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Nebulized Methylene Blue as an Anti-Pruritic Treatment Modality in Dogs with Atopic Dermatitis

Kerem URAL [1] Hasan ERDOĞAN [1] Songül ERDOĞAN [1] Serdar PAŞA [1] Alper SUNGUR [1] Tahir ÖZALP [1] ✉

[1] Aydin Adnan Menderes University, Veterinary Faculty, Department of Internal Medicine, Aydin/ Türkiye

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Abstract: Canine atopic dermatitis (cAD) is a chronic inflammatory dermotological disorder in dogs, primarily characterized by pruritus. Conventional treatment modalities involve topical and systemic agents among allergen-specific immunotherapy (ASIT); however, their effectiveness varies, and potential adverse effects necessitate alternative therapeutic approaches. Methylene blue (MB) is a cationic photosensitizer with antimicrobial and neuromodulatory effects, previously shown to alleviate pruritus in human medicine by blocking peripheral nerve endings. This study aimed to evaluate the therapeutic capability of nebulized MB in reducing pruritus severity in dogs with AD. For this propose a total of 13 dogs diagnosed with AD without any treatment for at least three weeks were enrolled to the study. All cases were assessed using the Canine Atopic Dermatitis Extent and Severity Index (CADESI-04) and Visual Analog Scale (VAS). MB was administered via nebulization in a single session, and pruritus scores were recorded on days 2, 4, 6, 8, and 10. The results demonstrated a marked reduction in pruritus scores following MB administration, with the most significant improvements observed on days 4, 6, and 8. no adverse effects were noted throughout the study. Given its neuro-modulatory and anti-inflammatory properties, MB appears to be a promising alternative approach for the management of pruritus associated with AD in dogs. In conclusion nebulized MB is a safe and effective treatment modality for pruritus management in cAD. These findings warrant further controlled studies to elucidate the underlying mechanisms and long-term therapeutic potential of MB in veterinary dermatology.

Keywords: Methylene blue, canine atopic dermatitis, pruritus, nebulization, veterinary dermatology

Detection of Serum Zonulin Levels in Association with Intestinal Permeability in Calves Mono- or Co-Infected with Rotavirus

Özet: Köpeklerde atopik dermatit (cAD), başlıca belirti olarak pruritus (kaşıntı) ile seyreden kronik bir inflamatuvar deri hastalığıdır. Geleneksel tedavi yöntemleri arasında topikal ve sistemik ajanların yanı sıra alerjene özgü immünoterapi (ASIT) yer almaktadır; ancak bu yöntemlerin etkinliği değişkenlik göstermekte ve olası yan etkileri nedeniyle alternatif tedavi yaklaşımlarına ihtiyaç duyulmaktadır. Metilen mavisi (MB), antimikrobiyal ve nöromodülatör etkileri bulunan katyonik bir fotosensitizer olup, insan tıbbında periferik sinir uçlarını bloke ederek pruritusun hafifletilmesinde olumlu sonuçlar göstermiştir. Bu çalışmanın amacı, nebulizasyon yoluyla uygulanan MB'nin atopik dermatitli köpeklerde kaşıntı şiddetini azaltmadaki tedavi edici potansiyelini değerlendirmektir. Bu amaçla, en az üç haftadır herhangi bir tedavi almamış ve atopik dermatit tanısı konmuş toplam 13 köpek çalışmaya dahil edilmiştir. Tüm olgular, Köpek Atopik Dermatiti Yaygınlık ve Şiddet İndeksi (CADESI-04) ve Görsel Analog Skala (VAS) kullanılarak değerlendirilmiştir. MB, tek seanslık bir nebulizasyon uygulamasıyla verilmiş ve pruritus skorları 2., 4., 6., 8. ve 10. günlerde kaydedilmiştir. Elde edilen veriler, MB uygulamasını takiben kaşıntı skorlarında belirgin bir azalma olduğunu göstermiştir; en dikkat çekici iyileşmeler ise 4., 6. ve 8. günlerde gözlenmiştir. Çalışma süresince herhangi bir yan etkiye rastlanmamıştır. Nöromodülatör ve antiinflamatuvar özellikleri göz önünde bulundurulduğunda, MB, atopik dermatitle ilişkili pruritusun kontrolünde umut vadeden bir alternatif tedavi seçeneği olarak öne çıkmaktadır. Sonuç olarak, nebulize MB uygulaması, cAD olgularında pruritusun yönetiminde güvenli ve etkili bir yöntemdir. Bu bulgular, veteriner dermatoloji alanında MB'nin etki mekanizmalarının ve uzun vadeli tedavi potansiyelinin daha kapsamlı şekilde araştırılmasını gerektirmektedir.

