

Effects of Dipyridamole on Histopathology of Salivary Glands in Rabbits

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Abstract

Aims: Dipyridamole is a well-known coronary vasodilator. It is an adenosine reuptake inhibitor leading to increased blood levels of adenosine. So, this study aims to investigate the effects of systemic administration of dipyridamole on the salivary gland tissues in the rabbit model. **Materials and methods:** Ten male rabbits with a body weight of 1.50 ± 0.25 kg were involved. The control group (5 animals) received no treatment, while the treatment group (5 animals) was treated with dipyridamole by gavage tube at an oral dose of 8 mg/kg once daily for 30 days. Then all animals were sacrificed and tissue sample sections from the salivary glands were subjected to a hematoxylin-eosin stain and evaluated for histopathological examination. **Results:** Histopathological slides of parotid and submandibular glands in the treatment group showed abnormal changes in the structure of acinar cells and the cells lining the striated ducts. Sections of parotid displayed necrosis of the cells of serous acini and the cells lining the striated ducts, with the presence of hemorrhage around these ducts. **Conclusions:** Increased levels of adenosine in the body microenvironment induced by systemic administration of the dipyridamole can cause salivary gland tissue disturbance.

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التأثيرات النسيجية والمرضية للدايبيريدامول الغدد اللعابية في الأرانب المخلص

الأهداف: ديبيريديامول هو موسع الأوعية الدموية التاجية المعروفة. وهو مثبط امتصاص الأدينوزين مما يؤدي إلى زيادة مستويات الأدينوزين في الدم. فلذلك هدفت الدراسة إلى دراسة تأثير الإعطاء الجهازى للدبيبيريدامول على الغدد اللعابية في نموذج الأرانب. **المواد وطرائق العمل:** تم استخدام عشرة أرانب ذكور بوزن 1.50 ± 0.25 كجم. لم تتلق مجموعة السيطرة (5 حيوانات) أي علاج، بينما في مجموعة العلاج (5 حيوانات) عولجت بالدبيبيريدامول عن طريق الفم بواسطة أنبوب تعقيمي بجرعة 8 ملغم/كغم/ مرة واحدة يومياً لمدة 30 يوماً. بعد ذلك تم التضحية بجميع الحيوانات وتم تعريض مقاطع الغدد اللعابية لصبغة الهيماتوكسيلين أيوزين ومن ثم تقييم التغيرات النسيجية المرضية. **النتائج:** أظهرت المقاطع النسيجية المرضية للغدد النكفية وتحت الفك السفلي في مجموعة العلاج تغيرات غير طبيعية في بنية الخلايا الغيبية والخلايا المبطنة للقنوات المخططة. أظهرت مقاطع الغدة النكفية نخر خلايا الأسيني المصلي والخلايا المبطنة للقنوات المخططة، مع وجود نزيف حول هذه القنوات. **الاستنتاجات:** زيادة مستوى الأدينوزين في البيئة الدقيقة للجسم الناجم عن الإدارة الجهازية للدبيبيريدامول يمكن أن يسبب اضطراب في الغدد اللعابية.

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INTRODUCTION

Many chemicals and drugs can cause tissue damage. Dipyridamole was presented in the 1960s as a vasodilator agent, it is also used widely and traditionally as an anti-platelet agent that is often given orally ⁽¹⁾. The mechanisms of the non-adenosine receptor can regulate the level of adenosine by inhibiting its transport, altering the intracellular pathway of metabolisms, and altering the generation pathways of adenosine. The commonly used vasodilator and antiplatelet drug, dipyridamole, was originally shown to reduce cyclic nucleotide phosphodiesterase's activity, possibly resulting in stimulation of adenosine receptors by increasing concentrations of local extracellular adenosine. Also, the tissue adenosine level is elevated by adenosine blocking effects of dipyridamole. The imbalance in adenosinergic signalling can cause some pathological conditions ⁽²⁾. Adenosine can induce vasodilation and increase oxygen supply in the heart, skeletal muscle, brain, oral mucosa, gingival, tongue, and salivary glands. So, it provides a negative feedback signal to preserve normal tissue oxygenation ⁽³⁻⁵⁾. Evidence supports the participation of adenosine in the regulation of various tissues ⁽⁶⁾. It can stimulate angiogenesis by poorly understood mechanisms, various studies have shown that the upregulation of endogenous adenosine (e.g., by dipyridamole) can promote vascular changes by the action of vascular endothelial growth factor in a variety of cell types (3).

