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Research Article

Biological Investigations of a New Natural Recipe Expected to Promote Healing of Superficial Burns

Nasef M[1], Kadry H A[2], Ashour M A[2], Nassar S L[2], Selim N M[2*], El-Raey M A[3]

[1]Department of Plastic and Burn Surgery, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Misr University for Science & Technology and Cairo University, Egypt.
[3]Department of Phytochemistry and Plant Systematic, National Research Centre, (NRC), Cairo, Egypt.

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ABSTRACT

Burns expose the deeper tissues of the skin or body to invasive microbes. Topical preparations for treating burn wounds, to be useful, should ideally have antibiotic power and promote healing by different mechanisms like their anti-inflammatory and antioxidant effects. Silver compounds have been the mainstay of topical burn treatment for decades. However, most chemical substances retard wound healing. Several natural agents such as honey and moist exposed burn ointment (β-sitosterol; MEBO ®) are believed to protect wounds from infection and promote healing of burns without causing any of the adverse effects of purified chemicals. In this study, we investigated the antimicrobial, anti-inflammatory and antioxidant characteristics of a new natural recipe expected to promote healing of superficial burns and also determined the safety of its topical pharmaceutical use. Moreover, we compared the healing properties of the recipe with the commercial drugs; silver sulphadiazine (SSD; Dermazin ®); a long-standing conventional burn dressing, and also with (β-sitosterol; MEBO ®); a herbal preparation of Chinese origin widely used in Asia and the Middle East. Biological investigations and trials had been made and conducted on experimental animals that were obtained from the animal house colony of National Research Centre, Dokki, Cairo, Egypt. All animals were kept on a standard laboratory diet and under the same hygienic and healthy conditions. They were acclimatized for two weeks in the laboratory in equal light and darkness periodicity, with liberal access to food and water. Animals were housed in separate hygienically maintained cages. For induction of superficial burns, one hundred and twenty adult male Murine albino rats weighing 130-150 g. were divided into four groups; each group consisted of 30 animals. They were housed in separate cages and received partial-thickness burn wounds on their dorsal skin in accordance to the Modified Murine model of partial thickness scald burn injury (A Standardized Model of Partial Thickness Scald Burns in Rats). Animals within each group were numbered then treated three times a day (t.i.d) with our new natural recipe, two times a day with (SSD; Dermazin ®), three times a day with (β-sitosterol; MEBO ®) in addition to three times a day treatment with a placebo plain gel (negative control). The burn wounds were visually inspected daily until day 14. It was found that animals in the first three groups were well preserved. No clinical infections occurred. Wound healing was at an advanced stage by the day 14 in all animals except the placebo group. Clinical examination showed that the three agents gave comparable protection and healing possibilities for the experimental animals. It is concluded that our new natural recipe is a suitable and efficacious alternative to conventional silver-based topical therapies and MEBO ® for treating partial-thickness burn wounds.

Keywords: Natural treatment of burns; Superficial burns; *Calendula officinalis*; *Cleome droserifolia*; Natural recipes.

INTRODUCTION

Burns are one of the most devastating conditions encountered in medicine. The injury represents an assault on all aspects of the patient, from the physical to the psychological. It affects all ages, from babies to elderly people, and is a problem in both the developed and developing world. All of us have experienced the severe pain that even a small burn can bring. However the pain and distress caused by a large burn are not limited to the immediate event. The visible physical and the invisible psychological scars are long lasting and often lead to chronic disability[1]. Burn injuries represent a diverse and varied challenge to medical and paramedical staff. Correct management requires a skilled multidisciplinary approach that addresses all the problems facing a burn patient[2]. Accidents resulting in serious burn injuries can have devastating consequences for the injured person.

Depending upon the severity of the burn, victims may require extended hospital care, numerous surgeries, and prolonged physical therapy to regain optimum function. Moreover, burn injuries can have enormous psychological, emotional and even economical consequences for the victim and their family culminating in social stigma and restriction in participation in society. Therefore, those individuals sustaining severe burn injury should seek the best medical care possible[3]. A burn wound is a skin injury

**Author for Correspondence: dr.mahmoudnasef@yahoo.com*

Figure 1: *C. officinalis* flowers (X=0.25).

Figure 2: *C. droserifolia* herb (X=0.25).

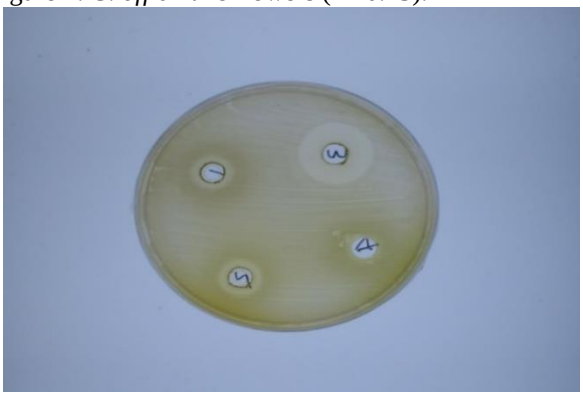

Figure 3: Antimicrobial sensitivity testing.

