensity Ki-67 positive immunoreactions in nearly all basal (B), some parabasal (Pb) and few prickle cells (PC) with some positive CT (red arrows) cells. (d)- Colchicine group: Low intensity few positive basal (B), parabasal cells (Pb) and prickle cells (PC) with rare positive CT (red arrows) cells (c & d; Anti-Ki-67 antibody, x200).

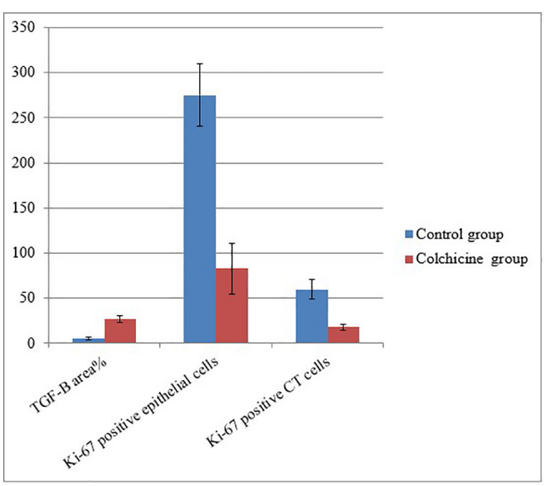

Fig. 3. Bar charts showing; comparison between groups according to area% of TGF-β and number of Ki-67 positive epithelial and CT cells.

Table 1: Comparison between the groups according to TGF-β area% and number of Ki-67 positive epithelial and CT cells

		Control group (n=20)	Colchicine group (n=20)	t-test	*p-value*
TGF-β area%	Mean ± SD	5.64 ± 1.57	27.54 ± 3.82	-23.697	<0.001**
	Range	2.98-8.65	20.23-34.36		
Ki-67 positive epithelial cells	Mean ± SD	275.00 ± 34.46	83.00 ± 27.90	19.367	<0.001**
	Range	227-323	31-120		
Ki-67 positive CT cells	Mean ± SD	60.00 ± 10.93	18.00 ± 3.09	16.534	<0.001**
	Range	44-78	13-25		

Data are expressed as Mean ± SD using: Independent Samples t-test. ***p-value* <0.001 indicates highly significant difference

DISCUSSION

Colchicine, a tricyclic alkaloid, has terminal half-life from 20 to 40 hours and bioavailability from 24% to 88%. It has a wide range of clinical usage including: familial Mediterranean fever, chronic cutaneous vasculitis, Behçet's disease, initial and recurrent pericarditis.[21]

In this study, simple conical filiform papillae in rats received oral high dose Colchicine for short duration showed epithelial and connective tissues histological changes. Our results agree with Takeda *et al*.,[22] who demonstrated that after Colchicine treatment, basal as well as suprabasal layers of epithelium in and surrounding circumvallate papillae contained condensed nuclei. Some epithelial cells enclosed vacuoles containing debris of cytoplasmic organelles. In addition, rat testis after Colchicine administration were examined and showed interstitial edema with fatty degeneration and spermatogoneal cells lining degeneration in some seminiferous tubules.[23] Furthermore, testis of rats in an earlier study showed Sertoli and Leydig cells degeneration with germinal epithelium irregular division and spermatid cells vacuolar degeneration.[24] It has been documented that the liver in rats received Colchicine revealed hepatocytes with deeply stained shrunken nuclei and some showed foamy appearance, as well as small hemorrhagic areas in the intestine.[13] Moreover, it has been observed that Colchicine induced hepatotoxicity with histopathological changes as pyknosis of hepatocytes nuclei, intracellular vacuolation and central vein dilatation[25]. Bekheet[17] noticed dilated and congested sub-capsular blood vessels in spleen after treatment with Colchicine.

