Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 254 (2021) 119639

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Spectrochemical and biochemical assay comparison study of the healing effect of the Aloe vera and Hypericum perforatum loaded nanofiber dressings on diabetic wound

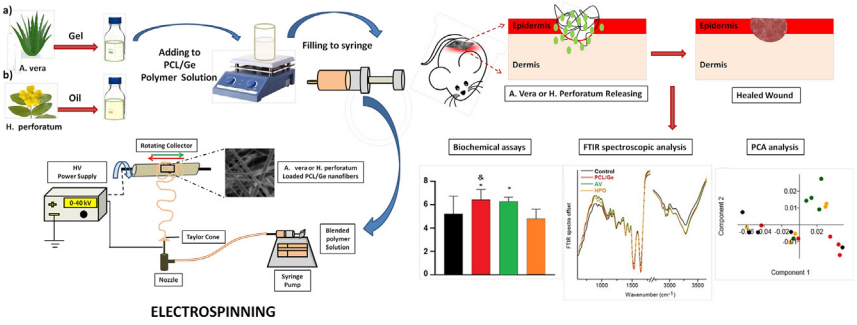
Zozan Guleken [a,*], Joanna Depciuch [b,*], Hasan Ege [c], Gül İlbay [d], Cevriye Kalkandelen [e], Dilek Ozbeyli [f], Huri Bulut [g], Goksel Sener [h], Nevzat Tarhan [i], Serap Erdem Kuruca [j]

[a] Department of Physiology, Uskudar University Faculty of Medicine, Istanbul, Turkey
[b] Institute of Nuclear Physics Polish Academy of Science, 31-342 Krakow, Poland
[c] Institute of Health Sciences, Department of Physiology Istanbul University Cerrahpaşa, Turkey
[d] Department of Physiology, Faculty of Medicine, Kocaeli University 41380 Kocaeli, Turkey
[e] Istanbul University Cerrahpaşa, Vocational School Technical Science Istanbul, Turkey
[f] Department of Medical Pathology Techniques, Vocational School of Health Services, Marmara University, Istanbul, Turkey
[g] Department of Medical Biochemistry, Faculty of Medicine Istinye University, Istanbul, Turkey
[h] Marmara University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey
[i] Uskudar University, Department of Psychiatry, Istanbul, Turkey NP Brain Hospital, İstanbul, Turkey
[j] Department of Physiology, Istanbul University Faculty of Medicine, Istanbul, Turkey

HIGHLIGHTS

- FTIR spectroscopy can monitor the biological events triggered by diabetic wound.
- Nanofiber dressings has a healing effect on DM skin wounds.
- HPO decreases ROS parameters on diabetic wound.
- HPO dressings are more effective than AV dressings on skin wound healing in the inflammation phase.
- IR spectroscopic analysis and biochemical assays, consistent and correlative with each other.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 September 2020
Received in revised form 12 February 2021
Accepted 20 February 2021
Available online 9 March 2021

Keywords:
Diabetic wound healing
Inflammation
Polymeric nanofibers dressings
Aloe vera extract
Hypericum perforatum oil
FTIR analysis
Biochemical assays

ABSTRACT

Diabetic wounds have a slow healing process and easy to be infected. In addition to current drug treatments, supportive approaches are needed for diabetic wound treatment. In this study, we aimed to load Aloe Vera (AV) and Hypericum perforatum oil (HPO) with PCL/Ge (Poly (ε-caprolactone)/Gelatine) polymeric biodegradable by electrospinning method into nanofiber dressings on an experimental diabetic wound model to compare the diabetic wound healing effect. Changes in the amount and chemical structure of phospholipids, proteins, and lipids were investigated in the blood and serum samples of the animals using Fourier transform infrared (FTIR) analysis. To evaluate biological events associated with the wound repair process in inflammatory phase we used oxidant and antioxidant status to determine the healing status of wounds such as Total antioxidant status (TAS), Total oxidant level (TOS) and tumor necrosis factor alpha (TNF-α) levels. TOS level increased in DM groups and decreased in the AV and HPO group. Oxidative stress index decreased and TNF-α level increased in the HPO group. FTIR spectra showed changes in the phospholipids, proteins, and carbon chain of lipids in the whole blood as well as serum of DM rats. FTIR spectra combined with Principal component analysis (PCA) showed, that

* Corresponding authors.
E-mail addresses: zozan.guleken@uskudar.edu.tr, zozanguleken@gmail.com (Z. Guleken), joanna.depciuch@ifj.edu.pl (J. Depciuch).

https://doi.org/10.1016/j.saa.2021.119639
1386-1425/© 2021 Elsevier B.V. All rights reserved.

treated DM rats by AV and HPO caused return chemical structure of blood and serum to this observed in control group. Higher similarity with control group for HPO rats was observed. HPO is better than AV in the alternative for healing on diabetic wound. Thus, we have demonstrated that IR spectroscopy and multivariate data analysis and biochemical assays are consistent and correlative with each other.
© 2021 Elsevier B.V. All rights reserved.

1. Introduction

Diabetes causes complicated pathogenesis with a major impact on the lifespan and well-being of individuals worldwide. The International Diabetes Federation reported that a total of 463 million peoples are estimated to be living with diabetes in 20–79 years. The authors explained that this number is expected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045 [1]. Hyperglycemia, which affects people who have diabetes, plays a key role in increased protein glycosylation [2]. Oxidation of glucose precursors causes the production of free radicals, which lead to oxidation of low-density lipoproteins or promote the oxidation of membrane lipids, which trigger the cellular membranes damages [3–4].

During the inflammatory phase of wound healing, damaged cells, pathogens, and bacteria are removed from the wound area. These white blood cells, growth factors, nutrients and enzymes create the swelling, heat, pain and redness commonly seen during this stage of wound healing. Inflammation is a natural part of the wound healing process and only problematic if prolonged or excessive. There is limited understanding of the specific role of altered redox signaling in the diabetic wound, although there is evidence that ROS are involved in the underlying pathology. Preclinical studies of antioxidant-based therapies for diabetic wound healing have yielded promising results. Redox-based therapeutics constitute a novel approach for the treatment of wounds in diabetes patients [5]. Recent studies had been shown that the production of high amounts of ROS could induce significant tissue damage, which could lead to the transformation of neoplastic cells. These cells have a capacity to induce cellular, DNA proteins, and lipids damage of the membrane structure of the impaired healing process [6].

The extract of AV has been regarded as additional treatment herbs for various diseases. It has been known that AV has anti-inflammatory, antibacterial, and antidiabetic activities [7]. Additionally, AV has important benefits such as an inhibitory role on the wound and contribution on the enhancement of immunomodulatory activity, and role in myogenic activities. In recent years, the active ingredients of Aloe vera are noticed in the field of biomaterials and nanotechnology-based medical applications. Researchers have reported that the addition of Aloe vera extract to the material, extensively improves the overall effectiveness of the product. However, they report that Aloe vera gel, combined with natural and synthetic polymers, in the form of films, membranes, and composites may be extremely useful for various wound and burn healing applications. Since these biomaterials obtained from Aloe vera gel extract are biodegradable, for this reason, they are available to eliminate the damage that commercially synthetic bandages cause to the new skin formed at the wound site [8]. The biological materials which include AV gel were commonly combined with the; bacterial cellulose, chitosan [9], alginate [10], gelatin [11], silk fibroin [12], and pectin- gelatin [13] collagen-chitosan combinations. Recent studies have been reported that chitosan-Aloe vera gel-based nano membranes have sufficient properties, for the usage of dressing wounds such as roughness, impairment rate, wettability, and mechanical properties [14].

HPO is another plant that is widely used because of its medical effects [15]. The topical application of this plant has been widely used to speed up the healing of burn and other type of wounds among people too. It is claimed that the plant may show some of its effects due to its antibacterial activity [16]. Suntar et al. [17] have been reported that HPO had an anti-inflammatory effect via increased resistance to infection, fibroblast migration, and providing collagen accumulation. There are also studies reporting that the wound healing activity of HPO is triggered by an increase in fibroblastic activity and collagen synthesis [18]. In the literature, there are several studies suggests the use of HPO's raw plant extract due to its antibacterial activity on wound treatment, treatment of skin diseases and infectious diseases [19].

