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Effect of Propolis Dressing Technique on the Healing of Septic Diabetic Foot Ulcers

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Abstract: **The aim** of this study was to evaluate the effect of propolis dressing technique on healing of septic diabetic foot ulcer patients. Design A true experimental design was utilized to fulfill the aim of the study. ***Design*** The study was carried out in vascular surgery department and outpatient clinic at Assiut university hospital. Subjects: Simple random sample of 60 patients with DFUs divided equally to control and study subjects. Three tools were used for data collection including Tool I. An interview questionnaire tool,two parts: Tool II. Diabetic foot ulcers (DFUs) parameter assessment tool.**Results**: this study was performed on 60 patients, their mean ages ranged between 32.63±10.65 and 32.17+11.29 respectively. 60% of the control and study subject at the beginning of the assessment had wound bed necrosis, as for the study subject who were dressed with propolis 63.3%were started granulation after the first week and 100% by the 3ed week dressed by propolis comparing to 6.7% of the control subject by the 3ed week were started granulation, (P value 0.00). Concerning exudates, the table illustrated that at the beginning of the study, all of patients of the control subject had purulent exudates (50%) and the majority of the study subject had sanguineous exudates (80%). However after the first week of dressing with propolis in the study subject 100% had serous exudates compared to none in the control subject, (P value 0.004). Also there was statistically significant difference (P value 0.005) between control and study subjects in the exudates amount where the amount of exudates was high, (63.3%, and 60%) at the start of the research. This percentage had changed to zero among the study subject after the first week dressing by propolis compared to (63.3%) in the control group. As regard exudates color &amount, odor, , wound pain ,surrounding tissue and grade of ulcer there were statistically significant difference (P value 0.004, 0.005.0.004,0.004,0.002ando.001) respectively between control and study subjects., At the end of 3rd week, there was a significant differences between two groups regarding to parameters of ulcer as well as total status of ulcer (P = 0.001). At the end of the follow up, propolis significantly decreased ulcer area (P = 0.01) and depth (P = 0.02) compared with control group. Majority of study subjects (76.6%) had complete ulcer healing, while 23.3% of them had partial healing. In control subjects, it was found that at the end of follow up period, no patients had complete ulcer healing and 66.66% had partial ulcer healing, while 33.3% of them complained of lack of healing. There were rapid improvement in the random glucose level throughout 3 weeks for study subjects compared to none for control subjects. **Conclusions:** It could be concluded that propolis accelerates wound healing and it is advisable to be used for DFUs dressing due to its clinical value and easy application. **Recommendations:** additional successful clinical evidence is required with validated laboratory findings to establish propolis as one of the most effective alternative topical medicines for treating diabetic wounds.

Keywords *-Propolis-Septic Wound-Diabetic Foot Ulcer(DFUs)-Healing.*

I. Introduction

Foot ulceration secondary to diabetes occurs in up to one quarter of people with diabetes [(1)] and it is the commonest cause of lower limb amputation [(2)]. Diabetes increases the risk of lower extremity amputation by 10 to 20 times [(3)] and the estimated cost to the US healthcare system of diabetic foot ulceration and related amputations is more than $10.9 billion annually [(4).] Thus diabetic foot ulceration is a cause of significant morbidity and financial burden.

Wound healing is an intricate, complex and dynamic process where the skin or other body tissue replacing devitalized and missing cellular structures after injury[(5)]. Diabetic foot ulcers (DFUs) are complex, chronic wounds, which have a major long-term negative impact onquality of patients' lives, morbidity, and mortality [(6)]. Unlike other chronic wounds, the development and progression of DFUs is often complicated by wide-ranging diabetic changes, such as neuropathy, vascular disease, altered neutrophil function, diminished tissue perfusion and defective protein synthesis [(7)].

Due to the impaired metabolic mechanisms in diabetic patient, there is an increased risk of infection and poor wound healing due to decreased cell and growth factor response, diminished peripheral blood flow and decreased local angiogenesis[8]. Thus, the feet are predisposed to peripheral vascular disease, damage of peripheral nerves, deformities, ulcerations and gangrene[9,].Without earlyand optimal intervention, the wound canrapidly deteriorate, leading to amputation ofthe affected limb[10].

Successful diagnosis and treatment of patients with DFUs involves a holistic approach that includes optimal diabetes control, effective local wound care, and infection control, pressure relieving strategies and restoring pulsatile blood flow. Amputations are generally used as a treatment of last resort when other measures fail as over prolonged antibiotic therapy[10].However, they may be also performed earlier to allow for earlier return to work or better functional status[11]. Infection is a major threat to DFUs much more so than to wounds as a critical part of wound-bed preparation which includes treating infection and aggressive wound cleansing with a prepared non-cytotoxic wound cleanser to reduce bacterial rate and allows the wound to move rapidly from chronic inflammation phase to proliferation phase [12].

Wound dressings represent a part of the management of diabetic foot ulceration. Ideally, dressings should alleviate symptoms, provide wound protection, and encourage healing[5]. No single dressing fulfills all the requirements of a diabetic patient with an infected foot ulcer. Choosing a dressing for an infected diabetic foot ulcer, several factors have to be taken into account[13]. Infected wounds tend to have heavyexudates that need to be controlled to prevent maceration of surrounding tissue. There may be considerable odor associated with infection that may be unpleasant and distressing for the patient and family [14].
A dressing must be comfortable and acceptable for the patient and should help alleviate or, at the very least, not worsen pain, especially at dressing changes. Ideally, the dressing should also aid in the management of the infection itself [15].Many cleansing and topical antimicrobial agents may be used in dressing for DFUs as Acetic Acid, povidine Iodine and Dakin solution (NaOcl) which may have no effect in heavy infection, its antimicrobial effect on wounds is debatable. Furthermore, some data have shown iodine solutions to be toxic to fibroblasts and keratinocytes. Also using Hydrogen Peroxide (H_2O_2) like to be toxic to many of the cells involved in wound healing cascade and so impede wound healing even at low concentrations has been shown to be toxic to granulocyte and monocytand results in decrease chemotaxis, it also capable of inhibiting lymphocytes function[16].

Propolis dressing enhances phagocytes, through providing substrates of glycolysis which is the major mechanism of energy production in the macrophage in addition its acidity (PH below 4) assist in the antibacterial action. So the wound will get rid of the infectious bacteria, bus, dead tissue and blood clot this lead to acceleration of healing process [17]. Its viscosity provides a barrier for cross infection of wounds also the higher osmolarity causes an out flow of lymphprovides. Propolis supply of glucose for leucocytes, which is an essential for production of the dominant component of antibacterial activity of macrophages H2O2[18]. Propolis reduce odor the often associated with diabetic foot wounds [19].The objectives of diabetic foot management not only to create optimum local condition for healing or reduce of malodour, pain, frequency of dressing change , deterioration of the wound but also hemodynamic improvement of the patient[20].

We have previously published in a preclinical, diabetic rodent model of full thickness cutaneous wound healing, that a single application of topical propolis normalized ulcer closure rate and reduced persistent neutrophil infiltration and elastase activity [21]. In humans, propolis has been described as a useful topical treatment for ulcers [22]).It is considered to have a low side-effect

profile [23]and is approved in many countries for treatment of ulcers and abrasions,being sold over the counter in many parts of the world including in Australasia [22].

Propolis is a resinous bee-hive product consisting of plant materials that are initially collected on the hindlegs ofworker bees. Thematerial is then masticated, salivary enzymes are added and mixed with wax toproduce propolis [24,25]. Its most biologically active fractions are flavonoids and esters of caffeic acid [26]. Propolis has multiple properties that make it an attractive agent for treatment of diabetic foot ulcers, including being anti-inflammatory, anti-oxidant [27] and anti-microbial [28] especially anti-bacterial [29], in its actions. Furthermore, propolis component caffeic acid, has potent activity to inhibit the proinflammatory proteinase, matrix metalloproteinase-9 (MMP-9), and MMP-9 is known to be increased in diabetic foot ulcers [30].

Propolis or bee glue is a natural resinous mixture that honey bee collect from tree buds, sap flows, or other botanical sources.It has a long history of medicinal use, back to 350 B.C., the time of Aristotle. Greeks have used propolis for abscesses; Assyrians have used it for healing wounds and tumors; and Egyptians have used it for mummification.[31]

Polyprenylated benzophenones artepillin C,propolins ,terpenes,zinc oxide, Polyphenols and flavonoids are components to propolis , While caffeic acid phenethyl ester (CAPE) is the active component, it has a lot of biological effects as antioxidant, anti-inflammatory, antitumor, antibacterial, antiviral, fungicide, immune modulatory, cardio protective, hepato protective, and anti-osteoporosis.[32]

Primary prevention is the aim of diabetes management, but secondary prevention is the goal of good foot-ulcercare and diabetic foot care is mainly a nursing role,it depends on Assessment, observation, recording and reporting that purposed to provide wound care, cleanse, select and evaluate dressing in addition providing emotional support and patient education for prevention of ulcer recurrence.[33]

It is essential to the nurse to know that diabetic foot ulcers receive the best possible wound management. Successfully treating a diabetic foot ulcer requires a comprehensive understanding of the wound, its cause, progression, risk, and treatment. But more than this, it takes a cross functional approach, where the patient also has an active role in the treatment process.The nurse must follow strict infection control protocol to prevent diabetic foot wound infection with closely monitor process of healing to prevent or reducing amputation[34,].

