

Mechanical Properties of Skin Wounds after Atropa Belladonna Application in Rats

**Tomáš TOPORCER¹, Tomáš GREDEL¹, Boris VIDINSKÝ¹, Peter GÁL¹,
Ján SABO¹, Radovan HUDÁK^{2*}**

¹*Department of Medical Biophysics, Faculty of Medicine, Pavol Jozef Šafárik University,
Trieda SNP 1, Košice, 040 11, Slovak Republic*

²*Department of Instrumental and Biomedical Engineering, Faculty of Mechanical Engineering,
Technical University of Košice, Letná 9, Košice, 042 00, Slovak Republic*

Abstract

Atropa belladonna is a topical agent used in the treatment of skin wounds in Slovak folk medicine. The aim of this study was to assess the effect of A. belladonna extract on wound tensile strength in Sprague-Dawley rats.

Twenty four rats were randomly divided into 3 groups of 8 animals. Two symmetrical skin incisions were performed on the back of each animal and immediately sutured. In the 1st group A. belladonna extract was not applied and the group served as a control. The animals in the 2nd group were treated by daily application of A. Belladonna extract during the first two days after surgery. The animals in the 3rd group were treated during all five days after surgery. The wound tensile strength of each group was measured 120 hours after surgery. A separate group of 14 rats was used to measure the tensile strength of unwounded skin.

The mean tensile strength of both A. belladonna extract treated groups (2nd group: 244±48 g; 2.09±0.42% of unwounded skin; 3rd group: 254±67 g; 2.18±0.58% of unwounded skin) was significantly higher ($p < 0.05$) than the untreated group (1st group: 194±31 g; 1.67±0.26 % of unwounded skin).

Results from our investigation suggest a positive effect of A. belladonna on aseptic surgical skin wound healing

Keywords: wound healing, tensiometer, phytotherapy, inflammation, proliferation, maturation

Introduction

Wound healing is defined as a complex process of the replacement of dead tissue by living one.⁽⁹⁾ It means not only the reproduction of cells but also the recovery of damaged extracellular matrix.⁽¹⁴⁾ Wound healing runs in three basic phases, inflammatory, proliferative and maturation phase.⁽⁶⁾ These phases are not strictly separated but overlap each other. The inflammatory phase is divided into two reactions. First one, vascular reaction includes hemostasis, platelet degranulation and activation of the complement

and clotting cascade, which provide scaffold for the wound healing process.⁽¹⁷⁾ Second one, cellular reaction is characterized by the presence of inflammatory cells. The beginning of the cellular reaction of the inflammatory process is characterized by the prevailing presence of polymorphonuclear leukocytes.⁽⁶⁾ Macrophages take the governing role during the later phase of the inflammatory process.⁽¹⁷⁾ The inflammatory phase is continually replaced by the proliferative phase. The last phase of the wound healing is the maturation phase that can take longer than two years.

*corresponding author: radovan.hudak@tuke.sk

With regards to the present stage of surgery, especially plastic surgery, a number of experimental studies deal with new approaches improving the process of wound healing.^(5, 17, 19) Earlier sutures resection assures uniquely better cosmetic results.⁽³⁾ Skin sutures can be removed as soon as no longer mechanical support is needed. Therefore, the tensile strength of the wound is the objective and preferred measurement for wound healing evaluation and is adequate for experimental as well as for clinical evaluation.⁽¹¹⁾

Many products based on plants have been shown to possess therapeutic potential as promoters of wound healing.^(10, 16) Many scientists who are trying to develop new drugs from natural resources are looking towards the Ayurveda – the Indian traditional system of medicine. Several drugs from plants are described in the Ayurveda because of their positive influences on wound healing under the term *Vranaropaka*.⁽²⁾ *Atropa belladonna* (AB) is a topical agent used in the treatment of skin wounds in Slovak traditional medicine. The plant is commonly known as “Nadragula”.

The aim of this study was to evaluate, from a biomechanical point of view, the effect of *Atropa belladonna* on wound tensile strength five days after surgery.

