

# Hippophae rhamnoides L. and dexpanthenol-bepanthenone on blood flow after experimental skin burns in rats using $^{133}\text{Xe}$ clearance technique

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## Abstract

The aim of the present experimental study was to determine and compare the effect of *Hippophae rhamnoides* L. extract (HRe-1) and of dexpanthenol on the blood flow of a wound region, in rats using xenon-133 ( $^{133}\text{Xe}$ ) clearance technique. **Methods:** Burn wounds were made on both thighs of rats and, HRe-1 and dexpanthenol were applied topically on the wound region only in the right thigh for a period of 8 days. The effect of HRe-1 and of dexpanthenol on blood flow of the wound region was assessed before and after their topical application by using the  $^{133}\text{Xe}$  clearance technique. **Results:** HRe-1 increased significantly blood flow of the wound region ( $P < 0.05$ ). Dexpanthenol showed a smaller increase in blood flow. **In conclusion,** our results in rats suggest that HRe-1 increases blood flow of the wound area and can be used for the treatment of skin wound healing, preferably than dexpanthenol.

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## Introduction

**H**ippophae rhamnoides L. is a perennial plant native in Europe and in Asia [1]. It is a member of the Elaeagnaceae family, and widely distributed in the fields of north and east Anatolia [2]. Its fruits contain carotenes ( $\alpha$ ,  $\beta$ ,  $\delta$ ), vitamins C, E, riboflavin, folic acid, tannins, sugar, glycerides of palmitic, stearic and oleic acids, polyphenols and some essential amino acids [3]. Fruits of *Hippophae rhamnoides* L. extract (HRe-1) have been used extensively in traditional medicine in Turkey as well as in China and former Soviet Republics, to treat constipation, gastric ulcer, skin wounds and influenza infections [4]. Beneficial effects of HRe-1 have been shown in experimental gastric ulcer models [5, 6] and antioxidant activity has been shown in vitro, in cell cultures and animal studies. Different fractions of HRe-1 fruit, inhibit 2,2-azobis (2,4-dimethylvaleronitrile) and ascorbate-iron induced lipid peroxidations in vitro [7]. HRe-1 decreases the malondialdehyde (MDA) content in hyperlipidemic rabbit serum cultured, smooth muscle cells [8]. Seed oil of HRe-1 inhibits MDA formation of liver induced by  $\text{CCl}_4$ , acetaminophen and ethyl alcohol and also prevents the acetaminophen-induced glutathione depletion in the liver [9]. The prevention of glutathione depletion by HRe-1 is also reported in gastric tissue in ethanol administered rats [5]. Additionally, HRe-1 has beneficial effects on nicotine-induced lipid peroxidation in rat blood [10] and brain [11]. However, according to our knowledge, no study has reported the effects of HRe-1 on tissue blood flow in wound region.

Dexpanthenol is the alcohol of pantothenic acid, a vitamin of the B complex and the inactive form of coenzyme A. It is used for topical application, which is characterized by good skin penetration and high local concentrations when administered in water-in-oil emulsions. Dexpanthenol is readily oxidized to pantothenic acid, stimulating regeneration of damaged permeability barriers of the skin and reducing local signs of inflammation [12, 13]. Dexpanthenol also increases fibroblast proliferations and accelerates re-epithelialization in wound healing [14, 15]. Moreover, topical anti-inflammatory effects have been observed in different situations [13, 14, 16].

The most thoroughly studied and often used blood flow technique is the xenon-133 ( $^{133}\text{Xe}$ ) clearance technique of Sejrsen [17].  $^{133}\text{Xe}$  is an inert lipophilic gas that can easily cross cellular membranes. The basic principle is that the clearance of a freely diffusible tracer from a tissue is determined only by blood supply to the tissue, therefore reflects local blood flow [18]. The  $^{133}\text{Xe}$  clearance technique has been used in many experimental and clinical investigations of blood flow changes [19-24].

Hence, the aim of this study was to determine and compare the effect of HRe-1 and dexpanthenol on the blood flow of experimental wounds in rats as part of the healing process using the  $^{133}\text{Xe}$  clearance technique.

## Materials and methods

### Animals

Sixteen male Sprague-Dawley rats (225±25g each), fed with standard laboratory chow and water, were used in this study and were randomly divided into Groups A (HRe-1, n=8) and B (dexpanthenol, n=8). The experiments were performed in an ethically proper way by following guidelines as set by the Ethical Committee of the University in accord with the international standards of experimental work on animals.