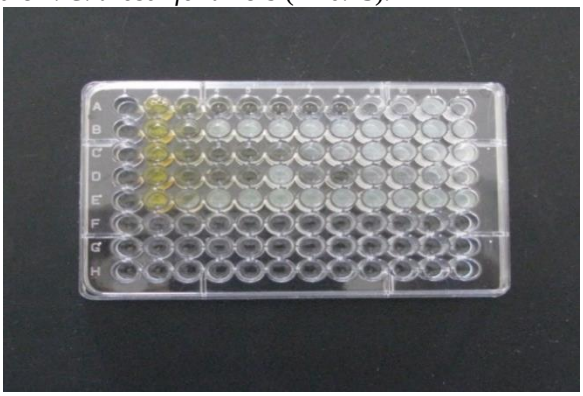

Figure 4: Microtitre broth dilution method.

Table 1: Results of LD_{50} of ethanol (70%) extract of *C. officinalis* flowers.

Group	Number of animals	Dose (mg/kg b.wt)	Number of Dead animals	z	d	Σ (z × d)
1	6	3708	0	0	0	0
2	6	4944	1	0.5	1236	618
3	6	6180	2	1.5	1236	1854
4	6	7416	5	3.5	1236	4326
5	6	8652	6	5.5	1236	6798
total						13596

Z= Mean of dead animals between 2 successive groups.
d= Difference between 2 successive doses.

Table 2: Results of LD_{50} of ethanol (70%) extract of *C. droserifolia* herb.

Group	Number of animals	Dose (mg/kg b. wt)	Number of Dead animals	z	d	Σ (z × d)
1	6	11124	0	0	0	0
2	6	14832	1	0.5	3708	1854
3	6	18540	3	2	3708	7416
4	6	22248	4	3.5	3708	12978
5	6	25956	6	5.5	3708	20394
total						42642

LD_{50} = 25956 – (42642) / 6 = 18849 mg/kg body weight. = 18.849 g/kg body weight.

Table 3: Results of antimicrobial sensitivity [inhibition zones diameters (mm)] caused by the dried ethanol (70%) extract of *C. officinalis* flowers represented by extract (A), and *C. droserifolia* herb represented by extract (B), versus standard antibiotic; using different standard bacterial species.

Bacterial species	*Staph. Aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*K. pneumonia* ATCC 700603	*Salmonella typhi*	*Shigela flexneri*
Plant extract	Inhibition zone (mm)					
A (10 mg)	9	10	9	10	11	9
B (10 mg)	14	15	13	14	17	16
Ciprofloxacin (5µg)	35	30	22	21	20	22

occurs when there is a direct or indirect contact between skin tissue & an energy sources such as heat (thermal), chemicals (e.g. Acids & alkalis), electrical current and radiation. According to the depth of the burn wounds; it may be superficial like sun burns, partial thickness which may be either superficial or deep and full thickness burns[2]. Burns expose the deeper tissues of the skin or body to invasive microbes. Topical preparations for treating burn wounds, to be useful, should ideally have antibiotic power and promote healing[4]. Devitalized materials in burned tissue serve as a nidus for bacterial proliferation. The delivery of systemically applied antibiotics is impaired by the occlusion of vessels in adjoining areas. Topical treatment of burn wounds is therefore of great importance, both in preventing death from invasive infection and in determining other treatment outcomes, such as the occurrence of scar hypertrophy and keloids[5,6]. Since the 1960s, silver compounds have been the mainstay of conventional topical burn treatment for decades. Over a period of many years it has come to be a standard against which non-silver therapies are evaluated. The main advantage of silver compounds is their lethal power against a wide range of microbes, including even fungi. However, most chemical substances retard wound healing[7-9]. On the other hand, bioactive or naturally occurring principles isolated from different plant material are of fewer side effects and are less hazardous; therefore appear to be one of the few available commercial alternatives for the treatment of burns. Several natural agents such as honey and moist exposed burn ointment (MEBO) ® are believed to protect wounds from infection and promote healing without causing any of the adverse effects of purified chemicals. (MEBO)® is an oil-based, natural preparation of Chinese origin widely used in Asia and the Middle East. It contains berberine oil and beta-sitosterol; a plant steroid. Oil soothes wounds; retain moisture, and relieve pain, while β-sitosterol promotes epithelialization[10-12]. The role of naturally occurring compounds in the treatment of various diseases related to the human kind can be of prime importance. Therefore, the bio-prospection and investigation of naturally occurring products having the ability to provide treatment and protection against some diseases are now strongly recommended[13]. Plant material namely; *Calendula officinalis* Linn. flowers (fig 1) and *Cleome droserifolia* (Forssk.) Del. herb (fig 2) growing in Egypt were chosen to serve as sources of various natural products, including poly-phenolic compounds, sterols, and triterpenes. Species of these plants were used in folk medicine for the treatment of several ailments e.g. skin diseases, sores, ulcers, wounds and several inflammatory conditions[13,15,16]. Flavonoids are poly-phenolic compounds showing several biological activities, such as anti-microbial and anti-oxidant activities which are explained by several mechanisms and may be responsible for the biological activities of the two plants under investigation[18]. There are some endogenous drugs used in folk medicine either as single or combined forms for treating different types of ailments with considerable success. Although the use of these drugs has a sound tradition, and their medicinal uses and general safety are well known to native peoples, their place has to be rationalized in modern therapy using the current methodology. Scientific studies have therefore, conducted to verify their safety and to judge their efficacy[17]. In this study, the bioactive compounds of the air dried and finely grinded *C. officinalis* flowers and *C. droserifolia.* herb were totally extracted by 70 % ethanol and examined for their anti-microbial, anti-inflammatory, anti-oxidant activities and subjected to a biological study aiming at finding out their healing effects on superficial burns and inflammatory conditions. The alcoholic plant extractives were formulated in a recipe using topical pharmaceutical base (gel). The role of this natural recipe in the enhancement of healing and treatment of superficial burns had been examined and biologically assessed on experimental animals (biological trials).