Concerning torn and separated keratin layer observed in the present research, it was documented that Colchicine affected the junctions between the epithelial cells where some cells showed broken desmosomes resembling half desmosomes which might explain our finding.[22]

In our results, the epithelial vacuolations that were detected may represent a sign of cellular response to injury which could be related to mitochondrial damage induced by Colchicine. Mitochondrial damage results in reactive oxygen species (ROS) accumulation and oxidative stresses as ascertain by a previous study.[26] This explanation come in accordance with Abbas *et al*.,[27] who detected increased number of cytoplasmic vacuolations and degenerated mitochondria in rat testis after high dose of Colchicine administration, and reported significantly lower values in Vardenafil co-administration with Colchicine group. It has been documented that Vardenafil administration during ischemia–reperfusion injury in testis and ovaries reduced cell damage by the reduction in oxidative stress which represent a crucial role in its cytoprotective action[28,29] Furthermore, local Colchicine administration in earlier studies disturbed the endogenous antioxidant defense mechanism in rat brain, leading to oxidative stress and free radical-induced toxicity. The oxidative stress was attenuated by using several agents with antioxidant property.[30-34]

The hyperchromatic nuclei presented in the current research agrees with an earlier research, recorded that Colchicine could induce neuronal apoptosis in both adult and developing rat brains, presenting apoptotic cells with extreme chromatin condensation and apoptotic bodies formation, in addition to their positive staining with In-situ end-labeling of nuclear Deoxyribonucleic acid (DNA) fragmentation.[35] As well as a previous publication documented an obvious increase in positive apoptotic cells after colchicine administration in taste buds of circumvallate papillae, some basal and suprabasal cells in trench wall besides dorsal epithelium.[22] Another study by Norma *et al*.[36] demonstrated apoptosis in the mouse duodenal crypt enterocytes after Colchicine administration. Additionally, significant rise in caspase 3 was found, indicating activated apoptotic pathway in hippocampus and cortex in rats received Colchicine as shown in a previous work, which attributed Colchicine-induced apoptosis to oxidative-nitrosative stress and mitochondrial dysfunction.[32]

The changes in lamina propria and CT papillae observed in this work could be attributed to an increase in matrix metalloproteinases (MMP), degrading the extracellular matrix that indicates the antifibrotic effect of Colchicine. A previous study revealed a significant increase in MMP1 expression in human periodontal ligament fibroblasts subjected to Colchicine than the control.[12] Furthermore, Colchicine induced a significant elevation in collagenase (MMP1) as well as stromelysin 1 (MMP3) levels with no influence on synthesis and activation of gelatinase A (MMP2) and tissue inhibitors of MMPs (TIMP) in human skin fibroblasts.[37] However, it has been recorded that TIMP 1 expression was decreased in cultured hepatic stellate cells received Colchicine.[38]

Regarding the inflammatory state as blood vessels dilation presented in the current experiment, the oxidative stress and ROS released from damaged mitochondria trigger inflammation through secretion of pro-inflammatory cytokines.[39,40] Another study displayed significant elevation of inflammatory mediators at rat hippocampus as well as cortex with Colchicine.[32]

In this research, the wide area% of positive TGF-β immunoreactivity with strong intensity in both epithelial and CT cells in Colchicine group that showed statistically significant higher values than the control coincides with the results of Nahm *et al*.,[12] investigating human periodontal ligament fibroblasts that was subjected to Colchicine at different time intervals. They supposed that TGF-β expression is probably sensitive to microtubules disruption induced by Colchicine. In addition, an experiment reviewed that ROS can influence the signaling of TGF-β, increasing its expression and activation from the latent complex.[41] As well as, it has been recorded that signaling pathway of TGF-β is a vital component of oxidative stress-induced damage, activating the antioxidative mechanisms of the cells.[42]

The epithelial and CT reductive changes of Colchicine in our study could be related to Colchicine-triggering apoptosis as mentioned earlier or reduction in cell proliferation. Herein, the inhibition of cell proliferation was statistically analyzed by significantly decreased number of ki-67 positive epithelial and CT cells in Colchicine treated group than the control. This is parallel to the results of Bekheet,[17] who recorded that Colchicine group revealed a significant decline in Proliferating cell nuclear antigen (PCNA) immune positive splenic parenchymal cells. Colchicine led to cell cycle arrest at G2/M phase by using Muse Cell Analyzer and the percent of cell populations in G0/G1, S as well as G2/M in human breast cancer cell line[43].

