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The Effectiveness of Nitric Oxide Loaded by Silica Nanoparticles with β-Sitosterol and Aloe Vera Gel for Treatment Burn Wound

Laith A. Younus

Department of Clinical Laboratory Sciences, Faculty of Pharmacy, Jabir Ibn Hayyan for Medical and Pharmaceutical Sciences, Al Najaf Al Ashraf, Iraq.
*Correspondence to: Laith A. Younus (E-mail: laith.ali@jmu.edu.iq)
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Abstract
Objective: To investigate the efficacy of nitric oxide nanoparticles (NO-np) and β-sitosterol, delivered via aloe vera hydrogel, as a topical treatment for burn wounds, leveraging nitric oxide's antibacterial, anti-inflammatory, and wound-healing properties.
Methods: Using a rat burn model, the study evaluated the antibacterial effect of NO against Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus in vitro. Burn healing was assessed by applying a combination of NO-np, β-sitosterol, and aloe vera gel to the wounds, comparing treated samples to control groups. Histological analysis was performed to assess tissue regeneration and angiogenesis in the treated areas.
Results: The NO-np exhibited significant antibacterial activity by inhibiting the growth of the tested bacteria. Additionally, the combination of NO-np, β-sitosterol, and aloe vera gel accelerated wound healing in burn injuries, with significant improvement in healing speed ($P < 0.05$) and enhanced angiogenesis compared to controls.
Conclusion: This study suggests that nitric oxide-loaded silica nanoparticles, especially when combined with β-sitosterol and delivered via aloe vera hydrogel, hold promise as an effective topical treatment for cutaneous burns. This combination therapy enhances wound healing, reduces burned tissue, and promotes angiogenesis, indicating potential for clinical application in burn management.
Key Words: Nitric Oxide, β-sitosterol, burn, silica nanoparticles, and aloe vera gel

Introduction

Nitric Oxide (NO)

Nitric oxide or nitrogen monoxide is small molecule and an essential compound with chemical formula of NO shown in Figure 1, nitric oxide is naturally produced free radical that plays role in host defense mechanisms against infection at various site,[1] and a diatomic hydrophobic compound.[2]

Nitric oxide (NO) has a molecular mass of 30.006 g / mol, a melting-point of -164°C and a boiling-point of -152°C.[3]

Numerous physiological and pathological processes, including endothelial vasorelaxation, cardiovascular functions, antimicrobial activity, wound healing, tissue repair, neurotransmission, immune processes, and blood pressure regulation, have been linked to NO, demonstrating its importance.[4,5]

The Roles of Nitric Oxide (NO)

There are many roles of Nitric Oxide as shown in Figure 2.

Nitric Oxide role in Inflammation and Immunity

As a poisonous defensive molecule against pathogenic pathogens, NO is significant. Additionally, it controls the development, proliferation, and death of a wide range of inflammatory and immunological cell types, including as neutrophils, mast cells, T-lymphocytes, macrophages, antigen-presenting cells, and natural killer cells.[6]

Nitric Oxide role as Vasodilation & Smooth Muscle Relaxant

NO is produced by vascular endothelium and swiftly diffuses into the bloodstream. Furthermore, it diffuses into vascular smooth muscle cells next to the endothelium, where it binds to and activates guanylyl cyclase.[7] This enzyme is responsible for catalyzing the conversion of GTP to cGMP, a second messenger that is necessary for several vital biological functions, chief among them being the signaling of smooth muscle relaxation. Many mechanisms, including increased intracellular cGMP, which decreases intracellular calcium concentrations and inhibits calcium entry into the cell, activation of K+ channels, hyperpolarization, and relaxation, are responsible for the relaxation of smooth muscle caused by cyclic GMP.[8] Additionally, it can start a cGMP-dependent protein kinase, which in turn activates the enzyme myosin light chain phosphatase, which dephosphorylates myosin light chains and relaxes smooth muscle...[9]

Material and methods

Study Design and Ethical Consideration

A clinical trials study design was started from (20 Mar, 2023) to the end of (20 Aug, 2023). has been adopted in order to achieve study objectives. The study subject included for the comparison between five identical groups of albino rats in age, weight and gender, the first group burned and treated by NO & SiNp & aloe vera gel, the second group burned and treated by NO & SiNp & β-sitosterol & aloe vera gel, the third group burned and treated by β-sitosterol & aloe vera gel, the fourth group burned and treated with aloe vera gel only, the fifth group burned without treatment (control). The ethical approvement was obtained from the Ethical committee in the Faculty of Pharmacy, Jabir Ibn Hayyan University for medical and Pharmaceutical Sciences.