The pathogenesis of slow healing in diabetics is not still exact. Wound infection is a common complication in diabetic patients. A diabetic wound causes disruption of membrane integrity and the wound becoming susceptible to infection [20]. However, researchers studied to improve the healing process via different types of plants to contribute in addition to chronic drug treatments of diabetics [21]. Additional multidisciplinary studies obtained by tissue engineering could help stimulating self-repair mechanism of wound healing in diabetic patients.

Determination of the healing status of diabetic wound plays has an importance in deciding suitable treatment. In order to understand the mechanism of wound repair there are examination of tissue biopsies. The techniques routinely used to study the mechanisms of wound tissue repair may include histological examination of tissue biopsies, immunohistochemistry, in situ hybridization, polymerase chain reaction, etc.

The aims of this study are firstly comparing the oxidative and antioxidative effect of AV and HPO loaded nanofiber dressings on diabetic wound in the process of healing. Secondly, we aimed to determine the healing status of diabetic wounds using FTIR signatures to evaluate the feasibility of Fourier transform infrared spectroscopy.

2. Materials and methods

In this study, as an experimental type 2 diabetes rat model was planned by using streptozotocin (STZ) to compose similar to metabolic abnormalities seen in humans which is comparatively easy to produce. Firstly, we produced nanofibers wound dressing patch with various polymers and to design it as a diabetic wound dressing material with AV and HPO loaded PCL/Ge blends antimicrobial properties. Next, these biocompatibilities and bio degradative patches were used as a wound dressing and the effect of two different nanofibers dressings were determined by biochemical methods such as TAS, TOS and TNF-α and FTIR spectroscopy. This is the first innovative study to combine AV and HPO with various polymers to design it as a diabetic wound dressing material.

2.1. Preparation of polymers

Poly(ε-caprolactone) (PCL) Mn: 80,000 and Porcine Gelatine (Ge) was purchased from Sigma-Aldrich. The solvent of Acetic acid and Formic acid was purchased from Merck. Hypericum perforatum plants were collected from the mountains of Izmir Kemalpasa. The Hypericum perforatum oil obtained from plants

were produced in our research center in Istanbul. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich. NIH3T3 mouse fibroblasts cell lines were obtained from American Type Culture Collection (ATCC). Dulbecco's modified Eagle medium (DMEM) and Fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). 3-(4, 5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium-bromide (MTT) was purchased from Sigma-Aldrich.

2.2. Extraction of Hypericum perforatum oil and of Aloe vera gel

Hypericum perforatum plants collected from Izmir / Kemalpaşa mountains were placed in a 1-liter jar and the pure olive oil was added on the plants. For about a week, the jar was left open and allowed to ferment in the sun. Afterward, the jar was closed and the mixture was kept in the sun for about 4 weeks until it got dark red. Hypericum perforatum oil (HPO) was then taken into bottles. Contact of the bottles with daylight has been prevented.

The leaves of the Aloe vera (AV) plant were gotten from Istanbul University botanical garden. The leaves were kept vertically for 2 h in a 2-liter beaker in the Pharmacy laboratory. The 4 leaves were weighted as 790 g. The AV gel was separated from the leaves and homogenized by waring blender for 3 min and it was weighted as 520 g. After homogenization, the gel was kept in the refrigerator at + 4 °C for overnight due to foam formation during homogenization. Thus, the upward foams were easily collected the next day. Then, leaf gel was cut into small pieces and weighed as 238 g. It was homogenized with 350 ml of Phosphate buffer saline (PBS P^H = 7.00, 600 ml) and kept at + 4 °C for overnight. Gel extract was filtered with cheesecloth. The obtained gel extract was placed in tubes and frozen at −20° C.

2.3. Preparation and characterization of electrospinning solutions

PCL and Ge were dissolved in a binary solvent system of 9:1 (v/v) Acetic acid (AA): Formic acid (FA) at room temperature with a magnetic stirrer for about 24 h in a concentration of 15% (w/v). After dissolving the polymers, Hypericum perforatum oil and Aloe vera gel were added separately to the PCL / Ge solutions at different concentrations of 15% (w / w) respectively. All solutions were mixed for 60 min with a magnetic stirrer at room temperature (23 °C).

2.4. Fabrication of HPO loaded nanofibers and Aloe vera gel loaded nanofibers by electrospinning method

Prepared HPO and AV included polymer solutions of PCL/Ge/15% HPO, PCL/Ge/15% AV was filled into 10 ml plastic syringes separately. The syringes were connected to the nozzle tip (ID: 1.88 mm) with plastic tubes for using of each electrospinning application. Infusing of the solutions in a controlled manner as ml/h each polymer solution was connected to the controllable syringe pumps (NE-300, New Era Pump Systems, Inc., USA). During electrospinning, a high voltage (0–30 kV) was applied among nozzle tip and collector drum by direct current (DC) power supply. Electrospun nanofibers were collected to the collector drum that covered with oily paper. All electrospinning parameters such as applied voltage and flow rates were optimized for each nanofiber type. The distance between the needle and the collector was kept constant at 15 cm. All the experiments were carried out at room temperature (23 °C). Their material compositions were investigated using spectroscopic and thermal techniques. The size and morphology of nanofibrous dressings were examined with scanning electron microscopy. Also, FTIR measurements were obtained to analyze the molecular contents of loaded fibrous dressings and to confirm the presence of AV and HPO. Fig. 1 illustrates the flow of the production process.

2.5. Animals surgery and experimental design

Wistar albino male rats (250–200 g), obtained from Kocaeli University Experimental Animals Research and Implementation Centre (KOÜ DETAB). The animals were fed with standard rat chow and able to access water ad libitum. They were housed in the standard cages one by one, under controlled environmental conditions with an ambient temperature of (22 ± 2 °C) a 12-h light/dark cycles and 60% humidity, during the experiment. Accordance to the Declaration of Helsinki and with the guidelines of the care and use of laboratory animals used for scientific purposes our study was accepted by properly appointed and approved by the Kocaeli University Animal Care and Use Committee (KOUHADYEK 5.1.2019). Male rats weighed (n = 20) 250 – 300 g were randomly divided into two main groups: sham-operated (n = 5) and Streptozotocin (STZ) induced (n = 15) group. STZ group was further divided into subgroups as vehicle-treated (n = 5), Aloe vera 15% AV gel treated and PCL/gel treated (n = 5), St. John's treated 15% HPO gel and PCL/gel treated groups (n = 5). Each group consisted of 5 rats. Experimental type 1 diabetes mellitus model was performed by injecting freshly prepared Streptozotocin solution in 0.1 mol/L sodium citrate buffer (pH 4.5) to 50 mg/kg (provided that it is stored in the icy environment) in rats by intraperitoneal injections as described previously [22]. We test the blood glucose level from the tail vein using a blood glucose strips to check for STZ injection-induced hyperglycemia. Rats with blood glucose levels greater than 300 mg/dl were considered diabetic and included in the experiment. We kept rats for 7 days after STZ administration to obtain a chronic hyperglycemic state. On the seventh day, the diabetic wound was performed as the surgically in the sham group with similar operative procedures. However, the surgeon wore clean scrubs, masks, and hair cap and used fresh sterile gloves for each rat. All the decontamination protocols were applied. For the diabetic wound model, we shaved the dorsal hair of diabetic rats completely and the decontamination of dorsal skin was provided with 10% povidone-iodine. A sterile biopsy skin punch with a diameter of 8.0 mm was utilized interscapular region of the upper back of each rat and a full-thickness wound to the deep fascia was created according to the template. Then the obtained 15% AV gel and 15% HPO gel-based polymeric nanofibers were applied on the interscapular region of the upper back of each rat where we applied the diabetic wound model. Wistar Albino male rats (n = 20) were divided into two main groups: sham-operated (n = 5) and Streptozotocin (STZ) induced diabetic model (DM) (n = 15). DM group was further divided into subgroups (each for 5 animals) depending on the treatment; as vehicle, AV, HPO, and PCL/Ge (Poly (ε-caprolactone)/Gelatine) patches treated groups. The wound and surrounding area were covered totally with the used material. We made wound care for 7 days in the phase of inflammation in wound dressing. Wound dressing applications began after 7th day of STZ inductions. After the postoperative 14th-day body trunk, blood was collected by cardiac puncture for the measurement of serum TNF-α, TAS, TOS, OS index and FTIR spectral analysis.

