ASSESSING THE SAFETY PROFILE OF POLYMERS IN TRANSDERMAL MICRONEEDLE PATCH FABRICATION: INSIGHTS FROM AN ACUTE TOXICITY STUDY

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The acute toxicity study is a crucial component of preclinical toxicology assessments conducted to evaluate the potential adverse effects of a substance or compound on albino rabbits. This study provides key findings and implications of an acute toxicity study of natural and synthetic polymers that are used in the preparation of transdermal microneedle patches (TMNPs) for sustained delivery of doxazocin mesylate. In this study, we divided the albino rabbits into three equal groups (n = 6). Group-I was labeled as a control group and group-II was treated with TMNPs-1 (HPMC, maltose) and group-III was treated with TMNPs-2 (PLGA, polyvinyl acetate). The results obtained from feed consumption, hematology profile, biochemical studies, vital organ weight, and histopathological changes in vital organs were compared with the control group, and proved the safety profile of the polymers involved in the fabrication of microneedle patches. Mechanical strength and dermal safety study established the facts about the safety of microneedle patches and potential risks to health as a carrier system for the delivery of drugs into the systemic circulation directly.

Keywords: acute toxicity, dissolvable microneedles patch, hydroxypropyl methylcellulose, maltose, safety profile, histopathology

INTRODUCTION

A transdermal drug delivery system (TDDS) is a novel approach to delivering medications/active pharmaceutical ingredients through the skin directly into the systemic circulation.[1-3] Gels, creams, and patches are the typical formulations that are available in the market to deliver drugs into the bloodstream.[4-7] TDDS takes advantage of the skin's ability to absorb certain substances. The skin consists of several layers, with the outermost layer known as the *stratum corneum*. Serving as a primary barrier, the *stratum corneum* plays a crucial role in preventing the entry of foreign substances into the body.[8] So, most of the drugs are unable to penetrate through the skin. We can overcome this problem with penetration enhancers like microneedles, iontophoresis, azone *etc.*[9-12] Microneedles are miniature, needle-like structures typically measuring less than 1 millimeter in length.[13-15] They are designed to puncture the outermost layer of the skin, known as the *stratum corneum*, in a minimally invasive manner.[16] Transdermal delivery offers several

advantages over other routes of drug administration. It provides a controlled release of medication over an extended period, reduces the need for frequent dosing, avoids first-pass metabolism in the liver, and allows for improved patient compliance and convenience.[17-19]

An acute toxicity study is a type of laboratory investigation conducted to assess the adverse effects that a substance or product may cause after a single or short-term exposure.[20-22] It is important to evaluate the safety of various substances, including those used in microneedle patches. Microneedle patches are a type of transdermal drug delivery system that consists of tiny needles that painlessly penetrate the skin's outermost layer to deliver medication or other substances. The study aims to determine the toxicity levels of a substance and identify any potential immediate harmful effects it may have on living organisms, such as animals (rabbits), and follow the OECD (Organization for Economic Co-operation and Development) guidelines.[23-26] The results of the acute toxicity study are documented and analyzed to assess the substance's toxic effects and determine its safety profile.[27-30] These findings are often presented in toxicological reports and may be submitted to regulatory authorities to comply with safety regulations and guidelines.[31]

Various polymers have been used for the fabrication of dissolvable microneedle patches to deliver drugs into the systemic circulation directly.[32-35] In this study, we fabricated doxazocin mesylate loaded dissolving microneedle patches with various natural and synthetic polymers, *e.g.* hydroxypropyl methylcellulose (HPMC), polyvinyl acetate, polylactic-co-glycolic-acid (PLGA), with different feed ratios that are required for the fabrication of microneedle patches. We used the solvent casting technique for the development of microneedle patches and determined their safety profile, which confirmed the safety of the carrier system that is used for the delivery of drugs, and also conducted stability studies. The findings of the acute toxicity study contribute to the overall hazard assessment of the substance and inform regarding subsequent safety evaluations.

EXPERIMENTAL

Materials

Hydroxypropyl methylcellulose (HPMC), polyvinyl acetate, polylactic-co-glycolic-acid (PLGA), and maltose were gifted by Remoxin Enterprises. A silicon mold was purchased from Micropoint Technologies Singapore. Distilled water was prepared in the lab of the University of Lahore.

Methods

Preparation of natural and synthetic polymer-based microneedle patches

Transdermal microneedle patches (TMNPs) were fabricated with the solvent casting method. To begin, we prepared a homogenous solution of each polymer, including 3% hydroxypropyl methylcellulose (HPMC), 15% maltose, 10% polylactic-co-glycolic-acid (PLGA) and 10% polyvinyl acetate, separately. Then, these prepared solutions were combined, mixed and labeled as Solution A and B. Solution A consisted of 3% HPMC and 15% maltose, while Solution B comprised 10% PLGA and 10% polyvinyl acetate. Next, each solution was poured separately into silicon molds and subjected to sonication for 2 h. Afterward, the solutions were left to dry at room temperature for 48 h. Once dried, the patches were carefully peeled from the mold and stored for future use. Figure 1 depicts a schematic presentation, illustrating the preparation process of transdermal microneedle patches.

