

# The histological changes attributed to intermittent negative pressure on mice skin

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## Abstract

Background: Modern applications of negative pressure in healthcare include wound therapy, assisted child delivery, sleep apnea, and liposuction. Studies in negative pressure effects in the skin are scarce but promising. Objectives: To study the changes that could occur to the skin and subcutaneous tissue after exposure to two weeks period of intermittent negative pressure, using the albino mice as a model. Study design: Experimental animal study. Materials and methods: Ten albino mice were subjected to two sessions/day of intermittent negative pressure (INP) for two weeks. The tested skin areas were then excised for histology examination and compared with a control patches from the same mice. Skin biopsies were stained with H&E (Harris Hematoxilin), Mayer's Hematoxilin, and Masson's trichrome stain. The slides were reviewed by pathologists and photographed. Results: The test slides showed marked loss of sebaceous glands and hair follicles, increased condensation of collagen, inflammatory features in the form of white blood cells infiltrations and edematous patches. Conclusion: Applying INP to the skin of albino mice results in the reduction of sebaceous glands and hair follicles, and evident signs of inflammatory changes.

**Keywords:** Intermittent Negative pressure, Sebaceous glands, Hair follicles.

## INTRODUCTION

Negative pressure is a general term used to describe a pressure that is lower than the atmospheric pressure [1]. Negative pressure was useful in medical practice thousands of years ago, when Chinese invented cubbing therapy [2], and spread to Europe [3], and then to the Middle East [4]. Started by manual suction by mouth and a horn, cubbing evolved till recent days which became performed by clean vacuum cups and automated machines [2]. Negative pressure constitutes the basis of negative pressure wound therapy NPWT used for healing of Diabetic wounds and other wounds of chronic etiology [5-10]. Other uses of negative pressure in medicine includes erectile dysfunction management, where the use of vacuum erection devices-VEDs, proved efficiency [11,12], also it is used in assisted childbirth [13], in reducing the prevalence of sleep apnea [14], in nasal septum defect correction [15], and in liposuction [16,17]. Studies on the lower limbs of healthy individuals revealed that application of intermittent negative pressure improved the microcirculation and increased the blood perfusion [18]; an explanatory finding to the negative pressure wound healing capacity [19]. Although it proved great aid to wound healing and circulatory improvement, the pain and

swelling during the suction site constitute a major complain from the candidates [20,21,22], nevertheless, the outcome is far greater in prospect than the transient pain and discomfort [21]. Despite the great results achieved by NPWT, still how it evokes healing and closure of wounds, is uncertain [23], but it is postulated that the vacuum process encourages wound healing and closure, presumably by increasing local hemodynamic circulation and vessels proliferation [5,23,24], isolating the skin area from bacteria, and stimulating cell regeneration [5,23]. It would be of a great value to detect the histological changes of skin and subcutaneous tissue after subjection to intermittent negative pressure (INP). Mierte et al. studied how the dermis and epidermis changes in structure within scar tissue post-vacuum [25]; they noticed a significant reduction in the epidermal thickness and increase in the dermis layer girth. Unfortunately, the changes were transient, and diminished after about two hours; nevertheless, this 2016 study gave hope about the possibility of vacuum to enhance the extracellular matrix (ECM) remodeling and proliferation. The writers themselves attribute the increased dermal thickness to edema [25], which is probably due to the prolonged time interval used in this study. This current experiment was held to study changes in the histological structure of healthy mammalian skin in response to

two weeks of exposure to INP, taking the albino mice as a model. Skin represents an optimal tissue to carry on a negative pressure experiment, being in direct contact with INP process.

