

## Effect of Propolis and Saline Dressing Materials on Diabetic Foot Ulcer

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**Abstract:** To assess the tolerability and safety of propolis dressing material compared to saline on wound healing patients, two groups of 30 patients each were treated with 7% propolis paste in group A and with normal saline in group B. All of them were under medical antibiotics.

Organisms isolated and identified from the swab culture of patients in, group A before and after treatment, *Staphylococcus aureus* 42.9% dropped to 5.3%, sterile growth 28.6% raised to 84.2%. *Klebsiella pneumoniae* 14.3% dropped to 5.3%, *E. coli* 7.1% dropped to 5.3%. *Streptococcus pyogenes* 3.6% dropped to zero and unidentified 3.6% were disappeared.

In group B organisms identified *Staph.* 41.4% dropped to 8.3%, *Staph.* 6.9% and *Pseudomonas* 3.45% were disappeared, *E. coli* 10.3% decreased to 4.2%, sterile growth 31.0% increased to 75% and *Klebs*, which was not detected before treatment appeared in 4.2%.

Duration of wound healing by propolis paste, two patients were completely healed at less than 6 weeks 13.3 %, after 6 weeks and before 3 months 53.3%, and after 3 months before 6 months 33.3%.

Only 2 patients were subjected to skin grafting out of 29 patients 6.9%. The outcome of the treatment with propolis paste, 14 patients healed with no amputation 8%, twelve kept on dressing and ulcer improvement 41.4%, one patient with minor amputation 3.4% and one patient was under major amputation 3.4%.

With normal saline, 2 patients were healed within 6 weeks 11.1 % eight between 6 weeks and 3 months 44.4% six between 3 and 6 months 33.% and 2 patients exceeded 6 months towards one year 11.1 %. Two patients treated with normal saline were subjected to skin grafting 3.3%.

The outcome of patients treated with normal saline, 16 were healed without amputation 57.1%, on dressing and ulcer improving 39.3% and with minor amputation 3.6%.

At the end of the treatment all patients treated with saline dressing material showed different stages of wound discharge with pus cells (53.6%)- with wound serious 7.1% and with serosanguinous 39.3% however, all patients were shifted to other dressing materials.

## INTRODUCTION

For propolis production, bees use, materials resulting from a variety of botanical processes in different parts of plants. These are substances actively secreted by plants as well as substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, gums, resins, lattices, etc. (Crane, 1988).

The chemical composition of propolis is quite complicated. Its compounds and biological activities depend on many different factors such as the geographical region, collecting time and plant source (Sforzin et al., 2000; Bankova et al., 2002). It contains about 55% resins and balsam, 30% wax, 10% etheric oils and 5% pollen.

Rich in amino acids, trace elements and vitamins, including bio-flavonoids and vitamin K.

These basic ingredients give propolis its dynamic bacteria destroying power (Krol et al., 1996). The ethanolic extract of raw propolis has some activities such as antibacterial (Granzeand Davey, 1990), antioxidant (Isla et al., 2001), antiviral (Amorose et al., 1994), anti-inflammatory (Strehl et al., 1993), anti-cancer (Frenkel et al., 1993), antibiotic and antifungal (Marcucci, 1995).

The objectives of the present work were to assess the tolerability and safety of propolis material and to evaluate the effect of propolis dressing compared to saline on wound healing in diabetic patients. This work was conducted in Jaber Abu Elezz Diabetic Center in Khartoum under full medical concern.

## **MATERIALS AND METHODS**

### **Pro polis collection**

Propolis was collected from El Faki Hashim Apiary, Khartoum State" according to Pidek (1987). Performed plastic plates (6 x 6 x 1 mm) were placed between combs and bottom boards of the colonies. Plastic plates systematically filled with propolis were emptied by freezing in the refrigerator for 7 days and weighed. Fifty grams of propolis were chopped into small pieces and extracted with 100 ml 70% ethanol with continuous magnetic stirring for 24 hours. After filtration (Wettman No. 41), the ethanolic extract was evaporated under vacuum at 50°C until dryness (Hegazi and Abdel Hady, 2002).

### **Inclusion criteria of patients**

- Both sexes of 18-65 years.
- Diabetic patients generally in good health and diagnosed to suffer from diabetic ulcers, naturopathic type. .
- Initial assessment including Hb%, renal liver functions.. etc.
- Physical assessment includin~ linear measures of the wound (cm) area (cm<sup>2</sup>), depth, volume (cm ), margins, discharge... etc.
- Granulation tissue, including level, color, consistency, discharge...etc.
- Microbiological assessment including organism, count of colony forming units... etc.
- Change to other dressing materials.

