Meandros Medical And Dental Journal
10.69601/meandrosmdj.1491717

ALTERATION OF SUBLINGUAL GLAND AFTER EXPOSURE TO 6-MERCAPTOPURINE IN MALE RAT: POTENTIAL EFFICACY OF PROPOLIS

Ahmad YAHYAZADEH[1,*]

[1]Department of Histology and Embryology, Faculty of Medicine, Karabuk University, Karabuk, Turkey

ABSTRACT

Objective: Chemotherapeutic drugs not only have a therapeutic effect, but also cause serious damage to healthy organs. In this study, we investigated on the possible efficacy of propolis (PS) on the sublingual gland tissues exposed to 6-mercaptopurine (6MR) in male rats.
Materials and Methods: Twenty-eight Wistar albino rats were allocated to four groups: control (KON), PS, 6MR, 6MR+PS. At 12 days of trial, sublingual glands of all rats were immediately dissected and analysed using the stereological technique and histological examination.
Results: Our findings revealed a significant increase in the total volume of stroma and significant decrease in the total volume of the total volumes of mucous acini, straited ducts, interlobular ducts, and intralobular ducts in the 6MR group than the KON group ($p<0.05$). A significant decrease was also found in the mucous cell number and the serous cell number in the 6MR group than the KON group ($p<0.05$). In the 6MR+PS group, we observed that the total volume of stroma, as well as the mucous cell number and the serous cell number were increased compared to the 6MR group ($p<0.05$). Besides, the total volumes of mucous acini, straited ducts, interlobular ducts, and intralobular ducts were significantly increased in the 6MR+PS group than the 6MR group ($p<0.05$).
Conclusion: : Our results showed that 6MR treatment caused toxicity in sublingual gland tissues, as well as PS application improved such changes in sublingual glands exposed to 6MR.

Keywords: 6-mercaptopurine, Male Rat, Propolis, Sublingual Gland

ÖZET

Amaç: Kemoterapötik ilaçlar sadece tedavi edici bir etkiye sahip olmakla kalmaz, aynı zamanda sağlıklı organlarda ciddi hasarlara neden olmaktadır. Bu çalışmada, 6-merkaptopurine (6MR) maruz bırakılan erkek sıçanların dil altı bezi dokularında propolisin (PS) olası etkisini araştırdık.
Materyal ve Metot: Yirmi sekiz Wistar albino sıçan dört gruba ayrıldı: kontrol (KON), PS, 6MR, 6MR+PS. On iki günlük denemede, tüm sıçanların dil altı bezleri hemen diseke edildi ve stereolojik teknik ve histolojik inceleme kullanılarak analiz edildi.
Bulgu: Bulgularımız, 6MR grubunda toplam stroma hacminde KON grubuna göre anlamlı bir artışın yanı sıra mukus asinüsler, çizgili kanallar, interlobüler kanalları ve intralobüler kanalların toplam hacimlerinde anlamlı bir azalma olduğunu ortaya koydu ($p<0.05$). 6MR grubunda müköz hücre ve seröz hücre sayısında da KON grubuna göre anlamlı bir azalma bulundu ($p<0.05$). 6MR+PS grubunda toplam stroma hacminin yanı sıra müköz hücre sayısı ve seröz hücre sayısının 6MR grubuna göre arttığını gözlemledik ($p<0.05$). Ayrıca 6MR+PS grubunda müköz asinüsleri, çizgili kanallar, interlobüler kanalları ve intralobüler kanalların toplam hacimleri 6MR grubuna göre anlamlı olarak arttığı saptandı ($p<0.05$).
Sonuç: Bulgularımız, 6MR tedavisinin dil altı bez dokularında toksisiteye yol açtığını, ayrıca PS uygulamasının 6MR'ye maruz kalan dil altı bezlerinde bu tür değişiklikleri iyileştirdiğini gösterdi.