Study animals

Nine male (1820 g to 1920 g) and nine female (1180 g to 1250 g) albino rabbits were taken from the animal house of the University of Lahore, Pakistan. All the albino rabbits were housed in clean cages in three groups (n = 6) separately under light/dark cycles of 12 h, at controlled humidity (55-65 ±5%) and temperature (23-26 °C ±2 °C). The rabbits were acclimatized for one week and had sufficient water/food supply. The animals used in this study were approved by the Institutional Research Ethics Committee (IREC) of the University of Lahore, Lahore, Pakistan, bearing letter no. DPH/22/FOP/2786, dated March 2, 2022.

Experimental design

Transdermal microneedle patches (TMNPs) were evaluated for the acute toxicity study as per OECD guidelines. A total of 18 male and female albino rabbits were divided into three groups (n = 6, 3 females, and 3 males/group) randomly. Group-I was labeled as control and other groups (G-II-III) were subjected to the treatment with different selected polymers, as mentioned in Table 1.

Body weight, clinical signs and food consumption

The body weight of all the albino rabbits was recorded on day 1 before treatment, day 7 during treatment, and after treatment on day 14 with the test sample before sacrifice. All the albino rabbits were closely monitored throughout the study for any changes concerning clinical signs and morbidity/mortality rate. The total feed consumed was determined by subtracting the water and food given to

the cages and their respective remnants. The obtained results were compared for the identification of any changes in the control group.

Hematology study

Hematology studies involve the analysis of blood samples to assess various parameters related to the cellular components of blood. These studies provide important information about the overall health and functioning of the blood cells. Blood samples were collected from the albino rabbits using appropriate techniques. Commonly used methods include venipuncture (drawing blood from a vein) from a marginal ear vein. The samples were collected and stored in an anticoagulant vial using sterile procedures to maintain sample integrity on days 0 and 14 of the study. Complete blood count, including RBCs, WBCs, Hb, and platelets, was determined by using an automated hematology analyzer manufactured by Sysmex Corporation, Japan, and the obtained results were compared with the control group to determine any change.

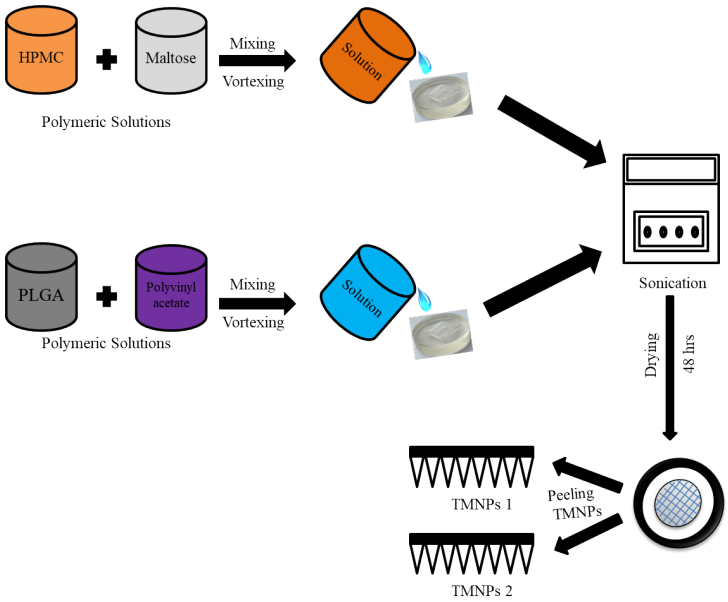

Figure 1: Layout plan for the preparation of microneedle patches

Table 1
Experimental design

Group	Drug concentration	Number	
		Male	Female
G-I	Control	3	3
G-II	3% HPMC and 15% maltose	3	3
G-III	10% PLGA and 10% polyvinyl acetate	3	3

Biochemical examination

Biochemical examination of blood involves analyzing various biochemical parameters to assess the functioning of organs, metabolic processes, and overall health status. The collected blood samples were processed to separate the serum or plasma from the cellular components. This is typically done by allowing the blood to clot and then centrifuging the sample to separate the liquid portion (serum or plasma) from the solid components (red blood cells, white blood cells, and platelets). The separated serum or plasma was then analyzed for various biochemical parameters, such as ALT, AST and creatinine, using an automated chemistry analyzer AU680 manufactured by Beckman Coulter, USA. The obtained results were recorded and analyzed to interpret the findings by comparing them with the control group to identify any deviation.

Urine analysis

Urine analysis, also known as urinalysis, involved the examination of urine on days 0 and 14 of the test to assess various parameters related to kidney function, metabolic processes, and overall health. Urine was collected into the jar and a refractometer was used to measure the specific gravity of urine. Specific gravity indicates the concentration of solutes in the urine and reflects the kidneys' ability to concentrate urine

properly. Test strips, also known as dipsticks, were dipped into the urine sample, and the color changes on the strip were observed. These strips contain various chemical reagents that react with specific substances in the urine, providing information about different parameters, such as pH, *etc*.

Effect of polymeric TMNPs on animal organ weight

Determining organ weights in an acute toxicity study involves the measurement of individual organ weight in animals following exposure to a substance. At the end of the 14 days, the animals were euthanized using approved methods, following ethical considerations and regulations. The animal's body was carefully dissected to access and organs of interest (liver, kidneys, heart, lungs, spleen, and brain) were removed. They were carefully cleaned to remove any excess blood or surrounding tissues. The organs were then blotted or gently dried to remove surface moisture. Each organ was weighed separately using a weighing balance and the weight of each organ was recorded in grams. The results were compared to control values to identify any significant changes or abnormalities in organ weights induced by the substance exposure.

