# TOXICOLOGICAL EVALUATION OF NATURAL AND SYNTHETIC POLYMER BASED DISSOLVABLE MICRONEEDLE PATCHES HAVING VARIABLE RELEASE PROFILES

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Acute toxicity studies of dissolvable microneedle (dMN) patches fabricated from a combination of polymers, *i.e*. thiolated chitosan (TCS), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP), were performed to determine the safety of polymers on white albino rabbits. The selected albino rabbits were categorized into four groups. Group I was a control group, whereas groups II, III and IV were administered different doses of polymeric dMN patches. The food and water uptake and the weight of the animals were observed before and after administration of the microneedle patch on days 1, 3, 5, 7 and 14. Hematological and serum biochemical changes were observed by taking blood samples of treated and control group rabbits on the 15$^{th}$ day of study. The weight of the main organs was determined and a histopathology study was also performed. Negligible alteration in body weight, meals and water uptake was observed. The control and treated animals displayed similar behavioral pattern. Moreover, the treated groups showed hematological and biochemical analysis results, which were comparable with the results of the control group animals. Animals from both control and treated groups did not present any significant difference in the results of relative organ body weight and urine analysis. A comparison of histopathology results for both treated and control animals' vital organs confirmed the absence of lesions. The findings of antioxidant effect, dermal and cardiac toxicity studies of chitosan, thiolated chitosan and the TCS/PVA/PVP combination were compared to those of the analysis of circulating oxidative levels, demonstrating that the combination of TCS/PVA/PVP showed improved antioxidant effect, as compared to those of chitosan and thiolated chitosan alone, and showed no significant effect on skin and heart. Thus, the findings of the study established the fact that the fabricated polymeric dMNs are a safe carrier system for different drugs.

***Keywords***: acute toxicity, antioxidant effect, dissolvable microneedle patch, thiolated chitosan, polyvinylpyrrolidone, polyvinyl alcohol, safety profile, histopathology

## INTRODUCTION

The delivery of drugs through the transdermal route is quite convenient and economical.[1] However, low bioavailability is a big drawback linked with this route.[2] This limitation can be tackled by fabricating microneedle patches for transdermal drug delivery.[3] A number of different types of microneedle patches have been explored by researchers in this regard,[4] *e.g.* dissolvable microneedle patches,[5-7] coated microneedle patches,[8-10] and hollow microneedle patches[11-13] *etc*. Among these types, dissolvable microneedle patches have emerged as the most advantageous and effective drug carrier system.[14,15] Therapeutically, this kind of dosage form reduces fluctuations in plasma drug concentrations, especially for drugs having short half-life.[12]

There are different ways to prepare microneedle patches, such as solvent washing, solvent casting,[16-18] reactive ion etching,[19-21] wet chemical etching[22,23] *etc*. In this study, the solvent casting method was selected, which utilizes a number of diverse natural, biodegradable, biocompatible and synthetic polymers for the fabrication of its base. Literature shows the

utilization of a vast number of polymers, such as polyvinyl alcohol (PVA),[24-26] chitosan (CS),[27-29] Eudragit,[30] hydroxypropyl methylcellulose (HPMC),[31] carboxymethylcellulose (CMC),[32,33] thiolated chitosan (TCS),[34] alginic acid,[35] polyvinylpyrrolidone (PVP),[36,37] *etc*. for fabrication of microneedle patches.

PVA and PVP are freely available on the market, whereas thiolated chitosan can be prepared by the thiolation of chitosan, as reported by Ahmad *et al*. Chitosan was successfully thiolated into TCS and reported in our previous study.[34] CS is the second most abundant naturally occurring marine biopolymer, which prevents soft tissue diseases and displays antioxidant, anti-inflammatory, anti-aging and antibacterial effects.[38,40-43] Modified forms of chitosan exhibit pH dependent drug delivery, as reported by Lv *et al*. They synthesized highly fluorescent graphene quantum dots (GQDs)-chitosan (CS) hybrid xerogel and reported that protonation/deprotonation of $–NH_2$ on CS result in a pH-dependent drug delivery.[39]

Keeping in view the benefits and usefulness of the above-mentioned natural and synthetic polymers in diverse fields, such as biomedicine, folk medicine, food and confectionary, we aimed to prove the transdermal safety of chitosan and thiolated chitosan taken separately, and the combination TCS-PVA-PVP through microneedle patches acting as drug carrier. To achieve this goal, acute toxicity studies and glutathione dependent antioxidant effect tests were performed on albino rabbits, while following the guidelines laid by the Organization for Economic Co-operation and Development (OECD, 2001) and National Institute of Health.