The antimitotic effect of Colchicine could be explained by its ability in reducing microtubule density, suppressing the microtubules dynamics in mitotic spindles and inhibiting microtubule polymerization that resulted in arresting cell division at metaphase.[44,45] Additionally, it has been suggested that ROS generated with Colchicine induced cancer cell arrest and apoptosis.[46] Moreover, the inhibition of cell proliferation may be indirectly influenced by the increase in TGF-β expression. A previous study indicated that at cellular level, the stimulation of TGF-β induces cytostasis of nearly all normal epithelial cells together with certain mesenchymal cell types.[47] It has been stated that TGF-β is involved in growth inhibition in all normal cell types.[48] Furthermore, recent studies indicated that TGF-β has a crucial effect in intermediating cell dormancy and it can induce cellular senescence which is irrevocably form of cell cycle arrest, under certain conditions.[49,50] At last, Baba *et al*.[51] documented that TGF-β acts as a tumor suppressor at initial onset, plays an important action for eliminating malignant cells by decreasing cells proliferation and their differentiation, inducing apoptosis. However, at a late stage of malignancy, TGF-β promotes tumorigenesis by enhancing transformation of cells and metastasis.

Although Colchicine in this work induced oxidative stress, apoptosis and inflammation, several studies confirmed its direct antioxidant, antiapoptotic and anti-inflammatory effects.[52-55] This conflict of findings could be attributed to the higher Colchicine dose used in our study than the latter mentioned ones.

CONCLUSIONS

Colchicine was able to induce degenerative changes in the filiform papillae of albino rats histologically. This was accompanied by highly significant increase of TGF-β besides highly significant decrease of Ki-67 immunohistochemical expression.

ABBREVIATIONS

TGF-β: Transforming growth factor-beta; **H&E:** Hematoxylin & Eosin; **PBS:** phosphate buffered saline; **CT:** connective tissue; **ROS:** reactive oxygen species; **MMP:** matrix metalloproteinases; **TIMP:** tissue inhibitors of MMPs.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003; 113: 685-700.
2. Massagué J. TGFβ signalling in context. Nat. Rev. Mol. Cell Biol. 2012; 13(10):616-630.
3. Yao Q Y, Xu B L, Wang J Y, Liu H C, Zhang S C, Tu CT. Inhibition by curcumin of multiple sites of the transforming growth factor-beta1 signalling pathway ameliorates the progression of liver fibrosis induced by carbon tetrachloride in rats. BMC Complement. Altern. Med. 2012; 12:156.
4. Gonzalez E J, Girard B M, Vizzard M A. Expression and function of transforming growth factor- isoforms and cognate receptors in the rat urinary bladder following cyclophosphamide-induced cystitis. Am. J. Physiol. Renal Physiol. 2013; 305: F1265-F1276.
5. Uxa S, Castillo-Binder P, Kohler R, Stangner K, Muller GA, Engeland K. Ki-67 gene expression. Cell Death and Differentiation. 2021; 28:3357-3370.
6. Sobecki M, Mrouj K, Colinge J, Gerbe F, Jay P, Krasinska L, Dulic V, Fisher D. Cell-Cycle Regulation Accounts for Variability in Ki-67 Expression Levels. Cancer Res. 2017; 77(10): 2722-2734.
7. Miller I, Min M, Yang C, Tian C, Gookin S, Carter D, Spencer S L. Ki67 is a Graded Rather than a Binary Marker of Proliferation versus Quiescence. Cell Rep. 2018; 24(5): 1105-1112.e5.

8. Jędrych M, Wawryk-Gawda E, Jędrych B J, Chylińska-Wrzos P, Jasiński L. Immunohistochemical evaluation of cell proliferation and apoptosis markers in ovarian surface epithelial cells of cladribine-treated rats. Protoplasma. 2013; 250:1025-1034.

9. Liu S, Huang L, Geng Y, He J, Chen X, Xu H, Li R, Wang Y, Ding Y, Liu X. Rapamycin inhibits spermatogenesis by changing the autophagy status through suppressing mechanistic target of rapamycin-p70S6 kinase in male rats. Mol. Med. Rep. 2017; 16:4029-4037.

10. Chagas D C, Barros-Oliveira M D C, Lopes-Costa P V, Pereira R D O, Melo M D A, Costa-Silva D R, Borges C S, Viana J L, Ribeiro dos Santos A, Facina G, Borges da Silva B. Effects of anastrozole on Ki-67 antigen expression in the vaginal epithelium of female rats in persistent estrus. Clinics. 2020; 75: e1643.

11. Elbatrick N A M, Halawa A M, Samir N, Hussein S I. The effect of Sofosbuvir administration on Albino rats' buccal mucosa (Histological and immunochemical study). EDJ. 2022; 68, 2321:2333.