Corresponding Author Ahmad Yahyazadeh, PhD Department of Histology and Embryology, Faculty of Medicine, Karabuk University, Karabuk, Turkey E-mail: yahyazadeh.ahmad@karabuk.edu.tr Recived:19.06.2023 Accepted:08.02.2024

Anahtar Kelimeler: 6-Merkaptopürin, Dil Altı Bez, Erkek Sıçan, Propolis

INTRODUCTION

The importance of cancer is known by everyone due to the increasing prevalence of cancers in our country and its impact on family, economy, and social psychology. Recently, these cancers have started to be seen frequently in children under the age of 15, and the increase in these cases has attracted the attention of researchers (1). There are many types of cancer treatments. Chemotherapeutic treatment is an ongoing process with the use of drugs containing strong chemicals to stop or kill uncontrolled cell growth in the body. Although chemotherapy is a very effective treatment method against cancer, it is known that it has many side effects. While some of these side effects are mild, some of them can cause serious complications to prevent their use. 6-mercaptopurine (6MR) is a common chemotherapeutic drug and immunosuppressive agent and has been found to play an important role in cancer treatments (2). Daily oral 6MR has been used as part of maintenance therapy for over 50 years. Following administration of 6MR, it is first metabolized intracellularly to an active form (3). This active metabolite inhibits the activities of enzymes in the de novo purine synthetic pathway. It is also converted to thioguanine, which is included in the nucleic acid and causes cytotoxicity (4). It acts on cancer cells by disrupting the replication and transcription processes of DNA (5). The lack of selective biological targets of 6MR can cause serious life-threatening effects such as myelosuppression, greatly affecting its clinical efficacy (6). In the treatment of children with acute lymphoblastic leukemia, 6MR is frequently interrupted due to liver toxicity, especially during the maintenance phase of treatment (7). There is a relationship between disruption of oxidative balance and complication in body organisms following exposure to 6MR. Therefore, this leads to oxidative damage to functional and architectural of vital organs. Sublingual gland tissues consist of serous and mucous acini, which may be disturbed by this chemotherapeutic agent, 6MR. Recently, numerous studies have focused on reducing oxidative stress and protecting healthy structures from the toxicity of anticancer drugs. Exogenous antioxidant administration may be one of the approaches to ameliorate the change in biosystem via reducing oxidative stress. Propolis (PS), as an antioxidative substance, is generally known as “bee glue” and is a general name given to the resinous substance that bees collect from different plant species. It has been reported that RR has a therapeutic effect on cytotoxicity (8). There is also a lot of research showing that PS has antiseptic, antioxidant, antimycotic, anti-inflammatory, antibacterial, antifungal, anticancer, immunomodulatory, and anti-ulcer properties (9). Recently, studies that contribute to the mitigation of the side effects of chemotherapeutic drugs have attracted attention. Our study investigated whether 6MR treatment would adversely affect the sublingual gland tissues of young male rats. Besides, we surveyed therapeutic efficacy of PS on 6MR-induced change in sublingual glands using stereological analysis and histological examination.

MATERIALS AND METHODS

In our study, 28 male Wistar albino rats, 5 weeks old and body weight of 60-80 g, were used. Ethical approval of the present investigation was granted by the Laboratory Animal Ethics Committee of Karabuk University (NO:2022/09/17-17.10.2022). The necessary care was taken to ensure the conditions of the ethics committee. All rats were maintained in plastic cages under 12:12 h day/night cycle at 22±2 temperature °C and 50±5% humidity with intake of water and food ad libitum. Besides, histological studies were carried out in the Histology and Embryology Department of Karabuk University. For the experiment, the animals were divided into 4 groups of 7 rats, then experimental procedure was performed as follows: 1. Control (KON) group (n:7): Healthy subjects received only olive oil for 12 days. 2. 6-mercaptopurine (6MR) group (n:7): Subjects received orally 5 mg/kg 6MR for 10 days from day 2 to day 12 (10). 3. Propolis (PS) group (n:7): Subjects received orally 150 mg/kg PS for 12 days (11). 4. 6-mercaptopurine + propolis (6MR+PS) group (n:7): Subjects received orally not only 150 mg/kg PS for 12 days, but also 5 mg/kg 6MR for 10 days from day 2 to day 12. After twelve days of treatment, all subjects were sacrificed under intraperitoneal anaesthesia with 60 mg/kg ketamine and 5 mg/kg xylazine, then sublingual gland tissues were dissected immediately. Histology All samples were fixed in 10% neutral formalin, followed by tissue processing (dehydration, clearing, and embedding) to prepare them for microscopic examination. Sublingual gland blocks were cut into 7 µm sections by means of a rotary microtome. Theses sections were then taken into a 40-45 °C water bath to vanish their wrinkles, followed by incubation in 60 °C to deparaffinized samples. The haematoxylin-eosin (H&E) dye was applied to the slides ready for staining. Finally, samples were used for unbiased stereological analysis and histopathological examination. Stereology We used the point-counting methods and Cavalieri principle to calculate the total volumes of mucous and serous acini, intercalated ducts, striated ducts, interlobular ducts, volume fraction ratio of serous and mucous acini to stroma (12). A pilot study was conducted to validate the point density of a grid. Photographs were taken from all sublingual gland sections, then calibrated grid was placed randomly on them. After the total number of the points that hit the region of interest was obtained, the volume of sublingual glands was calculated as:

