



Histological and clinical evaluation of gingival enlargement response to oral folic acid administration in rabbit

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Article information

Received: 5 May, 2021

Accepted: 25 June 2021

Available online: 30 September, 2022

Keywords

Folic acid

Gingival enlargement

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Abstract

Aims of the study: to clarify the clinical and histological evaluation of the oral folic acid effect on reduction of drug induced gingival enlargement. **Materials and methods:** The study was carried out in the college of dentistry /university of Mosul on twelve white local male Rabbits with gingival enlargement weights between (900 g±1.250g). six rabbits considered a control group and the other six rabbits considered a treated group which taking 15 mg orally folic acid once daily for about 14 days then depth of gingival sulcus(gingival pocket) evaluated clinically by a periodontal probe and euthanised for histological examination. **Result:** Clinically: after 14 days of drug administration, there is a high clinical difference between two group (mean +- SD), control group have (3-5mm) of gingival enlargement and the experimental group have (1-3 mm) of gingival enlargement. The Histological finding show significant differences in the form of gingival sulcus depth(gingival pocket) and inflammatory cell infiltration and connective tissue in the control group and experimental group. **Conclusion:** the effect of oral administration of folic acid have higher evaluation on reducing the drug induced gingival enlargement in rabbit

الخلاصة

الاهداف: لتوضيح وتقييم السريري والنسجي لتأثير حمض الفوليك الفموي على تقليل ارتفاع اللثة المزمن المزمن **المواد وطرائق العمل:** اجريت الدراسة في كلية طب الاسنان جامعة الموصل على اثنا عشر من ذكور الارانب التي تعاني من ارتفاع اللثة ويزن الارنب الواحد ما بين (٩٠٠-١,٢٥٠ غم) قسمت هذه الارانب عشوائيا الى مجموعتين: المجموعة الاولى تشمل على ستة ارانب لانتستخدم اي علاج وتعتبر كونترول والمجموعة الثانية ايضا تتكون من ستة ارانب ويتم اعطائها حامض الفوليك بجرعة ١٥ غم يوميا وتعتبر الكروب المعالج **النتائج:** بعد مضي حوالي اربعة عشر يوما تم قياس ارتفاع اللثة سريريا وكان هناك فرق معنوي بين النتائج وكانت قيمة الكروب الكنترول حوالي ٣-٥ ملم بينما مجموعة الثانية التي استخدمت عقار الفوليك كانت حوالي ١-٣ ملم اما من ناحية الفحص النسجي بينت الدراسة هناك فرق معنوي بين المجموعتين من ناحية ارتفاع اللثة النسجي بسبب الخلايا الالتهابية وبين كمية الخلايا الالتهابية التي ظهرت بكمية اقل في المجموعة الثانية. **الاستنتاجات:** حامض الفوليك اسيد عن طريق الفم له تأثير كبير في تقليل ارتفاع اللثة المزمن في الارانب.

DOI: [10.33899/rdenj.2022.130074.1103](https://doi.org/10.33899/rdenj.2022.130074.1103), © 2022, College of Dentistry, University of Mosul.

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INTRODUCTION

Gingival overgrowth is a side effect of several medications, indistinguishable from one another, where the lesions are clinically and histologically are observed in both human and animals ⁽¹⁾. Gingival enlargement is pathologic enlargement of the gingiva and has multiple etiologies among which drug-induced enlargement is a common side effect ⁽²⁾. Gingival enlargement can present as an increased gingival mass and volume of connective tissue that appear in clear way in Rabbit. It can range from mild to severe enlargement of papillary or marginal gingival tissues ⁽³⁾. It more commonly affects the anterior teeth than the posterior teeth and the buccal gingivae than the lingual/palatal gingivae of both human and animals ⁽⁴⁾ plaque-induced inflammatory process is the most common cause of gingival over growth, which can be modified by systemic disease or medications this can appear in human more than in rabbit because plaque induced is normally control by the animals ^(5,6). Drugs such as immunosuppressant's, antihypertensive, and antiepileptic's are the chief groups causing the hypertrophy ⁽⁷⁾. The depth of the gingival sulcus is lie between the junctional epithelium and the free gingivae that surround the tooth from the upper region ⁽⁸⁾ These depths is joined by the fiber of the connective tissue in the apical region and binds the free gingival margin in the carnal region⁽⁹⁾ the most common theory that explain the