Histopathological assessment

Histopathological examinations in an acute toxicity study involve the microscopic examination of tissue samples from various organs to assess any structural or cellular changes induced by exposure to a substance. At the end of the 14 days, the collected tissue samples were placed in fixative solution formalin, to preserve their structure and prevent degradation. The fixative helps maintain the cellular and tissue architecture for subsequent histological examination. After fixation, the tissue samples were dehydrated, cleared, and embedded in a solid medium such as paraffin wax. The processed tissue samples were then sectioned into thin slices, usually 4-6 micrometers thick, using a microtome. The tissue sections were mounted onto glass slides and treated with hematoxylin and eosin (H&E) staining technique to enhance tissue visibility and highlight specific cellular components or structures. The prepared slides were examined under a microscope to evaluate the cellular and tissue morphology, looking for any abnormal findings, such as tissue damage, inflammation, necrosis, hypertrophy, hyperplasia, or other pathological changes. The observations were recorded and compared with the control group.

Dermal safety

The rabbits were healthy and free from any pre-existing skin conditions or abnormalities. The flank region of the rabbits was selected and the fur in the test area was carefully removed with hair removal cream (EU cream) to expose the skin for application of the microneedle patches. The microneedle patches were placed firmly onto the skin surface, ensuring good contact with the prepared test area of the rabbit skin separately. After 48 h of the application period, the microneedle patches were removed from the skin and observed for immediate signs of irritation, such as redness, swelling, edema, or other visible changes. The observations were recorded and graded according to the Draize scoring system.

Mechanical strength

This test measures the force required to break the microneedles, which further confirms the mechanical strength of the needles required to penetrate the skin. A universal testing machine was used to determine the mechanical strength of the patch. In this machine, a fixture was used to hold the microneedle patch sample. The fixture allowed accurate alignment and positioning of the microneedles during the test. Next, we applied a constant rate of force or displacement to the microneedles until the microneedles broke. The force applied during the test is measured and compared with the literature. The moisture content was assessed utilizing a moisture analyzer. Additionally, the thickness of the prepared patches was measured using a digital micrometer (Mitutoyo, Japan) at multiple locations.

Statistical analysis

Each test was performed in triplicate and mean ± S.D was calculated. Graph Pad Prism software was applied and kept the level of significance at $p < 0.5$.

RESULTS AND DISCUSSION

Body weight, clinical signs and food consumption

By closely monitoring the body weight, clinical signs, and food consumption, researchers can detect early signs of toxicity, evaluate the severity of adverse effects, and assess the potential risks associated with the tested substance. These parameters provide important insights into the substance's overall toxicity profile, potential systemic effects, and its impact on physiological well-being. Monitoring changes in body weight helps assess the overall well-being and potential toxicity of the substance being tested. Significant weight loss or gain can indicate adverse effects on growth, metabolism, or organ function. Changes in body weight can also serve as an indicator of the substance's impact on appetite, digestion, or nutrient absorption (Fig. 2). Observing and recording clinical signs, such as changes in behavior, physical appearance, or physiological functions, provides valuable information about the substance's toxicity.

Monitoring food consumption helps evaluate the substance's impact on appetite and the potential for alterations in nutritional intake. Decreased food consumption could suggest adverse effects on palatability, taste aversion, or gastrointestinal disturbances caused by the substance. It is important to assess any potential changes in food consumption to understand if the substance affects the animals' feeding behavior or overall health. There were no significant changes in the body weight, feed consumption, and clinical signs of albino rabbits after 14 days of treatment.

Hematology study

The obtained results were recorded and analyzed by comparing them with those of the control group and identifying any deviations from normal values. Abnormalities in the blood cell parameters can provide important diagnostic information, indicating various conditions, such as anemia, infection, inflammation, or blood disorders. However, no changes were observed in the hematology profile of tested albino rabbits, as compared to the control group, on days 0 and 14, which confirmed the safety profile of these polymers and the absence of toxicity (Fig. 3).

Biochemical examination

Abnormalities in the biochemical parameters can provide diagnostic information about organ dysfunction, metabolic disorders, or specific diseases. There were no remarkable differences in creatinine, ALT, and AST of treated albino rabbits when compared with the control group on days 0 and 14 (Fig. 4). This indicates that the polymers used in the fabrication of microneedle patches are safe and have no toxicity in the chemistry profile.

Urine analysis

Specific gravity, urine color and pH of the urine were observed and compared with those of the control group to identify any abnormalities or potential health issues. There was no significant difference in the parameters of urine.

