VIETNAM MEDICAL ASSOCIATION
VIETNAM ASSOCIATION OF PHYSIOLOGY

VIETNAM JOURNAL

OF

PHYSIOLOGY

Tập 28, №3
9/2024

Vietnam Journal of Physiology
Volume 28, №3, September 2024

VIETNAM JOURNAL OF PHYSIOLOGY

Editorial Office:

First Floor, B2 Building, Hanoi Medical University
N°1 Ton That Tung Street, Dong Da District, Hanoi City
Tel: 84-4-3852-3798. Ext: 203, 205, 207
Email: tapchi@sinhlyhoc.com.vn

Contact Addresses:

First Floor, B2 Building, Hanoi Medical University
N°1 Ton That Tung Street, Dong Da District, Hanoi City
Tel: 84-4-3852-3798. Ext: 203, 205, 207
Email: tapchi@sinhlyhoc.com.vn

THỂ LỆ GỬI BÀI ĐĂNG TẠP CHÍ SINH LÝ HỌC VIỆT NAM

Tạp chí Sinh lý học Việt Nam là tạp chí chuyên ngành Sinh lý học. Tạp chí đăng tải các công trình nghiên cứu, các bài tổng quan, thông báo khoa học thuộc chuyên ngành Sinh lý học và các chuyên ngành có liên quan với Sinh lý học Y học, Sinh lý học Người và Động vật.

1. Quy định chung về bài đăng trên Tạp chí Sinh lý học Việt Nam

- Các thuật ngữ thống nhất theo tự điển Bách khoa Việt Nam.
- Bài gửi đăng phải đánh máy bằng tiếng Việt rõ ràng, phông chữ Unicode, kiểu chữ Arial, cỡ chữ 10, khổ giấy 26.5*19, lề trên 2cm, lề dưới 2cm, lề trái 2.5cm, lề phải 2.5cm, cách dòng 1.15 line. Các chữ viết tắt phải được chú thích các từ gốc của các chữ viết tắt đó. Thứ tự các đề mục đánh số Ả-rập, không đánh số La Mã (Thí dụ 1, 1.1, 1.1.1, 2, 2.2...).
- Bài đăng Tạp chí gửi qua trang web của Tạp chí Sinh lý học Việt Nam tại địa chỉ: https://tapchisinhlyhoc.com.vn
- Mỗi tác giả được phép đăng nhiều bài trong 1 số nhưng chỉ được đứng tên đầu ở 1 bài. Bài không đăng được, không trả lại bản thảo.
- Tác giả chịu trách nhiệm khoa học của bài báo phải ký vào văn bản cam kết về bản quyền của mình, các số liệu nghiên cứu, nội dung được đưa ra trong bài báo, các vấn đề về đạo đức nghiên cứu và gửi về địa chỉ Ban biên tập:

Văn phòng Hội Sinh lý học Việt Nam
Tầng 1, Nhà B2, Trường Đại học Y Hà Nội,
Số 1, Phố Tôn Thất Tùng, Quận Đống Đa, TP Hà Nội

2. Một số yêu cầu cụ thể về bài đăng công trình nghiên cứu khoa học

- Bài gửi đăng chưa được đăng ở bất kỳ Tạp chí quốc gia nào.
- Tổng số trang của bài đăng công trình không quá 8 trang, không quá 10 trang với bài tổng quan.
- Tổng số các đối tượng minh họa, kết quả (gồm hình, bảng, biểu) không quá 5 (gồm bảng, biểu, hình, ảnh, biểu đồ) và/hoặc 1/4 tổng số trang của bài báo. Tên các đối tượng được ghi theo số thứ tự cho mỗi loại (ví dụ hình 1, hình 2, bảng 1, bảng 2). Tên bảng được đặt ở trên, chính giữa bảng, tên hình, biểu đồ được đặt ở dưới, chính giữa hình, biểu đồ.
- Lệ phí đăng công trình nghiên cứu là 1.000.000 đồng/bài (quy trình 1, bài báo được chấp nhận đăng theo sắp xếp của tạp chí, đã bao gồm 4 quyển tạp chí) hoặc 2.000.000 đồng/bài (quy trình 2, bài báo được chấp nhận trong số gần nhất, đã bao gồm 4 quyển tạp chí). Kinh phí được thu khi tác giả gửi yêu cầu đăng bài và không hoàn trả kinh phí khi bài báo bị từ chối đăng. Thông tin tài khoản của Hội Sinh lý học Việt Nam như sau:

Chủ tài khoản: **Hội Sinh lý học Việt Nam**
Số tài khoản: **6 4567 6668**
tại **Ngân hàng Quân đội (MB), chi nhánh Thanh Xuân**

- Trình tự các mục trong bài:
  + Tên bài báo: Được viết ngắn gọn, thể hiện được nội dung chính của bài báo và bắt đầu bằng danh từ
  + Họ và tên các tác giả, địa chỉ cơ quan, nơi thực hiện công trình (không ghi học hàm, học vị, chức danh). Tác giả thực hiện chính được viết đầu tiên, tác giả chịu trách nhiệm khoa học về bài báo được viết cuối cùng nếu có (ví dụ tên thầy hướng dẫn). Cuối trang thứ nhất của bài báo cần ghi rõ tên tác giả chịu trách nhiệm khoa học về bài báo, kèm theo địa chỉ liên lạc, địa chỉ email và số điện thoại. Liệt kê đầy đủ tất cả các tác giả tham gia bài báo, đề nghị không viết "và cộng sự".
  + Tóm tắt tiếng Việt: Viết không quá 300 từ, viết dưới dạng bài văn xuôi thể hiện được mục tiêu, đối tượng nghiên cứu, phương pháp nghiên cứu, kết quả chính của nghiên cứu và kết luận. Từ khóa không quá 5 từ, cụm từ.
  + Tên bài báo và tóm tắt bằng tiếng Anh đặt ở cuối bài báo, sau tài liệu tham khảo, cần được dịch đầy đủ chính xác từ tên bài báo, tóm tắt và từ khóa bằng tiếng Việt.
  + Nội dung toàn văn gồm:

- √ Đặt vấn đề (bao gồm cả mục tiêu nghiên cứu của đề tài): Cần nêu rõ lý do hoặc giả thuyết nghiên cứu, mục tiêu nghiên cứu (không trùng lặp với tên bài báo).
- √ Đối tượng và phương pháp nghiên cứu: Viết ngắn gọn, đầy đủ thông tin bao gồm: đối tượng nghiên cứu, thiết kế nghiên cứu, công cụ nghiên cứu, phương pháp thu thập số liệu, phương pháp phân tích số liệu, đạo đức nghiên cứu.
- √ Kết quả nghiên cứu: được thể hiện bằng các bảng, biểu đồ, hình hoặc bằng lời.
- √ Bàn luận (bàn luận có thể viết chung với kết quả nghiên cứu, trong trường hợp viết chung thì đề mục cần ghi rõ "Kết quả và bàn luận"): tác giả cần so sánh kết quả nghiên cứu của mình với các tác giả khác và lý giải về kết quả thu được.
- √ Kết luận: viết ngắn gọn, trả lời đầy đủ mục tiêu đề ra.
- √ Khuyến nghị: nếu có.
- √ Lời cảm ơn: cảm ơn quỹ tài trợ, nơi thực hiện, cộng sự đóng góp cho công trình.
- √ Tài liệu tham khảo

3. Quy định về tài liệu tham khảo

- Tài liệu tham khảo (không quá 15 tài liệu) được xếp theo thứ tự vần chữ cái A, B, C..., tiếng Việt trước, tiếng nước ngoài sau.
- Nếu tài liệu là tạp chí thì ghi tên tác giả, năm xuất bản, tên bài, tên tạp chí, tập, số, trang (đầu và cuối). Ví dụ:
  + **Dean P, Michell IJ, Redgrave P (1988),** Responses resembling defensive behaviour produced by microinjection of glutamate into superior colliculus of rats. Neuroscience, 24(2):501-510.
- Trường hợp tài liệu tham khảo có từ 10 tác giả trở xuống thì ghi đầy đủ họ tên của 10 tác giả. Trong trường hợp có từ 11 tác giả trở lên thì ghi đầy đủ họ, tên của 5 tác giả đầu tiên, sau đó viết "và cs" nếu bài báo viết bằng tiếng Việt hoặc "et al" nếu bài báo viết bằng tiếng nước ngoài. Ví dụ:
  + **Dommett E, Coizet V, Blaha CD, Patricia G, Carol C et al (2005),** How visual stimuli activate dopaminergic neurons at short latency. Science, 307(5714):1476-1479.
- Nếu là sách chuyên khảo thì ghi tên tác giả, năm xuất bản, tên sách, nhà xuất bản, TP xuất bản, trang tham khảo. Ví dụ:
  + **Stein BE, Meredith MA (1993),** The merging of the senses. Cambridge, MA: MIT, pp.230-235.
- Nếu là một chương trong sách thì ghi tên tác giả của chương, năm xuất bản, tên chương, tên sách, tên người biên tập, thành phố xuất bản, nhà xuất bản, trang tham khảo. Ví dụ:
  + **Gerfen CR, Wilson CJ (1996),** The basal ganglia. In: Handbook of chemical neuroanatomy, Vol 12: Integrated systems of the CNS, Part III. (Swanson LW, Bjorklund A, Hokfelt T, eds), Amsterdam: Elservier, pp.371 - 468.
- Nếu tài liệu không thuộc hệ chữ Latinh thì phiên âm tên tác giả (theo tiếng Latinh) và dịch toàn bộ phần còn lại ra tiếng Việt, sau đó mở ngoặc ghi chú tiếng của tài liệu đó. Ví dụ: (tiếng Nga).
- Các tài liệu đưa ra phải được trích dẫn đầy đủ trong nội dung bài báo. Trong đó ít nhất 50% số tài liệu tham khảo cần xuất hiện trong phần bàn luận.

4. Yêu cầu đối với các bài tổng quan, thông báo khoa học và bài dịch

- Đối với các bài Tổng quan cần có đầy đủ các tài liệu tham khảo và nguồn số liệu được trích dẫn trong bài. Tác giả bài Tổng quan được ghi rõ chức danh khoa học, học vị, chuyên ngành, địa chỉ cơ quan (ghi ở cuối trang đầu của bài Tổng quan). Nếu bài tổng quan dài, Ban biên tập sẽ chia làm 2 kỳ, mỗi kỳ dài không quá 10 trang, kể cả hình ảnh, bảng, biểu và tài liệu tham khảo. Số tài liệu tham khảo không quá 20 tài liệu.
- Đối với các bài Thông tin khoa học, các bài dịch cần ghi rõ xuất xứ của nguồn dữ liệu được sử dụng để viết bài thông tin hoặc bài dịch. Đối với bài dịch cần photocopy toàn văn bản bài báo tiếng nước ngoài gửi kèm theo bản dịch.
- Đối với bài tổng quan và các bài thông tin khoa học, tác giả gửi đăng sẽ không phải nộp lệ phí khoa học.

TABLE OF CONTENTS

This journal is published under the approval of Ministry of Information and Communications, No. 138/GP-BVHTT dated 21/4/2014. Copyright deposit in September, 2024.

THE SKIN IRRITATION RISK OF CHILI FRUIT NANO GEL ON EXPERIMENTAL RABBIT SKIN

Do Thi Hang[1], Do Van Dung[1], Nguyen Van Hung[1], Nguyen Vu Hung[2], Mai Phuong Thanh[3], Le Anh Tung[3], Ho My Dung[4], Nguyen Thuy Dung[5], Phan Hong Minh[4]

[1]Phuc Yen Region General Hospital
[2]Institute for Applied Research of Traditional Medicine
[3]Hanoi Medical University
[4]VNU University of Medicine and Pharmacy
[5]Vietnam University of Traditional Medicine
Corresponding author: **Phan Hong Minh**
Email: phanhongminh.hmu@gmail.com
Received date: 28/7/2024
Reviewed date: 21/09/2024
Accepted date: 30/09/2024

SUMMARY

Chili is not only a popular spice in food preparation but is also used as a traditional medicine. In particular, when used topically, the active ingredient called capsaicin in chili peppers helps treat bone and joint pain. ***Objectives****: The study aims to evaluate the local irritation on the healthy skin of rabbits of a nano gel product containing chili fruit extract provided by the Institute for Applied Research of Traditional Medicine.* ***Methods****: The study was conducted according to the guidelines of the Vietnam Ministry of Health, ISO 10993-10, and OECD on skin irritation assessment for topical products. Three healthy white New Zealand rabbits were used. Animals were applied gauze which was soaked in nano gel containing chili fruit extract at a dose of 0.5 grams on a healthy skin area 2.5 x 2.5 cm for 4 hours. We evaluated the irritation index including erythema and edema on the skin at 24, 48, and 72 hours after gauze removal.* ***Results****: Three rabbits were still healthy, and living normally, there were no signs of erythema and edema on the rabbit's skin, and the irritation indexes were 0 during 72 hours of continuous monitoring.* ***Conclusion****: The nano gel containing chili fruit extract applied topically does not irritate healthy rabbit skin.*

Keywords*: nano gel, chili pepper, capsaicin, skin irritation*

1. INTRODUCTION

Chili has been an indispensable spice in food preparation daily for Asian and Latin Americans. In Vietnam, chili is a spice vegetable with high economic value, which is grown mainly in the Central and Southern provinces and expanded to some Red River Delta provinces such as Vinh Phuc in recent years. The scientific name of the chili plant is *Capsicum annuum* L. or *Capsicum frutescens* L., belonging to the Solanaceae family [1]. According to traditional medicine, chili peppers have a spicy and hot taste with the effect of dispelling cold, strengthening the spleen, eliminating food, reducing pain, and treating cancer... Therefore, chili has been used to treat stomach aches due to cold, poor digestion, joint pain, snake bites...[1]. Chili peppers contain abundant vitamins and mineral salts such as vitamin C, vitamin A, vitamin B1, B6, vitamin K, calcium, magnesium, folate, potassium, thiamin, iron, copper... [2,3]. The alkaloid capsaicin has been identified as the main component related to chili's spicy taste and some of its pharmacological effects. Capsaicin stimulates the brain to produce endorphin, an endogenous morphine, which is used as a pain reliever, especially for chronic arthritis and cancer [4,5]. Recently, the active ingredient capsaicin has appeared in some commercial products

as an adjunct therapy in the treatment of bone and joint pain when used topically [4]. In addition, chili peppers also have other effects such as reducing lipid accumulation, losing weight, and preventing cancer due to their antioxidant and anti-inflammatory effects...[1,5]. Although chili has a potential therapy, there has not been much research to develop products from chili. The ingredient capsaicin that creates chili's spicy taste can also cause skin-mucous irritation and burning. To reduce the irritation of chili, many researchers have created products with nano-coated films to control the drug release process as well as prevent direct contact of the drug with the skin, thereby reducing irritation skin reaction [6,7,8].

For drugs used topically on the skin, assessing the risk for skin irritation is necessary before testing for pharmacological effects. Therefore, to supply the scientific pieces of evidence before testing the pharmacological effects, the study was conducted to evaluate the skin irritation risk of chili nano gel on experimental rabbit skin.

2. MATERIALS AND METHODS

2.1. Materials

The research product is chili fruit nano gel that reached the basic standard provided by the Institute for Applied Research of Traditional Medicine.

2.2. Experimental animals

The study was conducted on three adult healthy New Zealand white rabbits, at 9-10 weeks of age, weighing 2.0 - 2.2 kg, without skin diseases. Rabbits were housed separately and raised in experimental conditions for 5 days to acclimatize before the research with room temperature of 25 – 30^0C, and humidity of 60 - 70%. Rabbits were supplied with standard food for research animals and ad libitum water.

2.3. Skin irritation testing

Research on the skin irritation of chili nano gel was conducted according to the guidelines of the OECD [9], Vietnam Ministry of Health [10], and ISO 10993-10 [11] on skin irritation assessment for topical products.

Preparation of animals: About 24 hours before the commencement of the investigation, all test animals had hair thoroughly removed using electric clippers from the dorsal area of their trunks without abrading the skin, with a dimension of about 10 × 15 cm. Based only on appearance, only rabbits with a healthy, intact epidermis were selected for the experiment.

Dosage preparation: Undiluted chili nano gel was applied directly in a single dose (0.5 grams) to the skin of experimental rabbits. The surrounding skin areas were applied with distilled water to serve as a control.

Test material application: 0.5 grams of the undiluted test gel formulation was applied to the 2.5 × 2.5 cm area while the distilled water was applied to the surrounding skin on the day of treatment. Each application site was covered with 6.25 cm^2 (2.5 × 2.5 cm) of cotton gauze. The test patches were affixed using non-irritating adhesive tape, and the entire trunk was covered with a cotton bandage. The test gel formulation came into contact with the skin for four hours. Following the contact period, the protective covering and patches were removed, the treated region was cleaned with distilled water and patted dry with tissue paper, and the local skin reactions were noted. Each animal's erythema and edema irritation ratings at all measurement intervals after patch removal were tabulated and displayed. To evaluate the test item's potential for irritation, each animal's mean score was calculated separately during three scoring periods (24, 48, and 72 hours after patch removal) for erythema/eschar grades and edema grades.

Interpretation of findings: After four hours of post-experiment patch removal and 72 hr, the principal irritating index of the test gel formulation was obtained by adding

up the erythema and edema scores for all three test rabbits, dividing the result by the number of observations. The Primary Irritation Index (PII) is a metric that measures how irritancy is divided into several categories as shown in Table 2.

Table 1. Evaluation of skin reaction

Erythema and eschar formation	**Score**
- No erythema at all	0
- Very light erythema that can be barely seen with the naked eye	1
- Defined erythema	2
- Kind of severe erythema	3
- Severe erythema and light eschar	4
Total possible erythema score	*4*
Edema formation	
- No oedema at all	0
- Very light edema that can be barely seen with the naked eye	1
- Light edema that distinguishes the marginal area due to swelling	2
- Normal edema (about 1 mm of swollen edema)	3
- Severe edema (it swells up more than 1 mm and extends out of the exposed area)	4
Total possible edema score	*4*

If there were any lesions on the skin, the rabbits were observed for up to 14 days to evaluate the reversibility of the lesions. During the observation period, if the rabbits showed any signs of severe pain at any time, the experiment was discontinued.

Table 2. Rating table of skin irritation toxicity

Rating	**Primary Irritation Index (PII)**
Non irritant	0.0 – 0.5
Light irritant	0.6 – 2.0
Moderate irritant	2.1 – 5.0
Strong irritant	5.1 – 8.0

2.4. Statistical analysis

The data were collected, cleaned, and statistically analyzed by Microsoft Excel 2010 software.

3. RESULTS

During the trial, no death or aberrant clinical symptoms were noticed in any of the animals.

At 24, 48, and 72 hr following exposure, the skin response was scored (after removal of the dressing, gauze patches, and test item).

Table 3. Skin reaction score of chili nano gel at various time intervals (3 treatment sites)

Reaction	**24hrs**		**48hrs**		**72hrs**	
	Control	**Nano gel**	**Control**	**Nano gel**	**Control**	**Nano gel**
Erythema	0	0	0	0	0	0
Edema	0	0	0	0	0	0

The findings of a skin tolerance test are displayed in Table 3. After using the chili fruit nano gel, the rabbits' skin edema and irritation symptoms were not observed. After the test substance was removed from each rabbit, their erythema and edema ratings were "0" at all points during the observation.

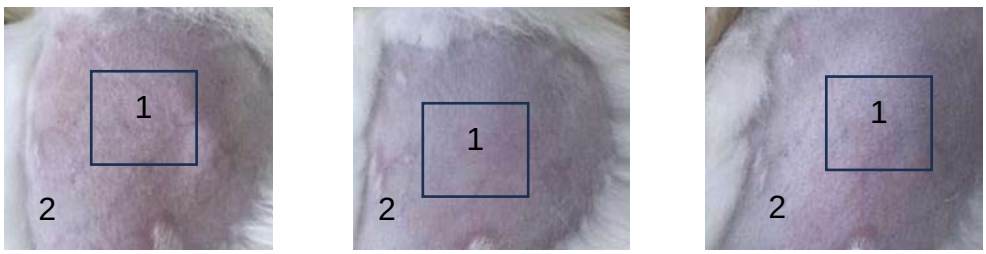
The Primary Irritation Index score for the skin was zero (Table 4).

Table 4. Primary Irritation Index (PII) on rabbit's skin

Animal identification number	Control	Nano gel
01	0	0
02	0	0
03	0	0
Total	**0**	**0**

Macroscopic images of rabbit skin at 72 hours following treatment did not differ between the chili nano gel applied area and the distilled water applied area.

The treated skin was intact; with no erythema, discoloration, inflammation, or corrosion compared to the untreated site (Figure 1).



Rabbit 1 ***Rabbit 2*** ***Rabbit 3***

Figure 1. Photographs of rabbit skin at 72 hours after removal of nano gel formulation

1: Applied with chili nano gel; 2: Applied with distilled water

4. DISCUSSION

Skin irritation assessment studies are generally recommended to be performed on skin that is prone to skin irritation. Rabbit skin is thinner and more sensitive than that of other rodents, and is therefore chosen in studies evaluating the skin irritation potential of an agent [9,10,11]. However, anatomically, rabbit skin has a thinner epidermis and is more susceptible to irritation than human skin. Therefore, if a drug is tested on rabbit skin and does not cause irritation, it is almost certain that the same result will be seen on human skin. To rule out the possibility of skin irritation on rabbits due to factors other than the reagent, we performed the same steps of applying gauze soaked in distilled water and bandaging on the adjacent healthy skin for comparison. The scoring assessment requires two investigators to work independently to eliminate subjective factors affecting the research results.

Erythema is a reddening of the skin or mucous membranes that disappears when pressed and reappears when released. This is one of the main manifestations of skin irritation caused by chemicals [12]. The substance applied to the skin's surface penetrated the stratum corneum and destroyed the underlying cell layers, which is the cause. Damaged keratinocytes release inflammatory mediators that act on the dermis, especially the stromal and vascular endothelium, causing vasodilation and increased vascular permeability, increasing blood flow (congestion), and causing erythema [12].

The study results did not record any erythema or scaling reactions in all experimental rabbits at 24 hours, 48 hours, and 72 hours after stopping exposure to the test gel (Table 3). The skin scales are formed to prevent the progression of erythema and appear in severe erythema reactions, so it is reasonable that no eschar was recorded.