### **Dressing materials**

Two groups of 30 patients each were treated with dressing materials.

Group A treated with 7% propolis paste in bee honey and group B treated with normal saline. All of them were under medical antibiotics and necessary medical treatments.

Diabetic patients with deep wound tissue were treated with propolis paste every day for the first two weeks, followed by dressing every other day till complete cureness of the wound. The effectiveness of the dressing materials were indicated by keeping treated wounds clean, being judged by a complete absence of pus, serous, serosanguinous and/or cellular exudates.

## **RESULTS**

### **Infection**

Treatments with propolis paste started with one patient grade 1 infection, 9 patients grade 2 and 19 patients grade 3. In saline dressing material on diabetic wounds, the treatment started with one patient grade 1, 16 grade 2, 12 grade 3 and one grade 4 (Table 1).

Valid	Propolis paste		Normal saline		Cumulative (%)
	Frequency	Valid (%)	Frequency	Valid (%)	
Grade 1	1	3.35	1	3.3	3.35
Grade 2	9	30.0	16	53.3	45.55
Grade 3	19	65.5	12	40.1	98.35
Grade 4	-	-	1	3.3	100.00
Total		100.00		100.00	
Missing system	31		30.0		
Total	60				

### Organism identified from the swab culture

The organisms isolated and identified from the swab culture of diabetic ulcer before and after treatment with propolis paste and saline are presented in Table 2. *Staphylococcus aureus* represented 42.9% in propolis dressing and 41.4% in saline dressing before treatment. This bacterial species dropped to 5.3% and 8.3% in propolis and saline dressing materials after treatments, respectively. Sterile ulcers represented 28.6% and 31.0% and were raised to 84.2% in propolis and 75.0% in the saline dressing. *Klebsiella pneumonia* which was absent in the saline group before treatment was detected in its swab culture after treatment in valid 4.2%. The same species represented 14.3% in propolis group and decreased to 5.3% after treatment. *E. coli* decreased from 7.1 % to 5.3% in propolis and from 10.4% to 4.2% in saline treatment. Streptococcus was completely disappeared in both treatments. Pseudomonas which was isolated in saline group only did not respond to the treatment. Another bacterial species was isolated from the swab culture in 3.6% in propolis in 6.9% in the saline group disappeared in propolis paste treatment and dropped to 4.2% in the saline treatment.

### Outcome of treatment

Table 3 depicts the outcome of the treatment with propolis paste and saline dressing materials. In propolis paste treatment, fourteen patients healed with no amputation in 48%, twelve patients kept on dressing and ulcer improvement (valid 41.4%), one patient with minor amputation (valid 3.4%) and only one patient was under major amputation (valid 3.4%) (Fig. 1).

Valid 13.3% patients were completely healed in less than 6 weeks by using propolis ptste. After 6 weeks and before 3 months 53.3% were successfully cured and after 3 months before 6 months, 33.3% were completely healed.

The outcome of wound healing of patients treated with normal saline, 16 patients were healed without amputation (valid 57.1 %). Patients on dressing and ulcer improving were 11 (39.3%) and only one patient with minor amputation (Table 3).

Regarding duration of wound healing treated with normal saline 11.1 % were healed within 6 weeks, 44.40;0 recovered between 6weeks and3- months, 33. % between 3 and 6 months (and 11.1 % patients exceeded 6 months towards one year.

Unfortunately, at the end of the treatment with normal saline dressing materials, all patients showed different degrees of wound discharge (Table 4). Fifteen patients with pus cells (valid 53.6%), two patients with wound serous (valid 7.1 %) and 11 patients with serosaninuous wound discharge (valid 39.3%).

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As a result of the above inconvenience, all patients treated with saline dressing material were shifted to other dressing material. The patients were treated with both propolis paste and saline in an alternating manner one day after another.

The outcome of the treatment was one patient subjected to major amputation and 2 patients to minor amputation, 52.6% healed with no amputation, 40.4% on dressing and ulcer improving, 1.8% others and 3 patients were considered missing system.

The duration of wound healing by using propolis paste and saline in an alternating manner ranged between 12.1% healed within 6 weeks, 60.6% cumulative effect between 6 weeks and 3 months and 93.9% cumulative effect over 6 months before one year.