Preparation of MacConkey, Mannitol, and Mueller-Hinton' Agar

MacConkey agar, Mannitol agar and Mueller-Hinton' belong to a himedia company were prepare to evaluate the antibacterial activity of NO.

Nitric oxide Nanoparticles

Additionally, nanoparticles (NPs) have the ability to act as carriers of NO, which may significantly improve the therapeutic NO's sustained release as well as its local delivery and solubility. Nano scale particles offer improved contact and penetration into the injured tissue because of their size.[11]

Experimental Groups and Treatment

All the rats were divided randomly to 5 groups (n = 40), each of which contained 8 rats. The first application was done directly after the burn as shown in Table 1.

Samples Collection for Histological Study

On 31 July 2023, using standard histology methods, the 6.7 cm^2 experimentally wounds and healed skin sample were taken from Albino rats as shown in Figures 3 and 4.

Statistical Analysis

Each experiment was done. Data was represented as mean ± SD. Data were also analyzed ANOVA to evaluate any significant differences between values obtained of the within and between the specimen groups. Differences were done using SPSS 20 statistical software. $P < 0.05$ was considered significant.

Result and Discussion

Evaluation of Antibacterial Activity of Nitric oxide powder (In-vitro)

On 18 May, the nitric oxide powder potential antibacterial activity was tested against *P. aeruginosa*, *K. pneumonia* and *S. aureus*, the results are shown in Figure (5). The nitric oxide inhibits the growth of bacterial colonies in the concentration of 100 mg per 5 ml, 10 ml, 15 ml, 20 ml and 25 ml of deionized water respectively as detailed in Table (2).

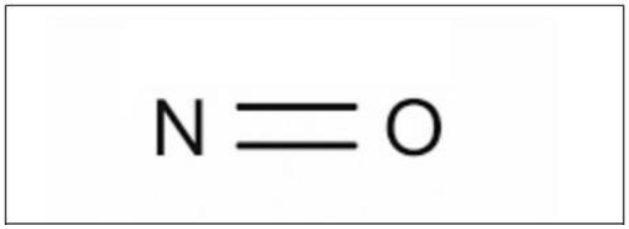


Fig. 1 **Structure and chemical formula of Nitric Oxide.**

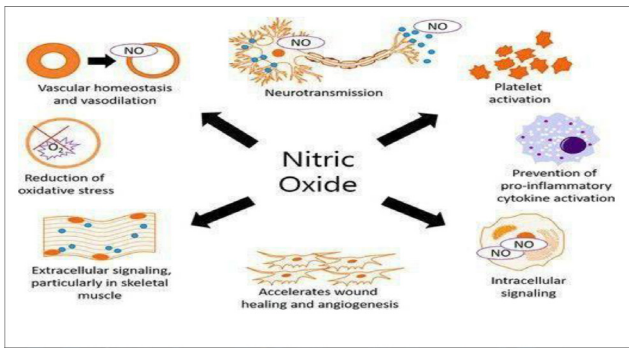


Fig. 2 **Main Physiological Functions of Nitric Oxide[10].**

Reactive nitrogen species (RNS) are elevated in the presence of nitric oxide. The nitrosation of cysteine thiols, suppression of ribonucleotide Reductase, an enzyme required for repairing bacterial DNA, and down regulation of Ferro protein, the exporter of cellular iron required for bacterial metabolism, are some of the putative mechanisms underlying the bactericidal effects of NO and RNOS.[12]

Table 1. **The treatment that gave for each group**

Group I	Treated with 112 mg of NO & 6.2 g of SiNp & 20 g of aloe vera gel.
Group II	Treated with 112 mg of NO & 6.2 g of SiNp & 10 g of β-sitosterol & 10 g of aloe vera gel.
Group III	Treated with 5.5 g of β-sitosterol & 5 g of aloe vera gel.
Group IV	Aloe vera gel only.
Group V	Control group (without treatment).