12. Nahm D S, Kim H J, Mah J, Baek S H. In *vitro* expression of matrix metalloproteinase -1, tissue inhibitor of metalloproteinase -1 and transforming growth factor –β1in human periodontal ligament fibroblasts. Eur. J. Orthod. 2004; 26:129-135.

13. Abd El Maguid A. Histological study of liver and intestine of rats treated with colchicine. Egypt. J. Hosp. Med. 2006; 23: 277- 286.

14. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri D C, Szopa A, Sharifi Rad J, Docea A O, Mardare I, Calina D, Cho W C. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. Cancer Cell Int. 2022; 22: 206.

15. Karakus S S, Engin H. Colchicine: where to use where not to use? Ann. Pharmacother. 2002; 36: 172-173.

16. Cocco G, Jerie P. Colchicine: Efficacy and safety issues. Journal of Symptoms and Signs. 2014; 3: 178-187.

17. Bekheet EA. Effect of colchicine administration on the structure of the spleen of adult male albino rat: histological and immunohistochemical study. EJH. 2020; 43: 519-530.

18. Bancroft JD, Suvarna K, Layton C. Bancroft's Theory and Practice of Histological Techniques, Ch. 6 & 10. 7th ed. Elsevier, Churchill Livingstone. 2013.

19. Rubert A, Manzanares M C, Ustrell J M, Duran J, Pérez-Tomás R. Immunohistochemical identification of TGF-beta1 at the maxillaries in growing Sprague-Dawley rats and after muscle section. Arch. Oral Biol. 2008; 53:304-309.

20. Nery-Aguiar A R, Aguiar Y Q, Conde Júnior A M, Alencar A P, Tavares C B, Lopes-Costa P V, Nazário A C, da Silva B B. Analysis of the Ki-67 index in the vaginal epithelium of castrated rats treated with tamoxifen. Clinics (Sao Paulo). 2016; 71:90-93.

21. Slobodnick A, Shah B, Pillinger M H, Krasnokutsky S. Colchicine: Old and New. Am J Med. 2015; 128(5): 461-70.

22. Takeda M, Suzuki Y, Obara N, Nagai Y. Induction of apoptosis by colchicine in taste bud and epithelial cells of the mouse circumvallate papillae. Cell Tissue Res. 2000; 302(3):391-395.

23. Abdel Motaal N A R. Effect of Colchicine on The Histology of Spleen and Testis of Albino Rats. Egypt. J. Hosp. Med. 2006; 23:268– 276.

24. Abdullah R A, Ismail H K, Ghanim A A. Impact of Colchicine on Histology of Testis in Rats. World Vet J. 2022; 12(3): 250-259.

25. Elshama S S, El-Kenawy A E, Osman H H. Hepatotoxicity and nephrotoxicity of colchicine prolonged use in the rats Int. J. of Adv. Res. 2014; 2: 1012-1023.

26. Sivitz W I, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxid. Redox Signal. 2010; 12: 537-577.

27. Abbas M, Hassan O, El-Fakahany H. Mitigating Effect of Vardenafil against Colchicine–Induced Testicular Toxicity in Adult Male Albino Rats Authors. AJFM. 2016; 27(2): 83-91.

28. Erol B, Tokgoz H, Hanci V, Bektas S, Akduman B, Yencilek F, Mungan G, Mungan A. Vardenafil reduces testicular damage following ischemia/ reperfusion injury in rats. Kaohsiung J. Med. Sci. 2009; 25(7): 374-380.

29. Baş H, Kara O, Kara M, Pandir D. Protective effect of vardenafil on ischemia reperfusion injury in rat ovary. Turk. J. Med. Sci. 2013; 43: 684-689.

30. Kumar A, Dogra S, Prakash A. Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats. J. Med. Food. 2010; 13 (4); 976-984.

31. Khurana S, Jain S, Mediratta PK, Banerjee B D, Sharma K K. Protective role of curcumin on colchicine-induced cognitive dysfunction and oxidative stress in rats. Hum. Exp. Toxicol. 2012; 31(7): 686-697.

32. Prakash A, Kumar A. Lycopene protects against memory impairment and mito-oxidative damage induced by colchicine in rats: an evidence of nitric oxide signaling. Eur. J. Pharmacol. 2013; 721(1-3): 373-381.