MATERIALS AND METHODS

Extraction of active constituents

Extraction of the active ingredients of each plant material was done by continuous maceration of the air-dried powdered plant in 70% ethyl alcohol till exhaustion, followed by filtration and water bath drying of the filtrate using a Heidolph Hei-VAP Series German rotatory evaporator.

Determination of LD_{50}

As a starting point for drug manufacturing, we must evaluate and prove the safety of the topical pharmaceutical use of the ethanol (70%) extracts of *C. officinalis* flowers and *C. droserifolia* herb by the determination of the LD_{50} (lethal dose which kills 50% of experimental animals). Thirty male Murine albino mice weighing 20-25 g. were used for this purpose, according to Karber's procedure[18]. The mice were divided into five groups each of six animals. Several doses were taken orally for each group. All groups of animals were observed for 24 hours, during which symptoms of toxicity and mortality rates in each group were recorded and the LD_{50} was calculated.

Investigation of the in vitro antimicrobial activity

The dried alcohol extract of each plant material was subjected to the following microbiological studies;

A- Antimicrobial sensitivity testing

Antibiotic susceptibility assay was performed in accordance to modified Kirby –Bauer disc diffusion technique[19]. A stock solution of each dried alcoholic plant extract was prepared by dissolving 100 mg plant extract in 1 ml of DMSO; Dimethyl Sulfoxide (Non antimicrobial solvent, used as a dissolution solvent). The standard antimicrobial disc and the filter paper discs saturated with 0.1 ml of each plant extract were placed on the agar surface of the inoculated plates containing the tested organism, using a sterile forceps. Each disc was gently pressed down to ensure even contact with the medium. The plates were placed in an incubator at 35°C within 30 minutes of preparation. After overnight (24 hours) incubation, the diameter of each inhibition zone (including the diameter of the disc) were measured and recorded in mm with a ruler on the under-surface of the plate without opening the lid (Fig. 3). The results were interpreted (Table 3) according to the critical diameters established by NCCLS (2002).

Table 4: Results of MIC and MBC values of *C. officinalis* flowers represented by extract (A), and *C. droserifolia* herb represented by extract (B), against standard antibiotic; using different standard bacterial species.

Bacterial species/ Antimicrobial agents	*Staph. Aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 700603	*Salmonella typhimurium*	*Shigela. flexneri*
Plant extract A						
MIC (µg/ml)	2048	512	512	2048	256	2048
MBC (µg/ml)	2048	1024	1024	2048	1024	2048
Plant extract B						
MIC (µg/ml)	256	>256	32	>256	32	128
MBC (µg/ml)	>256	>256	32	>256	256	128
Ciprofloxacin						
MIC (µg/ml)	≤0.09	≤0.09	≤0.09	≤0.09	≤0.09	≤0.09
MBC (µg/ml)	≤0.09	≤0.09	≤0.09	≤0.09	≤0.09	≤0.09

Table 5: Results of acute anti-inflammatory activities.

Edema	Control	Indomethacin	*Calendula* alc. ext.	*Cleome* alc. ext.
(1 h.) Edema rate %	73.11 ± 6.33	29.31± 4.21**	57.51 ± 4.11	49.30 ± 4.22
(1 h.) Edema inhibition%	----------	- 48.53 %	- 15.55 %	- 27.63 %
(2 hrs.) Edema rate %	80.76 ± 4.55	31.78 ± 3.99**	60.52 ± 5.42	45.63 ± 6.15**
(2 hrs.) Edema inhibition%	----------	- 51.36 %	- 18.33 %	- 39.50 %
(3 hrs.) Edema rate %	80.41 ± 7.31	31.21 ± 4.82**	62.70 ± 9.28	45.88 ± 3.90*
(3 hrs.) Edema inhibition%	----------	- 51.88 %	- 16.48 %	- 38.90 %
(4 hrs.) Edema rate %	71.46 ± 5.68	26.93 ± 3.67**	51.78 ± 3.93	42.74 ± 4.41*
(4 hrs.) Edema inhibition%	----------	- 52.60 %	- 19.11 %	- 35.60 %

Values represent the mean ± Standard Error (S.E.) of six animals for each group.
* P<0.05: Statistically significant from the corresponding control using oneway ANOVA (Dunnett's as post hoc. test).
** P<0.001: Statistically significant from the corresponding control using oneway ANOVA (Dunnett's as post hoc. test).

*B - Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)***;** **(**Table 4) by microtitre broth dilution method[20]. (fig. 4)

Investigation of the in vitro anti-inflammatory activity

For this purpose, 36 adult male Murine albino rats of Sprague Dawely Strain weighing 150-180 g. were divided into 6 groups (each contains 6 rats) were used, according to the method established by Winter C.A. *et al*[21]. Edema was induced in the left hind paw of all rats by subcutaneous (S.C) injection of 0.1 ml of 1% (w/v) carrageenan in distilled water (solvent) into their footpads. Plethysmometer was used to measure the paw volume of each rat before carrageenan injection and then hourly for 4 hours post administration of the plant extracts. Groups were classified into;

1st group (Control group)

was orally given the respective volume of the solvent (few drops of tween-80 in distilled water).