### Experimental protocol

Full-skin-thickness burns were produced by the method of Arons' burn model [23]. Under light ether anesthesia, rats were injected intraperitoneally with ketamin-HCl (20 mg/kg, Ketalar®). After careful shaving of the surface of both thighs, each rat was exposed to heated metal apparatus with a diameter of 1cm (90°C, 20s). Thus, a second-degree burn was formed in both thighs.

On the wound region in the right thigh of the rats in Group A and B HRe-1 and dexpanthenol (Bepanthen®) were applied topically for a period of 8 days, respectively. The left burned thigh was used as a control and was not treated.

### Measurement of blood flow

Two separate blood flow measurements were carried out one in the anaesthetized rats before the burn and the other after the two drugs were applied. Blood flow measurement of the wound of the left thigh used as control, was compared with the treated wound in the right thigh. A sterile saline solution containing 1.85MBq  $^{133}\text{Xe}$  (Dupont Pharma SA, Brussels, Belgium) was injected intradermally at the wound margins of both thighs. Immediately after  $^{133}\text{Xe}$  injection, the anesthetized rats were placed under a single-head gamma camera with a parallel-hole, low-energy, high-resolution collimator (GE-Starcam 4000 XR/T, St Albans, Hertfordshire, UK). A dy-

namic image series were acquired in a frame mode of 64x64 matrix at one frame per 30sec for 10min. Time-activity curves were generated from the regions of interest (ROI) at the sites of injection. The clearance half-time of  $^{133}\text{Xe}$  ( $T_{1/2}$ ) was measured from these curves. Blood flow,  $f$ , was calculated by the relation:

$$f = (\lambda \times \ln 2 \times 100 \text{g tissue}) / T_{1/2}$$

where

$f$  = cutaneous blood flow in ml/100g tissue/min

$\lambda$  = partition coefficient of  $^{133}\text{Xe}$  between tissue and blood (0.7 ml/g) [24]

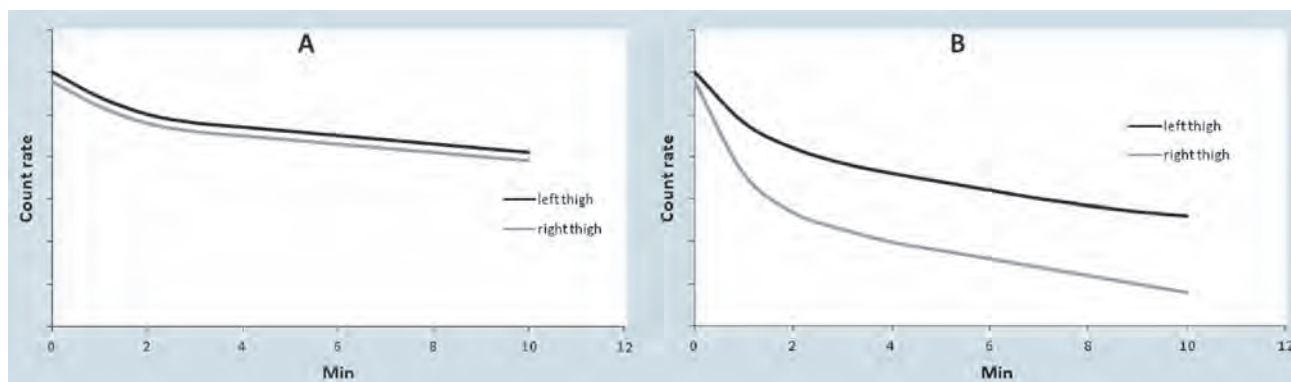
### Statistical analysis

The mean value of the two blood flow measurements from each treated wound in the right thigh and each untreated wound in the left thigh, was calculated. Statistical analysis for blood flow values between HRe-1 and dexpanthenol groups, before and after treatment, was done by using the Wilcoxon signed rank test and the Mann-Whitney U test. Differences with a  $P$  value less than 0.05 were accepted as significant, with data in the text presented as mean±standard deviation (SD). SPSS version 11.5 (SPSS Inc., Chicago, IL) software program, was used for statistical analysis.