## EXPERIMENTAL

### Preparation of polymeric dissolvable microneedle patches

A dissolvable microneedle (dMN) patch with thiolated chitosan was fabricated by the method reported in our previous study.[34] dMN patches with chitosan and combinations of polymers (TCS-PVA-PVP) were fabricated by using the solvent casting method, as reported in our previous study.

### Study animals

Twelve male (1840 g to 1940 g) and twelve female (1200 g to 1300 g) albino rabbits were purchased from Hameed Enterprises, Lahore, Pakistan. Both male and female rabbits were housed in four groups (n=6) separately in clean cages under 12 h dark/light cycles, controlled temperature (23-26 °C) and humidity (55-65%). The animals were given time to get acclimatized with the pleasant environment for a period of one week during which they were provided sufficient food and water. The study code was assented by the Institutional Research Ethics Committee of The University of Lahore vide letter no. DPH/21/FOP/4520a, dated October 6, 2021.

### Experimental design

The acute toxicity study of polymeric dissolvable microneedle (dMN) patches was carried out according to the OECD guidelines. A total 24 male and female rabbits were placed into four groups (G-I-IV) randomly (n=6, 3 males and 3 females/group). Group-I was used as control and other groups (G-II-IV) were treated with different concentrations of selected polymers, as explained in Table 1.

Table 1
Experimental design

| Group number | Drug concentration | Number | |
|---|---|---|---|
| | | Male | Female |
| G-I | Control | 3 | 3 |
| G-II | 2% Chitosan | 3 | 3 |
| G-III | 2% Thiolated chitosan | 3 | 3 |
| G-IV | 2% TCS, 10% PVA and 5% PVP/patch | 3 | 3 |

### Body weight, clinical signs and food consumption

The weight of all the rabbits was noted on day 1 before the administration of the test sample and later on the third, fifth, seventh and fourteenth days of the test, prior to sacrifice. Throughout the study, the animals were closely monitored for any clinical changes or mortality/morbidity rate. Feed consumption was calculated by subtracting the food and water supplies to the cages and their remnants and compared with the control group.

### Hematology study

The hematology study was carried out to evaluate the overall health of test animals. On days 0 and 15 of the study, blood specimens were drawn from the marginal ear vein of all the rabbits and stored in an

anticoagulant (EDTA) vial for analysis, followed by mixing on a tube roller mixer for 10 minutes. Evaluated hematology parameters included haemoglobin (Hb), red blood cells count (RBCs), platelets count (PLT) and white blood cells count (WBCs). These parameters were analyzed by an automated hematology analyzer XP series (Sysmex Corporation, Japan).

**Biochemical examination**

Biochemical analysis was performed by checking out the renal function (renal function test – RFT), lipid profile and liver function (liver function – LFT). Blood serum of all the collected samples was separated with the help of centrifugation at 5000 rpm for 10 minutes. Then, supernatant was carefully separated with the help of a pipette and stored in clotted vials for analysis. In the liver function analysis, levels of alanine transaminase (ALT) and aspartate transaminase (AST) were observed. In the renal function analysis, the creatinine level was observed. In the lipid profile analysis, cholesterol and triglyceride levels were determined by using an AU680 chemistry analyzer (Beckman Coulter, USA).

**Urine analysis**

On days 0 and 15 of the test, urine samples from all the test rabbits were collected in a urine jar and analysis was performed for urine color, specific gravity, RBCs, WBCs, urobilinogen, urine sugar, protein, uroketoneuria and pH using Combur-test® strips (Roche, USA).

**Effect of polymeric dMN patches on animal organ weight**

Fifteen days after the treatment with polymeric dMN patches, all the rabbits were slaughtered and all the vital organs, *i.e.* liver, spleen, heart and kidney, were observed macroscopically for lesions and weighed.

