In vivo and in vitro antibacterial assessment of *Nerium oleander* aqueous leaf extract against bacterial pathogens and its effect in treatment of wounds

Tareq Rifaaht Minnat
Coll. of Vet. Med. / Univ. of Diyala
email: tariq8222@yahoo.com
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Abstract

The aim of the study was to evaluate the antibacterial activities of aqueous leaf extract *Nerium oleander* against five identified bacteria namely Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and Gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis* and role of extract in treatment of wounds in laboratory animal. Antibacterial activity of aqueous extracts was determined by disc diffusion and well diffusion method on Muller–Hinton agar. Twelve laboratory animals (Rabbit) use to evaluate the role of aqueous leaf extract of *N. oleander* on healing of wound and divided into four equal groups. Control, Fucine ointment, *N. oleander* 80% and *N. oleander* 100 % groups. As the inhibitory zones were more at 60% and 80% in comparison with 2.5 % , 5 % and 10%, and the widest were at 100 % against all bacteria species and show significant difference at ($P<0.05$) between susceptibility of bacteria species to different concentration of aqueous leaf extract of *Nerium oleander* in agar well diffusion exhibit a significantly higher zones of inhibitions against all bacteria spp. in used at 100%, 80%, 60% in comparison with 2.5%, 5% and10% concentration more than in agar disc method, which did not exhibit any inhibitory zone against all bacteria spp. at 2.5% and the narrowest inhibitory zones was against *Bacillus subtilis*, and *Staphylococcus aureus.* at 5% and 10% Whenever, at 100% and 80% the widest inhibitory zones was against *Pseudomonas aeruginosa* and *Proteus mirabilis* and less than against *Staph. and Bacillus spp.* Hematological changes show significant difference at $P<0.05$ in erythrocyte indices and leukocyte count. Whenever, effect of extract on treatment of wound show complete healing at 6-7 days compared with local antibiotic fucine ointment without complication. In conclusion; Aqueous leaf extracts of *N. oleander* have inhibitory activity against both gram-positive and gram-negative bacteria this indicates of the presence antimicrobial compounds. Leaf extracts of *N. oleander* at 80-100 % can be used as local antibacterial and treatment of open wounds.

Key words: Antibacterial, *Nerium oleander*, blood parameters, wounds.

تقيم الفعالية المضادة للبكتريا للمستخلص المائي لأوراق نبات الدفلة ضد الجراثيم الممرضة وتأثيره على علاج الجروح (في التجارب المختبرية والحية)

طارق رفعت منت
كلية الطب البيطري/ جامعة ديالى

الخلاصة

هدفت الدراسة الى تقييم فعالية المضاد الجرثومي للمستخلص المائي لأوراق نبات الدفلة مختبريا ضد خمسة انواع من البكتيريا المسمية كالاتي الموجبة لصبغة كرام العصوية الرقيقة ، المكورات العنقودية الذهبية ، والبكتيريا السالبة لصبغة كرام الزائفة الزنجارية ، العصوية القولونية والمتقلبة الرائعة ، ودور المستخلص في شفاء والتئام الجروح في الحيوانات المختبرية. تم تحديد فعالية النشاط المضاد للبكتيريا للمستخلص المائي من خلال طريقة انتشار الأقراص وطريقة الحفر وعلى وسط مولر هنتون أجار. اثنا عشر حيوانا مختبريا (ارنباً) استخدم لتقييم دور المستخلص المائي لأوراق نبات الدفلة وتأثيره على شفاء الجروح وقسمت الى اربعة مجاميع متساوية. مجموعة السيطرة، مجموعة مرهم الفوسيين ومجموعة لمستخلص الدفلة بتركيز80٪ ومجموعة بتركيز 100٪. اظهرت الدراسة أن مناطق التثبيط للبكتريا كانت أكثر بالتراكيز

60٪ و 80٪ مقارنة مع 2.5٪ و 5٪ و 10٪، وعلى نطاق اوسع في التركيز 100٪ ضد كل انواع البكتيريا، وهناك فروق معنوية لحساسية انواع البكتريا ضد التراكيز المختلفة للمستخلص المائي لأوراق نبات الدفلة وبطريقة انتشار الحفر مقارنة بطريق انتشار الاقراص التي لم تظهر اي تثبيط واضح لأنواع البكتريا عند التركيز 2.5% واقل مناطق تثبيط نمو لبكتيريا العصوية الرقيقة والمكورات العنقودية الذهبية عند التركيز 5% و10%. بينما اظهرت التراكيز 80% و100% اوسع نطاق للتثبيط ضد البكتريا الزائفة الزنجارية والمتقلبة الرائعة واقل منها ضد بكتريا المكورات العنقودية والعصوية الرائعة وبطريقة انتشار الاقراص. اظهرت التغيرات الدمية فروق معنوية بمؤشرات كريات الدم الحمر والعدد الكلي لكريات الدم البيض .بينما تأثير المستخلص على علاج الجروح اظهرت شفاء تام للجروح باليوم 6-7 مقارنة مع المضاد الحيوي المستخدم وبدون حدوث مضاعفات. نستنتج من ذلك ان مستخلص أوراق نبات الدفلة المائي لها نشاط مثبط ضد كل من البكتيريا موجبة لصبغة كرام والسالبة لصبغة كرام وهذا يشير إلى وجود مركبات مضادة للبكتيريا. يمكن استخدام المستخلص المائي لأوراق نبات الدفلة بالتراكيز 80-100٪ كمضادات للبكتيريا ولعلاج الجروح المفتوحة.

