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THE EFFECT OF CRUDE *ALOE VERA* LEAF GEL IN PROMOTING WOUND HEALING AND AS AN ANTIBACTERIAL AGENT

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ABSTRACT
This study explored the effect of crude Aloe vera leaf gel (CAVLG) in promoting wound healing and its antibacterial effect against some pathogenic bacteria in comparison with standard antimicrobial agents as Gentamycin and other medicinal plants as *Nigella sativa* oil. Standard dilutions of Aloe vera leaf gel were made from 10-100%; its antibacterial effect was examined by seeded agar method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Gentamycin and *Nigella sativa* oil were used for comparison. To explore the effect of Aloe vera in vivo, twenty four local male rabbits were used and divided into 4 equal groups. Each animal was wounded from both sides of the back region. The first group was control. The second group was treated with crude Aloe vera gel twice daily for 10 days. The third group was wounded and infected with *Staphylococcus aureus* without treatment to observe the natural body defense. The last group was infected with the same bacteria and treated with crude Aloe vera. Left side wounds in all groups were self-control. Statistical analysis showed a significant difference ($P<0.05$) in comparison with *Nigella sativa* oil. Also, the effect of Aloe vera gel against *S. aureus* was more potent than *P. aeruginosa*. The Minimum Inhibitory Concentration (MIC) was 60mg/ml and the Minimum Bactericidal Concentration (MBC) was eighty mg/ml. Finally, a significant decrease in wound diameter was noticed in the groups treated with Aloe vera gel in comparison with the non-treated control group. It can be concluded that this study is a good step to show that crude Aloe vera leaf gel promotes wound healing and has an antibacterial effect in vitro and in vivo against *S. aureus* and *P. aeruginosa*.

KEY WORDS: Crude Aloe vera leaf gel (CAVG), *Nigella sativa* oil (NSO), wound healing and antibacterial agent.

INTRODUCTION

Aloe vera is a succulent plant contains thick green leaves with an inner gelatinous substance. It belongs to the lily family, which has been used over the years for a variety of medicinal purposes. *Aloe vera* is also called the "Nature's Miracle" and has been associated with myth magic and medicine.[6] There are about 400 species of *Aloe vera* but only four have nutritional value. One of these valuable species is *Aloe vera* of which *Aloe barbadensis* Miller is the most potent type.[8] *Aloe vera* has many medicinal properties, including the ability to treat wounds and burns.[9] Researchers have shown that *Aloe vera* works without any toxic or allergic effect because its nutrient and water content acts as buffers. Therefore, the theory of synergistic relationship (All chemical and physical components of the plant work together to add up a greater benefit than the sum total of each item) is supported by history and science.[13] In 1982, several studies compared *Aloe vera* with Prednisolone and Indomethacin, (common anti-inflammatory drugs), and found it to be as effective as these drugs without any long term toxicity or any side effect.[1]The world Health Organization, mentioned that the gel was very potent in the treatment of radiation burns.[20] *Aloe vera* was also used in the treatment of second degree burns and healing wounds in diabetic mice. [7]Therefore, this study was designed to explore the antibacterial action and the ability of crude *Aloe vera* gel (CAVG) in promoting wound healing on rabbits and mice. Also, investigate the antibacterial action of (CAVG) in vitro and compare it with standard antimicrobial agents as gentamycin and other medicinal plants as *Nigella sativa* oil. Finally, evaluate the activity of the plant according to histopathology point of view.

MATERIALS & METHODS

Plant material

(CAVG) was collected from home gardens and classified to be *Aloe barbadensis* Miler by Prof.Dr. Ali Al-Musawy, plant classification specialist- University of Baghdad Herbal Center. Direct gel extraction was used.[22] Leaves where washed with warm distal water at a temperature of 40c° then cut longitudinally, later the gel was pealed from the parenchymal tissue and blended in an electrical blender for 15 seconds and then collected in a jar previously sterilized.

Preparation of Standard dilutions of (CAVG)

Dilutions were prepared through mixing the desired concentrations of the plant extract and complete them with nutrient broth. Final volumes were completed to 10ml.

p^H measurement:

The pH of Crude *Aloe vera* leaf gel was measured using pHmeter.