**Histological assessment**

Histological assessment was performed to observe the changes brought about by the polymeric dMN patches on cellular structure. For this, vital organs, including spleen, liver, kidney, heart, brain and lungs, were separated after the sacrificing of rabbits and stored in a solution of formaldehyde 10% (v/v). A rotary microtome was used for the slicing procedure and slices were then affixed on glass slides. Next, they were stained by dipping in a solution of hematoxylin and eosin. Finally, the prepared slides were observed microscopically (100X) for any histological changes.

**Antioxidant effect of natural and synthetic polymers by the analysis of circulating oxidative levels**

On days 0 and 15, 2 mL of blood was taken from individual rabbits and the results of ALT, AST, glutathione and superoxide dismutase were examined. ELISA kits were purchased from Lifecare laboratories (Lahore, Pakistan).

**Dermal safety**

The hair on the dorsal surface of rabbits was removed by using hair removal cream (Veet cream) and dMN patches were applied on the albino rabbit skin in order to check any sort of irritation/edema by using the Draize scoring method. One side of the dorsal surface served as control and on the other side, the dMN patch was applied for 48 hours. Skin condition was evaluated by visual observation and compared in terms of any kind of redness/edema/irritation.[12,44]

**Cardiac safety**

According to the guidelines of the International Conference on Harmonization (ICH), all the excipients should be evaluated for pharmacological activity.[45] The safety of excipients can be verified with pharmacological studies that act as part of toxicological evaluation. The effect of polymeric solutions (that were poured into silicon molds for the preparation of dMN patches) on the isolated heart of rabbits was determined by using the previously reported procedure.[46] Langendorff's isolated cardiac perfusion method was followed with slight modification. Heparin 1000 IU was administered through the marginal ear vein of rabbits 30 minutes before dissection to avoid blood coagulation. The chest of rabbit was opened with Jorgensen thoracic scissors and the heart was isolated immediately with 1 cm of aorta from the pericardial sac to prevent ischemia. The heart was cleaned with Ringer solution, mounted in ice cold oxygenated Krebs-Henseleit solution, several times compressed for the removal of blood. A Radnoti Isolated Heart apparatus was used and aorta was tied up with a glass cannula. First, perfusion fluid was moved at greater flow rate. Second, the flow rate was decreased and maintained at the same level and a temperature of 37.5 ± 0.5 °C throughout the study. A clip was affixed to the apex of the heart to record the mechanical responses, including contraction force of the heart, by joining one end of the thread to the force displacement transducer and other end to the affixed clip, to determine the force of contraction of the heart. Transducers were connected to the Power Lab and signals were recorded before and after the study by using a computer running Chart 5.0 software. Polymeric solutions were administered after the stabilization of the heart and the force of contraction and perfusion pressure was observed and analyzed.

**Statistical analysis**

Each characterization was performed in triplicate and the mean ± S.D. was calculated. The statistics were applied using Graph Pad Prism software, keeping the level of significance at $p < 0.5$.

## RESULTS AND DISCUSSION

### Body weight, clinical signs and food consumption

All the rabbits were carefully observed for 14 days on daily basis, with respect to physical parameters, and there were no significant changes observed in the behavior of both control and treated groups. The mean body weight of male and female rabbits exposed to the dMN patches was not remarkably changed as compared to the control group (Fig. 1). However, a steady increase in body weight, indicating that all the rabbits were tolerated well the treatment. During this study, all the rabbits remained healthy and no mortality was noticed.

### Hematology study

Hematology parameters (RBCs, WBCs, haemoglobin and platelets) were observed in male and female rabbits after treating with polymeric dMN patches. On days 0 and 15, when compared with the control group, a negligible significant difference was observed in the results of RBCs, WBCs, haemoglobin and platelets of all four groups (Fig. 3). All the parameters remained within the normal range, showing absence of toxicity in the treated rabbits.