2nd and 3rd groups

were orally administered the 70% ethanol extract of *C. officinalis* flowers in doses of 0.25 and 0.5 g/kg body weight respectively, each dose was given 1 hour before carrageenan injection.

4th and 5th groups

were orally given the 70% ethanol extract of *C. droserifolia* herb in doses of 0.25 and 0.5 g/kg body weight, respectively; 1 hour before carrageenan injection.

6th group (Reference group)

was orally administered indomethacin (Indocid®) in a dose of 10 mg/ kg body weight. The rate of edema formation and inhibition for each group were calculated as follows:

Edema formation rate (E) % = $(V_t - V_o / V_o) \times 100$ Edema Inhibition rate (I) % = $(E_c - E_t / E_c) \times 100$ Where;

V_o is the volume before carrageenan injection (ml).

V_t is the volume at t hour after carrageenan injection (ml).

E_c is the edema rate of control group.

E_t is the edema rate of treated group.

Investigation of the in vitro antioxidant activity

The *in vitro* anti-oxidant activity (Free radical-scavenging ability) of the alcohol extract of each plant material under investigation was performed by the use of a stable DPPH radical. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicalscavenging activity was determined using the method proposed by *(Yen Gow-Chin and Chen Hui-Yin., 1995)*[22]. standard antioxidant (ascorbic acid, 1 mg/ml) was used for comparison as positive control. IC_{50} value was calculated using the dose inhibition curve. All determinations were performed in triplicate.*Pharmaceutical formulation*After we had proven the safety, antimicrobial, anti-inflammatory and the antioxidant activities of the 70% alcohol extract of each plant material under the investigation, we prepared a natural recipe in the form of a topical pharmaceutical dosage form, by overnight freeze drying of the alcoholic plant extractives at lyophilization conditions as follows; Temperature (-50 °C), pressure (133 x 10^{-3} m BAR) and time (24 hours) to yield fluffy spongy powders, which were then incorporated into a topical pharmaceutical base

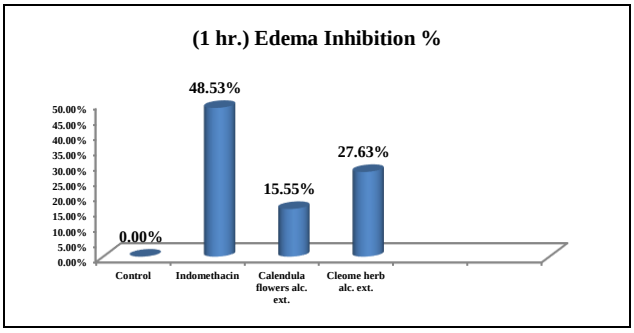

Figure 5: % Edema inhibition after (1 hr) by different samples versus negative control and Indomethacin (Indocid®) as a standard anti-inflammatory.

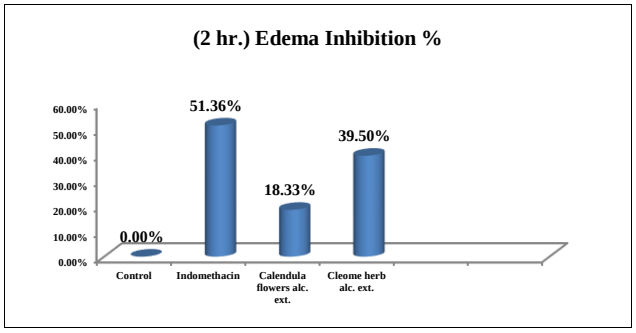

Figure 6: % Edema inhibition after (2 hr) by different samples versus negative control and Indomethacin (Indocid®) as a standard anti-inflammatory.

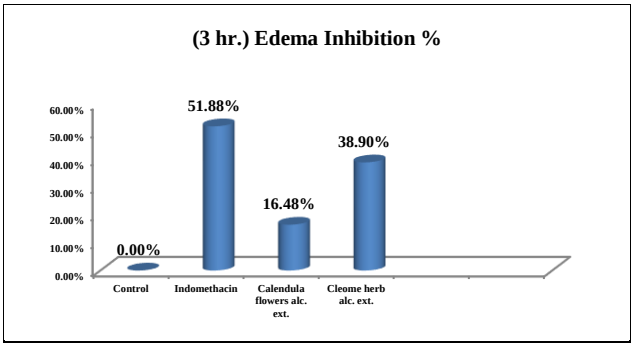

Figure 7: % Edema inhibition after (3 hr) by different samples versus negative control and Indomethacin (Indocid®) as a standard anti-inflammatory.