Materials and Methods

Male Sprague-Dawley rats ($n = 52$) of similar age from 8 to 10 months were used for experiments. A separate group of 14 animals were sacrificed and used to measure the tensile strength of unwounded skin, Table 1. The data of tensile strength of unwounded skin were obtained with an electrical shredder ZD 100. Twenty four animals were randomized into 3 groups of 8 animals (per group) and used for wound healing evaluation. A combination of ketamine, xylazine and tramadol was used to anesthetize the rats. Two 3.5 cm long parallel full thickness skin incisions were performed under aseptic conditions on the left and right side of each experimental rat spine and immediately sutured. Non-absorbable monofil material was used for intradermal suture (Chirafilon 5/0, Chirmax a.s., Czech Republic).

AB was collected from natural forestry places in August. Tops (30 – 40 cm long) were cut

and dried at normal temperature in the shade. Eight grams of medicinal herb in one liter of boiled water were used for the preparing of the water tincture. After cooling down the tincture was filtered and the pure extract was used for the wound treatment.

The animals in the 1st group were not treated and the group served as a control. The animals in the 2nd group were treated during the first two days after surgery by daily application of AB extract. The animals in the 3rd group were treated during all five days after surgery. (see Table 1). Each application of the tincture lasted 8 minutes and was performed by dint of gauze sponge. The temperature of tincture during application was 40°C.

Table 1. Scheme of extract application and the duration of wound healing in each group.

	time of A. belladonna extract application	time of healing (observation)
1 st group	0 hours	120 hours
2 nd group	48 hours	120 hours
3 rd group	120 hours	120 hours

Animals from the 1st, 2nd and 3rd group were sacrificed by ether inhalation 120 hours after surgery. The wounds were carefully excised immediately after euthanasia to prevent post mortal transformation. The sutures were removed and the skin strip was placed between the two clamps of the tensiometer and the clamps were secured to avoid any slippage of the sample. Pulling was performed perpendicularly to the original direction of the incision. Maximal breaking strengths [g] were registered for each sample using a personal computer. The similar method was used as in our previous study.⁽⁸⁾ The data of maximal breaking strength of the wounds were expressed as a percentage of the maximal breaking strength of the unwounded skin (MBS of wounds / MBS of unwounded skin).

The main construction unit of the tensile strength tester is the stand with the moving arm, which transfers force from the sample to connected

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Honeywell's piezoelectric sensor FSG15N1A, working with a frequency of 10 Hz. As sensor-computer interface an intelligent module ADAM 4011 (Advantech Company) was used. To achieve vertical tensile force a servomechanism compatible with the range of piezoelectric sensor and covering continual tensile force increase to the breaking point of the sample was used. Data transmission to the computer was realized through the serial port. Software solution of data processing, recording and analyzing has been realized by means of Matlab software. Data were recorded and processed into beforehand defined files with the possibility of graphical visualization.

Data were statistically evaluated using the ANOVA Tukey-Kramer test. The level of statistical significance was $p < 0.05$.

Results

The maximal breaking strength of unwounded skin was 11 633 g. 25% increase of maximal breaking strength after 120 hours of healing and 48 hours tincture-application (2nd group) as well as 30% increase of maximal breaking strength after 120 hours of healing and 120 hours tincture-application (3rd group) compared to control (1st group) were recorded. Table 2. However, between the 2nd and the 3rd group no statistical difference was found. (Figs 1 and 2)

Table 2. Results of measurements.

Group	Maximal breaking strength (g)	breaking strength (% unwounded skin)
1 st	194 ± 31	1.67 ± 0.26
2 nd	244 ± 48	2.09 ± 0.42
3 rd	254 ± 67	2.18 ± 0.58

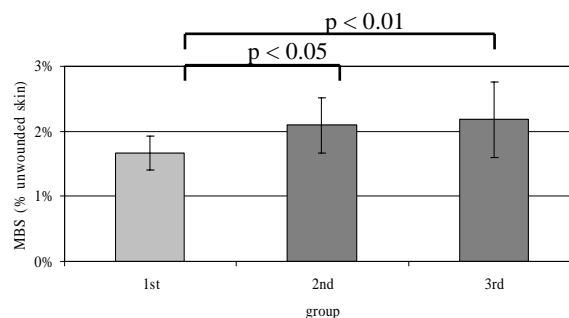


Figure 1. Comparison of maximal wound breaking strength between groups (MBS – maximal breaking strength).