causes of gingival enlargement is that reduced cation influx of folic acid (FA) active transport inside gingival fibroblasts which leads to decreased cellular folate uptake as shown in this study clearly on the Rabbits, which in turn leads to changes in Matrix Metalloproteinases metabolism and the failure to activate collagenase. Decreased availability of activated collagenase will lead to decreased degradation and accumulate of connective tissue which presents as Drug -induced gingival enlargement ⁽¹⁰⁾ in these cases, and to achieve greater levels of folic acid is administered systemically by oral administration. The present report shows that local White Rabbits are prone to developing DIGO, similar to the lesions observed in humans and dogs ⁽¹¹⁾

MATERIALS AND METHODS

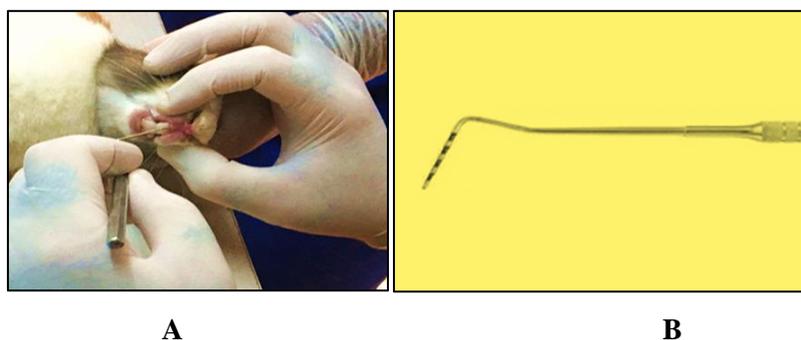
The study worked in the department of dental Basic Science, College of Dentistry, University of Mosul; with approved of Scientific Committee,A.L. 39/21 , the period was from 29/9/2020 to 1/5/2021 the study involve Twelve adult male local rabbits weighting between (900g-1.25kg) were included in this study. All animals with a good health to be used through the study Diagnoses were established according to the clinical findings of drug induced gingival enlargement. Research grouping classified as:

- 1- Control group (6 rabbit) with drug induce gingival enlargement are examined for clinical measurement and then euthanized for histological examination.
- 2- Experimental group (6 rabbit) with drug induce gingival enlargement taking oral folic acid 15mg daily

for 14 days then taking for clinical examination and euthanized for histological examination.

Clinical evaluation and measurement:

Clinical measurement of all rabbit in both group are done by using periodontal probe before and after treatment (Figure1)



(Figure 1) A) clinical measurement of gingival sulcus depth, B) periodontal prob.

Histological examination: The biopsy obtained from buccal side of two anterior teeth and separate the gingivae from bone by using periosteum elevator carefully to preserve any gingival tracing, then put in the solution of 10% neutral buffered formalin (NBF) for 24 hours and place in graduated increase of the alcohol concentration for 10 hours the concentration 70%, 80%, 90%, and 100% respectively for dehydration of spacemen. Xylol, used for 6 hours to remove the remaining alcohol, the biopsy embedded in paraffin wax to obtained block of wax ready for sliced to section of five microns

in series were cut by using microtome. these tissue section are stained by using hematoxyllin and eosin staining for good light microscopical examination .

Statistical analysis

The data were expressed as mean \pm SD, difference between control and experimental groups were statistically analyzed by using indended t-test and freedman test. The level of significance at $p \leq 0.05$.

RESULTS

1- Clinical finding:

The statically analysis of clinical finding obtained by using indended sample T test give significant relation between control and experimental group the result show mean of Gingival sulcus depth in

control group is about (4.92 +_0.73), where the mean of treated group is about (1.35+0.49) , this result show significant

differences between control and experimental group (P = 0.000*(Table 1)(Figure 2) p- Value ≤ 0.05.