Edema is defined as swelling relative to the surrounding skin and is likewise a primary manifestation of skin irritation [12]. Vasodilation and increased vascular permeability increase fluid leakage into body tissues and cause edema. The data in Table 3 also show that there were no other changes (changes in skin color, blisters, vesicles, dry skin...) on the rabbit skin in the skin areas where the samples were placed during 3 days of observation.

Thus, during the study period, all rabbits were normal, alert, and responded quickly to stimulation. The rabbit skin areas in contact with the test gel or distilled water did not show any signs of erythema, scaling, or edema at the time points before applying the test preparation, and after removing the gauze soaked with chili nano gel 24, 48, and 72 hours. The Primary Irritation Index of the test gel was determined to be 0 and corresponded to the classification of insignificant skin irritation.

Although chili has been used in folk medicine to treat many different diseases such as bone and joint pain, cancer, digestive disorders, snake bites, etc., chili has a spicy taste and often causes severe irritation and a burning sensation in the digestive tract as well as at the topical application [1]. A test by Winek CL et al. (1982) evaluating the toxicity of a chili sauce on animals showed that chili can cause mild irritation to the skin of New Zealand rabbits but moderate to severe irritation to the eyes [13]. The main active ingredient in chili that also creates the spicy taste of chili is capsaicin. Currently, there are some products with low capsaicin concentration including creams, gels, topical medications (0.025, 0.075, and 0.1%), and high capsaicin concentration patches (8%) have been developed to relieve pain such as bone and joint pain, neuropathic pain [4,14]. Products with high capsaicin concentrations (≥ 0.075% capsaicin) may be associated with a burning sensation, leading to severe skin irritation and patient noncompliance. This undesirable effect is because capsaicin is rapidly absorbed in the epidermis and binds to TRPV1 (transient receptor potential vanilloid subfamily member 1) receptors present on keratinocytes, simultaneously, the compound poorly penetrates the dermis, leading to capsaicin accumulation on the surface of the epidermis and causing skin irritation [15].

Recently, nanotechnology has been a strategy to reduce the irritation and allergy of active ingredients after application to the skin, by controlling the drug release and avoiding direct contact between the drug and the skin [6,7,8]. Studies have shown that the use of lipid nanoparticles carrying capsaicin is safe, without skin irritation, and local and systemic allergic manifestations. Anantaworasakul P et al. (2020) demonstrated that the lipid nanocarrier structure was more effective in encapsulating, and releasing capsaicin in vitro, and the permeability property enhanced the ability of capsaicin to be delivered into the dermis layer of the skin [6]. *In vitro*, skin irritation experiments showed that nanoparticles containing chili extract (high concentration of capsaicin 0.25%) minimized irritation compared to conventional chili extract. This was explained that lipid nanoparticles have a small structure and easy solubility in lipids, so it increased the penetration of capsaicin into the dermis, thereby minimizing irritation on the skin epidermis [6]. Other studies on lipid nanoparticles carrying capsaicin also showed similar results in reducing skin

irritation compared to normal capsaicin extract [7,8].

Evaluating the skin irritation of a topical product is a prerequisite step before assessing the efficacy of the drug. Using treatment products with inappropriate irritancy not only causes pain and discomfort to the patient but can also cause further damage. Rabbit skin is more sensitive than human skin. With the results of this study, it can be concluded that using chili nano gel will have almost no skin irritation risk in humans.

5. CONCLUSION

Evaluation of skin irritation of chili fruit nano gel on intact skin of experimental rabbits showed that the test gel did not cause erythema scabbing, or edema reaction after 24 hours, 48 hours, and 72 hours of gel removal. The primary irritation index was 0, corresponding to the classification of insignificant skin irritation, proving that chili fruit nano gel used topically was safe and did not cause skin irritation in experimental rabbits.

Acknowledgments

The research was funded by the project "Research on pain-relieving and fat-dissolving effects from chili fruits grown in Vinh Phuc", code: 16/ĐTKHVP/2023-2025

REFERENCES

1. **Do Huy Bich (2006).** Medicinal plants and medicinal animals in Vietnam, Volume II. Science and Technology Publishing House, Hanoi, 504-508 (in Vietnamese).
2. **Chakrabarty S, Islam AM, Islam AA (2017).** Nutritional benefits and pharmaceutical potentialities of chili: A review. Fundamentals and Applied Agriculture, 2(2):227-232.
3. **Pawar SS, Bharude NV, Sonone SS, et al (2011).** Chillies as food, spice and medicine: A Perspective. International Journal of Pharmacy and Biological Sciences, 1(3):311-318.
4. **Ilie MA, Caruntu C, Tampa M, et al (2019)**. Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions. Experimental and therapeutic medicine, 18(2):916–925.
5. **Sharma SK, Vij AS, Sharma M (2013)**. Mechanisms and clinical uses of capsaicin. European journal of pharmacology, 720(1-3):55–62.
6. **Anantaworasakul P, Chaiyana W, Michniak-Kohn BB, Rungseevijitprapa W, Ampasavate C (2020).** Enhanced Transdermal Delivery of Concentrated Capsaicin from Chili Extract-Loaded Lipid Nanoparticles with Reduced Skin Irritation. Pharmaceutics, 12(5):463. doi: 10.3390/pharmaceutics12050463.
7. **Wang XR, Gao SQ, Niu XQ, et al (2017).** Capsaicin-loaded nanolipoidal carriers for topical application: design, characterization, and in vitro/in vivo evaluation. International Journal of Nanomedicine, 12: 3881–3898.
8. **Raza K, Shareef MA, Singal P, et al (2014).** Lipid-based capsaicin-loaded nano-colloidal biocompatible topical carriers with enhanced analgesic potential and reduced dermal irritation. Journal of liposome research, 24(4), 290–296.
9. **OECD (2015).** Test No. 404: Acute Dermal Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.
10. **Vietnam Ministry of health (1999).** Methods of testing skin irritation (applicable to products used in medical and cosmetic applications, promulgated together with the Minister's Decision No. 3113/1999/QD-BYT of October 11, 1999) (in Vietnamese).
11. **International Organization of Standardization (2010).** Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization, ISO 10993-10

12. **Fluhr JW, Darlenski R, Angelova-Fischer I, Tsankov N, Basketter D (2008).**Skin irritation and sensitization: mechanisms and new approaches for risk assessment. 1. Skin irritation. Skin Pharmacol Physiol, 21(3):124-135.
13. **Winek CL, Markie DC, Shanor SP (1982).**Pepper sauce toxicity. Drug Chem Toxicol, 5(2):89-113.
14. **Reyes-Escogido Mde L, Gonzalez-Mondragon EG, Vazquez-Tzompantzi E (2011)**. Chemical and pharmacological aspects of capsaicin. Molecules, 16(2):1253-1270.
15. **Anogianaki A, Negrev NN, Shaik YB, et al (2007).**Capsaicin an irritant anti-inflammatory compound. J Biol Regul Homeost Agents, 21(1-2):1-4.

THE RISK OF SKIN IRRITATION OF CLARTÉ IN EXPERIMENTAL RABBITS

Nguyen Xuan Tuan[1], Nguyen Thuc Thu Huong[1], Ho My Dung[1], Mai Phuong Thanh[2], Phan Hong Minh[1]

[1]University of Medicine and Pharmacy, Vietnam National University

[2]Hanoi Medical University

Corresponding author: **Phan Hong Minh**

Email: phanhongminh.hmu@gmail.com

Received date: 28/7/2024

Reviewed date: 13/09/2024

Accepted date: 30/9/2024

SUMMARY

CLARTÉ is an external genital hygiene product, thus assessing skin irritation is an important test before commercializing the product for the community. ***Objectives:*** *The study evaluated the local irritation on healthy rabbit skin of CLARTÉ manufactured and supplied by VIHECO PHARMA., JSC.* ***Methods:*** *The study was conducted according to the guidelines of the Vietnam Ministry of Health, ISO 10993-10, and OECD on skin irritation assessment for topical products. Three healthy white New Zealand rabbits were shaved off the fur on the back. Animals were applied directly 0.5 mL of CLARTÉ solution on the back skin and covered the application sites with a 2,5 cm × 2,5 cm non-occlusive dressing for 4 hours. We evaluated the irritation index including erythema and edema on the skin at 1, 24, 48, and 72 hours after removing the patches.* ***Results:*** *Three rabbits were still healthy, and living normally. There was very slight or well-defined erythema and very slight edema on the rabbit's skin applied by CLARTÉ after the first 24-hour treatment. The irritation index of CLARTÉ was 0.44, corresponding to negligible skin irritation during 72 hours of continuous monitoring.* ***Conclusions:*** *CLARTÉ product applied topically caused negligible irritation on healthy rabbit skin.*

Keywords*: CLARTÉ', rabbit, skin irritation*

1. INTRODUCTION

Natural product application in treatment has a history of thousands of years and includes many plant-based products. They have been used both for internal and external treatment [1]. Many diseases are treated through the skin route. Presently, dermal natural products still have a mainly local effect [2]. In the previous time, due to the limitations of technology, medicine powders or crude herbal extracts were applied in formulations such as topical powders, and pastes for skin administration. Although it was hard to control the quality of those products, they were still widely utilized in some clinical cases because of their simple acquirement and convenient usage. Natural product dermal preparations are now widely utilized in the form of oils, ointments, patches, and gels thanks to advancements in pharmaceutical technology [1]. This route of administration is widely accepted by patients and physicians for its flexibility, first-pass hepatic metabolism avoidance, higher local efficacy, and fewer risks of systemic side effects [3].

CLARTÉ is manufactured and supplied by VIHECO Central Pharmaceutical Joint Stock Company. CLARTÉ is an external genital hygiene product that is used to gently clean the external genital area and help prevent bacteria and genital fungus. Its components are derived from natural extracts including Piper Betle extract, Rosa Damascena Flower extract, Aloe Vera extract, and Dimocarpus Longan extract; and combined with many other

chemical agents such as glycerin, PEG 400 (Polyethylene glycol 400), HEC (Hydroxyethylcellulose), CAB (Cocamidopropyl betaine), PEG 75 lanolin, saliguard EHGP… Some of its ingredients can easily cause skin irritation such as sodium lauryl sulfate [4], fragrances [5], and ethylhexylglycerin [6]. In addition, it also contains soothing ingredients to reduce skin irritation such as aloe vera [7], and panthenol [8,9]... Before conducting the research in humans, the product's safety and efficacy need to be proven in experimental animal models. In particular, the dermal irritation test in animals is one of the most important toxicity studies with a topical product. Thus, we conducted this study to investigate the skin irritation ability of CLARTÉ on healthy rabbit skin.

2. MATERIALS AND METHODS

2.1. CLARTÉ solution

CLARTÉ was an external genital hygiene product manufactured and supplied by VIHECO PHARMA., JSC. The product's ingredients included: Sodium lauryl sulfate, glycerin, and neo. Fer-Lactobacillus Ferment Lysate, PEG 400 (Polyethylene Glycol 400), HEC (Hydroxyethylcellulose), Sodium Chloride, CAB (Cocamidopropyl Betaine), PEG 75 Lanolin, Saliguard EHGP (Ethylhexylglycerin, Phenoxyethanol), Piper Betle Extract, Rosa Damascena Flower Extract, Tween 20, Aloe Vera Extract, Panthenol, Disodium EDTA, Menthol, Lactic acid, Dimocarpus Longan Extract, fragrance.

2.2. Experimental animals

Three adult healthy *New Zealand* White rabbits at 10 weeks of age, weighed 2.0 ± 0.2 kg, and had no skin diseases, were used in this study. They were acclimatized for 7 days before the research and maintained in specific standard conditions for animals throughout the study period in the Laboratory of the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University.

2.3. Methods

The experiment was carried out following the guidelines of the Vietnam Ministry of Health [10], OECD [11], and ISO 10993-10 [12] for skin irritation assessment of topical products.

Animal preparation: 24 hours before the experiment, rabbits were shaved to remove the fur on both sides of the spine for application and observation of the test sites (approximately 10 × 15 cm). The skin of rabbits was examined carefully to confirm that only animals with healthy and intact skin were used for the test.

Exposure period: 0.5 ml of CLARTÉ was applied to an area (approximately 2.5 × 2.5 cm) of the skin on one side of the back while 0.5 ml of distilled water was applied on the other side. Each application site was covered by a gauze patch (approximately 2.5 × 2.5 cm) and wrapped with a semi-occlusive bandage for 4 hours. After the exposure period, the residual test product was removed and the skin was carefully dried without altering the existing response of the skin.

Observation and assessment: The skin reaction for erythema and edema was described and scored according to the scoring system in Table 1 at 1 hour, 24 hours, 48 hours, and 72 hours after removing the patches. If there were any lesions on the skin, the rabbits were observed for up to 14 days to evaluate the reversibility of the lesions. During the observation period, if the rabbits showed any signs of severe pain at any time, the experiment was discontinued.

Table 1. Scoring system for skin reaction

Erythema and eschar formation	**Score**
- No erythema	0
- Very slight erythema (barely perceptible)	1
- Well-defined erythema	2
- Moderate erythema	3
- Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
- No oedema	0
- Very slight oedema (barely perceptible)	1
- Well-defined oedema (edges of area well-defined by definite raising)	2
- Moderate oedema (raised approximately 1 mm)	3
- Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	**8**
Other adverse changes at the skin sites shall be recorded and reported	

Rabbit skin reaction category: After scoring the rabbits, all erythema scores and edema scores at 24h, 48h, and 72h were separated for each test sample and control site for each rabbit. The Primary Irritation Index (PII) for a rabbit was calculated as the total erythema and edema scores divided by the number of observation times (two tests for each observation site, three-time points). Classification of the reaction on rabbit skin according to the Primary Irritation Index (PII) in Table 2.

Table 2. Rabbit skin reaction categories

Reaction category	**Primary Irritation Index (PII)**
Negligible	0 – 0.5
Slight	> 0.5 – 2.0
Moderate	> 2.0 – 5.0
Severe	> 5.0 – 8.0

2.4. Statistical analysis

The data were collected, cleaned, and statistically analyzed by Microsoft Excel 2010 software.

3. RESULTS

Symptoms of erythema and edema were assessed on 3 rabbits at 1 hour, 24 hours, 48 hours, and 72 hours after removing the test sample applied to the skin and evaluating the score according to Table 1. The results are described in Tables 3 and 4.

Table 3. Erythema score in experimental rabbits

Rabbit	**1h**		**24h**		**48h**		**72h**	
	T	**C**	**T**	**C**	**T**	**C**	**T**	**C**
1	0	0	0	0	0	0	0	0
2	0	0	1	0	0	0	0	0
3	0	0	2	0	0	0	0	0

T: CLARTÉ - applied area; C: Control area

As shown in Table 3, on the control skin area of all rabbits (applied distilled water), no erythema and any other abnormal signs were observed at all time points: 1 hour, 24 hours, 48 hours, and 72 hours. On the tested skin area (applied CLARTÉ), no erythema sign was observed at all the time points in Rabbit 1st, but there

was a sign of very slight erythema in Rabbit 2nd and well-defined erythema in Rabbit 3rd at 24 hours after removing CLARTÉ. The erythema reactions on the skin of two rabbits disappeared completely after 48 hours of observation.

Table 4. Edema score in experimental rabbits

Rabbit	1h		24h		48h		72h	
	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	1	0	0	0	0	0

T: CLARTÉ - applied area; C: Control area

Data in Table 4 have shown that no edema or any other abnormal signs were observed at all time points on the control skin area of all rabbits (applied distilled water). On the tested skin area (applied CLARTÉ), no edema sign was observed at all the time points in Rabbit 1st and Rabbit 2nd, however, there was a sign of very slight edema in Rabbit 3rd at 24 hours after removing CLARTÉ. The edema reactions on the skin of Rabbit 3rd disappeared completely after 48 hours of observation.

Table 5. Primary Irritation Index (PII) on rabbit skin

Rabbit	C	T
1	0	0
2	0	0.33
3	0	1.0
Total	0	0.44

T: CLARTÉ - applied area; C: Control area

The average irritation score of CLARTÉ on rabbit skin ranged from 0-1.0 and the overall Primary Irritation Index (PII) of 3 rabbits was 0.44 which was in the range of 0-0.5 and belonged to the level of negligible skin irritation (Table 5).

The signs of erythema and edema in Rabbit 3rd can be observed at the time points of 24 hours after CLARTÉ removal in Figure 1.

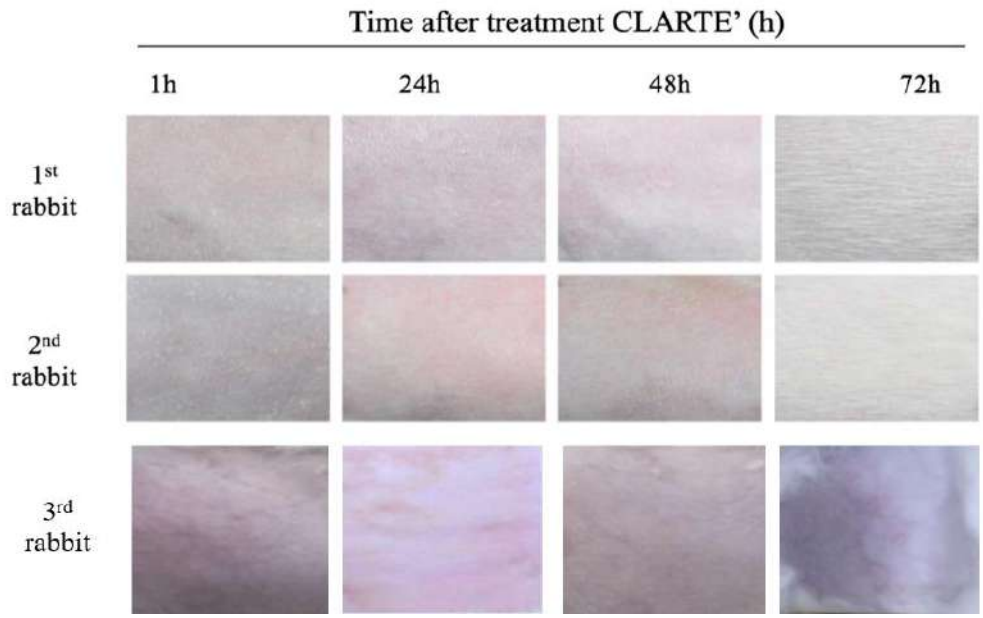


Figure 1. Images of the rabbit skin after applying CLARTÉ

In addition, observation of the rabbits' total heath during the follow-up period showed that there were no dead rabbits in the study. At the time points of the study, all rabbits were healthy, conscious, quick to respond to stimuli, and did not have digestive disorders (such as diarrhea, increased salivation, ...).

4. DISCUSSION

Assessment of irritation of pharmaceutical and cosmetic products with natural compounds is a significant step in the evaluation of their biocompatibility. The principle of a dermal irritation test is applying the test product to the skin of experimental animals and untreated skin sites of the animal as the control. The degree of irritation is observed and scored at specified intervals. If there is any sign of irritation observed, the study should be sufficient to evaluate the reversibility of the lesions [9]. In a dermal irritation test, the rabbit is the preferable experimental animal because the rabbit's skin is very sensitive to external factors and can easily detect the irritating potential of the investigational products [9,10]. However, the anatomical rabbit skin has a thinner epidermis and is more susceptible to irritation than human skin. Therefore, when the tested drug on the rabbit skin has no irritation, it is almost certainly similar to human skin. To limit the interference factors that affected the results, we carried out parallelly applying distilled water and bandage gauze on the healthy skin sites for comparison. During the study period, all rabbits were healthy and responded quickly to stimuli. The rabbit skin area exposed to the distilled water did not have erythema, scaling, or edema at 1, 24, 48, and 72 hours after the gauze removal.

Erythema is a phenomenon of red skin or mucous membranes that disappears if it is pressed and reappears if it is released. This is one of the main manifestations of skin irritation caused by chemicals [13]. The reason is that the test substance applied on the skin surface penetrates the stratum corneum and destroys the underlying skin layers. The damaged keratinocytes release inflammatory mediators on the dermis, especially the stromal and the vascular endothelium, causing vasodilation and increased vascular permeability, increasing blood flow and causing erythema [13]. The results showed that 2 out of 3 rabbits developed a very slight or well-defined erythema on the skin after the first 24 hours of applying CLARTÉ. However, the erythema completely disappeared after 48 hours of treatment. Scaling is formed to prevent the progression of erythema and appears in severe erythema (beet-redness), so it is reasonable to not record the eschar formation. The results were suitable to the skin irritation as a reversible skin injury since the repair mechanism was established immediately after the injury appearance [11]. This reversible manifestation indicated that the test drug is safe to a certain extent.

Edema is defined as swelling relative to the surrounding skin, which is also a major manifestation of skin irritation [13]. Vasodilation and increased vascular permeability increase fluid drainage into tissues and cause edema. The results showed that 1 out of 3 rabbits had very slight edema after 24 hours of treatment, but it completely disappeared after 48 hours of observation. The Primary Irritation Index (PII) of the CLARTÉ sample on 3 rabbits was 0.44, corresponding to a negligible skin irritation level according to the classification of rabbit skin irritation of the Vietnam Ministry of Health (Table 2) [10].

CLARTÉ contains some components that are able to skin irritation in clinical practice such as Sodium lauryl Sulfate [4] and ethylhexylglycerin [6]. To reduce skin irritation, many ingredients were added to the tested product such as

Aloe Vera, Panthenol…[7,8]. Various studies confirmed dexpanthenol's moisturizing and skin barrier-enhancing potential. It prevents skin irritation, stimulates skin regeneration, and promotes wound healing [8]. D-panthenol could improve the symptoms of skin irritation, such as dryness of the skin, roughness, scaling, pruritus, erythema, and erosion/fissures [9]. Research to evaluate the skin irritation of a topical product is a prerequisite before mentioning the effectiveness of the drug. Rabbit skin is more sensitive than human skin. With the results of this study, it can be confirmed that the use of CLARTÉ has almost negligible irritation on human skin.

5. CONCLUSIONS

The results of the evaluation of skin irritation of CLARTÉ on healthy rabbit skin showed that 2 out of 3 rabbits developed very slight or well-defined erythema on the skin after the first 24 hours of applying CLARTÉ. However, the erythema completely disappeared after 48 hours of treatment. 1 out of 3 rabbits had very slight edema after 24 hours of treatment, but it completely disappeared after 48 hours of observation. There were no cases of moderate to severe erythema, and no cases of well-defined to severe edema. The Primary Irritation Index (PII) of CLARTÉ on 3 rabbits was 0.44, corresponding to a negligible skin irritation level according to the classification of rabbit skin irritation.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University.