الكلمات المفتاحية: المضاد البكتيري ، نبات الدفلة ، المعايير الدمية ، الجروح.

Introduction

Plant materials used for medicines since ancient times, it have been indispensable source of both preventive and curative traditional medicine preparation for human beings as well as livestock (1). Various medical plants have been used in years in daily life to treat disease all over of the world. Defla (*Nerium oleander*) is a member of the families *Apocynaceae* (Dogbane family). Its ornamental shrub or small, densely branched tree, 1 to 10 m tall (2). Leaves opposite or whorled, every green, leathery, narrowly elliptic to linear entire. Flowers in terminal brunches each 2.5–5 cm, funnel–shaped with five lobes, fragrant, various colors from pink to red, white, peach and yellow. This plant grows outdoors in warmer regions, and in sometime is grown as a house plant. *Nerium oleander* has many therapeutic uses in different traditional medicine of the world (2). In ethno botanical literature it is mentioned to be effective in treatment of cardiac illness asthma, corns, cancer, and epilepsy and also used as diuretic (3). The leaves and flower are cardio tonic, diaphoretic, diuretic, emetic, and antibacterial, expectorant and have antiplatelet aggregation activity. It is various parts are reputed in therapeutic agent in treatment of swelling, leprosy, eye, and skin disease (4). Oleanderine is anti-inflammatory, antitumor, emollient, potentialises apoptosis (5). The plant also used for treatment of mange in rabbits (6). Antimicrobial activity has been screened in Nerium flower (essential oil) against various pathogenic organisms (7). Aqueous extract of Nerium sp. exhibit antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans* (8, 9). Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant crude extracts for their antimicrobial activity may provide new antimicrobial substances. Fucine ointment: Fusidic acid is potent topical antibacterial agent. It inhibit bacterial protein synthesis by interfering with amino acid transfer from amino acyl sRNA to protein on the ribosomes. It is bacteriostatic or bactericidal depending on inoculum size (10).The aim of this study was to evaluate the in vitro and in vivo antibacterial activities of *Nerium oleander* aqueous leaf extract on some bacterial pathogens and use of extract in treatment of wounds in laboratory animals.

Materials and methods

Antibacterial study of leaves of aqueous extracts of *Nerium oleander* were performed against five identified bacteria namely Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), and Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus spp.*) isolated from milk of cows suffering mastitis and identified in department of microbiology and clinical pathology of faculty of Veterinary Medicine of Diyala University. Leaves of *Nerium oleander* collected from Baqubah city of Diyala province. were placed in shade at laboratory temperature till complete dryness, then grinded by electrical mixer till obtain a powder (100g). Aqueous extract was prepared by filling a 100 g of shade dried, powder of *Nerium oleander* in a beaker contain 1000 ml distill water, using magnetic

stirrer mixer at 45^0C, and 800 speed for 72 hours. Then the extract was concentrated using rotary evaporator, after complete water evaporation, the extract was weighted and preserved at-20 ^{0}C in airtight bottles until use (11, 12, 13). The extract activities were determined by spreading of 0.1 ml of bacterial suspension prepared according (14) which contain $1x10^8$cell/ ml over the surface of Muller–Hinton agar plate, to obtain uniform growth, left the plate was to left dry for 5 minutes. Then the wells were prepared by using Pasteur pipette 5 mm diameter. These wells filled by 50 microliter concentrated extract of aqueous extract of *Nerium oleander* at a concentration used, 2.5%, 5%, 10%, 20%, 60%, 80% and 100% the medium was left for 1 hour in laboratory condition, then incubated for 24 h at 37 C^0 and the zone inhibition of antibacterial activity appeared around the well were measured the diameter by centimeter scale in mm (millimeter), each treatment consists of four repeat (15,16,17). Also the antibacterial activity of aqueous extracts was determined by disc diffusion method on Muller–Hinton agar (18). Sterile Whatman filter disc (5 mm diameter) were made by using sterile cork borer (5 mm), these disc impregnated in the 50 microliter of aqueous extract placed in Petri dishes according to concentration for 24 hours. Inoculums containing 10^8CFU/ml of bacteria were spread, with sterile swab moistened with the bacterial suspension. The disc also impregnated in 50 μL of solvent distilled water served as a standard control (18). Standard antibiotic disc; Rifampin 5 mcg; DO: Doxycycline 30mcg; AX: Amoxicillin 25 mcg; K: Kanamycin 30mcg; APX: Ampicillin Cloxacillin 5 mcg, NA: Nadalic acid, for antibacterial activity tests was carried out against bacterial strains used.