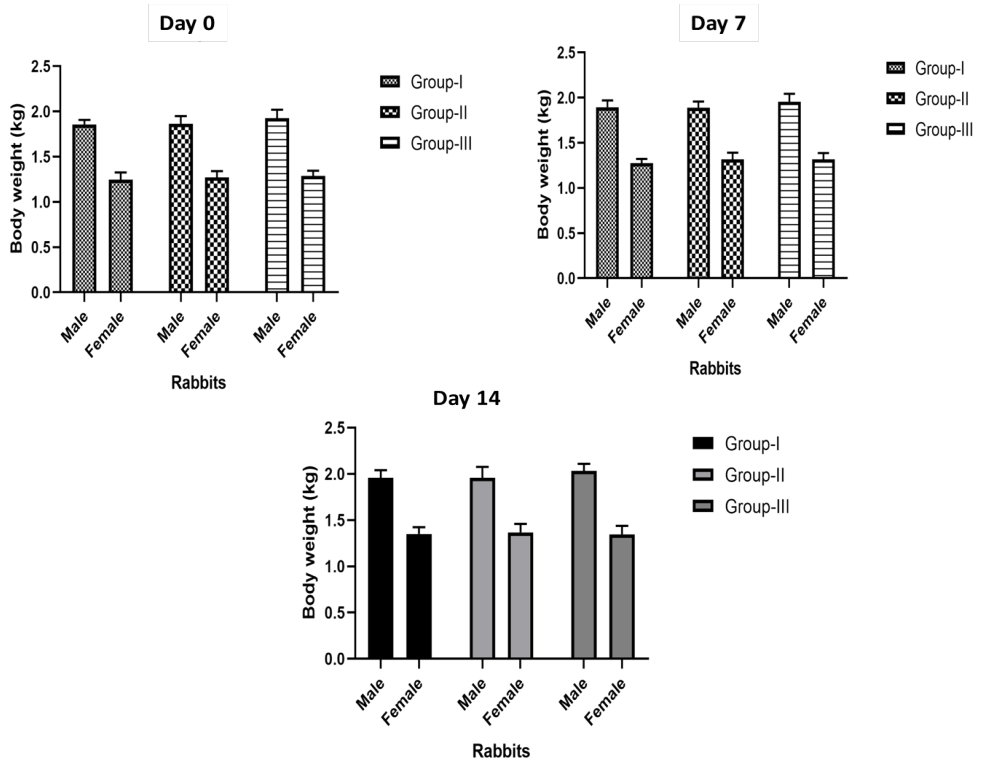

Figure 2: Body weight of albino rabbits on days 0 and 14

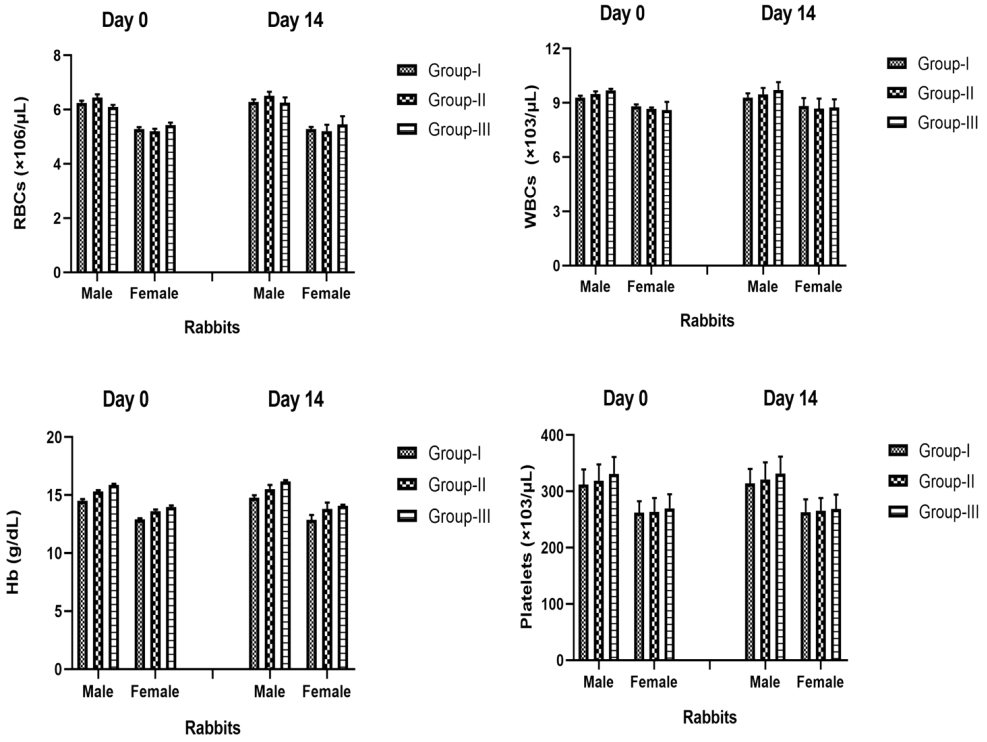

Figure 3: Hematology profile of albino rabbits on days 0 and 14

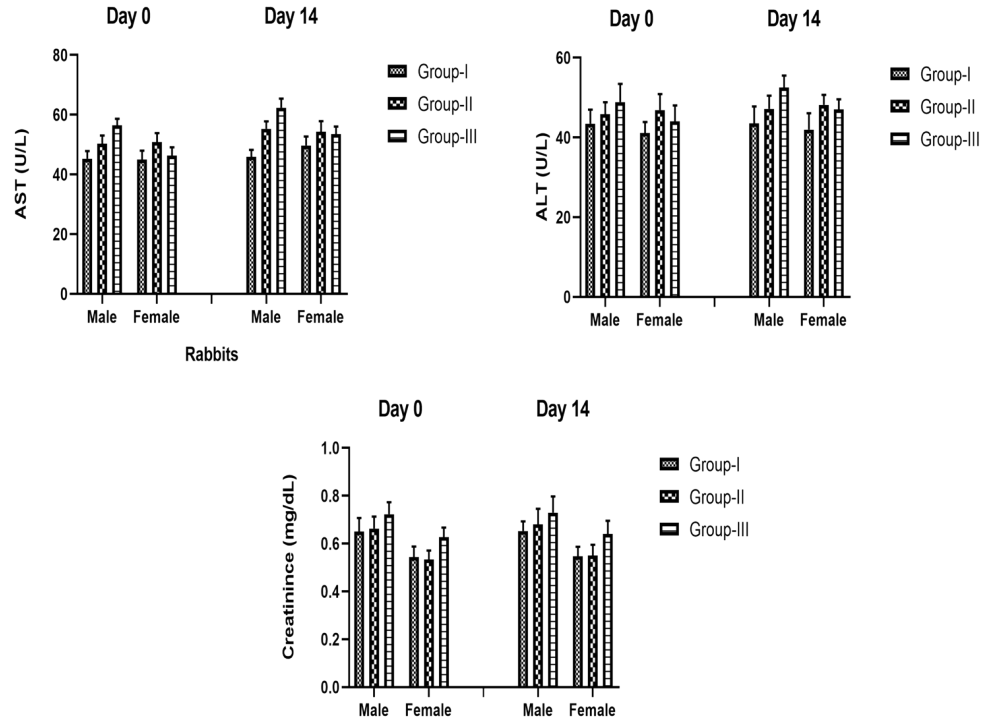

Figure 4: Biochemical examination of albino rabbits on days 0 and 14

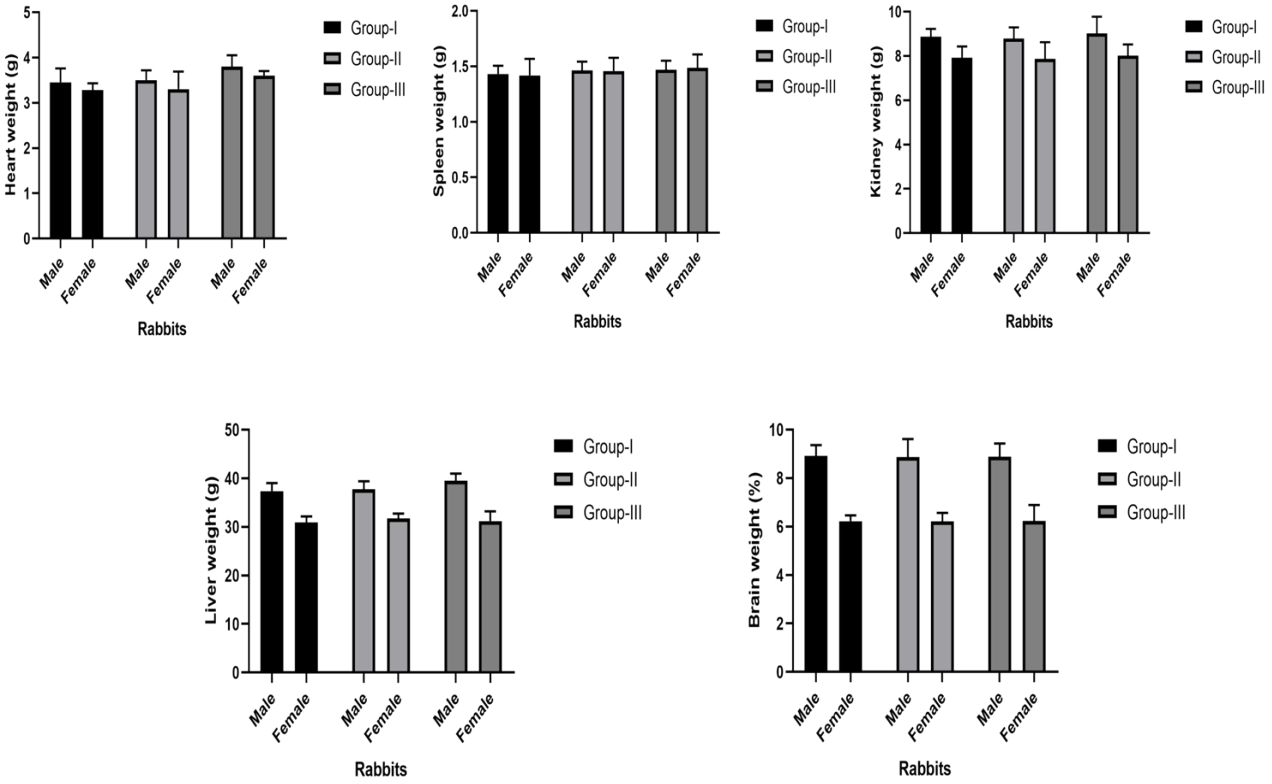

Figure 5: Weight of vital organs of albino rabbits on days 0 and 14

Effect of polymeric TMNPs on animal organ weight

These findings help assess the potential impact of the substance on organ size and function. The average weight of the vital organs of the treated groups was compared with the average weight of the vital organs of the control group. There were no significant differences in the weight of all groups (Fig. 5) after 14 days of treatment.