$$V_{(total)} = t \times \Sigma A$$

Here, “t” is the total thickness of all slices plus the intervals and “ΣA” is the total area of interest in all slices. “ΣA ” was also calculated as:

$$\Sigma A = a(p) \times \Sigma P$$

The physical disector was also utilized for estimating the number of both mucous and serous cells in 7 µm section pairs including reference and look-up (13). Consecutive sections were taken on numbered slides with a

1/100 interval based on the systematic and random sampling rule. This rule provides an equal chance of sampling to every part of the sampled structure so that the first section is determined randomly and then the other sections are systematically selected. Before starting the analyses, a suitable strategy was determined by conducting a pilot study, and then cell counting was carried out. All disector pairs were photographed, subsequently a counting frame was superimposed on pictures. All particles were then counted according to the physical disector rules and numerical density of particles was estimated as:

$$N_{(v)} = \frac{\Sigma Q^{-}}{\Sigma V^{Dissector}}$$

Here, "ΣQ^{-}'' refers to counted particles and "ΣV" refers to the total volume of the disector frames. Lastly, the total number of serous and mucous acini were estimated as:

$$TN(Total) = N_V \times V_{ref}$$

Statistical Analysis

Statistical analysis was carried out by IBM version 25.0 SPSS software (SPSS Inc., Chicago, IL, USA). We determined the normal distribution of the variable using the Shapiro-Wilk test, so the parametric test was chosen for data analysis of our all parameters. The One-Way ANOVA were utilized to test whether there was a significant difference between the means of 4 independent groups. Multiple group comparisons were carried out using Tukey's post hoc test. Levene's test was also conducted to assess for homogeneity of variance. The results were expressed as a mean ± standard deviation. P value < 0.05 was considered statistically significant.

RESULTS

Stereological result

Total volume of stroma The total volumes of stroma in all groups are shown in figure 1. Statistical comparison of the data revealed a significant increase in the total volume of stroma in the 6MR group than the KON and PS groups ($p<0.01$). In the 6MR+PS group, a significant reduction was detected when compared with the 6MR group ($p<0.05$). Our results also indicated a significant elevation in the 6MR+PS group than the KON and PS groups ($p<0.05$).

Total volume of mucous acini

The total volumes of mucous acini in all groups are shown in figure 2A. Our volumetric data showed that the total volume of mucous acini was significantly reduced in the 6MR group when compared with the KON and PS group ($p<0.01$). In the 6MR+PS group, a significant increase was found than the 6MR group ($p<0.01$). A significant reduction in the 6MR+PS group was found than the KON and PS groups ($p<0.05$).

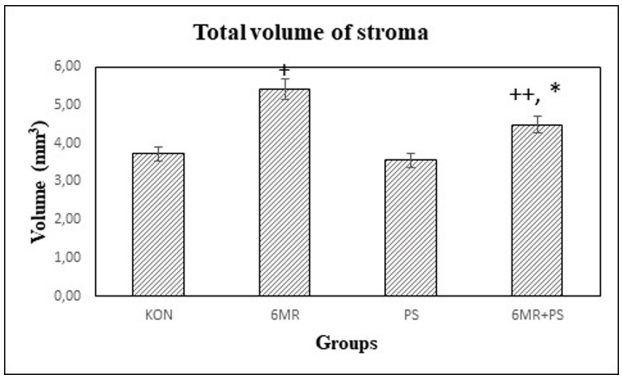

Figure 1: The total volume of stroma in all group. +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, *; significantly different from the KON and PS groups