REFERENCES

1. **Cheng YC, Li TS, Su HL, Lee PC, Wang HD (2020).** Transdermal Delivery Systems of Natural Products Applied to Skin Therapy and Care. Molecules, 25(21):5051. doi: 10.3390/molecules25215051.
2. **Kim B, Cho HE, Moon SH, et al (2020).** Transdermal delivery systems in cosmetics. Biomed Dermatol, 4:10. doi: 10.1186/s41702-020-0058-7
3. **Yin S et al (2021).** Transdermal Delivery of Chinese Medicine. In: Feng, N., Yang, Z. (eds) Novel Drug Delivery Systems for Chinese Medicines. Springer, Singapore. doi: 10.1007/978-981-16-3444-4_4
4. **Mei-Hsia Chan M, Tan LS, Leow YH, Goon AT, Goh CL (2019).** Comparison of Irritancy Potential of Sodium Lauryl Sulfate-free Aqueous Cream to Other Moisturizers: An Intraindividual Skin Occlusive Study. J Clin Aesthet Dermatol, 12(7):52-58.
5. **Pastor-Nieto MA, Gatica-Ortega ME (2021).** Ubiquity, Hazardous Effects, and Risk Assessment of Fragrances in Consumer Products. Curr Treat Options Allergy, 8(1):21-41.
6. **Aerts O, Verhulst L, Goossens A (2016).** Ethylhexylglycerin: a low-risk, but highly relevant, sensitizer in 'hypo-allergenic' cosmetics. Contact Dermatitis, 74(5):281-288.
7. **Han HJ, Park CW, Lee CH, Yoo CW (2004).** A study on anti-irritant effect of aloe vera gel against the irritation of sodium lauryl sulfate. Korean Journal of Dermatology, 42:413-419.
8. **Proksch E, de Bony R, Trapp S, Boudon S (2017).** Topical use of dexpanthenol: a 70th anniversary article. J Dermatolog Treat, 28(8):766-773.
9. **Ebner F, Heller A, Rippke F, Tausch I (2002).** Topical use of dexpanthenol in skin disorders. Am J Clin Dermatol, 3(6):427-433.
10. **Vietnam Ministry of Health (1999).** Skin irritation test method (applicable to medical and cosmetic products). Issued together with Decision No. 3113/1999/QD-BYT dated October 11, 1999, of the Minister of Health (in Vietnamese).

11. **OECD (2015).** Test No. 404: Acute Dermal Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris**.**
12. **International Organisation of Standardization (2010).** Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization, ISO 10993-10.
13. **Fluhr JW, Darlenski R, Angelova-Fischer I, Tsankov N, Basketter D (2008).** Skin irritation and sensitization: mechanisms and new approaches for risk assessment. 1. Skin irritation. Skin Pharmacol Physiol, 21(3):124-135.

SUBCHRONIC ORAL TOXICITY ASSESSMENTS OF "CAN HUYET VUONG" IN THE EXPERIMENT

Pham Thi Van Anh[1], Bui Thi Huong Thao[1], Dinh Thi Thu Hang[1]

[1] Department of Pharmacology, Hanoi Medical University
Corresponding author: **Dinh Thi Thu Hang**
Email: dinhthuhang@hmu.edu.vn
Received date: 09/09/2024
Reviewed date: 30/09/2024
Accepted date: 30/09/2024

SUMMARY

Nowadays, there has been a current trend for researchers to find out new natural ingredients which were safe and still effective in the treatment of diseases. "Can Huyet Vuong" was a herbal-derived product with two main ingredients including Curcuma longa L. (fresh turmeric) and Apis mellifera L. (honey bee) used as an oral medication. So far, the safety of this product has not been reported yet in Vietnam as well as in the world. Thus, this study was designed to assess the subchronic toxicity of "Can Huyet Vuong" in Wistar rats. The method used in this study followed the guidance of World Health Organization in rats with 2 oral doses of 1.8 g/kg b.w/day and 5.4 g/kg b.w/day for 30 days. As a result, "Can Huyet Vuong" did not caused deleterious impacts on general condition, hematological indexes, hepato-renal functions and microscopic images of liver and kidney. Hence, "Can Huyet Vuong" does not appear to produce subchronic toxicities in experimental animals.

Key words: *"Can Huyet Vuong", subchronic toxicity, Wistar rats.*

1. INTRODUCTION

The natural world has been a source of medicinal agents from ancient times and plenty of traditional medicines used in many countries worldwide have been formed from medicinal plants. [1] The exclusive use of herbal drugs for the prevention and treatment of a variety of diseases continues due to easy access, fewer adverse events, and economic reasons. According to the World Health Organization (WHO), traditional medicines are used by up to 80% of developing country populations for their primary health care. However, the biggest concern of medicinal plant use was the lack of evidence-based approaches and the lack of toxicological profiling of herbal preparations. Thus, evaluating their toxicity plays an important role in recognizing the toxicity of drug-derived herbal medicine, in helping to characterize them, to evaluate their risk for humans, and in recommending whether further human studies should be conducted and suggest appropriate dose levels for clinical trials. [2]

Toxicity refers to unwanted effects on the body's biological systems. In order to assess potential toxicity, it is crucial to choose the correct system, since no effects can otherwise be seen. Toxicity of a substance can be influenced by many factors, such as the route of exposure (topical application, oral administration, inhalation, or injection); the time of exposure (acute, subchronic, or chronic use); the number of exposures (a single dose or multiple doses over a period of time); the physical form of the toxin (solid, liquid, or gas); the organ system involved (cardiovascular, urinary, respiratory, nervous, hematopoietic system); and even the genome and physiological structure of the target cells or organisms. [3].

"Can Huyet Vuong" was a herbal-derived product containing two main

ingredients *Curcuma longa L.* (fresh turmeric) and *Apis mellifera L.* (honey). *Curcuma longa* L., was well known as turmeric, belongs to the Zingiberaceae family and used for traditional medicine as a remedy for various diseases. *Curcuma longa* L. has been used for supporting to treat liver obstruction and jaundice with oral administration, or ulcers and inflammation with topical administration. Moreover, it is used in other diseases such as gastrointestinal disorder, bronchitis, tumor, and hepatic disorders. [4] Curcuminoids from *Curcuma longa* L. have been assessed in a large number of *in vitro* and *in vivo* studies to exhibit potential biological activities. [5] Various beneficial effects of curcuminoids has been illustrated, including antioxidant and anti-cancer properties, antimicrobial properties, cardioprotective, hepatoprotective and neuroprotective properties. Honey is a product derived natural resources created by honey bees and stingless bees. Honey from both types of bee contain different types of phenolic and flavonoid compounds with variable biological activities and clinical applications. So far, honey can be used effectively for wound healing. Both honey bee and stingless bee honey were used for traditional and modern mecicine with various properties– such as the treatment of liver disorders, gastrointestinal tract diseases, neurological disorders and fertility disorders. [6] However, so far, there have been no reports available on the toxicity of a combination product from these components. Therefore, the present study aimed to validate the subchronic toxicity of “Can Huyet Vuong” in experimental animals.

2. MATERIALS AND METHODS

2.1. The preparation of “Can Huyet Vuong”

“Can Huyet Vuong” was manufactured by Vi Dieu Nam Medicine And Pharmacy Limited Company. This product contained two main ingredients as *Curcuma longa L.* (fresh turmeric) and *Apis mellifera L.* (honey bee). This product was prepared and offered in form of liquid extract.

2.2. Chemicals and laboratory equipments

Kits for testing enzymes and metabolites in blood: ALT (alanin aminotransferase), AST (aspartat aminotransferase), total bilirubin, albumin, total cholesterol, creatinine kits from Hospitex Diagnostics (Italy) và DIALAB GmbH (Austria) were used for Screen Master machine of Hospitex Diagnostics (Italy). Blood-testing solutions ABX Minidil LMG of ABX Diagnostics were used for Vet abcTM Animal Blood Counter. Chemicals for tests and histopathological examination.

2.3. Experimental animals

Healthy *Wistar* rats (180 ± 20 g) were used in this study. The animals were housed in cages (groups of ten rats/cage) under the standard conditions (temperature 25^0C ± 2^0C and relative humidity 80% ± 10%), 12 hours dark/light time. We fed the mice with standard animal feed and allowed free access to water. They were acclimated to housing for at 10 days prior to investigation at the Department of Pharmacology, Hanoi Medical University.

2.4. Subchronic toxicity study

Subchronic toxicity study were carried out according to WHO guidelines. [7]

The study was carried out in a continuous 30-day period. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control) was administered 1 mL distilled water/100 g b.w/day;

- Group 2 was administered “Can Huyet Vuong” at the dose of 1.8 g/kg b.w/day (equivalent to human recommended dose, conversion ratio 6);

- Group 3 was applied “Can Huyet

Vuong" at the dose of 1.8 g b.w/kg/day (3 times as high as the dose at group 2).

Animals were treated daily by oral route of administration once a day in the morning for 30 days and were observed once daily to detect signs of toxicity.

The signs and indexes were checked during the study including:

- General condition consists of mortality and clinical signs.
- Body weight changes
- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).
- Serum biochemistry: aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin, albumin, total cholesterol and creatinine levels.

The parameters were checked at these following times: before treatment, 15 days after treatment, and 30 days after treatment. At the end of experiment, all animals were subjected to a full gross necropsy. Liver and kidney of 30% rats of each group will be removed for histopathology examinations. The micro-histological examination was carried out at the Department of Pathology, Duc Giang General Hospital.

2.5. Statistical analysis

Data were analyzed using Microsoft Excel software version 2016. The levels of significance between the experimental groups and the control group were made using student's t-test and Avant-après test. Data was shown as mean ± standard deviation. All data were considered significantly at $p < 0.05$.

3. RESULTS

3.1. General condition

Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes when compared with the control group.

3.2. Body weight changes

Table 1. The effect of "Can Huyet Vuong" on body weight changes

Time	Body weight (g)		
	Group 1	**Group 2**	**Group 3**
Before treatment	162.00 ± 13.17	161.00 ± 14.49	160.00 ± 25.82
15 days after treatment	181.00 ± 17.92$^{\Delta}$	179.00 ± 22.34$^{\Delta}$	184.00 ± 34.06$^{\Delta}$
30 days after treatment	194.00 ± 11.74$^{\Delta}$	186.00 ± 24.59$^{\Delta}$	193.00 ± 40.84$^{\Delta}$

* $p < 0.05$, ** *p < 0.01,* *** *p < 0.001 compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *p < 0.01,* $^{\Delta\Delta\Delta}$ *p < 0.001 compared with the time point "before treatment"*

Table 1 showed that after 15 days and 30 days of treatment, there was a significant increase in body weight at all groups as compared with the time point "before treatment" ($p < 0.05$). No significant differences were observed between groups treated "Can Huyet Vuong" and control group ($p > 0.05$).

3.3. Effect on hematological examination

There was no significant difference in hematocrit, hemoglobin level, MCV and platelet count between groups treated "Can Huyet Vuong" and group 1 ($p > 0.05$) in Table 2.

In terms of red blood cell count, there was a significant decrease in the group treated "Can Huyet Vuong" at the dose of 1.8 g/kg/day as compared with the control group and the time point "before treatment" but red blood cell count was stil in normal range.

Table 2. Effect of "Can Huyet Vuong" on hematopoietic function

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
Red blood cell count (T/L)	Group 1	11.57 ± 0.97	12.29 ± 0.50	12.18 ± 0.82
	Group 2	12.28 ± 1.20	11.24 ± 0.47***$^{\Delta}$	10.98 ± 0.62**$^{\Delta\Delta}$
	Group 3	11.38 ± 1.13	11.56 ± 1.16	11.36 ± 0.98
Hemoglobin level (g/dL)	Group 1	13.69 ± 0.77	13.12 ± 1.05	13.79 ± 1.10
	Group 2	13.71 ± 1.45	12.42 ± 1.20	12.38 ± 2.51
	Group 3	13.64 ± 0.70	12.49 ± 1.40	12.88 ± 1.41
Hematocrit (%)	Group 1	61.59 ± 4.91	62.66 ± 4.16	60.67 ± 5.17
	Group 2	60.77 ± 4.94	58.59 ± 5.10	57.73 ± 4.58
	Group 3	61.65 ± 3.99	58.71 ± 4.30	58.50 ± 5.60
MCV (fL)	Group 1	53.20 ± 2.30	51.20 ± 2.74	51.10 ± 3.07
	Group 2	53.50 ± 3.72	51.20 ± 2.66	51.00 ± 3.13
	Group 3	54.00 ± 2.62	51.70 ± 3.02	52.90 ± 1.29
Platelet count (G/L)	Group 1	656.20 ± 175.28	707.80 ± 148.46	756.10 ± 143.25
	Group 2	635.40 ± 90.97	653.40 ± 164.26	702.10 ± 62.67
	Group 3	638.40 ± 80.53	669.60 ± 175.12	675.00 ± 134.92

MCV: Mean corpuscular volume

* p < 0.05, ** *p < 0.01, *** p < 0.001 compared with control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ *p < 0.01, $^{\Delta\Delta\Delta}$ p < 0.001 compared with the time point "before treatment*"

Table 3. Effects of "Can Huyet Vuong" on total WBC count and WBC differentials

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
Total WBC count (G/L)	Group 1	9.52 ± 1.31	8.98 ± 1.23	10.83 ± 1.74
	Group 2	9.01 ± 2.05	9.54 ± 2.79	9.91 ± 2.21
	Group 3	9.58 ± 2.17	8.22 ± 1.80	11.31 ± 1.43
Lymphocytes (%)	Group 1	74.27 ± 6.33	69.26 ± 7.40	71.68 ± 5.88
	Group 2	75.47 ± 4.23	73.07 ± 7.39	73.44 ± 5.11
	Group 3	74.46 ± 5.78	68.83 ± 5.38	71.09 ± 5.57
Neutrophils (%)	Group 1	12.07 ± 3.48	16.50 ± 4.50	13.18 ± 4.21
	Group 2	11.86 ± 2.50	14.17 ± 2.99	15.20 ± 3.67
	Group 3	12.98 ± 2.77	16.85 ± 4.23	15.71 ± 4.01

WBC: white blood cells

* p < 0.05, ** *p < 0.01, *** p < 0.001 compared with control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ *p < 0.01, $^{\Delta\Delta\Delta}$ p < 0.001 compared with the time point "before treatment*"

Table 3 demonstrated that at all time points, there was no significant difference in total WBC count, lymphocytes and neutrophils at groups treated “Can Huyet Vuong” as compared with group 1 and the time point “before treatment” ($p > 0.05$).

3.4. Effect on liver parameters

There were no significant diferences in total bilirubin, albumin concentration and total cholesterol concentration between groups treated “Can Huyet Vuong” and group 1 ($p > 0.05$). However, after 30 days of treatment, at group 2 and 3, aspartate amino transferase (AST) and alanine aminotransferase (ALT) levels increased significantly as compared with group 1 and the time point “before treatment” ($p < 0.001$).

Table 4. Effects of “Can Huyet Vuong” on liver parameters

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
AST level (UI/L)	Group 1	79.40 ± 14.06	70.90 ± 8.71	70.90 ± 15.47
	Group 2	91.00 ± 20.35	82.40 ± 18.42	72.90 ± 12.82
	Group 3	92.40 ± 19.55	79.90 ± 12.43	83.90 ± 21.34
ALT level (UI/L)	Group 1	31.80 ± 6.14	29.40 ± 7.24	31.00 ± 7.77
	Group 2	38.50 ± 9.05	36.20 ± 8.93	34.90 ± 7.39
	Group 3	35.30 ± 8.51	29.20 ± 8.93	38.00 ± 8.15
Total bilirubin (µmol/L)	Group 1	9.11 ± 0.67	9.50 ± 0.63	9.01 ± 0.34
	Group 2	8.93 ± 0.56	9.30 ± 0.71	8.94 ± 0.41
	Group 3	8.88 ± 0.43	9.47 ± 0.88	8.99 ± 0.48
Albumin concentration (g/dL)	Group 1	3.05 ± 0.16	3.42 ± 0.55	3.36 ± 0.37
	Group 2	3.21 ± 0.35	3.49 ± 0.39	3.04 ± 0.34
	Group 3	3.09 ± 0.39	3.21 ± 0.31	3.01 ± 0.38
Total cholesterol concentration (mg/dL)	Group 1	1.66 ± 0.25	1.75 ± 0.29	1.50 ± 0.17
	Group 2	1.65 ± 0.30	1.52 ± 0.24	1.42 ± 0.25
	Group 3	1.69 ± 0.21	1.68 ± 0.38	1.47 ± 0.26

* $p < 0.05$, ** *$p < 0.01$, *** $p < 0.001$ compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *$p < 0.01$,* $^{\Delta\Delta\Delta}$ *$p < 0.001$ compared with the time point “before treatment”*

3.5. Effect on kidney function

Table 5 illustrated that “Can Huyet Vuong” caused no significant differences in serum creatinine level between groups treated “Can Huyet Vuong” and group 1 ($p > 0.05$).

Table 5. Effects of “Can Huyet Vuong” on serum creatinine level

Days	Creatinine level (mg/dl)		
	Group 1	***Group 2***	***Group 3***
Before treatment	75.00 ± 6.90	74.22 ± 6.88	77.22 ± 11.28
15 days after treatment	74.60 ± 6.31	75.00 ± 5.96	79.70 ± 6.88
30 days after treatment	75.50 ± 6.72	74.40 ± 6.15	76.40 ± 4.25

* $p < 0.05$, ** *$p < 0.01$, *** $p < 0.001$ compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *$p < 0.01$,* $^{\Delta\Delta\Delta}$ *$p < 0.001$ compared with the time point “before treatment”*

3.6. Histopathological examination

No gross lesions or changes in size was observed when subjected all experimental rats to a full gross necropsy which examined of the hearts, livers, lungs, kidneys and abdominal cavities. There was no significant difference in histopathological examination of liver and kidney between groups treated “Can Huyet Vuong” and control group after 30 days of treatment (Figure 1 and 2).

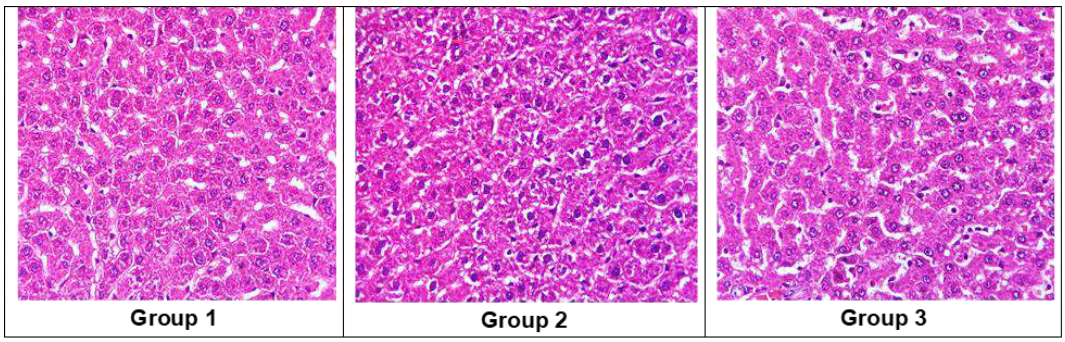


Figure 1. Histopathological images of liver (HE × 400)

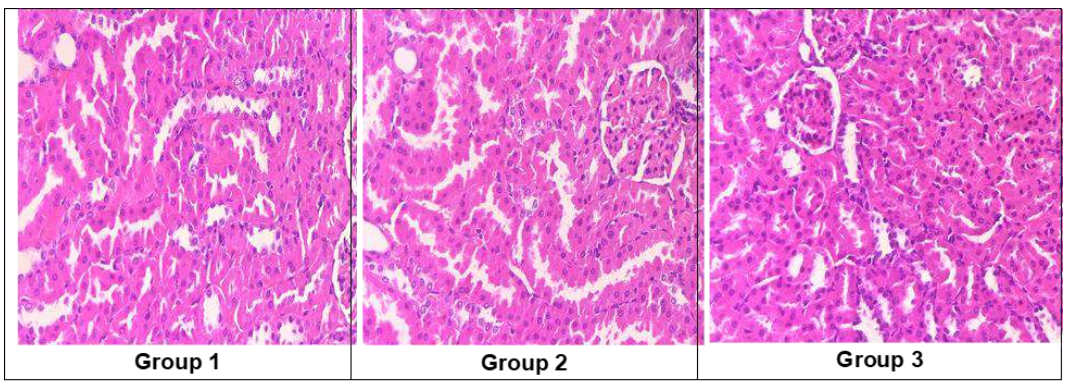


Figure 2. Histopathological images of kidney (HE × 400)

4. DISCUSSION

Toxicity is the negative impact to which a substance can harm the human body system. Toxicity can refer to the influence on a cell (cytotoxicity), an organ (e.g. nephrotoxicity or hepatotoxicity), or the whole organism. To assess the safety of drugs and herbal products for clinical use, various experimental animal models should be selected to conduct the toxicological evaluation for predicting toxicity and to provide recommendations for selecting ‘safe’ therapeutic dose levels in clinical. A subchronic toxicity study provides information on the impacts of repeated administration and can assess the need for further longer-term studies. [7], [8] Subchronic studies evaluate the undesirable effects of continuous exposure of traditional medicines or drugs over a part of the average life span of experimental animals, such as rodents. In particular, data from these studies provide toxicity information on target organs. [9]

The body weight changes serve as a sensitive index of the general health status of the animal. [10] Weights were observed in all animals treated with “Can Huyet Vuong”. It can be stated that “Can Huyet Vuong” did not interfere with the normal metabolism of animals as corroborated by the nonsignificant change from the control group.

The hematopoietic system is highly sensitive to influences from toxic compounds and is a vital index of physiological and pathological status in the body. Furthermore, when the data are translated from animal studies, the information about changes of the hematology system has higher predictive value for human toxicity. [7] After 15 days and 30 days of the treatment, there were no significant differences in hematocrit, hemoglobin level, platelet count, total WBC count and WBC differentials between groups treated “Can Huyet Vuong” with the control group. In terms of red blood cell count, there was a significant decrease in the group treated “Can Huyet Vuong” at the dose of 1.8 g/kg/day as compared with the control group and the time point “before treatment”, however, red blood cell count was stil in normal range. So it can be concluded that the administration of “Can Huyet Vuong” did not affect the hematological profile and blood formation process.

