AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 04, June, 2022

Investigation of Topical Nifedipine on Total Antioxidant Capacity and Mitochondrial Function During Facial Skin Wound Healing in Rabbits

Wasan J. Al-Dabbagh[1*], Faehaa A. Al-Mashhadane[2], Ayad A. Al-Sarraj[2]

Albatool Teaching Hospital, Alshifaa quarter Mosul, 41002, Ninevah Province, Iraq[1]
Department of Dental Basic Sciences, University of Mosul, Alhadbaa Street, Mosul, 41002, Ninevah Province, Iraq[2]

Corresponding Author: 1*

Keywords:
Nifedipine, Wound healing, Total antioxidant capacity, Lactate, mitochondrial function.

ABSTRACT

The objective of this study was to evaluate the impacts of 1% and 2% topical nifedipine ointments on total antioxidant capacity and mitochondrial function during the facial skin wound healing in rabbits. Nifedipine ointments 1% and 2% were prepared. Fifty healthy male rabbits were involved and distributed into two equal group 25 of each according to study period: group A (7 days) and group B (14 days). Each group was subdivided into five groups (5 rabbits/group): group I (Normal): rabbits were not subjected to any surgical procedure or treatments; group II (negative control): rabbits had surgical wound without treatment; group III (positive control): rabbits had surgical wound, treated with white petroleum; group IV(nifedipine 1%): rabbits had surgical wound, treated with nifedipine 1% ointment; and group V(nifedipine 2%): rabbits had surgical wound, treated with nifedipine 2% ointment. Blood samples were collected from all animals after euthanasia for analysis of biochemical parameters. The results revealed a significant increase of total antioxidant capacity levels in the nifedipine 1% group on the 7th and 14th days of study in comparison to the other groups. There is no substantial variance in lactate and pyruvate levels between the normal and nifedipine 1% groups, but there is a substantial variance when compared to the other groups. Topical nifedipine 1% ointment had a beneficial effect on the wound healing process. It had superior effect on total antioxidant capacity and promoted mitochondrial function during the healing process, whereas nifedipine 2% ointment had no such effect.

1. Introduction

Nifedipine (NFD) is a medicine that belongs to a class called calcium channel blockers in the dihydropyridine subclass. It lowers calcium ion inflow by inhibiting L-type calcium channels in myocardial and vascular smooth muscle cells, causing relaxation of these muscles and dilation [1].

Nifedipine has been used clinically for hypertension and angina pectoris treatment. Its valuable effect on endothelial function may similarly be due to its antioxidant capability by enhancing the production of nitric oxide, which prevents oxidative stress from inducing endothelial damage or decreasing nitric oxide bioavailability [2]. Some authors report that NFD is regarded to be beneficial in wound healing due to its vasodilation and antioxidant properties [3].

The wound healing process is quite complicated and consists of the following phases: hemostasis, inflammation, cell proliferation/granulation, and the remodeling [4]. Reactive oxygen species (ROS) are little oxygen-derived molecules primarily formed by the respiratory chain in mitochondria [5] and have an important function in the normal wound-healing reaction [6]. They are critical regulators at various phases of the healing process and they are involved in intracellular signaling during wound healing, cell proliferation and migration, pathogen sterilization of the wound region, and scar tissue closure [7]. They may be present in high quantities at wound sites, causing oxidative stress production, which leads to numerous cell damage and dysregulates the healing process [8]. During wound healing, cells multiply quickly to motivate the glycolytic pathway, leading to lactate buildup in the interstitial fluids up to 5–15 mM. Lactate is produced during glycolysis through the conversion of pyruvate by lactate dehydrogenase (LDH) in anaerobic or aerobic circumstances. However, increasing evidence suggests that lactate is required for energy and redox homeostasis. A greater level of serum lactate has been considered as a predictive factor of muscle exhaustion, often connected with tissue hypoxia and bad clinical prognosis [9].

The aim of this study focused on the effect of topical NFD ointment 1% and 2% on total antioxidant capacity and mitochondrial function. Also to study a new aspect of skin wound healing which is facial skin in order to find out the ability of these prepared ointments to help in such type of healing and to prevent scar formation in such important area.

2. Material and Methods

The experimental work for this research was agreed by the Research Ethics Committee and Scientific Committee/Department of Dental Basic Science/College of Dentistry/University of Mosul (approval No.: UoM.Dent/A.L.6/22).