2.6. TAS and TOS measurement:

Total Antioxidant Status (TAS) levels were measured by the spectrophotometric method using a commercial kit (Rel Assay, Turkey). Thermo Scientific Microplate Reader (USA) was used to perform assay. The changes on samples total antioxidants level were measured at absorbance on 660 nm. TAS activities were expressed as mmol Trolox Equiv/L of samples.

Total Oxidant Status (TOS) levels were measured by the spectrophotometric method using a commercial kit (Rel Assay, Turkey). Thermo Scientific Microplate Reader, USA device was used to per-

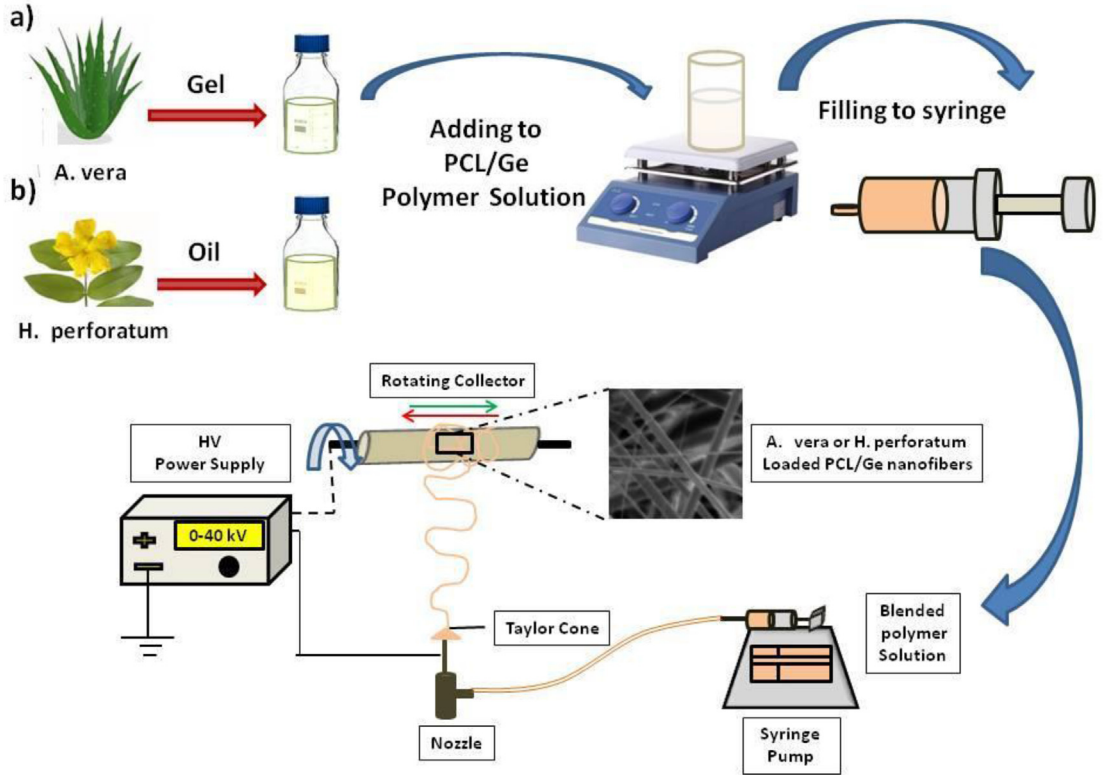


Fig. 1. Schematic illustration of production processes of a) Aloe vera (AV) loaded PCL/Ge blend nanofibers, and b) Hypericum perforatum (HPO) loaded PCL/Ge blend nanofibers by electrospinning method.

form assay. Results are indicated in terms of μm H_2O_2 Equiv/L. Oxidative Stress Calculation (OSI): OSI) level for each patient calculated by using (TOS (μmol·H2O2.equiv/L) × 100)/ (TAS (μmol.Trolox.equiv/L)) formula.

2.7. TNF-α ELISA

TNF-α ELISA kit (Elabscience C., Cat no: E-EL-R0019, China) was used for the quantitative measurement of TNF-α in serum samples. Samples and standards were pre-coated with the Anti-Human monoclonal antibody before incubation. Biotin was added to all wells, and combined with Streptavidin-HRP to form an immune complex; then carry out incubation again and washed to remove the uncombined enzyme. Optical density was read on a standard automated plate reader at 450 nm (Thermo Scientific Microplate Reader). The detection range of the kit was between 78.13 and 5000 pg/mL.

2.8. Sample preparation and spectral acquisition of IR spectroscopic measurements

Chemometric analysis of serum samples were made with an FTIR spectrometer (Jasco, FT/IR 4700) device with serial number C034661788 and licensed at Marmara University (JASCO Corporation 2967–5 Ishikawa-machi, Hachioji-shi, TOKYO, JAPAN). The detection was made with liquid-nitrogen cooled mercury cadmium telluride (MCT) Jasco, containing diamond with Gladi attenuated total reflection (ATR) TM, crystal plate, using the appropriate software JASCO Spectra Manager version 2. To acquire IR spectra, 4 cm^{-1} spectral resolution, with on zero-filling of the interferogram giving 4 cm^{-1} data spacing equivalent to 3684 data points within the 4000 cm^{-1} to 450 cm^{-1} regions with 64 scans. All samples were scanned two times. To compare the control group with others, all spectra were normalized using vector normalization. To obtain chemometric analysis, all features were averaged for serum samples spectral data. Raw spectra were ATR from 4000 to 600 cm^{-1} spectral regions, baseline-corrected, vector normalized in the pre-processing stage prior to multivariate analyses. Twenty-five points smoothed with Savitzky–Golay to eliminate sharp bands in the spectral regions. Moreover, smoothing using 9 points were done. Spectra were analyzed using OPUS 7.0. software.

2.9. Statistic

We compared all groups with each other with using analysis of variance (ANOVA) with 95% confidence interval and Bonferroni's post hoc test. To compare the groups, we used *t*-test (Graph Pad Prism 6.01 (Graph Pad Software, Inc.). We expressed results as mean ± SD (standard deviation). The statistical significance was signified as less than or equal to $p < 0.05^{*}$ and $p < 0.01^{**}$.

The number of obtained data from FTIR spectra was very large, therefore, to determine if is possible to distinguish FTIR spectra from control and non-control groups, and consequently to determine if the chemical structure of blood and serum collected from non-healthy rats treated with hypericum perforatum oil and of aloe vera oil return to this characteristic for control group, principal component analysis (PCA) was performed for the fingerprint region (between 800 cm^{-1} to 1800 cm^{-1}) for five FTIR spectra form each group. For this purpose, 518 points from the FTIR spectra were analyzed using Past 3.0 software.

