Effects of Turkish Propolis and Silver Sulfadiazine on Burn Wound Healing in Rats

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SUMMARY

This experiment was conducted to compare 50 % propolis cream and silver sulfadiazine (SSD) for healing of burned wounds in rats. Sixty male Wistar albino rats were divided into three equal groups. A burned model was constituted on the back of all rats. The burned areas in the first, second and third groups were covered daily with 50 % propolis, SSD skin cream and cold cream (control) respectively. Ten and 21 days later the rats were anaesthetized and the burned skin tissue samples were collected from rats for histopathological examinations. In conclusion, application of 50 % propolis cream is significantly effective in healing of burned skin wounds in rat model.

Keywords : propolis - silver sulfadiazine - burn - rat.

RÉSUMÉ

Effets du Propolis turc et de la Sulfadiazine argentique sur la cicatrisation des brûlures cutanées chez les rats. Par M. C. HAN, A. S. DURMUS, E. Karabulut et I. Yaman.

L'efficacité du propolis dans le traitement des brûlures cutanées a été comparée à celle de la sulfadiazine argentique (SSD). L'étude a été réalisée sur soixante rats mâles Wistar brûlés en région dorsale. Les rats ont été divisés trois lots égaux. Le premier lot a été traité quotidiennement avec la préparation contenant le propolis, le deuxième lot avec la préparation contenant le SSD et le dernier lot (lot contrôle) avec la crème cold. Dix et 21 jours plus tard les rats ont été anesthésiés et des échantillons de peau brûlée ont été réalisés pour des examens histopathologiques. En conclusion, l'étude montre, de façon significative, que le propolis favorise la cicatrisation des brûlures cutanées dans le modèle de rat.

Mots-clés : propolis - sulfadiazine argentique - brûlure - rat.

Abbreviations :

SSD : silver sulfadiazine ; SOD : superoxide dismutase ; PGs : prostaglandins ; LTs : leucotrienes ; NSAIDs : non-steroidal antiinflammatory drugs ; CAPE : caffeic acid phenethyl ester.

1. Introduction

Topical application of honey and propolis to burn wounds and other wounds has been found to be effective in controlling infection and producing a clean granulating bed [4,5,10,11]. Propolis is a natural product from the honey bee (bee glue). The extract is an extremely complicated mixture of natural substances. It contains aminoacids, phenolic acids, phenolic acids esters, flavanoids, cinnamic acid, terpens and caffeic acid. These substances have been shown to exert a variety of medical properties, such as anti-microbial activity [4,13].

Silver sulfadiazine (SSD) is the topical agent of choice in 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 which include neutropenia, erythema sultiforme, crystalluria and methaemoglobinemia [6,7,21].

The purpose of this study was to compare healing rates of wounds treated with propolis dressing and with silver sulfadizine clinically and histologically in rat model.

2. Materials and Methods

2.1. MATERIALS

Experiment was conducted in the Animal Hospital of Veterinary Faculty of the Firat University of Turkey in accordance with usual guidelines.

Turkish propolis used in this study was obtained from The Civan Bee Farm in Bursa, Turkey.

Extracts of propolis sample was prepared and used throughout this work as described by ISLA *et al.* [9] with minor modifications. Propolis was frozen at -20°C, and ground in a chilled mortar. Then, the round powder was extracted with ethanol (15 ml of 80 % ethanol/g of propolis) with continuous stirring at room temperature for 24 h. The suspension was let to sediment for 3 days. The supernatant was then concentrated in a evaporator under reduced pressure at 40°C and the residue was mixed within same amount of 50 % cold cream (Botafarma, cold creme, 12.5 % spermaceti + 12 % white wax + 56 % liquid paraffin + 0.5 % borate of soda + 19 % distilled water).

2.2. EXPERIMENTATION

Experiment was carried out in 60 male Wistar albino rats weighing between 250 and 300 g. The rats were obtained from the Veterinary Control and Research Institue in Elazig, Turkey. Animals were housed at 21°C with a day/night cycle of 12 h. They had free access to water and standard rodent feed.

