

NASAL IRRITATION OF MUI XOANG NHAT NHAT SPRAY IN EXPERIMENTAL ANIMALS

Pham Thi Van Anh, Nguyen Thi Thuy, Nguyen Quoc Hoai
and Dau Thuy Duong✉

Hanoi Medical University

Mui xoang Nhat Nhat (MXNN) spray is a herbal combination of Angelica dahurica, Xanthium strumarium L., Ageratum conyzoides L, which is intended to use as the supportive treatment of stuffy nose, runny nose in some upper respiratory diseases such as rhinitis and sinusitis. This study was carried out to evaluate the nasal mucosa irritation of MXNN spray in experimental animals. Rabbits were divided into 3 groups: group 1 administered 0.9% sodium chloride, group 2A and 2B administered MXNN spray 2 puffs for each nostril, 3 times a day for 5 days. The investigational product was discontinued for 7 days for Group 2B rabbits to evaluate the recovery from the lesions. At the end of the experimental period, the rabbits were grossly and microscopically evaluated. Our results showed that MXNN spray caused slight irritation, which might cause vascular congestion in the submucosa layer and could be mostly recovered in a week in rabbits.

Keywords: Mui Xoang Nhat Nhat, nasal spray, irritation, nasal mucosa, rabbits.

I. INTRODUCTION

Over the last decades, there has been a huge increase in the use of traditional medicine across the world.¹ With the development of manufacturing process, herbal plants are not only used in the traditional forms of decoctions, tinctures, but are also prepared in modern dosage forms. This trend is considered an advance in traditional medicine because it not only contributes to the safety and efficacy of herbal products but also improves the confidence and compliance of patients.² The herbal medicinal product's administration routes include oral, rectal, topical, parenteral, respiratory, nasal, ophthalmic and otic.² Nasal administration is a route in which drugs are insufflated through the nose.³ This route is a high-potential way with good patient compliance because the nose is an easy-to-access, noninvasive and versatile

location for absorption.⁴ However, the nasal mucosa is relatively vulnerable, and the nasal products may cause some irritation and even affect the normal function of the nose.^{5,6}

In Vietnam, many systemic toxicity studies of herbal products have been carried out, but research on local irritation or local toxicity of topical routes (such as nasal administration) has not been focused. It is necessary to comprehend the importance of local irritation tests of the products administered intranasally to provide complete safety profile of these preparations before clinical use.

Mui Xoang Nhat Nhat (MXNN) nasal liquid spray is a herbal medicinal product prepared from some herbal plants including *Angelica dahurica*, *Xanthium strumarium L.*, *Ageratum conyzoides L.* It is intended to be used as a supportive treatment of stuffy nose, runny nose in upper respiratory diseases such as rhinitis and sinusitis. There has been no study report on the safety of nasal sprays composed of the above ingredients. Consequently, this study

Corresponding author: Dau Thuy Duong

Hanoi Medical University

Email: dauthuyduong@hmu.edu.vn

Received: 26/03/2024

Accepted: 09/04/2024

was carried out to evaluate the nasal mucosa irritation of MXNN spray in experimental animals.

II. SUBJECTS AND METHODS

1. The investigational product

Mui Xoang Nhat Nhat nasal spray is a herbal product prepared by Nhat Nhat Pharmaceutical Company from herbal plants including: *Angelica dahurica*, *Xanthium strumarium* L., *Ageratum conyzoides* L.

2. Experimental animals

Healthy adult *New Zealand* rabbits, weighed 2.0 ± 0.2 g, were individually housed in cages and fed a standard certified diet. They were acclimated to housing one week before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

3. Methods

The nasal irritation test of Mui xoang Nhat Nhat spray (MXNN spray) was carried out in rabbits.^{6,7}

A total of 09 rabbits were randomly divided into 3 groups:

- Group 1 (control group): were administered 0.9% sodium chloride 3 times a day (2 puffs for each nostril) for 5 consecutive days.