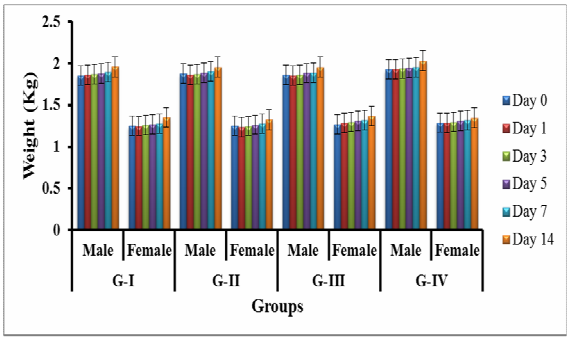


Figure 1: Effect of polymeric dMN patches on body weight of male and female rabbits

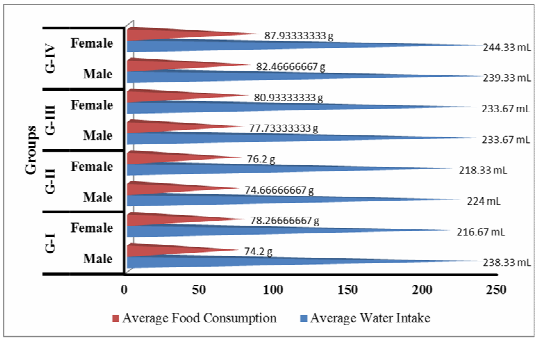


Figure 2: Effect of polymeric dMN patches on water intake and feed consumption of rabbits

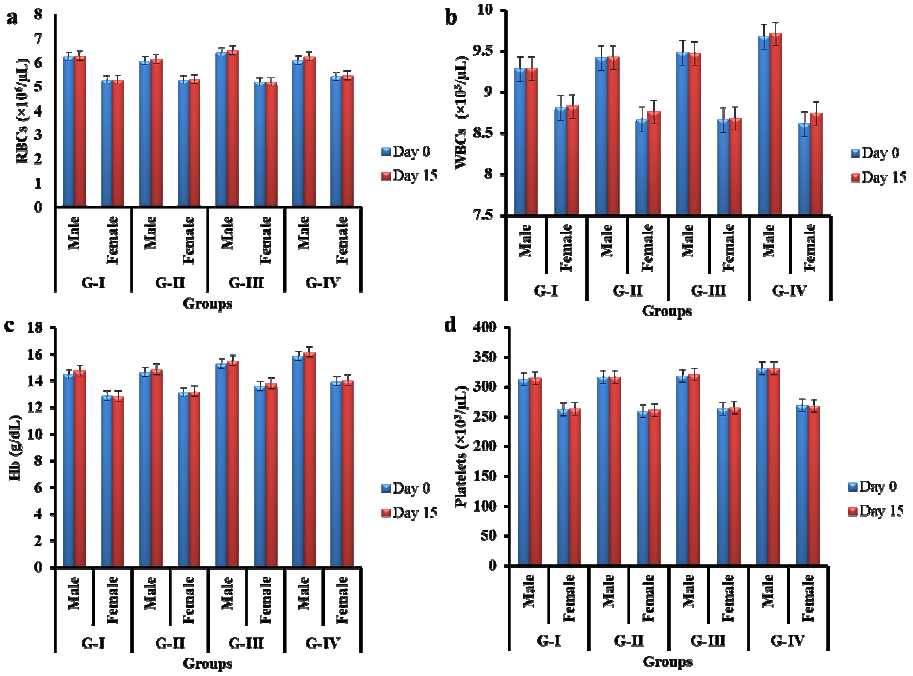


Figure 3: Effect of polymeric dMN patches on hematology of rabbits on days 0 and 15