Anahtar Kelimeler: Calf, Diarrhea, Intestinal Permeability, Rotavirus, Zonulin

1. Introduction

Canine atopic dermatitis (cAD) is a chronic inflammatory skin disorder with a predominance of pruritus (itching) that develops as a result of the interaction of genetic predisposition and environmental allergens. Pollen, molds, house dust mites, dietary components and secondary infections can increase the severity of the disease (1). In cAD, which has no curative treatment, it is recommended to manage on an individual patient basis and to determine sustainable treatment strategies in the long term. Topical and systemic agents are widely used in the treatment of cAD (1). Systemic agents include cyclosporine, oglacitinib oclacitinib and lokivetmab, which act by suppressing inflammation and

✉: tozalp@adu.edu.tr

mediators of pruritus (2). Allergen-specific immunotherapy is considered as a technique that supports the development of long-term immunotolerance in dogs with cAD (3).

Alternative therapeutic approaches include mesenchymal stem cell (MSC) applications, mast cell inhibitor masitinib, and agents such as luteolin that reduce the expression of proinflammatory cytokines (4). In addition, lactoferricin/verbascoside compounds prevent secondary infections, and vaccines against IL-31 are effective in controlling pruritus (5,6). Cannabidiol and its derivatives alleviate pruritus by acting through the endocannabinoid system, although probiotics may play a supportive function in the management of clinical signs with their immunomodulatory properties (7). Nutritional supplements such as vitamin D and E show anti-inflammatory effects, while agents such as pentoxifylline and misoprostol might improve symptoms by suppressing TNF-α and inflammatory cytokines (8,9).

Methylene blue (MB) is a cationic dye which could be stimulated by red light in photodynamic applications, generating reactive oxygen species in presence of molecular oxygen (10). However, beyond aforementioned usage area, MB has therapeutic potential in various medical applications due to its antimicrobial, antioxidant, and neuromodulatory properties. In veterinary medicine, MB has been reported to be effective against a broad spectrum of microorganisms, especially in treatment of superficial infections (10). Its ability to inactivate spores and hyphae of dermatophyte agents makes MB a safe and economical option for the management of skin infections (11). Another importance of MB in the biomedical field is management of pruritus. In medicine, pruritus ani is another important symptom following hematochezia in anorectal diseases and its pathogenesis is explained by “itch-scratch-itch” term (12). The constant stimulation of sensory nerves causes patients to exhibit repetitive behaviors that increase pruritus and cause damage to skin barrier (13). Studies medicine have shown that MB reduces pruritus by blocking the nerve endings of unmyelinated C-fibers in the perianal region, that it has been suggested on MB injections might be an effective method in the management on treatment-resistant idiopathic pruritus ani (13,14).

In veterinary medicine, effective management of pruritus in cAD is of great importance to improve the life quality. In this context, the aim of this study to evaluate the Visual Analog Score (VAS) outcomes of using methylene blue in the management of pruritus in dogs with atopic dermatitis.