Bacterial culture

Bacterial stock cultures previously isolated from skin infections of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were prepared. Bacteria was activated , then each type of bacteria was transferred into four sterilized tubes containing brain agar infusion broth then incubated

for 24-72Hrs at 37°C. Total bacterial count was measured using spectrophotometer; the percentage of light transmittance was 26% at a wave length of 580 nanometer while the light transmittance was 100% for the nutrient broth used to prepare the bacteria.[12]

In vitro experiment

1- Identification of the experimental bacteria by studying the culture properties using mannitol salt agar to distinguish *Staphylococcus aureus* bacteria, while macConkey agar was used to distinguish *Pseudomonas aeruginosa*.[21] Also, the microscopic properties were studied using gram stains. At last, a number of biochemical tests were conducted including gelatinase test , oxidase test, catalase test and finally the pigment production test.

2- The antibacterial effect of (CAVG) was explored using sensitivity test for both (filtrated and nonfiltrated). This test was conducted through seeded agar method[1-11]. 0.6ml of the bacteria stock (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) was seeded in to 100ml of nutrient agar at 45°C in a final concentration of 10^6 CFU/ ml and poured into a depth of 4mm into sterile Petri-dishes. Then, three wells if 6mm diameter were made on the surface of each agar plate. Finally, these wells were filled with different concentrations of (CAVG) from 10-100%. These plates were incubated at 37 °C for 24 hours; the presence of zones of inhibition was regarded as the presence of antimicrobial activity. Results were determined by measuring the diameter of these inhibition zones with a ruler.[16] In addition, *Nigella sativa* oil (NSO) was use in comparison with (CAVG). Standard dilutions of (NSO) were prepared from 10-100% using standard ethylene glycol solution which is inactive against microorganisms and tested for antibacterial activity.[4] These very same methods were also used to measure the sensitivity against gentamycin to be compared with (CAVG).

3- The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of (CAVG) was determined.[2] Stock solutions for CAVG were diluted to concentrations of (2.5, 5, 10, 30, 40, 50 and 60)% with nutrient broth, and then 1ml of each concentration was placed in seven tubes with 1 ml of *Staphylococcus aureus* suspension in phosphate buffer saline. Tubes were completed to a volume of 5ml through adding nutrient broth, the plant extract final concentrations were (0.5, 1, 2, 6, 8, 10 and 20) % respectively. After manual shaking for few minutes, tubes were incubated for 18 hours at a temperature of 37°C. The growth of the bacteria was determined visually. The (MIC) was represented by the first clear tube while the (MBC) was that which when cultured on nutrient agar and incubated for 27hours at 37°C shows the absence of bacterial growth.[17-19]

In vivo experiments:

Determining concentrations of (CAVG) used in this study:

A pilot study was conducted under the same circumstances for the main experiment based on results obtained from the vitro study.

Experiment Animals

Experiment 1:

Twenty four adult male rabbits were used. Aged between six months to one year, obtained from the local market and placed in cages subjected to constant environmental conditions.

Experiment 2: Twelve adult male mice were used.

Infective Bacteria

Staphylococcus aureus was used for the *vivo* experiment. The same stock used for the vitro experiment was reactivated every 15 days.

Induction of wounds

Experiment 1

Surgical preparations were made on each side of the upper back region after clipping, shaving and washing the area with tap water and hen drying. Then, standard longitudinal incisions ($1x2cm^2$) were implemented using surgical scalpel, One side of the wound was directly infected with *Staphylococcus aureus* using a sterile cotton swab socked in the bacterial suspension (3 ml of bacteria in brain heart infusion broth + 3ml normal saline).

To verify the identity of the infective bacteria, swabs were taken from the wound area after 24 hours and cultured in mannitol salt agar.

Experiment 2

Mice were shaved from the back region, then two holes were induced (6 cm diameter/ 2cm depth) via a bone morrow puncture from both sides of the vertebral column.

Experiment 1

Twenty four adult male rabbits were used and divided into four equal groups. The effect of (CAVG) was evaluated visually and through histopatholigical changes.

First group: Was considered as control group, each rabbit was wounded and left without treatment.

Second group: Was considered as treatment group, rabbits were wounded and treated from one side with (CAVG) while the other side was considered as self control.

Third group: Was considered as infected group. Wounds were infected with *Staphylococcus aureus* as mentioned above.

Fourth group: Infected wounds were treated with (CAVG).