Twelve rabbits were used to evaluate the role of aqueous leaf extract of *N. oleander* on wound healing, divided into four equal groups. Control, Fucine ointment, *N. oleander* 80% and *N. oleander* 100 % groups respectively. Antiseptic preparation of animals by clipping, shaving and disinfecting the area of incision were done prior to inducing 6 cm open wound by scalpel in each animal and the experiment lasted for 7 days. *N. oleander* group: Two concentration of *N. oleander* 80% and 100 % applied twice daily, were used in treatment of wound. Fucine ointment group; The ointment (Each 1gram contains Sodium fusidate 20 mg) applied on the wound twice daily. 2.5 milliliters of blood was collected from the marginal ear vein of each rabbits into tubes containing ethylenediaminetetra-acetic acid (EDTA) as the anticoagulant (1 mg/ml). 1-The samples were examined on the day of collection and the hematocrit (PCV) was determined in a microhematocrit centrifuge by spinning at 12000 rev/min for 6 min. 2-The erythrocyte count evaluated by hemocytometer technique and diluting fluid (Hayem's solution), and leukocyte count evaluated by hemocytometer technique and diluting fluid (Truck's solution) and one hundred leukocytes were counted on each blood smear stain by Giemsa stain to evaluate differential leukocytes (19). 3-The hemoglobin (Hb) was assayed using the Sahli's method (acid hematin method). 4-Erythrocyte indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), means corpuscular hemoglobin concentration (MCHC) measured manually (19).

Results

The present study show that the inhibitory zones were more at 60% and 80% in comparison with 2.5 %, 5 % and 10%, and the widest were at 100 % against all bacteria species. The present study show significant difference at ($P<0.05$) between susceptibility of bacteria species to different concentration of aqueous leaf extract of *Nerium oleander* in each disc diffusion and well diffusion method (table 1, and 2).

The leaf extract of *Nerium oleander* in well diffusion method showed inhibition zone in all concentration more than in disc method (Fig. 1, 2, 3, 4, 5). The aqueous extract of *N. Oleander* in agar well diffusion method exhibit a significantly higher zones of inhibitions against all bacteria spp. in used at 100%, 80%, and 60% in comparison with 2.5%, 5% and10% concentration (table 1).

Table (1): Agar well diffusion methods: Comparison between susceptibility of bacteria species to different concentration of aqueous leaf extract of *Nerium oleander*.

Bacteria species	Concentration of extract							
	2.5% M ± SE	5% M ± SE	10% M ±SE	20% M ±SE	40% M ±SE	60% M±SE	80% M ± SE	100% M±SE
Staphylococcus aureus	6.75±0.25 a*	9.75±0.25 b*	12.0±0.66 c*	16.75±2.25 d*	19.0±1.33 e*	22.75± 4.92 f*	23.0±0.66 g*,**	27.5±0.33 h*,**
Bacillus subtlis	7.5±0.33	9.0±0.33 b*	13.0±1.33 c*	17.5±1.0 d*	19.0±0.66 e*	22.75±4.9	23.0±0.66 g* ,**	27.5±0.33 h*,**
Pseudomonas aeruginosa	6.25±0.25 a*	10.5±0.33 b*	12.25±4.75 c*	15.25±2.75 d*	18.0±0.0 e*	19.0± 1.33	21.5±152.0 g**	26.75±6.75 h*,**
Escherichia coli	6.5±0.33 a*	8.0±2.66 b*	12.5±1.0 c*	13.5 ± 3.0 d*	19.25±0.92	20.0±2.66 f*	27.25±0.25 g*,**	32.0± 0.66 h*,**
Proteus mirabilis	9.5±0.33 a*	11.25± 0.75 b*	14.5± 1.0 c*	18.0±2.67	18.5±1.0 e*	20.75±0.92 f*	23.5±0.30 g*,**	28.25±1.58 h*,**

Values: M± SE.: a, b, c, d, e, f, g, and h; significantly different level at P<0.05; a* between 2.5% and 5%. Concentration, b* between 5% and 10%, c* between 10% and 20%, d* between 20% and 40%, e* between 40% and 60%, f* between 60% and 80%, g* between 80% and 100%, g between 2.5% and 80%, h* between 2.5% and 100%, h** between 60% and 100%.**

Table (2): Agar disc diffusion methods: Comparison between susceptibility of bacteria species to different concentration of aqueous leaf extract of *Nerium oleander*.

Bacteria species	Concentration of extract							
	2.5% M± SE	5% M± SE	10% M±SE	20% M± SE	40% M± SE	60% M± SE	80% M± SE	100% M± SE
Staphylococcus aureus	0.0±0.0 NS	6.0±0.0 b*	8.0±2.66 c*	9.0±1.33 d*	12.75±0.92 e*	15.5±0.33 f*	18.0±0.66 g*,**	19.75±0.92 h*,**
Bacillus subtlis	0.0±0.0 a*	5.75±0.25 NS	6.25±0.25 NS	9.25±0.25 d*	13.75±0.92 e*	15.0±1.33 f*	17.25± 0.25 NS	19.75±0.25 h*,**
Pseudomonas aerginosa	0.0±0.0 a*	8.75±0.92-b*	10.5± 0.33 NS	11.5± 0.33 NS	12.5± 0.33 NS	15.5± 0.33 NS	19.5± 0.33 g*, **	22.25±0.25 h*,**
Escherichia coli	0.0±0.0 a*	6.75±0.25 b*	7.5± 0.33 c*	11.0±1.33 d*	12.5±0.33– e*	16.0±0.0 f*	18.75±0.25– g*, **	20.75±0.92 h*,**
Proteus mirabilis	0.0±0.0 a*	7.0±1.33-b-NS	7.25±2.25 c*	10.5±0.33 NS	12.5±0.33– e*	13.75±0.25 NS	19.75±0.25– g*, **	21.5± 0.33 h*,**

a* significance between 2.5% and 5% concentration, b* between 5% and 10%, c* between 10% and 20%, d* between 20% and 40%, e* between 40% and 60%, f* between 60% and 80%, g* between 80% and 100%, g between 2.5% and 80%, h between 2.5% and 80%, h** between 2.5% and 100%, h*** between 60% and 100%, NS=No Significant difference at P< 0.05.**

Table (3): Standard antibiotic disc sensitivity test.