Table 2. **Diameters of the Inhibition zone (mm) measurements for *P. aeruginosa*, *S. aureus* and *K. pneumonia* exposure to nitric oxide powder (mg/ml)**

Nitric Oxide Powder (mg/ml)	Inhibition Zone (mm)		
	S. aureus	*K. pneumonia*	*P. aeruginosa*
1.32 mg/ml	50	47	0
100 mg/5ml	28	42	39
100 mg/10ml	27	40	32
100 mg/15ml	25	45	30
100 mg/20ml	26	25	29
100 mg/25ml	27	30	35

Fig. 3 **Collection the Samples.**

Fig. 4 **Samples in Formaldehyde.**

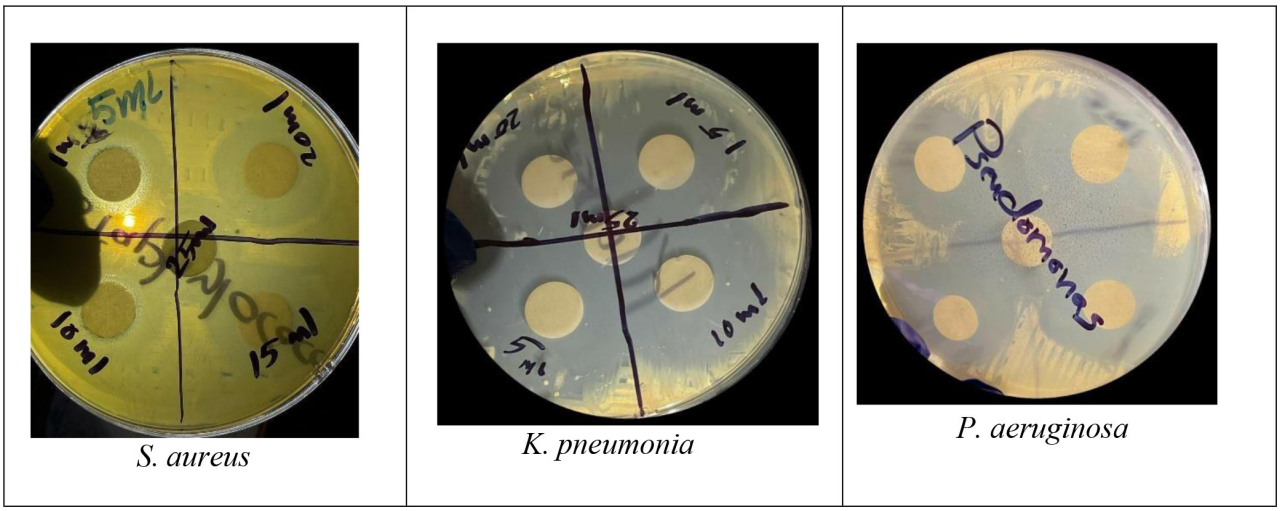


Fig. 5 **Zone of Inhibition of Nitric Oxide Powder in Different Concentrations.**