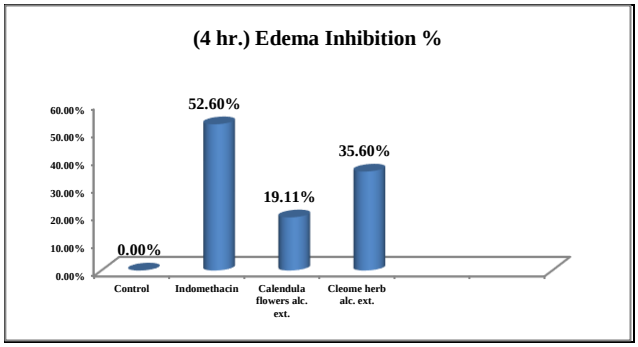

Figure 8: % Edema inhibition after (4 hr) by different samples versus negative control and Indomethacin (Indocid®) as a standard anti-inflammatory.

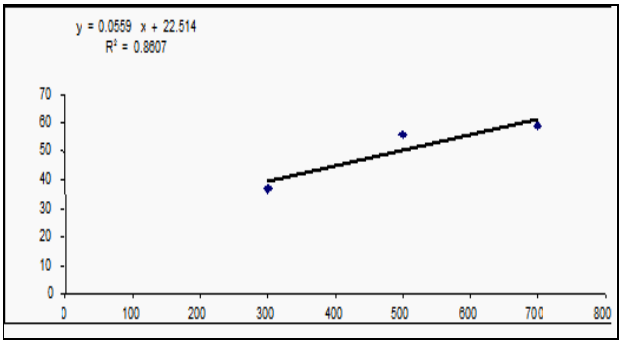

Figure 9: Anti-oxidant activity of *C. officinalis* Linn. flowers ethanol (70%) extract.

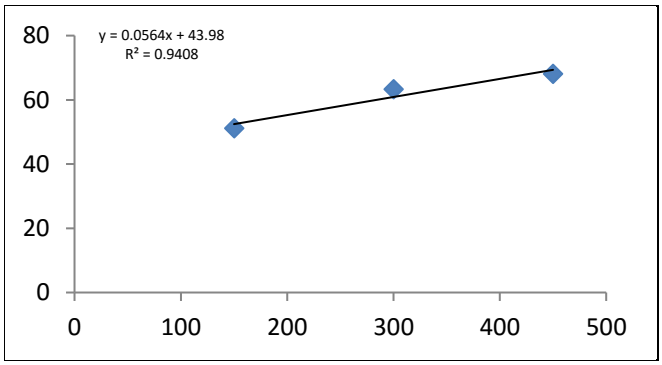

Figure 10: Anti-oxidant activity of *C. droserifolia* herb ethanol (70%) extract.

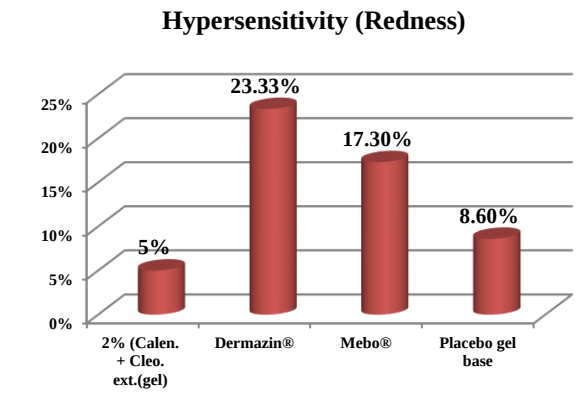

Figure 11: Average results of the pre-healing hypersensitivity.

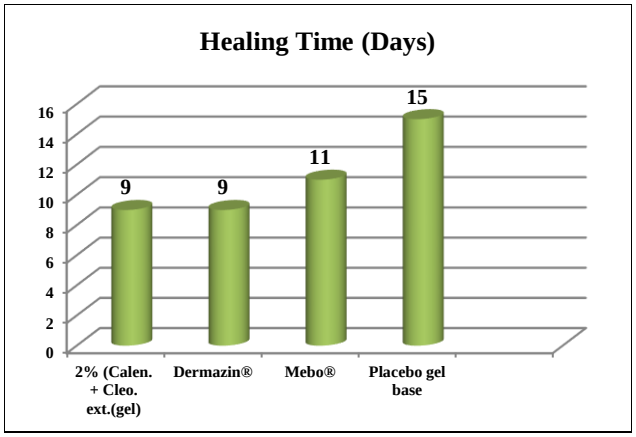

Figure 12: Average results of the healing time.

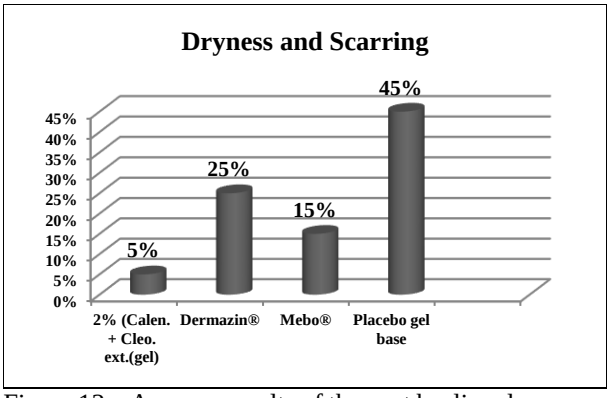

Figure 12a: Average results of the post healing dryness and scarring.