2. Materials and Methods

2.1. Research era / Triage criteria

The present study was conducted at the Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine. In a total of 13 dogs (n=5 female, eight male, at the age of 2 to 9 years, three crossbred and 10 pure breeds) were enrolled, with primary inclusion criteria as pruritus were tentatively diagnosed with a presumed cAD based on through history, clinical signs, dermatoscopy (DermLite, DL4 Dermatoscopy), epidermal corneometry (Callegari Soft Plus, Italy), Polycheck in vitro Allergy Test (Germany), Quantum Pet Bioresonance Assay (China) and relevantly dermatological interpretation, similar to previous studies by the same researchers (15-19). All dogs participated based on written owner consent and available ethical guidelines (Aydın Adnan Menderes University, Animal Experiments Local Research Committee (ADU-HADYEK document no: 2017/008). During the trial, all dogs were subjected to low glycemic index commercial food. As an inclusion criterion, none of the dogs were allowed to receive any drug for at least 3 3-week duration. An entire physical analysis was performed on days 0 and 10. The Canine Atopic Dermatitis Extent and Severity Index version 4 (CADESI-04) was performed and fulfilled in an attempt to follow the clinical findings. Although the CADESI-04 was used to support the clinical diagnosis of cAD and determine baseline status, it was not used as a primary outcome measure in this study. Pet owners were instructed to systematically evaluate and document pruritus severity utilizing a 10 cm Visual Analog Scale (VAS), following standardized assessment protocols described in the literature. (20-21).

2.2. Methylene blue application

Aropi Methylene Blue Solution 1%, 50 ml (Aropi Chemistry, formula: C16H18ClN3S, molarity mass: 319,85 g/mol) was preferred and prescribed by the present investigators, in which animal owners were easily purchased from available online stores. Briefly a single dose of MB was administered by inhalation at 2 mg/kg. The total dose was diluted in lactated ringer solution of 5 ml was added on to carrier chamber of nebulizator device (Mesilife Br-cn116 Compressor Nebulization Device Cn116) which were was then by usage of air mask the dogs were inhaled/nebulized. Every dog participated received this MB solution by inhaler route for 1 session solely. There was no side effect noticed.

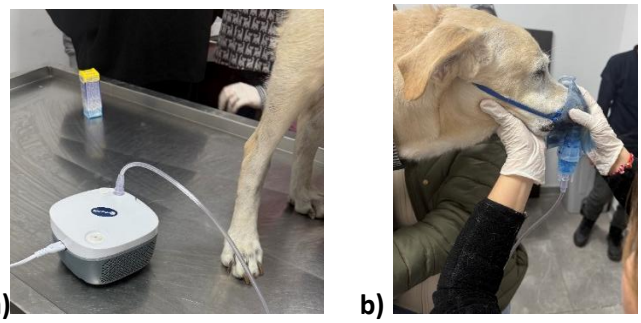

Figure 1. a. Mesilife Br-cn116 Compressor Nebulization Device Cn116, MB solution as shown on the table and **b)** inhaled MB by use of airway mask.

2.3. Statistical analyses

All statistical analyses were conducted using SPSS 29.0 (IBM, USA). Descriptive statistics, including mean, median, standard dev, and interquartile ranges, were calculated for each time point to summarize the distribution of VAS (Visual Analog Scale) values across different days. To assess differences in VAS values over time, the Friedman test, was applied to define the statistically significant alterations on repeated measurements. Pairwise comparisons between baseline (Day 0) and subsequent days (Days 1, 2, 3, 4, and 5) were performed using the Wilcoxon signed-rank test to identify specific time points. A significance level of $p < 0.05$ was considered statistically significant for all analyses.

3. Results

The results of the descriptive statistics performed to evaluate the change in VAS scores between days were presented in a table. Friedman analysis was performed to determine the statistical difference between days. According to the results of Friedman analysis, VAS scores showed a statistically significant difference between days ($p < 0.001$). To evaluate the difference between days in detail, pairwise comparisons between day 0 and the following days were analyzed with Wilcoxon paired rank test. As a result of these comparisons, a statistically significant difference was found between day 0 and all other days ($p < 0.05$). Especially the p-values obtained on days 4, 6 and 8 were at the $p < 0.001$ level (Table 1).

