Effects of *extractum cepae*, heparin, allantoin gel and silver sulfadiazine on burn wound healing: an experimental study in a rat model

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ABSTRACT: These experiments were conducted in order to compare the effects of *extractum cepae*, heparin, allantoin gel (CTBX) and silver sulfadiazine (SSD) cream on burn wound healing in rats. Thirty six adult, female Wistar albino rats were divided into three equal groups. A burn was made on the back of all rats. The burned areas in the first, second and third groups were covered with cold cream (control), SSD skin cream and CTBX twice a day, respectively. Seven and 14 days later, the rats were sacrificed and burned skin tissue samples were collected from the rats for histopathological examinations. Histopatological evaluations on the 7^{th} and 14^{th} days showed burn healing to be better in the CTBX and SSD groups with respect to the control group. The best burn wound healing was observed in the CTBX group ($P < 0.001$). Wound healing was significantly different between the groups at days 7 and 14 ($P < 0.001$). In conclusion, application of CTBX has significant positive effects on the healing of burn wounds in a rat model.

Keywords: *extractum cepae*; heparin; allantoin; silver sulfadiazine; burn; rat

Silver sulfadiazine (SSD) is the topical agent of choice for severe burns and is used almost universally today in preference to compounds such as silver nitrate and mafenide acetate. SSD cream, while being effective, causes some systemic complications including neutropenia, erythaema multiforme, crystalluria and methemoglobinaemia (Gracia 2001; Hosnuter et al. 2004; Han et al. 2005; Durmus et al. 2009).

The plant *Allium cepae* Linn. belongs to the family Liliaceae and contains the active agents kampferol, β-sitosterol, ferulic acid, myritic acid and prostaglandins (Prakash et al. 2007). *Allium cepae* Linn. have been proven to exert antidiabetic, anti-oxidant, anti-hypertensive, anti-thromobotic, hypoglycaemic and antihyperlipidaemic effects (Compos et al. 2003; Sakai et al. 2003; Dhanprakash and Garima 2007; Shenoy et al. 2009; Venkatanarayana et al. 2010). When applied to scars, significant improvements in collagen organisation have been shown in previous experimental studies (Willital and Heine 1994; Hosnuter et al. 2007).

Heparin has also been reported harbour beneficial properties with regard to wound healing. Studies have revealed that heparin has antiinflammatory, neoangiogenic, collagen-restoring, epithelialising, antiadhesive and anticoagulating effects (Saliba 1997; Reyes et al. 2001; Venakatachalapathy et al. 2007; Durmus et al. 2011). Heparin strengthens the anti-inflammatory effects of *extractum cepae* and can enhance collagen restoration (Saliba 2001). Moreover, no negative effects upon using heparin to treat burn wounds in humans have been reported.

Allantoin, meanwhile, has been shown to promote cell proliferation (removal of necrotic tissue) and epithelialisation (skin growth) and thereby hastens the growth of new healthy tissue over wounds and sores. Allantoin is also a counterirritant that helps alleviate skin irritation. Allantoin has emollient and soothing properties and can reduce inflammation (Hosnuter et al. 2007; Campanati et al. 2010; Muangman et al. 2011).

Contractubex (Merz Pharma, Frankfurt, Germany) (CTBX) is a gel containing primarily *Allium cepae*

(onion extract) as well as 50 IU sodium heparin and 1% allantoin. In clinical practice, this drug is generally used for hypertrophic scars and keloids (Hosnuter et al. 2007; Koc et al. 2008; Karagoz et al. 2009).

The purpose of this study was to compare the effects of *extractum cepae*, heparin, allantoin gel (CTBX) and silver sulfadiazine (SSD) cream on clinical and histological healing rates of skin burn wounds in a rat model with the aim of evaluating the effectiveness of the tested agents for the healing of burn wounds in domestic animals.

MATERIAL AND METHODS

Experimental animals and protocol design

This study was carried out in thirty six, adult, female Wistar albino rats (five months old, weight between 200 and 220 g) housed in a climate-controlled animal care facility. The rats had free access to water and to standard rodent feed. Guidelines for the care and use of animals approved by the relevant institution were followed and the local ethics committee approved this study.