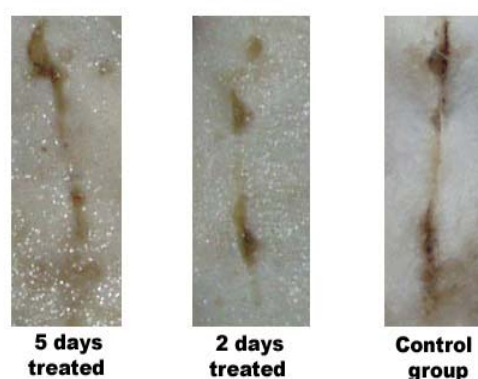


Figure 2. Pictures of wounds 120 hours after surgery (5 days treated – left, 2 days treated – middle, non-treated – right)

Discussion

There are numerous reports of positive effects of different medicinal herbs published in literature. Biswass, *et al.* (2003) in his study is looking toward the Ayurveda, the Indian traditional system of medicine. It reports about 130 medicinal herbs that have positive influence on wound healing, however, *A. belladonna* is not included. In our folk medicine *A. belladonna* is often used to improve wound healing and so this herb was chosen to assess its effect, from a biomechanical point of view, in our experimental study excluding the placebo effect. Folk medicine uses *A. belladonna* especially for septic posttraumatic wounds.⁽⁷⁾ We decided to use it for aseptic surgical skin wounds to differ the effects of antibiotic and immunogenic.

The measurement of maximal breaking strength (MBS) is the preferable method for wound healing evaluation.⁽¹¹⁾ The tensile strength of a one week healing wound is 3 – 5% of unwounded skin.^(9, 11, 14, 17) In our study the MBS after 5 days of healing in the control group was 1.67% of unwounded skin. This comparison shows that results of our study are similar with results of other published studies. However, different components are responsible for the increase of tensile strength of skin wounds during the healing time. In the first two days the major factor is fibrin, created in the process of haemostasis.⁽⁴⁾ According to Hudák *et al.* (2004) of mechanical tensile strength measurement of rat skin wounds the maturation phase of skin wound healing starts on the 6th day where the significant difference between the tensile strength of 5th and 6th day was recorded. Therefore the 5th day was chosen to evaluate if the tincture is able to accelerate the beginning of maturation. In our study statistical significant differences between the 3rd and the 1st group and between the 2nd and 1st group were recorded, after that we suggest that the beginning of the maturation phase in the 2nd and 3rd group was accelerated.

The increase of the wound tensile strength in the 2nd and 3rd group after 120 hours of healing can be caused by different mechanisms. According to Balzariny *et al.* (2000) *A. belladonna* can precipitate in wound healing by anti-inflammatory effect. The faster course of inflammatory phase can induce the earlier initiation of collagen production and this may explain the increase of wound tensile strength on the 5th day. However, there are also other mechanisms of the effect of this herb on wound healing. Antioxidant activities can improve the proliferation of cells into the injured area and so accelerate the synthesis of collagen.⁽¹⁸⁾ Other authors report about acceleration of the healing process by contraction of the wound area and increasing the wound tensile strength.⁽¹³⁾ Rane, *et al.* (2003) refers the increase of amount of hydroxyproline in granulation tissue in excisional wounds that indicates rapid collagen turnover and leads to accelerated healing.

Conclusions

This study demonstrates the positive effect of *Atropa belladonna* on aseptic surgical wound healing. Other studies are needed to explain the

specific mechanism of action and to negate the possible toxic effects of topical application.

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