Histopathological assessment

The histopathological findings were recorded and analyzed. The observed changes were compared to the control group to determine the presence and severity of toxicological effects induced by substance exposure. The histopathological examination provides valuable information on the specific organs or tissues affected the nature of the changes, and the potential mechanisms of toxicity. No remarkable signs of degeneration/abnormality were found in any vital organ of albino rabbits (Fig. 6). Results indicated that the TMNPs did not affect the various tissues of the organs, which confirmed the safety of the used polymers.

Dermal safety

The recorded observations and grading scores were analyzed to evaluate the irritation potential of the microneedle patches. The irritation or any sign of erythema was observed at 0 hours and after 48 hours of TMNPs application. Observations proved that TMNPs prepared by using these polymers are considered to be non-irritant, non-toxic and safe on the skin.

Mechanical strength

The maximum force required to break the tips of the needles was recorded to prove the sufficient strength of the needles that are required for penetration into the skin. Specifically, TMNPs 1 and TMNPs 2 needles broke at 1.39 and 1.89, respectively (Fig. 7). These findings were compared to existing literature, affirming that the observed strength is adequate for skin penetration.[36,37] The obtained results are presented in Figure 8. Additionally, the moisture content of TMNPs-1 (composed of 3% HPMC and 15%

maltose) and TMNPs-2 (comprising 10% PLGA and 10% polyvinyl acetate) was found to be 5.4% and 4.9%, respectively.

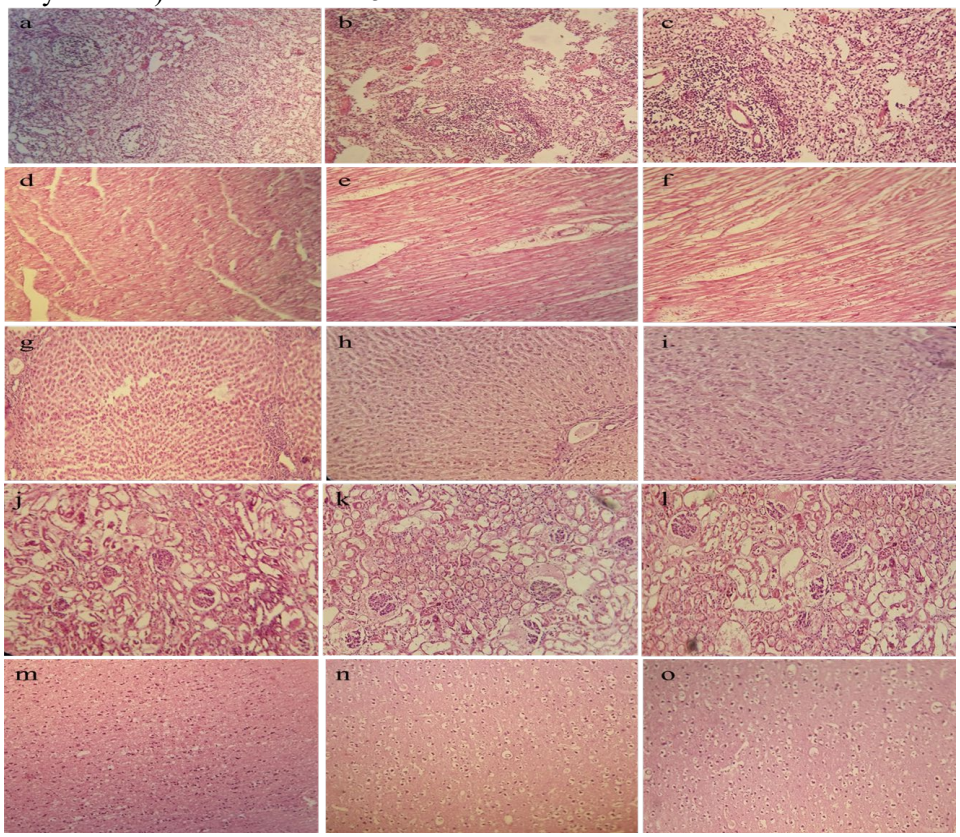

Figure 6: Histopathological changes of vital organs after 14 days of microneedle applications; a) Spleen (control), b) Spleen treated with TMNPs-1, c) Spleen treated with TMNPs-2, d) Heart (control), e) Heart treated with TMNPs-1, f) Heart treated with TMNPs-2, g) Liver (control), h) Liver treated with TMNPs-1, i) Liver treated with TMNPs-2, j) Kidney (control), k) Kidney treated with TMNPs-1, l) Kidney treated with TMNPs-2, m) Brain (control), n) Brain treated with TMNPs-1 and 0) Brain treated with TMNPs-2