Table1: statistical analysis of clinical examination of gingival sulcus depth

Group	Mean +-SD	Sig.(paired- 2tailed)
Control	4.92 +-0.73	0.000*
Experimental	1.35+- 0.49	

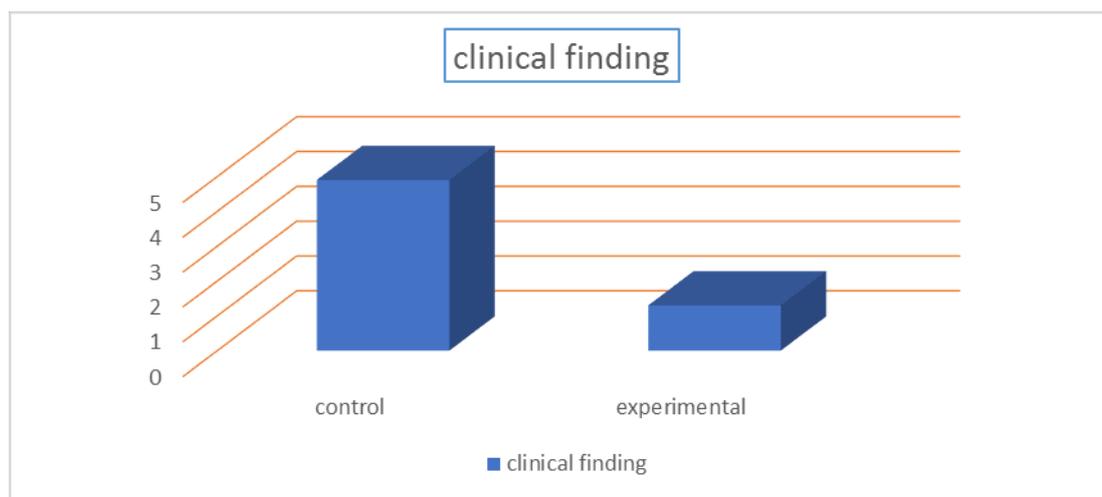


Figure 2: descriptive analysis of clinical measurement OF Gingival sulcus depth.

2- Histological finding of gingival sulcus depth:

In histological examination of control group show obvious overgrowth of gingival sulcus depth with mean (12.9+-1.49). While in experimental group there is an obvious reduction in gingival sulcus depth with mean about (8.6+_ 1.3).

The statistical analysis of this indended sample according to t- test, show significant differences between two group in evaluation of gingival sulcus depth(P=0.000*) p- value ≤0.05 (Table 2) (Figure 3)

Table (2): statistical analysis of histologically examination of gingival sulcus depth

Group	Mean +-SD	Sig.(paired- 2tailed)
Control	12.9+- 1.49	0.000*
Experimental	8.6+- 1.3	

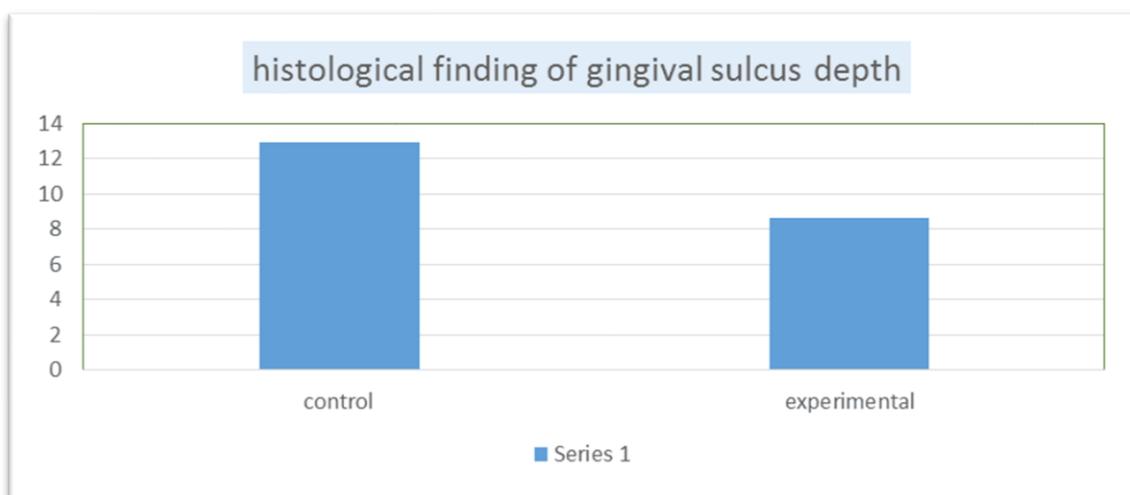
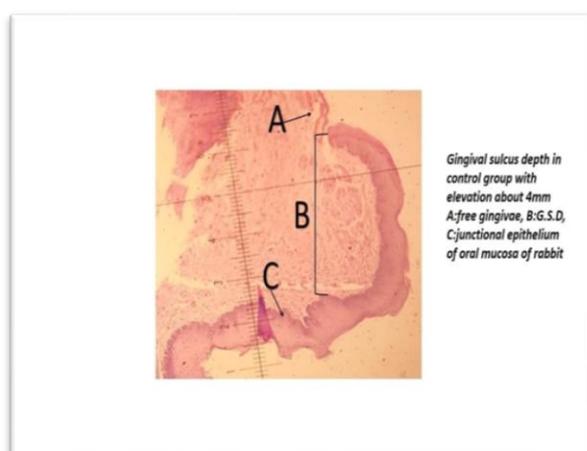