Histopathological Study

Specimens were taken from day one, two, four, five, six and twelve. Animals were anesthetized using (Xylazine and Ketamine) in a dose of 5mg/Kg and 15 mg/Kg respectively. Later, the specimens were kept in bounes solution and examined.

Experiment 2

This experiment was designed to backup experiment #1 in the ability of (CAVG) in promoting wound healing. The effect of the plant was determined by the diameter of the hole using Vernier® caliper on days one, four and seven.

First group: Considered as control group, was left without treatment.

Second group: Was treated with (CAVG).

RESULTS

In vitro experiment

Bacterial growth, Microscopic and biochemical results

The special culture media helped to distinguish between the two types of the experiment bacteria. Morphological

examination of the bacteria showed two types of cells, gram positive bacteria which were spherical in shape which resembles *Staph.aureus*, while the other type of cells were gram negative bacteria which were rod in shape.

The following tests were carried out oxidase test, gelatinase test, catalase test and pigment production test; the two isolates gave positive result for catalase and gelatinase. While, one isolate gave a negative result for oxidase with golden color and these properties resembled the general characteristics of *Staph. aureus*, the other isolate gave a positive result for oxidase test with green color, these properties resembled *Pseudo.aeruginosa.*

Sensitivity Test

Results showed that both (CAVG) and (NSO) had inhibitory effect on the bacteria. Non filtrated (CAVG) was the most potent. The sensitivity of the previously mentioned bacteria gradually increased with the increase of concentrations. Results were verified in the following tables and figures.

TABLE 1: In *vitro* activity of non-filtered (CAVG) against *Staph.aureus* measured by the diameter of inhibition zones against concentrations. Statistical analysis showed there was a significant difference between each concentration P<0.05.

Concentrations (%)	Diameters of inhibition zones (mm)			Mean ± SE
10	30	29	32	30.33±0.88
20	35	33	36	34.66±0.88
30	37	35	38	36.66±0.88
40	38	38	39	38.33±0.33
50	39	39	40	39.33±0.33
60	40	40	42	40.66±0.32
70	41	40	42	41.00±0.57
80	42	42	44	42.66±0.32
90	43	42	46	43.66±0.88
100	46	45	46	45.66±0.33

TABLE 2: In *vitro* activity of non-filtrated (CAVG) against *Pseudo. aeruginosa* measured by the diameter of inhibition zones against concentrations. Statistical analysis showed there was a significant difference between non filtrated (CAVG) and *Nigella sativa* oil P<0.05.

Concentrations (%)	Diameters of zones of inhibition			Mean ± SE
10	13	15	16	14.66±0.88
20	20	20	22	20.66±0.32
30	19	22	23	21.33±0.88
40	22	23	24	23.00±0.57
50	24	24	26	24.00±0.32
60	22	25	25	24.00±0.33
70	24	26	28	26.00±0.88
80	25	27	26	26.00±0.57
90	27	28	28	27.66±0.33
100	30	30	31	30.00±0.33

TABLE 3: In *vitro* activity of *Nigella sativa* oil against *Staph. aureus* measured by diameter of inhibition zones against concentrations. Statistical analysis showed there was a significant difference between each concentration P<0.05.

Concentration (%)	Diameters of zones of inhibition (mm)			Mean ± SE
10	9	8	8	8.33±0.33
20	10	9	9	9.33±0.33
30	11	13	12	11.33±0.32
40	13	13	14	13.33±0.32
50	15	15	16	15.33±0.32
60	16	17	17	16.66±0.32
70	18	20	19	18.33±0.33
80	20	23	21	20.33±0.32
90	21	22	23	22.33±0.32
100	22	24	25	24.33±0.32

TABLE 4: In *vitro* activity of *Nigella sativa* oil against *Pseudo. aeruginosa* measured by diameter of inhibition zones against concentrations. Statistical analysis showed there was a significant difference between each concentration P<0.05.