Table 1 [a,b]. Values with different letters in the same column indicate statistical differences between day 0 and other days.

Days	Mean	Std	Median	Minumum	Maximum
Day 0	8.43[a]	1.28	8.0	6.0	10.0
Day 2	7.29[b]	1.44	7.0	5.0	10.0
Day 4	5.64[b]	1.87	6.0	2.0	8.0
Day 6	3.71[b]	1.68	3.5	0.0	6.0
Day 8	2.14[b]	1.29	2.0	0.0	5.0
Day 10	1.07[b]	1.07	1.0	0.0	3.0

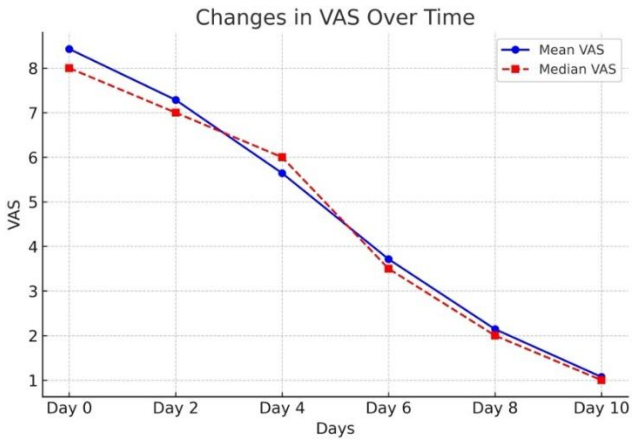

Figure 2. Alterations among VAS pruritus scores to those of dogs with cAD linked to trial days.

4. Discussion and Conclusion

In this study, inhaled MB significantly ($p < 0.001$) reduced pruritus scores associated with atopic dermatitis. The findings support that MB may be an effective agent in the management of pruritus. In the literature, it has been reported that MB has been used in various medical conditions with different application routes and successful results have been obtained (22,23). For example, it was reported that MB was successfully used in a dog with dermatophytosis by topical photodynamic therapy and the lesions healed in a short time (24). Prolonged administration of antifungal agents has been associated with significant adverse effects, including hepatotoxicity. Therefore, MB may serve as a safer alternative to mitigate the risk of systemic toxicity (22). In the present study during nebulization with MB, there was no side effects noticed.

In our study, inhaled administration of MB provided a statistically significant decrease in pruritus scores. Significant decreases in pruritus scores, especially on days 4, 6 and 8, were found to be remarkable in terms of the early efficacy of MB. These data suggest that MB may be considered as an alternative strategy in the management of cAD. Moreover, this novel treatment modality could substitute old and unfashioned drug trials.

The effects of MB on pruritus have historically been associated with its ability to stain nerve terminals and disrupt their function. Ehrlich's discovery in 1890 that high concentrations of MB target nerve terminals and disrupt their function with local application paved the way for the use of this compound in the treatment of neurological diseases and rheumatic conditions (25,26). In the present study MB was preferred to be given in inhaler route which could have hastened clinical recovery among dogs enrolled.

In a study conducted in 1968, MB was shown to be effective in the treatment of refractory idiopathic pruritus ani. In clinical and electron microscopic examinations, it was

determined that MB injection caused local nerve terminal necrosis and this effect blocked sensory transmission for approximately 26-28 days (27). This mechanism reduces the symptoms by breaking the itch-scratch-itch cycle, which is one of the most important triggers of pruritus. In addition, it has been reported that MB causes a reversible change in nerve tissue and does not cause any disruption in normal tissue structure in long-term follow-up (28). Although histopathological assessment could not be performed in this study, the absence of adverse reactions and observed clinical improvement suggests that MB might utilize its effects without harmful impact on tissue integrity. These data were in accordance with our results obtained herein. Vicious itching cycle was disrupted solely with MB inhalation in the present study. This was supported by the results we obtained.