The administration of Dipyridamole, causes an increased level of cyclic adenosine monophosphate (cAMP) and cyclic guanosine

monophosphate (cGMP), in the tissue leading to elevated protein kinase and regulates multiple cells signalling pathways depending on many conditions like cells type ⁽⁷⁾. Dipyridamole has been used in medicine effectively but little is known about it in the context of dentistry and this warrants further studies. This work was to study the effects of adenosine administration on the tongue and salivary glands in rabbits.

MATERIALS AND METHODS

The scientific committee in the Department of Dental Basic Science at the College of Dentistry, University of Mosul, and the Research Ethics Committee, UoM.Dent/A. L 17/21 on 23/2/2021 approved this research.

This study will be carried out on ten apparently healthy 6-month-old male rabbits with a body weight of 1.5 ± 0.25 kg. They will randomly be divided into two groups. The control group (5 animals), not receive any treatment, while the treatment group (5 animals) will be treated by dipyridamole orally by gavage tube in dose of 8 mg/kg/ once daily for 30 days (11). All rabbits will be sacrificed at the end of the study. For histological investigation tissue samples from the salivary glands will be isolated and fixed in 10% formalin, embedded in paraffin, cut into 5 μ m sections, and stained with H&E. These sections will then be examined under a light microscope for histological changes by a pathologist.

RESULTS

Microscopic Examination of salivary glands

For the control group: The parotid and submandibular glands sections of this group seemed to have normal

histology. Sections of serous acini of the parotid gland appear to be lined by pyramidal cells with eosinophilic cytoplasm with deeply basophilic and round-shaped nuclei, positioned in the basal layer of the cells. The striated duct with distinctive borders is lined by one layer of columnar epithelium. Sections of the submandibular gland, a serous and mucous acinus, appear larger in diameter than those of the parotid gland and the borders of cell lining are clearer. The diameter of striated ducts of the parotid gland is smaller, and lower in number than those of the submandibular gland, Figures (1& 2).

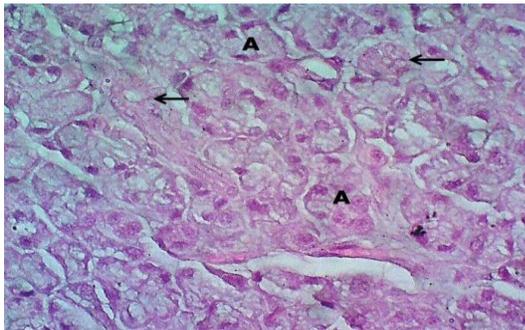


Figure 1: Digital micrograph of a histological section of the parotid salivary gland of the control group. Note the striated ducts (arrows) and serous acini (A). H&E, 400x.

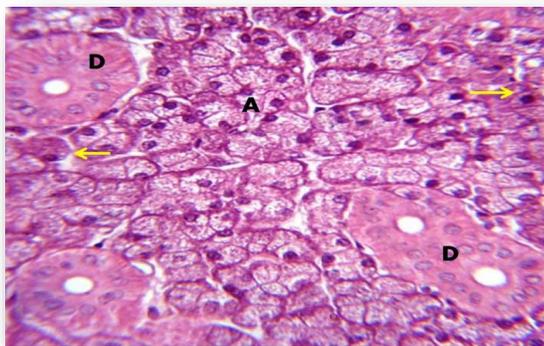


Figure 2: Digital micrograph of a histological section of the submandibular salivary gland of the control group. Note the striated ducts (D) serous-acini (A) and mucous-acini (arrows). H&E, 400x.

The histological section stained by H.& E. of submandibular and parotid of treated groups showed

the same tissue alterations. there is a marked disturbance in the architecture of acinar cells and the cells lining the striated ducts. Figures (3 & 4): Parotid sections showed necrosis of the cells of serous acini and the cells lining the striated ducts, with the presence of hemorrhage around these ducts.