Concentration (%)	Diameters of zones of inhibition(mm)			Mean ± SE
10	7	7	8	7.33±0.33
20	8	9	9	9.33±0.32
30	9	10	10	10.33±0.32
40	12	12	13	12.33±0.32
50	12	13	14	13±0.57
60	14	14	15	14.33±0.32
70	16	17	17	16.66±0.33
80	19	19	18	18.66±0.33
90	20	21	21	20.66±0.33
100	22	22	23	22.33±0.33

TABLE 5: In *vitro* activity of Gentamycin (30µg) against the test bacteria

Test bacteria	Diameters of zones of inhibition			Mean ± SE
Staphreus.aureus	13	14	14	13.66±0.33
Pseudo.aeruginosa	14	15	15	14.33±0.33

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of (CAVG): The Minimal Inhibitory Concentration (MIC) was 60 mg/ml. while the Minimal Bactericidal Concentration (MBC) was 80 mg/ml.

pH of (CAVG): Results obtained from measuring pH of crude *Aloe vera* leaf gel was 4.5.

In vivo experiments:

Experiment 1:

Pilot study: Three concentrations of (CAVG) were used based on sensitivity test results 100%, 80% and 70%. Concentrations were administrated twice daily during treatment. Visual healing signs were noticed, and showed that (CAVG) in a concentration of 100% showed best results from day one.

Visual and histopathological observations

First group (Wounding without treatment): Day one: Within 24 hrs from wounding there was a clear appearance on inflammatory signs at the wound area, with swelling at the wound edges. Fibrous threads took place and new epidermal layer formed the marginal ends started to thicken. **Day two:-** Inflammatory signs existed including: redness, edema and an obvious swelling of wound edges. The most prominent type of cells in the wound area was neutrophils. There was also a clear migration of epidermal cell. **Day four and five:** Inflammatory signs still exist. The main histopathological feature was the appearance of inflammatory cells mainly macrophages. **Day six:** Inflammatory signs started to disappear gradually with obvious wound healing. Collagen fibers formation were organized and there was also thickening of epithelial layer from the marginal edges of the wound area and started to connect underneath the provisional matrix. **Day twelve:** There were no signs of inflammation. There was also an increase in collagen proliferation. **Day fourteent**h: Wound was completely sealed with scar formation. The junction of wound edges is tightly connected and there was an obvious increase in collagen.

Group two (wounding and treatment with (CAVG))

Day one and two:-Healing signs were very clear from day one. There were no inflammatory signs. There were a large number of macrophages, skin started to heal and the basal layer in epidermis was developed into vertical cells, there was an increase in collagen synthesis. **Day four and five:-**The complete disappearance of inflammatory signs. Collagen fibers formation was well organized and there was also thickening of the epithelial layer from the marginal edges of the wound area and started to connect underneath the provisional matrix. **Day six:-**Wound edges started to meet. There was an increase in collagen proliferation and formation of granulation tissue. Complete closure of wound was on day twelve.

Group three (wounding and infecting without treatment)

Day one:-Within the first 24 hours from infecting the wound there redness, edema and swelling of the edges were noticed; moreover there was a rise in temperature and the formation of clot in the wound. The most important feature was the migration of epidermal cells from the skin edges to damaged area (wound surface). Moreover, there was an increase in the number of white blood cells at the wound site (neutrophils). In addition, a significant number of clots at the wound area. Day two: There were redness and swelling of wound edges was very obvious with the appearance of exudates due to the infection with *Staphylococcus aureus*. There was thickening of the epidermal layer around the wound area. Also, the migration of the epidermal cells was backed up with multiplication forming a finger like projection, the main feature was the massive multiplication of lymphocytes also collagen production with the appearance of inflammatory cells mainly macrophages. Day four: Inflammatory signs were more obvious; wound area was filled with pus due to the infection with *Staphylococcus aurous.* There was more thickening of the epidermal layer around the wound area and the dermis was characterized by the development of granulation tissue. In addition there was a clear increase in the number of inflammatory and white blood cells, at the end of day four, the development and distinguishing of the granulation tissue which was the beginning of healing. Day five:-Inflammatory signs started to become less severe, and the swelling started to shrink. Skin started to heal and the basal layer in the epidermis was developed into vertical cells, also the upper corneum layer loosed their nucleic and formed a thick layer of necrotic cells. Day six:- Inflammatory signs started to

decrease, less pus, no swelling, and the main feature was the meeting of wound edges. The main feature was the presence of a projection developed from the regenerated epidermis and grows upward to be transformed later to hair follicles observed very clearly at day twelve. Day twelve: Absence of inflammatory signs. There was an increase in collagen proliferation. **Day fourteen:** There were complete closure of the wound with scar tissue, and there were no signs of inflammation. Junction between wound edges were very strong; also there was an increase in collagen which tended to orientate parallel to the skin surface and crossing the axis of the wound.