2.1 Preparation of nifedipine ointment

Two different concentrations of nifedipine ointment were prepared by mixing (1 & 2 g) of nifedipine powder (Shandong Look Chemical Co., Ltd.) in 100 gm of white petroleum to provide an ultimate dosage (1%, 2%) W\W with continuous mixing, utilizing a spatula and glass plate, until identical ointments were made. They were kept in opaque containers and stored at 25°C ± 2°C till used [10], [11].

2.2 Experimental Animals:

Fifty local, healthy, mature male rabbits weighing 1.25–1.5 kg and aged 10–12 months were purchased from the local market and included in the study. The animals were housed individually in special cages under standard conditions (room temperature of about 25±2°C with a 12:12 hr. light-dark cycle) [12], and given free access to water and a standard diet. The rabbits were randomly distributed into two equal groups, 25 of each, according to euthanizing day:

Group A was euthanized on the 7^{th} day. Group B was euthanized on the 14^{th} day. Each group was subdivided into five groups (5 rabbits/group):Group I (Normal): Rabbits were not subjected to any surgical procedure or treatments. Group II (negative control): Rabbits were subjected to a surgical procedure without receiving any treatment. Group III (positive control): Rabbits were subjected to a surgical procedure and

ISSN: 0005-2523
Volume 62, Issue 04, June, 2022

were treated twice daily with white petroleum from the first day of the surgical procedure to the euthanizing day. Group IV (NFD1%): Rabbits were subjected to a surgical procedure and were treated twice daily with NFD1% ointment from the first day of the surgical procedure to the euthanizing day. Group V (NFD 2%): Rabbits were subjected to a surgical procedure and were treated twice daily with NFD 2% ointment from the first day of the surgical procedure to the euthanizing day.

2.3 Surgical procedure

Animals except (normal group (were anesthetized by administering an intramuscular dose of xylazine hydrochloride and ketamine hydrochloride at 5, 50 mg/Kg, respectively [13]. The anesthetized animal was laid on its ventral side on the surgical board, and the surgical area (forehead) was shaved using scissors and a surgical scalpel, then rinsed with tap water and sterilized with povidone–iodine solutions. A full-thickness circular (1 cm in diameter) excision was carefully created using surgical blade no. 15 and forceps [14]. After that, the wounds were left open without sutures to heal by secondary intention and treated topically according to their grouping twice daily till the day of euthanasia. The animals did not receive any antibiotics.

2.4 Blood Samples Collection:

The rabbits were euthanized before being killed in accordance with the American Veterinary Medical Association's ethical rules [15]. Five milliliters of blood samples were collected in plain tubes from the jugular vein during the sacrifice and kept for 30 minutes at room temperature, then centrifuged at 3000 rpm for 15 min. Serum samples transferred to an eppendorf tube using a micropipette and stored at (-20 C) until utilized for biochemical parameter analysis [16].

2.5 Statistical Analysis

The records were stated as mean ± SD, variance between five experimental sets were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. P values ≤ 0.01were considered to be significant.

3. Result

3.1 Clinical observation

The animals were observed frequently following the topical application of NFD1% and NFD2% ointment. No toxicity, no mortality, no abnormal signs and symptoms in the activity, behavioral pattern, postural irregularities, or any other clinical observations were recorded throughout the experimental period in all groups.

3.2 Comparisons of biochemical markers among study groups

- The 1st week:

The results in table (1) showed no substantial variance in the serum TAC among normal, negative control, and NFD 2% groups. While a substantial rise in the serum TAC was found in the NFD 1% group when compared to the other groups. Also, there was a substantial variance between the positive control group and the other groups.

For serum lactate and pyruvate, there was no substantial variance between the NFD 1% group and (normal, NFD 2% groups), although it was clear that the NFD 1% group has more value than the normal group and less value than the NFD 2% group. There was a substantial rise in lactate and pyruvate levels in the negative control and positive control groups when compared to the other groups. There was no substantial

variance between negative and positive control groups, although it was clear that negative control has more value than the positive control group. There was a substantial variance between the normal group and (negative control, positive control, and the NFD 2% groups).