33. Komal, Sharma P, Khan F, Lubhan, Garg V K. To Investigate the Neuroprotective Effect of Crocin against Colchicine and Aluminium Chloride Induced Cognitive Disfunction and Oxidative Damage in Rodents. Adv. Pharmacol. Clin. Trials. 2019; 4(3): 000163.

34. Bhardwaj J S, Manandhar S, Chatterjee S, Hari G, Priya K, Raviteja S, Sreedhara K, Pai R. Protective Effects of Gossypin in Colchicine-induced Cognitive Dysfunction and Oxidative Damage in Rats. Research J. Pharm. and Tech. 2020; 13(11):5189-5196.

35. Pozas E, Aguado F, Ferrer I. Localization and expression of Jun-like immunoreactivity in apoptotic neurons induced by colchicine administration in *vivo* and in *vitro* depends on the antisera used. Acta Neuropathol. 1999; 98(2): 119-128.

36. Norma V G, Badrán A F, Barbeito C G. Daily variations in colchicine-induced apoptosis in duodenal crypts. Chronobiol. Int. 2005; 22(1): 79-88.

37. Mathisen B, Loennechen T, Gedde-Dahl T, Winberg J. Fibroblast heterogeneity in collagenolytic response to colchicine. Biochemical pharmacology. 2006; 71: 574-583.

38. Zesong L, Shaoxi C, Yuan J, RuiJun G, Wen Z. Colchicine inhibited the expression of tissue inhibitor of metalloprotenase-1 and interleukin-6 in cultured activated hepatic stellate cells. Wuhan Univ. J. Nat. Sci. 2006; 11, 720–724.

39. Zhou R, Yazdi A, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011; 469, 221-225.

40. Biswas S K. Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? Oxid. Med. Cell. Longev. 2016; 2016: 5698931.

41. Krstić J, Trivanović D, Mojsilović S, Santibanez J F. Transforming Growth Factor-Beta and Oxidative Stress Interplay: Implications in Tumorigenesis and Cancer Progression. Oxid. Med. Cell Longev. 2015; 2015:654594.

42. Chen H Y, Chou H C, Ho Y J, Chang S J, Liao E C, Wei Y S, Lin M W, Wang Y S, Chien Y A, Yu X R, Kung H Y, Yang C C, Chen J Y, Chan H L, Ko M L. Characterization of TGF-β by Induced Oxidative Stress in Human Trabecular Meshwork Cells. Antioxidants (Basel). 2021; 10(1):107.

43. Bakar-Ates F, Özmen N, Kaya-Sezginer E, Kurt E E. Effects of colchicine on cell cycle arrest and MMP-2 mRNA expression in MCF-7 breast adenocarcinoma cells. Turk Hij Den Biyol Derg. 2018; 75(3): 239-244.

44. Wilson L, Jordan M A. Microtubule dynamics: taking aim at a moving target. Chemistry & Biology.1995; 2:569-573.

45. Prins K W, Tian L, Wu D, Thenappan T, Metzger J M, Archer, S L. Colchicine depolymerizes microtubules, increases junctophilin-2, and improves right ventricular function in experimental pulmonary arterial hypertension. Journal of the American Heart Association. 2017; 6(6): e 006195.

46. Oh J, An H J, Yeo H J, Choi S, Oh J, Kim S, Kim J M, Choi J, Lee S. Colchicine as a novel drug for the treatment of osteosarcoma through drug repositioning based on an FDA drug library. Front. Oncol. 2022; 12:893951.

47. Siegel P M, Massague´ J. Cytostatic and apoptotic actions of TGF-b in homeostasis and cancer. Nat Rev Cancer. 2003; 3: 807-820.

48. Huang S S, Huang J S. TGF-beta control of cell proliferation. J Cell Biochem. 2005; 96(3):447-62.

49. Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina H G, Schewe D M, Aguirre-Ghiso J A. TGFb 2 dictates disseminated tumour cell fate in target organs through TGF-b-RIII and p38 a/b signalling. Nat Cell Biol. 2013; 15: 1351-U1210.

50. Zhang Y, Alexander P B, Wang X F. TGF-β Family Signaling in the Control of Cell Proliferation and Survival. Cold Spring Harb Perspect Biol. 2017; 9(4): a022145.

51. Baba A B, Rah B, Bhat G R, Mushtaq I, Parveen S, Hassan R, Hameed Z M, Afroze D. Transforming Growth Factor-Beta (TGF-β) Signaling in Cancer-A Betrayal Within. Front Pharmacol. 2022; 13:791272.