3. Results

3.1. Synthesis of nanofibrous by electrospinning method

Nanofibrous scaffolds were produced through the blending of a synthetic polymer, polycaprolactone (PCL), and a natural polymer,

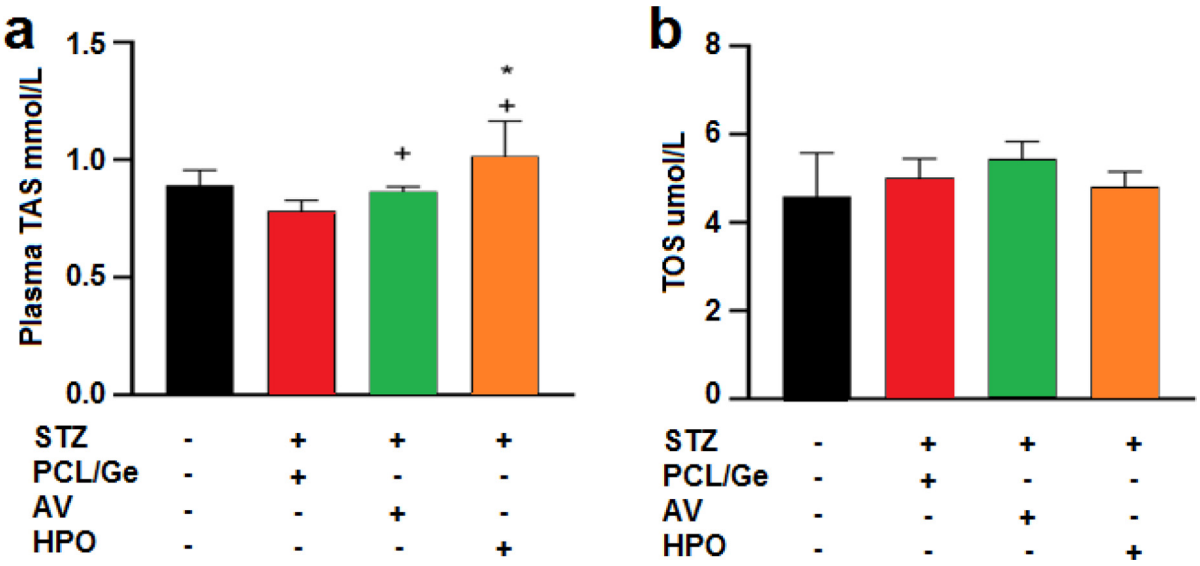


Fig. 2. Total antioxidant status (TAS) (a) and Total oxidant level (TOS) (b) levels on the serum of male rats which were either control or DM with or without Streptozotocin (STZ) induced diabetes mellitus model, and which were vehicle-, PGL/Ge, HPO, or AV treated. $^{+}P < 0.05$, compared to DM group; $^{*}P < 0.05$, compared to HPO group. Each group consists of 5 animals: control (black color) vehicle, PCL/ Ge (red color) porcine gelatin, AV (green color) Aloe v., HPO (orange color) Hypericum p. treated groups. (+means exist, - means non exist).

gelatin (GE), using an electrospinning technique. Then the extracts of AV and HPO were loaded to these polymers Fig. 1 oxidative .

3.2. Biochemical analysis: Total antioxidant status (TAS) and Total oxidant levels (TOS), tumor necrosis factor alpha (TNF-α) and Oxidative stress index (OSI)

We measured total status of serum to investigate the systemic effect of healing, (Fig. 2). We compared all groups with each other with One-way ANOVA statistics via Bonferroni's multiple comparisons test. From Fig. 2a it can be seen that compared to the DM (0.781 ± 0.044) group with mean ± SD the TAS value of HPO (1.01 ± 0.149) group was significantly increased ($p < 0.05$). There was no significance among other groups. However, when we compare groups with a *t*-test, there was a decrease TAS level of DM (0.781 ± 0.044) group compared to the control (0.89 ± 0,06) group but this decrease was not significant. Additionally, when we compared control to AV (0.864 ± 0.021) and HPO (1.016 ± 0.149) groups, there was a tendency to increase but these results were statistically with non-significant. On the other hand, when we compared the DM group to AV (0.864 ± 0.021) and HPO (1.016 ± 0.149), there was a significant increase in the TAS level of both AV and HPO groups ($p < 0.05$). Fig. 2b showed results obtained for TOS level measurement. There was none significant value with the mean ± SD when the Control group (4.58 ± 0.99), DM (5.01 ± 0.429), AV (5.441 ± 0.398), HPO (4.812 ± 0.337) among groups and compared to each other. However, as it is seen in Fig. 2b there was an increased TOS level on DM groups' serum and there was a tendency of decrease in AV and HPO group compared to Control group. Additionally, when we compared HPO and DM group there was a decrease on the HPO group compared to the AV group but with a non-significant statistical result.

Oxidative stress attributed to an imbalance between oxidant and antioxidant substances in the body. OSI marked as a marker of the capacity of antioxidant defense exaggeration by the production of reactive oxygen substances (ROS). OSI based on the ratio of ROS and antioxidant capacity. Pro-oxidants mark changing in the ROS during the DM. Changes in ROS during the study is attributed to an increase in the oxidant species after DM. Fig. 3a illustrates the statistical comparison of the OS index between groups. Statistically, there was a significant increase in DM group (6.443 ± 0.856) compared to the Control group (5.21 ± 1.51). Although there was a tendency of decrease in the AV group (5.292 ± 0.349) and HPO group (4.81 ± 0.798) compared to the Control group, the results were statistically non-significant. On the other hand, when we compared the OS index of the DM group with HPO, there was a significant decrease in the HPO group ($p < 0.05$). Finally, there was another significant between AV and HPO groups. OS index of HPO was significantly decreased compared to the AV group ($p < 0.05$).

During diabetic wounds, some inflammatory cells and liver tissues release inflammatory mediators and cytokines, which influence the whole process of inflammation. The most important cytokines about this process are TNF-alpha, interleukins, and transforming growth factor [23]. We measured the TNF-alpha level to present the degree of inflammatory cytokines rise to link the severity of the disease. In Fig. 3b**,** we compared with mean ± SD the DM (1028 ± 72.74), AV (1142 ± 81.93), and HPO (1017 ± 64.66) group with Control (964.6 ± 204.6) between groups. However, there was a significant reduction in the pro-inflammatory mediator of the TNF-α level of the HPO group ($p < 0.01$) compared to the DM groups. Table 1 summaries the statistical results of TAS, TOS TNF- α and OS index of our measurements.

3.3. FTIR spectroscopy analysis

To determine the chemical changes induced by diabetic disease, FTIR measured blood (Fig. 4a) and serum (Fig. 4b) collected from rats were performed. In the obtained FTIR spectra, peaks corresponding to phospholipids, proteins, and lipids vibrations, were analyzed, because changes in these structures are observed in people suffering from diabetic diseases [24] The peaks at 1057 cm^{-1} and 1249 cm^{-1} wavenumbers correspond to symmetric and asymmetric vibrations of PO^{2-} groups in phospholipids [25]. The other peaks present in this spectrum are attributed to proteins [26]. The peaks at 1314 cm^{-1} are assigned to amide III, at 1395 cm^{-1} to symmetric vibration of the COO group, at 1456 cm^{-1} to the C-C bond, at 1544 cm^{-1} to bending vibration of N-H and stretching vibration of the C-N group from – amide II [27] and finally at