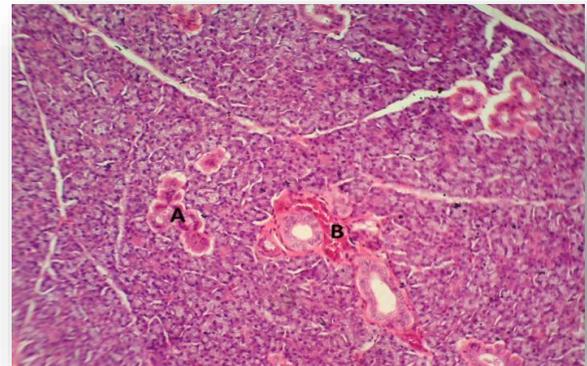


Figure 3: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of epithelial cells lining the striated ducts (A) and presence of haemorrhage around these ducts (B). H&E, 100x.

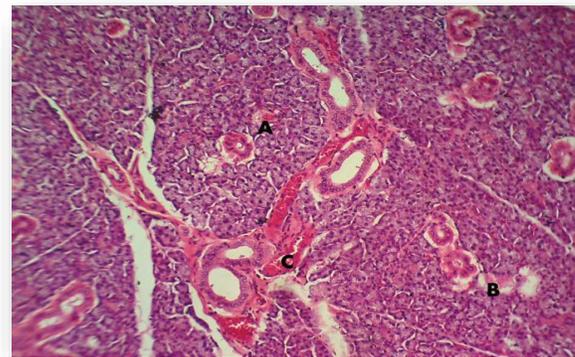


Figure 4: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of epithelial cells lining the striated ducts (A) and serous acini (B) and presence of hemorrhage around these ducts (C). H&E, 100x.

Figures (5 & 6): Parotid sections presented vacuolations and necrosis of serous acini with the

appearance of edema and hemorrhage. The epithelial cells necrosis of lining the striated ducts with the demonstration of macrophage cells and edema.

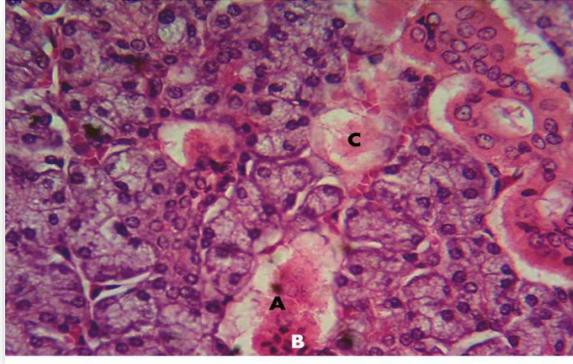


Figure 5: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of epithelial cells lining the striated ducts (A) with a demonstration of macrophages and edema (B), and necrosis and edema of serous-acini (C). H&E, 400x.

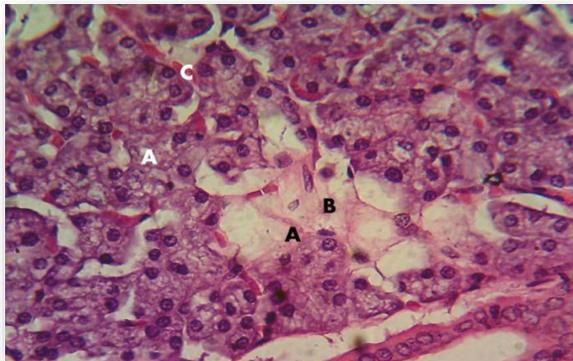


Figure 6: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis and vacuolation of serous-acini (A) with a demonstration of hemorrhage (B), and oedema (C). H&E, 100x.

Figures (7 & 8): Parotid sections showed necrosis of cells of serous acini and the cells lining the striated ducts with stenosis of the lumen and presentation of macrophages, presence of

hemorrhage and congestion of blood vessels around the interlobular ducts.