Significant of the study

Recent reports from doctors and nursing staff in plastic surgery department and outpatient clinic at Assiut university hospital and also from the patients pointed out to diabetic foot problems have a significant financial impact on the National Health Service(NHS) through outpatient costs, increased bed occupancy and prolonged stays in hospital. In addition, diabetic foot problems have a significant impact on patients' quality of life as, reduced mobility that may lead to loss of employment, depression and damage to or loss of limbs. A delay in diagnosis and management increases morbidity and mortality and contributes to a higher amputation rate. So, this study will be the first study in this geographical location which will help such group of patient to prevent or reduce amputation. The number of patient with diabetic foot ulcer following up in plastic surgery department and outpatient clinic at Assiut university hospital in the last year was 9000 case according to the Hospital statistical record (2014).

Aim of the study:

This study aimed to evaluate the effect of propolis dressing technique on the healing of a septic diabetic foot ulcers (DFUs) patient compared to routine hospital care in vascular surgery department and outpatient clinic at Assiut university hospital.

Research Hypothesis:

Diabetic patient with septic foot ulcer subjected to propolis dressing promote less inflammatory reaction, faster healing improve peripheral sensation than routine hospital dressing technique

Subjects and method

Research Design:

A True experimenta (pretest-posttest) control group design was utilized to fulfill the aim of the study.

Setting:

The study was carried out in vascular surgery department and outpatient clinic at Assiut university hospital.

Subjects:

A convenience sample of 60 patients who were admitted in vascular surgery department and follow up at outpatient clinic at Assiut university hospital between June 2014 till December 2014, and willing to participate in the study were recruited. They were randomly assigned into two equal groups, study and control group, (30 patients each).

Inclusion criteria:

Patient's age from 18- ≤65 years, both sexes, Conscious and alert, Body Mass Index (BMI) of 18 to 35, complaining of DFUs with grade 1 and 2 of Wagener's classification [35] on toes, soles, heels or dorsum of the feet since 3 months without healing, not receiving drugs that lead to delay of wound healing such as corticosteroids, immunosuppressive and cytotoxic drugs and free from any chronic diseases other than diabetes that may affect the healing of ulcers such as cancers, congestive heart failure, end stage renal disease and liver failure. Patients with foot gangrene that needed amputation as well as osteomyelitis that needed antibiotic therapy were excluded from the study. Also patients who preferred to receive the treatment out of the study, inappropriate follow up by the patients (missing follow-up more than two times), and the patient's desire to withdraw in each phase of the study were considered as others exclusion criteria.

Tools of data collection: Tow tools were used for data collection

Tool I. An interview questionnaire tool: It developed by the researchers to assess patient's medical history, includes two parts: Part I: Patients Socio-demographic data (age, gender, education level, occupation). Part II: Patient's related medical data include structured items to identify patient's related medical characteristics { body mass index ,type of diabetes, onset of diabetes , weight (kg), height (cm), ,site and grade of ulcer and HA1c, random blood glucose level.

Tool II. Diabetic foot ulcers (DFUs) parameter assessment tool: It was developed by the researcher it includes:

- wound assessment parameters as wound bed (Necrosis, Slough, Granulation, and epithelization), exudates (color, amount odor) wound pain frequency, surrounding tissues (Intact, Fragile, Dry Macerated, Erythema, Edema),ulcer healing assessment scale and grade of ulcer according to Wagner system, it was used for ulcer classification that its reliability and validity was proven by (Wagner.) Based on this system, ulcer classify into 5 grades. Grade 1 ulcers are superficial ulcers involving the full skin thickness, but no underlying tissues. Grade 2 ulcers are deeper, penetrating down to ligaments and muscle, but not involving bone or abscess formation. Grade 3 ulcers are deep ulcers with cellulites or abscess formation, often complicated with osteomyelitis. Ulcers with localized gangrene are classified as Grade 4, and those with extensive gangrene involving the entire foot are classified as Grade 5. Photograph Picture: Photograph were taken to compare ulcer healing process before starting the propolis dressing technique, after 1 week, after 2 weeks, after 3 weeks and before discharging.

- Lab investigation: such as Tumor necrosis factor as (TNF- α) , NFRs, Anti TNF, Cytokines 1L-6 , Cytokines 1L-12, C Reactive protein(CRP), NAD(P)H &H2O2 , to identify the effect of propolis dressing on endothelial dysfunction in addition to HA1c and random blood sugar level
- Bacteriological culture: It was taken by the researcher to identify types of microorganism that causes DFU infection dressing technique, after 1 week, after 2 weeks, after 3 weeks and before discharging.

Method:

Administrative approval: An official was forwarded from the dean of the faculty of Nursing, South Valley University explaining the aim of the study, and requesting a permission to conduct the study. A written approval was obtained from the director of vascular surgery department at Assuit University Hospital to carry out the study. The study was approved by an institutional ethics committee.

The study tools were developed by the researchers after extensive review of the relevant literature. The tools were tested for content validity by 5 experts of academic medical and nursing staff at Assuit University. Modifications were done accordingly, and then the tools were designed in its final format and tested for reliability using internal consistency for the tools was measured using &-cronbach test which were reliable (0.75, 0.71, and 0.81 respectively).

Ethical consideration: The study was approved by an institutional ethics committee. Informed consent was obtained from patients to participate in the study. The researchers initially introduced themselves to all potential subjects and they were assured that the collected data were absolutely confidential. They were informed that participation is voluntary and they can withdraw at any time of the study.

Pilot study: A pilot study was conducted before starting data collection on (6) patients who was included in the sample to test the clarity, and applicability of the tool and to estimate the time required to fill the sheet. Modifications were done as needed.

Data collection:

The data collection was done through the following phases:

1- Assessment phase:

The researcher interviewed the patients in both groups individually and gets their written consent to participate and an interview questionnaire tool I. was applied which is concerned by Patients Socio-demographic and clinical characteristics including: age, Gender, educational level, body mass index was calculated by measuring patients' height and body weight and then use the following equation [BMI = weight / (height^2) = Kgm/m^2]. ,type of diabetes, onset of diabetes , ,site of ulcer and a 5 ml blood sample was obtained from all patients , after an overnight fasting and the blood samples were investigated once for HA1c to define the controlled and uncontrolled diabetes as regards the random blood glucose level, It was measured 4 times (Baseline,1st week,2nd week, 3rd week)

. Diabetic foot ulcers (DFUs) parameter assessment tool II. Was applied which is concerned by assessment of the DFUs grade according to Wagner system**(35),** Ulcer size was considered as ulcer surface area it was measured by ruler and ulcer depth was measured at the deepest part of the ulcer with a sterile probe . Ulcer healing assessment scale [(34)]evaluated 8 ulcer's parameters including degree, colour, surrounding tissues, exudates ,pain frequency, wound bed, oder and peripheral sensation, as well as total ulcer status weekly. Based on this scale, each parameter obtained 100 scores, and the total ulcer status scores range from 100 to 800. Based on this scale, the higher the score the better healing .The status of ulcer healing at the end of the 3rd week was considered as complete, partial, lack of healing and deterioration according to the scores that was obtained by ulcer healing assessment scale at the end of follow up. Complete ulcer healing was considered as cases that their ulcers had 800 scores, and partial ulcer healing was considered as increase of ulcers scores at least 60 scores more than the baseline Ulcer healing assessment. Lack of healing was considered as lack of any changes in ulcers scores and deterioration of ulcer was considered as decrease of ulcers scores at least 20 scores less than the baseline, peripheral sensation to touch, vascular insufficiency was made clinically on the basis of absence of both pedal pulses of the involved foot, pain frequency was assessed daily during treatment , weekly ulcer parameter assessment was done, and at the end of 3rd week; photograph was taken and measurement of ulcer size was estimated in both groups by the researcher. Both pre- and post-assessment and scoring was done

2- Implementation phase:

Patient who was met the inclusion criteria were assigned randomly to either the study subjects who were subjected every other day dressing technique by propolis, or control subjects who were subjected to conventional dressing technique.Initial aassessment of the patient condition was done using the developed tool I considered as baseline, Study subject were dressed by propolis technique ones every other day till healing occur or good epithelization reach optimal level to cover.

Propolis collecting trap:

White glass slides with 48 cm length 5 cm width and 5 mm thickness. These slides were arranged contiguous to each other and were put onto the top bar of the combs, with an elevation at approximately 3 mm in between. So, seven glass slides were put in one colony containing 10 combs.

The used traps were placed on top bars of the hive frames. These traps were replaced monthly where they take to laboratory for propolis collection and new ones were used.

A sharp scraping knife was used to scrap the pellets, pieces of propolis periodically every month. The collected propolis samples were put in a small nylon bags, then were kept in a freezer at OoC

Propolis Ethyl Extract (PEE) :

Five g of the crude material was dissolved in 50 ml of ethyl alcohol 70%. The mixture was shaken for half hour and left in the laboratory for 24 hours. This procedure was repeated five times. After five days the extract was filtered by filter paper Watman No. 4. The obtained extract propolis ethyl extract (PEE) was evaporated to a thick mass on a water path under vacuum, and hardened after cooling to give a gummy matter of propolis. Except the evaporation process was achieved through leaving the extraction to the room temperature for 48 hours that was enough for the alcohol to evaporate. [36].