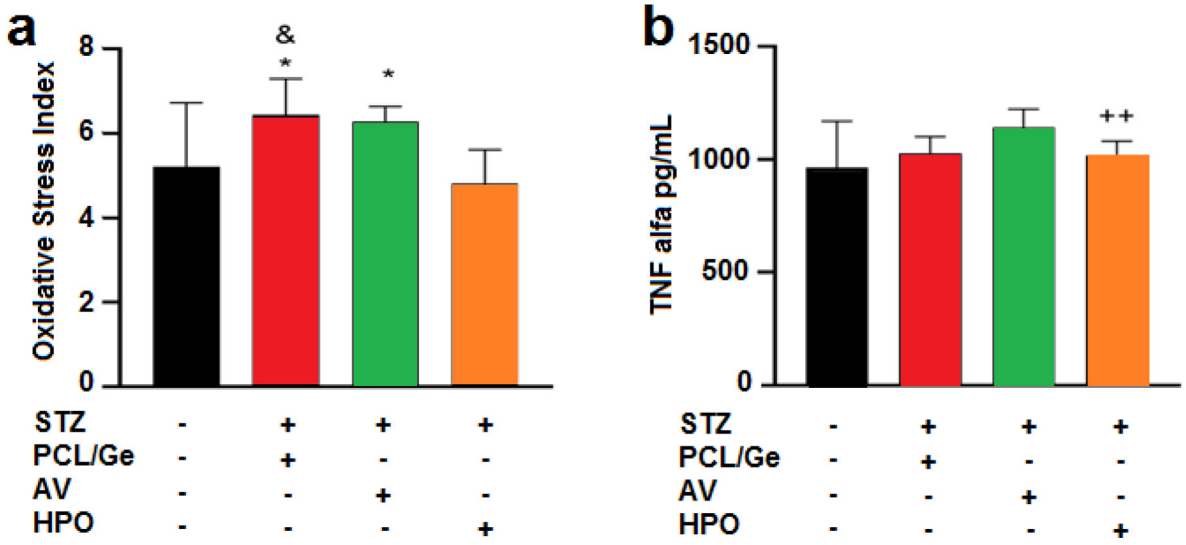


Fig. 3. Oxidative Stress Index of TAS (Total oxidant status) to Total oxidant level (TOS) levels rate (a); Tumor necrosis factor alpha (TNF-α) levels (b) on the serum of male rats which were either control or DM with or without Streptozotocin-induced diabetes mellitus model (DM), and which were vehicle-, PGL/Ge, HPO, or AV treated. Each group consists of 5 animals. $^{\&}P < 0.05$, compared to Control group; $^{*}P < 0.05$, compared to HPO group, $^{++}P < 0.01$compared to DM group. Each group consists of 5 animals: control (black color) vehicle, PCL/ Ge (red color) porcine gelatin, AV (green color) Aloe v., HPO (orange color) Hypericum p. treated groups. (+means exist, - means none exist).

Table 1
Serum TAS. TOS. TNF- α and OSI (Oxidative stress index) level with Mean ± Std. Deviation, One-way ANOVA Bonferroni's multiple comparisons test results.

	Control	DM	AV	HPO
TAS	0.89 ± 0.064	0.781 ± 0.044	0.864 ± 0.021	1.016 ± 0.149
TOS	4.58 ± 0.99	5.01 ± 0.429	5.441 ± 0.398	4.812 ± 0.337
TNF-α	964.6 ± 204.6	1028 ± 72.74	1142 ± 81.93	1017 ± 64.66
OSI	5.21 ± 1.51	6.443 ± 0.856	5.292 ± 0.349	4.81 ± 0.798

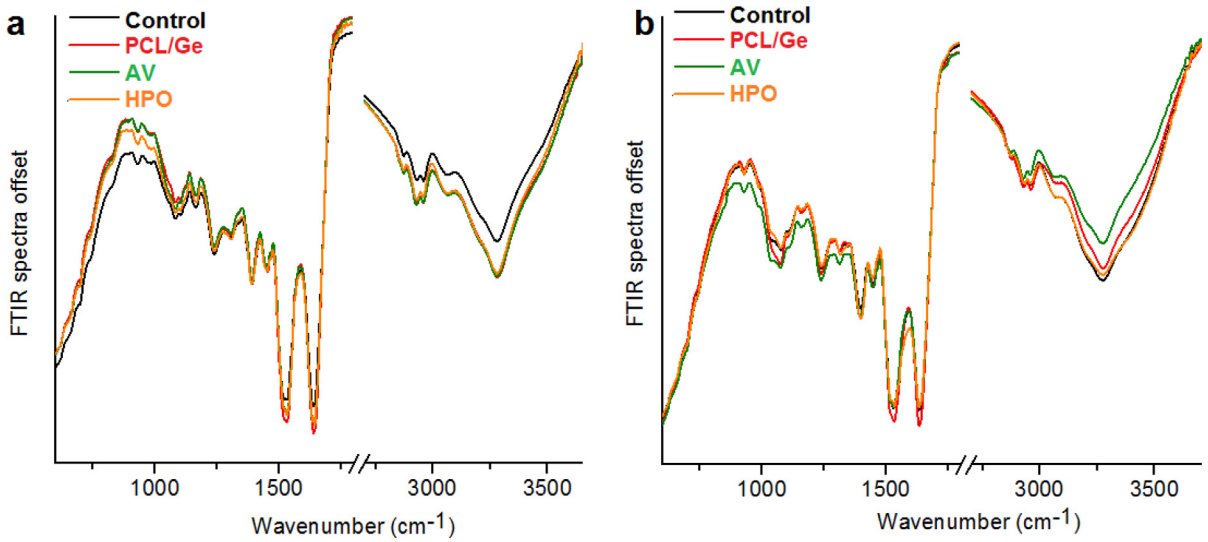


Fig. 4. FTIR spectra of blood (a) and serum (b) of male rat groups: control (black color) vehicle, PCL/ Ge (red color), (red color), AV (green color), HPO (orange color) treated groups. PCL/Ge, AV and HPO groups are STZ induced DM animals.

1648 cm^{-1} to stretching vibrations of amide I [28]. Moreover, C = O stretching vibrations building lipids structure were noticed in 1740 cm^{-1} wavenumber. Characteristic CH_2 and CH_3 vibrations originating from carbon chain from lipids were visible in the IR region between 2800 and 3000 cm^{-1} [29–31].

In the FTIR, spectra of blood (Fig. 4a) recorded for diabetic rats a decrease in intensity of dressings originating from phospholipids is noticed, in comparison to the control group. Moreover, a very similar level of amide III vibrations in blood collected from DM, AV, HPO, and control rats, is visible. Furthermore, when we compared control and diabetic rats, an increase of amide II, amide I, and lipids CH_2 and CH_3 vibrations are observed in the FTIR spectra collected from three diabetic groups. In the case of FTIR spectra of serum, a similar level of vibrations corresponding from symmetric and

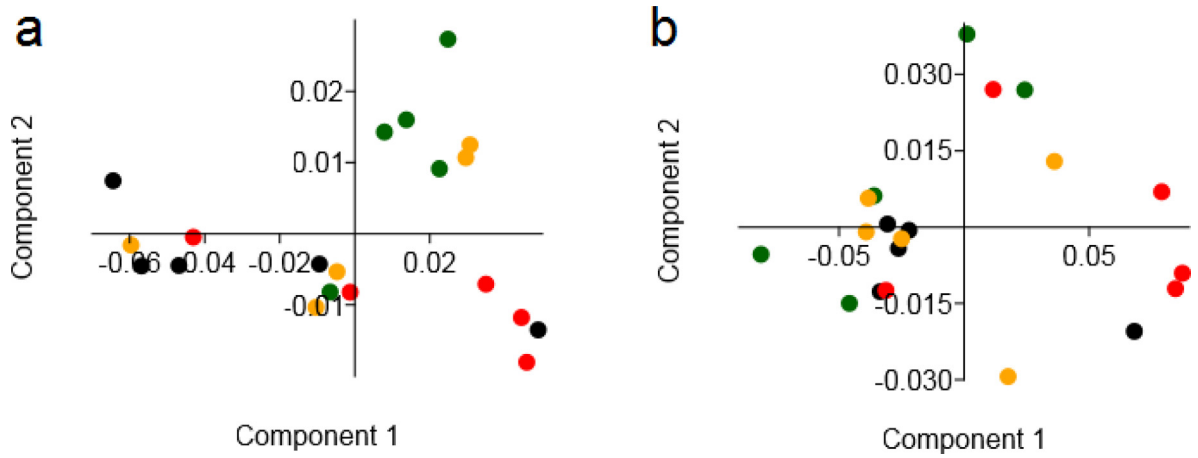


Fig. 5. PCA analyses of blood (a) and serum (b) collected from male rat's groups: control (black color) vehicle, PCL/ Ge (red color), AV (green color), HPO (orange color) treated groups. PCL/Ge, AV and HPO groups are STZ induced DM animals. The IR range between 800 and 1800 cm^{-1}.

asymmetric of PO^{2-} groups, amide III and lipids (the region between 2800 and 3000 cm^{-1}) in the Control, DM, and HPO is noticed, while in the case of AV rats, the levels of phospholipids and proteins functional group are lower, but the level of CH_2 and CH_3 vibrations are higher, in comparison with control group. Moreover, an increase of amide I and amide II vibrations in STZ rats are visible, when we compared this group with control one. Furthermore, the structure of peaks corresponding to PO^{2-} vibrations differ in serum collected from diabetic rats.