Rats were divided randomly into three equal groups. They were anaesthetized with single intramuscular injection of 85 mg/kg ketamine hydrochlorure (Parke-Davis, Ketalar, ketamine hydrochlorure, 50 mg/ml) and 6 mg/kg xylazine hydrochloride (Bayer, Rompun, Xylazine hydrochloride, 23.32 mg/ml). The backs of the rats were shaved and prepared with

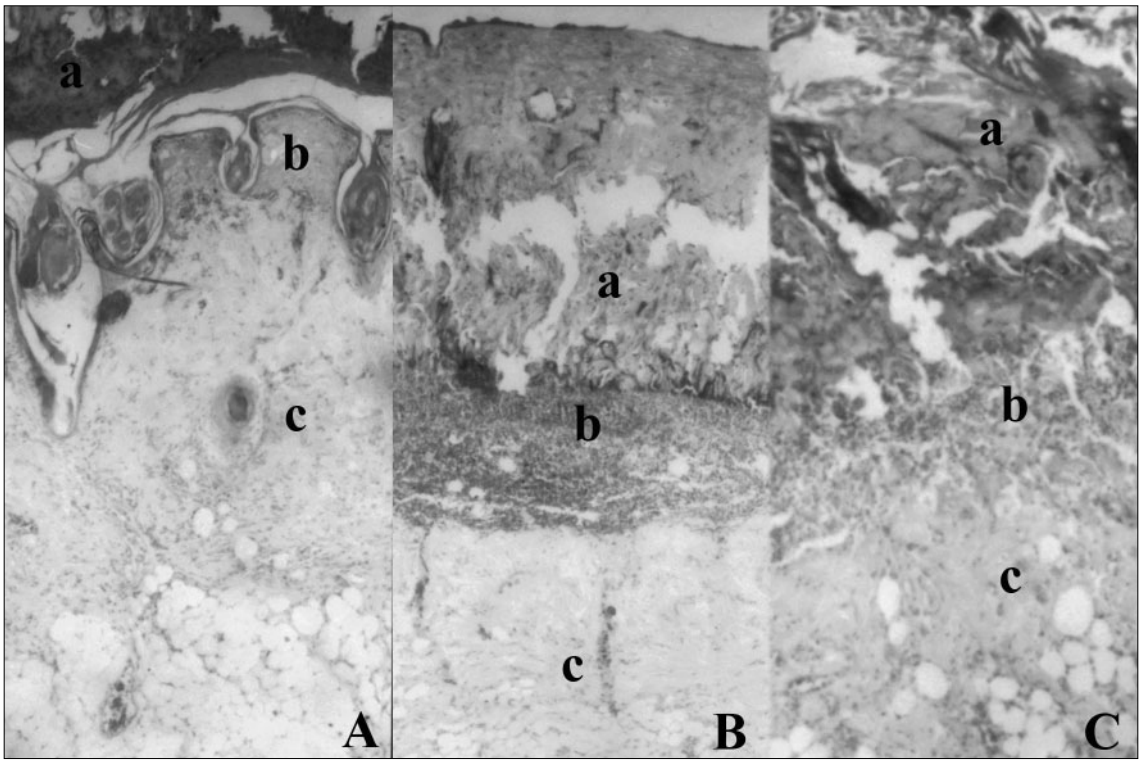

FIGURE 1. — Microscopic appearance of burned skin on the 10th day. - **A.** Propolis skin cream group a) Scab b) Epidermis c) Dermis **B.** SSD group a) Scab b) Infiltration of mononuclear cells c) Dermis. - **C.** Control group a) Scab b) Infiltration of mononuclear cells c) Dermis, (H E x 40).