Bacteria species	Concentration of antibiotic disc					
	RA 5mcg	DO 30mcg	AX 25mcg	K 30mcg	APX 5mcg	NA
Staphylococcus aureus	0 mm	12 mm	31mm	14mm	0 mm	10 mm
Bacillus subtlis	40 mm	38 mm	22mm	30 mm	0 mm	0 mm
Pseudomonas aerginosa	23 mm	20 mm	8.5mm	22 mm	6 mm	16 mm
Escherichia coli	10 mm	22 mm	6 mm	0 mm	4 mm	16 mm
Proteus mirabilis	12 mm	6 mm	18mm	22 mm	0 mm	30 mm

RA: Rifampin 5 mcg, DO: Doxycycline 30mcg, AX: Amoxicillin 25 mcg, K: Kanamycin 30mcg, APX: Ampicillin cloxacillin 5 mcg, NA: Nadalic acid.

The aqueous extract of *N. oleander* in agar disc method shows no significant difference at 2.5 % and did not exhibit any inhibitory zone against all bacteria species. While at 5% and 10% the narrowest inhibitory zones were against *Bacillus subtlis* and *Staphylococcus aureus.* Whenever at 100% and 80% the widest inhibitory zones were against *Pseudomonas aeruginosa, and Proteus mirabilis* and less than against *Staphylococcus aureus and Bacillus subtlis* (table 2) (Fig. 7, 8). Different antibiotic disc use to evaluate the sensitivity test and compared with each disc diffusion and well diffusion method. *Bacillus subtlis* show highest inhibition zone and more sensitive to rifampin, doxycycline than amoxicillin when compared with the *Staphylococcus aureus* show lowest inhibition zone. On the other hand the *Pseudomonas aerginosa* shows inhibition zone more than *Escherichia coli* and *Proteus mirabilis* (table 3).

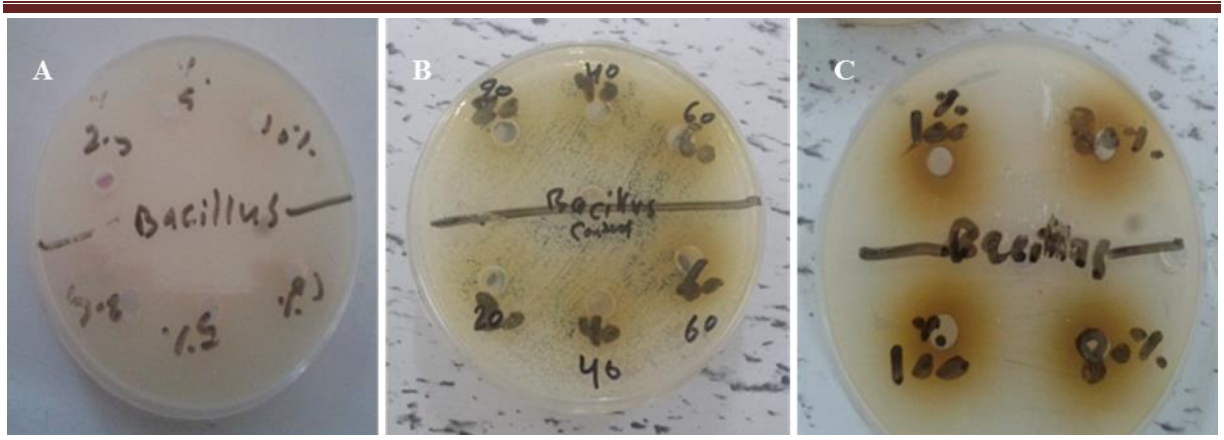

Fig. (1): Inhibitor zone of *N. Oleander* at [(A) 2.5% ,5%,10%; (B) 20%, 40%, 60%; and 80%, 100% (C)] concentration on *Bacillus subtlis* (Well diffusion method).