In our study, MB administered by inhalation was found to be effective in reducing pruritus due to atopic dermatitis. In the literature, it has been reported that MB reduces itching in the perianal region by creating local nerve blockage when administered by injection (28,29). Consistent with previous findings, this study demonstrated that inhaled MB administration effectively alleviated pruritus associated with cAD. Considering the neuromodulatory effects of MB, it is thought that this compound may reduce itching sensation by suppressing the neuroinflammatory processes underlying pruritus. The regulatory effects of methylene blue on inflammatory processes and ROS have been investigated in different clinical situations. In the literature, it has been reported that MB suppresses inflammation by inhibiting cytokine production (30). In studies in COVID-19 patients, inhaled MB has been shown to reduce inflammatory markers and decrease oxygen demand (31). In the referenced study, a notable reduction in inflammatory markers, including C-reactive protein (CRP) and lactate dehydrogenase (LDH), was observed in groups receiving inhaled MB treatment.

Atopic dermatitis is a disease associated with high levels of proinflammatory cytokines (IL-4, IL-13, IL-31) and Th2 response and is recognized as one of the main causes of chronic pruritus. In the literature, it has been reported that MB suppresses inflammation by providing large-scale cytokine inhibition (30). MB has also been suggested to contribute to the regulation of inflammatory processes by modulating nitric oxide (NO) production (32). It has been suggested that NO metabolites are at high levels in COVID-19 patients and MB may regulate these mechanisms with its antioxidant effects (33). Regarding skin survival among burn models of rats, MB diminished necrosis, in association with lowered oxidative stress via NO blockage (34). Although we did not measure NO levels compared to those of dogs enrolled in this study, we could speculate that MB was capable of inhibiting NO levels at least at the skin anatomy. As a well-known neurotransmitter, NO has been linked as a pruritus mediator (35), contributing to its etiology. Keratinocytes, fibroblasts, and mast cells are whole selected examples of skin cells that could emit NO (36). All aforementioned explanations might be responsible for the clinical recovery obtained at the present study herein reported, in which MB was probably capable of suppressing NO levels among the cutaneous environment of dogs with atopic dermatitis.

In Conclusion, VAS scores exhibited a statistically significant difference between days ($p < 0.001$) (table 1 and figure 2). To evaluate the difference between days in detail, pairwise comparisons between day 0 and the following days were analyzed with Wilcoxon paired rank test. As a result of these comparisons, a statistically significant difference was found between day 0 and all other days ($p < 0.05$). Especially, the p-values obtained on days 4, 6, and 8 were at the $p < 0.001$ level (Table 1). Mean and median Vas pruritus scores on day 0 vs. day 10 were 8.43 vs 8 and 1.07 vs 1.0, indicating the efficacy of MB as a therapeutic response against pruritus in dogs with atopic dermatitis.

Authors' contributions

All authors contributed equally and were involved at all stages of the manuscript.

Competing interests

There is no conflict of interest between the authors.