3.4. Multivariate analysis such as principal component analysis (PCA)

To determine the possibility to distinguish FTIR spectra of control and non-healthy groups treated and non-treated by Aloe vera (L.) and St. John's Wort oils, PCA (Fig. 5) analyses of blood and serum collected from four rats group, were performed and presented below.

PCA analysis of blood (Fig. 5a) showed that FTIR spectra of AV and STZ rat's group are separated from the FTIR spectra of the control group. Moreover, PCA analysis of blood showed, that more than half of rats from the HPO group have a similar chemical composition of blood to this observed in the Control group. The same similarity in the case of HPO and Control groups was observed in the serum collected from rats, Fig. 5b. Moreover, in this case, FTIR spectra of serum collected from AV rats are more similar to FTIR spectra collected from the Control group, than in the case of FTIR blood spectra.

4. Discussion

Diabetes mellitus is a metabolic disorder, which affects more and more people every year in worldwide. Unfortunately, effective treatment for this disease has not yet been invented, and complications that cause diabetes are often very severe and difficult to treat. Diabetic wound is one of the most widespread diabetes related complication influencing 25% of diabetes patients [32], it is very difficult to treat wounds on the body of people with diabetes compared to the healthy individuals. Therefore, in this study, we produced nanofibers with A. vera (AV) and H. perforatum (HPO) loaded PCL/Ge blends to stimulate self-repair mechanism on the patients. Next, these nanofibers were used as wound dressings and the effect of two different nanofibers was determined by biochemical methods such as TAS, TOS, TNF-α and FTIR spectroscopy. Diabetes with diabetic wounds causes a burst of free radicals that interact with various biomolecules, leading to the destruction of cellular components, inflammatory reactions, increased oxidative stress in the cells, and delay in healing, antioxidants prevent the oxidation of biomolecules by eliminating overproduced ROS, removing inflammation products in the process of healing. As it is known that wound healing has four stages. Hemostasis which is the process of the wound being closed by clotting. Inflammatory Phase is the second stage of wound healing and begins right after the injury, Proliferative Phase, wound healing is when the wound is rebuilt with new tissue made up of collagen and extracellular matrix. Maturation Phase is the remodeling stage of wound healing. In this study, we mainly aimed to see the effect of patches on DM wounds especially in inflammatory phase. In our study, we observed HPO patches are more the efficienct than AV patches on wound healing. As it was described in previous studies, HPO has impact on wound healing process either in our study. The extract of this plant has tannins, hyperin, hypericin, hyperforin, amentoflavone, flavonoids, and xanthones [33]. It is also demonstrated that amentoflavone and hypericin have anti-inflammatory activities also; hyperforin has antibacterial potential [34]. Additionally, we found the HPO treated STZ induced Diabetes group had increased TAS level and decreased TOS level compared to AV treated DM group. These may be because of the extracts of HPO that reduces lipid peroxidation slowing cell necrosis and improving vascularity [35].

Additionally, Aloe V. extracts contains polyphenols, anthranoids, steroids, fiber, salicylic acid, mineral components, as well as vitamins. AV showed its antioxidative effect on DM animals treated with these patches too. Nevertheless, AV extract was not efficient as HPO patches. Our results Hes et al, [36] had been showed that despite the numerous health-promoting properties of aloe and a rich range of the bio-active compounds, Aloe vera extract does not confirm a high antioxidant activity as in our results. We showed that Aloe extract has scavenging effect antioxidant effect but its effect is weaker than H. Perporatium.

Very characteristic for diabetes is an increase of reactive oxygen species, which are reproduced in various tissues under diabetic conditions [37]. In our study, we also observed a higher level of ROS parameters, Figs. 2, 3. Obtained results conforms, that diabetes model, which we used are proper. Moreover, in people suffering from diabetes diseases, dysregulation of glucose levels is observed [24]. These dysregulations have very wide-ranging consequences for lipids balance because metabolism of fats and carbohydrates are intimately intertwined [38,39]. Indeed, when comparing the results obtained by FTIR measurements of the blood as well as

serum of STZ rats, changes in the amount and structure of phospholipids, proteins, and lipids were observed, Fig. 4a, b. Moreover, other researchers, which used FTIR spectroscopy to determine the chemical changes induced by diabetes diseases, also showed, that the amount and structure of lipid components in the erythrocyte is different in healthy and non-healthy cells [40]. Caixeta et al. showed changes in the structure of phospholipids, proteins, and lipids in the FTIR spectra collected from animals suffering from diabetes mellitus [41]. Moreover, they also noticed a correlation between changes in the proteins and lipids. Furthermore, they monitored the treatment process of diabetes mellitus by FTIR spectroscopy and they showed that it is possible to use this method as to provide information about the overall biochemical status of the examined sample based on the vibrations of membrane structural unit as many other studies [42,43,44]

Herbs or plant oils are very often used in disease therapy [45]. In diabetic, they are used in wounds treatment. Aloe vera showed antidiabetic, anti-inflammatory, and antibacterial activities [21] and antioxidant properties [46], which are also noticed in our study. Figs. 2 and 3 showed a decrease of TOS and TAS levels in the serum collected from STZ rats in comparison with animals without Aloe vera treatment. In the literature information, that this plant caused fibroplasia, collagen formation, and contraction that culminated in faster healing in the diabetic animal model [47]. This is means that Aloe vera extract is helpful in wound healing and should be caused that the chemical compositions of tissues, body fluids. Indeed, our FTIR measurements (Fig. 4) combined with PCA analysis (Fig. 5) showed, that chemical composition of blood and serum collected from AV rats (green spectrum) are more similar to this observed in the control group (black spectrum) than in PCL/Ge treated group (red spectrum). However, in this study also HPO, was investigated. Therapy of STZ rats using this oil caused a decrease of ROS parameters (Figs. 2, 3) and return chemical compositions of blood and serum to this characteristic for the control group (Figs. 4, 5). Moreover, for HPO higher decrease of TOS and TAS levels as for Aloe vera extract, was observed. This could be because HPO contains hyperforin, flavonoids, and hypericin, which are very strong antioxidants [48]. Moreover, Farsak et al. claimed H. perforatum and olive oil decreased the oxidative stress in the diabetic tissue permitting better healing [49]. Adollah-Damavandi et al. showed, that the number of fibroblast cells increased in diabetic rats that were treated with HPO compared to the STZ rats [35].

This study clearly shows that there is a significant effect of HPO dressings on the improvement of diabetic wounds compared to the AV dressings. These wound dressing dressings may be applied as a potential for the healing of DM wound. The obtained results definitely show that this absorbable band plays a very important role in inflammation and pathophysiology of DM wounds. These dressings directly affect inflammation parameters. It has high biocompatibility, low toxicity, and excellent mucoadhesion properties. Depending on the composition, the selected herb extracts act as for cell proliferation and provides tissue generation in skin membrane structure.

5. Conclusion

In summary, we investigated nano dressings conjugation on polymeric nanofiber formed from gelatin and polycaprolactone composed by Aloe vera and Hypericum perforatum oil dressings in STZ induced diabetic model on Wistar rats. Furthermore, in the light of our results, these nano dressings with the efficacy of both these plants can serve as a valid alternative for healing on surgical diabetic wound healing and treatment.

Our study showed that Hypericum perforatum and Aloe vera oils caused an increase of the TOS level in STZ rats. However, in the case of oxidative stress index higher decrease of this parameter was observed for the HPO group, than form AV rats, in comparison with the diabetic model on Wistar rats. Also, FTIR spectra showed, that the chemical structure of blood and serum collected from STZ rats is more similar to this observed in control group after Hypericum perforatum oil used than after treated using Aloe vera oil. These differences are visible especially in PO^{2-} groups from phospholipids and in amide- I, II, III vibrations.