Table 1. The level (mean± SD) of total antioxidants capacity TAC, lactate and pyruvate in the serum of study groups at the end of the 1st week

Group	Total antioxidants capacity(TAC) mmol/L	Lactate µmol/L	Pyruvate µmol/L
Normal group	0.7332±0.01327A	870.000±69.64194A	39.4000±5.17687A
Negative control	0.6280 ±0.05552A	2220.00±303.31502C	145.400±22.78815C
Positive control	0.9432±0.02605B	2000.00±387.29833C	125.200±27.03146C
NFD 1%	1.1424±0.08021C	996.000±140.46352AB	53.8000±1.92354AB
NFD 2%	0.6750±0.14953A	1239.40±81.09131B	74.8600±1.66973B
P-value	0.0001**	0.0001**	0.0001**

Each group consists of 5 animals. Data expressed as Mean ± Stander error
** Highly Significant at P ≤ 0.01
The various letters in the column indicate there are substantial variance between groups at P ≤ 0.01

The 2nd week:

The results in table (2) showed no substantial variance in the serum TAC between normal and NFD 2% groups. Also, there was no substantial variance between negative control and positive control groups. There was a substantial rise in TAC level in the NFD 1% group when compared to the other groups, but the level was higher than the first week. There is a substantial variance between (negative control, positive control groups) and other groups.

For serum lactate, there was no substantial variance between the NFD 1% group and (normal, and NFD 2% groups), although it is clear that the NFD 1% group has more value than normal but less value than the NFD 2% groups. There was a substantial variance in lactate levels in the negative control and positive control groups when compared to the other groups, but the levels were lower than the first week. There was no substantial variance between the negative control and positive control groups. There was a substantial variance between the normal group and (negative control, positive control, and NFD 2% groups).

For serum pyruvate, there was no substantial variance between the normal and NFD 1% groups. There was a substantial variance between (negative control, positive control groups) and the other groups, but the levels were lower than the first week. There was no substantial variance between the negative control and positive control groups. There was a substantial variance between the normal group and (negative control, positive control, and NFD 2% groups).

Table 2. The level (mean± SD) of total antioxidants capacity TAC, lactate and pyruvate in the serum of study groups at the end of the 2nd week.

Group	Total antioxidants capacity(TAC) mmol/L	Lactate µmol/L	Pyruvate µmol/L
Normal group	0.7078±0.04392A	830.000±89.44272A	35.8000±4.26849A
Negative control	0.8602 ±0.05647B	1668.00±271.97426C	94.0000±14.84924C
Positive control	0.9106 ±0.07648B	1619.00±330.99094C	98.4000±16.13382C
NFD 1%	1.8306±0.16126C	922.000±45.49725AB	41.3000±1.78885A
NFD 2%	0.7280±0.04822A	1134.00±54.58938B	57.2000±4.43847B
P-value	0.0001**	0.0001**	0.0001**

Each group consists of 5 animals. Data expressed as Mean ± Stander error
** Highly Significant at P ≤ 0.01
The various letters in the column indicate there are substantial variance between groups at P ≤ 0.01

ISSN: 0005-2523
Volume 62, Issue 04, June, 2022

4. Discussion

Wound-healing is the normal outcome of tissue injury that leads to the restoration of damaged tissue to its normal state. Healing a wound in the shortest possible time could improve the quality of life. The wound-healing process can be accelerated by many chemical agents [17], and local therapy can support the healing process and minimize systemic side effects [18]. Topical NFD for the treatment of skin wound is prescribed in medicinal practice in few case reports, with no standard concentrations, i.e., it is recommended off-label [19] . It is superior to oral therapy in its ability to potentiate local effects without significant systemic drug distribution because of the poor percutaneous penetration and rapid metabolism of nifedipine in the skin [20].

Based on macroscopic observation and biochemical analysis, the topical application of NFD 1% ointment significantly accelerated wound healing in rabbits and decreased the days needed for complete healing compared to other groups. However, NFD 2% ointment produced a less beneficial effect.