References

1. Olivry, T., DeBoer, D. J., Favrot, C., Jackson, H. A., Mueller, R. et al. Treatment of canine atopic dermatitis: 2015 updated guidelines from the International Committee on Allergic Diseases of Animals (ICADA). BMC Vet Res. 2015; 11: 210.
2. Gonzales, A. J., Bowman, J. W., Fici, G. J., Zhang, M., Mann, D, et al. Oclacitinib (APOQUEL®) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. J Vet Pharmacol Ther. 2014; 37(4): 317–324.
3. Mueller R. Allergen-specific immunotherapy. In: Noli C, Foster A, Rosenkrantz W, eds. Veterinary Allergy. Oxford: John Wiley & Sons; 2014: 85–89.
4. Kaur, G., Ramirez, A., Xie, C., Clark, D., Dong, C., Maki, C. et al. A double-blinded placebo-controlled evaluation of adipose-derived mesenchymal stem cells in treatment of canine atopic dermatitis. Vet Res Commun. 2022; 46(1): 251–260.
5. Bachmann, M. F., Zeltins, A., Kalnins, G., Balke, I., Fischer, N, et al. Vaccination against IL-31 for the treatment of atopic dermatitis in dogs. J Allergy Clin Immunol. 2018; 142(1): 279–281.
6. Biasibetti E, Bruni N, Bigliati M, Capucchio MT. Lactoferricin/verbascoside topical emulsion: A possible alternative treatment for atopic dermatitis in dogs. Nat Prod Res. 2018; 32(17): 2107–2110.
7. Loewinger, M., Wakshlag, J. J., Bowden, D., Peters-Kennedy, J., & Rosenberg, A. The effect of a mixed cannabidiol and cannabidiolic acid-based oil on client-owned dogs with atopic dermatitis. Vet Dermatol. 2022; 33(4): 329–e77.
8. Klinger, C. J., Hobi, S., Johansen, C., Koch, H. J., Weber, K, et al. Vitamin D shows in vivo efficacy in a placebo-controlled, double-blinded, randomised clinical trial on canine atopic dermatitis. Vet Rec. 2018; 182(14): 406–406.