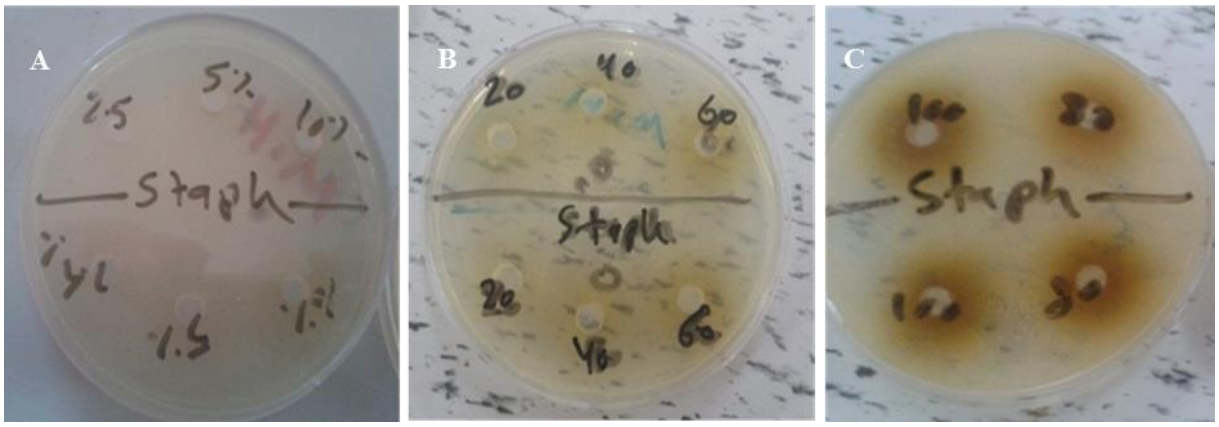

Fig. (2): Inhibitor zone of *N. Oleander* at [2.5%, 5%, 10% (A); 20%, 40%, 60% (B), and 80%, 100% (C)] concentration on *Staphylococcus aureus* (Well diffusion method).

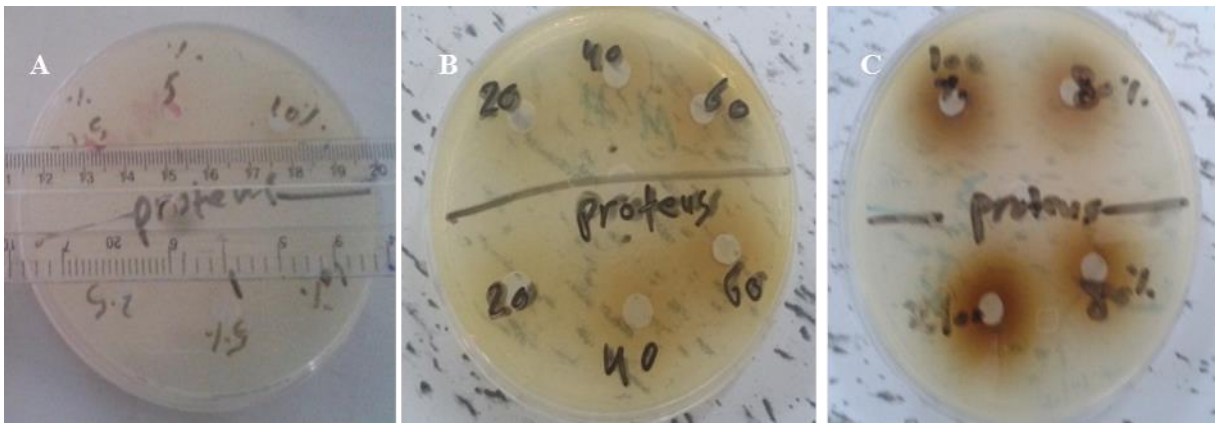

Fig. (3): Inhibitor zone of *N. Oleander* at [2.5%, 5%, 10% (A); 20%, 40%, 60% (B) and 80%, 100% (C)] concentration on *Proteus mirabilis* (Well diffusion method).

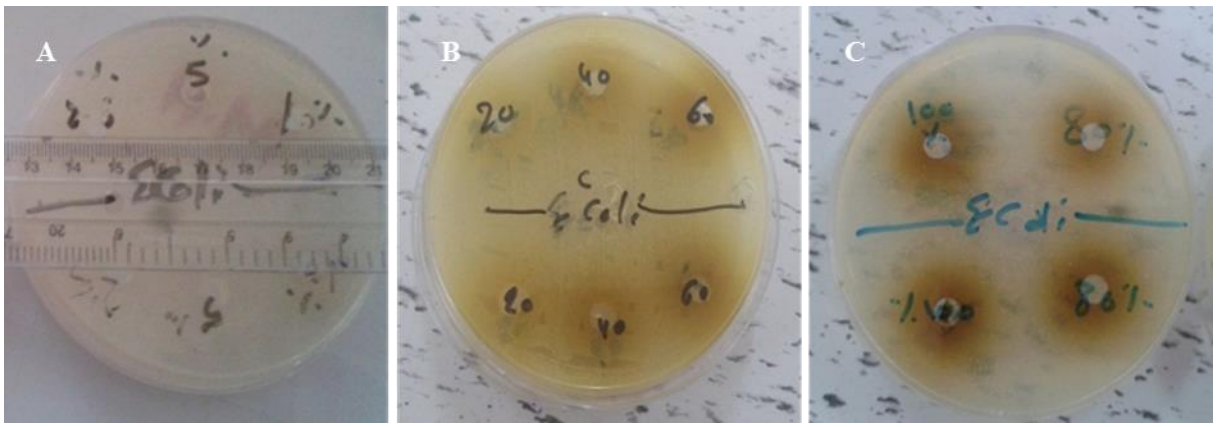

Fig. (4): Inhibitor zone of *N. Oleander* at [2.5%, 5%, 10% (A); 20%, 40%, 60% (B) and 80%, 100% (C)] concentration on *Escherichia coli* (Well diffusion method).

Table (4): Comparison between normal blood parameter and treated groups (M ± SD).