(gel) by the use of Polyethylene glycols (PEGs) gel base, following the standard pharmaceutical formulation procedures suitable for compounding plant extractives[13]. A ratio of 40:50 % (w/w) of PEG 1500: PEG 400 was selected to give the best *in vitro* medication release rate. PEG 1500 was placed into a porcelain dish over water bath adjusted at (70 °C) till melting, and then PEG 400 was added with continuous stirring by a glass rod. The drug in its desired concentration (2% w/w) was mixed with the molten base, distilled water was added to 100% w/w and the mixture was stirred till it congealed at room temperature to give the topical natural recipe (gel) (fig. 13).

Induction of burn wounds

Induction of superficial burns was done in accordance to the Modified Murine model of partial thickness scald (boiling fluid) burn injury (A Standardized Model of Partial Thickness Scald Burns in Rats); a simple protocol which provides a standardized thermal wound with consistent depth of injury, low mortality, and complete wound re-epithelialization by proper topical treatment was seen within two weeks post-burn[23]. Murine models (fig 15) refer to rats or mice from the Murinae families that are used in experiments like drug testing and development (fig. 14). They are used because they have similar characteristics to humans. The model is typically studied to get ideas which may be transferred to situations relating to humans. Briefly, 120 adult male albino rats of Sprague Dawely Strain weighing 130-150 g. were secured in a burn template allowing exposure to a homemade scald-producing apparatus (50 ml plastic syringe). Continuous water (60°C) stream emission (approximately 0.5-1 ml / second) was used to create scald injury on an area of a 2 x 3 cm of the shaved dorsum (back area) in male rats for 45 seconds (proper scalding time for the experiment), followed by 4°C water application for 45 seconds. The nozzle of the syringe was aligned perpendicularly to the back of mice, 2 cm above the skin surface. Previous small mammals (mice) models of partial thickness burn injury have failed to demonstrate complete re-epithelialization in less than three weeks after scald induction. Reference commercial drugs were used as positive controls for the treatment of induced scald burns. They were SSD; Silver Sulphadiazine (Dermazin) ® (made by Sandoz Co.) and β-sitosterol (Mebo) ® (made by Gulf Pharmaceutical Industries; Julfar Co. UAE). Both drugs came from a donated stock.

Treatment with topical agents

Animals were divided into four groups of thirty rats each.

1st Group: animals were treated with our natural recipe only, every 8 hours. (fig. 16)

2nd Group: animals were treated with SSD only, every 12 hours. (fig. 18)

3rd Group: animals were treated with MEBO only, every 8 hours. (fig. 17)

4th Group: animals were treated with plain gel of Polyethylene glycol PEG gel base (placebo) only, every 8 hours.

All burn wounds were exposed to air and visually inspected daily for evidence of clinical infection.

Investigation parameters for the biological trials

The gross condition of burn wounds was visually inspected by the naked eyes immediately after injury, also during treatment and after healing; in order to determine the proper required time and condition of healthy healing (without or with minimal local complications) for the experiment. Investigation parameters for evaluation of biological activity of the prepared natural recipes were chosen to provide pre and post-healing assessment of healthy (non-complicated) healing.

Three investigation parameters for evaluation of superficial burn healing had been chosen, they were;

1- Hyper-sensitivity (Pre-healing redness). (fig. 19)

2- Average healing time in days (Healing power).

3- Dryness & scar formation (Post-healing complication). (fig. 20)

RESULTS

1- Results of determination of the (LD_{50})

A- For ethanol (70%) extract of C. officinalis flowers

LD_{50} (g/kg body weight) = the largest dose; (which kills all experimental animals) - $\Sigma (z \times d) / n$

LD_{50} = 8652 – (13596) / 6 = 6386 mg/kg body weight. = 6.386 g/kg body weight.*B- For ethanol (70%) extract of C. droserifolia herb***.**

2- Results of the in vitro antimicrobial activity:

A- Antimicrobial sensitivity of the ethanol (70%) plant extracts

B- Determination of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC) by microtitre broth dilution method.

Results are shown in table (4).

3- Results of the in vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activity of the ethanol (70%) extracts of both plants under the study versus Indomethacin (Indocid®) had been investigated. From the statistical point of view, there was significant suppression of the inflammatory process as indicated by ANOVA ($P<0.001$) at tested dose (0.25-0.5 g./Kg) of the alcohol extract of *C. droserifolia* herb when compared with the control (tween-80 in distilled water), and by ANOVA ($p<0.05$) at tested dose of (0.25-0.5 g./Kg) of the alcohol extract of *C. officinalis* flowers when compared with the control (tween-80 in distilled Water).

4- Results of the in vitro antioxidant activity

Figure 13: Natural recipe (gel) expected to promote healing of superficial burns.

Figure 14: Murine model rat.

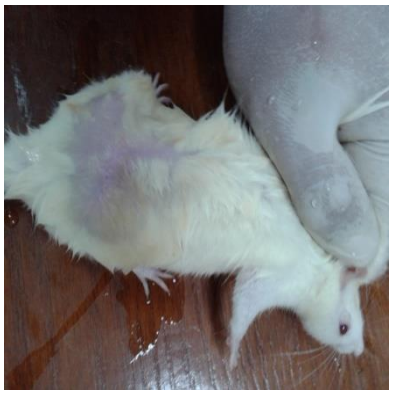

Figure 15: Superficial (scald) burn induction.

Figure 16: Treatment with the natural recipe (gel).