Total volume of serous acini

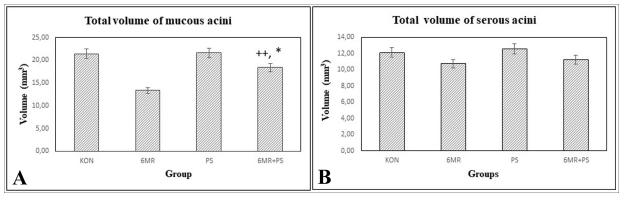

Figure 2: The total volumes of mucous acini (A) and serous acini (B) in all group. +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, *; significantly different from the KON and PS groups

The total volumes of serous acini in all groups are shown in figure 2B. The total volume of serous acini was reduced in the 6MR group than the KON group, but no significant difference was detected. There was also no significant difference among the KON, PS, and 6MR+PS groups.

Total volume of intercalated ducts

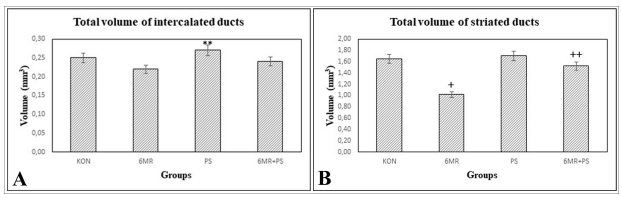

Figure 3: The total volumes of intercalated ducts (A) and striated ducts in all groups (B). +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, **; significantly different from the 6MR group

The total volumes of intercalated ducts in all groups are shown in figure 3A. Our results indicated no significant difference between the 6MR group and the KON group. There was also no significant difference among the KON, PS, and 6MR+PS groups. We detected a significant increase in the PS group than the 6MR group ($p<0.05$).

Total volume of striated ducts

The total volumes of striated ducts in all groups are shown in figure 3B. The total volume of striated ducts was significantly decreased in the 6MR group when compared with the KON and PS groups ($p<0.01$). In the 6MR+PS group, a significant reduction was observed compared to the 6MR group ($p<0.01$).

Total volume of interlobular ducts

The total volumes of interlobular ducts in all groups are shown in figure 4A. We detected that the total volume of interlobular ducts was significantly lower in the 6MR group when compared with the KON and PS groups ($p<0.01$). In the 6MR+PS group, a significant increase was found than the 6MR group ($p<0.01$). The present quantitative data revealed a significant reduction in the 6MR+PS group when compared with the KON and PS groups ($p<0.01$).

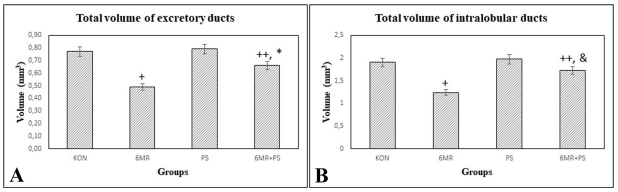

Figure 4: The total volumes of interlobular ducts (A) and intralobular ducts (B) in all groups. +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, *; significantly different from the KON and PS groups, &; significantly different from the PS group

Total volume of intralobular ducts

The total volumes of intralobular ducts in all groups are shown in figure 4B. The total volume of intralobular ducts was significantly lower in the 6MR group when compared with the KON and PS groups ($p<0.01$). To the contrary, a significant elevation was detected in the 6MR+PS group than the 6MR group ($p<0.01$). Although there was no significant difference between the 6MR+PS group and the KON group, decreased volume was detected in the 6MR+PS group than the PS group ($p<0.05$).

Volume fraction ratio of serous acini to stroma The volume fraction ratios of serous acini to stroma in all groups are shown in figure 5A. Comparison of data revealed that a significant reduction was detected in the 6MR group than the KON and PS group ($p<0.01$). In the 6MR+PS group, the volume fraction ratio of serous acini to stroma was higher than the 6MR group ($p<0.01$). Our results also indicated a reduction in the 6MR+PS group than the KON and PS groups ($p<0.01$).