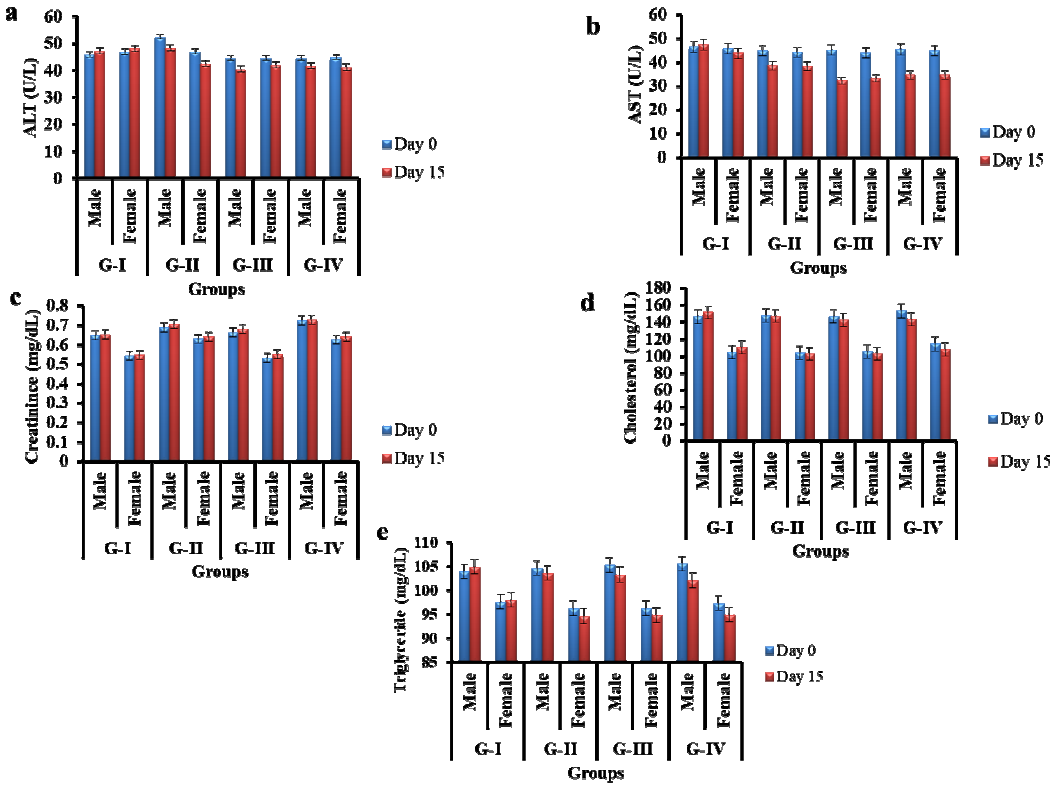


Figure 4: Effect of polymeric dMN patches on blood biochemistry on days 0 and 15

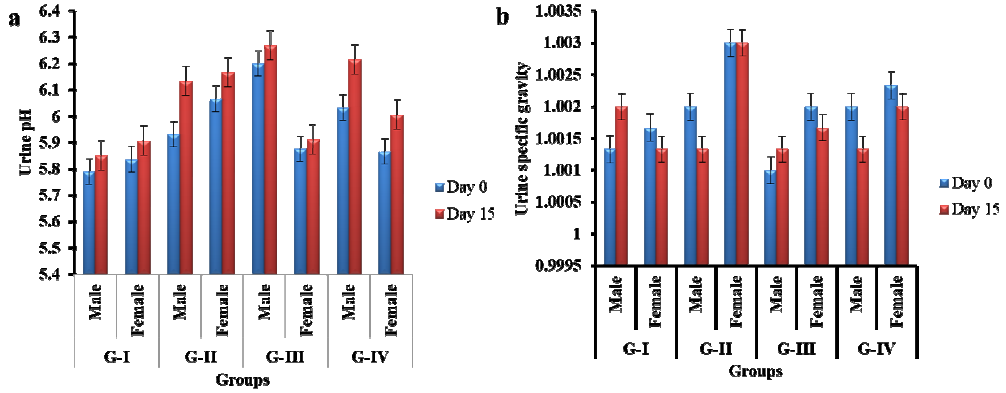


Figure 5: Effect of polymeric dMN patches on rabbit urine on days 0 and 15

**Biochemical examination**

Polymeric dissolvable microneedle patches did not affect the blood biochemistry of male and female rabbits. On days 0 and 15, the results of ALT, AST and creatinine did not show any statistically significant difference between rabbits of different genders. Moreover, cholesterol and triglyceride levels were also observed in the control group and the test groups, but there was no statistical difference. However, as compared to the control group, the results of blood biochemistry fell within normal ranges, pointing out the harmless behavior of patches in treated rabbits.

**Urine analysis**

All rabbits showed yellowish urine, with absence of RBCs, WBCs, urobilinogen, urine sugar, protein and uroketoneuria. No remarkable changes were noticed in the urinary parameters of male and female rabbits in the control and treated groups (Fig. 5).