Preparing propolis solution:

One g of a gummy matter of propolis was dissolved in1 cm ethyl alcohol 70%, and then kept in closed glass tubes for dressing technique.

Bee venom collection:

The deposited bee venom on the glass plate was scrapped by means of a scraping knife .The collected bee venom was weighted according to each colony.

Keeping the bee venom material:

The dried collected bee venom was kept in dark clean screw-tubes which were kept in a freezer running of a zero degree.

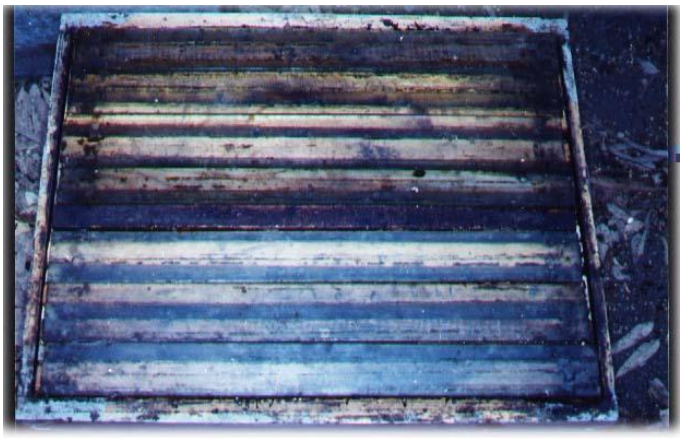


Fig.(1): Models of trap type designed for collecting propolis

a thick layer of propolis were stretched over it, this was directly applied over the ulcer every other day at 9 Am. a paste consisting of myrrh, bee propolis and honey (MPH) was applied to the wound. Following treatment, the wound settled and healed well

Procedure:

The procedure were carried out by the researcher: remove old dressing, then gently scrub the ulcer from tissue exuded using gauze soaked with normal saline(NaCl 0.9%),ulcer photographed, dressing were wet with normal saline (NaCl 0.9%) then use of the Propolis

The wound culture: done using sterile swab at the start then every week before dressing with propolis technique and before discharge, result of local swab cultures interpreted that for both groups then record of abnormalities of occurred.

Ulcer healing: were evaluated every week using evaluation sheet (tool 3), with measuring depth and lengths of the ulcer for all patients of each group.

Photograph pictures: were taken before start, after the first week, after second week, after third week and before discharge to evaluate the healing process for both subjects, to compare between the two subjects (study and control).

Lab investigation: blood sample was taken before start then every week to every patient of each subject (study and control) before start then every week tile discharge for comparing between the both subjects to identify the effect of propolis dressing on endothelial dysfunction

Pain: at each visit, patients were asked to report their pain as it happens in the following forms: none, only during dressing, intermittent, or continuous.

Exudates: DFU exudates amount was observed and recorded as dry, scant wound tissue moist (no measurable drainage),small/ minimal wound tissue very moist or wet(drainage <25%of bandage),moderate wound tissue wet (drainage involved25-75% bandage) and Large/copious wound tissues filled with fluid **(**drainage >75% of bandage) at the start ,then at each visit for dressing as well as odor was assessed and recorded as none, only when dressing was removed or before and after dressing was removed.

3-Evaluation phase includes:

The follow-up of the two subjects was done in vascular surgery unit and outpatient clinic, Diabetic foot ulcers (DFUs) parameter assessment was applied during every assessment standard parameters of wound healing, pain, odor, exudates amount and color were evaluated.

Data Analysis:

The data obtained were reviewed prepared for computer entry, coded, analyzed and tabulated. Descriptive statistics as (number and percentage, mean and stander deviation) was done using computer program SPSS version (17). Chi-square, P-value and T-value used to compare differences in the distribution of frequencies between the two groups (study and control)

I. Results

Table 1: show that control and study subjects, their mean ages ranged between 34.63 ± 9.65 and 34.17+8.29 respectively. They were 39 male and 21 female, however more than half of subjects 73.3%and 66.6% respectively were diabetic since more than 10 years. Also 33.3%, 26.6% respectively of control and studied subjects their medication were combination of oral and insulin as a hypoglycemic agents , Also half 50% of the control and study subject was class II obesity. , While 80% of all subjects had uncontrolled diabetes HA1c more than7%

Table II &figure 1: Showed that 60% of the control and study subject at the beginning of the assessment had wound bed necrosis, as for the study subject who were dressed with propolis 63.3%were started granulation after the first week and 100% by the 3ed week dressed by propolis comparing to 6.7% of the control subject by the 3ed week were started granulation, (P value 0.00). Concerning exudates, the table illustrated that at the beginning of the study, all of patients of the control subject had purulent exudates (50%) and the majority of the study subject had sanguineous exudates (80%). However after the first week of dressing with propolis in the study subject 100% had serous exudates compared to none in the control subject, (P value 0.004). Also there was statistically significant difference (P value 0.005) between control and study subjects in the exudates amount where the amount of exudates was high, (63.3%, and 60%) at the start of the research. This percentage had changed to zero among the study subject after the first week dressing by propolis compared to (63.3%) in the control group. As regard exudates color &amount, odor, , wound pain ,surrounding tissue and grade of ulcer there were statistically significant difference (P value 0.004, 0.005.o.oo4,0.004,0.002ando.oo1) respectively between control and study subjects.

Table 3: shows ulcer parameters and total ulcer status scores in both studied subjects at baseline and during follow up period. Based on this table, results indicated no significant difference between the two groups regarding to all ulcer parameters and total ulcer status at baseline, while after 1stweek of follow up a significant difference was seen regarding to ulcer color and surrounding tissues. Furthermore, a significant difference was

seen regarding to ulcer degree after 2nd week of follow up between the two groups. In addition, regarding to ulcer exudates results showed significant difference between studied groups in the 3rd weeks of follow up. Total ulcer status scores showed significant difference between the two groups at the end of the 1st, 2nd and 3rd weeks of follow up it is related to anti microbial effect of propolis.

Table (4) Illustrated that, baseline ulcer surface area (cm2) was 1.17±0.69 and 0.87±0.26 in control and study groups respectively. Baseline ulcer depth (cm) was 0.34±0.07 and 0.24±0.05 in control and intervention groups respectively. Initial surface area (P=0.66) and depth (P=0.28) were not statistically different between the two groups. At the end of the follow up, propolis significantly decreased ulcer area (P=0.01) and depth (P=0.02) compared with control group. At the end of the study period, the ulcer area had changed by −54.7±28.8% in the study subjects (P=0.02 vs at the start of the study) and by +2.7±47.2% in the control group (P=0.18). The ulcer depth had changed by −60.1±13.8% in the propolis subjects (P=0.004 vs at the start of the study) and −29.6±12.6% in the control group (P=0.04).

Table (5) shows comparison between the two studied subjects according to ulcer healing status after 3rd week (the end of follow up). Majority of study subjects (76.6%) had complete ulcer healing, while 23.3% of them had partial healing. In control subjects, it was found that at the end of follow up period, no patients had complete ulcer healing and 66.66% had partial ulcer healing, while 33.3% of them complained of lack of healing.

Table (6): Illustrated that, in the beginning of the study, the majority of patients had high level of tumor necrosis factor (93.3%, 86.6 %) among the study and control subjects respectively. However, after two weeks100% of the study subject was low level compared to none of the control subject. Also, difference between the two subjects were statistically significant (p value 0.001) related to cytokines (1L-6, 1L-12).There was a significant difference between control and study subjects in C-Reactive Protein (CRP), where, the majority of both subjects (60%) were in high increased risk but after the first week the majority of the study subject (83.3%) were normal range compared to non in the control subject. As regard NAD(P)H &H_2O_2 Product, the table show difference between the two subjects were statistically significant (p value 0.004).