The inflammatory phase is an important stage in wound healing. However, a prolonged and severe inflammatory reaction frequently results in a delayed wound [21]. Dressing wounds with antioxidant preparations is beneficial for scavenging free radicals and reducing inflammation at the injury site [22], as free radicals can stimulate nuclear factor kappa-B (NF-κB), a redox-sensitive transcription factor that activates inflammatory genes and causes the production of inflammatory mediators [23]. The results showed that in the NFD 1% treated group, there were significant increases in TAC levels in the first and second weeks when compared to the other groups. The increase in TAC levels was greater in the second week, which might explain the speed of wound healing when compared to the other groups. whereas NFD 2% ointment has no such effect on TAC levels. This result is consistent with the findings of [24], who reported that oxidative stress plays an essential role in wound healing and many cellular enzymatic antioxidants, such as superoxide dismutase, hasten the healing process by destroying free radicals. Several studies have reported that NFD reduces oxidative stress in hypertensive patients and increases superoxide dismutase activity [25], which is recognized as the first line of defense against oxidative stress due to its ability to neutralize ROS with pro-inflammatory potential that can induce tissue damage [26]. In vitro, NFD has been shown to improve the function of endothelial progenitor cells (EPCs) [27], an important cell type implicated in angiogenesis [28], by upregulating superoxide dismutase [27], allowing EPCs to resist oxidative stress by scavenging mitochondrial reactive oxygen species (mtROS) [28]. Therefore, the antioxidant properties of NFD1% ointment could hasten wound healing process.

lactate and pyruvate are useful indicators for mitochondrial function and any variations in cellular respiration are reflected by variations in both serum levels [29]. Lactate is produced during glycolysis by the reduction of pyruvate in anaerobic or aerobic circumstances. Local hypoxia as the consequence of a disrupted vascular network and reduced supply during injury, increases lactate production by activating the glycolytic pathway, resulting in lactate buildup in the interstitial fluids [9], [30]. In addition, under hypoxic conditions, oxidative phosphorylation (OXPHOS), a metabolic pathway that occurs in the mitochondria to produce ATP, is hindered, and pyruvate is not oxidized, resulting in energy deficits and the accumulation of protons and lactate. This causes cellular acidification, which can affect the activity and/or integrity of mitochondrial enzymes, reducing mitochondrial respiration and consequently aerobic ATP production [31].

Reducing the availability of energy has a negative influence on wound healing because injured tissues demand more energy throughout the healing process. Furthermore, decreased energy output reduces angiogenesis in wounds, resulting in impaired wound healing. In addition, hypoxic circumstances promote the production of reactive oxygen species (ROS), which exacerbates tissue damage and has a negative

impact on wound healing [32]. Lactic acid plays an important for NOD-like receptor protein 3 (NLRP3) inflammasome activation [33], which is a multiprotein complex that mediates caspase-1 activation and the secretion of proinflammatory cytokines interleukins-1β/ interleukins-18 in response to microbial infection and cellular damage [34]. It has been demonstrated that inflammasomes participate in the innate immune response and cause inflammation [35]. Mitochondrial dysfunction is regarded as one of the mechanisms capable of activating the NLRP3 inflammasome [36]. Also under hypoxic condition, there is a significant increase in cytosolic calcium levels [37], [38]. Calcium influx, in particular, causes mitochondrial damage, which may boost mitochondrial reactive oxygen species (mtROS) generation and mitochondrial DNA release, hence amplifying NLRP3 inflammasome activation [39], [40].

The results of this study showed that the serum levels of lactate and pyruvate in the NFD1% treated group were near to those of the normal group (no significant difference), suggesting that NFD1% ointment improves microcirculation, limits injury-induced hypoxia, and reduces hypoxia-induced mitochondrial dysfunction and inflammation during injury [37]. The reduction in inflammatory response allows damaged tissue go in the later phases of healing, which include cell proliferation and tissue remodeling, contributing to the hypothesis that the reduction in inflammation enhances healing processes [41]. This finding supports prior studies that described NLRP3 inflammasomes' negative regulation as a potential therapeutic target for enhancing wound healing [37]. The significant increase in lactate and pyruvate levels in the NFD2%, negative control, and positive control groups compared to the normal group might delay the wound healing process. However, further investigations are required to elucidate the mechanisms involved in the healing property of NFD1% and why NFD2% has no such effect.

5. Conclusions

Topical application of NFD1% ointment showed more positive healing activity in full thickness wound model through increase in TAC, improved mitochondrial respiratory chain during wound healing, whereas nifedipine 2% ointment had no such effect.

Acknowledgment: Authors sincerely appreciate the support provided by the University of Mosul/ College of Dentistry to reach the best quality of this study.