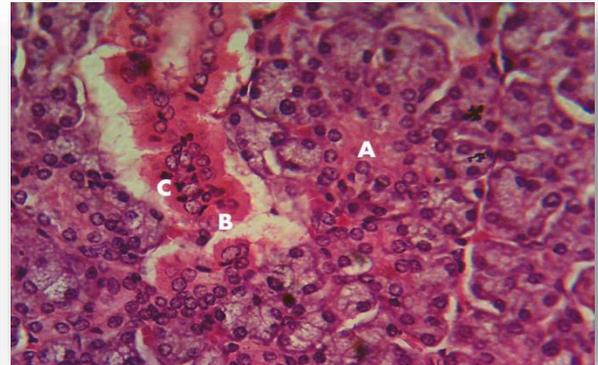


Figure 7: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of serous-acini (A) and striated ducts (B) with stenosis of the lumen and demonstration of macrophages (C). H&E, 400x.

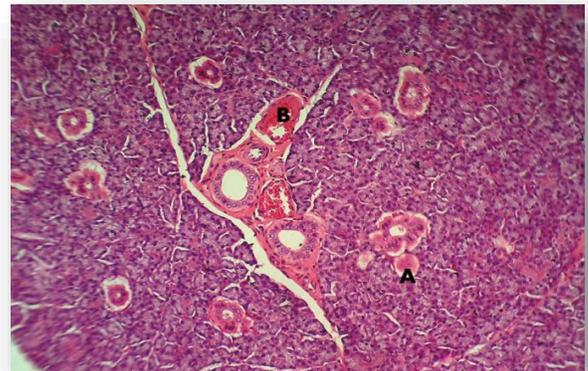


Figure 8: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of epithelial cells lining the striated ducts (A) and presence of hemorrhage and congestion of blood vessels around interlobular ducts (B). H&E, 100x.

Figures (9 & 10): Parotid sections showed destruction, granular cytoplasm, and vacuolation of serous-acini. Necrosis of the striated ducts, pycnotic nuclei with stenosis of the lumen by oedema and aggregation of macrophages.

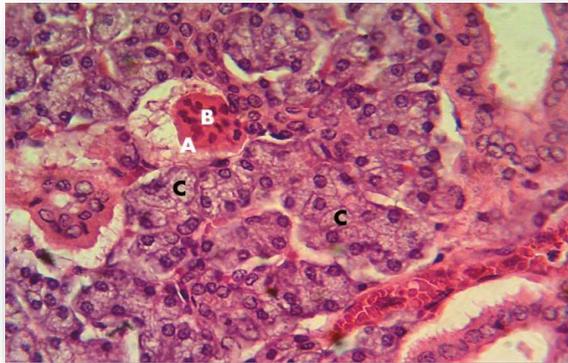


Figure 9: Digital micrograph of a histological section of the parotid salivary gland of the treated group. The striated ducts- necrosis (A) with aggregation of macrophage cells (B), granular cytoplasm, and vacuolation of serous acini (C). H&E, 400x.

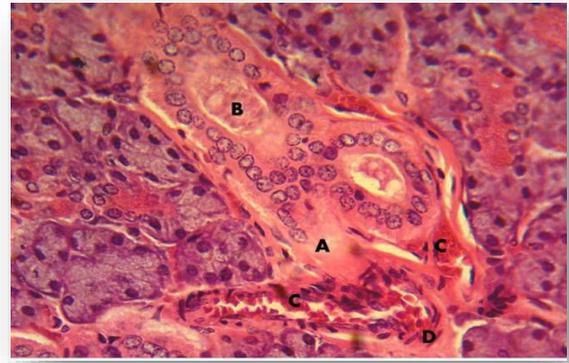


Figure 11: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of striated ducts (A), presentation of oedema in the lumen (B) severe hemorrhage (C) congestion (C), and proliferation of fibrous tissue (D). H&E, 400x.

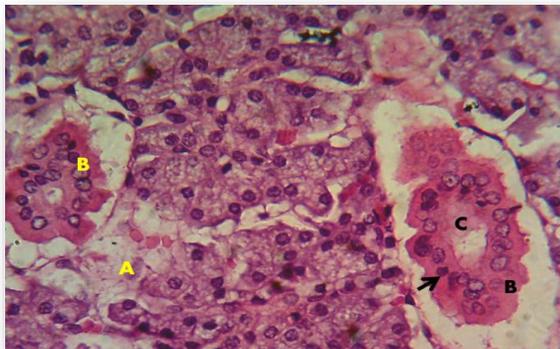


Figure 10: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Necrosis of serous acini (A) and striated ducts (B) (pycnotic nuclei →) with stenosis of the lumen by oedema (C). H&E, 400x.