Volume fraction ratio of mucous acini to stroma

The volume fraction ratios of mucous acini to stroma in all groups are shown in figure 5B. In terms of the volume fraction ratio of mucous acini to stroma, our quantitative data indicated a significant reduction in the 6MR group than the KON and PS groups ($p<0.01$). To the contrary, a significant increase was detected in the 6MR+PS group than the 6MR group ($p<0.01$). Our results also indicated a significant reduction in the 6MR+PS group than the KON and PS groups ($p<0.01$).

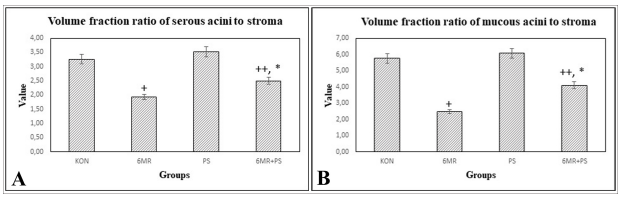

Figure 5: The volume fraction ratios of mucous acini to stroma (A) and the volume fraction ratios of serous acini to stroma (B) in all groups. +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, *; significantly different from the KON and PS groups

Total number of mucous cells

The total number of mucous cells in all groups are shown in figure 6A. The present stereological results revealed a significant reduction in the mucous cell number in the 6MR group than the KON group ($p<0.01$). We also observed the increased mucous cell number in the 6MR+PS group than the 6MR group ($p<0.01$). A significant reduction in the 6MR+PS group was detected than the KON ($p<0.05$) and PS ($p<0.01$) groups.

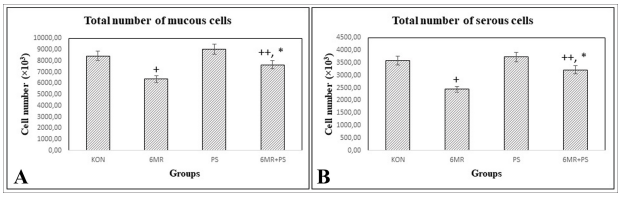

Figure 6: The total number of mucous cells (A) and serous cells (B) in all groups. +; significantly different from the KON and PS groups, ++, significantly different from the 6MR group, *; significantly different from the KON and PS groups

Total number of serous cells

The total number of serous cells in all groups are shown in figure 6B. The serous cell number in the 6MR and group was significantly reduced than the KON group ($p<0.01$). A significant increase in the 6MR+PS group was detected than the 6MR group ($p<0.01$). In the 6MR+PS group compared to the KON and PS groups, the serous cell number was significantly lower ($p<0.05$).

Histopathological result

Our histological examination revealed valuable results in all groups. While the architectures of the sublingual glands appeared normal in the KON and PS groups (Figures 7A-C and 8A-C), marked alterations were observed in the 6MR group (Figure 7D-F). We detected separation of acini from each other, and degeneration of acinar cells, as well as rupture of interlobular ducts in the 6MR group. Discrete interlobular and intralobular ducts from acini were also prominent. In the 6MR+PS group, structural alterations were detected, but less than the 6MR group (Figure 8D-F).