## MATERIALS AND METHOD

Ten male albino mice were used in this experiment. First, Mice were sedated by inhalation of ether in a sealed fiberglass chamber. Two diamond shaped patches, each of them was 2×2 cm, were shaved in the lateral abdominal wall of each mouse; one patch used as a control and the other were used as a test to perform INP. Negative pressure was applied on an interval of 10 seconds pump and ten seconds for rest, using a 50 ml syringe: the syringe was trimmed and cut by the edges to offer a wider space at the tip; see figure? The pressure was in the range of (-20 to -25) mmhg. Calibration of pressure was through a pressure gauge, by which, the grading within the syringe shaft was marked at (-20 to -25) mmhg. Each mouse was subjected to the INP cycle continuously for five minutes, two times daily for two weeks. The skin of both test area and control was excised in a quick and clean manner. Wounds healed within eight days through contraction, and hair grew back in the experiment site. Skin samples were put in 10% formaldehyde, and blotted in paraffin within three days of preservation. Slides were obtained from the samples by Leica® microtome. Thirty clear slides were prepared. The following histological stains were applied for test and controls:

1. Hematoxylin and Eosin (H&E) stain, particularly Harris Hematoxylin.
2. Mayer's Hematoxylin to highlight the nuclei.
3. Masson's Trichrome stain for collagen.

The slides were photographed through a microscope by the pathologist, who commented on the findings. The study underwent for two weeks, after which, the mice were returned to the center of veterinary researches, Khartoum, Sudan. All the procedures were performed according to the Institutional Animal Care and Use Committee guidelines [26].

## RESULTS

H&E stain showed features of inflammatory reaction including the presence of edema and increased white blood cells (WBCs). There was marked reduction in the number of hair follicles and sebaceous glands; See figure (1)

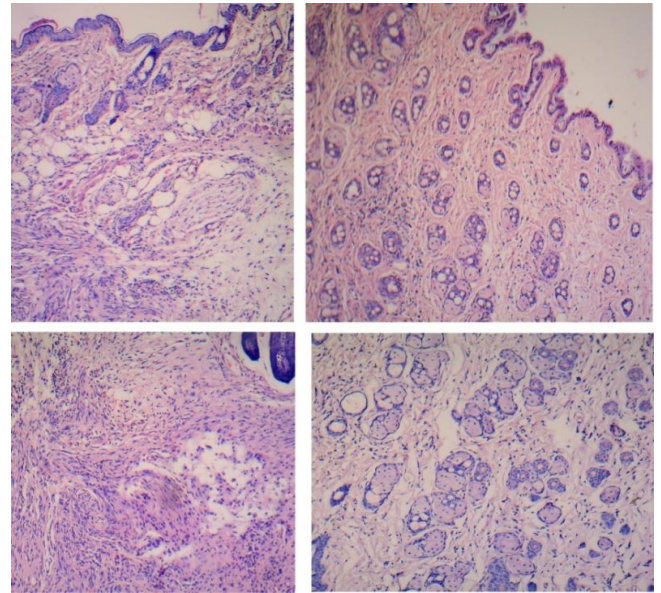
Mayer's hematoxylin stain revealed the normal appearance and positioning of nuclei within the cytoplasm. See figure (2).

Masson's trichrome staining showed that Collagen appears denser and better organized in the test slides when compared to the control; revise figure (3).