Evaluation of kidney and liver functions plays an important role in the toxicity evaluation of plant extracts as they are both necessary for the survival of a living organism. Analyses of biochemistry indexes were performed to assess the possible alterations in the function of the hepato-renal system impacted by herbal medicines. [7] The liver releases aspartate transaminase (AST) and alanine transaminase (ALT) and an elevation in serum levels of these enzymes is an indicator of hepatocellular damage. [11] The results showed that there was no subtantial change in the AST and ALT levels between the group treated “Can Huyet Vuong” and the control group. These results indicated that “Can Huyet Vuong” had no deleterious effect on liver function.

Creatinine level is a critical important index used in assessing the function of the kidney.**Error! Reference source not found.** No significant differences were observed in the creatinine level between control group and groups treated “Can Huyet Vuong” at various dose levels ($p > 0.05$). Therefore, “Can Huyet Vuong” did not affect kidney function.

The histopathological examination revealed the alteration in the microstructure of organs and the signs of the disease when viewed under brightfield microscopy. The further histological study could provide additional information regarding the hepatotoxicity and nephrotoxicity of “Can Huyet Vuong”. Our study showed that there was no significant difference in histopathological examination of the liver and kidney between groups treated “Can Huyet Vuong” and the control group.

Overall, the findings of this study indicated that no significant differences were observed in blood profile, biochemistry parameters and histopathological observations of liver and kidney tissues between the groups treated “Can Huyet Vuong” and the control group.

Our study was consistent with the previous report about the toxicity of components in “Can Huyet Vuong”. According to the study of Murugan S et al (2021), 90-day subchronic oral toxicity study, *Wistar* rats were given the administration of dose levels of 250, 500, and 1000 mg/kg of extract of Curcuma longa. The results showed that no deaths, behavioral changes, or signs of toxicity from body weight gain, food consumption, ocular and neurological examination, and hematological, blood biochemical, hormone, urine analysis or gross pathological and histopathological examination. [12] A 28-day subacute oral toxicity study was conducted to evaluate the toxicity of honey in *Wistar* rats. The results revealed that the administration at levels of 3, 6, 12, and 24 g/kg body weight/day of honey to the rats for a consecutive 28 days did not cause

significant change in clinical observations, hematological, chemical and histopathological examination as compared with the control group. [13]

5. CONCLUSION

"Can Huyet Vuong" at doses of 1.8 g/kg b.w/day and 5.4 g/kg b.w/day administered orally during continuous 30 days did not make any toxic signs or symptoms of toxicities on general indexes, hematopoietic, liver and kidney function in *Wistar* rats.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from the Department of Pharmacology, Hanoi Medical University.

REFERENCES

1. Krishnan S. (2018). TRADITIONAL HERBAL MEDICINES - A REVIEW. **5**, 611–614.
2. WHO global report on traditional and complementary medicine 2019. <https://www.who.int/publications/i/item/978924151536>, accessed: 09/06/2024.
3. Venkatasubbu G.D., Ramasamy S., Gaddam P.R., et al. (2015). Acute and subchronic toxicity analysis of surface modified paclitaxel attached hydroxyapatite and titanium dioxide nanoparticles. *Int J Nanomedicine*, **10 Suppl 1**(**Suppl 1**), 137–148.
4. Debnath B., Singh W.S., and Manna K. (2023). A phytopharmacological review on Ananas comosus. *ADV TRADIT MED (ADTM)*,**23**(**2**),291–298.
5. Jyotirmayee B. and Mahalik G. (2022). A review on selected pharmacological activities of Curcuma longa L. *International Journal of Food Properties*, **25**(**1**), 1377–1398.
6. Lu B., Wu X., Tie X., et al. (2005). Toxicology and safety of anti-oxidant of bamboo leaves. Part 1: Acute and subchronic toxicity studies on anti-oxidant of bamboo leaves. *Food and Chemical Toxicology*, **43**(**5**), 783–792.
7. Organization W.H. (2007), *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues*, World Health Organization.
8. World Health Organization *Working group on the safety and efficacy of herbal medicine*, Report of regional office for the western pacific of the World Health Organization; 2000.
9. Adamou A. (2022). An Overview of Subchronic and Chronic Toxicology. *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays*. Springer International Publishing, Cham, 1–14.
10. Agents C., Toxicology B., Studies D., et al. (2006), *Toxicity testing for assessment of environmental agents: Interim report*, .
11. Kalas M.A., Chavez L., Leon M., et al. (2021). Abnormal liver enzymes: A review for clinicians. *World J Hepatol*, **13**(**11**), 1688–1698.
12. Murugan S., Solanki H., Purusothaman D., et al. (2021). Safety Evaluation of Standardized Extract of Curcuma longa (NR-INF-02): A 90-Day Subchronic Oral Toxicity Study in Rats. *BioMed Research International*, **2021**.
13. Du H., Zhang P., Zheng S., et al. (2023). A 28-day subacute oral toxicity study of Apis cerana (Fabricius) honey in Wistar rats. *International Food Research Journal*, **30**, 1481–1494.

SUB-CHRONIC ORAL TOXICITY ASSESSMENTS OF BANIKHA HARD CAPSULE IN *WISTAR* RATS

Bui Thi Huong Thao[1], Pham Thi Van Anh[1], Dang Thi Thu Hien[1],
[1] Department of Pharmacology, Hanoi Medical University
Corresponding author: **Dang Thi Thu Hien**
Email: thuhien@hmu.edu.vn
Received date: 08/09/2024
Reviewed date: 30/09/2024
Accepted date: 30/09/2024

ABSTRACT

Banikha is a formula originating from herbal medicines used to improve and treat some diseases such as immunostimulatory activity and improve physical strength; however, to our knowledge, no systematic study concerning its toxicity profile has been reported. Therefore, this study aimed to evaluate the potential sub-chronic toxicity of Banikha hard capsules in experimental animal. The sub-chronic toxicity study was carried out in Wistar rats for 12 consecutive weeks by oral administration at doses of 0.385 and 1.155 g/kg/day (n = 10/group). The general behavior and body weight of the rats was observed daily. Biochemical, hematological, macroscopically, and histopathological examinations of several organs were conducted at the end of the treatment period. After treatment, Banikha induced no mortality or treatment-related adverse effects with regard to body weight, general behavior, or hematological, and biochemical parameters. Histopathology assessment did not show any significant variation between control and treatment groups during the study period. In conclusion, the present study revealed that oral administration of Banikha hard capsule for 12 weeks, at dosages did not induce toxicological damage in rats.

Keywords: *Sub-chronic toxicity, Banikha hard capsule, experimental animals.*

1. INTRODUCTION

The use of traditional medicine plays a vital role in the treatment of various diseases. These products offer a rich source of bioactive compounds with potential therapeutic benefits. Recently, there has been a shift from using only synthetic medications to combining them with traditional herbal drugs to control various conditions. [1] According to the World Health Organization (WHO), up to 80 % of the world's population uses herbal medicine for their health care because of its effectiveness, availability, cost, and minimization of side effects. [2] As the usage of herbal medicine increases, more scientific evidence regarding the efficacy and safety of herbal products is required. Several studies have reported the efficacy of medicinal plants in treating various diseases. They are generally considered safe, which might have contributed to the lack of toxicology evaluations of various herbal plants and phytoconstituents in current literature.

In Vietnam, several traditional medicines have been widely used to treat and improve the clinical symptoms of conditions. Banikha is a well-characterized formulation prepared by mixing components including *Cordyceps militaris, Red ginseng extract, Ganoderma lucidum, and Yeast extract* in a precise ratio and given to patients in hard capsule forms. According to folk experiments and documents on traditional medicine, the researchers found that each of these medicines had shown pharmacological activities like antioxidant, anti-cancer, antihyperlipidemic, anti-diabetic, anti-fatigue, anti-aging, hypocholesterolemic, hypotensive, vasorel. [3] Despite studies of each of these traditional

medicines having already started broadly many years ago, the safety of their combination in this hard capsule has not been assessed. With a full toxicity profile, its development and optimal use can be further promoted. Herein, we evaluated the sub-chronic toxicity of Banikha hard capsule in animals to predict their safety in humans and promote further development.

2. MATERIAL AND METHODS

2.1. Materials

Banikha was supplied by Medistar Vietnam Co., LTD. It was prepared in hard capsule form, including Cordyceps militaris 500 mg, Red ginseng extract, Ganoderma lucidum, and Yeast extract, and other synthetic ingredients enough for one hard capsule. The expected dose in clinical is 6 hard capsules per day (equivalent to 3,21 g per day)

2.2. Animals

Wistar rats of either sex of 8 weeks weighing 150−210 g were procured from the Laboratory Animal Center, Dan Phuong district, Hanoi.

The animals were kept in cages in laboratory conditions (25^{o}C, 12:12 dark/light cycle) of the Department of Pharmacology. Experimental protocols adopted were based on World Health Organization Guidelines for the care and use of laboratory animals. They were acclimatized for 5 - 7 day weeks before the experiments and fed with a normal pellet diet and water ad libitum.

2.3. Methods

The experiment was conducted according to the guidance of the World Health Organization. [4] Banikha was administered once daily orally for 12 consecutive weeks. Thirty rats were randomly distributed into three groups (I, II, and III) ten rats in each group, and their weights were recorded. Before treatment, rats were handled individually and carefully examined for abnormal behavior and appearance. The Banikha dissolved in distilled water, was administered orally once a day for 12 consecutive weeks. Group1 (Control rats) received distilled water; Group 2 (Banikha 0.385 g/kg/day- low dose- equivalent to clinical dose) received extract at a dose of 0.385 g/kg; Group 3 (Banikha 1.155 g/kg/day - high dose - 3 times-equivalent to clinical dose) received the extract at a dose of 1.155 g/kg. The animals were observed daily during the experimental period for mortality or morbidity, overall conditions, changes in posture, changes in the fur of the skin, eyes, mucous membranes, and behaviors. At the end of the treatment, the animals were fasted overnight but had free access to the water. The blood samples were drawn from the vein before and after administration and at week 4; week 8 and on the day of autopsy in a 12-week study for biochemistry and hematology parameters measured. After blood collection, the rats were sacrificed by cervical dislocation. Organs were collected, washed immediately in NaCl (0.9 %), and examined macroscopically. Histopathological findings were evaluated on the tissues (liver, kidney) of 30% of the studied rats.

2.4. Statistical analysis

Data sets were entered and analyzed using Excel 2013 software. Results were expressed as the Mean value ($\underline{X}$) ± Standard Deviation (SD) or the percentage (%). The level of significance was considered at values of $p < 0.05$ The two arms of the recovery group were analyzed by the Student t-test. Unless otherwise noted, 'significant' means that it has statistical significance compared with the control group.

3. RESULTS

3.1. Sub - chronic toxicity experiments

General observation: During the experiment period, rats in all groups displayed normal activities, good eating, agility, bright eyes, and dry stools. There were no abnormal clinical signs recorded regarding the capsules.

The body weight: 12-week oral administration of Banikha did not alter the feed and water consumption in rats compared to the respective control animals. The body weight of rats in all groups (control group and 2 treatment groups) significantly increased compared to before the experiment However, there is no statistically significant weight difference between the treated and the control group (p > 0.05). As shown in Table 1.

Table 1. Effect of 12-week treatment with Banikha on the body weight of rats.

	Week	Control (n=10, $\underline{X}$ ± SD)	Banikha (n =10, $\underline{X}$ SD)	
			0.385 g/kg	1.155 g/kg
Body weight (g)	**Before treatment**	169.00 ± 16.63	172.00 ± 13.98	161.50 ± 18.27
	Week 4	173.00 ± 24.97	205.50 ± 24.32**,b	184.50 ± 33.37*
	Week 8	201.00 ± 35.10*	242.00 ± 22.01***,b	201.00 ± 35.73**
	Week 12	226.00 ± 48.12**	249.00 ± 22.83***	220.00 ± 30.18***

*Note: *p < 0.05, **p < 0.01, ***p < 0.001 were significant changes compared to before treatment*

[a]p < 0.05, [b]p < 0.01, [c]p < 0.001 were significant changes compared to control

3.2. Effect of Banikha on hematological parameters in rats

The results in Table 2 and Table 3 showed that all the hematological parameters in treated groups were no significantly different from the control group and there was no significantly different comparison between the time before and after the experiment (p> 0.05).

Table 2. Effect of Banikha on rat's hematological parameters

Parameters	Groups (n=10)	Initial ($\underline{X}$ ± SD)	After treatment ($\underline{X}$ ± SD)		
			Week 4	Week 8	Week 12
RBC (T/l)	Control	8.89 ± 1.05	8.95 ± 1.43	8.57 ± 0.80	8.33 ± 0.90
	Group I	9.27 ± 1.55	9.17 ± 1.20	9.25 ± 0.66	9.54 ± 1.61
	Group II	8.99 ± 1.12	8.95 ± 1.71	9.89 ± 2.12	9.23 ± 1.08
HGB (g/dL)	Control	11.22 ± 1.64	11.31 ± 1.73	11.44 ± 1.34	11.68 ± 1.03
	Group I	11.83 ± 2.13	11.20 ± 1.70	12.09 ± 1.12	12.55 ± 2.64
	Group II	11.83 ± 2.55	10.51 ± 1.89	12.56 ± 2.85	13.30 ± 2.32
HCT (%)	Control	43.47 ± 3.13	44,21 ± 4,18	41,70 ± 2,51	41,16 ± 4,15
	Group I	45,29 ± 4,18	43,69 ± 6,55	42,82 ± 4,80	42,16 ± 4,18
	Group II	44,79 ± 3,44	42,49 ± 4,82	44,46 ± 5,02	43,56 ± 5,84
MCV (fL)	Control	48,30 ± 3,13	47,80 ± 4,69	47,50 ± 3.63	45.35 ± 5.31
	Group I	47.50 ± 3.21	47.60 ± 2.22	45.80 ± 4.10	46.30 ± 5.07
	Group II	48.80 ± 2.30	47.40 ± 4.14	48.88 ± 2.64	49.33 ± 3.40
PLT (g/L)	Control	689.80 ± 100.28	700.60 ± 116.82	758.20 ± 117.99	657.50 ± 114.95
	Group I	738.40 ± 128.86	750.60 ± 133.44	762.60 ± 130.90	690.60 ± 102.00
	Group II	691.50 ± 136,80	699,90 ± 135,39	742,20 ± 99,19	718,70 ± 113,72

Table 3. Differential white blood cell count values of rats in the subchronic toxicity Banikha hard capsule

Weeks	Groups (n=10)	Differential white blood cell ($\underline{X}$ ± SD)		
		WBC (T/l)	Lym (%)	Neu (%)

	Control	11.35 ± 1.76	66.99 ± 4.31	15.71 ± 3.17
Initial	Group I	10.35 ± 2.19	69.47 ± 6.09	15.77 ± 3.64
	Group II	10.04 ± 2.61	68.01 ± 5.63	14.62 ± 3.47
	Control	10.29 ± 2.18	67.03 ± 5.49	15.74 ± 2.60
Week 4	Group I	10.31 ± 2.45	66.89 ± 7.01	19.00 ± 5.24
	Group II	9.40 ± 2.95	69.23 ± 6.49	15.55 ± 4.46
	Control	10.65 ± 2.24	71.49 ± 5.73	13.64 ± 1.59
Week 8	Group I	11.08 ± 2.20	72.87 ± 6.68	12.51 ± 3.80
	Group II	10.04 ± 2.77	72.40 ± 5.66	13.82 ± 3.96
	Control	10.64 ± 1.70	68.19 ± 3.90	14.03 ± 2.11
Week 12	Group I	10.24 ± 2.40	68.50 ± 6.08	15.69 ± 4.47
	Group II	9.08 ± 2.49	71.40 ± 6.96	14.31 ± 4.38

*Note: *p < 0.05. **p < 0.01. ***p < 0.001 were significant changes compared to before treatment*
ap < 0.05. bp < 0.01. cp < 0.001 were significant changes compared to control

3.3. Effect of Banikha on Serum biochemical parameters

The sub-chronic oral administration of Banikha (daily for 12 weeks), albumin, total cholesterol, creatinine, total bilirubin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are shown in Table 4, Figure 1. Clinical chemistry results did not show significant differences in values between treated groups and control ones.

Table 4. Effect of orally administration of Banikha on serum biochemical parameters in rats

Parameters	Groups (n=10)	Initial	After treatment		
			Week 4	Week 8	Week 12
Albumin (g/dL)	Control	3.13 ± 0.38	3.16 ± 0.29	3.10 ± 0.35	3.20 ± 0.32
	Group I	3.12 ± 0.29	2.99 ± 0.42	2.96 ± 0.13	3.00 ± 0.34
	Group II	3.14 ± 0.38	3.31 ± 0.35	3.34 ± 0.33	3.28 ± 0.36
Total cholesterol (mmol/L)	Control	1.46 ± 0.22	1.35 ± 0.18	1.41 ± 0.17	1.49 ± 0.36
	Group I	1.58 ± 0.32	1.54 ± 0.35	1.41 ± 0.26	1.17 ± 0.34
	Group II	1.52 ± 0.20	1.51 ± 0.22	1.48 ± 0.29	1.25 ± 0.22
Total bilirubin (mmol/L)	Control	13.38 ± 0.32	13.22 ± 0.75	13.29 ± 0.55	13.45 ± 0.43
	Group I	13.44 ± 0.34	13.38 ± 0.51	13.47 ± 0.45	13.45 ± 0.29
	Group II	13.43 ± 0.23	13.38 ± 0.51	13.28 ± 0.54	13.50 ± 0.40
Creatinine (mg/dL)	Control	0.77 ± 0.13	0.81 ± 0.19	0.80 ± 0.16	0.84 ± 0.14
	Group I	0.80 ± 0.14	0.83 ± 0.13	0.88 ± 0.10	0.86 ± 0.10
	Group II	0.779 ± 0.13	0.83 ± 0.13	0.75 ± 0.15	0.82 ± 0.18

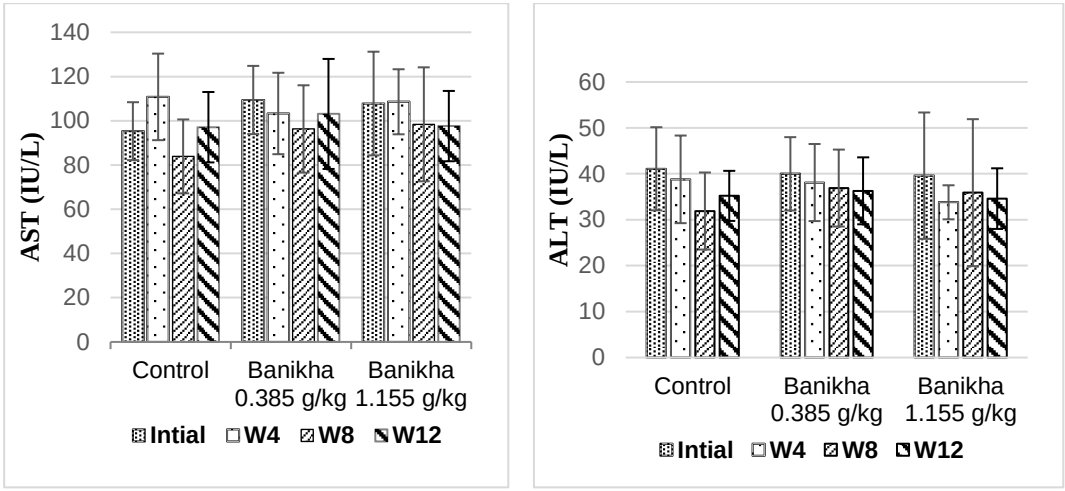
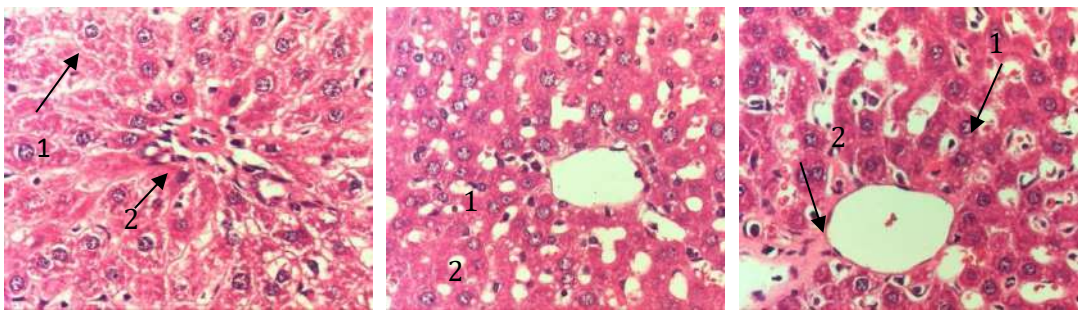


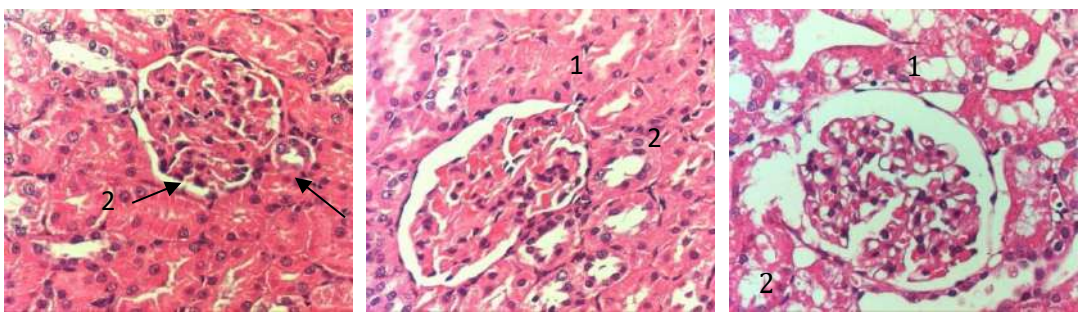
Figure 1. Effect of orally administration of Banikha on serum biochemical parameters

3.4. Effect of Banikha hard capsule on experimental animal histopathology



a. Control group b. Banikha 0.385 g/kg c. Banikha 1.155 g/kg

Figure 2: Liver sections of control rats (a) and rats treated daily with Banikha at two doses of 0.385g/kg (b), and Banikha 1.155 g/kg (c). (1) hepatocyte (2) portal venule
(Selected microphotographs HE staining magnification × 400)



a. Control group b. Banikha 0.385 g/kg c. Banikha 1.155 g/kg

Figure 3: Kidney sections of control rats (a) and rats treated daily with Boga- TN at two doses of 0.77g/kg (b), 1.155 g/kg (c). (1) convoluted tubule; (2) renal corpuscle
(Selected microphotographs HE staining magnification × 400)

Gross anatomical examination of the vital organs (liver, kidney, heart, lung, and spleen) in sub-chronic oral toxicity study did not reveal any gross pathological lesions. The effects of Banikha on the histopathology of the liver and kidney at the termination of treatment are shown in Figure 2 – 3. Histological evaluations of the livers and kidneys in rats showed no changes when compared to the control.