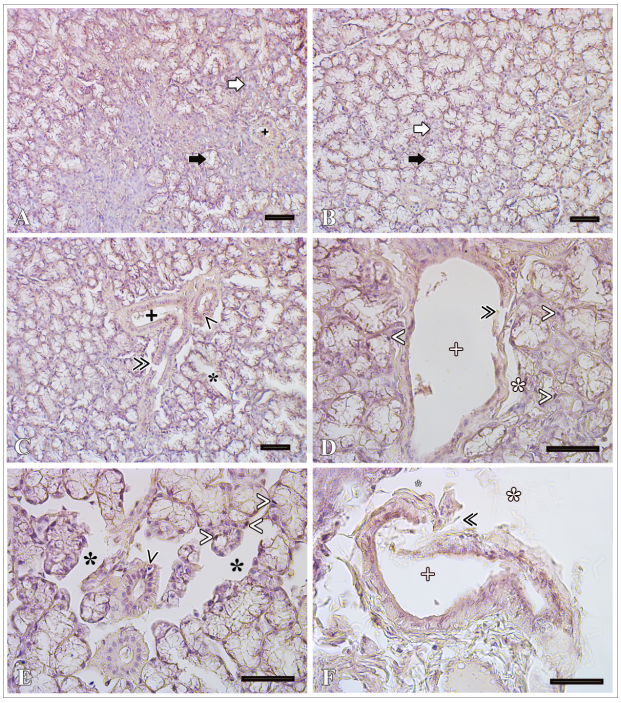

Figure 7: Micrographs of sublingual gland tissues in the KON (A-C) and 6MR (D-F) groups. White arrow, serous cell; black arrow, mucous cells; black pluses, intralobular ducts, white pluses, interlobular ducts; black asterisks, separation of acini from each other; white asterisks, separation of interlobular and intralobular ducts from acini; arrow heads, degenerated cells; double arrowhead, rupture of interlobular ducts

DISCUSSION

Recently, multiple drug applications used in childhood cancers have increased the chance of success in treatment. 6-MP, which is an essential drug in the current treatment of some acute lymphoblastic leukaemia and lymphoma types, should be used for more than one year in accordance with chemotherapy protocols. In such long-term use of the drug, damage to vital organs is often inevitable as side effects, and the solution to this situation is usually discontinuation of the drug. However, such interruptions in chemotherapy reduce the chance and success of treatment. Since it is vitally important to continue the treatment uninterruptedly in anti-cancer protocols, there is a need for methods and practices that will ensure uninterrupted continuation of treatment by minimizing drug side effects (14). To the best of our knowledge, this study investigated the deleterious effect of 6MR on the sublingual gland tissue by the stereological technique for first time. The other aim was to survey the ameliorative efficacious of PS on 6MR-induced structural change in the sublingual gland tissue. Stereological methods as reliable tools provide accurate data regarding the parameters of the sublingual glands. Our quantitative data revealed that 6MR treatment in the 6MR group significantly increased the total volume of stroma than the KON group. Given our results, the total volumes of mucous acini, straited ducts, interlobular ducts, and intralobular ducts were significantly reduced in the 6MR group than the KON group. Besides, administration of 6MR significantly reduced the total number of serous cells and mucous cells than the Kon group. These findings showed the harmful effect of 6MR on the sublingual glans. Free radicals in the organism can be formed both as a by-product of normal metabolism and from endogenous and exogenous sources with the effect drugs and other harmful chemicals. If the oxidative balance in the body is disturbed for any reason, it can cause damage to vital organs. In other words, disturbance of oxidative balance can result in high ROS generation, subsequently oxidative stress (12, 15). When free radicals are also formed at rates that exceed the capacity of the antioxidant defence mechanisms, they cause disorders in various structures of the organism. A study reported an association between 6MR treatment and increased oxidative stress in the analysis of blood samples on day 14 of the experiment (7). 6MR therapy was found to induce increased malondialdehyde and decreased endogenous enzyme level. High ROS generation can also lead to microvessel barrier dysfunction (16). An association has been found between ROS-induced oxidative stress and increased vascular permeability. Excessive ROS production also can cause deteriorative impact on pericytes and endothelial cells (17). Therefore, vascular leakage occurs, which may be the main cause of the increased stroma volume of the sublingual gland exposed to 6MR. Interaction between free radicals and unsaturated fatty acids in the cell membrane can also contribute to lipid peroxidation, resulting in oxidative damage (18). Moreover, oxidative stress can induce damage to intracellular molecules such as DNA, carbohydrates, and proteins (19, 20). 6-methylmercaptopurine, as an active metabolite of 6MR, has been found to cause toxicity in vital organs (14). Physiological and histological profiles of organs can be altered following exposure to 6MR (21).