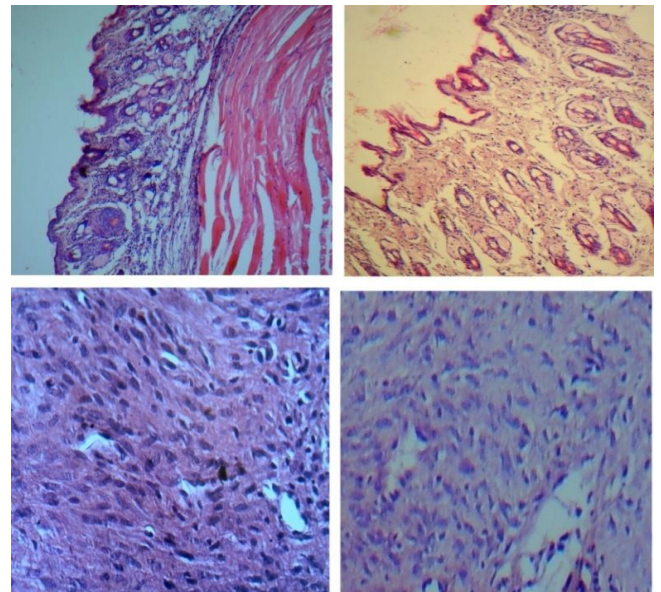
## DISCUSSION

To summarize the findings, it is comprehensive to say that inflammatory changes were documented by the edema and increased inflammatory WBCs. Clues of cellular distortions noticed as patchy areas of epithelial disorganization. There was a distinctive reduction in the number of both sebaceous glands and hair follicles. Edema and infiltration of white blood cells could be attributed to the traumatic effect of INP; trauma is known to elicit an immune response [27]. Another possible cause of the inflammatory signs could be a concomitant inflammatory pathology such as an infection, although is very unlikely to have infectious incidents in all the thirty samples. Epithelial cells are distorted mainly because they are in direct contact with the negative suction power. A good possible

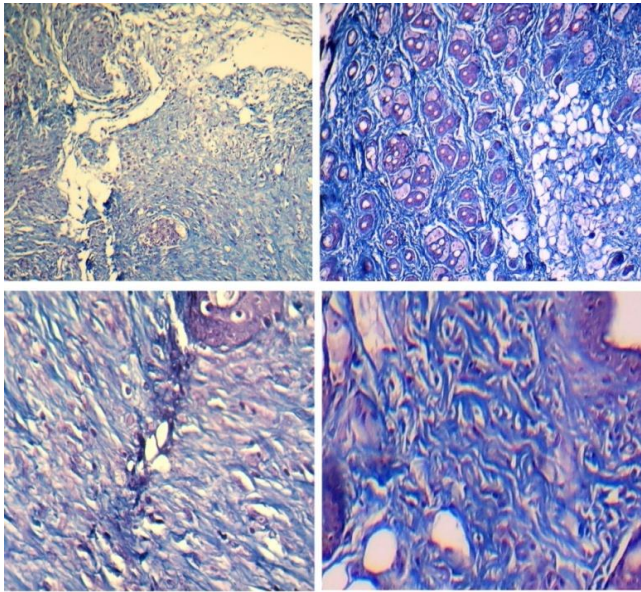
explanation for the marked reduction in number of the sebaceous glands and hair follicles is the reduced blood perfusion due to the INP, which lead to the atrophy of their cells. Sebaceous glands could be absent because of an autoimmune disease or reaction, as also for the hair follicles, but the two speculations are arbitrary, since the tissue samples were extracted from several mice, and no visible changes-such as skin dryness or alopecia patches, were noticed. Against what was predicted, the collagen concentrations did not increase (significantly), even active fibroblasts were not detected, and this applies to all histological sections it could be because tissues were subjected to INP for only three to four weeks and then immediately excised for histology; complete collagen organization needs more than that time.



**Figure 1:** H&E stained slides, where the left is the test and the right x4 (& x20) shows the control; notice irregular connective tissue alignment, and the ill defined cells with areas of edema and signs of inflammation. The hair follicles and sebaceous glands are almost absent.



**Figure 2:** Mayer's hematoxylin stain-for the nuclei and cytoplasm. No differences were detected concerning nuclei appearance, shape, or positioning within the cytoplasm. Left sides are the test slides, and right sides are the controls.



**Figure 3:** Masson's trichrome staining in test slides -left, and control-right. Notice the thicker and more ordered collagen (appear as blue streaks) on the test slides.

## CONCLUSION

When a healthy mouse skin is subjected to intermittent negative pressure of (-20 to -25) mmhg for two weeks, the number of both sebaceous glands and hair follicles are expected to decrease and the thickness of collagen increases and changes its shape to a more organized arrangement. Features of inflammation such as WBCs infiltration could be transiently noticeable.

## Conflict of Interest

All authors declare that there are no conflicts regarding the article.

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None declared.

## Author contributions

Dr. Osama S. Abbadi designed the study, and carried the practical procedure; Dr. Dalal S. Idris participated in the development of the practical procedure, specimen preparation, and discussion writing. Dr. Khalid Nafie Fadelemula is the histopathology counselor and the critical reviewer of the experiment.

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