**Effect of polymeric dMN patches on animal organ weight**

On day 15th, rabbits from the control and treated groups were sacrificed and the average weight of key organs (heart, spleen, liver and kidney) was noted. Negligible difference in the weight of the heart, spleen, liver and kidney between the four groups of male and female rabbits was observed (Fig. 6).

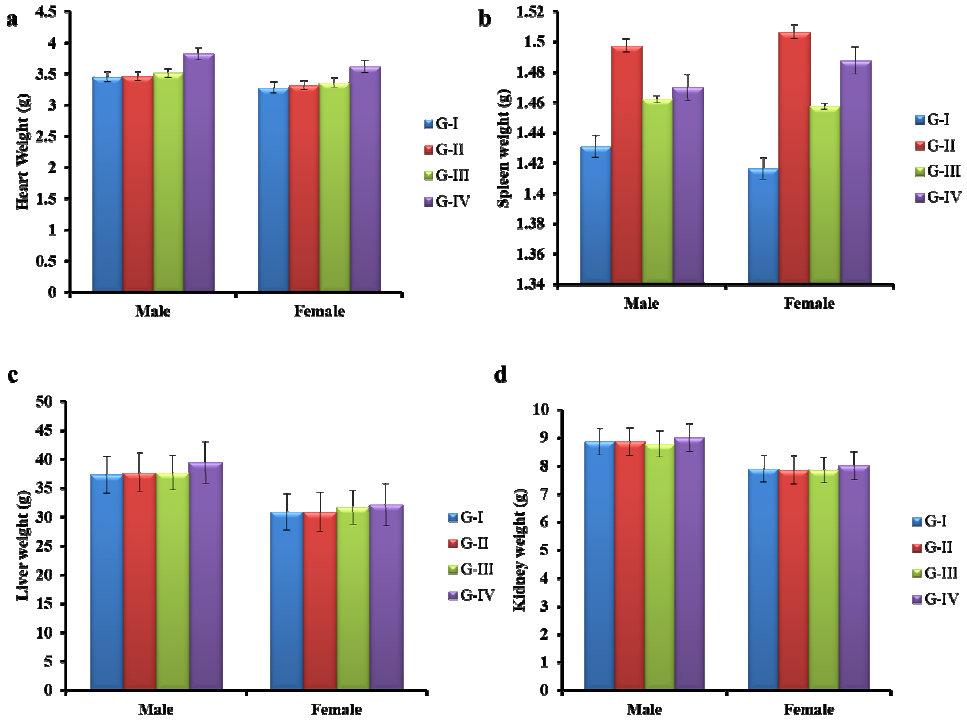


Figure 6: Effect of polymeric dMN patches on organ weight

**Histological assessment**

The histopathological findings for the vital organs of treated rabbits were similar to those for the control group. There was no sign of degeneration or remarkable abnormality observed in any organ (heart, spleen, liver, kidney, brain and lungs) of male and female rabbits of all groups (Fig. 7), indicating that the treatment with polymeric dMN patches did not cause damage to various tissues and organs of rabbits.

**Antioxidant effect of natural and synthetic polymers by the analysis of circulating oxidative levels**

On day 0, there was no remarkable difference between the control and the treated group, with respect to biomarkers of antioxidant and oxidative stress. After 15 days of therapy, the circulating levels of glutathione (GSH) and superoxide dismutase (SOD) increased (Fig. 8), and the levels of AST and ALT also increased (Fig. 4) in the treated group (G-II to G-IV), when compared with the control group (G-I). The results indicated that thiolated chitosan caused an increase in antioxidant properties, as compared to chitosan, and the combination of TCS/PVA/PVP led to an increase in antioxidant properties, as compared to thiolated chitosan alone: Chitosan < Thiolated chitosan < Combination of TCS/PVA/PVP.