4. DISCUSSION

Traditional medicine has a long history and toxicological study strategy prevailed in the 19th and 20th centuries. The new drug development process must continue through several stages to make a medicine that is safe, effective, and has approved all regulatory requirements. According to FDA guidances, before trialing a drug in humans, researchers must assess its possibility to cause any serious toxicity which can be performed in vitro and in vivo. [5], [6] Toxicity research is an important step in the development of traditional and complementary medicine. The first principle in medicine is to do no harm and safety is always a fundamental principle in the provision of any health-care treatment and procedures. WHO has consistently advocated for the integration into national health systems of traditional medicine practices and products that meet the standards of quality, safety, and efficacy and before any pharmacological validation and the development of a phytomedicine of any medicinal plant, toxicity is mandatory according to standard guidelines. Nevertheless, the latest surveys have indicated that traditional medicine plants contains pharmacologically active ingredients, some of which have been associated with adverse effects. [7] Since safety continues to be a major issue with the use of medicinal plants, it is important to conduct toxicity studies on them to ascertain their safety profile.

As many drugs are species-specific, it is essential to select appropriate animal species for toxicity studies. In the present study, *Wistar* rats were used to evaluate the safety of the Banikha with estimates of physical signs, and biochemical, hematological, and histopathological vital organs in subchronic toxicity study. The choice of administration routes depends on the intended clinical route and current knowledge of the oral bioavailability of the test substance. The subchronic toxicity study evaluated for 12 weeks with two doses of 0.385 and 1.155 g/kg/day was conducted. No dead or moribund animals were reported in the treated groups. After 12 weeks of oral administration of Banikha, food and water consumption was not affected. It indicates that the capsule did not effect on appetite or adverse effects on the growth and body metabolism of the animalsHematological and clinical chemistry parameters are good indicators in determining toxicity. [8] The results revealed no major haematological changes in rats administered with the test dose of Banikha. The roles of the liver and kidney functions function are vital, with one being used for the metabolism of ingestion and the other for excretion of the waste product respectively. The biochemical biomarkers showed that the capsule is not toxic at the doses studied which correlated well with the gross observation and the histopathology findings.

The component of Banikha was analyzed to detect within it the presence or absence of toxic compounds. *Cordyceps militaris (C. militaris)* is a parasitic fungus that grows on the larvae of Lepidoptera which is a well-known fungus with immunomodulatory activity and improved physical strength. A 90-day subchronic toxicity study of *Cordyceps militaris* in rats showed no systemic toxicity attributable to dietary exposure to the powder of C. militaris submerged mycelial culture at the highest dose of 4000 mg per kg BW per day in

rats. [9] Ginseng is a well-known traditional medicine used in Asian countries for thousands of years which produced a variety of pharmacological and therapeutic effects on central nerve system disorders, cardiovascular disease, endocrine secretions, aging, and immune function. According to the research of Sang-Jin Park et al, Sprague-Dawley rats administrated at dose levels of 500, 1,000, and 2,000 mg/kg/day for four weeks were no observation of toxicity. Neither deaths nor clinical symptoms were observed in any group during the experiment. Furthermore, no abnormalities in body weight, food consumption, ophthalmology, urinalysis, hematology, serum biochemistry, gross findings, organ weights, or histopathology were revealed related to the administration of the test article in either sex of any dosed group. [10] *Ganoderma lucidum* has been reported that no detailed toxicological assessments on oral safety/toxicity (Jianjun Zhang et al (2016). [11] Besides, *Yeast* extract at the dose of 0, 500, 1000, and 2000 mg/kg in SD rats for 26 weeks with a 4-week recovery period. Banikha was administrated *via* gavage did not alter weight, food intake, urinalysis parameters, hematological analysis parameters, organ weight, organ-to-weight ratio, microscopic and macroscopic examination of organs. [12]

5. CONCLUSION

Banikha with a dose equivalent to the proposed clinical dose and 3 times the clinical dose did not cause any significant toxicity resulting in death, or produce any hematological, serum chemical alteration, and histo-pathological derangements. these observations suggest that *Banikha hard capsule* may be safe.

Acknowledgments

The authors duly acknowledge Department of Pharmacology – Hanoi Medical University and Medistar Vietnam Co., LTD, Thien phuc herbal medicine for their support.

REFERENCES

1. Nasim N., Sandeep I.S., and Mohanty S. (2022). Plant-derived natural products for drug discovery: current approaches and prospects. *The Nucleus*, **65**(**3**), 399–411.
2. WHO (World Health Organization) B. (2011). The World Traditional Medicines Situation, in Traditional medicines: Global Situation, Issues and Challenges. *Geneva 3*, 1–14.
3. Das G., Shin H.-S., Leyva-Gómez G., et al. (2021). Cordyceps spp.: A Review on Its Immune-Stimulatory and Other Biological Potentials. *Front Pharmacol*, **11**, 602364.
4. WHO (2000). General guidelines for methodologies on research and evaluation of traditional medicine. 28–29.
5. FDA (2019), *The Drug Development and Approval Process*, .
6. Deore A., Dhumane J., Wagh R., et al. (2019). The Stages of Drug Discovery and Development Process. *Asian J Pharm Res Dev*, **7**, 62–67.
7. Posadzki P., Watson L.K., and Ernst E. (2013). Adverse effects of herbal medicines: an overview of systematic reviews. *Clin Med*, **13**(**1**), 7–12.
8. Petterino C. and Argentino-Storino A. (2006). Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Exp Toxicol Pathol*, **57**(**3**), 213–219.
9. Jhou B.-Y., Fang W.-C., Chen Y.-L., et al. (2018). A 90-day subchronic toxicity study of submerged mycelial culture of Cordyceps militaris in rats. *Toxicol Res*, **7**(**5**), 977–986.
10. Park S.-J., Lim K.-H., Noh J.-H., et al. (2013). Subacute Oral Toxicity Study of Korean Red Ginseng Extract in Sprague-Dawley Rats. *Toxicol Res*, **29**(**4**), 285–292.
11. Zhang J., Gao X., Pan Y., et al. (2016). Toxicology and immunology of

Ganoderma lucidum polysaccharides in Kunming mice and Wistar rats. *Int J Biol Macromol*, **85**, 302–310.

12. Gao Y., Chen X., Li C., et al. (2020). Toxicological evaluation of, red rice yeast extract, Xuezhikang: Acute, 26-week chronic and genotoxicity studies. *Regul Toxicol Pharmacol*, **114**, 104654.

IMMUNOENHANCEMENT EFFECTS OF THE HERBAL FORMULA ANTI-U200 ON CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION IN MICE

Pham Thi Van Anh[1], Bui Thi Huong Thao[1], Mai Phuong Thanh[1]

[1]Department of Pharmacology, Hanoi Medical University

Corresponding author: **Pham Thi Van Anh**

Email: maiphuongthanh@hmu.edu.vn

Received date: 09/09/2024

Reviewed date: 30/09/2024

Accepted date: 30/09/2024

ABSTRACT

Anti-U200 is an herbal blend comprising Ganoderma lucidum, Hedyotis diffusa, and Scutellaria barbata is known to have immunomodulatory effects. ***Objectives:*** *We examined the immuno-enhancing effect of this herbal blend on cyclophosphamide (CYP)-induced immunosuppression.* ***Methods:*** *Swiss mice were assigned to one of five groups: the intact control and four CYP treatment groups (one control, one reference (levamisole), and two with the application of Anti-U200 at different dosages; 1.92 or 5.76 capsules/kg). Mice received one of the experimental treatments after being injected with CYP to induce myelosuppression and immunosuppression.* ***Results:*** *Anti-U200 therapy improved lymphoid organ atrophy in histological assessments and ameliorated the CYP-induced decreases in spleen and thymus weights in immunosuppressed animals. It also led to beneficial changes in leucocyte counts and boosted TNF-α and IgM serum levels.* ***Conclusions:*** *We have proven that Anti-U200, a combination of three immunomodulatory herbs, is an efficient immunomodulatory agent that can strengthen the immune system.*

Keywords*: Anti-U200, cyclophosphamide, immunomodulatory, mixed herbal, mice.*

1. INTRODUCTION

Nowadays, one of the main concerns with chemotherapy is the adverse effects connected to the delivery of chemotherapeutic medicine [1]. Myelosuppression and immunosuppression are brought on by both cytotoxic and immunomodulatory chemotherapy, which impair bodily processes and lower quality of life [2]. Chemotherapy for an extended period may also lead to immunodeficiency, which increases vulnerability to infections and lowers immunosurveillance against cancer [3]. Fever, flu-like symptoms, and allergic responses are among the known side effects of certain immunomodulatory medications, even though they can be used to avoid these side effects [4]. Natural herbal remedies, on the other hand, are thought to be potentially useful immunomodulators with negligible or no side effects.

Anti-U200 is a herbal blend that consists of three medicinal plants containing dry extracts from *Scutellaria barbata*, *Hedyotis diffusa*, and *Ganoderma lucidum*. Anti-tumor and immunomodulatory effects have been demonstrated for herb constituents in the Anti-U200 preparation [5-7]. Nevertheless, there is a lack of study on the impacts of these immunomodulatory herbal mixtures on immunological modulation, despite the assumption that herbal formulations are more synergistic than individual immunomodulatory herbal components. Therefore, it is anticipated that the combination of these herbs in Anti-U200 would have a more pronounced immunomodulatory effect. In this work, we investigated the immune-enhancing

effects of Anti-U200 on cyclophosphamide-induced immunosuppression in mice, which have been employed as a useful animal model for assessing the immunomodulatory effects of natural substances.

2. MATERIALS AND METHODS

2.1. Chemicals

Anti-U200 was supplied by the Institute of Traditional Medicine Research and Development (Hanoi, Vietnam). This product comprises a 93% herbal blend (*Scutellaria barbata*, *Hedyotis diffusa*, and *Ganoderma lucidum*). Other constituents include magnesium carbonate, 7% PVP K30/ethanol 96%, aerosil, and talc. The lowest dose of Anti-U200 used in this study was 1.92 capsules/kg, which was calculated by multiplying the recommended dosage for humans (8 capsules per day) by the conversion rate of animal doses to human-equivalent doses (12-fold), and we also assessed the effects of 5.76 capsules/kg, representing three-fold increases in the administered dosage.

Cyclophosphamide (Endoxan 200 mg, Baxter Oncology GmbH, Germany) was used as the immunosuppressive agent. Levamisole was obtained from Sigma (Aldrich) Chemicals Pvt. Ltd. USA and used as a positive control in this experiment. Sheep red blood cells (SRBC) and OA solution (ovalbumin + $Al(OH)_3$) were used as the delayed-type hypersensitivity inducer. Cytokine ELISA kits and antibodies were purchased from Cloud-Clone Corp (CCC, USA).

2.2. Experimental animals

50 mice of either sex (20–30 g; 2-3 months old) were used. The animals were supplied by the National Institute of Hygiene and Epidemiology (Hanoi, Vietnam). The acclimatization of test animals was carried out by placing mice in metal cages with standard environmental conditions for 7–14 days at room temperature and with adequate ventilation before the experiment. The animals were provided with a standard pellet diet and water ad libitum.

2.3. Methods

The experiment was carried out following our previous publication [8].

After being adapted to the environment for 7-14 days, these mice were randomly divided into 5 groups consisting of 10 mice each. Mice were treated as Table 1. The groups were the non-CTX group (Normal), immunosuppressive model group (CYP), positive control group (100 mg kg^{-1} levamisole), 1.92 capsules kg^{-1} Anti-U200 group, and 5.76 capsules kg^{-1} Anti-U200 group. Except for the normal group, the other groups were injected 200 mg kg^{-1} CYP on the 4th day to establish the immunosuppressive mice model. In the Anti-U200 experimental group, mice were given different doses of Anti-U200 by gavage for 7 days. On the 8th day, mice were anesthetized by chloral hydrate (250 mg kg^{-1} i.p.) and then euthanized to collect blood samples, spleen, and thymus to evaluate immune parameters.

Table 1. Mouse treatment schedule.

Group	Treatment	Duration
Normal	Distilled water (0,2 mL/10 g bw, gavage)	7 days
CYP	200 mg kg^{-1} CYP (inject)	Day 4
Levamisole	100 mg kg^{-1} Levamisole (gavage) 200 mg kg^{-1} CYP (inject)	7 days Day 4
Low-dose Anti-U200	1.92 capsules kg^{-1} Anti-U200 (gavage) 200 mg kg^{-1} CYP (inject)	7 days Day 4
High-dose Anti-U200	5.76 capsules kg^{-1} Anti-U200 (gavage) 200 mg kg^{-1} CYP (inject)	7 days Day 4

Determination of thymus and spleen indices

Mice were sacrificed 24 hours after the last intragastric administration, and then each mouse's thymus and spleen were aseptically removed and weighed. The organ index was calculated as follows:

$$Index\ (‰) = \frac{weight\ of\ spleen\ or\ thymus\ (mg)}{body\ weight\ (g)}$$

Leukocyte Counts

On the day of sacrifice, blood samples were collected from carotid arteries to determine the total WBC, lymphocytes, neutrophils, and monocytes using a HORIBA® ABX MircoES60 blood analyzer.

Determination of Cytokines in Serum by ELISA

Serum samples were prepared by centrifuging the whole blood at 3500 rpm at 4^0C for 10 min. The levels of immunoglobulin (IgM) and TNF-α in the serum were measured according to the ELISA kit instructions.

Delayed type hypersensitivity (DTH) test

On the second day of the experiment, animals were given an i.p. injection of 0.5 ml of 5% (v/v) sheep red blood cells and injected subcutaneously OVA 0,1 mL into the nape of the neck site. DTH reaction was elicited 5 d later by the injection of OVA 50 µL into right the hind paw and physiological saline into the left one after measuring the thickness of the footpad in the center of walking pads using an INSIZE® digital external caliper (Code: 2312–20). After 24 h, the paw volume was measured again to assess the swelling degree through differential percentage between the two footpads.

Histopathological analysis

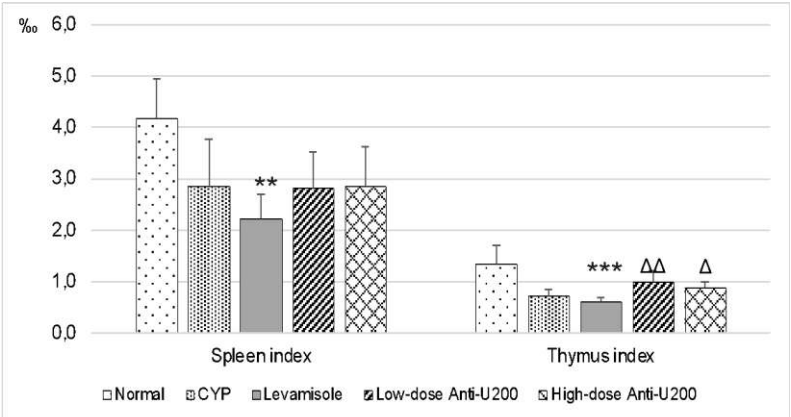
A histopathological examination was performed in a total of 15 spleens/thymi: 3 in each group. After their removal, the organs were weighed using a digital scale (Precisa®, Swiss, Model 321 LX Type 2200 C), and then preserved in 10% formalin for fixation. Sections stained with hematoxylin and eosin and examined under a light microscope. The images were photographed digitally at 100x.

2.4. Statistical analysis

Data are shown as mean ± SD. The statistical significance of the differences between various groups was determined by Student's t-test using Microsoft Excel version 2010. Values of $p < 0.05$ were considered statistically significant.

3. RESULTS

3.1. Effects of Anti-U200 on organ indices



***p<0.01, ***p<0.001 vs. Normal group; $^{\Delta}$p<0.05, $^{\Delta\Delta}$p<0.01 vs. CYP alone.*

Figure 1. Effect of Anti-U200 on immune organ indices

The spleen and thymus indices of the normal and immunosuppressive mice are depicted in Figure 1. Compared with the normal group, the spleen and thymus

indices of the CYP-exposed group were significantly decreased. The relative thymus weights were not affected by Anti-U200 capsules. However, there was an increase in relative spleen weight in mice treated with test capsules, which was obvious in both tested doses.

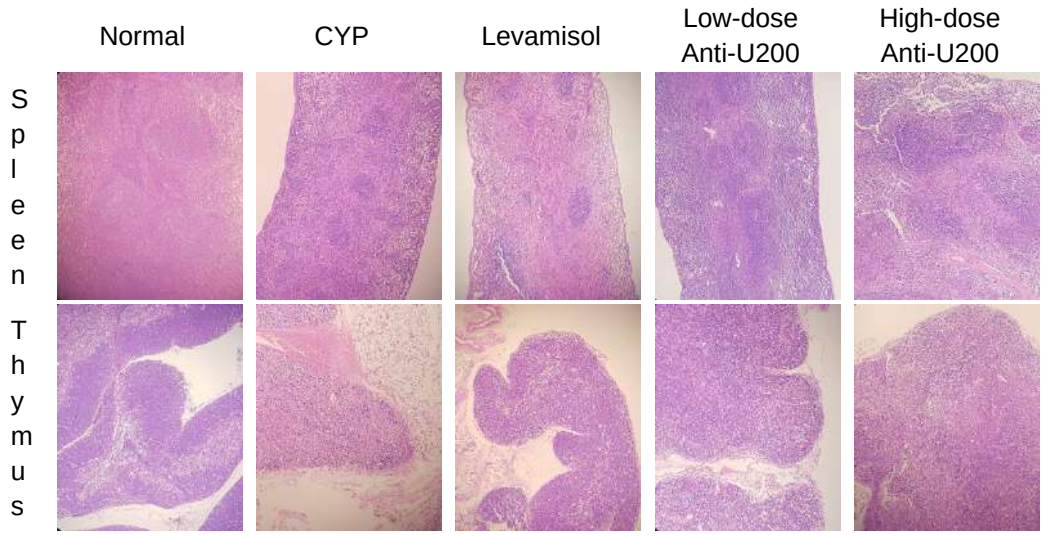


Figure 2. Representative histopathological images of the spleen and thymus

In the present study, the histomorphology of the spleen and thymus by HE staining was examined with an optical microscope (Figure 2). In the spleen, white pulp atrophy was easily observed in the model group, and red pulp and white pulp were intermixed as well when compared with those in the normal group. In the thymus, the clear tissue structures including cortex, medulla, and thymic corpuscle were observed in the normal group, which were much destroyed in the model group. In the model group, we also observed a decrease in the number of splenocytes and thymocytes. Anti-U200 administration significantly inhibited the pathological alterations in the mouse spleen and thymus induced by CYP. Besides, as the positive control, the pathological alteration of the levamisole group was also much rescued, similar to those in the Anti-U200 groups.

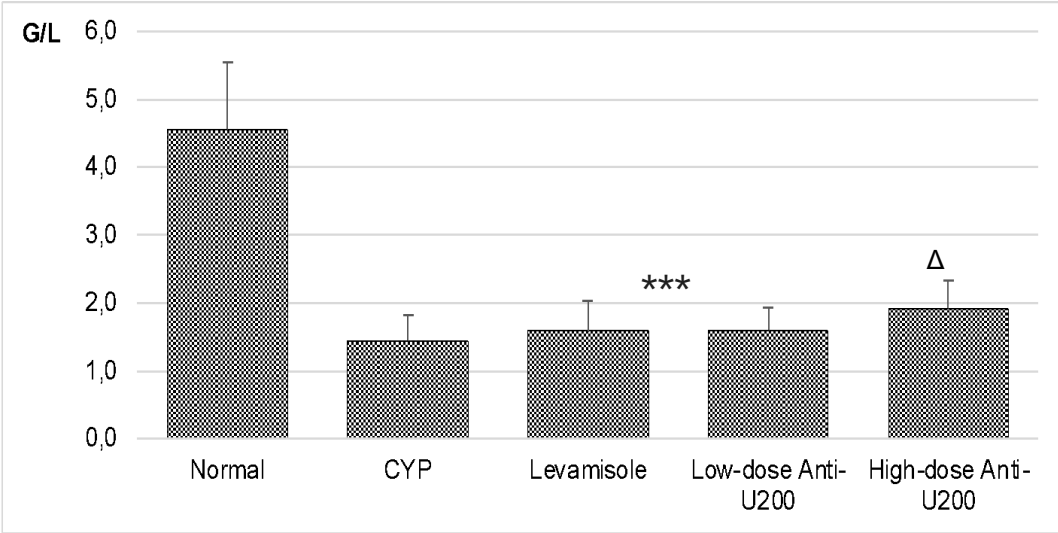
3.2. Effect of Anti-U200 on leukocyte counts

As shown in Table 2 and Figure 3, compared with the normal group, the number of peripheral blood WBCs was significantly decreased in the CYP group. When treated with Anti-U200, the number of peripheral blood WBCs tended to increase, especially in the 5.76 capsules/kg group.

Table 2. Effects of Anti-U200 on the absolute number of peripheral white blood cells

Groups	Lymphocyte (cells/mm^3)	Neutrophil (cells/mm^3)	Monocyte (cells/mm^3)
Normal	2140 ± 610	1840 ± 520	580 ± 170
CYP	700 ± 210***	580 ± 170***	170 ± 50***
Levamisol	720 ± 190	620 ± 200	240 ± 70$^{\Delta}$
Low-dose Anti-U200	940 ± 230$^{\Delta}$	500 ± 100	160 ± 50
High-dose Anti-U200	710 ± 130	930 ± 230$^{\Delta\Delta}$	270 ± 80$^{\Delta\Delta}$

****p<0.001 vs. Normal group; $^{\Delta}$p<0.05, $^{\Delta\Delta}$p<0.01 vs. CYP alone.*



****p<0.001 vs. Normal group*; $^{\Delta}$*p<0.05 vs. CYP alone.*

Figure 3. Effects of Anti-U200 on WBCs

3.3. Effect of Anti-U200 on Delayed-Type Hypersensitivity (DTH) Response

Administration of CYP (200 mg kg−1, i.p) showed a decrease in the DTH response (Table 3). There was a tendency to increase the paw volume in the group treated with Anti-U200 at two studied doses, but there was no significant difference.