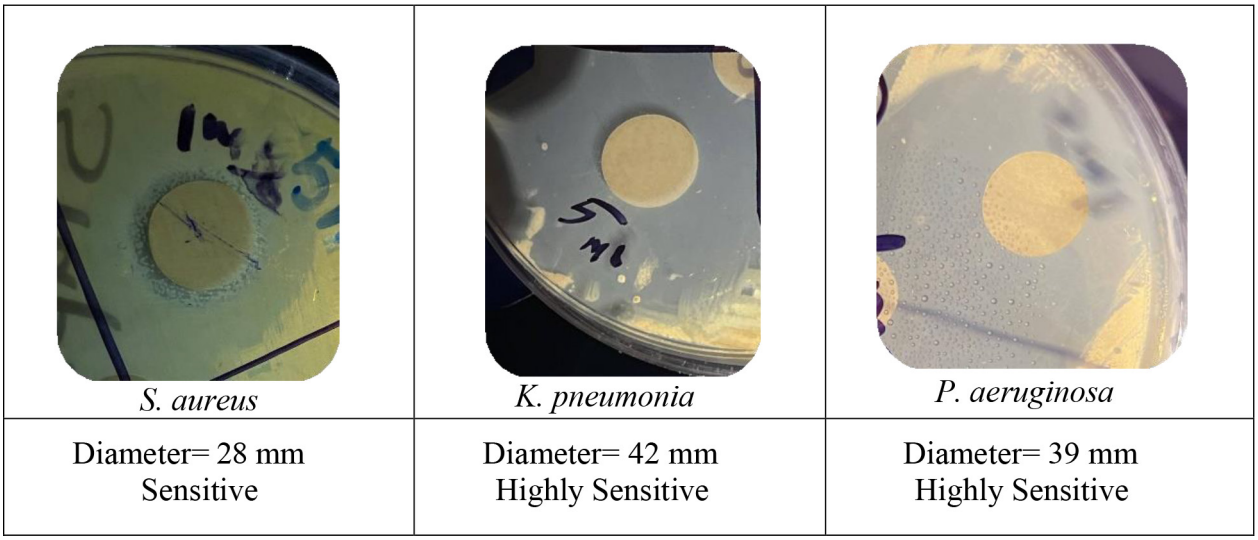


Fig. 6 **100 mg NO/ 5 ml of deionized water.**

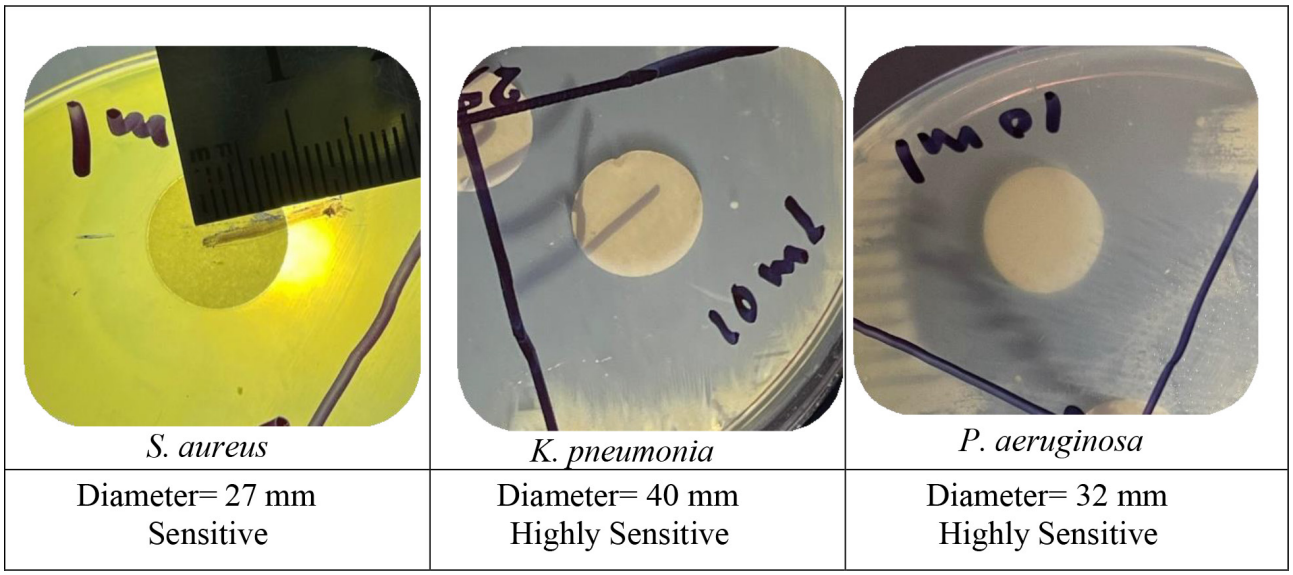


Fig. 7 **100 mg NO/ 10 ml of deionized water.**

While In *pseudomonas aeruginosa* NO demonstrated to reduce cGMP level by stimulation the activities of phosphodiesterase responsible for the hydrolysis of cGMP,[13] a decrease in intracellular cGMP level lead low Ca^{+2}, which it plays an important role in regulation and increases gene expression and increases biofilm formation in *P. aeruginosa.*[14]