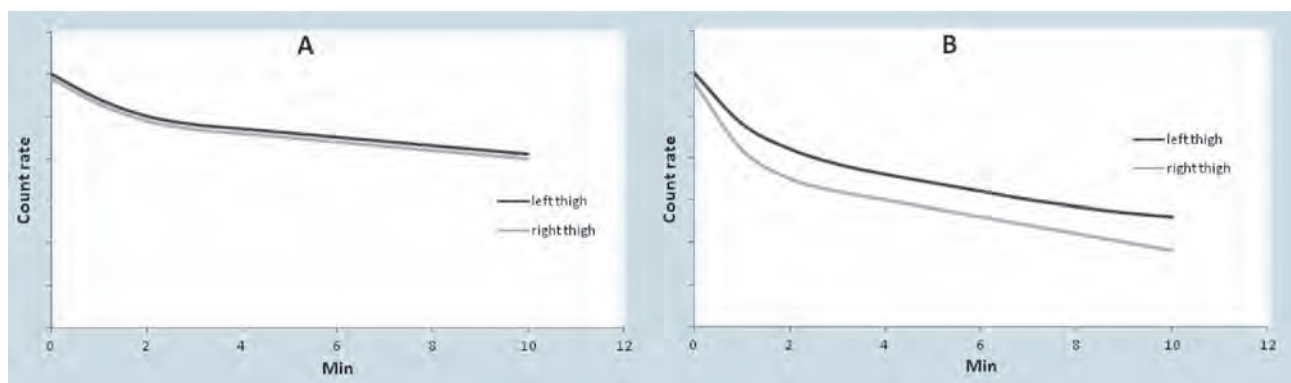
## Results

The clearance curves of  $^{133}\text{Xe}$  from treated and untreated wound regions before and after HRe-1 and dexpanthenol treatments, are shown in Figures 1 and 2. Table 1 shows the detailed statistical results. The blood flow of treated wound regions before treatment, was the same in both groups (0.09±0.01ml/100g/min). After treatment, the blood flow of treated wound regions was 0.25±0.07 and 0.16±0.43ml/100g/min for HRe-1 and dexpanthenol groups, respectively. These values for HRe-1 were significant ( $P<0.05$ ) while for dexpanthenol were not ( $P>0.05$ ).

Blood flow of untreated wound regions as a control at the beginning of the study was 0.08±0.01 and 0.09±0.01ml/100g/min, and at the end of the study was 0.12±0.01 and 0.13±0.02 ml/100g/min for HRe-1 and dexpanthenol groups, respectively, not significant by different for both Groups ( $P>0.05$ ).



**Figure 1.** Xenon-133 clearance curves from treated (at the right thigh) and untreated (at the left thigh) wound regions, before (A) and after (B) HRe-1 treatment.



**Figure 2.** Xenon-133 clearance curves from treated (at the right thigh) and untreated (at the left thigh) wound regions, before (A) and after (B) dexpantenol treatment.

**Table 1.** The blood flows of treated and untreated wound regions before and after HRe-1 and dexpantenol treatments. Data in the text presented as mean±standard deviation (SD).

	HRe-1			Dexpantenol		
	Before	After	P	Before	After	P
Treated wound region (the right thigh)	0.09±0.01	0.25±0.07	<0.05	0.09±0.01	0.16±0.43	>0.05
Untreated wound region (the left thigh)	0.08±0.01	0.12±0.01	>0.05	0.09±0.01	0.13±0.02	>0.05
P	>0.05	<0.05		>0.05	>0.05	

## Discussion

The process of wound healing is essential to prevent inflammation and to partially or completely reform the damaged tissue. Wound healing process involves granulation, fibrogenesis, neo-vascularization, wound contraction and epithelialization [25, 26]. Up to now, no study has reported the effects of HRe-1 and dexpantenol on blood flow in the wound region. The beneficial effects of HRe-1 have been shown in experimental gastric ulcer models [5, 6] and in wound healing [26-29]. Antioxidant activity of HRe-1 has also been shown *in vitro*, in cell culture and in animal studies [5, 7-11, 26].

Dexpantenol stimulates the regeneration of damaged permeability barriers of the skin reducing local signs of inflammations [12, 13]. Dexpantenol also increases fibroblasts proliferation and accelerates re-epithelialization in wound healing [14, 15]. Moreover, anti-inflammatory effects have been observed in different clinical situations [13, 14, 16].

*In conclusion*, our results in rats suggest that HRe-1 increases blood flow of the wound area and can be used for the treatment of skin wound healing, preferably than dexpantenol.

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