- Group 2A (3 rabbits): were administered MXNN spray 3 times a day (2 puffs for each nostril) for 5 consecutive days. Twenty-four hours after the last dose, the rabbits were grossly and microscopically evaluated.

- Group 2B (3 rabbits): were administered MXNN spray 3 times a day (2 puffs for each nostril) for 5 consecutive days and then discontinued administering the investigational product for 7 days to evaluate the recovery from the lesions. After a 7-day recovery, the rabbits were grossly and microscopically evaluated.

Right before each administration of 0.9% sodium chloride solution or MXNN spray, rabbits were observed the nasal area externally to detect any abnormal symptoms. The interval between sprays was 4 hours \pm 10 minutes.

At the end of the study period, rabbits were evaluated grossly and microscopically:

+ Gross examination: The nasal area was examined externally in all rabbits for signs of erythema, redness, swelling, edema, discharge or any other abnormal symptoms.

+ Microscopic examination: The rabbits were sacrificed. Nasal septa were separated and stained with HE for microscopic observation. Epithelial changes, loss of cilia, presence of cellular infiltration, vascular conditions (such as congestion, hyperplasia), glandular proliferation and other signs were investigated.

III. RESULTS

1. Gross examination

No erythema, redness, swelling, edema, discharge or any other abnormal symptoms were seen during the study period in the external nasal area of all rabbits in 3 groups (Figure 1-3).



Figure 1. Images of rabbit's external nasal area in the control group (group 1)

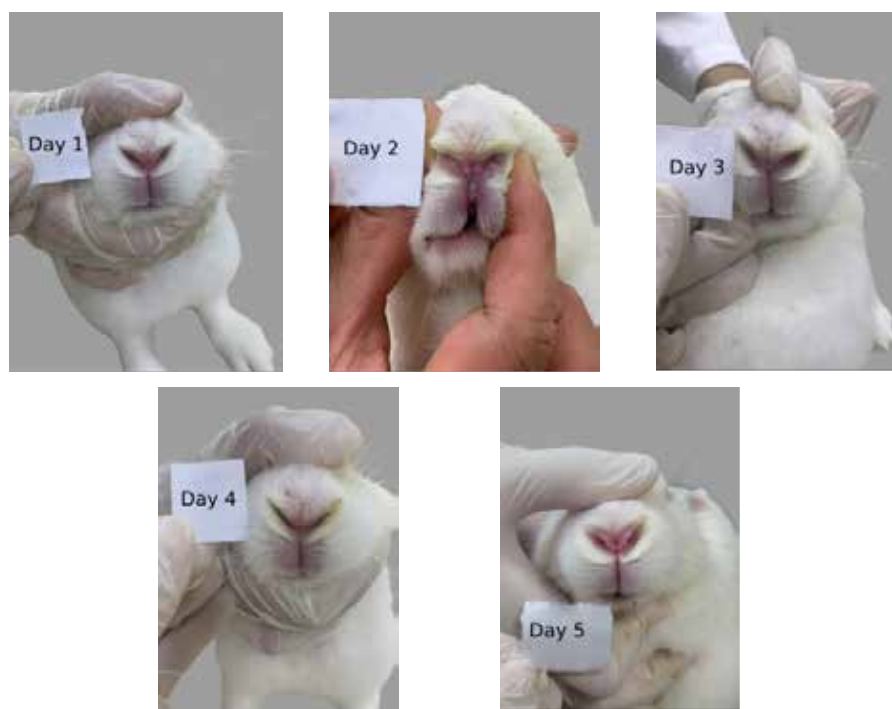


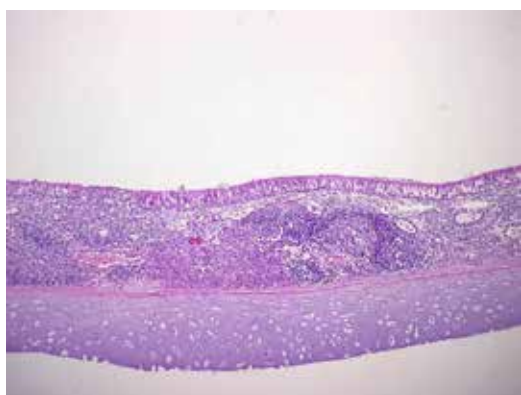
Figure 2. Images of rabbit's external nasal area in MNXX treated group (group 2A)