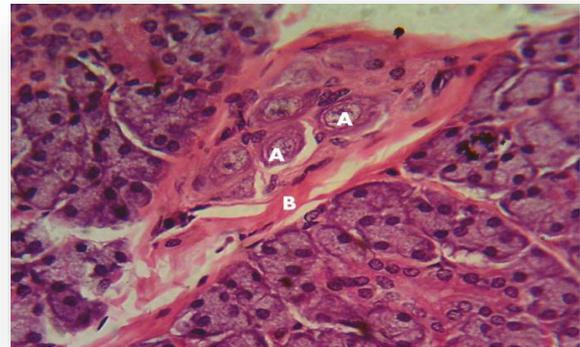


Figure 12: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Presence of many sialolithiasis or deposition of salts (A), surrounded by a proliferation of fibrous tissue (B). H&E, 400x.

Figures (11 &12): Parotid sections showed necrosis of cells of striated ducts, presence of oedema in the lumen, severe haemorrhage, congestion and proliferation of fibrous tissue, and presence of many sialolithiasis or deposition of salts.

Figures (13,14): Submandibular sections showed vascular degeneration of mucous and serous acini, degeneration of striated ducts, and interlobular duct with the presentation of haemorrhage around the striated ducts.

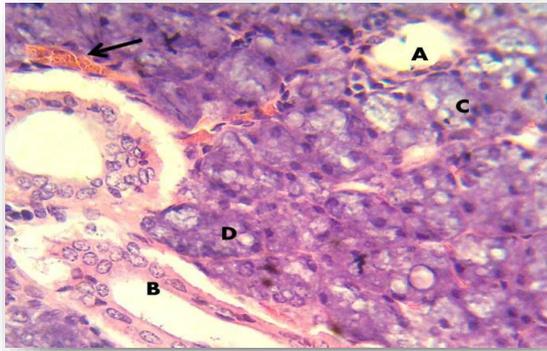


Figure 13: Digital micrograph of a histological section of the parotid salivary gland of the treated group. Note the degeneration of striated duct (A) and interlobular duct (B), vascular necroses of mucous-acini (C) and serous-acini (D), haemorrhage (arrow). H&E, 400x.

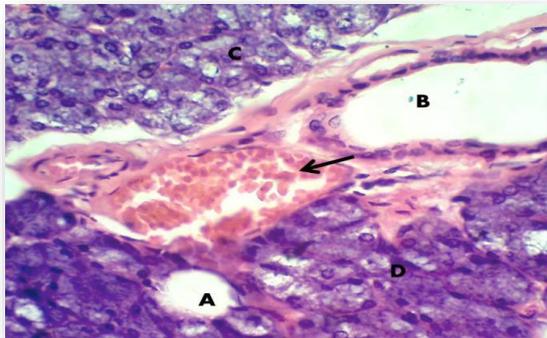


Figure 14: Digital micrograph of a histological section of the submandibular salivary-gland of the treated group. Note the degeneration of striated duct (A) and interlobular duct (B), vascular necroses of mucous-acini (C) and serous-acini (D), haemorrhage (arrow). H&E, 400x.

DISCUSSION

Adenosine is a nucleoside molecule that provokes numerous physiological responses in tissues and organs ⁽⁸⁾. Dipyridamole impedes reuptake of adenosine by platelets, erythrocytes, and endothelial cells leading to elevated plasma levels of adenosine ⁽⁹⁾. dipyridamole has been used to reduce the re-uptake of adenosine and inhibit Cyclin D1 (D1) and

c-Myc levels ⁽¹⁰⁾, the precise effects and the relevant role of dipyridamole in interfering with the regulatory function of different cells are yet to be elucidated ⁽⁷⁾. Alfakje, 2021, said that adenosine accumulation in great quantity and for one-month duration can cause tissue oxidative damage ⁽¹¹⁾.