Test	Normal	Fucine ointment group		*N. oleander* 80% group		*N. oleander* 100% group	
		2-3days	7 days	2- 3days	7 days	2-3days	6 days
Hematocrit (HCT) %	36.5± 0.58	36.5 ±0.5	36.5 ± 0.5	35.75± 0.5	35.75± 0.5	35 ±0.816	35 ±0.816
Hemoglobin (Hb) g/dl	11.5± 0.3	11.25± 0.191	11.25± 0.191	11.25 ± 0.19	11.25 ± 0.19	10.9±0.346	10.9± 0.346
RBC (per/µL)	5750000± 182574.18	5501750± 816510	5501750± 816510	5504000± 128859.09	5504000± 128859.09	5528250 ±95834.49	5528250 ±95834.49
MCV (fl)	63.5±1.11	58.24± 14.47	58.24± 14.47	64.91± 1.92	64.91± 1.92	63.21±0.77	63.21± 0.77
MCH (pg)	20.005± 0.451	20.45± 0.760	20.45± 0.76	23.35± 5.41	23.35± 5.41	19.98±0.70	19.98± 0.70
MCHC (g/dL)	31.5025±0.342	31.46± 0.260	31.46± 0.26	31.46 ±0.26	31.46± 0.26	28.37±5.72	28.37± 5.72
WBC (per/µL)	6337.5± 137.68	7111.25±71.92*	6250± 61.73	7205± 145.2*	6362.5± 149.30	7230± 72.57*	6537.5± 149.30
Hertophils %	44.25± 107.78	49.75 ±4.787 *	43.75± 0.5	49.75 ±1.70 *	44.75 ±2.21	51±2.58*	43.5±1.29
Eosinophils%	5±0.82	5 ±0.618	4± 0.816	6.25±0.95*	5 ± 0.81	5.75±0.5*	5.25±0.95
Basophils %	2.5 ±0.58	2 ±0.816	2.5±0.577	2.75±0.95	2±0.81	2.75±0.95*	2± 0.81
Monocyte %	12.75± 0.956	9.75± 1.707	11.75± 0.95	11.75 ±1.70	13.75± 0.95*	11.75±0.95	11.75± 0.95
Lymphocyte%	35.5± 1.914	33.5± 3.415	38±0.816	29.5± 0.577	26.5±2.88	28.75±2.06	37.5 ±1.0

***significant difference at P<0.5**

The hematological change showed slight increase in total WBC at 2-3 day and return to normal range at 7 days after recovery stage with significant difference at (P<0.05). On the other hand slight decrease in hemoglobin concentration (Hb), erythrocyte count and MCHC (table 4). The aqueous extract of *N. oleander* at 80% and100% concentration able to healing of open wound at 2-3 days without complication and complete healing at 6-7 days and 5-6 days respectively compared with Fucine ointment (Fig. 8,9) (table 5).

Table (5): Comparison between dosage of treatment and period of healing.

Animal group	Treatment dosage	Period of healing
N. oleander	80%	6-7 days
N. oleander	100%	5-6 days*
Fucine ointment	20 mg	6-7 days

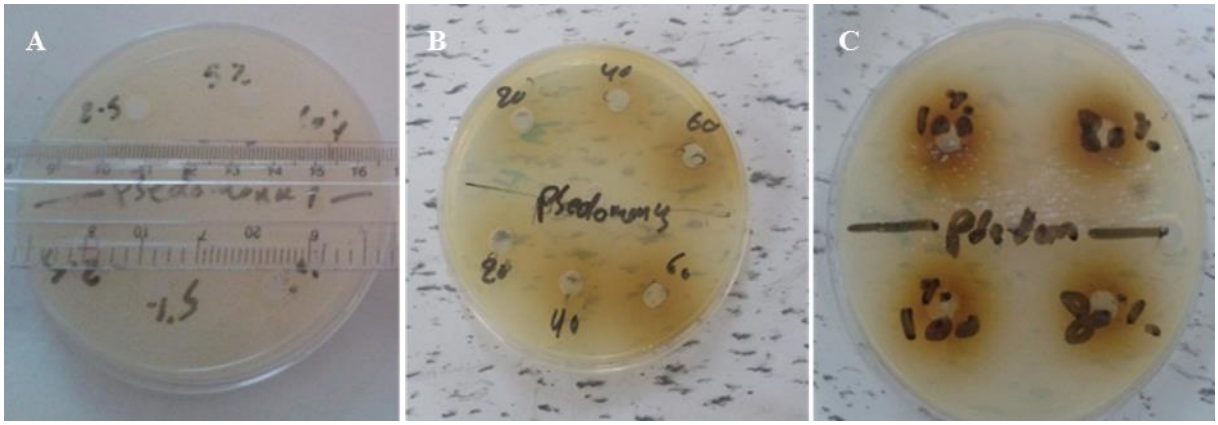

Fig. (5): Inhibitor zone of *N. Oleander* at [2.5%, 5%, 10% (A); 20%, 40%, 60% (B) and 80%, 100% (C)] concentration on *Pseudomonas aeruginosa* (Well diffusion method).