Conflict of interest
None

6. References

[1] Mahgoub, S., El-Sayed, M. I. K., El-Shehry, M. F., Awad, S. M., Mansour, Y. E., & Fatahala, S. S. (2021). Synthesis of novel calcium channel blockers with ACE2 inhibition and dual antihypertensive/anti-inflammatory effects: A possible therapeutic tool for COVID-19. Bioorganic chemistry, 116, 105272. https://doi.org/10.1016/j.bioorg.2021.105272

[2] Pereira, D. A., Sandrim, V. C., Palei, A. C., Amaral, L. M., Belo, V. A., Lacchini, R., ... & Luizon, M. R. (2021). NAMPT single-nucleotide polymorphism rs1319501 and visfatin/NAMPT affect nitric oxide formation, sFlt-1 and antihypertensive therapy response in preeclampsia. Pharmacogenomics, 22(8), 451-464. https://doi.org/10.2217/pgs-2021-0006

[3] Lo, Y., Lin, L. Y., & Tsai, T. F. (2021). Use of calcium channel blockers in dermatology: a narrative review. Expert review of clinical pharmacology, 14(4), 481-489. https://doi.org/10.1080/17512433.2021.1894128

ISSN: 0005-2523
Volume 62, Issue 04, June, 2022

[4] Miricescu, D., Badoiu, S. C., Stanescu-Spinu, I. I., Totan, A. R., Stefani, C., & Greabu, M. (2021). Growth Factors, Reactive Oxygen Species, and Metformin—Promoters of the Wound Healing Process in Burns?. International Journal of Molecular Sciences, 22(17), 9512. https://doi.org/10.3390/ijms22179512

[5] Comino-Sanz, I. M., López-Franco, M. D., Castro, B., & Pancorbo-Hidalgo, P. L. (2021). The role of antioxidants on wound healing: A review of the current evidence. Journal of Clinical Medicine, 10(16), 3558. https://doi.org/10.3390/jcm10163558

[6] Zhu, J., Jin, Q., Zhao, H., Zhu, W., Liu, Z., & Chen, Q. (2021). Reactive oxygen species scavenging sutures for enhanced wound sealing and repair. Small Structures, 2(7), 2100002. https://doi.org/10.1002/sstr.202100002

[7] Alver, E. N., Kaltalioglu, K., & Coskun-Cevher, S. (2021). Reactive Oxygen Species and Wound Healing. Research & Reviews in Science and Mathematics, 65.

[8] Dunnill, C., Patton, T., Brennan, J., Barrett, J., Dryden, M., Cooke, J., ... & Georgopoulos, N. T. (2017). Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. International wound journal, 14(1), 89-96. https://doi.org/10.1111/iwj.12557

[9] Lee, T. Y. (2021). Lactate: a multifunctional signaling molecule. Yeungnam University Journal of Medicine, 38(3), 183. https://doi.org/10.12701/yujm.2020.00892

[10] Friciu, M., Chefson, A., & Leclair, G. (2016). Stability of Hydrocortisone, Nifedipine, and Nitroglycerine Compounded Preparations for the Treatment of Anorectal Conditions. The Canadian Journal of Hospital Pharmacy, 69(4), 329. https://doi.org/10.4212/cjhp.v69i4.1578

[11] Katsinelos, P., Papaziogas, B., Koutelidakis, I., Paroutoglou, G., Dimiropoulos, S., Souparis, A., & Atmatzidis, K. (2006). Topical 0.5% nifedipine vs. lateral internal sphincterotomy for the treatment of chronic anal fissure: long-term follow-up. International journal of colorectal disease, 21(2), 179-183. https://doi.org/10.1007/s00384-005-0766-x

[12] Nazir, T., Shakir, L., Rahman, Z. U., Najam, K., Choudhary, A., Saeed, N., ... & Khanum, A. B. (2020). Hepatoprotective Activity of Foeniculum Vulgare Against Paracetamol Induced Hepatotoxicity in Rabbit. J. Appl. Pharm, 12, 2376-0354.

[13] Ricci, F., Bresesti, I., LaVerde, P. A. M., Salomone, F., Casiraghi, C., Mersanne, A., ... & Lista, G. (2021). Surfactant lung delivery with LISA and InSurE in adult rabbits with respiratory distress. Pediatric research, 90(3), 576-583. https://doi.org/10.1038/s41390-020-01324-2

[14] Ragab, G. H., Zaki, F. M., Hassan, F. E., & Safwat, N. M. (2021). Comparable Study of Different Materials (Silver Nanoparticles, PRP and its Mixture) That Enhance Surgical Excisional Skin Wound Healing in New Zealand Rabbits; Histopathological Evaluation. Annals of the Romanian Society for Cell Biology, 25(6), 15966-15975.