Table (7) shows that, there is a positive correlation between (TNF- α) and C Reactive protein (CRP), also between serum insulin level and CRP. While negative correlation between(TNF- α) and NAD(P)H &H_2O_2 …and H2O2.There are a positive correlation between Anti TNF and NAD(P)H &H_2O_2.While negative correlation between Anti TNF , C Reactive protein(CRP) and serum insulin level. Result of local swab cultures interpreted that for both groups at start and end of the study. There were 5 different types of microorganisms in this study according to the culture results Proteus, Klebsella, and Psydomonus. There were a statistically significance decrease in quantitative reduction of micro-organisms between using porpolis dressing technique and routine dressing as a dressing therapy in treating infected diabetic foot ulcers DFUs (P value 0.0001) (Fig 4)

Table 1: **Table (1): Socio-demographic and clinical characteristic for control and study subjects**

Socio-demographic &clinical characteristic	Group				P.value
	Control (n=30)		Study (n=30)		
	No	%	No	%	
Age(years) • 40-<50 • 50-≤60	 10 20	 33.3 66.6	 12 18	 40.0 60.0	0.353
Mean+ SD	34.63 ± 9.65		34.17+8.29		
Gender • Male • Female	 20 10	 66.6 33.3	 19 11	 63.3 36.6	0.705
Educational level • Illiterate • Less than a diploma • Diploma • University	 22 4 4 0	 73.4 19.3 19.3 0.0	 16 6 4 4	 53.4 20.0 13.3 13.3	0.445
Occupation • Housewife • clerical work • Not working • Retirement	 18 6 2 4	 60.0 20.0 6.7 13.3	 16 6 4 4	 53.4 20.0 13.3 13.3	0.822
Onset of diabetes ≤ 10 years >10 years	 8 22	 26.6 73.3	 10 20	 33.3 66.6	0.235
Mean+ SD	13.70 ±5.2		14.70 ±4.2		
Medications • Insulin • Oral hypoglycemic • Hypoglycemic agents (insulin +oral)	 20 0 10	 66.6 0 33.3	 17 5 8	 56.6 16.6 26.6	0.065* 0.143
BMI • 18<25(desirable) • 25<30(class I) • 30-35 (classII)	 9 6 15	 30.0 20.0 50.0	 6 9 15	 20.0 30.0 50.0	0.655
Site of ulcer • Beg toe • Heal of feet • Dorsum of the feet	 8 11 11	 26.6 36.6 36.6	 7 9 14	 23.3 30.1 46.6	0.946
HA1c ≤ 7% >7%	 6 24	 20 80	 6 24	 20 80	0.044*

*: Significant (P< 0.05).

Table (2): Frequency and percentage distribution of the sample according to wound assessment among control and study subjects at the end of week.

Ulcer Parameter	Control group(n=30)				Study group(n=30)				X^2	P
	baseline	1 st Week	2 ed Week	3ed Week	baseline	1 st Week	2 ed Week	3ed Week		

	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Wound bed																		
-Necrosis	25	83.3	22	60	21	56.7	19	56.7	25	60	18	0	0	0	0	0		
-Slough	30	100	28	40	24	33.3	20	33.3	30	33.3	20	0	0	0	0	0		
-Granulation	0	0	0	0	3	6.7	3	0	0	0	19	63.3	25	50	30	100		
-epithelisation	0	0	0	0	0	0	0	0	0	0	11	36.7	25	50	26	86.6	9.2	0.00 **
Exudates(color)																		
-serous	0	0	0	0	0	0	0	0	1	3.3	15	50	20	75	30	100	8.5	0.004 *
-serosanguinous	0	0	0	0	0	0	0	0	5	16.7	15	50	10	25	0	0		
-sanguinous	0	0	0	0	0	0	15	50	24	80	27	90	4	13.3	0	0		
-purulent	30	100	30	100	30	100	15	50	30	100	0	0	0	0	0	0		
Exudates(amount)																		
-dry/none	0	0	0	0	0	0	0	0	0	0	15	50	25	83.3	25	83.3	9.0	0.005 *
-Scant	0	0	0	0	0	0	0	0	1	3.3	15	50	5	16.7	5	16.7		
-Small	0	0	0	0	3	10	3	10	5	16.7	0	0	0	0	0	0		
-Moderate	11	36.7	11	36.7	11	36.7	12	40	6	53.3	0	0	0	0	0	0		
-Large/copious	19	63.3	19	63.3	16	53.3	15	50	18	60	0	0	0	0	0	0		
Odor:																		
• None	0	0	0	0	2	6.7	2	6.7	0	0	30	100	30	100	30	100	8.8	0.004 *
• only at dressing	9	30	9	30	11	36.7	11	36.7	9	30	4	13.3	0	0	0	0		
• fill the room	21	70	21	70	17	56.7	17	56.7	26	86.7	0	0	0	0	0	0		
Wound pain (frequency)																	8.2	0.004 *
-only at dressing	27	90	26	86.7	26	86.7	25	83.3	15	50	25	83.3	0	0	0	0		
-none	3	10	4	13.3	4	13.3	5	16.7	15	50	5	16.7	30	100	30	100		
Surrounding tissues: Intact	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0	20	0	9.0	0.002 *
Fragile	0	0	0	0	0	0	0	0	0	0	19	63.3	21	70	25	83.3		
Dry	0	0	0	0	0	0	0	0	0	0	10	33.3	20	66.6	27	90		
Macerated	30	100	30	100	25	83.3	22	73.3	30	100	10	33.3	5	16.6	0	0		
Erythema	30	100	30	100	28	93.3	21	70	30	100	11	36.6	5	16.6	0	0		
Edema	30	100	30	100	26	86.6	20	66.6	30	100	9	30	3	1	0	0		
• **Grade of ulcer** • Superficial	0	0	0	0	0	0	3	1	0	0	0	0	10	33.3	28	93.3	9.0	0.001 *
• Partial	5	16.6	7	23.3	7	23.3	7	23.3	3	1	8	26.6	10	33.3	2	6.6		
• Deep	25	83.3	23	76.6	23	76.6	20	66.6	27	90	22	73.3	10	33.3	0	0		

Scant wound tissue moist (no measurable drainage) *Significant (P< 0.05).
Small/ minimal wound tissue very moist or wet (drainage <25%of bandage)
Moderate wound tissue wet (drainage involved25-75% bandage)
Large/copious wound tissues filled with fluid **(**drainage >75% of bandage)

Figure (1): Comparison length of tissue healing (epithelization rate) / week for control and study subjects

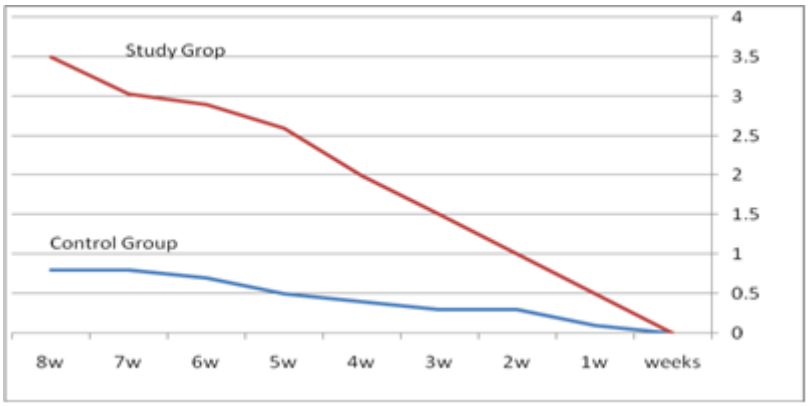


Table (3): Comparison of M±SD of Ulcer Parameters at the baseline and during follow-up for each group=30

Ulcer parameter	Study M±SD	Control M±SD	P value
Wound bed:			
• Baseline	67.0± 15.32	84.0 ±16.81	0.651
• 1st wks	81.33± 12.31	76.66± 14.34	0.025
• 2nd wks	90.30± 9.72	62.66± 13.34	0.011
• 3rd wks	97.33± 4.57	64.00± 10.55	0.001*
P value	0.004		
Oxudate:			
• Baseline	68.0 ±14.54	84.0± 16.81	0.730
• 1st wks	93.33± 9.75	76.66± 14.34	0.001*
• 2nd wks	97.33 ±7.03	62.66 ±13.34	0.005*
• 3rd wks	98.86± 316	64.00 ±10.55	0.017*
P value	0.004		
Oder:			
• Baseline	66.0± 9.10	68.0 ±13.68	0.001
• 1st wks	85.0± 9.85	72.33± 8.16	0.450
• 2nd wks	91.0± 10.1	70.34 ±12.22	0.007*
• 3rd wks	97.33± 4.57	84.0 ±14.33	0.004*
P value	0.004		
Pain frequency:			
• Baseline	68.09± 13.54	84.0± 16.81	0.210
• 1st wks	84.0 ±9.83	73.33 ± 8.16	0.117
• 2nd wks	90.0 ±99.72	71.31± 11.68	0.19
• 3rd wks	97.33 ±4.57	86.40± 13.15	0.04*
P value	0.005		
Surrounding tissues			
• Baseline	67.0 ±15.32	69.0 ±11.68	0.691
• 1st wks	81.33± 12.31	73.33± 8.16	0.045
• 2nd wks	90.30± 9.72	79.33± 12.22	0.011
• 3rd wks	97.33± 4.57	83.0± 13.33	0.001*
P value	0.019		
Degree			
• Baseline	69.0± 11.38	61.0 ± 17.54	0.154
• 1st wks	79.33 ±10.15	69.33± 17.30	0.064
• 2nd wks	87.33± 9.79	74.33± 17.20	0.017
• 3rd wks	92.23 ± 6.17	80.0 1± 6.47	0.019
P value	0.004		
Peripheral sensation			
• Baseline			
• 1st wks	60.09± 13.54	87.0± 16.81	0.210

• 2nd wks • 3rd wks	83.0 ±9.83 89.0 ±99.72 95.33 ±4.57	75.33 ± 8.16 72.31± 11.68 84.40± 13.15	0.117 0.19 0.04*
P value			
Total ulcer status: • Baseline • 1st wks • 2nd wks • 3rd wks	 588.00 40.52 742.00 33.36 765.00 29.82 791.33 15.05	 577.33 35.55 601.67 35.55 625.00 43.20 648.00 43.08	 0.450 0.004* 0.007* 0.001*
P value	0.004		

*Significant (P< 0.05).