In the 6MR+PS group, we detected a significant reduction in the total volume of stroma, volume fraction ratio of mucous acini to stroma, and volume fraction ratio of serous acini to stroma, as well as a significant elevation in the total volumes of mucous acini, straited ducts, interlobular ducts, and intralobular ducts than the 6MR group. The mucous cell number and the serous cell number in the 6MR+PS group was also significantly increased than the 6MR group. These results indicated that PS exerted the ameliorative efficacy on the sublingual gland tissues. PS has been reported to possess low or no toxicity to healthy cells due to its selectively toxic properties against tumour cells (22). PS also contributes to a selective cytotoxic effect on cancer cells by inducing apoptosis, endoplasmic reticulum stress and caspase activity (23). Another study suggested an association between PS treatment and reduction in inflammation and oxidative stress due to attenuating total oxidant status and improving total antioxidant capacity (24).

Histopathological results exhibited the toxicity of 6MR on the sublingual architectures. In the 6MR+PS group, administration of PS also improved such changes detected in the 6MR group. This histopathological finding confirmed our accurate stereological result. In the next study, other

experimental parameters should be investigated to identify other unknown facts.

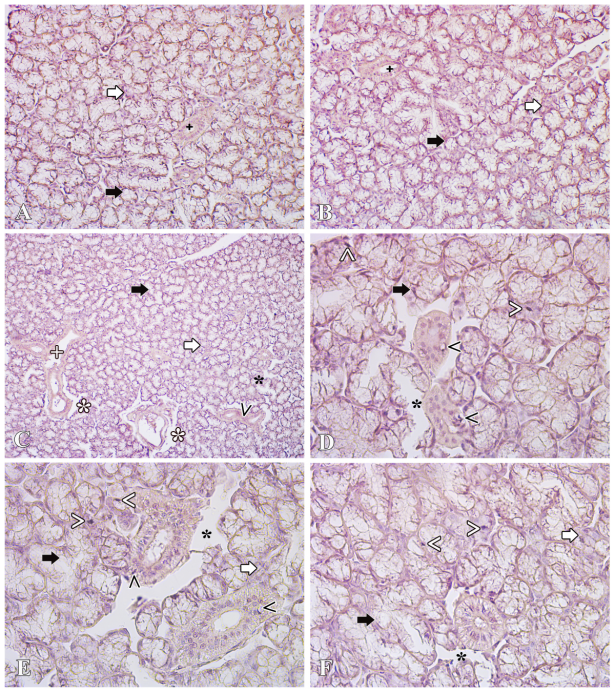

Figure 8: Micrographs of sublingual gland tissues in the PS (A-C) and 6MR+PS (D-F) groups. White arrow, serous cell; black arrow, mucous cells; black pluses, intralobular ducts, white pluses, interlobular ducts; black asterisks, separation of acini from each other; white asterisks, separation of interlobular and intralobular ducts from acini; arrow heads, degenerated cells.

CONCLUSION

Our findings revealed that 6MR caused a significant elevation in the total volume of stroma, as well as a significant reduction in volume fraction ratio of mucous acini to stroma, and volume fraction ratio of serous acini to stroma, as well as the mucous cell number and the serous cell number in the 6MR group to the KON group. We also detected a significant elevation in the total volumes of mucous acini, straited ducts, interlobular ducts, and intralobular ducts in the 6MR group to the KON group. In the 6MR+PS group than the 6MR group, 6MR-induced changes in the sublingual gland tissues were improved following PS administration. We suggested that PS might have been as an alternative therapeutic agent to alleviate the toxicity of 6MR in human.

Ethics

The authors declared that this study received no financial support.

Authorship Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Declaration of competing interest

No conflict of interest was declared by the author.