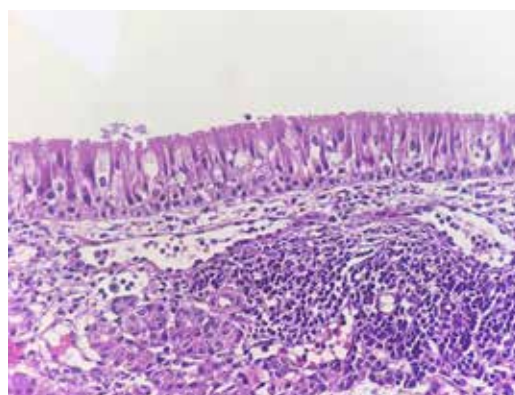
Figure 3. Images of rabbit's external nasal area in the recovery group (group 2B)

2. Microscopic examination

Nasal histological examination after 5-day



HE x 100



HE x 400

Figure 4. Histological images of rabbit nasal septa in the control group (group 1)

- Group 2A:

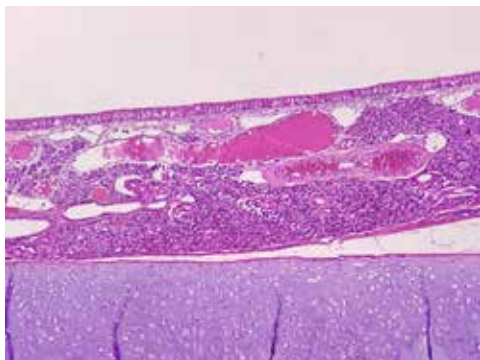
+ 2 of 6 test samples in group 2A had normal nasal septum structure similar to the control group.

+ Vasocongestion in the submucosa layer was observed in 4 of 6 test samples. The nasal septum had a complete structure of tissue layers including: mucosa, submucosa and hyaline cartilage. The mucosa was covered by

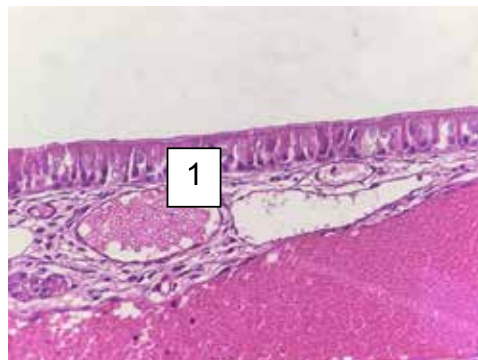
MXNN administration

- 100% of the samples in group 1 had normal nasal septum structure. The nasal septum had a complete structure of tissue layers including: mucosa, submucosa and hyaline cartilage. The mucosa was covered by ciliated pseudostratified columnar epithelium with no epithelial damage. The submucosal layer was a layer of loose connective tissue rich in blood vessels and serous secreting glands with no signs of damage to blood vessels and glands. No damage of cartilage tissue was observed (Figure 4).

ciliated pseudostratified columnar epithelium with no epithelial lesions. The submucosal layer was a layer of loose connective tissue with many dilated congested blood vessels, the lumen contained many red blood cells and serous secreting glands, with no signs of gland damage. There was no cartilage tissue damage observed (Figure 5).