Our results showed, that both Hypericum perforatum oil, as well as Aloe vera oils, could be used alternative for healing on surgical diabetic wound healing and treatment, however, Hypericum perforatum oil gives better results for bio-nano application.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

SE supervised the study, ZG and JD made data curation, software, FTIR formal analysis, investigation, writing - review & editing. GI, made animal experiments, HE made the nanofiber dressings by electrospinning and visualization, ZG, HB, GS, DO made the biochemical assays, CK obtained FTIR spectroscopy. NE contributed the resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, D. Bright, R. Williams, Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition, Diabetes Res. Clin. Pract. 157 (2019). https://doi.org/10.1016/j.diabres.2019.107843.

[2] M. Brownlee, The pathobiology of diabetic complications: a unifying mechanism, Diabetes. 54 (2005), https://doi.org/10.2337/DIABETES.54.6.1615.

[3] N. Alvarado-Vásquez, P. Zamudio, E. Cerón, B. Vanda, E. Zenteno, G. Carvajal-Sandoval, Effect of glycine in streptozotocin-induced diabetic rats, Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 134 (2003) 521–527, https://doi.org/10.1016/S1532-0456(03)00046-2.

[4] O.O. Oguntibeju, S. Meyer, Y.G. Aboua, M. Goboza, Hypoxis hemerocallidea Significantly Reduced Hyperglycaemia and Hyperglycaemic-Induced Oxidative Stress in the Liver and Kidney Tissues of Streptozotocin-Induced Diabetic Male Wistar Rats, Evidence-Based Complement. Altern. Med. 2016 (2016), https://doi.org/10.1155/2016/8934362.

[5] B. Kunkemoeller, T.R. Kyriakides, Redox Signaling in Diabetic Wound Healing Regulates Extracellular Matrix Deposition, Antioxid. Redox Signal. 27 (2017) 823–838, https://doi.org/10.1089/ars.2017.7263.

[6] R. Bahramsoltani, M.H. Farzaei, R. Rahimi, Medicinal plants and their natural components as future drugs for the treatment of burn wounds: An integrative review, Arch. Dermatol. Res. 306 (2014) 601–617, https://doi.org/10.1007/s00403-014-1474-6.

[7] A.G. Pirbalouti, S. Azizi, A. Koohpayeh, B. Hamedi, Wound healing activity of Malva sylvestris and Punica granatum in alloxan-induced diabetic rats., Acta Pol. Pharm. 67 (2010) 511–6. [PubMed] [Google Scholar].

[8] A. Balaji, M.V. Vellayappan, A.A. John, A.P. Subramanian, S.K. Jaganathan, M. Selvakumar, A.A. Bin Mohd Faudzi, E. Supriyanto, M. Yusof, Biomaterials based nano-applications of Aloe vera and its perspective: a review, RSC Adv. 5 (2015) 86199–86213, https://doi.org/10.1039/c5ra13282g.

[9] S.S. Silva, M.B. Oliveira, J.F. Mano, R.L. Reis, Bio-inspired Aloe vera sponges for biomedical applications, Carbohydr. Polym. 112 (2014) 264–270, https://doi.org/10.1016/j.carbpol.2014.05.042.

[10] R. Pereira, A. Tojeira, D.C. Vaz, A. Mendes, P. Bártolo, Preparation and characterization of films based on alginate and aloe vera, Int. J. Polym. Anal. Charact. 16 (2011) 449–464, https://doi.org/10.1080/1023666X.2011.599923.