Group four (Infected wounds treated with (CAVG))

Day one and two: Healing signs were very clear; there were no inflammatory signs, no swelling, and no redness. Histopathological changes mostly were the same for the previous group except for the noticeable increase in collagen production and stimulation of macrophages and monocytes. **Day four and five:** Absence of any inflammatory signs, there was no swelling or any discharge and wound edges started to meet. There were vasodilatation and vessels permeability. **Day six:** Wound area was filled with granulation tissue and the wound was almost closed. There was a noticeable increase in collagen proliferation. The complete closure of wound occurred on day twelve and scar was less.

In vivo experiment 2:

Results showed that *Aloe vera* gel has the ability to improve wound healing in holes on both sides of vertebral column on mice shaved back. Table below shows the effect of tropical treatment in mice over a period of a week. Wounds in the treated group demonstrated a 4.14 ± 0.2 mm reduction in diameter of the induced holes, as compared with untreated wounds diameter reduction (2.13 ± 0.11 mm). This difference was significant at $p<0.05$. It was also observed that the untreated animals had generally appeared unclean wounds; however, the treated animals had clean wounds with a healthy granulation tissue.

TABLE 6: Topical effect of (CAVG) on wound healing on mice

	Decrease in wound diameter (mm)			Mean ±SE
	Day 1	Day 4	Day 7	
Untreated group	1.9	2.2	2.3	2.13±0.11
Treated group	3.8	4.2	4.5	4.14±0.2

DISCUSSION/ CONCLUSION

The gel before extraction was a clear transparent thick substance but when pealed from the parenchyma layer then blended became a thick semi liquid, viscous, pale green in color. Sensitivity test was carried out to determine the antibacterial effect of (CAVG) against some pathogenic bacteria. Seeded agar method was used successfully in determining the inhibitory effect of the gel. Results were used for comparison with standard antimicrobial agent as gentamycin and another plant extract as *Nigella sativa* oil. The purpose of using different concentrations was to obtain a broad image of the effect of different concentrations on the growth of pathogenic bacteria and the proportion between the increases in concentrations with the increase of size of zones of inhibition, which gave a positive ascending result (Tables 1,2). The antibacterial result may refer to the activity of certain concentrations to the nature of the active constituents of (CAVG) including salicylic acid, saponins and tannins.[18] In addition, results indicated that the gel in low concentration could give an opposite effect and become a nutrient medium for bacteria; this effect may refer to the high nutritional value of the plant.[6] Results of sensitivity test for filtrated AVG demonstrated no antibacterial effect and gave a nutrient effect for the test bacteria this result may refer to the presence of the active ingredients in the non filtrated components.This result indicated that the gel can give an inhibitory and nutrient effect on bacteria depending on its concentration. This result is a start to determine the therapeutic concentration of (CAVG). In the present study (CAVG) has possessed greatest inhibitory effect on *Staph.aureus*, this may be the cause of the popular use of (CAVG) in the treatment of many skin diseases as acne.[10] *Pseudo. aeruginosa* was more resistant to (CAVG) as it contains an outside capsule.[15] Histopathological and visual results indicated that (CAVG) supported wound healing by increasing collagen formation and deposition. Moreover, animals that had not received topical treatment had unclean, hard and crusty wounds. While treated wounds appeared clean with healthy granulation formation. Also, treated wounds had firmer tissue and the appearance of more vascularization. The proliferation and migration of epithelial cells depends on the oxygen supply. Therefore the increased presence of oxygen caused by (CAVG) improved wounds microcirculation, therefore helped in the wound healing process.[3] Crude *Aloe vera* gel contains substantial ingredients for wound healing as vitamin C, E and zinc.[6] These ingredients give an antioxidant effect and are necessary for collagen synthesis. It was found from this study that (CAVG) decreased wound healing time; all inflammatory signs disappeared from day one from treatment. Moreover, AVG acted as a moisturizer for the wounded skin so it can be suggested to use the gel in cosmetics. Table 6 depicts the effect of (CAVG) administrated topically on holes induced in both sides of the vertebra column and, was found it has the ability to improve wound healing compared to wound diameter for non treated control groups. From the forgiving results, it has been concluded that CAVG can be used in promoting and providing a better wound healing and can be used as an antimicrobial agent.

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