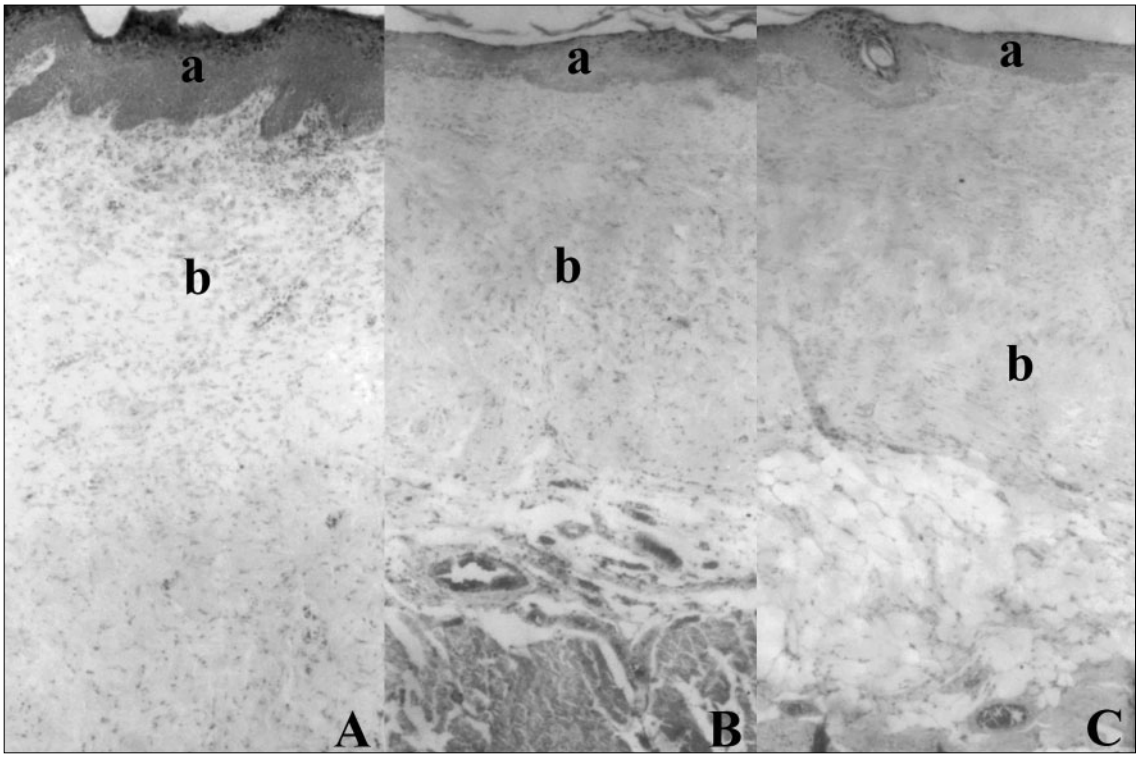

FIGURE 2. — Microscopic appearance of burned skin on the 21th day. - **A.** Propolis skin cream group a) Epidermis b) Dermis - **B.** SSD group a) Epidermis b) Dermis - **C.** Control group a) Epidermis b) Dermis, (H E x 40).

10 % antiseptic povidone-iodine solution (Kim-Pa, Poviiodeks, 10 % povidone-iodine) and a 1 cm diameter burns were made.

Skin burns were made as described by HOSNUTER *et al.* [8]. Animals were subjected to a 1 cm diameter surface area full-thickness third-degree skin burns by brass probe. The brass probe, was immersed in boiling (100°C) water until thermal equilibrium was achieved, then it was placed without pressure for 20 s on the back of the rats. All animals were resuscitated immediately with lactated Ringer's solution (2 ml/100 g body weight) intraperitoneally. Following the burning, each animal was placed in a separate cage.

Immediately after burn, the burned areas in the first, second and third groups were covered with 1 ml 50 % propolis, SSD skin cream and cold cream (control) respectively. These applications were repeated every day. Ten and 21 days later the rats were anaesthesied and the burned skin tissue samples were collected from rats for histopathological examinations. The samples was fixed in 10 % neutral buffered formalin, and were cut into 5 µm sections and stained with hematoxylin and eosin (H&E), and examined by light microscopy.

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 of the mean. The differences between 10 and 21th days 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 accomplished by using statistical analysis system configured for computer (SPSS, Relase 10.0, SPSS. Inc).

3. Results

No mortality was seen in the animals during the study.