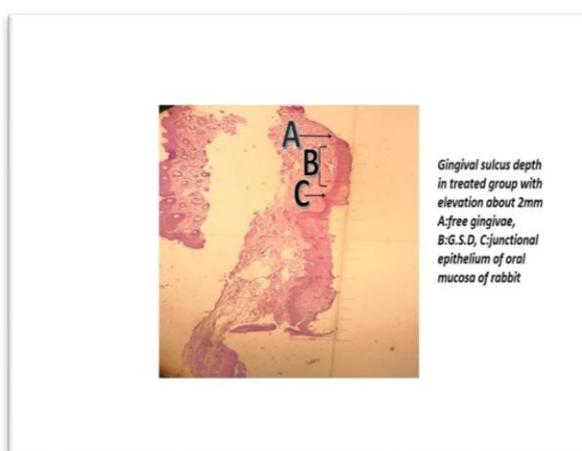
Figure (3): descriptive analysis of histological finding of gingival sulcus depth

The histological finding of upper gingivae of rabbit show higher elevation of free gingivae from the junctional

epithelium in the control group while reduce this elevation in experimental group (Figure 4)



1



2

Figure (4): digital imaging of histological analysis of control group (1) and experimental group (2) of gingival sulcus depth A: Free gingivae, B: Gingival Sulcus Depth, C: junctional epithelium

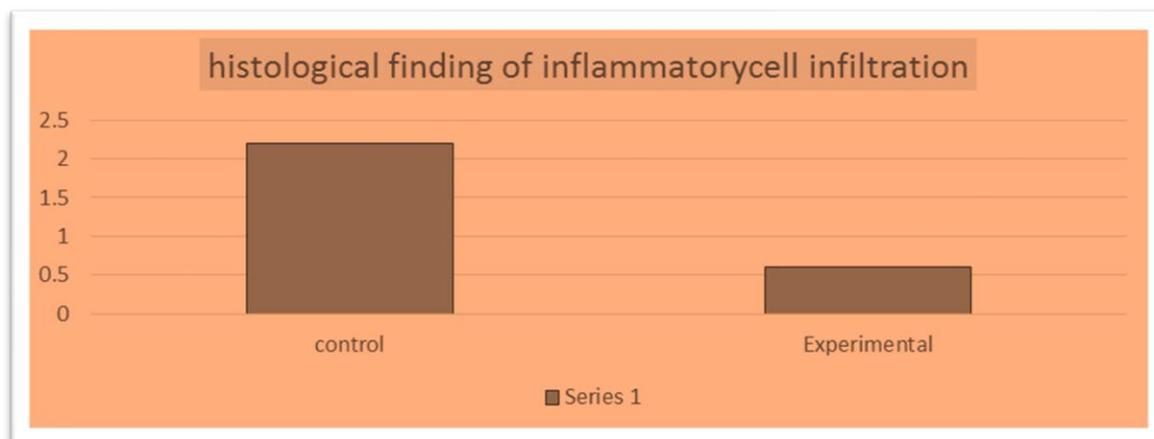
1- Histological finding of inflammatory cell infiltration

According freedman test of statistically analysed criteria of inflammatory cell infiltration the mean is (1.96) in control

group compare to a mean of experimental group that equal to (1.04), with significant differences ($p = 0.000^*$) between two groups (p - value ≤ 0.05). (Table 3) Figure 5))

Table (3): statistical analysis of inflammatory cell infiltration.