The rats were anaesthetised with single intramuscular injection of 6 mg/kg xylazine hydrochloride (Rompun, Bayer, 23.32 mg/ml) and 85 mg/kg ketamine hydrochlorure (Ketalar, Parke-Davis, 50 mg/ml).

The backs of the rats were shaved and prepared with 10% antiseptic povidone-iodine solution (Kim-Pa, Poviiodeks, 10% povidone-iodine) and burns of 1 cm in diameter were established. Skin burns were created bilaterally as described by Hosnuter et al. (2004), Han et al. (2005), Durmus et al. (2009) and Yaman et al. (2010). Animals were subjected to full-thickness second-degree skin burns with 1 cm surface area diameter with the use of brass probes. The brass probe was immersed in boiling (100 °C) water until thermal equilibrium was reached and it was then placed without pressure for 20 s on the backs of the rats. All animals were immediately resuscitated with lactated Ringer's solution (2 ml/100 g body weight) applied intraperitoneally.

The rats were randomly divided into three equal groups. Immediately after the burns were made, the burned areas in the first (n = 12), second (n = 12) and third groups (n = 12) were covered with cold cream (Botafarma, 12.5% spermaceti + 12% white wax + 56% liquid paraffin + 0.5% borate of soda + 19% distilled water) (Control), SSD skin cream (Silverdin, Deva, Silver sulfadiazine, 10 mg/g) and CTBX gel (Contratubex, Merz, 100 mg *extractum cepae* + 50 IU heparin + 10 mg allantoin per 1 g gel) twice a day, respectively. These applications were repeated every day. The wounds were clinically observed in all groups every day. Seven and 14 days later, the rats were sacrificed after being anaesthetised.

Histopathological examination

Burned skin tissue samples were collected after sacrificing the rats for histopathological examination purposes. These tissue samples were fixed in 10% neutralbuffered formalin solution, embedded in parafin wax, cut into 5 μm-thick sections and stained with haematoxyline-eosin and Masson's trichrome stain for examination by light microscopy.

Statistical analyses

The thickness of granulation tissue was examined and recorded at the center of each wound. Statistically, all data are expressed in milimeters as mean ± standard error. The differences between Days 7 and 14 were compared using the Mann-Whitney U test. The differences between groups were compared using the Kruskal-Wallis test. Statements of statistical significance are based on $P < 0.05$. These analyses were carried out using SPSS statistical analysis system (Relase 10.0, SPSS. Inc).

RESULTS

No mortality was observed throughout the course of the study.

Wounds in the control group displayed a greater degree of inflammation on the basis of the three clinical signs of the inflammatory process: heat, redness and swelling, which appeared to be less in wounds treated with SSD and CTBX.

A scab formed by necrotic tissue remnants and mononuclear cell infiltration was present in all groups on the 7th day of the trial. Inflammatory cell infiltration without an epithelial layer was noted under the scab. Vessels were hyperaemic in the dermis and there were no hair follicles, seba-