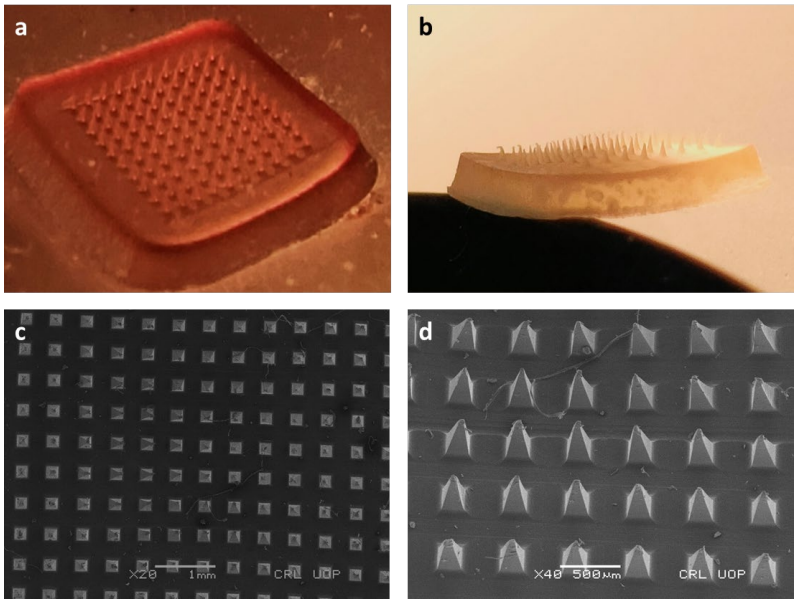

Figure 7: Mechanical strength of prepared patches; a) TMNPs-1, b) TMNPs-2, c) SEM image with top view, d) SEM image with side view after the test

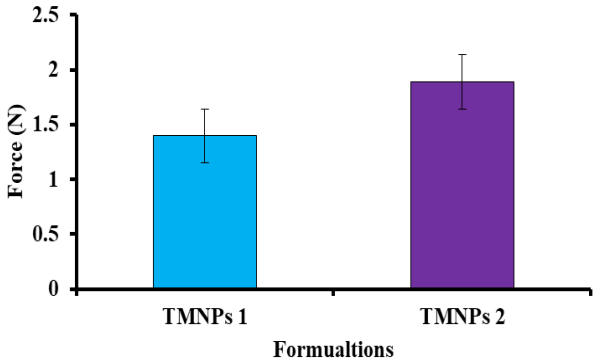

Figure 8: Graphical representation of mechanical strength of microneedle patches

The thickness of both MNP-1 and MNP-2 was 45.23µm and 50.55µm, respectively. Moreover, the morphology of the microneedle patch was confirmed using scanning electron microscopy, revealing pyramid-shaped needles, with a height of 500 µm and a base width of 200 µm.

CONCLUSION

This acute toxicity study provided important insights into the potential hazards associated with the different polymers involved in the fabrication of microneedle patches. By evaluating the adverse effects of various polymers on albino rabbits within a short period, the study contributes to our understanding of the substance's acute toxicity profile. The findings from the acute toxicity study enable us in decision-making regarding the safe handling, use, and regulatory classification of the polymers (HPMC, maltose, PLGA and polyvinyl acetate). It also serves as a foundation for further investigations, such as subchronic and chronic toxicity studies, to comprehensively assess the substance's long-term effects. Ultimately, the acute toxicity study aids in protecting human health and the environment by facilitating the appropriate management and control of potentially hazardous substances.

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REFERENCES

[1] E. Keleb, R. K. Sharma, E. B. Mosa and A. A. Z. Aljahwi, *Int. J. Adv. Pharm. Sci.*, **1**, (2010), https://doi.org/10.5138/09750657.1918

[2] C. Tiwari, M. Choudhary, P. Malik, P. K. Jaiswal and R. Chauhan, *J. Drug Deliv. Ther.*, **12**, 179 (2022), https://doi.org/10.22270/jddt.v12i6.5914

[3] S. Sadab, S. Sahu, S. Patel, R. Khan, B. Khare *et al.*, *Asian J. Dent. Health Sci.*, **2**, 40 (2022), https://doi.org/10.55567/ajdhs/2022/2.4/005

[4] D. Ramadon, M. T. C. McCrudden, A. J. Courtenay and R. F. Donnelly, *Drug Deliv. Transl. Res.*, **12**, 758 (2021), https://doi.org/10.1007/s13346-021-01041-x

[5] Zulcaif, N. Zafar, A. Mahmood, R. M. Sarfraz and A. Elaissari, *Micromachines*, **13**, 1304 (2022), https://doi.org/10.3390/mi13081304

[6] Z. Ahmad, M. I. Khan, M. I. Siddique, H. S. Sarwar, G. Shahnaz *et al.*, *AAPS PharmSciTech*, **21**, 1 (2020), https://doi.org/10.1208/s12249-019-1611-9

[7] J. Arya and M. R. Prausnitz, *J. Control. Release*, **240**, 135 (2016), https://doi.org/10.1016/j.jconrel.2015.11.019

[8] Z. Khatti, S. M. Hashemianzadeh and S. A. Shafiei, *Adv. Pharm. Bull.*, **8**, 169 (2018), https://doi.org/10.15171/apb.2018.020

[9] R. F. Donnelly and E. Larrañeta, *Drug Discov. Today*, **23**, 181 (2018), https://doi.org/10.1016/j.drudis.2017.10.013

[10] T. Waghule, G. Singhvi, S. K. Dubey, M. M. Pandey, G. Gupta *et al.*, *Biomed. Pharmacother.*, **109**, 1249 (2019), https://doi.org/10.1016/j.biopha.2018.10.078

[11] R. Donnelly and D. Douroumis, *Drug Deliv. Transl. Res.*, **5**, 311 (2015), https://doi.org/10.1007/s13346-015-0250-2

[12] J. W. Lee and M. R. Prausnitz, *Drug Deliv.*, **15**, 541 (2018), https://doi.org/10.1080/17425247.2018.1471059

[13] Z. Sartawi, C. Blackshields and W. Faisal, *J. Control. Release*, **348**, 186 (2022), https://doi.org/10.1016/j.jconrel.2022.05.045