9. Saridomichelakis MN, Olivry T. An update on the treatment of canine atopic dermatitis. Vet J. 2016; 207: 29–37.
10. Sellera FP, Nascimento CL, Ribeiro MS. Photodynamic therapy in veterinary medicine: From basics to clinical practice. In: Sellera FP, Nascimento CL, Ribeiro MS, eds. Cham: Springer; 2016: 169–196.
11. Li C, Jia X, Bian Y, Qi D, Wu J. Different susceptibility of spores and hyphae of Trichophyton rubrum to methylene blue mediated photodynamic treatment in vitro. Mycoses. 2021; 64(1): 48–54. https://doi.org/10.1111/myc.13182
12. Abramowitz L, Benabderrahmane M, Pospait D, Philip J, Laouénan C. The prevalence of proctological symptoms amongst patients who see general practitioners in France. Eur J Gen Pract. 2014; 20(4): 301–306.
13. Hsieh, J. C., Hagermark, O., Stahle-Backdahl, M., Ericson, K., Eriksson, L, et al. Urge to scratch represented in the human cerebral cortex during itch. J Neurophysiol. 1994; 72(6): 3004–3008.
14. Etter L, Myers SA. Pruritus in systemic disease: Mechanisms and management. Dermatol Clin. 2002; 20(3): 459–472.
15. Ural, K., Gültekin, M., Erdoğan, H., Erdoğan, S., Gül, G, et al. Could short-term altered calendar probiotic treatment relieve itching in dogs with atopic dermatitis? Turk Klin J Vet Sci. 2020; 11: 1–8. https://doi.org/10.5336/vetsci.2019-71390
16. Ural K. Fecal microbiota transplantation capsule therapy via oral route for combatting atopic dermatitis in dogs. Ankara Univ Vet Fak Derg. 2022; 69(2): 211–219. https://doi.org/10.33988/auvfd.822971
17. Ural, K., Erdoğan, H., Erdoğan, S., Camkerten, İ., & Şahin, N. Circulating serum zonulin levels before and after probiotic enema treatment in dogs with atopic dermatitis: Randomized clinical study. Turk Klin J Vet Sci. 2021; 12(2): 70–78.
18. Ural, K., Erdoğan, S., Erdoğan, H., Gültekin, M., Gül, G., et al. Köpeklerde Atopinin İyi, Kötü ve Çirkin Yönleri: Çoklu Olgu Serisi ile Retrospektif Çalışma. Türkiye Klinikleri Journal of Veterinary Sciences, 2020, 11(1).
19. Ural K, Gültekin M, Erdoğan S, Erdoğan H. Antipruritic armamentarium with short term nutritional support solution involving silymarin and curcumin for atopic dermatitis in dogs. MAE Vet Fak Derg. 2021; 6(1): 1–7.
20. Cosgrove SB, Wren JA, Cleaver DM, et al. A blinded, randomized, placebo-controlled trial of the efficacy and safety of the Janus kinase inhibitor oclacitinib (Apoquel®) in client-owned dogs with atopic dermatitis. Vet Dermatol. 2013; 24: 587–597.
21. Hill PB, Lau P, Rybnicek J. Development of an owner-assessed scale to measure the severity of pruritus in dogs. Vet Dermatol. 2007;18:301-308.
22. Moriello K, Coyner K, Paterson S, Mignon B. Diagnosis and treatment of dermatophytosis in dogs and cats: Clinical consensus guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2017;28(4):266–e68. https://doi.org/10.1111/vde.12440
23. Paryuni A, Indarjulianto S, SW S. Dermatophytosis in companion animals: A review.Vet World. 2020;13(6):1174–1181. https://doi.org/10.14202/vetworld.2020.1174-1181
24. Cabral FV, Sellera FP, Ribeiro MS. Methylene blue-mediated antimicrobial photodynamic therapy for canine dermatophytosis caused by Microsporum canis: A successful case report with 6 months follow-up. Photodiagnosis Photodyn Ther. 2021;36:102602.
25. Oz M, Lorke DE, Hasan M, Petroianu GA. Cellular and molecular actions of methylene blue in the nervous system. Med Res Rev. 2011;31(1):93–117.
26. Wainwright M, Crossley KB. Methylene blue: cA therapeutic dye for all seasons? J Chemother. 2002;14(5):431–443.
27. An R. Atlas of the operations on the rectum and colon. Moscow Meduch Posovie; 1968.
28. Eusebio EB, Graham J, Mody N. Treatment of intractable pruritus ani. Dis Colon Rectum. 1990;33(9):770–772.
29. Kim JH, Kim DH, Lee YP. Long-term follow-up of intradermal injection of methylene blue for intractable, idiopathic pruritus ani. Tech Coloproctol. 2019;23(2):143–149.
30. Scigliano G, Scigliano GA. Methylene blue in COVID-19. Med Hypotheses. 2021;146:110455. https://doi.org/10.1016/j.mehy.2020.110455
31. Patidar V, Sharma A, Bhoraskar S, Tripathi AP, Dhaneriya S. The role of nebulized methylene blue (NMB) in the management of COVID-19 cases: An observational study. Int J Med Arts. 2022;4(2):2129–2132.
32. Alamdari DH, Moghaddam AB, Amini S, Keramati MR, Zarmehri AM, et al. Application of methylene blue-vitamin C-N-acetyl cysteine for treatment of critically ill COVID-19 patients: Report of a phase-I clinical trial. Eur J Pharmacol. 2020;885:173494. https://doi.org/10.1016/j.ejphar.2020.173494
33. Evora PR. Methylene blue is a guanylate cyclase inhibitor that does not interfere with nitric oxide synthesis. Tex Heart Inst J. 2016;43(1):103. https://doi.org/10.14503/THIJ15-5629
34. Rosique MJ, Rosique RG, Faria FM, Oliveira CC, Farina JA, et al. Methylene blue reduces progression of burn and increases skin survival in an experimental rat model. Burns. 2017;43(7):1702–1708.
35. Ostadhadi S, Haj-Mirzaian A, Azimi E, Mansouri P, Dehpour AJ. Involvement of nitric oxide in serotonin-induced scratching in mice. Clin Exp Dermatol. 2015;40(6):647–652.
36. Roosterman D, Goerge T, Schneider SW, Bunnett NW, Steinhoff M. Neuronal control of skin function: The skin as a neuroimmunoendocrine organ. Mol Pathol Biol. 2006;86(4):1309–1379.