[11] O.M. Grace, Current perspectives on the economic botany of the genus Aloe L. (Xanthorrhoeaceae), South African J. Bot. 77 (2011) 980–987, https://doi.org/10.1016/j.sajb.2011.07.002.
[12] J.E. Kim, J.H. Lee, S.H. Kim, Y. Jung, Skin Regeneration with Self-Assembled Peptide Hydrogels Conjugated with Substance P in a Diabetic Rat Model, Tissue Eng. - Part A. 24 (2018) 21–33, https://doi.org/10.1089/ten.tea.2016.0517.
[13] M. Tummalapalli, M. Berthet, B. Verrier, B. Deopura, M. Alam, B. Gupta, Composite wound dressings of pectin and gelatin with aloe vera and curcumin as bioactive agents, Int. J. Biol. Macromol. 82 (2016) 104–113, https://doi.org/10.1016/j.ijbiomac.2015.10.087.
[14] S.P. Miguel, M.P. Ribeiro, P. Coutinho, I.J. Correia, Electrospun polycaprolactone/Aloe Vera_chitosan nanofibrous asymmetric membranes aimed for wound healing applications, Polymers (Basel). 9 (2017), https://doi.org/10.3390/polym9050183.
[15] D.K. Kim, B.R. Sim, G. Khang, Nature-Derived Aloe Vera Gel Blended Silk Fibroin Film Scaffolds for Cornea Endothelial Cell Regeneration and Transplantation, ACS Appl. Mater. Interfaces. 8 (2016) 15160–15168, https://doi.org/10.1021/acsami.6b04901.
[16] Z. Saddiqe, I. Naeem, A. Maimoona, A review of the antibacterial activity of Hypericum perforatum L, J. Ethnopharmacol. 131 (2010) 511–521, https://doi.org/10.1016/j.jep.2010.07.034.
[17] I.P. Süntar, E.K. Akkol, D. Yilmazer, T. Baykal, H. Kirmizibekmez, M. Alper, E. Yeşilada, Investigations on the in vivo wound healing potential of Hypericum perforatum L, J. Ethnopharmacol. 127 (2010) 468–477, https://doi.org/10.1016/j.jep.2009.10.011.
[18] A. Ozturk, L. Demirsoy, H. Demirsoy, S. Ozturk, Ozturk et al, Afr J Tradit Complement Altern Med. 12 (2015) 63–69, https://doi.org/10.4314/ajtcam.v12i5.9.
[19] I. Süntar, O. Oyardı, E. Küpeli Akkol, B. Ozçelik, Pharmaceutical Biology Antimicrobial effect of the extracts from Hypericum perforatum against oral bacteria and biofilm formation Antimicrobial effect of the extracts from Hypericum perforatum against oral bacteria and biofilm formation, Pharm. Biol. 54 (2016) 1065–1070, https://doi.org/10.3109/13880209.2015.1102948.
[20] A. Sofowora, Recent trends in research into African medicinal plants, J. Ethnopharmacol. 38 (1993) 197–208, https://doi.org/10.1016/0378-8741(93)90017-Y.
[21] O.O. Oguntibeju, Medicinal plants and their effects on diabetic wound healing, Vet. World. 12 (2019) 653–663. https://doi.org/10.14202/vetworld.2019.653-663.
[22] K.K. Wu, Y. Huan, Streptozotocin-Induced Diabetic Models in Mice and Rats 5.47.1-5.47.14 Curr. Protoc. Pharmacol. 40 (2008), https://doi.org/10.1002/0471141755.ph0547s40.
[23] Z. Guleken, D. Ozbeyli, MS Oktay Acikel-Elmas, B. Alev, S. Sirvanci, A. Velioglu Ogunc, Cakir O. Kasimay, The effect of estrogen receptor agonists on pancreaticobiliary duct ligation induced experimental acute pancreatitis, J. Physiol. Pharmacol. 68 (2017) 847–858, PMID: 29550797.
[24] A.I.S. Sobczak, C.A. Blindauer, A.J. Stewart, Changes in plasma free fatty acids associated with type-2 diabetes, Nutrients. 11 (2019), https://doi.org/10.3390/nu11092022.
[25] P. Garidel, Mid-FTIR-microspectroscopy of stratum corneum single cells and stratum corneum tissue, Phys. Chem. Chem. Phys. 4 (2002) 5671–5677, https://doi.org/10.1039/b207478h.
[26] R.W. Hendler, S.M. Barnett, S. Dracheva, S. Bose, I.W. Levin, Purple membrane lipid control of bacteriorhodopsin conformational flexibility and photocycle activity: An infrared spectroscopic study, Eur. J. Biochem. 270 (2003) 1920–1925, https://doi.org/10.1046/j.1432-1033.2003.03547.x.
[27] C. Jung, Insight Into Protein Structure and Protein-Ligand Recognition by Fourier Transform Infrared Spectroscopy, J. Mol. Recognit. 13 (2000), https://doi.org/10.1002/1099-1352(200011/12)13:6<325::AID-JMR507>3.0.CO;2-C.
[28] J. Depciuch, M. Parlinska-Wojtan, Qualitative and quantitative changes in phospholipids and proteins investigated by spectroscopic techniques in olfactory bulbectomy animal depression model, J. Pharm. Biomed. Anal. 148 (2018) 24–31, https://doi.org/10.1016/j.jpba.2017.09.016.
[29] J.L.R. Arrondo, F.M. Goñi, Infrared spectroscopic studies of lipid-protein interactions in membranes, New Compr. Biochem. 25 (1993) 321–349, https://doi.org/10.1016/S0167-7306(08)60242-2.
[30] J. Depciuch, M. Sowa-Kucma, G. Nowak, M. Papp, P. Gruca, P. Misztak, M. Parlinska-Wojtan, Qualitative and quantitative changes in phospholipids and proteins investigated by spectroscopic techniques in animal depression model, Spectrochim, Acta Part A Mol. Biomol. Spectrosc. 176 (2017) 30–37, https://doi.org/10.1016/j.saa.2016.12.007.
[31] R. Mendelsohn, G. Anderle, M. Jaworsky, H.H. Mantsch, R.A. Dluhy, Fourier transform infrared spectroscopic studies of lipid-protein interaction in native and reconstituted sarcoplasmic reticulum, BBA - Biomembr. 775 (1984) 215–224, https://doi.org/10.1016/0005-2736(84)90173-1.
[32] D.G. Armstrong, A.J.M. Boulton, S.A. Bus, Diabetic foot ulcers and their recurrence, N. Engl. J. Med. 376 (2017) 2367–2375, https://doi.org/10.1056/NEJMra1615439.
[33] S. Rafiee, A. Samadnejad, F. Zarifi, Q. Asgari, S. Ashkani-Esfahani, A. Noorafshan, Taurine improves the wound healing process in cutaneous leishmaniasis in mice model, based on stereological parameters, Adv. Biomed. Res. 3 (2014) 204, https://doi.org/10.4103/2277-9175.142314.
[34] C.M. Schempp, K. Pelz, A. Wittmer, E. Schöpf, J.C. Simon, Antibacterial activity of hyperforin from St John's wort, against multiresistant Staphylococcus aureus and gram-positive bacteria, Lancet. 353 (1999) 2129, https://doi.org/10.1016/S0140-6736(99)00214-7.
[35] S. Yadollah-Damavandi, M. Chavoshi-Nejad, E. Jangholi, N. Nekouyian, S. Hosseini, A. Seifaee, S. Rafiee, H. Karimi, S. Ashkani-Esfahani, Y. Parsa, M. Mohsenikia, Topical Hypericum perforatum Improves Tissue Regeneration in Full-Thickness Excisional Wounds in Diabetic Rat Model (2015), https://doi.org/10.1155/2015/245328.
[36] M. Hęś, K. Dziedzic, J. Le Thanh-Blicharz, D. Kmiecik, D. Górecka, Antioxidant activity of true aloe (Aloe vera) extract in model systems, Nauk. Przyr. Technol. 10 (2017). https://doi.org/10.17306/j.npt.2016.4.53.
[37] S. Fakhruddin, W. Alanazi, K.E. Jackson, Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal, Injury (2017), https://doi.org/10.1155/2017/8379327.
[38] G. Boden, Obesity, insulin resistance and free fatty acids, Curr. Opin. Endocrinol. Diabetes Obes. 18 (2011) 139–143, https://doi.org/10.1097/MED.0b013e3283444b09.
[39] M.K. Lee, K. Han, M.K. Kim, E.S. Koh, E.S. Kim, G.E. Nam, H.S. Kwon, Changes in metabolic syndrome and its components and the risk of type 2 diabetes: a nationwide cohort study, Sci. Rep. 10 (2020) 1–8, https://doi.org/10.1038/s41598-020-59203-z.
[40] S.S. Mahmoud, The Impact of Elevated Blood Glycemic Level of Patients with Type 2 Diabetes Mellitus on the Erythrocyte Membrane: FTIR study, Cell Biochem. Biophys. 58 (2010) 45–51, https://doi.org/10.1007/s12013-010-9092-1.
[41] D.C. Caixeta, E.M.G. Aguiar, L. Cardoso-Sousa, L.M.D. Coelho, S.W. Oliveira, F.S. Espindola, L. Raniero, K.T.B. Crosara, M.J. Baker, W.L. Siqueira, R. Sabino-Silva, Salivary molecular spectroscopy: A sustainable, rapid and non-invasive monitoring tool for diabetes mellitus during insulin treatment, PLoS One. 15 (2020), https://doi.org/10.1371/journal.pone.0223461 e0223461.
[42] Z. Guleken, S.E. Kuruca, B. Ünübol, S. Toraman, R. Bilici, D. Sarıbal, O. Gunduz, J. Depciuch, Biochemical assay and spectroscopic analysis of oxidative/antioxidative parameters in the blood and serum of substance use disorders patients. A methodological comparison study, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 240 (2020), https://doi.org/10.1016/j.saa.2020.118625 118625.
[43] Z. Güleken, B. Ünübol, S. Toraman, R. Bilici, O. Gündüz, S.E. Kuruca, Diagnosis of opioid use disorder with high sensitivity and specificity by advanced computational analysis of Fourier transform infrared spectroscopy, Infrared Phys. Technol. 105 (2020), https://doi.org/10.1016/j.infrared.2020.103218 103218.
[44] Z. Guleken, B. Ünübol, R. Bilici, D. Sarıbal, S. Toraman, O. Gündüz, S. Erdem Kuruca, Investigation of the discrimination and characterization of blood serum structure in patients with opioid use disorder using IR spectroscopy and PCA-LDA analysis, J. Pharm. Biomed. Anal. 190 (2020), https://doi.org/10.1016/j.jpba.2020.113553 113553.
[45] A.A. Boadu, A. Asase, Documentation of Herbal Medicines Used for the Treatment and Management of Human Diseases by Some Communities in Southern Ghana (2017), https://doi.org/10.1155/2017/3043061.
[46] M. Hęś, K. Dziedzic, D. Górecka, A. Jędrusek-Golińska, E. Gujska, Aloe vera (L.) Webb.: Natural Sources of Antioxidants – A Review, Plant Foods Hum. Nutr. 74 (2019) 255–265, https://doi.org/10.1007/s11130-019-00747-5.
[47] P. Chithra, G.B. Sajithlal, G. Chandrakasan, Influence of aloe vera on the healing of dermal wounds in diabetic rats, J. Ethnopharmacol. 59 (1998) 195–201, https://doi.org/10.1016/S0378-8741(97)00124-4.
[48] A. Altan, M.H. Aras, I. Damlar, H. Gokce, O. Ozcan, C. Alpaslan, The effect of Hypericum Perforatum on wound healing of oral mucosa in diabetic rats, Eur. Oral Res. 52 (2019) 143–149. https://doi.org/10.26650/eor.2018.505.
[49] M. Farsak, G. Özdağli, D. Özmüş, Ü. Çömelekoğlu, S. Yalın, R. Bozdoğan Arpacı, R. Gen, A. Kanık, D. Ümit Talas, Effects of Hypericum perforatum on an Experimentally Induced Diabetic Wound in a Rat Model, Wounds a Compend. Clin. Res. Pract. 29 (2017) E10–E17. https://europepmc.org/article/med/28272017 (accessed June 20, 2020).