Table (4) Distribution of M±SD of Ulcer size at the baseline and at the end of follow-up for study and control subjects :(n=30)

Ulcer Size	Study M±SD	Control M±SD	P value
Ulcer surface area($cm^{2)}$ • Baseline • 3th wks	 0.87 ±0.26 -54.7 28.8%	 1.17 ±0.69 +2.7 47.2%	 0.66 0.01
P value	0,02	0.18	
Ulcer Depth(cm) • Baseline • 3th wks	 0.24 ±0.05 - 60.1±13%	 0.34 ±0.07 -29.6±12.6%	 0.28 0.02
P value	0.004	0.04	

* Significant (P< 0.05).

Table (5) Comparison of two studied groups according to ulcer healing status after 3 weeks (the end of follow up).

	Study		Control	
Ulcer healing status	n	%	n	%
• Complete	23	76.66	0.0	0.0
• Partial	7	23.33	20	66.66
• Lack	0.0	0.0	10	33.33

Table (6): Distribution of Serum laboratory values among control and study subjects

variable	Control Group						Study Group						P-value
	1 week		2 week		3 week		1 week		2 week		3 week		
	No	%	No	%	No	%	No	%	No	%	No	%	
Tumor necrosis factor -alfa (TNF- α)(3.8-4.8)													
TNFRs													
- Low	1	3.33	1	3.33	1	3.33	2	6.66	30	100	30	100	0.003
- High	28	93.3	28	93.3	28	93.3	26	86.6	0	0	0	0	**
- Normal	1	3.33	1	3.33	1	3.33	2	6.66	0	0	0	0	
Anti TNF													
- Low	5	16.6	5	16.6	5	16.6	4	13.3	28	93.3	28	93.3	0.004
- High	23	76.6	23	76.6	23	76.6	22	73.3	2	6.66	2	6.66	**
- Normal	2	6.66	2	6.66	2	6.66	4	13.3	0	0	0	0	
Mean+ SD	22.94+25.2						215.8+119.58						0.000*
Cytokines 1L-6													
- Low	9	30.0	9	30.0	9	30.0	11	36.6	24	80.0	24	80.0	0.003
- High	21	70.0	21	70.0	21	70.0	18	60.0	2	6.66	2	6.66	**
- Normal	0	0	0	0	0	0	1	3.33	4	13.3	4	13.3	

Mean± SD	63.98± 2.09						96.24± 2.49						0.001*
Cytokines 1L-12 - Low - High - Normal	9 21 0	30. 0 70 0	9 21 0	30.0 70 0	9 21 0	30.0 70 0	10 20 0	33.3 66.6 0	2 4 2 4	80.0 6.66 13.3	24 2 4	80.0 6.66 13.3	0.001 **
Mean± SD	22.94±25.2						96.24± 2.49						0.001*
C Reactive protein(CRP) - Normal range(lower 10mg/l	0	0	0	0	0	0	1	33.3	25	83.3	25	83.3	0.004 **
- Low inflammation risk(< 1.00mg/l)	3	10.0	3	10.0	3	10.0	3	10.0	5	16.6	5	16.6	
- Average increased risk (1.00-3.00mg/l)	9	30.0	9	30.0	9	30.0	10	33.3	0	0	0	0	
- High increased risk (> 3.00mg/l)	18	60.0	18	60.0	18	60.0	16	53.3	0	0	0	0	
NAD(P)H &H_2O_2 Product - Low range	20	66.6	20	66.6	20	66.6	22	73.3	0	0	0	0	0.004
- Moderate range	9	30.0	9	30.0	9	30.0	8	26.6	6	20	6	20	**
- High range	1	3.33	1	3.33	1	3.33	0	0	24	80	24	80	
Random glucose level - 120-149	3	10	3	10	2	6.66	2	6.66	16	53.3	23	76.6	0.005
- 150-179	3	10	5	16.6	5	16.6	5	16.6	9	30.0	6	20	**
- 180- 209	5	16.6	3	10	4	13.3	4	13.3	5	16.6	1	33.3	
- 210- 249	10	33.3	12	40	11	36.6	11	36.6	0	0	0	0	
- 250- 280	9	30	7	23.3	8	26.6	8	26.6	0	0	0	0	

*Significant (P< 0.05).

Fig 2 &3: Show that there was rapid improvement in the random glucose level throughout 3 weeks for study group compared to none for control subject.

Figure (2): control subject blood sugar level/week

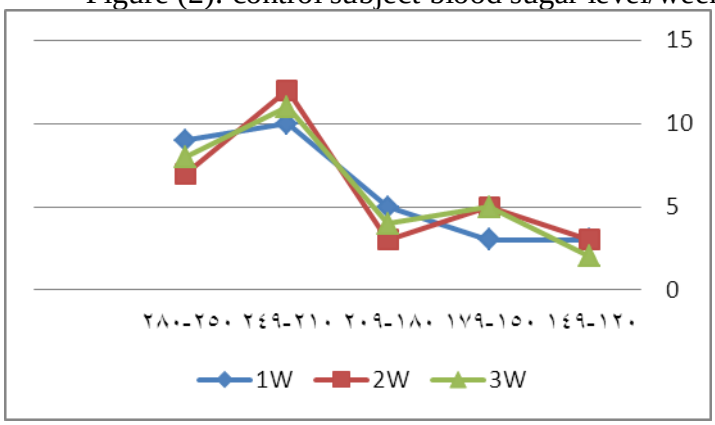


Figure (3): study subject blood sugar level/week

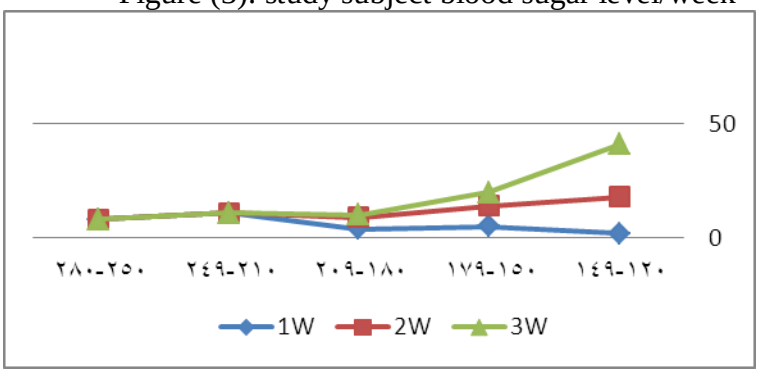
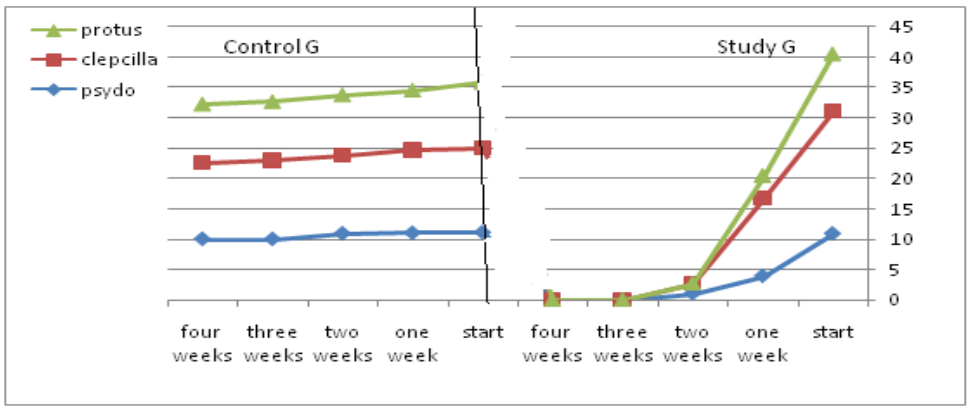


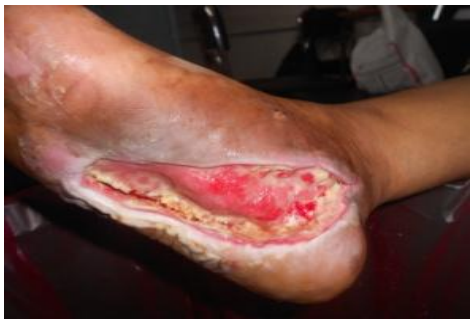
Table (7): Correlation coefficient between inflammatory cytokines, endothelial dysfunction in the form of [CRP, serum insulin, NAD (P)H oxidase & H_2O_2] for control and study subjects.