Measurement of the Inhibition Zone

The diameter of the inhibition zone can be found in figures below, which involves measuring the diameter of the inhibition area using a ruler and recording measurements in millimeters (mm) in concentration 100 mg per ml of deionized water (5 ml, 10 ml, 15 ml, 20 ml and 25 ml), the inhibition zone shows even in small concentration (1.32 mg/ml), as shown in Figures (6–11), which illustrated in Tables 2 and 3.

In Vitro Wound Healing Evaluation

After burn the five rat groups were followed up in all times of study as shown in Figure 3.8 then the evaluation of wound healing in all five groups were shown in Figure 12 and in Tables 4–7.

The area of skin wound in experimental group (group II) decreased under (NO, SiNp, β-sitosterol and aloe vera gel) treatment much faster than other groups. The $P < 0.05$ Significant differences in surface area of burn wound compression with time (4, 8, 10) day in group II and other groups no significant differences $P > 0.05$. While the percentages of healing in Table 7 showed the Group II have difference significant in healing ($P < 0.05$) in (4^{th}, 8^{th}, 10^{th}) day comparison with percentage in other groups, as in Table 8 and Figure 13 which show the angiogenesis percent

The finding of this study was supported by the study done by *Tavares G,*[15] *Lee J,*[16] and *Igrunkova A,*[17] these studies found that nitric oxide (NO) loaded by silica nanoparticles use to treated burns.

In our study show there is significantly difference ($P < 0.05$) in group II. Rather than these studies *Ben-Yehuda Greenwald M,*[18] *Dou J,*[19] and *Li M*[20] found that there is significant difference

Table 3. **The statistical analysis of antibacterial activity of nitric oxide**

Conc..	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	Mean	SD	*P*-value
20%	39 mm	42 mm	28 mm	36.333	7.3711	**$P < 0.001$**
10%	32 mm	40 mm	27 mm	33	6.5574	**$P < 0.001$**
7.5%	30 mm	45 mm	25 mm	33.333	10.408	**$P < 0.001$**
5%	29 mm	25 mm	26 mm	26.667	2.0817	**$P < 0.001$**
4%	35 mm	30 mm	27 mm	30.667	4.0415	**$P < 0.001$**
1.32%	0	47 mm	50 mm	32.333	28.042	**$P < 0.001$**
0.00%	0	0	0	0	0	***P*-value**
Mean	23.57142857	32.71428571	26.14285714	Mean	Mean	Mean
SD	16.44036844	16.48953492	14.4848032	SD	SD	SD
***P*-value**	***P*-value = 0.5486 ($P > 0.05$)**			***F*-value = 0.6208**		

$P < 0.001$ highly statistically significant difference.