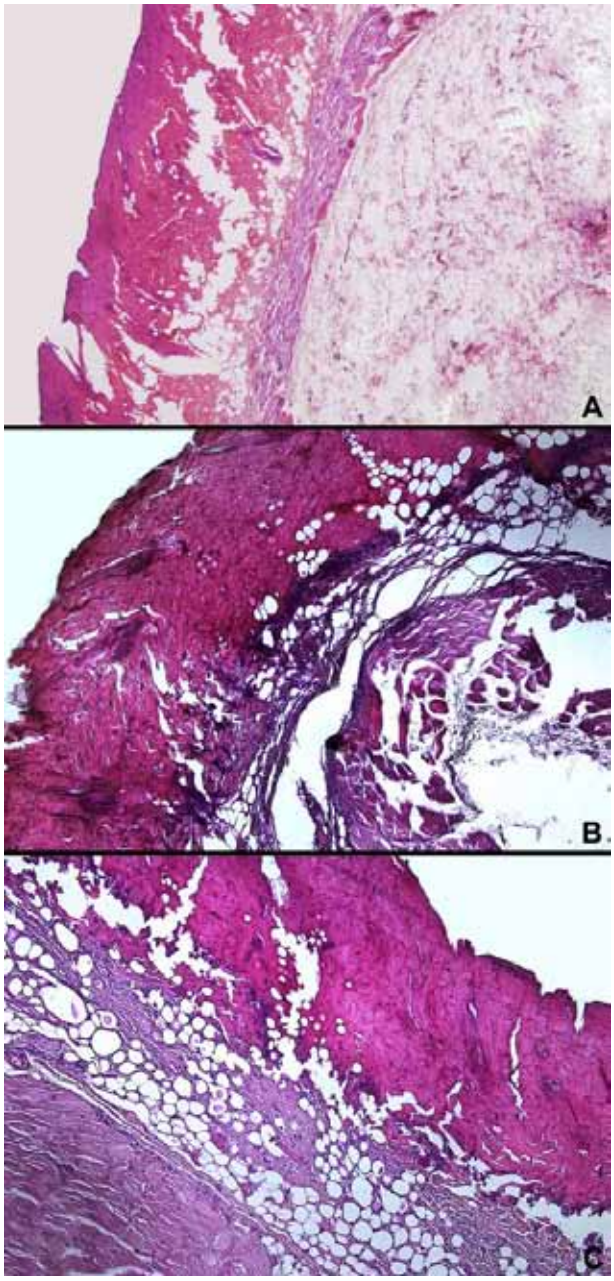

Figure 1. Microscopic appearance of burned skin on the 7th day. A = control group; B = SSD group; C = CTBX group (H&E, 100×)

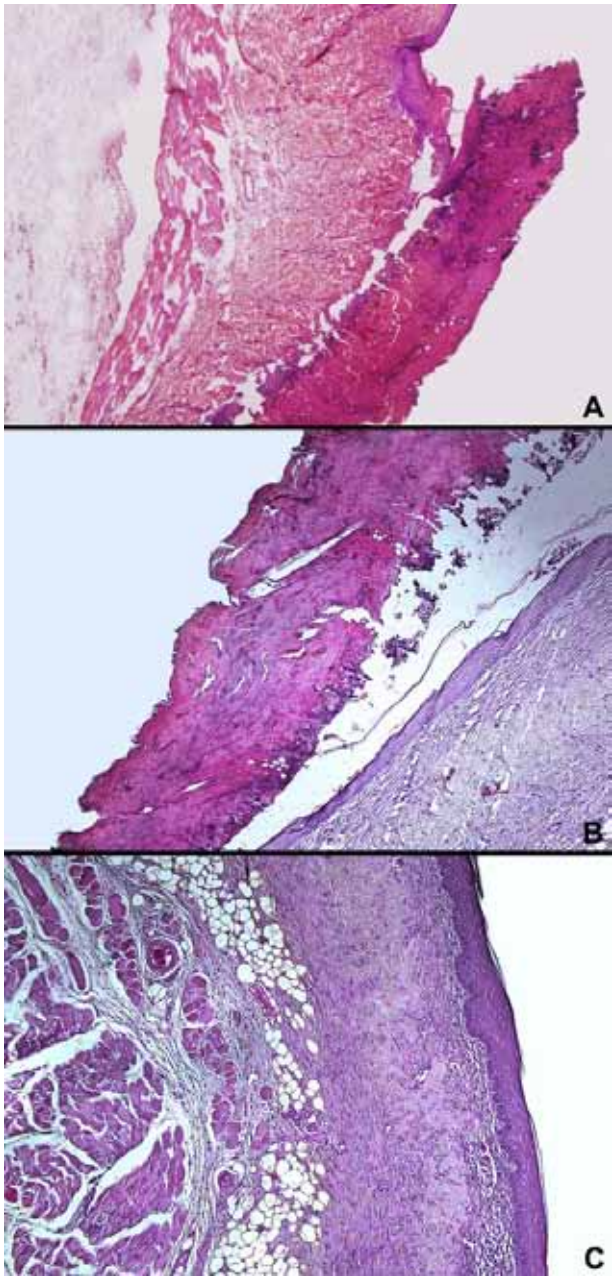

Figure 2. Microscopic appearance of burned skin on the 14th day. A = control group; B = SSD group; C = CTBX group (H&E, 100×)

ceous or sweat glands in rats from any of the groups (Figure 1).