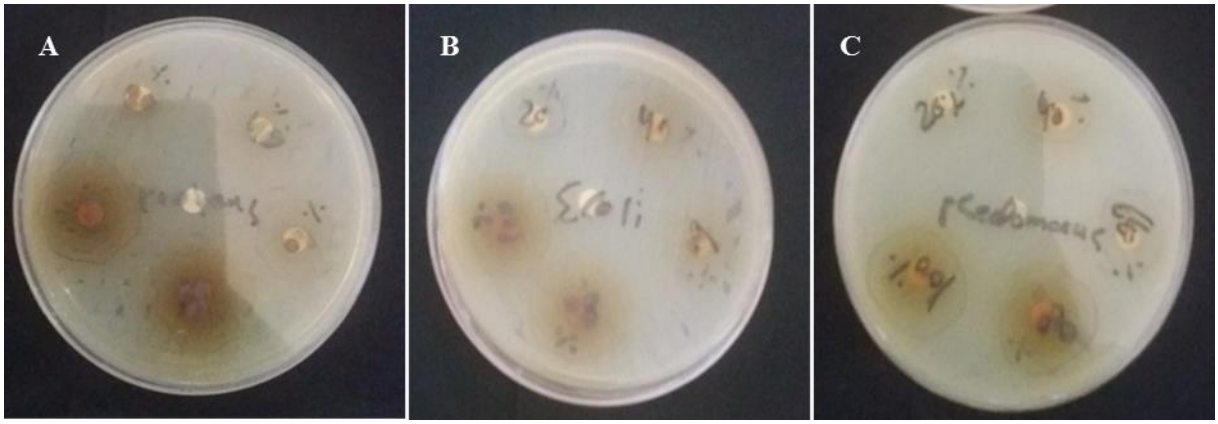

Fig. (6): **Inhibitor zone of *N. Oleander* (disc diffusion method) [(A) *Proteus mirabilis*; (B) *E. coli*; and (C) *Pseudomonas aeruginosa*.**

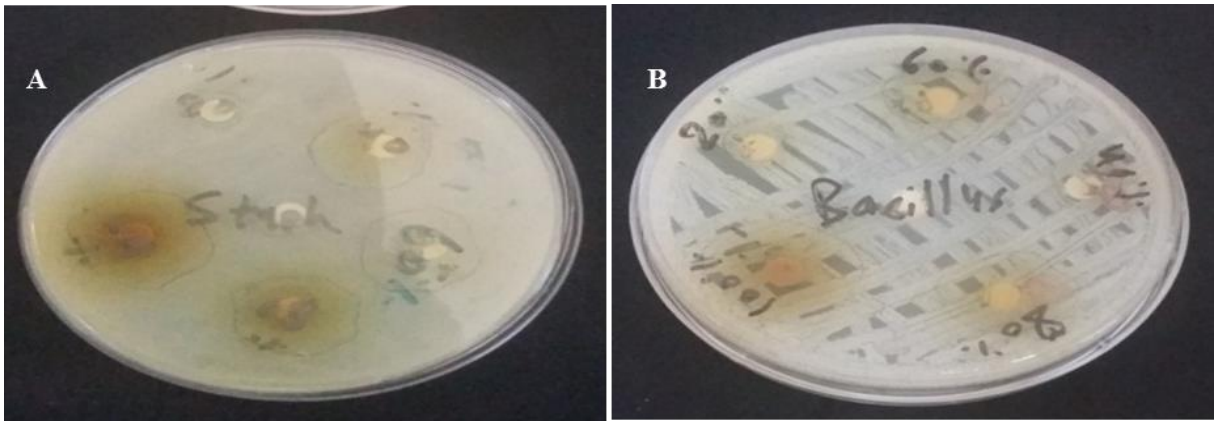

Fig. (7): **Inhibitor zone of *N. Oleander* (disc diffusion method) at [2.5%, 5%, 10%; 20%, 40%, 60% and 80%, 100% concentrations: (A) *Staphylococcus aureus*; (B) *Bacillus subtlis*.**

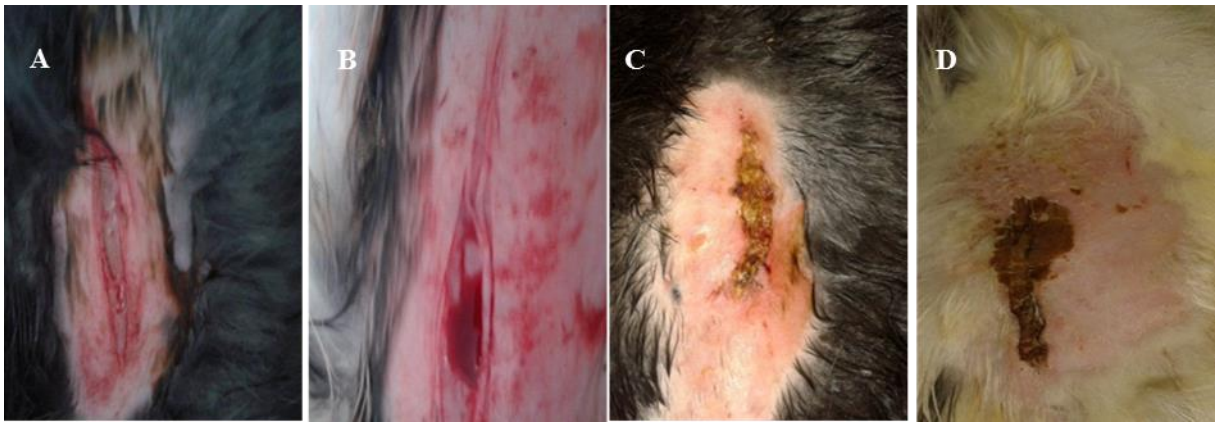

Fig (8): A, and B open wound without treatment (zero time); C: Fucine ointment after 2-3 days; D: *N. oleander* treatment 2-3 days without complications.