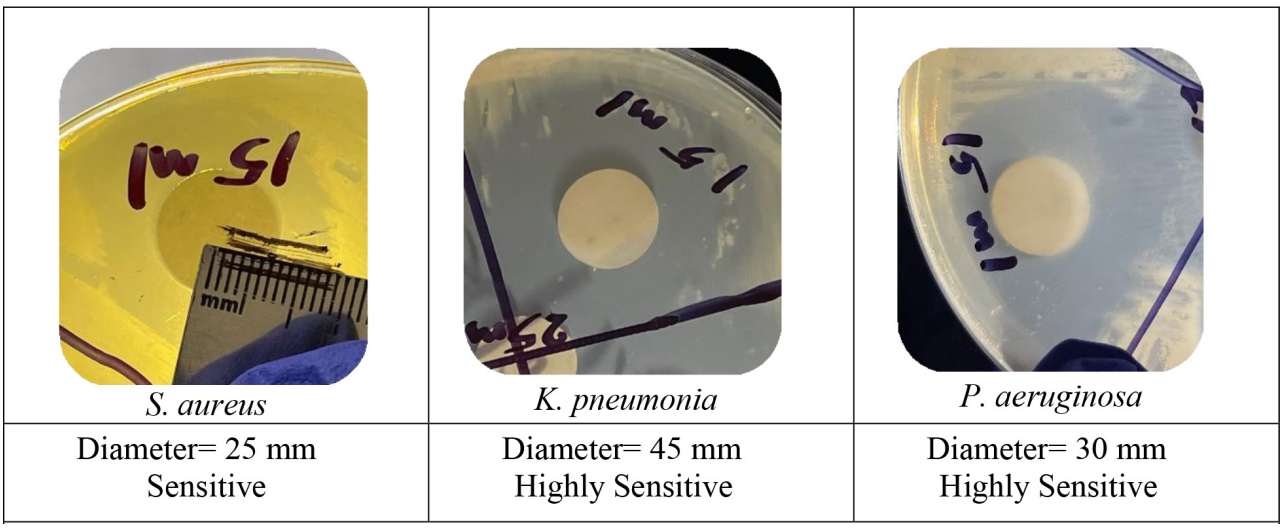


Fig. 8 **100 mg NO/ 15 ml of deionized water.**

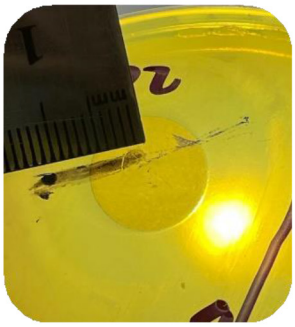
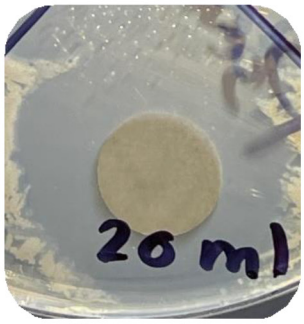
 *S. aureus*	 *K. pneumonia*	 *P. aeruginosa*
Diameter= 26 mm Sensitive	Diameter= 25 mm Sensitive	Diameter= 29 mm Sensitive

Fig. 9 **100 mg NO/ 20 ml of deionized water.**

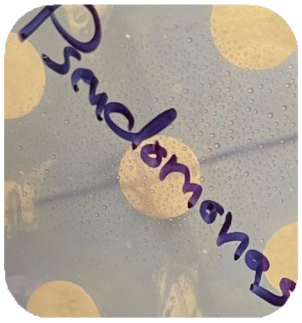
 S. aureus	 K. pneumonia	 P. aeruginosa
Diameter= 27 mm Sensitive	Diameter= 30 mm Sensitive	Diameter= 35 mm Highly Sensitive

Fig. 10 **100 mg NO/ 25 ml of deionized water.**

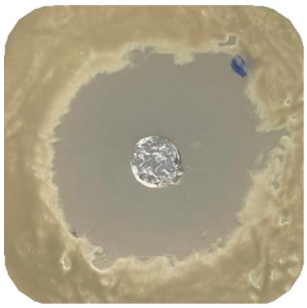
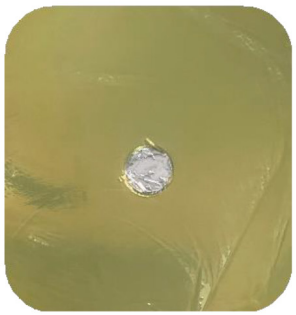
 *S. aureus*	 *K. pneumonia*	 *P. aeruginosa*
Diameter= 50 mm Highly Sensitive	Diameter= 47 mm Highly Sensitive	Diameter= 0 mm This concentration not effective