HE x 100



HE x 400

Figure 5. Histological images of rabbit nasal septa in MXNN treated group (group 2A)

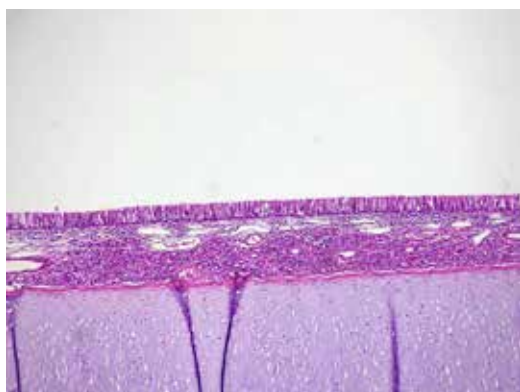
1: Vasocongestion in the submucosa layer

Nasal histological examination after 7-day recovery

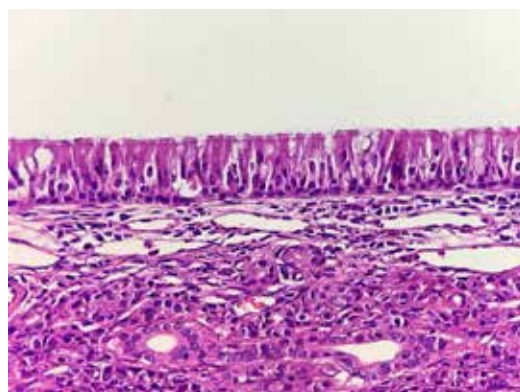
- 4 of 6 test samples in group 2B had normal nasal septum structure similar to the control group.

- Vasocongestion in the submucosa layer was observed in 2 of 6 test samples. The nasal septum had a complete structure of tissue layers including: mucosa, submucosa and

hyaline cartilage. The mucosa was covered by ciliated pseudostratified columnar epithelium with no epithelial lesions. The submucosal layer was a layer of loose connective tissue with many dilated congested blood vessels, the lumen contained many red blood cells and serous secreting glands, with no signs of gland damage. There was no cartilage tissue damage observed (Figure 6).



HE x 100



HE x 400

Figure 6. Histological images of rabbit nasal septa in the recovery group (group 2B)

IV. DISCUSSION

Many medications can be given into the nose for local and systemic effects. Most of them are used to treat problems within the nose and sinus area.³ Apart from conventional dosage forms

(such as nasal drops), nasal sprays are used frequently, where the active ingredients can be formulated as nasal liquid sprays (a solution or suspension) or as nasal powders.⁴

MXNN nasal liquid spray is a herbal product which is prepared from the herbal ingredients including *Angelica dahurica*, *Xanthium strumarium* L. and *Ageratum conyzoides* L.. These medicinal herbs have been used for a long time in traditional medicine to treat problems of the upper respiratory system. Each medicinal ingredient in this product and some active ingredients extracted have been demonstrated to have anti-inflammatory, antioxidant, anti-histamine, anti-viral and antibacterial effects.⁸⁻¹² Thus, combining these ingredients in a product for local treatment of upper respiratory symptoms of rhinitis and sinusitis such as stuffy nose and runny nose has a scientific basis. Furthermore, the preparation of this herbal combination in a modern dosage form of nasal spray could increase the product's efficacy, the patient's compliance and the convenience in use. The advantage of the nasal administration is the direct administration to the target site, so the active ingredients in herbal plants can act rapidly and effectively at low doses and therefore cause less risks of adverse effects than the systemic administration.³

However, the use of herbal products in nasal cavities can cause nasal irritation and result in alterations on nasal mucosa. Consequently, It is necessary to evaluate the potential effects (including nasal mucosa irritation) of herbal products used intranasally prior to marketing. An animal test is usually required prior to human testing and rabbits are the preferred test animals in irritation tests.⁸

Nasal mucosa irritation testing in rabbits was performed to help identify whether the MXNN spray can be a potential nasal mucosal tissue irritant. A nasal mucosa irritant is a material which produces changes in the nasal septa characterized by the presence of epithelial

changes, loss of cilia, cellular infiltration, vascular conditions (such as congestion, hyperplasia) and glandular proliferation.