52. Aral C A, Aral K, Yay A, Özçoban O, Berdeli A, Saraymen R. Effects of colchicine on gingival inflammation, apoptosis, and alveolar bone loss in experimental periodontitis. J Periodontol. 2018; 89:577-585.

53. Bakhta O, Blanchard S J, Guihot A L, Tamareille S, Mirebeau-Prunier D. *et al*. Cardioprotective Role of Colchicine Against Inflammatory Injury in a Rat Model of Acute Myocardial Infarction. Journal of Cardiovascular Pharmacology and Therapeutics. 2018; 23 (5), pp.446-455.

54. Zalar D M, Pop C, Buzdugan E, Kiss B, Stefan M G, Ghibu S, Crisan D, Buruiana-Simic A, Grozav A, Borda I M, Mogosan C I. Effects of Colchicine in a Rat Model of Diet-Induced Hyperlipidemia. Antioxidants. 2022; 11, 230.

55. Zhang D, Li L, Li J, Wei Y, Tang J, Man X, Liu F. Colchicine improves severe acute pancreatitis-induced acute lung injury by suppressing inflammation, apoptosis and oxidative stress in rats. Biomed. Pharmacother. 2022; 153: 113461.

الملخص العربى

التعبير الهستوكيميائي المناعي لعامل النمو المتحول- بيتا و صبغة Ki-67 في الحليمات الخيطية للفئران البيضاء البالغة المعالجة بالكولشيسين

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المقدمة: يعتبرعامل النمو المتحول- بيتا واحدا من المنظمات متعددة الوظائف لنمو الخلية و تحولها. ويعتبر Ki-67 بروتين نووي معروف على نطاق واسع مصحوب بنمو الخلية و إنقسامها. يعد الكولشيسين مانع تقليدي للإنقسام المتساوي للخلية و يستخدم في علاج العديد من الأمراض.

هدف البحث: هو تقييم تأثير عقارالكولشيسين على أنسجة الحليمات الخيطية و التعبير الهستوكيميائي المناعي لعامل النمو المتحول- بيتا و صبغة Ki-67 .

طرق و مواد البحث: إستخدم في هذا البحث أربعين فأرا ذكرا أبيضا بالغا (٢٠٠-٢٥٠ جرام) و تم تقسيمهم إلى مجموعتين: المجموعة الضابطة التي لم تتلقى أي علاج و المجموعة المعالجة بالكولشيسين التي اخذت عقار الكولشيسن (٣ مجم / كجم) لمدة سبعة أيام. بعد التضحية بالفئران, تمت معالجة العينات و صبغتها بالهيماتوكسيلين و الإيوسين و عامل النمو المتحول- بيتا و صبغة Ki-67 . و تم عمل التحليل الإحصائي للنسبة المئوية الموجبة للمساحة لعامل النمو المتحول – بيتا و عدد الخلايا الإيجابية لصبغة Ki-67 .

النتائج: ظهرت الحليمات الخيطية هستولوجيا في المجموعة المعالجة بالكولشيسين قصيرة و رفيعة مع زيادة المسافات بين الحليمات. أظهر النسيج الطلائي بعض الخلايا بنواة غامقة اللون و بعض الخلايا الجوفاء. كما قلت تخانة النسيج الضام مع وجود بعض الأوعية الدموية المتسعة. كما أظهرت الحليمات زيادة في صبغة عامل النمو المتحول- بيتا في خلايا النسيج الطلائي و النسيج الضام و نقصان في عدد الخلايا الموجبة لصبغة Ki-67 في النسيج الطلائي و ندرتها في خلايا النسيج الضام. أظهر التحليل الإحصائي زيادة عالية ملحوظة في النسبة المئوية الموجبة لصبغة عامل النمو المتحول- بيتا و نقصان عالي ذات دلالة احصائية في عدد الخلايا الموجبة لصبغة Ki-٦٧ في النسيج الطلائي و النسيج الضام بالمقارنة مع المجموعة الضابطة.

الإستنتاجات: أدى إستخدام الكولشيسين إلى تغيرات هستولوجية في الحليمات الخيطية بالإضافة إلى زيادة التعبير الهستوكيميائي المناعي لصبغة عامل النمو المتحول- بيتا و نقصان صبغة Ki-67 .