Fig. 11 **1.32 mg NO/ml of deionized water.**

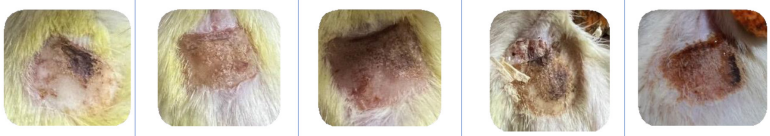
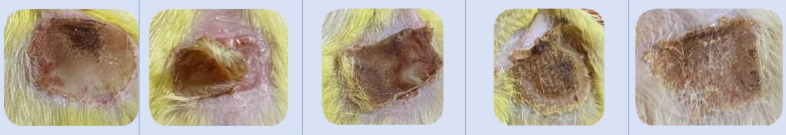
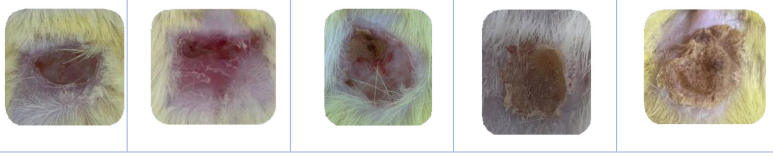
Days	Group I: Treated with NO & SiNp & aloe vera gel.	Group II: Treated with NO & SiNp & β-sitosterol & of aloe vera gel.	Group III: Treated with β-sitosterol & aloe vera gel.	Group IV: aloe vera gel only	Group V: Control group
Day0					
Day4					
Day8					
Day10					

Fig. 12 **Figure (12) Photographs of Burn Development in the Five Groups through 10 Days.**

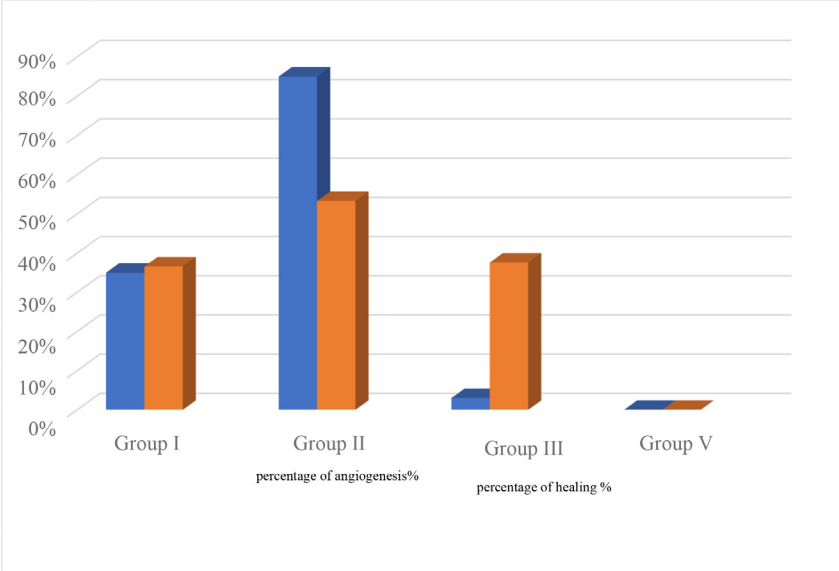


Fig. 13 **Percentage of Healing and Angiogenesis.**

Table 4. **Measurement Wound Surface Area (cm²)**

Group	4th day	8th day	10th day
Group I	5.32 cm²	4.25 cm²	3.68 cm²
Group II	4.5 cm²	3.68 cm²	2.8 cm²
Group III	5.13 cm²	4.68 cm²	3.74 cm²
Group IV	6 cm²	4.86 cm²	4.42 cm²
Group V	6 cm²	6 cm²	6 cm²

Table 5. **Percentage of Wound Contraction Healing%**

Group	4th day	8th day	10th day
Group I	11.3%	29.1%	36.6%
Group II	25%	36%	53.3%
Group III	14.5%	22%	37.6%
Group IV	0%	19%	26%
Group V	0%	0%	0%