Table 3. Effect of Anti-U200 on the swelling degree of footpads

Group	Swelling of foot-pads (%)
Normal	14.36 ± 2.99
CYP	7.74 ± 1,95***
Levamisol	8.32 ± 1.80
Low-dose Anti-U200	10.05 ± 3.07
High-dose Anti-U200	7.90 ± 1.75

****p<0.001 vs. Normal group.*

3.4. Effect of Anti-U200 on serum IgM and TNF-α concentrations

Table 4. Effect of Anti-U200 on serum IgM and TNF-α concentrations

Groups	TNF-α (pg/mL)	IgM (ng/mL)
Normal	30.89 ± 7.08	408638.88 ± 79384.54
CYP	17.10 ± 4.15***	100721.81 ± 19799.21***
Levamisol	25.32 ± 7.10$^{\Delta\Delta}$	127198.58 ± 24118.03$^{\Delta}$
Low-dose Anti-U200	21.63 ± 3.88$^{\Delta}$	103012.33 ± 26255.44
High-dose Anti-U200	28.04 ± 7.19$^{\Delta\Delta}$	108668.64 ± 21020.91

****p<0,001 vs. Normal group;* $^{\Delta}$*p<0.05,* $^{\Delta\Delta}$*p<0.01 vs. CYP alone.*

CYP injection caused a significant reduction of cytokine TNF-α levels in serum. Similarly, we found that the concentrations of IgM in serum were noticeably decreased in the model group when compared with that in the normal control group. As shown

in Table 4, Anti-U200 improved the CYP-induced reduction in the cytokine TNF-α and immunoglobulin (IgM) in the sera, in which, statistically significant differences were observed in TNF-α concentrations increased.

4. DISCUSSION

The immune system plays a role in protecting the body against foreign pathogens and infections, thus playing a role in the body's homeostasis. Regulation of the immune system by stimulation or inhibition helps maintain a disease-free state in an individual [9]. Therefore, immunomodulators have been widely used in medicine to control diseases. This study was conducted to evaluate the immunomodulatory activity of Anti-U200 by evaluating the effects of the test capsules on WBC and differential counts, spleen and thymus weight, delayed-type hypersensitivity response, TNFα, and IgM levels. Immunodeficiency in experimental animals was induced by intraperitoneal injection of a single dose of 200 mg/kg of cyclophosphamide, a potent immunosuppressive agent that can suppress both humoral and cell-mediated immune responses [10].

Monitoring the weight of the spleen and thymus can indirectly assess the immune system's ability to respond to protect the body against antigen invasion. The thymus is the main place where T lymphocytes are concentrated, while the spleen is mainly B lymphocytes [9]. A single intraperitoneal injection of CYP at a dose of 200 mg/kg significantly reduced the relative weight of these two organs, corresponding to the microscopic image of a severe reduction in the size of the white pulp of the spleen and the number of lymphocytes in the thymus, which clearly shows the suppressing effect of CYP on both humoral and cell-mediated immunity. Comparing the relative thymus weight in the Anti-U200 groups with the model group, it can be seen that the test capsules significantly increased the weight of the thymus compared to the model group. There was no change in the relative spleen weight in all Anti-U200 groups compared to the model group (Figure 1). However, microscopic images of the spleen and thymus showed a partial structural improvement of both lymphoid organs in the presence of Anti-U200, specifically an increase in the size of the white pulp of the spleen and the number of lymphocytes of the thymus in the Anti-U200 groups compared to the model group (Figure 2), in other words, the mixed herbal preparation demonstrated initially a stimulating effect on cell proliferation in these lymphoid organs. Corresponding to the efficacy of Anti-U200 on increasing the weight of the above lymphoid organs was a differential increase in the concentration of TNFα ($p < 0.05$), a cytokine representing a cell-mediated immune response originating mainly from T lymphocytes concentrated principally in the thymus, and a tendency to increase the concentration of IgM antibodies ($p > 0.05$) representing humoral immunity originating primarily from B lymphocytes condensed mainly in the spleen, when compared to the model group.

The above-denoted research results suggested that Anti-U200 at doses of 1.92 capsules/kg/day and 5.76 capsules/kg/day treated orally continuously for 7 days exhibited an immune-stimulating effect on CYP-induced immunosuppressive mice by increasing the relative thymus weight, WBCs, TNFα concentration in serum, and at the same time improve microscopic images of spleen and thymus compared to the model group.

Polyherbal formulations are created by combining extracts from different medicinal plants to provide synergistic effects or to heighten desired effects. The modulation of humoral and cellular-mediated immunity in the CYP-induced immunosuppressive model by combined extracts of *Ganoderma lucidum*, *Hedyotis*

diffusa, and *Scutellaria barbata* may suggest a positive interaction between the bioactive components of the three plants, leading to the stimulatory effect. The immunostimulatory effect of combined extracts of *Scutellaria barbata*, *Hedyotis diffusa*, and *Ganoderma lucidum* indicates their potential to be developed into a promising nutraceutical for the modulation of immune responses for the prevention and treatment of various diseases, such as immunodeficiency and cancer. The side effects of biological agent immunotherapy in cancer have been documented in several studies [11]. As a result, there is a tremendous deal of potential to develop the combined plant extracts into a safe and efficient cancer treatment and prevention agent. However, more research is required to explore the molecular mechanisms underlying the synergistic effects to elucidate the immunostimulatory effect of the combined extracts.

5. CONCLUSIONS

This study concluded that polyherbal formulation Anti-U200 exhibited immunomodulatory effects in CYP-induced immunosuppressed mice by improving the relative weight of lymphoid tissues likewise the levels of WBCs and differential. In addition, the extract demonstrated a noteworthy increase in the levels of TNF-alpha and IgM in CYP-injected mice. These findings indicated the potential use of the mixed herbal extract capsule as an alternative immunomodulatory agent in the management of diseases related to immune deficiency.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from the Department of Pharmacology, Hanoi Medical University, and the Center for Research and Early Detection of Cancer (CREDCA), Tri Duc Hospital.

REFERENCES

1. **Nurgali K, Jagoe RT, Abalo R (2018).** Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? Front Pharmacol, 9:245. doi: 10.3389/fphar.2018.00245.
2. **Kurtin S (2012).** Myeloid toxicity of cancer treatment. J Adv Pract Oncol, 3(4):209-224.
3. **Ruiz R, Kirk AD (2015).** Long-Term Toxicity of Immunosuppressive Therapy. Transplantation of the Liver, 2015:1354-1363. doi: 10.1016/B978-1-4557-0268-8.00097-X.
4. **Lee SJ, Chinen J, Kavanaugh A (2010).** Immunomodulator therapy: monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins. J Allergy Clin Immunol, 125(2 Suppl 2):S314-323.
5. **Gong T, Wang CF, Yuan JR, Li Y, Gu JF, Zhao BJ, Zhang L, Jia XB, Feng L, Liu SL (2015).** Inhibition of Tumor Growth and Immunomodulatory Effects of Flavonoids and Scutebarbatines of *Scutellaria barbata* D. Don in Lewis-Bearing C57BL/6 Mice. Evid Based Complement Alternat Med, 2015:630760. doi: 10.1155/2015/630760.
6. **Chen R, He J, Tong X, Tang L, Liu M (2016).** The *Hedyotis diffusa* Willd. (Rubiaceae): A Review on Phytochemistry, Pharmacology, Quality Control and Pharmacokinetics. Molecules, 21(6):710. doi: 10.3390/molecules21060710.
7. **Wu S, Zhang S, Peng B, Tan D, Wu M, et al (2024).** *Ganoderma lucidum*: a comprehensive review of phytochemistry, efficacy, safety and clinical study. Food Science and Human Wellness, 13(2):568-596.
8. **Van Pham AT, Luong MH, Dinh HTT, Mai TP, Trinh QV, Luong LH (2021).** Immunostimulatory Effect of *Moringa oleifera* Extracts on Cyclophosphamide-induced Immunosuppressed Mice. Journal of

Herbs, Spices & Medicinal Plants, 27(4):377-385.

9. **Justiz Vaillant AA, Sabir S, Jan A (2024).** Physiology, Immune Response. [Updated 2024 Jul 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm nih.gov/books/NBK539801/
10. **Ogino MH, Tadi P (2023).** Cyclophosphamide. [Updated 2023 Jul 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553087/.
11. **Rahman MM, Behl T, Islam MR, Alam MN, Islam MM, et al (2022).** Emerging Management Approach for the Adverse Events of Immunotherapy of Cancer. Molecules, 27(12):3798. doi: 10.3390/molecules27123798

SYSTEMIC TOXICITY FROM TOPICALLY APPLIED VIRGIN COCONUT OIL IN BURNED EXPERIMENTAL ANIMALS

Pham Thi Van Anh[1], Bui Thi Huong Thao[1], Nguyen Thi Thanh Loan[1]
[1]Hanoi Medical University
Corresponding author: Nguyen Thi Thanh Loan
Email: nguyenthanhloan@hmu.edu.vn
Received date: 09/09/2024
Reviewed date: 30/09/2024
Accepted date: 30/09/2024

SUMMARY

The healing properties and potential benefits of virgin coconut oil (VCO) for treating burned skin lesions have been demonstrated. Nevertheless, prolonged topical use can lead to systemic effects, especially when applied to open wounds. Currently, the systemic toxicity of topical VCO in burned experimental animals has yet to be fully elucidated. ***Objective****: The present study investigated the systemic toxicity from topically applied VCO in burned experimental animals.* ***Methods:*** *All animals, except the normal control group, were subjected to thermal burns on the back of each rat by using a standard burning technique. Following the burning, each animal was placed in a separate cage, and the affected areas were covered with VCO* 0.2 or 0.4 ml/day *twice a day for 21 consecutive days. Hematological and biochemical analyses were determined. Macroscopic and histopathological examinations* of the liver and kidney were conducte*d at the end of the treatment period.* ***Results:*** *Our results indicated that topical administration of VCO caused no significant change in animals' general status, hematological parameters, and renal and hepatic functions. Additionally, it did not alter the liver and kidney histology in animals.* ***Conclusion:*** *These findings suggest that the topical application of VCO was considered safe for the wounded animals.*

Keywords*: Virgin coconut oil, burn, systemic toxicity, experimental animals.*

1. INTRODUCTION

The skin, the most significant human organ, is the primary mechanical barrier between the body and the external environment. It protects against harmful agents, regulates temperature, and maintains water and electrolyte balance [1]. According to the World Health Organization, millions of people with pathological wounds require medical attention each year. Currently, there are several treatments for skin injuries, comprising surgical procedures, non-surgical therapies, and pharmacologic agents, with costs of $12 billion annually [2]. Virgin coconut oil (VCO) is a natural oil extracted from the mature kernel of the coconut fruit through different mechanical and natural methods. VCO has been traditionally used as a moisturizer for centuries in the tropical regions. It offers numerous health benefits due to its retained vitamins, antioxidants, and antimicrobial and antiviral properties [3].

We have demonstrated the beneficial effects and potentials of VCO in terms of healing effects in burned skin lesions [4]. However, long-term topical application can also have systemic effects, mainly when applied to open wounds [5]. Additionally, evaluating the possible toxic effects of herbal products, including toxicity tests in experimental animal models, is essential before conducting clinical studies in humans. To date, the systemic toxicity of topical VCO in burned experimental animals has not been fully elucidated. Thus, the present study investigated the systemic toxicity from topically applied VCO in burned experimental animals.

2. MATERIALS AND METHODS

2.1. Preparation of VCO

VCO was produced by Vi Dieu Nam Medicine and Pharmacy Limited Company, reaching the manufacturer's standard. VCO was made by cold-pressing the liquid from the solid endosperm of mature coconut (*Cocos nucifera* L.). The 120 ml bottle of VCO adheres to ISO 22000:2018 standards.

2.2. Experimental animals

Healthy *Wistar albino* rats of either sex, weighing 180±20 gam, were used. Animals were acclimatized to the laboratory conditions (constant temperature of 24±1^{o}C, with a 12-light/dark cycle) for seven days before the study commenced. All experiments, as well as animal care and handling, followed the relevant guidelines and regulations.

2.3. Evaluation of systemic toxicity of topical administration of VCO in burned animals

The 30 rats were randomly divided into three groups of 10 animals. The rats were anesthetized with a single intraperitoneal injection of 250 mg/kg chloralhydrate. As preparation, they were shaven at the dorsum with an electric shaver and later sterilized with 70% alcohol. All animals, except the normal control group, were subjected to thermal burns on the back of each rat by using a standard burning technique [6]. Burn wounds were formed by pressurelessly applying a 200-gram cylindrical stainless-steel rod (2.5 cm diameter), which was pre-heated to 100°C in boiling water with the thermal equilibrium confirmed by a monitoring thermometer, onto the shaven skin for 35 seconds. All animals were resuscitated immediately with Lactated Ringer's solution (2 ml/100 g body weight) intraperitoneally. Following the burning, each animal was placed in a separate cage, and the affected areas were covered with VCO 0.2 or 0.4 ml/day twice a day for 21 days. The vehicle-treated burned rats topically received sterile distilled water.

The signs and parameters were checked during the study:

- General conditions, body weight changes;
- Evaluation of hematological parameters: red blood cell count, hemoglobin, hematocrit, total white blood cells (WBC), and platelet count;
- Evaluation of liver damage through aspartate aminotransferase level (AST) and alanine aminotransferase level (ALT);
- Evaluation of liver function through total bilirubin, albumin, and total cholesterol;
- Evaluation of kidney function through creatinine level.

Follow-up parameters were checked at the time points before, after 10 and 21 days of applying VCO in thermal burn rats. At the end of the experiment, rats were euthanized after blood collection, and the internal organs (heart, liver, spleen, kidney, and lungs) were removed and observed for any gross lesions. The liver and kidney of 30% of the animals in each group were preserved in a 10% buffered formaldehyde solution for histopathological studies. The sections were stained with hematoxylin and eosin (H&E) and checked for histopathological assessment by the blinded researcher.

2.5. Statistical analysis

Sigmaplot 12.0 (SYSTA Software Inc, Richmond, CA, USA) was used for statistical analysis. Obtained data were expressed as the mean ± S.D and compared with either one-way-ANOVA followed by the post hoc Student-Newman-Keuls test for multiple comparisons. Statistically significant differences were considered when the p-value was less than 0.05.

2.6. Research location

The research was conducted at the Department of Pharmacology, Hanoi Medical University.

3. RESULTS

3.1. General condition and body weight changes

During the experiment, animals in all groups had normal locomotor activities, good feedings, agility, skin, fur colors, bright eyes, and dry stools. As shown in Figure 1, after 10 and 21 days of treatment, the body weight of treated rats increased compared to before treatment ($p<0.05$). There was no significant difference between the treated and control groups ($p>0.05$).

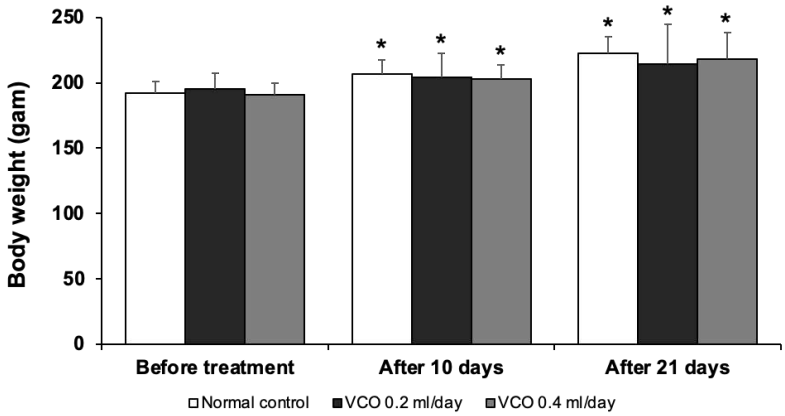


Figure 1. The effect of VCO on body weight changes

*p<0.05, **p<0.01, ***p<0.001: as compared with the time point "Before treatment"

3.2. Effect of VCO on hematological parameters

There were no significant differences in red blood cell count, hematocrit, hemoglobin level, or mean corpuscular volume between the VCO-treated groups and the normal control group ($p>0.05$) (Table 1).

Table 1. The effect of VCO on red blood cell count, hematocrit, hemoglobin level, mean corpuscular volume

Parameters	Group	Before treatment (1)	After treatment	
			10 days (2)	21 days (3)
Red blood cells count (T/L)	Normal control (a)	10.59 ± 0.87	11.18 ± 0.65	11.06 ± 0.96
	VCO 0.2 ml/day (b)	10.25 ± 0.80	10.86 ± 0.91	10.42 ± 0.87
	VCO 0.4 ml/day (c)	11.42 ± 1.37	11.35 ± 1.57	10.54 ± 1.37
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Hemoglobin (g/dl)	Normal control (a)	12.47 ± 1.25	13.23 ± 1.30	13.07 ± 1.13
	VCO 0.2 ml/day (b)	12.39 ± 0.98	13.42 ± 1.14	12.63 ± 1.41
	VCO 0.4 ml/day (c)	13.39 ± 1.65	13.68 ± 1.21	12.73 ± 1.76
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Hematocrit (%)	Normal control (a)	52.51 ± 8.14	55.26 ± 7.50	57.09 ± 8.92
	VCO 0.2 ml/day (b)	51.51 ± 4.60	54.48 ± 4.90	51.90 ± 4.75
	VCO 0.4 ml/day (c)	56.66 ± 7.53	57.26 ± 5.66	53.42 ± 7.35
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Mean corpuscular volume (fL)	Normal control (a)	51.70 ± 2.11	52.60 ± 1.51	52.30 ± 1.25
	VCO 0.2 ml/day (b)	51.10 ± 1.10	51.60 ± 1.35	51.00 ± 2.21
	VCO 0.4 ml/day (c)	49.90 ± 2.69	50.80 ± 2.86	50.50 ± 3.44
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			

As shown in Figure 2, there were no significant differences in total WBC count and platelet count between the VCO-treated groups and the control group (p>0.05) (shown in Fig. 2).

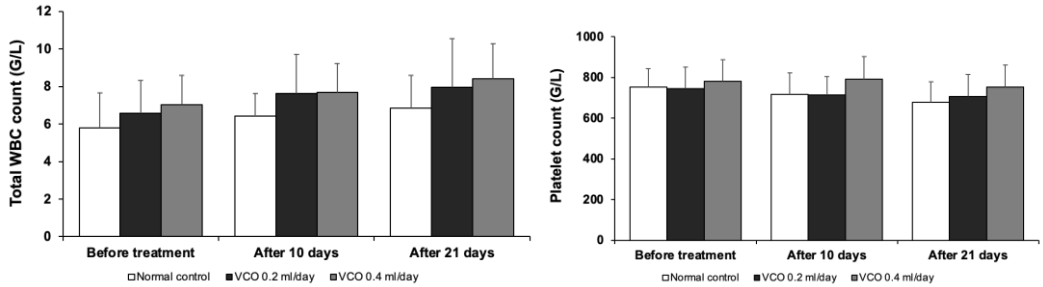


Figure 2. The effect of VCO on total WBC and platelet count

3.3. Effect of VCO on liver damage

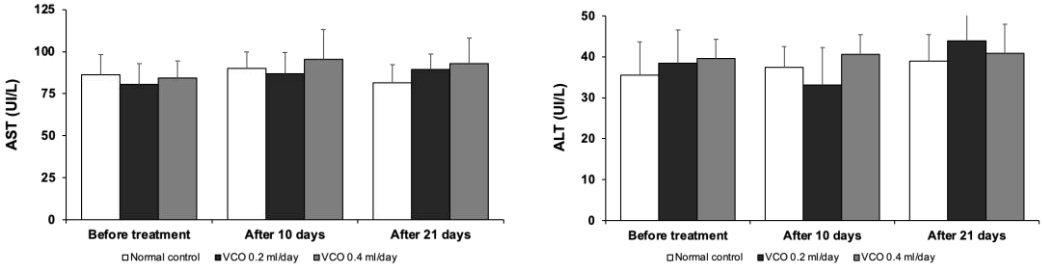


Figure 3. Effect of VCO on AST and ALT level

Figure 3 demonstrates that after 10 and 21 days of treatment, VCO at doses of 0.2 ml/day and 0.4 ml/day did not cause statistical differences in AST and ALT levels when comparing the treated groups to the control group (p>0.05).

3.4. Effect of VCO on liver function

Table 2 illustrates that after 10 and 21 days of treatment, there was no statistical difference in total bilirubin, albumin, and total cholesterol concentration in the all-treated groups compared to the control group (p>0.05).