Group	Mean	Sig.(paired- 2tailed)
Control	1.96	0.000*
Experimental	1.04	



(Figure 5): Descriptive analysis of histological findings of inflammatory cell infiltration

The histological finding of the upper gingivae of rabbit shows higher number of fibroblast cell with dense connective tissue in basement membrane in tissue section of

control group while there is less number of fibroblast with less density connective tissue in experimental group (figure 6)

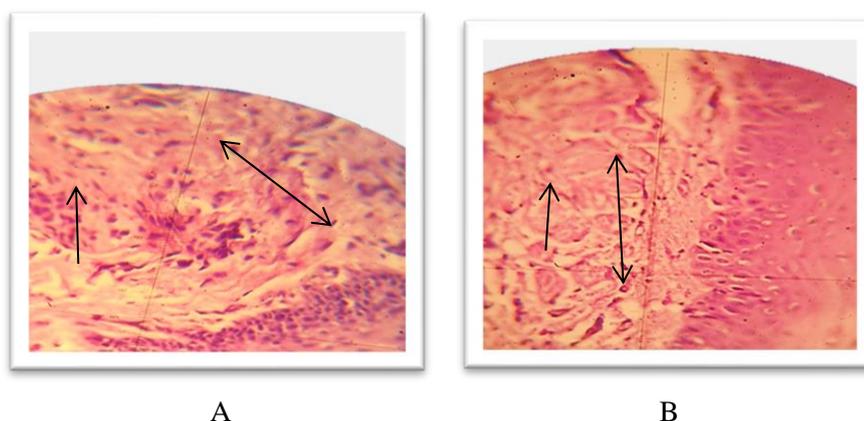


Figure (6): digital imaging of oral mucosa in control group (A) and experimental group(B) after 14 days of folic acid administration.

This figure show high amount of inflammatory cell and dense connective tissue in control group and little amount of inflammatory cell with less density of connective tissue in experimental group. Inflammatory cell (↖) connective tissue (↗)

DISCUSSION

Drug induced gingival over growth shown mostly with increased incidence of elevation gingival sulcus clinically and increase connective tissue density by histological examination this show occur in 30% in human and 40% in animals ^(12,13)

Folic acid supplement shows in this study the higher efficacy in reducing gingival over growth as shown through the day of treatment in experimental group and evident by clinical examination there is a higher difference between two group.

Lee et al, (2020) who showed that drug induce gingival enlargement (amlodipine) use in rabbits study also increased inflammatory cells infiltrate which is a very common well-documented feature of gingival enlargement and this in agreement with our study but show lower incidence in case of use folic acid orally because reduce dense connective tissue and reduce number of inflammatory cell.⁽¹⁴⁾

Tungare and paranjpe , (2019) showed reduced gingival enlargement when used folic acid supplement in patient used drug induced gingival enlargement and this result in agreement with our study in which there is high differences in gingival hyperplasia in group that use folic acid with other group of gingival enlargement especially when use large dose of oral folic acid ⁽¹⁵⁾

Koirala et al, (2017) used topical Folic acid mouthwash (1 mg/ml) on patient have gingival enlargement and showed reduce recurrence of nifedipine induced gingival overgrowth but in small amount Topical folate may reduce gingival inflammation by binding to plaque-derived endotoxins' this in agreement with our study in which folic acid largely reduced gingival enlargement by reduced collagen dense fiber because systemic use of oral folic acid . ⁽¹⁶⁾

Makino et al ,2019 who show in oral administration of FA, there is a significant reduction particularly seen with the highest FA concentration. Downward trends in the histological scores of inflammatory responses in study on mouse model to show inflammatory response on submucosal dermatitis this in agreement with this study in reducing inflammatory response of oral mucosa and reduce fibroblast cell infiltration as seen in experimental group ⁽¹⁷⁾

Alkassimi et al 2018 also noted that gingival inflammation and overgrowth could be reduced to some degree, but not completely, by simply removing gingival sutures and reduce inflammatory cell when used folic acid supplement with cyclosporine for 14 days in mice and compared it with mice taking only cyclosporine for induce gingival enlargement. This study also in agreement with our study on folic acid effect on gingival enlargement. ⁽¹⁸⁾

CONCLUSION

Folic acid supplement is show are effective in reducing the incidence of gingival overgrowth are effective in preventing the recurrence of gingival enlargement.

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