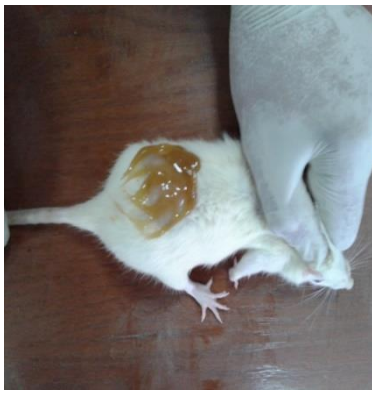

Figure 17: Treatment with Mebo® (ointment).

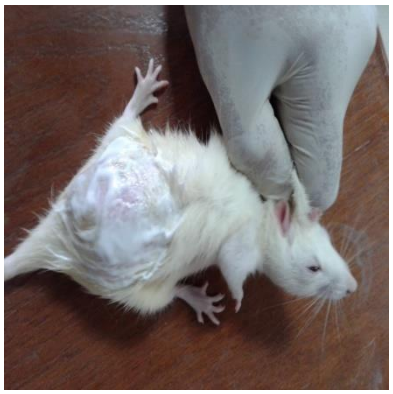

Figure 18: Treatment with Dermazin® (cream).

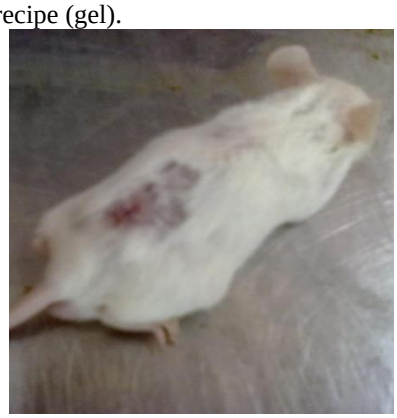

Figure 19: Hypersensitivity (pre-healing complication).

Figure 20: Dryness / scarring (post healing complication).

Figure 21: Shaved dorsum showing healthy healing of superficial burn by the natural recipe.

A- Results of the in vitro anti-oxidant activity of C. officinalis flowers.

DISCUSSION AND CONCLUSION

The sample showed antioxidant activity with IC_{50} = 492 µg/ml, while the IC_{50} value of the reference standard ascorbic acid was 58.92 µg/ml.

B- Results of the in vitro anti-oxidant activity of C. droserifolia herb. The sample showed antioxidant activity with IC_{50} = 107.5 µg/ml, while the IC_{50} value of the reference standard ascorbic acid was 58.92 µg/ml.*5- Results of the biological trials (superficial burn induction)*

The animals were generally well preserved and active, feeding within 2 hours of the procedure. No clinical infection was recorded except in the placebo group. By day 3, there was a hard dark-brown eschar over the burned areas. By day 8, the eschar had spontaneously detached, leaving pink tender skin in all groups except the placebo one.

The aim of work is to promote healthy healing of superficial burn wounds by the use of a new natural recipe (based on the available data from the literature review), so as to avoid progress of the superficial burn wounds into deep ones with serious general and local complications. Burn wounds, even of partial depth, can present challenging clinical problems if the surface involved is sufficiently extensive. Excision and early wound closure

Table 6: Results of the anti-oxidant activity of the ethanol (70%) extract of *C. officinalis* flowers.

Sample Conc. (µg)	300	500	700
DPPH scavenging %	36.68	55.64	59.03

Table 7: Results of the anti-oxidant activity of the ethanol (70%) extract of *C. droserifolia* herb.

Sample Conc. (µg)	150	300	450
DPPH scavenging %	51.21	63.34	68.12

are the recommended treatment at the present time. However, topical treatment is required in most burn patients with superficial burns. It is also needed in deep burns pending more definitive closure modalities because it plays a vital role in preventing microbial invasion[24]. The strategy of treatment of burns depends on the depth, area and location of the burn wound. For healthy healing (with minimal or without complications), agent(s) for initial treatment of superficial burns require(s) all of the following properties;

- Antimicrobial property[25].
- Anti-inflammatory property[26].
- Anti-oxidant property[15].
- Analgesic property or pain relieving power[27].