Table 2. The effect of VCO on liver function

Parameters	Group	Before treatment (1)	After treatment	
			10 days (2)	21 days (3)
Total bilirubin (mmol/l)	Normal control (a)	8.65 ± 0.52	8.74 ± 0.65	9.04 ± 0.57
	VCO 0.2 ml/day (b)	8.73 ± 0.55	9.23 ± 0.60	9.30 ± 0.75
	VCO 0.4 ml/day (c)	8.54 ± 0.47	9.01 ± 0.90	9.11 ± 0.87
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Albumin concentration (g/dl)	Normal control (a)	3.17 ± 0.33	3.30 ± 0.21	3.46 ± 0.28
	VCO 0.2 ml/day (b)	3.18 ± 0.26	3.11 ± 0.34	3.24 ± 0.31
	VCO 0.4 ml/day (c)	3.30 ± 0.21	3.12 ± 0.19	3.27 ± 0.29
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Total cholesterol concentration (mmol/l)	Normal control (a)	1.40 ± 0.16	1.34 ± 0.20	1.37 ± 0.17
	VCO 0.2 ml/day (b)	1.44 ± 0.14	1.36 ± 0.15	1.42 ± 0.10
	VCO 0.4 ml/day (c)	1.46 ± 0.11	1.42 ± 0.13	1.36 ± 0.14
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			

3.5. Effect of VCO on kidney function

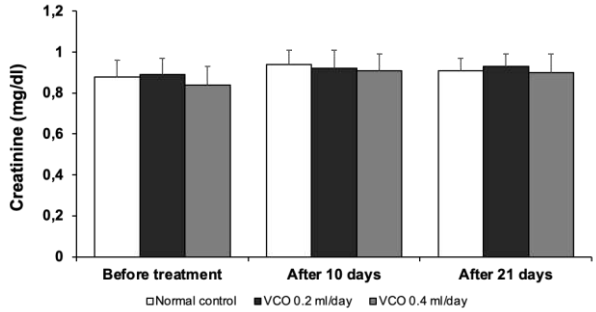


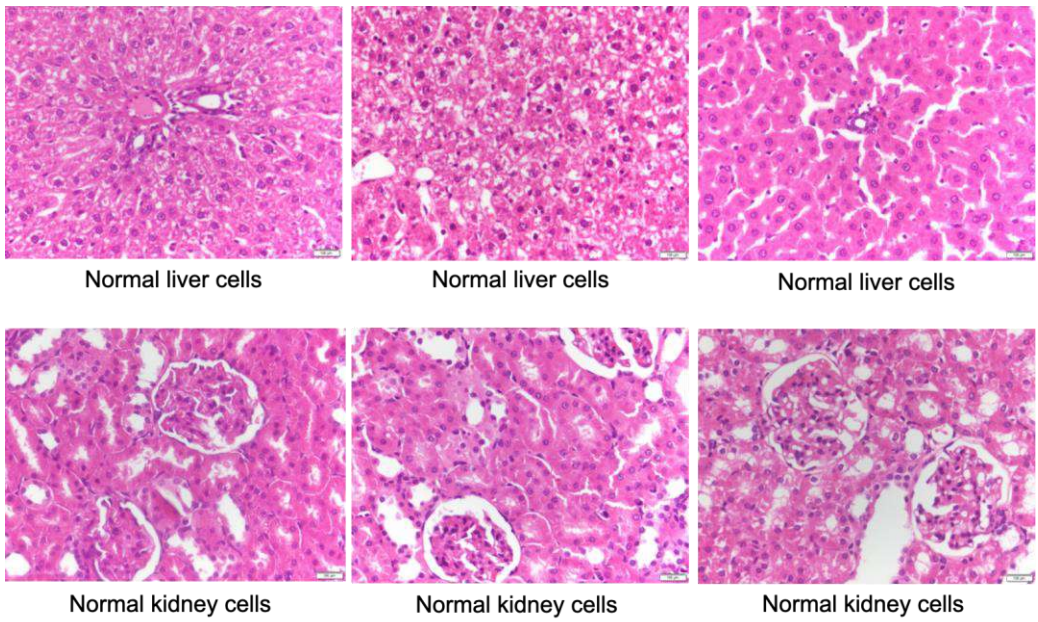
Figure 4. Effect of VCO on creatinine level

Figure 4 demonstrates that after 10 and 21 days of treatment, VCO at doses of 0.2 and 0.4 ml/day did not cause a statistical difference in creatinine levels between the treated and the control groups ($p>0.05$).

3.6. Histopathological examination

No gross lesions or changes in size were observed when all experimental rats were subjected to a complete gross necropsy, which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There were no significant differences between VCO-treated rats and the control group in histopathological examinations of livers and kidneys.



Normal control (rat #02, HE x 400) **VCO 0.2 ml/day** (rat #13, HE x 400) **VCO 0.4 ml/day** (rat #28, HE x 400)

Figure 5. Histopathological morphology of liver and kidney

4. DISCUSSION

VCO was used in traditional medicine based on theories, beliefs, and experiences. We have demonstrated the potential of topical VCO in terms of healing effects in burned skin lesions [4]. However, long-term topical application can also affect the systemic effects, mainly applied to open wounds [5]. Currently, there is no adequate data about the safety of VCO. In this study, we evaluated the systemic toxicity after applying topical VCO on thermal burns. Overall, the findings of this study indicated that topical administration of VCO caused

no significant change in the general status, hematological parameters, or renal and hepatic functions. Additionally, they did not alter the liver and kidney histology in animals.

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is essential to choose the correct system since no effects may otherwise be seen [7]. The toxicity study provides information on the undesirable effects of continuous or repeated exposure to plant extracts or compounds over a portion of the average life span of experimental animals [8]. Regarding the assessment of repeated-dose toxicity tests, they were performed to investigate the general conditions (body weight, appearance, and behavior) and the potential toxic effects on the animals' hematology, biochemical parameters, and histopathology.

The changes in body weight are the most basic index to reflect the general health status of animals and the combined effects of xenobiotics on the body [8]. Our study observed weight gains in all animals topically administered with VCO. In addition, none of the *Wistar* animals in all treated groups showed any macroscopic or gross pathological changes compared to the control group. It can be stated that VCO did not interfere with the normal metabolism of animals, as corroborated by the non-significant difference in the control group. The World Health Organization identifies hematological parameters as sensitive and reliable indicators for assessing the toxicity of herbal extracts [8]. The blood circulatory system performs essential functions. The hematopoietic system is one of the most sensitive targets of toxic compounds and is a critical parameter in animal physiological and pathological status [9]. Furthermore, analyzing changes in the hematological system is essential for risk evaluation, as these changes can provide valuable predictions about human toxicity. Our results showed that, after 21 days of treatment, there were no significant differences in total red blood cells, hematocrit, hemoglobin level, platelet count, and total WBC count between the VCO-treated groups and the normal control group. All parameters were within the normal reference range of the species [10]. Thus, it can be concluded that the VCO does not affect the hematological system.

Additionally, analysis of the liver is critical in the toxicity evaluation of drug tests as it is necessary for the survival of an organism. The biochemistry analyses evaluated the possible alterations in hepatic function influenced by the product [11]. Total bilirubin, albumin, and total cholesterol are valuable indices of the excretory function of the liver. Besides, ALT and AST are valuable indices for identifying inflammation and necrosis of the liver. Accordingly, the liver releases AST and ALT, and an elevation in plasma concentration indicates liver damage. Moreover, the kidneys are the body's excretory organs, and renal parenchyma cells are highly vulnerable to endogenous and exogenous substances. When a compound is introduced into the body, it can induce toxicity and damage the kidneys, affecting kidney function. Assessment of renal function after taking a drug is usually achieved by a quantitative blood creatinine test [12]. Creatinine is the most stable protein in the blood, almost independent of diet or physiological changes but only dependent on the ability of the kidneys to excrete it. When the glomeruli are damaged, blood creatinine levels rise earlier than urea. Blood creatinine is a more reliable and essential indicator than blood urea, which is currently used to assess and monitor kidney function [13]. Our results demonstrated that the blood biochemistry levels of VCO-treated groups presented no significant differences compared to the control group. This evidence shows that VCO did not affect liver and kidney functions. Furthermore, histopathological

examination of the liver and kidney of the control and all treated groups did not reveal any morphological differences. This is consistent with the results of biochemical parameters in VCO-treated groups.

Our findings showed that VCO caused no significant differences in general conditions, hematological parameters, liver and kidney function tests, and histopathological examination compared to untreated rats. A previous study evaluated VCO's safety in some Southeast Asian countries. The acute oral toxicity was assessed at the dose of 5000 mg/kg, and the sub-chronic and chronic oral toxicity was evaluated at the doses of 175, 550, and 2000 mg/kg [14]. The results revealed that the treated rats were safe with the research doses. Overall, our results showed the safety of topical VCO in burned animals.

5. CONCLUSION

VCO at doses of 0.2 and 0.4 ml/kg/day did not cause detrimental effects on the body weight, hematological and biochemical parameters, as well as macromorphology and micromorphology of the livers and kidneys of the treated animals compared to the control group.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from the Department of Pharmacology, Hanoi Medical University.

REFERENCES

1. **Ahmad, Z, Hasham, R, Nor, NA, et al (2015).** Physico-chemical and antioxidant analysis of virgin coconut oil using West African tall variety, Journal of advanced research in materials science. 13(1), 1-10.
2. **Monavarian, M, Kader, S, Moeinzadeh, S, et al (2019).** Regenerative scar-free skin wound healing, Tissue Engineering Part B: Reviews. 25(4), 294-311.
3. **Mansor, T, Che Man, Y, Shuhaimi, M, et al (2012).** Physicochemical properties of Virgin Coconut Oil extracted from different processing methods, International Food Research Journal. 19(3).
4. **Nguyen Dinh Ninh, VDL, Pham Thi Van Anh et al. (2024).** The burn wound healing property of Dau dua Lao nha que on experimental thermal burns, Journal of Medical Research.
5. **Alikhan, FS,Maibach, H (2011).** Topical absorption and systemic toxicity, Cutaneous and Ocular Toxicology. 30(3), 175-186.
6. **Durmus, A, Han, MC,Yaman, I (2009).** Comperative evaluation of collagenase and silver sulfadiazine on burned wound healing in rats, Firat Universitesi Saglik Bilimleri Veteriner Dergisi. 23(3), 135-139.
7. **Saganuwan, S (2017).** Toxicity studies of drugs and chemicals in animals: an overview, Bulgarian Journal of Veterinary Medicine. 20(4).
8. **Organization, WH (2000).** Working group on the safety and efficacy of herbal medicine, Report of regional office for the western pacific of the World Health Organization. 200.
9. **Etim, NN, Williams, ME, Akpabio, U, et al (2014).** Haematological parameters and factors affecting their values, Agricultural science. 2(1), 37-47.
10. **Vigneshwar, R, Arivuchelvan, A, Mekala, P, et al (2021).** Sex-specific reference intervals for Wistar albino rats: hematology and clinical biochemistry, Indian Journal of Animal Health. 60(1), 58-65.
11. **Olson, H, Betton, G, Robinson, D, et al (2000).** Concordance of the toxicity of pharmaceuticals in humans and in animals, Regulatory toxicology and pharmacology. 32(1), 56-67.
12. **Grenier, N, Merville, P,Combe, C (2016).** Radiologic imaging of the renal parenchyma structure and function,

Nature Reviews Nephrology. 12(6), 348-359.

13. **Moore, JF,Sharer, JD (2017).** Methods for quantitative creatinine determination, Current protocols in human genetics. 93(1),A.3O.1-A.3O. 7.
14. **Ibrahim, AH, Khan, MSS, Al-Rawi, SS, et al (2016).** Safety assessment of widely used fermented virgin coconut oil (Cocos nucifera) in Malaysia: Chronic toxicity studies and SAR analysis of the active components, Regulatory Toxicology and Pharmacology. 81, 457-467.

NUTRITIONAL STATUS AND SOME RELATED FACTORS IN CHILDREN UNDER 24 MONTHS AT THE GENERAL CLINIC OF HOA BINH PROVINCIAL CENTER FOR DISEASES CONTROL 2023-2024

To Thi Quyen[1], Phan Thi Minh Ngoc[2], Pham Van Phu[2]
[1]Hoa Binh Provincial Center for Diseases Control
[2]Hanoi Medical University
Corresponding author: To Thi Quyen
Email: quyenhbskss@gmail.com
Received date: 02/09/2024
Reviewed date: 30/09/2024
Accepted date: 30/09/2024

SUMMARY

Introduction: *During the first two years of life, children have a high demand for essential nutrients that support physical growth and the gradual maturation of organ systems. Hoa Binh is a mountainous province in the Northwest region of Vietnam, characterized by complex terrain and difficult transportation.* ***Objectives:*** *1. To assess the nutritional status of children under 24 months at the General Clinic of the Center for Disease Control, Hoa Binh Province, in 2023-2024. 2.To describe some factors related to the nutritional status of children under 24 months at the General Clinic of the Center for Disease Control, Hoa Binh Province, in 2023-2024.* ***Methods:*** *Cross-sectional descriptive study on Children under 24 months of age who visit the clinic or vaccination center in the study area and their moms.* ***Results:*** *The prevalence of underweight, stuning and wasting were 16.1, 13.8 and 8.7%, respectively. Birth weight less than 2500 grams, children older than 6 months old, not vaccinated enough for their age, and not breastfed have a statistically significant higher rate of underweight malnutrition. Maternal age over 35 and no breastfeeding early after birth have a statistically significant higher rate of stunting. Families with more than two children, those who are not fully vaccinated according to age, and those who do not breastfeed are groups with a significantly higher rate of wasting.* ***Conclusion:*** *The prevalence of malnutrition of study subjects was still high, some related factors of each type were showed. May be there is an urgent need to implement good interventions to improve children health.*

Keywords: *nutritional status, children, under 24 months, Hoa Binh provincial center for diseases control.*

1. INTRODUCTION

Nutrition at each stage of life has significant impacts on a child's development, from the fetal stage to adulthood. During the first two years of life, children have a high demand for essential nutrients that support physical growth and the gradual maturation of organ systems. Proper nutrition during the first 1,000 days of life is recognized as one of the key practices for preventing malnutrition in children. If children are not properly and adequately nourished, it can lead to malnutrition, affecting their physical, mental, and intellectual development, and resulting in severe consequences for society. [1],[3]

In Vietnam, according to statistics from the National Institute of Nutrition, the prevalence of underweight malnutrition among children under five years old in 2010 was 17.5%, with grade I malnutrition accounting for the highest proportion at 15.4%. The prevalence of stunting malnutrition was 29.3%. By 2018, the prevalence of underweight malnutrition had decreased to 12.8%, with grade I malnutrition comprising 11.4%, and the prevalence of stunting malnutrition stood at 23.2%. Malnutrition is not only caused by a

lack of food resources, poor socioeconomic conditions, and inadequate healthcare, but also by a lack of knowledge among mothers and family members, as well as inappropriate and unscientific child-rearing practices. [2],[3]

Hoa Binh is a mountainous province in the Northwest region of Vietnam, characterized by complex terrain and difficult transportation. It is home to many ethnic minorities. At the same time, Hoa Binh is undergoing industrialization and modernization, with active industrial zones and a significant proportion of women of reproductive age. To contribute to the nutritional care of children under 24 months in Hoa Binh, we conducted this study with two objectives: (1) To assess the nutritional status of children under 24 months at the General Clinic of the Center for Disease Control, Hoa Binh Province, in 2023-2024. And (2) To describe some factors related to the nutritional status of children under 24 months at the General Clinic of the Center for Disease Control, Hoa Binh Province, in 2023-2024.

2. METHODS

2.1. Study Subjects

- Children under 24 months of age who visit the clinic or vaccination center in the study area.
- Mothers with children under 24 months of age (mothers of selected children) who are present in the area during the study period.

2.2. Study Design: Cross-sectional descriptive study.

2.3. Research Tools:

- Length board for measuring children under 2 years of age.
- SECA scale provided by UNICEF.
- Interview questionnaire.

2.4. Data Analysis Method

- WHO Anthro 2006 software was used to calculate children's Z-Scores.
- Data analysis was conducted using SPSS software version 18.0.

2.5. Research Ethics

- Mothers caring for children were fully informed and clearly explained the objectives and procedures of the study. Families had the right to decline participation. Children were only included in the study with the consent of their parents.
- The study solely aimed to assess nutritional status and did not impact on the health of the children.
- During data collection, participants had the right to withdraw from the study at any time.

3. RESULTS AND DISCUSSION

3.1. Nutritional status of children

Table 1. Information on average weight and average height distributed by age group and gender (n=298)

		Age group (month)			
		0 – 5 (n=120)	**6 – 11 (n=89)**	**12 – 17 (n=57)**	**18 – 23 (n=32)**
Weight (kg)	Male	6.1 ± 1.4	7.7 ± 1.1	9.5 ± 1.9	10.4 ± 2.1
	Female	6.0 ± 1.2	7.3 ± 1.4	8.3 ± 1.4	9.7 ± 1.1
	General	6.1 ± 1.3	7.5 ± 1.3	9.1 ± 1.8	10.2 ± 1.8
	p	0.708	0.046**	0.012**	0.748
Height (cm)	Male	59.9 ± 4.1	67.9 ± 2.9	75.1 ± 2.5	82.0 ± 3.8
	Female	58.4 ± 3.3	65.7 ± 3.9	72.8 ± 3.5	81.1 ± 3.1
	General	59.4 ± 3.9	67.0 ± 3.5	74.3 ± 3.1	81.7 ± 3.5
	p	0.05*	0.003*	0.005*	0.520

*: t – test independent **: Mann-Whitney

Table 1 shows that, at the age of under 18 months, the height of male children is statistically higher than that of female children; at the ages of 6-11 months

and 12-17 months, the weight of male children is higher. statistically significant than female children. These indicators all tend to be lower than the average level of children in the world [11]. This could be attributed to Hoa Binh being a mountainous province with underdeveloped economic and social conditions, as well as a lower educational level, which contribute to certain limitations in childcare and upbringing.

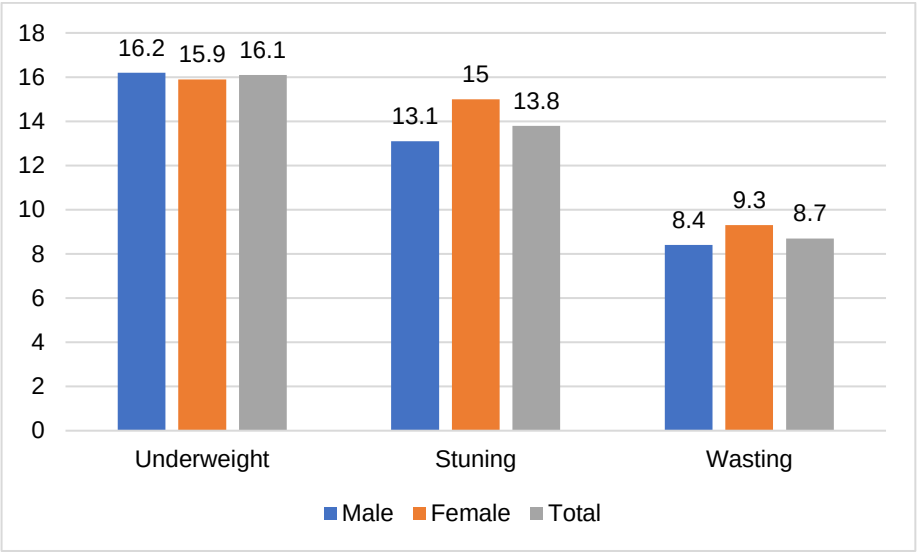


Figure 1. Malnutrition rate by type and gender (n=298)

Figure 1 shows that the most common form of malnutrition is underweight, and the least common is wasting. In 2016, according to estimates by WHO, UNICEF and the World Bank, from 1990 to 2014, malnutrition in children under 5 years old has decreased significantly: stunting malnutrition decreased from 39.6% to 23%, 8% (from 255 million children to 159 million children); Underweight malnutrition decreased from 25.0% to 14.3% (from 160.5 million children to 95.5 million children). Regarding wasting (acute malnutrition), in 2014 there were 50 million children (7.5%) worldwide, with 16 million children suffering from severe wasting, of which 67% of children in this group lived in Asian countries and 28% live in African countries [12],[13]. The obtained results were lower than the prevalence of malnutrition in children aged under 5 years admitted to the Pediatric Departmentof the National Lung Hospital in Hanoi, Vietnam, from August to December 2018, which was 30.1%, 25.7%, and 17.8% respectively for underweight, stunting and wasting [10].

The highest prevalence of wasting occurs in young children (6–23 months). The prevalence of stunting and wasting among children aged 6–24 months were 58.1 and 17.0%, respectively [9]: The prevalence of stunting, wasting, and underweight was 20.1%, 4.3%, and 8.0%, respectively in Children living in Roma settlements in Central and Eastern Europe. Malnutrition is an important indicator of child health. A significant contributing factor to infant and child mortality, poor nutritional status during childhood also has implications for adult economic achievement and health. [8]

3.2. Some factors related to children's nutritional status

3.2.1. Some factors related to underweight children

Table 2. Some factors related to underweight (n=298)

Nutrition status	Underweight	OR (95%CI)	p

Factor		Yes (n, %)	No (n, %)		
Birth weight	≥ 2500gr	39 (14.5%)	230 (85.5%)	2.7 (1.1 – 6.7)	0.037*
	< 2500gr	9 (31.0%)	20 (69.0%)		
Age in months	< 6 months	7 (5.8%)	113 (94.2%)	3.0 (1.2 – 7.6)	0.019*
	≥ 6 months	41 (23.0%)	137 (77.0%)		
Vaccination	Enough	20 (9.7%)	186 (90.3%)	2.6 (1.3 – 5.4)	0.007*
	Not enough	28 (30.4%)	64 (69.6%)		
Breast feeding	Yes	35 (13.7%)	221 (86.3%)	2.9 (1.3 – 6.1)	0.007*
	No	13 (31.0%)	29 (69.0%)		

*: Multivariable logistic regression

Table 2 shows that birth weight less than 2500 grams, children older than 6 months old, not vaccinated enough for their age, and not breastfed have a statistically significant higher rate of underweight malnutrition. Underweight refers to having a low weight for one's age. It can be a result of wasting, stunting, or both. In some Southeast Asian countries, particularly in rural and impoverished areas, the rate of underweight children remains high, although there has been some progress in recent years. Underweight children may either be wasted, stunted, or both [12],[13]. Underweight is the most dependable growth indicator for overall child growth. Tanzania has the highest rate of underweight children in East Africa, with 1.27 million children under the age of five suffering from the condition. A secondary analysis on a sample of 4,327 children aged 0–23 months in Tanzania showed significantly higher risks of underweight were found for children born with a lower birth weight compared to other children born with an average or higher than average weight. Children in the age group of 18–23 months were significantly more likely to be underweight compared with other groups. This could be attributed to increased physical activity at this age when children can walk and run, as well as a decrease in mothers' care for these children due to the assumption that they are already grown up. Therefore, more training and counseling for mothers/child caretakers on proper and consistent feeding of all children under the age of 2 years when they attend postnatal clinics is needed to guarantee that children acquire weight (0.5 kg per month) as suggested by WHO. [6]

3.2.2. Some factors related to stunting children

Table 3. Some factors related to stunting (n=298)

Nutrition status / Fator		Stuning		OR (95%CI)	p
		Yes (n, %)	No (n, %)		
Mother's age	18 - 35	33 (11.8%)	246 (88.2%)	7.9 (2.4 – 26.2)	0.001*
	> 35	8 (42.1%)	11 (57.9%)		
Early breastfed	Yes	21 (11.3%)	165 (88.7%)	2.7 (1.2 – 6.1)	0.020*
	No	20 (17.9%)	92 (82.1%)		

*: Multivariable logistic regression

Table 3 shows that maternal age over 35 and no breastfeeding early after birth have a statistically significant higher rate of stunting. Stunting occurs when a person has a low height for their age. It is caused by prolonged periods of insufficient nutrition, usually beginning in early childhood or even before birth due to poor maternal nutrition. This is one of the most prevalent forms of malnutrition in Southeast Asia. In 2022, 22.3% of children under five globally were stunted [12],[13]. Stunting is a result of chronic undernutrition and often begins in early childhood or even during

pregnancy, affecting long-term growth and development. According to nutritionists' recommendations, the baby should be breastfed within the first hour after birth to help reduce the risk of malnutrition [4]. Stunting is a severe public health problem in the predominantly rural northwest Ethiopia. Mother's occupation, postnatal vitamin-A supplementation, source of family food and household wealth status were identified as determinants of severe stunting in Ethiopia. The prevalence of stunting among children aged 6–24 months were 58.1% [95% CI; 50.3, 65.9%]. Besides, the burden of stunting among children aged 12–24 months was 44.6, while it was 13.5 in infants aged 6–11 months [9].