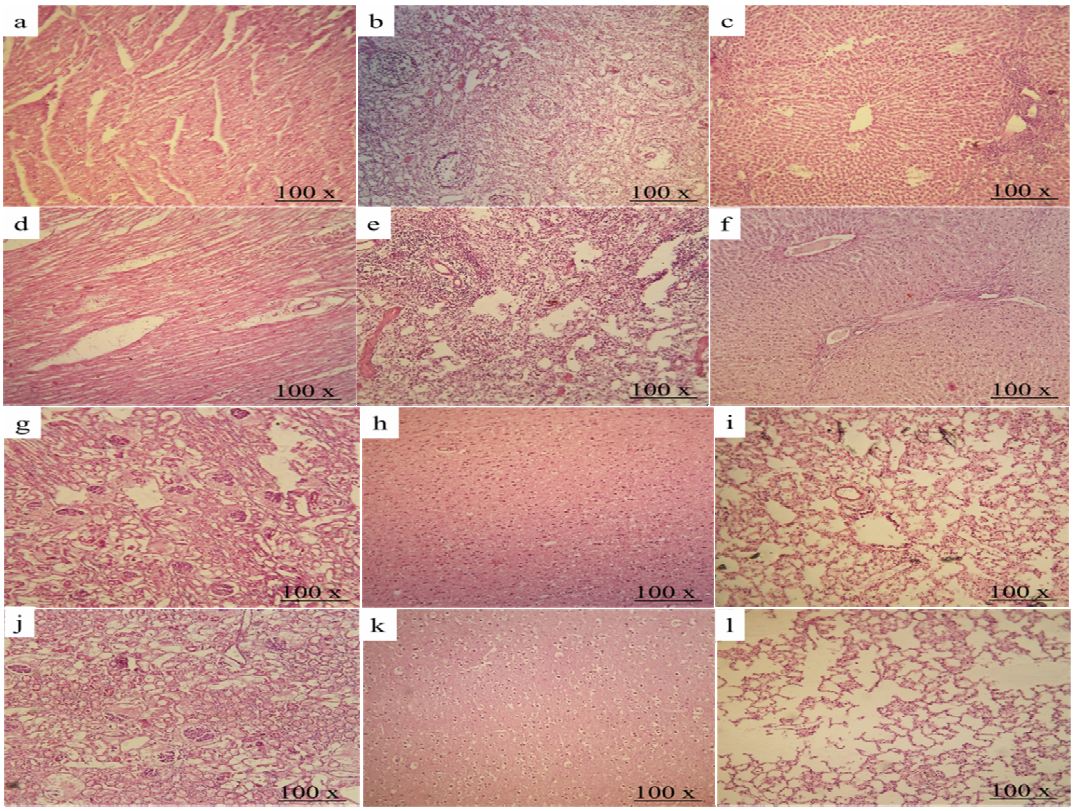


Figure 7: Effect of polymeric dMN patches for 15 days on histological section (hematoxylin and eosin staining, 100X) of vital organs; a) heart (control), b) spleen (control), c) liver (control), d) heart (treated), e) spleen (treated), f) liver (treated), g) kidney (control), h) brain (control), i) lungs (control), j) kidney (treated), k) brain (treated) and l) lungs (treated)

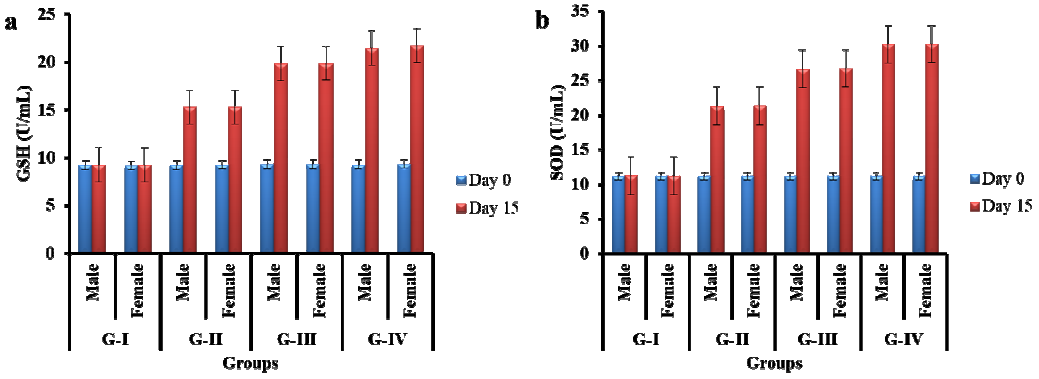


Figure 8: Effect of polymeric dMN patches on GSH and SOD on days 0 and 15

**Dermal safety**

Observations were recorded at 0 hour and after 48$^{th}$ hours. Slight edema was noticed upon applying dMN patches (Fig. 9a). However, after 48 h, no signs of skin allergy, erythema or edema were recorded (Fig. 9b). These findings proved that the developed dMN patches were biocompatible, non-toxic and non-irritant at the site of application.