Inflammatory cytokines / Endothelial dysfunction	(TNF- α)		Anti TNF	
	T- value	P-Value	T-value	P-Value
CRP	4.73	<0.001**	0.26	1.00 ns
Serum insulin level	2.01	< 0.05*	0.16	1.00 ns
NAD(P) H&H_2O_2	2.98	ns	6.73	<0.001**

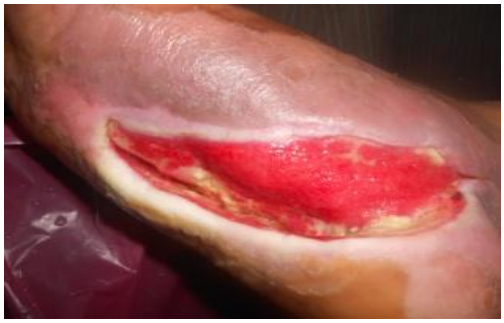
* : Significant (P< 0.05). ns: No significant (P>0.05)

(Fig 4)Microorganism means scores in both groups at start and end of the study

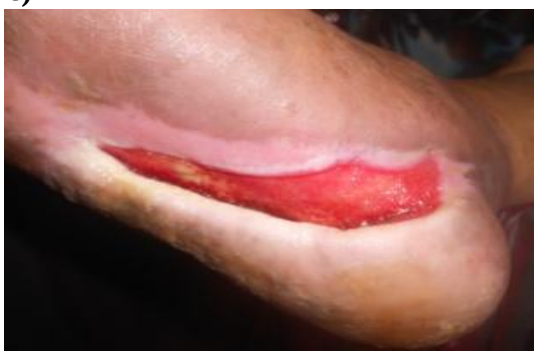




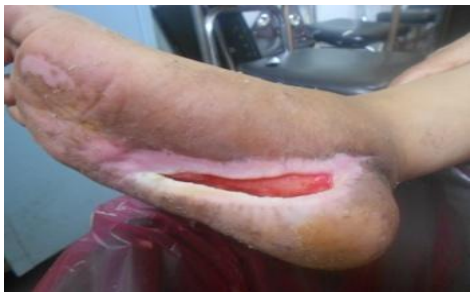
At start (baseline)

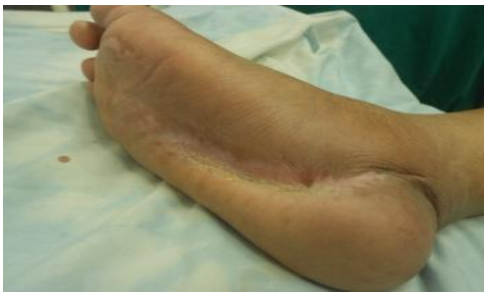


After 1 week



After2weeks





After 3 weeks

II. Discussion

The known anti-inflammatory and anti-bacterial properties of propolis combined with positive preclinical data in diabetic ulcers [(21)] make it a natural target for a human wound healing study in diabetes. Previous studies of propolis have indicated that it has low allergenicity to humans [(37)], low financial cost and shows wound healing acceleration in a diabetic rat model [(21)]. DFU is a common, expensive and debilitating problem among diabetic patients that may lead to infection and amputation **[(38)].** Therefore, today several different methods have been studied to achieve better results in the treatment of this sort of ulcers [**(34)**]. Recently, studies have shown therapeutic effects of some natural products on healing of ulcer in patients with DFU [**(39)**]. In consistent with previous studies [**(40)**]**,** findings of this research showed that propolise as an natural product could be effective in healing of DFU. Regarding to sociodemographic data and clinical characteristics our results shows that significant differences were found between study and control subjects the incidence of diabetic foot ulcer was found to be among diabetic patients more than 10 years ago and at the age of 50 years and more. These results were consistence with Bethesda who repoted that the incidence of diabetic foot ulceration increases in the middle age and in older person with diabetes.also the majority of patients were uncontrolled diabetes. Based on our results, total ulcer status showed significant difference between both subjects at the end of the 3rd week, So, it shows the potential efficacy of topical propolis as routine wound care on healing of DFUs ,it has anti-inflammatory, antimicrobial effects which lead to rapid improvement of healing. In this regard, our result is to some extent similar to Lotfy and Alenzi[(42)], study which assessed the effects of the propolis dressing technique on the healing of superficial and

deep DFUs. Based on the results of their study, at the end of the 1st, 2nd and 3rd weeks of follow up period the propolis dressing technique had significant effect on ulcer healing than routine dressing technique (P<0.05). In another similar study that was performed by [40], results indicated that, at the end of the 1st week of follow up period the propolis dressing had significant effect on ulcer healing than routine dressing technique (P<0.05) .Henshaw *et al*,[43] which is compatible with our result in the 1st week, while is not in line with our result in the 2nd and 3rd week of follow up.

In present study, 76.66% of the patients in the study subjects had complete ulcer healing as compared to 0.0% in the control subjects at the end of the follow up, which reveals that patients who treated with propolis had better healing process. In this regard, our result is to some extent consistent with the results of to Henshaw *et al,* which indicated that 60% of patients in the study group had complete ulcer healing at the end of follow up period as compared to 0% in the control group[42]) .While is not in line with Sheehan et al, and colleagues reported that achieving 50% in wound healing area by week 4 is a robust predictor of long term healing prognosis [44]. In addition, in the control group, healing rates were greater at weeks 2 and 3 in the wounds **[45]**, which is not in accordance with our result in control group. It is logical that these wounds in the control group healed more rapidly because they lacked the complicating factor of infection. Regarding to partial ulcer healing, our results showed that 23.33% of the patients in the study group had partial ulcer healing as compared to 66.66% in the control group at the end of the follow up, which is in line with the results of to Abu-Ahmed that showed most of patients (66.7%) in the control group had partial ulcer healing at the end of follow up[45]. In present study, no patients complained of lacking ulcer healing in the study subjects, while 13.3% of the patients in the control group had unhealed ulcer at the end of follow up period, which is consistent with the results of to Khadem, and Abu-Ahmed that showed 33.3% and 4.0% of patients respectively complained of lacking ulcer healing in control groups at the end of follow up. The discrepancies between our results and above mentioned studies may be due to administration of different type of propolis and using different questionnaires. Beside, in our study so that care techniques are different among health care providers, all intervention was done by trained researcher but in Khadem, study the intervention was done by patients. So, it's may be another reason for differences between our results.

Regarding to wound bed, our results showed that. 63.3%were started granulation after the first week and 100% by the 3ed week in study subjects were epithelization compared to 3.0% of the control subject by the 3ed week were stared granulation, (P 0.001). Which is in accordance with result of to **[45]** that indicated that after 1st week of treatment, 70% of clinically treated wounds with propolis showed clean healthy bright red surface with no infection and marked decrease in wound dimensions. Alam etal[46] stated that it is possible to increase the oxygen release rate from hemoglobin by lowering the wound pH via honey application, thus increasing tissue granulation and improving the wound healing rate in diabetic patients. Moreover, acidifying a wound through honey application can potentially reduce the protease activity and provide a suitable environment for increasing fibroblast activity, consequently promoting wound healing[46]. After 2-3 weeks of treatment, wound sizes showed marked reduction and significant increase in wound contraction 100% with granulation tissue formation, in addition, showed no exudates.

As regarding to ulcer size, the our present study demonstrated that, there is statistical significant differences decreased in ulcer surface area and depth by the propolis group compared with the control group at week 1 (P < 0.001), and at week 3, respectively (P < 0.05), Our result is to some extent similar to [43], which indicated that Ulcer surface area was reduced in the propolis group compared with control group at 1st week(p<0.001),and(p <0.05) respectively. Alam et al[46] mentioned that a previous study investigated the effects of honey dressing following two weeks of application on a non-healing ulcer by collecting measurements of the change in wound surface pH and the ulcer size. A statistically significant reduction in the wound pH and size was observed[46].

According to fluids exudate, the current study showed that wound fluid active MMP-9 was significantly reduced, by at the end of 3rd week from baseline in propolis treated ulcers vs. controls (P < 0.001), as were bacterial counts (P < 0.005).

Propolis is a natural product that has been recently introduced in modern medical practice. Propolis antibacterial properties and its effects on wound healing have been thoroughly investigated. This is in line with [47]Who state that Honey that contains <20% water is hyperosmolar which creates an unfavorable environment for the growth and survival of microorganisms .High osmolarity substrates such as honey, glucose, and sugar pastes can inhibit microbial growth because water molecules are chemically tied to the sugar molecules, thus creating a nonconductive environment for organism

survival, leading to death . Therefore, the hyperosmolar condition created by propolis is also important for treating infections because it prevents the growth of bacteria and encourages rapid wound healing[47]. Laboratory studies and clinical trials have shown that propolis is an effective broad-spectrum antibacterial agent[42] .

Propolis as a natural product could be effective in healing of DFUs. Propolis is the most antibiotic man has ever discovered. The old Egyptians, Greeks and Romans reported the use of propolis in popular medicine [49]. 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[50]., Due to impairment in physiological synchronization of events that lead to rapid healing, foot ulcers do not follow an orderly and reliable wound healing process. For this reason, the current study evaluate whether propolis dressing technique would bring an improvement in DFUs healing more than routine method .

It is notable that the effect of propolis was most clearly seen within some weeks of its first topical application. Advantage of propolis on ulcer healing rate was seen at 3weeks of follow up with decreased levels of random blood glucose for study subjects on week 3. These quite rapid effects are consistent with the known potent anti-inflammatory effects of propolis, and its efficacy of diabetic ulcer healing.[51].