Wounds treated with SSD appeared to display a greater degree of inflammation as notable by the three clinical signs of the inflammatory process : heat, redness, and swelling which appeared to be lessened in wounds treated with propolis skin cream.

Histopatologic evaluations, on the 10th day showed that burn healing was better in propolis than the other groups. A scab (formed from necrotic tissue remnants and mononuclear cell infiltration) was observed in propolis group. Regenerative and reparative attempts were in epidermal layer. Not epithelium but inflammatory cell infiltration was observed below of the scab in the SSD and control groups. Vessels were hyperemic in the dermis and there was no hair follicularis, sebace and sweat glands in all groups (Figure 1). On the 21th day, development of the epidermis was observed in all groups (Figure 2). Wound healing was significantly different in each groups at 10 and 21th days ($p<0.001$). Thickness of granulation tissue was significantly different between each groups ($p<0.001$). The mean values of thickness of granulation tissue in the center of the wounds for propolis cream, SSD and control are shown in table I.

4. Discussion

A wide range of antioxidants have been used in the treatment of burn injury both clinically and experimentally. Among them ; glutathione, Vitamins A, C, and E, melatonin, exogenous superoxide dismutase (SOD), selenium, ceruloplasmin, uric acid, dimethyl sulfoxide, β-carotene, ubiquinol, coumarine, caffeic acid phenethyl ester (CAPE), and glycolic acid could be mentioned [2,8].

Propolis is a natural remedy that has been employed extensively since ancient times. The drug was employed as an antiseptic and cicatrizant in wound treatment and as mouth disinfectant, with these uses being perpetuated in the Middle Ages and among Arab physicians. Applied externally, propolis relieves various types of dermatitis caused by bacteria and fungi [3].

KHAYYAL *et al.,* [13], reported that aqueous propolis extract possesses significant antiinflammatory properties and has successfully reduced oedema in both acute and chronic models of inflammation. Propolis inhibits the release of prostaglandins (PGs) and leucotrienes (LTs) from sensitized guinea-pig lung. It is possible that inhibition of both cyclooxygenase and lipoxygenase will offer greater therapeutic benefits than the current range of non-steroidal antiinflammatory drugs (NSAIDs), which are selective inhibitors of cyclooxygenase. It was reported that the anti-inflammatory activity [1], and antimicrobial activity [12,15], of propolis is due to CAPE. Some investigators [9,16,17,18,19,20], demonstrated that antioxidative activity of propolis.

SUBRAHMANYAM [21] reported that honey, as a topical agent, does not adhere to the surface. In comparison to silver sulfadizine, honey seems to be better as a topical treatment for superficial burns because it appears to promote fast re-epithelialization and decrease inflammatory reaction. In addition, honey treatment of superficial burns is cost effective because it shortens the duration of treatment. DURGUN and DURMUS [4] reported that propolis extract have been antiinflammatory effects in treatment of anal sacculitis in dogs. GREGORY *et al.* [7] reported that propolis skin cream appears to have a beneficial effects on the healing burn wounds. If dressings had been changed more frequent the antimicrobial and wound healing effects would have been enhanced. Similar to these results, results of current study showed that it is clear that 50 % propolis cream plays a role in healing of burned skin wounds, its antioxidant, antiinflammatoir and antimicrobial effects. Comparison of the three groups histopathologically indicated that healing of burned skin wounds was better in the 50 % propolis cream group ($p<0.001$).

Consequently, results of the present study indicated that propolis skin cream could be applied in treatment of burns and it may be an alternative to the use of SSD.

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Groups	Days	
	10	21
Propolis cream (n=20)	1.48 ± 0.04^{aA}	2.29 ± 0.08^{aB}
SSD (n=20)	1.03 ± 0.13^{bA}	1.52 ± 0.08^{bB}
Control (n=20)	0.54 ± 0.12^{cA}	1.01 ± 0.11^{cB}

abc Values in the same column with different superscripts are significantly different ($P < 0.001$).
AB Values in the same row with different upper letters are significantly different ($P < 0.001$).

TABLE I. — Thickness (mm) of Granulation Tissue in the Center of the Wound.

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