On the 14th day of the trial, scabs became thinner in the control and SSD groups, but had totally exfoliated in the CTBX group. A thin epithelial layer under the scab in the SSD group and no epithelial layer in the control group were observed. However, this layer was observed to be fully formed in the CTBX group (Figure 2).

Histopathological evaluations on Days 7 and 14 showed the burn healing to be better in the SSD and CTBX groups with respect to the control group. The best healing was observed in the CTBX group.

Wound healing was significantly different among the groups at Days 7 and 14 ($P < 0.001$). The thickness of the granulation tissue was significantly different between each group on the 7th and the 14th days of the trial ($P < 0.001$). The mean values

Table 1. Thickness (mm) of granulation tissue in the centre of the wound

Groups (n = 12)	Days	
	7	14
Control	0.38 ± 0.01^{aA}	0.41 ± 0.01^{aB}
SSD	0.59 ± 0.02^{bA}	1.51 ± 0.01^{bB}
CTBX	0.93 ± 0.01^{cA}	2.05 ± 0.06^{cB}

^{abc}values in the same column with different superscripts are significantly different ($P < 0.001$)

^{AB}values in the same row with different case letters are significantly different ($P < 0.001$).

of thickness of granulation tissue in the centre of the wounds for the control, SSD and CTBX groups are shown in Table 1.

DISCUSSION

Burns remain a public health issue, especially in terms of morbidity and long-term disability, throughout the world. Burn wounds are painful maladies and they entail a complex healing process. Moreover, they cause severe discomfort and are prone to infection and other complications.

Test animals such as the rat have been used in various studies (Hosnuter et al. 2004; Han et al. 2005; Gal et al. 2008; Durmus et al. 2009; Yaman et al. 2010; Sabol et al. 2012). The model used here is simple and reproducible. The burn wound healing model provides an *in vivo* approach for studying the healing of burn-related wounds in domestic animals.

The backs of the rat appears to be a suitable model for the study of burn healing and we have utilised this model because of its simplicity. The methods in this study were similar to those in previous experimental and clinical burn studies (Hosnuter et al. 2004; Han et al. 2005; Durmus et al. 2009; Yaman et al. 2010).

Antioxidants scavenge free radicals and are associated with a reduced risk of cancer and cardiovascular diseases. *Allium cepae* L. has been reported to have antimicrobial, antispasmodic, anticholesterolaemic, hypotensive, hypoglycaemic, antiasthmatic, anticancer and antioxidant properties. Polyphenols, anthocyanins, flavonoids, quercetin, kaempferol and their glycosides have been reported in onions (Compos et al. 2003; Sakai et al. 2003; Dhanprakash and Garima 2007; Prakash et al. 2007; Venkatanarayana et al. 2010). Shenoy et al. (1997) reported that in an excision wound model the increased rate of wound contraction and decrease in period of epithelisation in the animals treated with an alcohol extract of *Alium cepae* may be attributed to their broad spectrum antibacterial activity. The effect of Contractubex gel (Merz Pharma, Frankfurt, Germany) (components: 100 mg *extractum cepae* + 50 IU heparin + 10 mg allantoin per 1 gram of gel) on collagen synthesis and its loosening effect on collagen structure may promote physiological scar development (Willital and Heine 1994).