Our results showed that no abnormal symptom was observed in the external area of the nose in all rabbits. The nasal septa of all rabbits were examined histologically. For physiological saline group (group 1), epithelial cells were arranged normally, no loss of cilia, no change in vascular or glandular conditions was observed. For MXNN spray treated group, vascular congestion was observed in 4/6 samples but no other changes of vascular conditions were identified. No epithelial changes, loss of cilia, cellular infiltration, glandular proliferation, cartilage tissue damage or any other abnormal signs were observed in all treated rabbits.

It was necessary to have a recovery group that discontinued the investigational product for an appropriate period to assess the late toxicity or the ability to recover from treatment-related lesions. In our study, after 7-day recovery, no additional lesion was identified. Moreover, the vasocongestion in rabbits was mostly repaired after 7 days of recovery.

It could be concluded that MXNN nasal spray produced a slight irritation on the rabbit's nasal mucosa. The results of nasal irritation study along with the evidence of pharmacological effects of the herbal ingredients of MXNN nasal spray illustrated its potential for drug product formulation and clinical use in patients.

V. CONCLUSION

Our results indicated that MXNN spray had slight irritation, which might cause vascular congestion in the submucosa layer, but it was not a permanent damage, which could be mostly recovered shortly after discontinuing the product.

REFERENCES

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014; 4:177.
2. Doris kumadoh, Kwabena ofori-kwakye. Dosage forms of herbal medicinal products and their stability considerations-an overview. *J Crit rev.* 2017; vol 4, issue 4, 1-8.
3. Savic, Snezana & Milić, Jela. Nasal preparations: Conventional and advanced pharmaceutical dosage forms. *Arhiv za Farmaciju.* 2011; 61, 177-193.
4. Scherließ R. Nasal formulations for drug administration and characterization of nasal preparations in drug delivery. *Ther Deliv.* 2020; 11(3):183-191.
5. Cankaya H, Ozen S, Kiroğlu F, Yurttas V.. Effects of topical chlorhexidine applied to the rabbit nasal mucosa, *Auris Nasus Larynx.* 2003; 30(1):65-9.
6. Min Wang, Shan Wang, Yi Zhang. Studies of Mucosal Irritation and Cellular Uptake Mechanisms of Xingnaojing Nanoemulsion, *Braz. J. Pharm.* 2022; 58: e20241.
7. Wang AJ, Li X, Wang H. Nasal mucosa and mucociliary toxicity study of compound tetramethylpyrazine. *Pharmacol Clin Chin Materia Medica.* 2016; 32(1):141-145.
8. Zhao H, Feng YL, Wang M, Wang JJ, Liu T, Yu J. The *Angelica dahurica*: A Review of Traditional Uses, Phytochemistry and Pharmacology. *Front Pharmacol.* 2022; 13.
9. Li D, Wu L. Coumarins from the roots of *Angelica dahurica* cause anti-allergic inflammation. *Exp Ther Med.* 2017; 14(1): 874-880.
10. Qianhua Peng, RuiXiang Chen. Biosynthesis of gold nanoparticles using Caffeoylxanthiazonoside, chemical isolated from *Xanthium strumarium* L. fruit and their anti-allergic rhinitis effect- a traditional Chinese medicine. *Journal of Photochemistry and Photobiology B: Biology.* 2019; Volume 192, 13-18.
11. Peng W, Ming QL, Han P, Zhang QY, Jiang YP, Zheng CJ, Han T, Qin LP. Anti-allergic rhinitis effect of caffeoylxanthiazonoside isolated from fruits of *Xanthium strumarium* L. in rodent animals. *Phytomedicine.* 2014;21(6):824-9.
12. Kamboj, Anjoo & Saluja, Ajay Kumar. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy.* 2008; 2.10.