This result in congruent with [52]. who stated that infection causes a stress response in the body by increasing the amount of certain hormones such as cortisol and adrenaline. These hormones work against the action of insulin and as a result, the body production of glucose increases, which results in high blood sugar levels. also (46) stated that some of the properties of honey (acidity, osmosis, hydrogen peroxide, and nitric oxide) contribute to its antimicrobial activities against diabetic wounds

A major concern during the period of treatment illustrated that the pain only at dressing, p=0.004. Fortunately, similar to the previous studies showed that, propolis and some of its components produce anesthesia, which in some studies were shown to be 3 times as powerful as cocaine and 52 times that of procaine [53]. The anaesthetic effect of propolis may relief wound pain. Also (46) stated that
Propolis dressings which provided for a moist wound environment facilitated autolytic debridement, Honey contains protease enzyme that induces wound tissues to start autolytic debridement and removing dead, damaged, or infected wound tissues , slough and necrotic tissue without any feeling of pain.

AS regards wound Odor it was observed that no Odor after first week of treatment with a statistical significant difference (P value 0.004) between control and study subjects. This results was supported by [46] reported that propolis has the potential ability to minimize offensive-smelling wounds through its strong osmotic action which draws exudates and lymph fluid from the wound out towards the surface to add the moisture needed for autolytic debridement, a decrease in wound odor has been reported during the treatment of diabetic foot and leg ulcers.
propolis can deodorize wound odor through two mechanisms. First, the presence of some anaerobic bacteria such as *Bacteroides* spp., *Peptostreptococcus*
spp., and *Prevotella* spp. is documented to produce malodor. Second, wound odor is produced by the creation of amino acids through the decomposition of serum, tissue proteins, and dead cells by bacteria. Honey acts by providing an abundance of glucose as a substrate in preference to amino acids for bacterial metabolism .Therefore, glucose is converted to lactic acid by bacteria in the presence of honey instead of the malodor-producing ammonia, amines, and sulfur compounds typically produced by the metabolism of amino acids.
Furthermore, a significant difference was seen regarding to grade of ulcer after second week of follow up between both subjects and the majority of study subjects showed remarkable improvement following application of propolis. This result in congruent with [54]. who reported that honey dressing was beneficial and safe for Wagner's grade II diabetic foot ulcers.

In chronic wounds, the reestablishment of a normal repair pattern by topical propolis is apparently related mainly to the ability of its Polyphenols to down regulate the activation of TNF the master key of the genetic regulation of immunity and inflammation – induced by bacterial molecules, inflammatory mediators, and oxygen/nitrogen reactive species; and with its iron chelating capability[55].

And this concomitant with the current result where, the researcher observed that laboratory studies showed that the levels of inflammatory mediators were TNFα (TNFRs, Anti TNF), Cytocines 1L-6, 1L-12, CRP, NAD(p)H and H_2O_2 product, scientifically reduced by propolis dressing technique due to the effect of flavonoids and caffeic acid. Additionally elevated concentrations of inflammatory mediators in the wound case pain receptors nearby amplifying pain mechanism thus local analgesic effect displayed by propolis[56].

A relationship has been found to exist between inflammation and endothelial dysfunction. It is believed that tumor necrosis factor-α (TNF-α) is one of the most important cytokines that mediate inflammation as well as endothelial dysfunction. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, uncoupled endothelial nitric oxide synthase. TNF- α is one of the stimuli to directly activate NADPH oxidase and TNFR may provide additional benefit in the patient with diabetes type I and II [55]. Endothelial dysfunction in diabetes increased Cytokine TNF through stimulation secretion of TNFR1 so increased secretion NAD(p) H and H_2O_2[58].Unfortuntely,we couldn't find similar study to compare our result.

In the present study showed that highly significant differences in number of microorganism between control and study subjects. These results came in line with Daleprane(59) tested the bactericidal activity of propolis towards 20 staphylococcus,10 streptococcus and 10 E. coli cultures using concentrations of 1.25-5 mg propolis/ml, it showed strong inhibitory activity against 25 of tested bacterial species. (Bacillus cereus, B. subtitles, Staphylococcus aureus, and S. epidermis and Streptococcus pyogenes) of the Gramnegative species, Enterobacter cloacae and Proteus vulgaris were inhibited, and Pseudomonas aeruginosa, Serratia marcescens, and Serratia sp. The other 3 species (Eschericia coli, Klebsiella pneumoniae and Salmonella typhymurium) were inhibited. Propolis dressings provided for a moist wound environment,facilitated autolytic depridement and healthy granulation tissue formation,were painless and easy to use and to remove without trauma to the wound[38]

As virtually all bacterial pathogens require iron to survive and develop virulence factors, reducing it availability by chelating is a valid antipathogenic strategy, particularly against staphylococcus aureus and psedudomonas aeruginosa [61]. So, the ability of propolis as an iron chelator seems to be the leading cause for significantly reducing biofilm formation in turn, this reduction improves ulcer healing outcomes. Additionally propolis dressing technique showed a high powerful oxidizing and nitrating moliecule concomitantly, wound healing rate and re-epithelialization improved .

A major concern during the treatment with herbal medications is the unpredicted side effects such as allergic reactions. Fortunately, similar to the previous studies (Khadem,Koushan and Asgharzadeh) no significant side effects were observed in patients treated with propolis which indicates that this could well become a part of routine therapy for DFU. So, the propolis should be more assess by researcher in educational and research centers due to it is found abundantly in Egypt and compared to the chemical drugs, has no adverse effects and is quite cheap.

Some limitations: in this study should be noted. Firstly, follow up time in this study was too short and we couldn't follow up patients until complete healing. Secondary, this study is limited by virtue of a small patients' population that may create a low power of statistical analysis. So, it's suggested that future study pay more attention to these limitations and conduct their investigations with propolis and treat and follow up patients until complete healing.

III. Conclusion And Recommendations

Conclusion: Our results concluded that, topical propolis is more effective than routine dressing; it accelerates wound healing and is without any side effect. Propolis dressing was effective, safe and inexpensive to treat poor healing diabetic wounds However; further studies are required in the future to confirm these results.

Recommendation: additional successful clinical evidence is required with validated laboratory findings to establish propolis as one of the most effective alternative topical medicines for treating diabetic wounds.

References

[1]. Bentley, J., & Foster, A. (2007). Multidisciplinary management of the diabetic foot ulcer.British Journal of Community Nursing, 12, S6–S10

[2]. Boulton, A. J., Vileikyte, L., Ragnarson-Tennvall, G., & Apelqvist, J. (2005). The global burden of diabetic foot disease. The Lancet, 366, 1719–1724.