The findings in this study using CTBX were similar to those in previous experimental and clinical burn studies that established the rationale and method of using heparin (Saliba 1997, 2001; Reyes et al. 2001). It was reported that heparin relieved pain, reduced inflammation, prevented burn extension, shortened healing, and resulted in smooth healed skin without scars or contractures. Heparin also has anti-inflammatory effects and helps water to bind to scar tissue. Further, heparin helps to increase microcirculation (Temiz et al. 2009). Other notable benefits upon use of heparin included shortened revascularisation time, reduction in swelling and a reduction in the number of procedures (Reyes et al. 2001). The erythema, swelling, and heat signs characteristically associated with inflammation were reduced in the rats of the CTBX group (Figures 1 and 2). Thus, in this study, anti-inflammatory effects were evident with CTBX, as reported in previous studies (Saliba 1997, 2001; Reyes et al. 2001).

Heparin stimulates capillary endothelial cells to migrate into ischaemic tissues where they multiply and form new capillary blood vessel systems which, on perfusion with blood, restor blood flow into the ischaemic tissues (Saliba 2001; Venakatachalapathy et al. 2007). Heparin was reported to initially accelerate collagen production and deposition, and in the second phase decelerated and reabsorbed collagen, which would tend to inhibit fibrin accumulation and scar formation (Venakatachalapathy et al. 2007). In response to heparin application, the burn blisters, which are not removed and rarely become infected, function as natural skin grafts that required no further care. Smooth new skin is evident beneath the dried thin blister usually within 7–14 days (Saliba 2001; Venakatachalapathy et al. 2007). Several burn studies (Saliba 1997, 2001; Reyes et al. 2001) have tested large doses of heparin

topically and parenterally and significant therapeutic effects have been reported. This study showed that the topical use of CTBX can be reccomended for burn healing and that the benefits continue into final healing with the result of smoother skin. CTBX enhanced revascularisation, shortened healing, and resulted in smooth skin without scars or contractures.

Allantoin is known to have supportive effects on the primary and secondary wound healing process (Hosnuter et al. 2007; Koc et al. 2008; Karagoz et al. 2009; Muangman et al. 2011). It was shown that heparin, *cepae extract* and allantoin mixtures decrease dermal scar formation more effectively than steroids (Beuth et al. 2006; Temiz et al. 2009). Temiz et al. (2009) reported that this mixture also decreased the inflammatory reaction of tissue both in acute and chronic phases.

In conclusion, burn care was improved and simplified by the topical use of CTBX. CTBX is readily available and affordable and, when expediently administered, is an effective and affordable treatment for burn wounds.

REFERENCES

Beuth J, Hunzelmann N, Van Leendert R, Basten R, Noehle M, Schneider B (2006): Safety and efficacy of local administration of contractubex to hypertrophic scars in comparison to corticosteroid treatment. Results of a multicenter, comparative epidemiological cohort study in Germany. In Vivo 20, 277–283.

Campanati A, Savelli A, Sandroni L, Marconi B, Giuliano A, Giuliodori K, Ganzetti G, Offidani A (2010): Effect of allium cepa-allantoin-pentaglycan gel on skin hypertrophic scars: clinical and video-capillaroscopic results of an open-label, controlled, nonrandomized clinical trial. Dermatologic Surgery 36, 1439–1444.

Compos KE, Diniz YS, Cataneo AC, Faine LA, Alves MJ, Nobeli EL (2003): Hypoglycemic and antioxidant effect of onion, Allium cepa: diatary onion addition, antioxidant activity and hypoglycic effect on diabetic rats. International Journal of Food Sciences and Nutrition 54, 241–246.

Dhanprakash BN, Garima U (2007): Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 102, 1389–1393.

Durmus AS, Han MC, Yaman I (2009): Comperative evaluation of collagenase and silver sulfadiazine on burned wound healing in rats. Firat Universitesi Saglik Bilimleri Veteriner Dergisi 23, 135–139.

Durmus AS, Yildiz H, Yaman M, Simsek H (2011): The effects of heparin and pentoxifylline on prevention of intra-abdominal adhesions in rat uterine horn models: histopathological and biochemical evaluations. Revue de Medecine Veterinaire 162, 198–203.

Gal P, Kilik R., Mokry M, Vidinsky B, Vasilenko T, Mozes S, Bobrov N, Tomori Z, Bober J, Lenhardt L (2008): Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semi-quantitative and quantitative histological assessments. Veterinarni Medicina 53, 652–659.