[14] B. H. J. Gowda, M. G. Ahmed, A. Sahebkar, Y. Riadi, R. Shukla *et al.*, *Biomacromolecules*, **23**, 1519 (2022), https:// doi.org/10.1021/acs.biomac.1c01691

[15] Y. Yang, R. Luo, S. Chao, J. Xue, D. Jiang *et al.*, *Nat. Commun.*, **13**, 6908 (2022), https://doi.org/10.1038/s41467-022-34716-5

[16] W. Ma, X. Zhang, Y. Liu, L. Fan, J. Gan *et al.*, *Adv. Sci.*, **9**, 2103317 (2022), https://doi.org/10.1002/advs.202103317

[17] A. Roy, K. Nishchaya and V. K. Rai, *Expert Opin. Drug Deliv.*, **19**, 303 (2022), https://doi.org/10.1080/17425247.2022.2045944
[18] F. Sabbagh and B. S. Kim, *J. Control. Release.*, **341**, 132 (2022), https://doi.org/10.1016/j.jconrel.2021.11.025
[19] S. Priya and G. Singhvi, *Biomed. Pharmacother.*, **155**, 113717 (2022), https://doi.org/10.1016/j.biopha.2022.113717
[20] S. N. Hussain Shah, Zulcaif, A. Syed, A. Aslam, N. Zafar *et al.*, *Polym. Bull.*, **2023**, (2023), https://doi.org/10.1007/s00289-023-05004-z
[21] F. Cappa, D. Baracchi and R. Cervo, *Sci. Total Environ.*, **837**, 155714 (2022), https://doi.org/10.1016/j.scitotenv.2022.155714
[22] S. K. Rada and A. Kusuma, *Trop. J. Nat. Prod. Res.*, **7**, 2965 (2023), https://doi.org/10.26538/tjnpr/v7i5.19
[23] R. Wang, H. Wang, G. Jiang, Y. Sun, T. Liu *et al.*, *Biomater. Sci.*, **11**, 1704 (2023), https://doi.org/10.1039/D2BM01836E
[24] V. G. S. S. Jyothi, H. Veerabomma, K. P. Tryphena, D. K. Khatri, S. B. Singh *et al.*, *Biochem. Biophys. Res. Commun.*, **660**, 88 (2023), https://doi.org/10.1016/j.bbrc.2023.04.001
[25] A. Agrawal, G. T. Kulkarni and Lakshmayya, *J. Ethnopharmacol.*, **315**, 116685 (2023), https://doi.org/10.1016/j.jep.2023.116685
[26] S. Hajare, R. S. Ingole, M. Kumar, V. Ingawale, V. Borekar *et al.*, *J. Phytopharmacol.*, **11**, 57 (2022), https://doi.org/10.31254/phyto.2022.11201
[27] A. Alhalmi, S. Amin, Z. Khan, S. Beg, O. A. L. Kamaly *et al.*, *Pharmaceutics*, **14**, 1771 (2022), https://doi.org/10.3390/pharmaceutics14091771
[28] S. K. Paswan, P. Verma, S. Srivastava and C. V. Rao, *Drug Chem. Toxicol.*, **46**, 625 (2022), https://doi.org/10.1080/01480545.2022.2075378
[29] X. Yang, J. Li, S. Chen, L. Xiao, D. Cao *et al.*, *Evid. Based Complement. Alternat. Med.*, **2022** (2022), https://doi.org/10.1155/2022/9869293
[30] Y. Liu, X. Zhu, S. Ji, Z. Huang, Y. Zang *et al.*, *Drug Deliv.*, **29**, 2984 (2022), https://doi.org/10.1080/10717544.2022.2122632
[31] D. Tusé, M. Reeves, J. Royal, K. T. Hamorsky, H. Ng *et al.*, *J. Pharmacol. Exp. Ther.*, **380**, 162 (2022), https://doi.org/10.1124/jpet.121.000904
[32] A. Khalid, H. S. Sarwar, M. Sarfraz, M. F. Sohail, A. Jalil *et al.*, *Saudi Pharm. J.*, **31**, 669 (2023), https://doi.org/10.1016/j.jsps.2023.03.007
[33] X. Y. Jiang, Y. Zeng, W. W. Zhang, C. Wang and W. Li, *Acta Mater. Med.*, **2**, 1 (2023), https://doi.org/10.15212/AMM-2022-0042
[34] C. Ryall, S. Chen, S. Duarah and J. Wen, *Int. J. Pharm.*, **627**, 122221 (2022), https://doi.org/10.1016/j.ijpharm.2022.122221
[35] M. I. Abdul Jalal, K. S. Ooi, K. C. Foo, A. A. Hamzah, C. S. Goh *et al.*, *J. Clin. Med.*, **11**, 5291 (2022), https://doi.org/10.3390/jcm11185291
[36] G. Anbazhagan, S. B. Suseela and R. Sankararajan, *Drug Deliv. Transl. Res.*, **13**, 1813 (2023), https://doi.org/10.1007/s13346-023-01296-w
[37] L. Zheng, D. Zhu, W. Wang, J. Liu, S. T. G. Thng, *et al.*, *Actuators B Chem.*, **372**, 132626 (2022), https://doi.org/10.1016/j.snb.2022.132626