[15] Altaay, O. Y. Y., & Al-Haaik, A. G. (2022). Histomorphometrical and histochemical postnatal development of cornea in indigenous rabbits. Iraqi Journal of Veterinary Sciences, 36(2), 291-

296.https://doi.org/10.33899/ijvs.2021.130031.1722.

[16] Naji, A. H., Al-Watter, W. T., & Taqa, G. A. (2022). The Effect of Xylitol on Bone Alkaline Phosphatase Serum Level and Bone Defect Diameter in Rabbits. Journal of Applied Veterinary Sciences, 7(1), 6-10.https://doi.org/10.21608/javs.2021.97815.1105

[17] Naghavi, M., Tamri, P., & Asl, S. S. (2021). Investigation of healing effects of cinnamic acid in a full-thickness wound model in rabbit. Jundishapur Journal of Natural Pharmaceutical Products, 16(1).

[18] Mustafa, E. A., Al-Mashhadane, F. A., & Taqa, G. A. (2020). The Activity of Alcoholic Extract of Urtica Urens Against Staphylococcus Aureus and Oral Wound Healing. Journal of International Dental and Medical Research, 13(3), 957-963.

[19] Brasileiro, A. C. L., Oliveira, D. C. D., Silva, P. B. D., & Rocha, J. K. S. D. L. (2020). Impact of topical nifedipine on wound healing in animal model (pig). Jornal Vascular Brasileiro, 19. https://doi.org/10.1590/1677-5449.190092

[20] Zolfagharnezhad, H., Khalili, H., Mohammadi, M., Niknam, S., & Vatanara, A. (2021). Topical nifedipine for the treatment of pressure ulcer: a randomized, placebo-controlled clinical trial. American Journal of Therapeutics, 28(1), e41-e51.https://doi.org/10.1097/MJT.0000000000000936

[21] Du, H., Jiang, D., Song, G., Cao, C., Zhang, D., Yu, P., ... & Jin, X. (2021). Wound Healing Activity of Phage-Sisplayed TGF-β1 Model Peptide in Streptozotocin-Induced Diabetic Rats. International Journal of Peptide Research and Therapeutics, 27(2), 1079-1094. https://doi.org/10.1007/s10989-020-10152-1

[22] Hassan, M. A., Tamer, T. M., Valachová, K., Omer, A. M., El-Shafeey, M., Eldin, M. S. M., & Šoltés, L. (2021). Antioxidant and antibacterial polyelectrolyte wound dressing based on chitosan/hyaluronan/phosphatidylcholine dihydroquercetin. International Journal of Biological Macromolecules, 166, 18-31.https://doi.org/10.1016/j.ijbiomac.2020.11.119

[23] Alsaidya, A., Ismail, H. K., & Mostafa, E. S. (2022). Efficacy of Urtiea dicica extract for amelioration of brain lesions induced by ethylene glycol in male rabbits. Iraqi Journal of Veterinary Sciences, 36(2), 485-488. https://doi.org/10.33899/ijvs.2021.130563.1848

[24] Ahmad, S. U., Binti Aladdin, N. A., Jamal, J. A., Shuid, A. N., & Mohamed, I. N. (2021). Evaluation of wound-healing and antioxidant effects of Marantodes pumilum (Blume) Kuntze in an excision wound model. Molecules, 26(1), 228.https://doi.org/10.3390/molecules26010228

[25] Takase, H., Toriyama, T., Sugiyama, M., Nakazawa, A. I., Hayashi, K., Goto, T., ... & Dohi, Y. (2005). Effect of nifedipine on C-reactive protein levels in the coronary sinus and on coronary blood flow in response to acetylcholine in patients with stable angina pectoris having percutaneous coronary intervention. The American journal of cardiology, 95(10), 1235-1237.https://doi.org/10.1016/j.amjcard.2005.01.054

[26] Ołdakowska, M., Ściskalska, M., Kepinska, M., Marek, G., & Milnerowicz, H. (2022). Association of Genetic Variants in IL6 Gene (rs1800795) with the Concentration of Inflammatory Markers (IL-6, hs-CRP) and Superoxide Dismutase in the Blood of Patients with Acute Pancreatitis—Preliminary Findings. Genes, 13(2), 290.https://doi.org/10.3390/genes13020290