Gracia CG (2001): An open study comparing topical silver sulfadiazine and topical silver sulfadiazine-cerium nitrate in the treatment of moderate and severe burns. Burns 27, 67–74.

Han MC, Durmus AS, Karabulut E, Yaman I (2005): Effects of Turkish propolis and silver sulfadiazine on burn wound healing in rats. Revue de Medecine Veterinaire 156, 624–627.

Hosnuter M, Gurel A, Babuccu O, Armutcu F, Kargi E, Isikdemir A (2004): The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns 30, 121–125.

Hosnuter M, Payasli C, Isikdemir A, Tekerekoglu B (2007): The effects of onion extract on hypertrophic and keloid scars. Journal of Wound Care 16, 251–254.

Karagoz H, Yuksel F, Ulkur E, Evinc R (2009): Comparison of efficacy of silicone gel, silicone gel sheeting, and topical onion extract including heparin and allantoin for the treatment of postburn hypertrophic scars. Burns 35, 1097–1103.

Koc E, Arca E, Surucu B, Kurumlu Z (2008): An open, randomized, controlled, comparative study of the combined effect of intralesional triamcinolone acetonide and onion extract gel and intralesional triamcinolone acetonide alone in the treatment of hypertrophic scars and keloids. Dermatologic Surgery 34, 1507–1514.

Muangman P, Aramwit P, Palapinyo S, Opasanon S, Chuangsuwanich A (2011): Efficacy of the combination of herbal extracts and a silicone derivative in the treatment of hypertrophic scar formation after burn injury. African Journal of Pharmacy and Pharmacology 5, 442–446.

Prakash D, Singh BN, Upadhyay G (2007): Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 102, 1389–1393.

Reyes A, Astiazaran JA, Chavez CC, Jaramilla F, Saliba MJ (2001): Burns treated with and without heparin: Controlled use in a thermal disaster. Annals of Burns and Fire Disasters 14, 183–191.

Sabol F, Dancakova L, Gal P, Vasilenko T, Novotny M, Smetana K, Lenhardt L. (2012): Immunohistological

changes in skin wounds during the early periods of healing in a rat model. Veterinarni Medicina 57, 77–82.

Sakai Y, Murakami T, Yamamoto Y (2003): Antihypertensive effect of onion on NO synthase inhibitor induced hypertensive rats. Bioscience Biotechnology and Biochemistry 67, 1305–1311.

Saliba MJ Jr (1997): The effects and uses of heparin in the care of burns that improves treatment and enhances the quality of life. Acta Chirurgiae Plasticae 39, 13–16.

Saliba MJ Jr (2001): Heparin in the treatment of burns: a review. Burns 27, 349–358.

Shenoy C, Patil MB, Kumar R, Patil S (2009): Preliminary phytochemical investigation and wound healing activity of Allium cepa linn (liliaceae). International Journal of Pharmacy and Pharmaceutical Sciences 2, 167–175.

Temiz C, Temiz P, Sayin M, Ucar K (2009): Effect of cepea extract-heparin and allantoin mixture on epidural fibrosis in a rat hemilaminectomy model. Turkish Neurosurg 19, 387–392.

Venakatachalapathy TS, Mohan Kumar S, Saliba MJ (2007): A comparative study of burns treated with topical heparin and without heparin. Annals of Burns and Fire Disasters 20, 189–198.

Venkatanarayana D, Kumar AS, Lakshmi SM (2010): Review on natural wound healing agents. International Journal of Phytopharmacy Research 1, 1–4.

Willital GH, Heine H (1994): Efficacy of Contractubex gel in the treatment of fresh scars after thoracic surgery in children and adolescents. International Journal of Clinical Pharmacology Research 14, 193–202.

Yaman I, Durmus AS, Ceribasi S, Yaman M (2010): Effects of Nigella sativa and Silver sulfadiazine on burn wound healing in rats. Veterinarni Medicina 55, 619–624.

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