Table 6. **The statistical analysis of surface area of burn wound**

Days of Examining	Day0	Day4	Day8	Day10	Mean ±SD	P-value
Group I	6 cm^2	5.32 cm^2	4.25 cm^2	3.68 cm^2	4.8125 ± 1.0435	N S
Group II	6 cm^2	4.5 cm^2	3.68 cm^2	2.8 cm^2	4.245 ± 1.3604	$P < 0.05$
Group III	6 cm^2	5.13 cm^2	4.68 cm^2	3.74 cm^2	4.8875 ± 0.941	N S
Group IV	6 cm^2	6 cm^2	4.86 cm^2	4.42 cm^2	5.32 ± 0.8055	N S
Group V	6 cm^2	6 cm^2	6 cm^2	6 cm^2	6 ± 0	N S

A significant difference $P < 0.05$

Table 7. **The statistical analysis of percentage of healing**

Days of Examining	Day0	Day4	Day8	Day10	Mean ± SD	*P*-value
Group I	0	11.3%	29.1%	36.6%	16.651 ± 19.25	N S
Group II	0	25%	36%	53.3%	22.329 ± 28.575	$P < 0.05$
Group III	0	14.5%	22%	37.6%	15.656 ± 18.525	N S
Group IV	0	0	19%	26%	13.301 ± 11.25	N S
Group V	0	0	0	0	0 ± 0	N S

A significant difference $P < 0.05$

Table 8. **Percentage of angiogenesis formation**

Group	Percent of angiogenesis %	Percent of healing %
Group I	35%	36.6%
Group II	85%	53.3%
Group III	3%	37.6%
Group V	0%	0%

Fig. 14 **shows the histology of control new burned (a) and after 10 days of burn (b). histopathological observation on (a) showed a complete loss of superficial Epithelium, and crust formation while the histopathological observation on (b) showed a complete loss of superficial Epithelium, sever inflammation with pus cells and crust formation.**

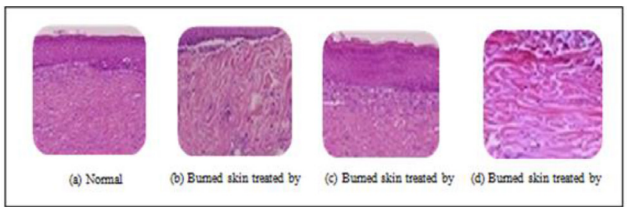


Fig. 15 **Histopathological observation (Rat skin section stained with Hematoxylin and Eosin Magnification x 40) of the normal rat skin and healing site of the skin burn in different groups on day 10 of the research.**

($P < 0.01$), the reason for these results is the larger sample size that used in our study.

Histopathological Observations

Histological processing. At 10th day after burned the rats and treatment with all combinations as above, excision the burned tissues the rats, dropped in 10% formalin. Then, Rats' skin sections stained with Hematoxylin and eosin, magnification × 40 and get histopathological observations shown in Figures 14 and 15.

In Figure 3.10 shows the histology of: (a) normal rat skin which show normal cells with clear superficial epithelium. The histopathological observation on (b) showed an acute inflammation with mild angiogenesis and little superficial epithelium was returned, while the histopathological observation on (c) showed highly angiogenesis and the formation of granulation tissue, collagen deposition and epidermal regeneration were observed. and a fully re-epithelialization was shown. Finally, the histopathological observation on (d) showed an acute inflammation and loss of superficial epithelium with pus cells. The fourth group doesn't have histological observations because it died.

GI treated by NOnp plus aloe Vera gel.
GII treated by NOnp plus aloe Vera gel plus β-sitosterol.
GIII treated by aloe Vera gel plus β-sitosterol.
GV control group without treatment.

The Conclusion

At the end of this study we can conclude that:

1. Nitric Oxide is an important therapeutic agent that used to treat burns due to its physiologic role such as anti-inflammatory, antioxidants, anti-bacterial and vasodilation.

2. The antibacterial activity of Nitric Oxide was shown a potent activity against *Staphylococcus aureus*, *pseudomonas aeruginosa* and *Klebsiella pneumoniae* which are the most common pathogenic causes infection in burns.
3. Loading Nitric Oxide by silica nanoparticles can maintain its stability and enhance its releases in addition to increase the penetration in to the wound tissue.

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Conflict of interest

There is no Conflict of interest.

Authors' Contribution

This article with one author only myself.

Reference

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