Adenosine-generated reactive oxygen species induce chronic inflammation in the renal tissue that contributes to the interstitial infiltration of mononuclear cells and the development of pro-fibrotic mediators that come from mononuclear cells (macrophages). These macrophages have a vital role in interstitial fibrosis of renal tissues by the formation of fibrogenic factors that enhance the myofibroblast cells induction to form an extracellular matrix of other tissue bodies like the kidney ^(12 & 13).

When drugs, like adenosine, enter the tissues, they are carried out into the cells through organic cation and anion transporters, then eliminated by efflux transporters may cause the drug to accumulate within the tissue cells, which later on results in drug-induced toxicity characterized by necrosis and epithelial cell sloughing. Thus, dipyridamole that enhances adenosine accumulation might produce such an effect ⁽¹⁴⁾.

Another study suggests that dipyridamole acts in the vasculature through different actions, including inhibition of endothelial proliferation, anti-inflammatory actions, and oxidation action. The selected dose of dipyridamole was to be considered for replication of the entire spectrum that can be found with various therapeutic applications ⁽¹⁵⁾.

In our research therapeutic dose was used, a larger dose may be needed to produce greater effects. The

antioxidant effect of the study drug is therefore significant and observable at doses obtained during clinical therapeutic usage. High dipyridamole levels, clinically possible with high intravenous doses, are needed for the recruitment of the antioxidant capacity⁽¹⁶⁾. There is developing confirmation that adenosine signaling through its receptors may cause severe inflammatory changes in chronic lung disease⁽¹⁷⁾.

Various histological changes where changes were observed in rabbit salivary glands, including infiltration of inflammatory cells, hyperplasia of the interstitial tissue and fibrous deposits, necrosis, severe congestion in the blood vessels, haemorrhage. Adenosine released during injury can engage the adenosine receptor in macrophages and mast cells of alveolar tissues to encourage the construction of MMP-9 then results in stimulation of TGF- B1 and aids in the magnification of lung fibrosis⁽¹⁸⁾. This gives a novel way by which factors produced in response to destruction can impact inflammation and subsequent fibrosis⁽¹⁹⁾. Diffuse haemorrhage can result from different factors including drug reactions⁽²⁰⁾. In the tissue remodeling process, adenosine may also be involved⁽²¹⁾. Mucous gland hyperplasia, sub-epithelial fibrosis, smooth muscle hypertrophy, and angiogenesis are pathogenic hallmarks of tissue remodeling⁽²²⁾. Damage that caused by adenosine and dipyridamole, which induce adenosine accumulation, is suggested to be due to direct injury similar to that induced by catecholamines⁽²³⁾.

Some drugs, like dipyridamole, encourage oxidative stress and result in changes in several micro RNAs (miRNAs) expression, including miR-1 and miR-2. An increase of miR-2 is tangled in myocardial

hypertrophy⁽²⁴⁾ and fibrosis⁽²⁵⁾. An increase in miR-2 has also been associated with the up-regulation of the protein lakase C (PKC) S⁽²⁶⁾. In disagreement with the results of this study, it was found that the administration of dipyridamole attenuated oxidative stress, inflammation, and tissue necrosis⁽²⁷⁾.

The loss of mitochondrial integrity due to oxidative damage eventually interrupts cell functions, makes cells more sensitive to stress, and triggers irreversible pathological consequences that potentially cause cell death. Evidence suggests that tissue damage may linked to increased mitochondrial Ros's formation and oxidative stress production^(28 & 29).

Adding to anti-platelet and antithrombotic activities through the generation of adenosine, dipyridamole provokes vasodilation and through the combination of these functions mostly improves tissue perfusion with possible haemorrhage and blood vessel congestion^(30 & 31).

CONCLUSIONS

Although dipyridamole is effective in inhibiting the formation of blood clots, the resulting oxidative stress plays an important role in causing histopathological changes in healthy organs. The main factors that determined the damage degree of tissue caused by adenosine and dipyridamole were the dose and time intervals of experiments. The data collected from this vivo study indicated that the administration of dipyridamole can destroy tissues of the salivary glands.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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