[3]. Wrobel, J.S., Mayfield, J. A., & GE, R. (2005). Geographic variation of lower-extremity major amputation in individuals with and without diabetes in the Medicare population. Diabetes Care,24,860–864.
[4]. Shearer, A., Scuffham, P., Gordois, A., Oglesby, A., & Tobian, J. A. (2003). The health care costs of diabetic peripheral neuropathy in the US. Diabetes Care, 26, 1790–1795
[5]. Bren H., and Tomic Canic M. (2012):Cellular and molecular basis of wound healing in diabetes. J Clin Invest 117:1219-22.
[6]. Median A. (2012):Patholophsilogy of chronic non healing wounds. J Burn Care Rehabil 26:306-19:
[7]. Chen WY., and Rogers AA. (2013):Recent in sights into the causes of chronic leg ulceration in venous diseases and implications in other types of chronic wounds. Wound Rep Reg 15:434-49..
[8]. Hartoch RS. (2010):Emergency management of chronic wounds. Emerg Med Clin N Am 25:203-21..
[9]. Wolcott R. and Dowd S. (2011):The role of biofilms:Are we hitting the right target? Plast Reconstructr Surg 127(suppl): 28S-35S;
[10]. Afshani M., Bastan Hagh MH., Pajouhi M., and Baradar Jalili R. (2014): Prevalence of lower limb amputation in patients with diabetic foot ulcer. Journal of Medical Council of I.R.I:23(1):25-29.
[11]. Wieman TJ., and Smiell JM.(2013): Effect of local epidermal growth factor on wound healing in diabetic foot. International Journal of Clinical Practice 61(11): 1931-38.
[12]. Gilchrist B.(2014): Sampling bacterial flora: a review of literature. Journal of Wound Care 5:8 386-88.
[13]. Oyibo A. (2009):coparison of two diabetic foot ulcer classification systems: The Wagner and the University of Texas. Wound classification systems. Diabetes Care.:24(1):84-8.
[14]. Nelson EA. (2013):update: Compression bandaging in treatment of venous leg ulcer. Journal of Wound Care 5:9 415-18.
[15]. Boyce ST. (1999): Cytotoxicity to cultured human keratinocytes of topical antimicrobial agents. J Surg Res; 84:188-98.
[16]. Zapata-Sirvent RT., and Hansbrough JF. (2013): Cytotoxicity to human lekocytes by topical antimicrobial agents used for diabetes care. J Diabtes Care Rehabil; 14(2):132-80.
[17]. Abdulsalam K. S; Mohamed , M. I. ; El-Nawawy M.A. (2013):Effect of Propolis on some bacterial species.
[18]. Krol W.,Scheller S. Shani J., Pietsz G.,Czuba Z. (2012):Synergistic effect of ethanolic extract of propolis and antibiotics on the growth of staphylococcus aureus. Arzneimittel Forschung 43-1 (5) 607-9.
[19]. Morales F., and Garbarino L.J.(2014):Clinical Evaluation of a new hypoallergenic formula of propolis in dressings. In: Bee products. Properties, applications and apitherapy Mizrahi and Y Lensky Eds), pp 101-105. Plenum Press, New York,
[20]. The Diabetes Control and Complications Trial Research Group. (2013):The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med;329:977-986.
[21]. McLennan, S. V., Bonner, J., Milne, S., Lo, L., Charlton, A., Kurup, S., et al. (2008). The anti-inflammatory agent propolis improves wound healing in a rodent model of experimental diabetes. Wound Repair and Regeneration, 16, 706–713.
[22]. Wagh, V. D. (2013b). Propolis: A wonder bees product and its pharmacological potentials. Advances in Pharmacological Sciences, 2013, 308249.
[23]. Gallo, E., Lucenteforte, E., Firenzuoli, F., Menniti-Ippolito, F., Maggini, V., Pugi, A., et al. (2014). Herbalists' perception of risks involving commonly sold medicinal plants in Italy. Complementary Therapies in Medicine, 22, 81–86.
[24]. Bufalo, M. C., Ferreira, I., Costa, G., Francisco, V., Liberal, J., Cruz, M. T., et al. (2013).Propolis and its constituent caffeic acid suppress LPS-stimulated pro-inflammatory response by blocking NF-kappaB and MAPK activation in macrophages. Journal of Ethnopharmacology, 149, 84–92.
[25]. Wagh, V. D. (2013a). Propolis: A wonder bees product and its pharmacological potentials. Advances in Pharmacological Sciences, http://dx.doi.org/10.1155/2013/308249.
[26]. Banskota, A. H., Nagaoka, T., Sumioka, L. Y., Tezuka, Y., Awale, S., Midorikawa, K., et al.(2002). Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. Journal of Ethnopharmacology, 80, 67–73.
[27]. Talas, Z. S., Ozdemir, I., Ciftci, O., Cakir, O., Gulhan, M. F., & Pasaoglu, O. M. (2014). Role of propolis on biochemical parameters in kidney and heart tissues against L-NAME induced oxidative injury in rats. Clinical and Experimental Hypertension [Ahead of print; PMID: 24490594].
[28]. Gekker, G., Hu, S., Spivak, M., Lokensgard, J. R., & Peterson, P. K. (2005). Anti-HIV-1activity of propolis in CD4(+) lymphocyte and microglial cell cultures. Journal of Ethnopharmacology, 102, 158–163.
[29]. Astani, A., Zimmermann, S., Hassan, E., Reichling, J., Sensch, K. H., & Schnitzler, P. (2013).Antimicrobial activity of propolis special extract GH 2002 against multidrugresistant clinical isolates. Pharmazie, 68, 695–701.
[30]. Liu, Y., Min, D., Bolton, T., Nubé, V., Twigg, S. M., Yue, D. K., et al. (2009). Increased matrix metalloproteinase-9 predicts poor wound healing in diabetic foot ulcers. Diabetes Care, 32, 117–119.
[31]. Mirzoeva OK., and Calder PC.(2011): The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukot Essent Fatty Acids*, 55:441-449.
[32]. Dwtott K., and Buthelezi S.(2010):Anti-inflammatory and antibacterial Profiles of selected compounds found in South African Propolis .South African Journal of signs.105(11- 12):470-72.
[33]. Shahbazian H., Yazdanpanah L., and Latifi SM.(2013): Risk assessment of patients with diabetes for foot ulcers according to risk classification consensus of International Working Group on Diabetic Foot (IWGDF). Pak J Med Sci; 29: 730-734.
[34]. Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG,Woo K, Boeni T, Ayello EA, and Kirsner RS. (2014): Diabetic foot ulcers: Part II. Management.J Am Acad Dermatol; 70: 21.
[35]. Mohanny,K.M.(2005): Investigations on propolis and bee venom Produced by two hybrids of honeybee with reference to a new device for bee venom collection.
[36]. Wagner FW.(2009):Supplement,algorithms of foot care .The diabetic foot,3rded,St,Louis,MO,CV;Mosby; p:291-302.
[37]. Rajpara, S., Wilkinson, M. S., King, C. M., Gawkrodger, D. J., English, J. S., Statham, B. N., et al. (2009). The importance of propolis in patch testing—A multicentre survey. Contact Dermatitis, 61, 287–290
[38]. Driver VR, Fabbi M, Lavery LA, Gibbons G. The costs of diabetic foot: the economic case for the limb salvages team. J Vasc Surg. 2010; 52(3 Suppl):17-22.
[39]. Li S, Zhao J, Liu J, Xiang F, Lu D, Liu B. Prospective randomized controlled study of a Chinese herbal medicine compound Tangzu Yuyang Ointment for chronic diabetic foot ulcers: a preliminary report. J Ethnopharmacol. 2011; 133:543-50. PubMed Abstract | Publisher Full Text
[40]. Khadem Haghighian H, Koushan Y, Asgharzadeh AA. Treatment of Diabetic Foot Ulcer with Propolis and Olive Oil: A Case Report. Knowl Health. 2012; 6:35-8.
[41]. BethedaM.New diabetic foot wound treatments,reduce the risk of amputations.Journal of the americanpodiatricmedicalassociation.2000.Feb.(14)

[42]. Lotfy, M., Badra, G., Burham, W., & Alenzi, F. Q. (2006). Combined use of honey, bee propolis and myrrh in healing a deep, infected wound in a patient with diabetes mellitus. British Journal of Biomedical Science, 63, 171–173.
[43]. Henshaw FR, Pfrunder L, McKew, GL, Macleod , McLennan SV. Twigg SM,BoltonTV, Nube, A, Veldhoen D.(2013): Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study.Journal of Diabetes and Its Complications 28 (2014) 850–57.
[44]. Sheehan, P., Jones, P., Giurini, J. M., Caselli, A., & Veves, A. (2006). Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Plastic and Reconstructive Surgery,17, 239S–244S.
[45]. Abu-Ahmed, H, Abdel-Wahed R.E, El-Kammar M.H. and. El-Neweshy2M.S(2013) Evaluation of the Effectiveness of Propolis Compared with Honey on Second Intention Wound Healing in the Equine. Middle-East Journal of Scientific Research 14 (10): 1292-98.
[46]. Alam F, Islam. A,1 Gan S H,1 and Eddy K J. J, Gideonsen M.D., and Mack G.P., (2014):" propolis: A Potential Therapeutic Agent for Managing Diabetic Wounds.vol 2014.
[47]. Eddy J. J.,. Gideonsen M. D, and. Mack G. P, (2008):Practical considerations of using topical propolise for neuropathic diabetic foot ulcers: a review,"Wisconsin Medical Journal, vol. 107, no. 4, pp. 187–190, 2008. View at Google Scholar · View at Scopus
[48]. Grunberger, D., Banerjee, R., Eisinger, K., Oltz, E. M., Efros, L., Caldwell, M., et al. (1988).Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia,44, 230–232.
[49]. Hegazi, A.G.; Abdel Hady, F.K. and Abdallah, F.A.M. (2000). Chemical composition and antimicrobial activity of European propolis. Z. Naturforsch. 55: 70-75.
[50]. Salatino, A.; Teixeira, E.W.; negi, T.G and Message, D. (2005). Origin and chemical variation of Brazilian propolis. Evid. Based Complement Alternal. Med. 2(1): 33-38
[51]. Sehn E., Hernandes L., Franco S.L, Goncalves C.C., and Baesso M.L .(2013): Dynamics of re-epithelization and penetration rate of a bee propolis formulation during cutaneous wound healing. Analytical Chimica Acta; 635: 115.
[52]. Dire DJ. (2009): Evaluation of antimicrobial activity of a new superoxidised water for disinfection of diabitic foot ulcer. Clinical Practice Guide, 19(6): 801-7
[53]. Efem, S "Clinical observations on the wound healing properties of propolis," British Journal of Surgery, vol. 75, no. 7, pp. 679–681, 1988. View at Publisher · View at Google Scholar · View at Scopus
[54]. Zasshi, Y. (2005). Effects of propolis on blood glucose, blood lipid and free radials in rats with Diabetes meflitus. Pharmacal. Res. 5(2): 147-52
[55]. Ghesalbert, E.L. (1979). Propolis review. Bee world. 60; 59-84.
[56]. Barber SM. (2010): Molecular and cellular aspects of wound healing. The north American veterinary conference.
[57]. Farouk A., Hassan T., Kashef H., Khalid SA., Mutawalia I., and Wadi M. (2012): Studies on Sudanese bee honey: laboratory and clinical evaluation. Int. J. Crude Drug Res; 26 (3):161.
[58]. Ahdieh, M., Vandenbos T., and Youakim A.. (2011): Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-. Am. J. Physiol. 281:C2029.
[59]. Focht, J. ; Hansen, S.H. ; Nielsen, J. V. ; Berg- Segers, A. Van Den; Riezler, R. (2013): Bactericidal Effect of Propolis in Vitro against agents causing upper respiratory tract infections. Arzneimitte Forschung 43-II (8) 921-923.
[60]. Daleprane J.(2012):Anti-Atherogenic activities of polyphenole from propolis.Journal of National biochemistry. 23 (6):557-66.
[61]. Zicaardi P., Nappo F., Giugliano G.,.Esposito K., Martella R., Ciuffi. M . (2013): Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation., 105, 804-9.