ISSN: 0005-2523
Volume 62, Issue 04, June, 2022

[27] Passacquale, G., Desideri, G., Croce, G., Murgo, S., Mancarelli, M. M., Zazzeroni, F., ... & Ferri, C. (2008). Nifedipine improves the migratory ability of circulating endothelial progenitor cells depending on manganese superoxide dismutase upregulation. Journal of hypertension, 26(4), 737-746.https://doi.org/10.1097/HJH.0b013e3282f4d1bd

[28] Kaushik, K., & Das, A. (2019). Endothelial progenitor cell therapy for chronic wound tissue regeneration. Cytotherapy, 21(11), 1137-1150. https://doi.org/10.1016/j.jcyt.2019.09.002

[29] Al Hafidh, N. M., Aljammas, E. K., & Al-Kataan, M. A. (2020). Vitamin D Status and Mitochondrial Function in Children. J Med J.2020; 45 (4),189-196.

[30] Haller, H. L., Sander, F., Popp, D., Rapp, M., Hartmann, B., Demircan, M., ... & Kamolz, L. P. (2021). Oxygen, pH, Lactate, and Metabolism—How Old Knowledge and New Insights Might Be Combined for New Wound Treatment. Medicina, 57(11), 1190. https://doi.org/10.3390/medicina57111190

[31] Huynh, K. W., & Pamenter, M. E. (2022). Lactate inhibits naked mole-rat cardiac mitochondrial respiration. Journal of Comparative Physiology B, 1-11. https://doi.org/10.1007/s00360-022-01430-z

[32] Gupta, S., Mujawdiya, P., Maheshwari, G., & Sagar, S. (2022). Dynamic Role of Oxygen in Wound Healing: A Microbial, Immunological, and Biochemical Perspective. Archives of Razi Institute, 77(2), 513-523.https://doi.org/10.22092/ARI.2022.357230.2003

[33] Lin, H. C., Chen, Y. J., Wei, Y. H., Lin, H. A., Chen, C. C., Liu, T. F., ... & Chen, L. C. (2021). Lactic acid fermentation is required for NLRP3 inflammasome activation. Frontiers in Immunology, 12.https://doi.org/10.3389/fimmu.2021.630380.

[34] Kelley, N., Jeltema, D., Duan, Y., & He, Y. (2019). The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. International journal of molecular sciences, 20(13), 3328.https://doi.org/10.3390/ijms20133328

[35] Qing, L., Fu, J., Wu, P., Zhou, Z., Yu, F., & Tang, J. (2019). Metformin induces the M2 macrophage polarization to accelerate the wound healing via regulating AMPK/mTOR/NLRP3 inflammasome singling pathway. American journal of translational research, 11(2), 655.

[36] Wan, L., Bai, X., Zhou, Q., Chen, C., Wang, H., Liu, T., ... & Xie, L. (2022). The advanced glycation end-products (AGEs)/ROS/NLRP3 inflammasome axis contributes to delayed diabetic corneal wound healing and nerve regeneration. International journal of biological sciences, 18(2), 809.doi: 10.7150/ijbs.63219.

[37] Manohar, K., Gupta, R. K., Gupta, P., Saha, D., Gare, S., Sarkar, R., ... & Giri, L. (2021). FDA approved L-type channel blocker Nifedipine reduces cell death in hypoxic A549 cells through modulation of mitochondrial calcium and superoxide generation. Free Radical Biology and Medicine, 177, 189-200.https://doi.org/10.1016/j.freeradbiomed.2021.08.245

[38] Liu, Y., Yang, X., Liu, Y., Jiang, T., Ren, S., Chen, J., ... & Chen, Z. (2021). NRF2 signalling pathway: New insights and progress in the field of wound healing. Journal of Cellular and Molecular Medicine, 25(13), 5857-5868.https://doi.org/10.1111/jcmm.16597

[39] Choi, T. G., & Kim, S. S. (2019). Physiological functions of mitochondrial reactive oxygen species. Free Radic. Med. Biol.

[40] Patergnani, S., Bouhamida, E., Leo, S., Pinton, P., & Rimessi, A. (2021). Mitochondrial Oxidative Stress and "Mito-Inflammation": Actors in the Diseases. Biomedicines, 9(2), 216.https://doi.org/10.3390/biomedicines9020216

[41] Al-Jureisy, I. T., Al-Wattar, W., & Taqa, G. A. A. (2019). The Topical Effect of Coenzyme Q10 on Wound Healing in Mice. Al-Rafidain Dental Journal, 19(1), 42-51.https://doi.org/10.33899/rden.2018.163600.