Table 3 .Outcome of treatment with propolis paste and saline dressing materials on diabetic foot ulcer

Valid	Propolis paste		saline		Cumulative (%)	
	Frequency	Valid (%)	Frequency	Valid (%)	Frequency	Valid (%)
Healing with no amputation	14	48.3	16	57.1	30	52.6
Major amputation	1	3.4	-	-	1	54.4
Minor amputation	1	3.4	1	3.6	2	57.9
Other	1	3.4	-	-	1	59.6
On dressing and ulcer improving	12	41.4	11	39.3	23	100.0
Total	29	100.0	28	100.0		
Missing system	31		32			
Total	60		60			

Table 4. Wound discharge of treatment with saline dressing material

Valid	Frequency	Valid (%)	Cumulative (%)
Pus	15	53.6	53.6
Serous	2	7.1	60.7
Serosanguinous	11	18.3	100.0
Total	28	100.0	
Missing system	32		
Total	60		

## DISCUSSION

Propolis is the most antibiotic man has ever discovered. The old Egyptians, Greeks and Romans reported the use of propolis in popular medicine (Hegazi et al., 2000). Propolis started gaining appreciation as a means of treatment of health problems in the 1950's and 1960's in the former Soviet Union and countries of North and South America and in Japan propolis did not acquire popularity until the 1980's (Salatino et al., 2005).

In Sudan, no proper research work has hitherto been carried out on propolis except or some in published data by the authors concerning the chemical composition of various geographical propolis samples.

In the present study all bacterial species detected in swap culture of diabetic foot ulcer are often antibiotic resistant complicating the choice of therapy and may lead to diabetic wound amputation. However, they were susceptible to Sudanese propolis, *Staphylococci* and *Streptococci* constituted the main groups of medically important gram-positive cocci. The two groups are difficult to treat, especially those contracted in hospitals, because of the remarkable ability of *Staphylococci* to become resistant to antibiotics. *Staphylococci* are rather fastidious, facultatively anaerobic and are routinely clustered on enriched media containing blood. The most virulent *S. aureus* produces catalase and secretes coagulase, the enzyme that causes citrated plasma to clot. Moreover, *S. aureus* is hardly resistant to heat and drying, thus can persist long periods on formats, which can then serve as sources of infection. Moreover, *S. aureus* is largely the result of actual invasive infection that is colonize and overcome host defense mechanisms and the protection of extra cellular substances which facilitate invasion, toxinoses and/or a combination of invasive infection and intoxication.

Although *Pseudomonas aeruginosa*, may colonize healthy humans without causing disease, it is also a significant opportunistic pathogen and a major cause of hospital acquired infections, especially in immuno-compromised hosts.

The suggested theoretic activities of the Sudanese propolis may be due to the presence of flavonoids, esters and other organic constituents detected in the propolis samples collected in Al Faki Hashim Apiary (Khartoum North).

Twenty phenolic compounds were detected when HPLC analysis was used and other 18 different constituents were resulted when GC/MS was carried out (unpublished data of the authors).

The most virulent constituents were: pinocembrin, galangin, caffeic acid, ferulic acid, sclerodin and hepta-deutero-meso-dimethylamino-methyl-etioporphyrin.

The last compound was detected in high concentration 16.13%. These results came in line with Ghisalberti (1979) who found that propolis has been shown to stimulate various enzymes, cell metabolism, circulation and collagen formation and proved healing of bum wounds. The flavonoid has been shown to promote the immune system (Wleklik et al., 1997), as oxygen radical scavenger and therapy that protecting lipids and their cell compounds from being oxidized or destroyed (Yashimoto, 2006).

In China, Zasshi (2005) proved that propolis decreased levels of blood glucose, fructosamine and increase serum levels and high density lipoprotein cholesterol and superoxide dismutase. These findings suggest that propolis can control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased outputs of lipid peroxidation and scavenge the free radicals, resulting in complete cureness of diabetic foot ulcers.

Propolis and some of its components produce anaesthesia, which in some studies were shown to be 3 times as powerful as cocaine and 52 times that of procaine (Ghisalberti, 1979). The anaesthetic effect of propolis may relieve wound pain.

Brago *et al.* (2000) concluded that propolis presented bacterial cell division and also broke down, bacteria walls - and cytoplasm. This may suggest another scientific evidence for curing foot ulcers.

Although the antimicrobial properties of propolis have been the subject of many investigations, it is very difficult to compare the results of different studies, due to the difference of propolis composition or different methods used for the evaluation of propolis antibacterial activities.

## ACKNOWLEDGEMENTS;

The authors express their appreciation and gratitude to all those who contributed to the conduction of this work. Very special thanks have to go to Prof. EI Rasheed A. Abdallah, Director of Jaber Abu Elezz Diabetic Wound Centre and to all the staff who provided technical advice.

Without the support and help of Professor Zuheir AI-Fadil Abjer, Director of the Sudan Academy of Science this work would not have been possible.

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