References

1. El-Sabagh ME, Ramadan KS, El-slam IMA, Ibrahim AM. Antioxidants Status in Acute Lymphoblastic Leukemia Patients. 2011;1(1):1-6.
2. Schmiegelow K, Nielsen SN, Frandsen TL, Nersting J. Mercaptopurine/Methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. J Pediatr Hematol Oncol. 2014;36(7):503-17.
3. Bradford K, Shih DQ. Optimizing 6-mercaptopurine and azathioprine therapy in the management of inflammatory bowel disease. World J Gastroenterol. 2011;17(37):4166-73.
4. Karran P. Thiopurines, DNA damage, DNA repair and therapy-related cancer. Br Med Bull. 2006;79-80:153-70.
5. Gaynon PS. Mercaptopurine in childhood acute lymphoblastic leukaemia. Lancet Oncol. 2017;18(4):425-6.
6. Xie C, Yue LJ, Ding H, Ren YF, Yang CL, Zheng MM. [Correlations between 6-mercaptopurine treatment-related adverse reactions in children with acute lymphoblastic leukemia and polymorphisms of thiopurine methyltransferase gene]. Zhongguo Dang Dai Er Ke Za Zhi. 2014;16(5):499-503.
7. Tulumen T, Ayata A, Ozen M, Sutcu R, Canatan D. The protective effect of Capparis ovata on 6-mercaptopurine-induced hepatotoxicity and oxidative stress in rats. J Pediatr Hematol Oncol. 2015;37(4):290-4.
8. Laaroussi H, Bakour M, Ousaaid D, Aboulghazi A, Ferreira-Santos P, Genisheva Z, et al. Effect of antioxidant-rich propolis and bee pollen extracts against D-glucose induced type 2 diabetes in rats. Food Res Int. 2020;138(Pt B):109802.
9. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. Oxid Med Cell Longev. 2017;2017:1259510.
10. Abdelbaky NW, Abdelazem AZ, Hashem KS. Thymoquinone Attenuates 6-Mercaptopurine Induced Testicular Toxicity in Albino Rats: Possible Mechanisms are Involved. Advances in Animal and Veterinary Sciences. 2020;8(6):653-60.
11. Swamy M, Suhaili D, Sirajudeen KN, Mustapha Z, Govindasamy C. Propolis ameliorates tumor nerosis factor-alpha, nitric oxide levels, caspase-3 and nitric oxide synthase activities in kainic acid mediated excitotoxicity in rat brain. Afr J Tradit Complement Altern Med. 2014;11(5):48-53.
12. Aktas I, Yahyazadeh A. Protective potential of misoprostol against kidney alteration via alleviating oxidative stress in rat following exposure to paclitaxel. Tissue Cell. 2022;79:101966.
13. Tufek NH, Yahyazadeh A, Altunkaynak BZ. Protective effect of indole-3-carbinol on testis of a high fat diet induced obesity. Biotech Histochem. 2023;98(1):1-12.

14. Kamojjala R, Bostrom B. Allopurinol to Prevent Mercaptopurine Adverse Effects in Children and Young Adults With Acute Lymphoblastic Leukemia. J Pediatr Hematol Oncol. 2021;43(3):95-100.
15. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19.
16. He P, Talukder MAH, Gao F. Oxidative Stress and Microvessel Barrier Dysfunction. Front Physiol. 2020;11:472.
17. Zhu L, He P. fMLP-stimulated release of reactive oxygen species from adherent leukocytes increases microvessel permeability. Am J Physiol Heart Circ Physiol. 2006;290(1):H365-72.
18. Unsal V, Cicek M, Sabancilar I. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev Environ Health. 2021;36(2):279-95.
19. Hanukoglu I. Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. Drug Metab Rev. 2006;38(1-2):171-96.
20. Hasegawa M, Wilson G, Russell LD, Meistrich ML. Radiation-induced cell death in the mouse testis: relationship to apoptosis. Radiat Res. 1997;147(4):457-67.
21. Govindappa PK, Joladarashi D, Hallur RLS, Sanganal JS, Phani AR. Toxicity evaluation of 6-mercaptopurine-Chitosan nanoparticles in rats. Saudi Pharm J. 2020;28(1):147-54.
22. Xuan H, Li Z, Yan H, Sang Q, Wang K, He Q, et al. Antitumor Activity of Chinese Propolis in Human Breast Cancer MCF-7 and MDA-MB-231 Cells. Evid Based Complement Alternat Med. 2014;2014:280120.
23. Demir S, Aliyazicioglu Y, Turan I, Misir S, Mentese A, Yaman SO, et al. Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutr Cancer. 2016;68(1):165-72.
24. Soleimani D, Miryan M, Hadi V, Gholizadeh Navashenaq J, Moludi J, Sayedi SM, et al. Effect of propolis supplementation on athletic performance, body composition, inflammation, and oxidative stress following intense exercise: A triple-blind randomized clinical trial. Food Sci Nutr. 2021;9(7):3631-40.