The discrepancy could be explained by the depth of the studies in that the latter reports were national with larger number of children. The multivariate logistic regression analysis detected that child age, maternal postnatal vitamin-A supplementation, latrine availability, main source of family food and household wealth status were remained significantly and independently associated with stunting. [7].

3.2.3. Some factors related to wasting in children

Table 4. Some factors related to wasting (n=298)

Factor	Nutrition status	Wasting		OR (95%CI)	p
		Yes (n, %)	No (n, %)		
Number of children in their family	≤ 2	19 (7.3%)	243 (92.7%)	19.0 (1.9 – 188.5)	0.012*
	> 2	7 (19.4%)	29 (80.6%)		
Vaccination	Enough	10 (4.9%)	196 (95.1%)	2.6 (1.0 – 6.4)	0.043*
	Not enough	16 (17.4%)	76 (82.6%)		
Breastfed	Yes	16 (6.2%)	240 (93.8%)	5.0 (2.0 – 12.3)	0.000*
	No	10 (23.8%)	32 (76.2%)		

*Multivariate Logistic Regression

Table 4 shows that families with more than two children, those who are not fully vaccinated according to age, and those who do not breastfeed are groups with a significantly higher rate of wasting. Wasting occurs when a person has a low weight for their height and is often the result of acute food shortages or severe illness. As an indicator of acute malnutrition, wasting affected approximately 13.7 million children globally in 2022 [12],[13]. Southeast Asia has countries with some of the highest rates of wasting, indicating recent or severe weight loss, often due to insufficient food intake or repeated infections. For children under 12 months of age, breast milk is the most valuable food that no artificial food can compare to. Many studies have shown that the rates of malnutrition and respiratory infections or diarrhea are significantly higher in children whose mothers have insufficient breast milk. Children need careful attention regarding hygiene, expanded vaccination, growth monitoring, love, education, and proper nutritional care when ill, such as during diarrhea or respiratory infections. The condition of having many children in a family may also limit the care provided, leading to potential malnutrition in children. Bivariate and multivariate analyses indicated that only diarrheal morbidity remained significantly and independently associated with wasting [7]. A study to estimate the prevalence of wasting in children aged 6-23 months in the Sahel region of Burkina Faso and to identify its associated factors. a total of 956 children participated in the study. The prevalence of wasting was 25% in the Sahel region. Only 24.37% of children received a minimum meal frequency and 13.38% received a minimum dietary diversification the day

before the survey. In the multivariate analysis, being male, breastfeeding the day before the survey and having a history of illness significantly increased the risk of acute malnutrition [5]. The prevalence of wasting among children aged 6–24 months were 17.0% [95% CI; 11.1, 22.9%]. Besides, the burden of wasting among children aged 12–24 months was 11.6%, while it was 5.4% in infants aged 6–11 months [9]. The discrepancy could be explained by the depth of the studies in that the latter reports were national with larger number of children. May be there is an urgent need to implement good interventions to improve children health.

4. CONCLUSION

The height and weight of children under 24 months at the General Clinic of the Hoa Binh Provincial Center for Disease Control in 2023-2024 are lower than global standards. For children under 18 months, the height of male children is statistically significantly higher than that of female children. In the age groups of 6-11 months and 12-17 months, the weight of male children is also statistically significantly higher than that of female children.

Several factors related to the nutritional status of children under 24 months at the General Clinic of the Hoa Binh Provincial Center for Diseases Control in 2023-2024 include: (1) Birth weight under 2500 grams, children older than 6 months, not receiving full vaccinations according to age, and not breastfeeding have a high rate of underweight malnutrition. (2) Mothers older than 35 and early breastfeeding after birth have a high rate of stunted malnutrition. (3) Families with more than two children, those who are not fully vaccinated according to age, and those who do not breastfeed are groups with a high rate of wasting.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from General Clinic of the Center for Disease Control, Hoa Binh Province.

REFERENCES

1. Phạm Văn Hoan. Cải thiện kiến thức, thực hành của người chăm sóc và tình trạng dinh dưỡng trẻ em thông qua can thiệp khả thi tại vùng khó khăn tỉnh Quảng Bình. Tạp Chí Dinh Dưỡng Và Thực Phẩm. 2008;4(2):33-39.
2. Đinh Thanh Huề. Tình hình suy dinh dưỡng ở trẻ dưới 5 tuổi người dân tộc thiểu số huyện Bắc Trà My, tỉnh Quảng Nam năm 2009. Tạp Chí Y Học Thực Hành. 2009;6(666):50-52.
3. Hà Huy Khôi và Nguyễn Công Khẩn (2010). Tính thời sự của suy dinh dưỡng thể thấp còi và hỗ trợ gia tăng tăng trưởng ở người Việt Nam. Tạp chí Dinh dưỡng và Thực phẩm, 4(1), 3-7.
4. Hoàng Thị Liên. Nghiên cứu tình hình suy dinh dưỡng trẻ dưới 5 tuổi sau 5 năm thực hiện chương trình phòng chống suy dinh dưỡng tại xã Thủy Phù. Luận án chuyên khoa II. Đại học Y Dược Huế; 2005. (2022).
5. Boyo Constant Paré, Désiré Lucien Dahourou, Ahmed Kabore et al, Prevalence of wasting and associated factors among 6 to 23 months old children in the Sahel Region of Burkina Faso, Pan African Medical Journal, Vol. 34 No. 1 (2019)
6. Cypriana Cyprian Moshi, Penina Joseph Sebastian, Devotha Gabriel Mushumbusi, et al, Determinants of underweight among children aged 0–23 months in Tanzania, Food Science and Nutrition, Volume10, Issue4, April 2022, Pages 1167-1174
7. Derso, T., Tariku, A., Biks, G.A. et al. Stunting, wasting and associated factors among children aged 6–24 months in Dabat health and demographic surveillance system site: A community based cross-sectional study in Ethiopia. BMC Pediatr 17, 96 (2017). https://doi.org/10.1186/s12887-017-0848-2 (WASTING) STUNING2.

8. Janevic T, Petrovic O, Bjelic I, Kubera A. Risk factors for childhood malnutrition in Roma settlements in Serbia. BMC Public Health. 2010;10(1):509. doi:10.1186/1471-2458-10-509.
9. Tariku, A., Biks, G.A., Derso, T. et al. Stunting and its determinant factors among children aged 6–59 months in Ethiopia. Ital J Pediatr 43, 112 (2017).https://doi.org/10.1186/s13052-017-0433-1 (STUNING).
10. Trần CQ, NGHIEM NT, Trần QB, Nguyễn TN, Lê DT. MALNUTRITION IN PEDIATRIC PATIENTS WITH LOW RESPIRATORY TRACT INFECTIONS AT NATIONAL LUNG HOSPITAL, VIETNAM. Tạp chí Dinh dưỡng và Thực phẩm. 2022;18(2E):44-53. doi:10.56283/1859-0381/105.
11. https://www.who.int/tools/child-growth-standards/standards/weight-for-age
12.https://www.who.int/data/gho/data/themes/topics/joint-child-malnutrition-estimates-unicef-who-wb
13. https://www.who.int/news/item/31-03-2020-unicef-who-wb-jme-group-new-data

THE EFFECTIVENESS OF COMBINED GROUP TASK THERAPY IN MOTOR REHABILITATION FOR PATIENTS WITH ISCHEMIC STROKE AT SON TAY GENERAL HOSPITAL, HA NOI

Pham Van Minh[1,2], Nguyen Thi Hoai Thu[3], Phan Thi Kieu Loan[1,2]
[1]Hanoi Rehabilitation Hospital
[2]Hanoi Medical University
[3]Son Tay General Hospital, Hanoi
Corresponding author: **Pham Van Minh**
Email: pvminhrehab@yahoo.com
Received date: 21/09/2024
Reviewed date: 28/09/2024
Accepted date: 30/09/2024

SUMMARY

Objective: *To evaluate the effectiveness of combined group task therapy in motor rehabilitation for patients with ischemic stroke.* ***Methods:*** *Interventional study, comparing before and after treatment on 32 patients with ischemic stroke treated at Son Tay General Hospital, Ha Noi from May 2024 to October 2024. Rehabilitation intervention includes physical therapy: 20 minutes/day, occupational therapy: 20 minutes/day combined with group task therapy for balance and walking 60 minutes/day, 05 sessions/week, evaluated after 4 weeks of treatment.* ***Results:*** *After 4 weeks of treatment, there was an improvement in balance ability (BBS), Berg score increased by 19 ± 4 points (p<0.001). There was an improvement between the time to stand and walk (TUG) before and after the intervention of 8.51 ± 3.84 seconds (p<0.001). Improvement in walking speed (10MWT) increased by 0.26 ± 0.11 m/s (p<0.01). Improvement in distance traveled in the 2-minute walk test (2MWT) increased to an average of 41.32 ± 0.75 m (p<0.001).* ***Conclusion:*** *Intervention of motor rehabilitation combined with group task therapy in 32 patients with ischemic stroke at Son Tay General Hospital after 4 weeks of intervention, we found an improvement in BBS score, standing up and walking time before, and after TUG intervention. After intervention, patients had improved 10 m walking speed and improved distance traveled in the 2-minute walking test.*

Keywords: *Ischemic stroke, group task therapy, rehabilitation.*

I. INTRODUCTION

According to the World Health Organization, 15 million people worldwide suffer from strokes annually, of which 5 million die, and another 5 million are permanently disabled. The reduction in stroke mortality rates has led to an increase in the number of patients with motor disabilities after discharge[1]. Globally, many scientists have studied stroke, especially regarding rehabilitation for hemiplegic patients, confirming that this remains a significant challenge for healthcare systems.

Rehabilitation is an essential and urgent need for all types of disabilities, particularly post-stroke hemiplegia, to minimize sequelae and help individuals regain independence. For stroke patients and their families, the ability to achieve independence in daily living activities is a primary concern. Restoring mobility is particularly important, as it is often crucial for daily activities and increases the likelihood of discharge.

Researchers have observed positive

cortical reorganization in both animals and humans post-stroke, stimulated by activity and repetitive task-specific actions. Moreover, studies show that motor recovery after stroke is best facilitated through intensive, task-specific therapy.

Providing task-oriented therapy to stroke patients in group settings has been proposed as a way to increase the time patients actively participate in task practice.

In Vietnam, numerous studies have been conducted on motor rehabilitation for hemiplegic stroke patients; however, group task training in rehabilitation remains a novel approach with limited research. For these reasons, we conducted the study, *"The Effectiveness of combined Group Task Therapy in Motor Rehabilitation for Patients with Ischemic stroke at Son Tay General Hospital, Hanoi."*

II. STUDY SUBJECTS AND METHODS

2.1. Study Subjects

2.1.1. Inclusion Criteria

- Patients are diagnosed with ischemic stroke.
- Patients with sufficient ability to participate in group task-oriented therapy:
    - Ability to follow instructions.
    - The ability to sit without support.
    - Ability to stand with the assistance of one person.
- Patients without cognitive impairment as assessed by the Mini-Mental State Examination (MMSE) (score ≥ 24).
- Consent from both patients and their families to participate in the study.

2.1.2. Exclusion Criteria

- Patients with cerebellar lesions.
- A history of any neurological disorders (except for prior strokes).
- Regular use of mobility aids (except for single-point canes) or reliance on personal assistance before the stroke.
- Currently experiencing trauma or recovering from orthopedic surgery on the lower limbs.
- Severe visual impairment.
- Development of new brain lesions during the treatment period.

2.2. Research Methods

2.2.1. Study design

An interventional, prospective study without a control group.

2.2.3. Sample Size and Sampling

A total of 32 patients meeting the inclusion criteria were admitted simultaneously and divided into groups of 4 patients per group, resulting in 8 patient groups.

2.2.4. Study Location and Duration

- **Location**: Son Tay General Hospital, Hanoi.
- **Duration**: From April 2024 to September 2024.

2.2.5. Rehabilitation Intervention Details

- Physical therapy: 20 minutes/day.
- Occupational therapy: 20 minutes/day.
- Group task therapy: 60 minutes/day.

Group Task Therapy: Patients participated in rehabilitation sessions 5 days/week, with 60 minutes/day over 4 weeks. The exercises focused on balance and gait rehabilitation, including:

- Strengthening exercises for the lower limb muscles and balance.
- Sit-to-stand practice and walking exercises.
- Postural control exercises while standing.
- Exercise for the upper limbs and hands.

2.2.6. Research Outcome Evaluation

- **Balance assessment**: Using the Berg Balance Scale (BBS) for scoring and evaluation.
- **Timed Up and Go (TUG) Test**: For scoring and outcome evaluation.
- **10-Meter Walk Test (10MWT)**: For

scoring and outcome evaluation.

- **2-Minute Walk Test (2MWT)**: For scoring and outcome evaluation.

2.2.7. Data Processing

- Data was collected based on study-specific medical records and processed using medical statistical methods with SPSS 20.0 software.
- Study results were considered statistically significant at p<0.05.

III. RESEARCH RESULTS

Table 1. Characteristics of Study Subjects by Age Group

Age Group	n	Rate (%)
<60	4	12.5%
60 -69	16	50.0%
≥ 70	12	37.5%
Total	32	100%
Mean ± standard deviation	69.50 ± 8.92	100%

The most common age group experiencing strokes was 60–69 years, with 16 patients (50.0%). The group aged ≥70 years included 12 patients (37.5%), while the least affected was the group aged <60 years, with 4 patients (12.5%).

3.1.2. Characteristics of Study Subjects by Gender

Female patients accounted for most of the study group, making up 65.6%. Male patients were fewer, comprising 34.4% of the total participants.

3.2. Effectiveness of Group Task-Oriented Therapy in Rehabilitation

Table 2. Balance Ability Based on the Berg Balance Scale (BBS)

Subject	BBS score	p
Pre	24 ± 7 (8-38)	0.000
Post	43 ± 6 (23-52)	
Δ	19 ± 4	

The BBS score improvement among patients was 19 points. The improvement in BBS scores was statistically significant with p<0.001.

Table 3. Movement Time Using the Timed Up and Go (TUG) Test

Subject	TUG (second)	p
Pre	20.37 ± 6.32	0.000
Post	10.84 ± 3.39	
Δ	8.51 ± 3.84	

A significant difference was observed in the time taken to stand up and move before and after the intervention (p<0.001). The average improvement after the intervention was 8.51 ± 3.84 seconds (p<0.01).

Table 4. Walking Speed Using the 10-Meter Walk Test (10MWT)

Subject	10MWT (m/s)	p
Pre	0.33 ± 0.11	0.001
Post	0.59 ± 0.22	
Δ	0.26 ± 0.11	

After the intervention, patients showed an improvement in walking speed, increasing by 0.26 ± 0.11 m/s. This improvement was statistically significant with p<0.01.

Table 5. Walking Distance Using the 2-Minute Walk Test (2MWT)

Subject	2MWT (m)	p
Pre	43.81 ± 19.18	0.000
Post	85.13 ± 19.93	
Δ	41.32 ± 0.75	

The walking distance during the 2-minute test increased by an average of 41.32 ± 0.75m for the study group. This improvement was statistically significant ($p<0.001$).

IV. DISCUSSION

4.1. General Characteristics of Study Subjects

4.1.1. Age Characteristics

According to Table 3.1, the average age of study subjects was 69.50 ± 8.92 years. Patients aged 70 and older accounted for 37.5%, followed by those aged 60–69 (50%) and those under (12.5%). These findings align with the study by Edzie et al. (2021) [2] on 840 first-time stroke patients, which reported an average age of 62.45 ± 14.45 years.

Our study is consistent with previous research and aligns with statistics from the Centers for Disease Control and Prevention (CDC), which indicate that 3 out of 4 stroke cases occur in individuals aged over 65.

4.1.2. Gender Characteristics

In our study, female patients accounted for 65.6%, while males comprised 34.4%. According to several authors, gender differences in stroke epidemiology depend on patient age, as the impact of gender on stroke risk and outcomes changes over a lifetime. According to Cámana et al. (2020), in subjects under 75 years, stroke incidence is higher in men, but this pattern reverses in those over 75 years[3].

The overall lifetime risk for stroke is calculated as 1.6 and 1.5 times higher in men than women, respectively. This difference can be explained by factors such as higher rates of hypertension in younger male patients and lifestyle habits such as smoking and alcohol consumption. Meanwhile, women tend to live longer and have unique risk factors, including contraceptive use, pregnancy history, and menopause. However, when considering first-time stroke patients, the gender distribution shows no significant difference.

4.2. Outcomes of Rehabilitation on Mobility and Balance

4.2.1. Improvement in Balance Evaluated Using the Berg Balance Scale (BBS)

According to Table 3.2, the pre-intervention BBS score was 24 ± 7 points. After four weeks of intervention, the score improved to 43 ± 6 points, with a difference of 19 ± 4 points ($p<0.05$). The BBS is a validated and reliable scale for assessing balance, particularly in stroke patients.

Our study showed a 19-point improvement in BBS scores after group task-oriented therapy. This result is consistent with the findings of Nguyễn Thanh Duy et al. (2022) [4], who studied 54 post-stroke patients and observed significant BBS score improvements in the group receiving task-oriented therapy compared to a control group ($p<0.001$). This suggests that group therapy is at least as effective, if not more so, than conventional rehabilitation in improving balance.

4.2.2. Reduction in Fall Risk Assessed Using the TUG Test

As shown in Table 3.3, the average time to complete the TUG test decreased from 20.37 ± 6.32 seconds pre-intervention to 10.84 ± 3.39 seconds post-intervention, with a significant reduction of 8.51 ± 3.84 seconds ($p<0.05$).

This result aligns with Ain et al. (2018) [5], who studied the effectiveness of group task therapy on gait and mobility in 30 participants. Over six weeks, the TUG scores improved from 26.01 ± 8.5 seconds

to 20.87 ± 8.02 seconds, with the group therapy participants showing more significant improvement ($p<0.05$).

The improved TUG performance in our study is likely due to repeated practice of transitions, such as moving from sitting to standing and walking, which were emphasized more in the group therapy sessions than in conventional rehabilitation.

4.2.3. Improvement in Speed and Time in the 10-Meter Walk Test

According to Table 3.4, the average walking speed increased from 0.33 ± 0.11 m/s pre-intervention to 0.59 ± 0.22 m/s post-intervention, with a significant improvement of 0.26 ± 0.11 m/s. Dean et al. (2000) [6] similarly observed improvements in walking speed at four weeks and two months post-intervention ($p<0.05$).

This result is consistent with the meta-analysis by English et al. (2017)[7], which included 8 studies with 744 participants, demonstrating significant improvements in walking speed with task-oriented group interventions. Thus, group therapy effectively enhances walking speed compared to conventional rehabilitation, likely due to the increased practice time during group sessions.

4.2.4. Improvement in Endurance Using the 2-Minute Walk Test

According to Table 3.5, the average walking distance in the 2MWT increased from 43.81 ± 19.18 m to 85.13 ± 19.93 m ($p<0.05$). This aligns with Song et al. (2015) [8], who reported significant differences in the 2MWT between groups ($p<0.001$). In their study, the group receiving task-oriented therapy improved from 57.6 ± 20.5 m to 73.9 ± 27.2 m, while the conventional therapy group showed a decline from 76.6 ± 33.1 m to 74.1 ± 27.00 m. Our findings align with previous research, demonstrating that group task-oriented therapy is more effective in improving walking endurance than conventional rehabilitation.

V. CONCLUSION

After four weeks of combined motor rehabilitation and group task therapy for 32 ischemic stroke patients at Son Tay General Hospital, significant improvements were observed in BBS scores, TUG test times, 10-meter walking speeds, and 2-minute walking distances.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from Son Tay General Hospital, Hanoi City, Vietnam.

REFERENCES

1. **Yen HC, Jeng JS, Chen WS et al.** Early Mobilization of Mild-Moderate Intracerebral Hemorrhage Patients in a Stroke Center: A Randomized Controlled Trial. *Neurorehabil Neural Repair*. 2020;34(1):72-81.
2. **Edzie EKM, Gorleku PN, Dzefi-Tettey K et al.** Incidence rate and age of onset of first stroke from CT scan examinations in Cape Coast metropolis. *Heliyon*. 2021;7(2):e06214. doi:10.1016/j.heliyon.2021.e06214
3. **Soto-Cámara R, González-Bernal JJ, González-Santos J et al.** Age-Related Risk Factors at the First Stroke Event. *J Clin Med*. 2020;9(7):2233. doi:10.3390/jcm9072233
4. **Nguyễn Thanh Duy, Hoàng Thị Huyền Trang.** Hiệu quả của chương trình tập luyện xoay vòng so với tập thường quy trên khả năng thăng bằng và di chuyển của người bệnh đột quỵ. *Hội nghị khoa học vật lý trị liệu Việt Nam lần thứ 1*. Published online 2022.
5. **Ain QU, Malik AN, Haq U, Ali S.** Effect of task specific circuit training on gait parameters and mobility in stroke survivors. *Pak J Med Sci*. 2018;34(5). doi:10.12669/pjms.345.15006
6. **Dean CM, Richards CL, Malouin F**. Task-related circuit training improves performance of locomotor tasks in chronic stroke: A randomized, controlled pilot trial. *Archives of Physical Medicine and Rehabilitation*. 2000;81(4):409-417. doi:10.1053/mr.2000.3839.

7. English CK, Hillier SL, Stiller KR, Warden-Flood A. Circuit class therapy versus individual physiotherapy sessions during inpatient stroke rehabilitation: a controlled trial. *Arch Phys Med Rehabil*. 2007;88(8):955-963.

8. Song HS, Kim JY, Park SD. The effect of class-based task-oriented circuit training on the self-satisfaction of patients with chronic stroke. *J Phys Ther Sci*. 2015;27(1):127-129. doi:10.1589/jpts.27.127.