The results of determination of the mean lethal dose (LD_{50}) proved that both plants comprising our new natural recipe were safe for even internal use in animals and might be safe for topical application in humans. The results of biological investigations of our new natural recipe were very promising for the treatment of superficial burns and the results of the biological trials for induced superficial burns on experimental animals showed that the three applied treatments produced comparable healing effects. The dried alcohol extracts of both plants under investigations showed pronounced antimicrobial activity at tested doses (Table 3); enhanced inhibition zones were observed for *C. droserifolia* herb extract. The inhibition zones of the plant extracts were compared to the inhibition zones of the standard antibiotic disc; ciprofloxacin disc (5µg) against the same bacterial species. Both plant material under investigation having the potential to be used as antimicrobial agents. *C. droserifolia* herb dried alcohol extract showed pronounced antibacterial activity, as expected from inhibition zones values; it also showed promising MIC values with all tested bacterial species. The Results of the anti-inflammatory activity showed that alcohol extracts of both plants under investigation had anti-inflammatory activity and could be used for this purpose at tested doses. However, they exhibited less anti-inflammatory activity than the standard anti inflammatory agent; Indomethacin. The anti-inflammatory activity was attributed to the presence of triterpenoids in both of the investigated plant material[28-30]. The Results of the anti-oxidant activity of both plants under investigation showed that, ethanol (70%) extracts of both plants had anti-oxidant activity at tested doses, compared with the control, hence; could be used for this purpose. However, they had less anti-oxidant activity than ascorbic acid as a standard anti-oxidant. By comparing the biological results of average healing days &/or complications of different therapeutic agents under the study; it was found that, the 2% concentration of the dried ethanol (70%) extract was promising and effective with minimal side effects (animal trials) and minimal required time of healing, especially when incorporated in a gel form, this result was in agreement with the previous findings, which - was contributed to Jurga Bernatonienel *et al.* – where an ethanol (70%) extract of C. *officinalis* flowers was investigated against experimentally induced thermal burns in rats, the daily application of 2% C. *officinalis* gel resulted in enhanced wound healing, due to its antimicrobial and antioxidant property[31]. Superficial burn healing power of the natural recipe of 2% of the dried ethanol (70%) extract of both (combined) plants under investigation in the gel form was very promising, since healthy healing (without or with minimal local complications) was observed within 9 days. This biological result may be attributed to the presence of sterols and triterpenoidal compounds (anti-inflammatory effects) in the two plants under investigation[32]. The biological activity of a plant alcohol extract may be also explained by the fact that, water miscible solvent such as 70% ethanol has the ability to extract most of plant active ingredients e.g. flavonoids and flavonoid derivatives, as well as saponins and saponin derivatives (antimicrobial and antioxidant effects), which may be responsible for the plant healing power[32]. Superficial burn healing power of the commercial drug Dermazin® (silver Sulphadiazine) cream was comparable or nearly similar to that of our new natural recipe, since the average healing days was similar. However, in spite of the well known healing power of Dermazine® cream, local complications were observed in the form of hypersensitivity and dryness (disadvantages). There are many reported local & general complications of Dermazine® cream. Pseudo membrane formation on the burn wound is a common reported local complication of Dermazine® cream; which sometimes is being difficult & painful upon its removal from the burn wound[33]. Neutropenia is another famous reported systemic complication of the drug. These complications were considered as major drawbacks[12]. On the other hand, superficial burn healing power of the commercial drug Mebo® ointment (β-sitosterol) was next to all therapeutic reagents used in the experiment, since average healing time was 11days. However, the ointment has an advantage of lacking the dryness and scarring produced by other agents. It soothes wounds by the action of a moisture-retaining oil, berberine, one of its active agents. It also suppresses microbial proliferation and promotes rapid re-epithelialization.[11] This is believed to be aided by β-sitosterol, a member of a family of plant steroids found in several plants, especially soya. MEBO reduces exposure of the burn surface, which - as shown by Smahel J. – limits tissue damage and leads to better healing outcomes, even if healing times may be altered as a consequence[34]. Although *in vitro* antimicrobial activity of Mebo® ointment has not been demonstrated, it has been shown that in experimental and clinical use it prevents wound

Table 8: Average results of healing power and post-healing local complications of the natural recipe (Gel of 2% *Calendula* and *Cleome* alcohol extract) expected to promote healing of superficial burns.

Investigation parameter	2% (Calendula + Cleome) 70% alcohol extract (gel)	Dermazin® cream	Mebo® ointment	Placebo Gel base
Hypersensitivity	5 %	23.3%	17.3 %	8.6 %
Average healing time	9	9	11	15
Dryness & Scarring	5 %	25%	15 %	45 %

infection just as much as SSD, possibly by blocking penetration of the eschar by virulent microbes[35]. Although several researchers have studied MEBO's wound healing properties, only a few controlled animal trials were found in the literature we reviewed. This study thus compares the efficacy of a new natural recipe with MEBO® and with that of SSD in partial-depth burn wounds in rats. Mortality rates of animals among time of healing elapsed were low, maximum mortality rates were observed at the first few hours after scald induction and the first week of therapy (common in placebo group; probably due to microbial invasion and colonization, continuous pain generation and the subsequent neurogenic shock), then it was diminished by time[36]. The promising results were noticed in the synergy of action of the combination of both plant materials under investigation, incorporated and formulated in the form of gel, rather than single plant formulation. Conventional moisture-retaining devices such as Sofra-tulle and Tegaderm are time-consuming and labour intensive to apply. Although the mean healing times were virtually identical for our new natural recipe and SSD in this study, a number of other studies show that SSD may delay healing, especially in deeper burns. In one such study, healing time was normal when SSD was used in split-thickness donor sites but significantly slower in deeper wounds[37,38]. The mechanism by which this unique effect of delayed healing occurs is not yet clear. Invasive wound infection continues to be a route for much of the mortality from burns, especially in children[39]. The search for suitable topical burn therapy will continue to focus on preparations that can kill microbes and promote tissue regeneration while limiting tissue damage. Concern about the toxicity of purified chemicals will continue to make people turn to complementary and alternative medicines (CAM) such as our new natural recipe and MEBO®. Low toxicity, cheaper costs, easier handling, and availability are all factors that may promote the use of CAM such as our new natural recipe and MEBO®[10]. Our study showed that the investigated new natural recipe will be found to be an effective burn treatment, at least in partial-thickness burns and may be a useful alternative to conventional therapies such as SSD and MEBO®, especially in cases where infection is a major consideration. Fig (21)

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