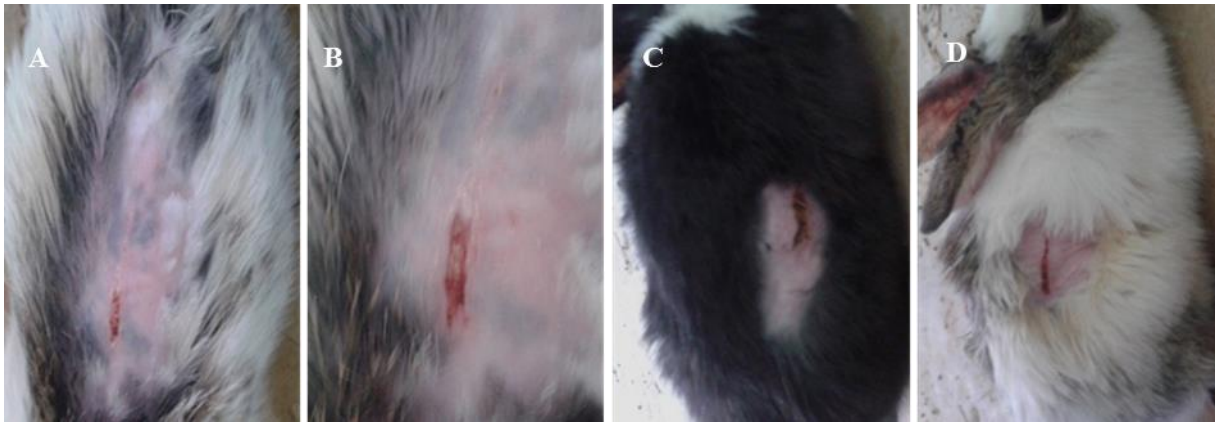

Fig. (9): A; wound treated with Fucine ointment after 4-5 days, B: treated with *N. oleander* after 4-5 days without complications, C and D; Complete healing of wound after 6-7 days use of *N. oleander* treatment 80-100% concentrations.

Discussion

The results in the present study indicated that extract showed antibacterial activity in all tested gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis* used in this study because presence active compound (Oleandrin which is toxic cardiac glycoside, tocopherol, ursolic acid) this finding agreement with other authors (20,21,22), and with (23) who mention the compound act as anticancer, anti-inflammatory and antimicrobial activity. Comparison between susceptibility of

bacteria spp in this study to different concentration of aqueous leaf extract of *Nerium oleander* showed that aqueous extract in agar well diffusion method exhibit a significantly higher zones of inhibitions against all Gram positive and Gram negative used bacteria at, 100%, 80%, 60% and 40% in comparison with 2.5%, 5%, 10% and 20%. *Staphylococcus aureus* and *Bacillus subtilis* showed remarkable growth inhibition against the extract which was in accordance with previous study of (24), which showed 13mm zone of inhibition with 25mg/disk concentration of *N. oleander* extract. In case of Gram negative bacterial strains used during this study only *E. coli* and *Proteus sp*. showed high inhibition zone at 80% and 100% compared with *Staphylococcus aureus* and *Bacillus subtilis*. This result disagreement with result of (23) who reported (*E. coli, P. aeruginosa* and *Salmonella spp*.) showed no inhibition zones against *N. oleander* extract. Whenever, present study agreement with (25).They reported that extract of roots of *Nerium oleander* showed high antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* while it have moderate activity against *Staphylococcus epidermis*. This difference in tolerance against *N. oleander* extract may also be attributed to the differences in the bacterial physiology and cell wall structure in Gram positive and negative bacterial strains and the method of extraction use for *Nerium oleander* (11). Comparison between normal blood parameter and treated groups showed slight difference in erythrocyte count and erythrocyte indices at 2 day compared with control this may be explained to bleeding at incision time and the erythrocyte indices return to normal value at recovery stage 6-7 days. On the other hand increase in total white blood cell count and neutrophils cell at 2 day may explained to stage of inflammation and role of neutrophils in healing of wound (24). Comparison between dosage of treatment and period of healing showed significant difference at $P<0.05$ between aqueous leaf extract of *N. oleander* and commercial Fucine ointment. This finding explained to role of oleanderine as anti-inflammatory, antibacterial and treatment of skin lesion, this agreement with other authors (3, 4). **In conclusion**; The results of the antibacterial activity of the study were in agreement with the findings of previous studies. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal health and provide biochemical tools for the study of infectious diseases. Aqueous leaf extracts of *N. oleander* have activity against both gram-positive and gram-negative bacteria this indicates of the presence of broad spectrum antimicrobial compounds. Leaf extracts of *N. oleander* can be the good candidates for further process of isolation, and character-ization of active compounds in the extracts. Leaf extracts of *N. oleander* at 80-100 % can be used as local antibacterial and treatment of open wounds.

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