

Effect of *Atropa belladonna* on wound healing in rats

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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 and collagen maturation in wistar rats. Wistar rats of either sex were divided into 2 groups. Group I: Animals served as wounded control, treated with normal saline. Group II: Animals were daily administered *Atropa belladonna* extract (0.3ml /100gm /rat) orally once daily from day 0 to 9 via intragastric tube. Dead space wound model was used for this study and granulation tissue was used to analyze the biochemical parameters of wound healing such as hydroxyproline, hexosamine and hexuronic acid. Significant increase in hydroxyproline and glycosaminoglycans content in granulation tissue were observed in rats treated with the extracts of *Atropa Belladonna*. The mean tensile strength of both *A. belladonna* extract treated groups was significantly higher than the untreated group. Present result shows that *Atropa* extract has significant wound healing promoting activity indicating collagen maturation as indicated by increase in hydroxyproline, hexosamine and hexuronic acid levels in the granulation tissue. The wound breaking strength also increased significantly.

Keywords: *Atropa belladonna*.

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INTRODUCTION

Wound healing is a process where the skin or other body tissue repairs itself after injury, where there is replacement of dead tissue by living one. It not only involves the reproduction of cells but also the recovery of damaged extracellular matrix.¹ Wound healing comprises of three basic phases, inflammatory, proliferative and maturation phase.² The inflammatory phase involves two reactions. First one being vascular reaction includes hemostasis, platelet degranulation, activation of the complement and clotting cascade, which provide basis for the wound healing process.³ Second one, cellular reaction

is characterized by the initial presence of polymorphonuclear leukocytes. Macrophages play a major role during the later phase of the inflammatory process.¹ The inflammatory phase is replaced by the proliferative phase. During **proliferation**, the wound is rebuilt and it involves angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction.⁴ **Maturation** is the final phase and occurs once the wound has closed. During maturation collagen is realigned and cells that are no longer needed are removed by apoptosis.⁵ The wound healing process is not only complex but also fragile, and it is susceptible to interruption or failure leading to the formation of non-healing chronic wounds.⁶ Many plant based products have wound healing promoting property. Considering economic aspects, applying natural products may be a feasible option of treatment in many regions of the world. In this respect, the *Atropa belladonna* L. (AB) aqueous extract has been used for promoting wound healing in the traditional medicine of Slovakia.⁷ But the effect of this herb is never being extensively verified experimentally thus prompted us to conduct this investigation. The aim of this study was to evaluate, from a biomechanical point of view, the effect of *Atropa*

belladonna on wound tensile strength and collagen maturation based on in hydroxyproline, hexosamine and hexuronic acid levels in the granulation tissue.

MATERIALS AND METHOD

All experimental protocols involving animals were conducted in accordance with the guidelines from Committee for the purpose of control and supervision of experiments on animals. The study designs were evaluated and were approved by the Institutional Animal Ethical Committee of Kasturba Medical College, Karnataka, India.

Animals: Wistar rats (150-250g) of either sex, bred locally were used. They were housed individually in polypropylene cages, fed pelleted rat chow (Hindustan lever rat pellets) and water ad libitum.

Wound Model: Dead space wound model was used for this study. Wounding procedures were carried out under light ether anesthesia. Dead space wounds were created by a small transverse incision in lumbar region. A polypropylene tube (2.5 x 0.5 cm) was implanted subcutaneously beneath the dorsal lumbar paravertebral skin. Granulation tissue formed around the polypropylene tube was harvested by careful dissection on 10th day and used for various investigations.

Experiment and Drugs: The animals were divided into 2 groups of 8 animals each. They were treated as described below.

Group I: Animals served as wounded control, treated with normal saline.

Group II: Animals were daily administered *Atropa belladonna* extract (0.3ml /100gm /rat) orally once daily from day 0 to 9 via intragastric tube.

Parameters: The harvested wet granulation tissue tensile strength was noted and was cut into two halves and their weights were noted. One piece was dried in the oven at 60 °C overnight and the dry weight was noted. Acid hydrolysate of dry tissue was used to determine hydroxyproline (OH-P), hexosamine (HA) and hexuronic acid (HUA).

Statistical analysis: The results were analyzed by using one-way ANOVA followed by Post hoc Scheffe's test using SPSS package and Students "t" test. P values <0.05 was considered statistically significant.

RESULT

Effect of *Atropa belladonna* extract on dry wt. tensile strength, hydroxyproline (OH-P), hexosamine(HA) and hexuronic acid (HUA) of granulation tissue.(Values are mean±SEand Number of animals in each group =8)

| Drug | Dry weight (mg/100 g) | Tensile strength (g) | OH-P(mg/g) mg/gdry tissue | HA(mg/g) mg/g dry tissue | HUA (mg/g) mg/g dry tissue |
|---------|-----------------------|----------------------|---------------------------|--------------------------|----------------------------|
| Control | 36.4 ± 2.4 | 254 ± 32 | 24 ± 3.2 | 11.92 ± 2.3 | 10.8 ± 2.4 |
| Atropa | 40.5 ± 1.2 | 326 ± 12 * | 32 ± 2.5 * | 16 .05 ± 1.3* | 15.3 ± 1.6* |

*P<0.05

DISCUSSION

There are large number of studies that been published which projects the positive effects of different medicinal herbs. Research on wound healing drugs is a developing area and scientists are trying to develop newer drugs from natural resources.⁸ There are studies which have mentioned large number of medicinal herbs with positive influence on wound healing.^{8,9} Biswass, *et al.* (2003) in his study is *A. belladonna* is not included. *A. belladonna* is often used in folk medicine of Slovakia to improve wound healing so this herb was chosen. The results of the present study clearly indicate that the *Atropa belladonna* extract possesses a definite wound healing property. Stimulation of wound strength is one of the most important factors in the wounds healing process.¹⁰ It depends upon the amount of newly deposited collage and also on the degradation of preformed collagen.¹¹ Tensile strength indicates how much the repaired tissue resists to break under tension and may indicate in part the quality of the repaired tissue.¹² In the present study, the tensile strength values of experimental group were higher than

the control group and were statistically significant. Tensile strength reflects the subdermal organization of the collagen fibers in the newly deposited collagen, higher values indicate increase in collagen concentration and stabilization of the fibers.¹⁰ Collagen is the extracellular protein in granulation tissue of a healing wound and is responsible for strength and integrity of tissue matrix. Hydroxyproline, product of collagen breakdown is used as the indicator of collagen turnover.¹³ In the study higher hydroxyl proline content of the granulation tissue represents the higher collagen content and turnover promoting wound healing with the concurrent increase in the tensile strength of the treated wound. Hexosamine (HA) and Hexuronic acid are synthesized from fibroblast, are matrix molecules are involved in the synthesis of new extracellular matrix. These are involved in the stabilization of collagen fibers and also control their ultimate alignment and characteristic size. In the present study these parameters were significantly increased in the study group. The increase in the tensile strength of the wound which is observed may not be only due to increased collagen deposition but may also be attributed

to its proper alignment and deposition. The present study observes that *Atropa belladonna* extract has wound healing promoting property as there is increase in the tensile strength which might be due to increased turnover of collagen evident from increased hydroxyl proline levels or by aiding the stabilization and alignment of the collagen, which is indicated by increased levels of hexosamine (HA) and hexuronic acid in study group or by both.

CONCLUSION

Atropa extract showed significant wound healing promoting activity indicating collagen maturation as indicated by increase in hydroxyproline, hexosamine and hexuronic acid levels in the granulation tissue. The wound breaking strength also increased significantly.

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