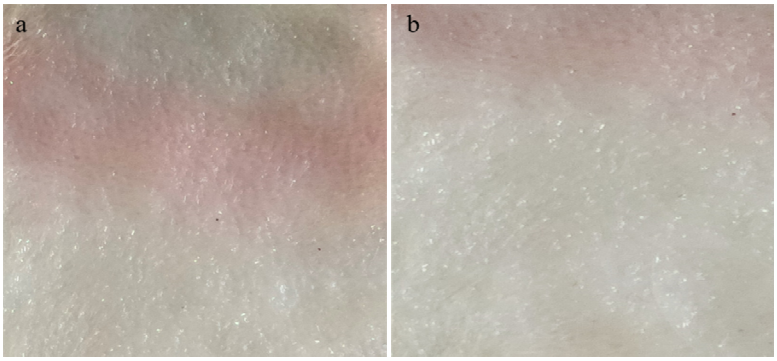


Figure 9: Effect of polymeric dMNs patches on skin a) at day 0 b) at day 15

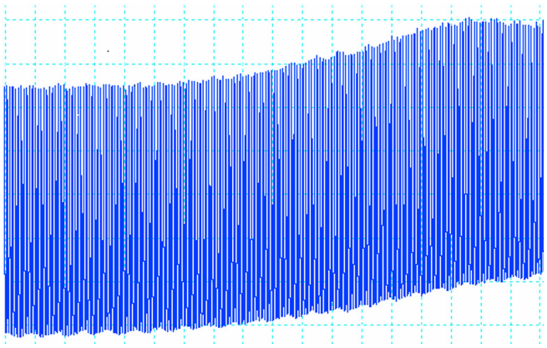

Figure 10: Effect of polymer solutions on force of contraction

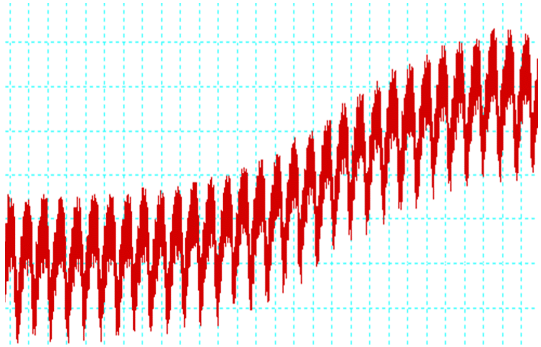

Figure 11: Effect of polymer solutions on perfusion pressure

**Cardiac safety**

ICH guidelines (2005) established the safety procedure for excipients to assess cardiac safety. The effect of the polymeric solution on the force of cardiac contraction and perfusion pressure was evaluated in the albino rabbit heart (Figs. 10 and 11). It was observed that after the application of polymeric solutions, there was only a slight decrease in the force of contraction of the heart muscles. However, there was not any significant change in perfusion pressure.

**CONCLUSION**

This study showed that different physical parameters, namely, the overall health of treated albino rabbits, their behavioral pattern, body weight and food intake, were absolutely fine. No abnormalities that might have resulted from the acute toxicity study of polymeric dMN patches were observed. Biochemical analysis, histopathology studies of key organs and cardiac safety testing in the treated animals displayed no abnormality of any sort. Moreover, excellent antioxidant property was observed and the application of the dMN patches onto the skin showed no sign of irritation and edema. Thus, the results revealed the possibility of using such dMN patches as a safe mode of treatment and a possible alternative to conventional dosage forms in the pharmaceutical industry. In conclusion, the fabricated polymeric dissolvable microneedle patches based on different polymer combinations can be considered quite safe for transdermal drug delivery of various actives. Moreover, such patches can also be utilized for delivery of actives in skin products. However, more detailed toxicology studies are suggested to be carried out in the future to calculate LD50.

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