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A STUDY OF THE POSSIBLE HEALING AND ANTI-INFECTION EFFECTS OF *PORTULACA OLERACEA* PLANT ON WOUNDS IN RATS

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ABSTRACT

Portulaca oleracea (purslane), known as Regla in Egypt, is a nutritious vegetable used for human consumption. Microbial infections of various types of wounds are a challenge to the treatment of wounds and wound healing. The study was to investigate the wound healing, antimicrobial and antioxidant properties of aqueous extract of Portulaca oleracea plant. An exisional wound was performed in rats and inoculated by staphylococcus aureus organism. The influence of Portulaca oleracea extract on rate of wound contraction was investigated in infected and non-infected wounds. The antioxidant activity (GSH& MDA) was evaluated in wound tissues in addition to histopathological studies. In vitro antimicrobial activities of Portulaca oleracea plant were determined against Gram-positive and Gram-negative bacteria, fungi and yeast. The results showed significant increase in the rate of wound healing by Portulaca oleracea (95.72±0.676) in a duration of 15 days meanwhile by applying the plant in infected wound the rate of wound contraction was declined to (87.11 ±1.893). Portulaca oleracea reversed the decrease in GSH and increased MDA in skin induced by *staphylococcus aureus* infection. In vitro studies, revealed the highest inhibition zone to *Aspergillus Niger* Fungi. The high content of Portulaca oleracea extract to alkaloids, tannins, flavonoids and other constituents may explain its action in healing of wounds with anti-infective and antioxidant properties.

KEYWORDS: P Ortulaca Oleracea, Wound Healing, Antibacterial, Antioxidant.

INTRODUCTION

Portulaca oleracea (purslane) is a useful herb in curing diseases of the bladder, kidneys, spleen, lungs and blood system and has many medicinal uses (Chan et al., 2000). Recent studies demonstrated that the herb had anti-inflammatory and anti-oxidation effects (Abd El-Azime et al.2014). Scientific analysis of its chemical components has shown the presence of high content of antioxidants such as (vitamins A and C, alpha-tocopherol, beta-

carotene, glutathione and omega-3 fatty acids. Some of the biologically active compounds isolated from the plant include free oxalic acids, alkaloids, coumarins, flavonoids, cardiac glycosides, and anthraquinone glycosides (Ezekwe et al., 1999)

The herb possesses natural cooling properties that soothe the inflammation of skin .Topical application of the aqueous extract on to the skin is effective as antibacterial and antifungal (Leung ,1996) .Purslane has wound healing properties and a poultice made from the leaves of the plant is applied to draw the pus out of infected sores, useful for burns and skin diseases. (Rashed et al., 2003).

Purslane demonstrated antibacterial and antifungal activity. The antimicrobial activity of the plant may be attributed to the presence of tannins (Fabry et al. 1998).Studies found that ethanolic crude extract of purslane had maximum effects of sensitivity on staph. aureus and klebsiella pneumonia by agar diffusion methods (Hanumatappa et al., 2014).

The present study aims to investigate the healing and anti-infective effects of Portulaca oleracea as skin remedy in wound healing

Materials and Methods

1. Plant juice extract:

Portulaca oleracea plant was obtained from a farm in Minufiya .The juice of fresh portulaca oleracea plant was extracted by water (2kg of plant: 1 Liter water) in warring blender and filtered from tuff fiber, packed in polyethylene pouches then stored in fridge till analysis. To prepare plant emulsion, Plant extract was filtrated, dried on air and the semisolid mass was homogeneously mixed with pure Vaseline to make a Vaseline-based (0.6% ointment)to be applied locally in wounds.

2. Animals:

Adult male albino rats weighing 180-200 g were used in the present study. They were obtained from the breeding colony maintained at the animal house of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Animals had free access to food and water *adlibitum*. They were maintained at (25 ± 2) °C and 40–60% relative humidity with 12-h light–dark cycle. Animals were adapted for one week in the animal house before experimentation. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the

Ethical Committee of Faculty of Pharmacy, Ain Shams University, Egypt and comply with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996).

3-Bacterial suspension

Staphylococcus aureus bacterial strain was obtained from Microbiology department in the National Organization for Drug Control and Research. Bacteria were grown on nutrient agar for an incubation period of 24 hours at 37º C in aerobic conditions. The colonies were verified for their viability and purity.

4-ExcisionWoundModel:

Rats were anaesthetized with 300mg kg of chloral hydrate via intraperitoneal injection. The dorsal surface of rat was shaved, cleaned with 70% ethanol. Excision wounds were made by cutting out a pre-determined dorsal area (20 mm in diameter) of skin from the shaved area .The entire wound was left open (Esimone et al., 2005 and Perumal-Samy et al., 2006).The infection was induced in wound by inoculation with15 µl of bacterial suspension of *staphylococcus aureus*. The entire wound was left open for 24 h. before any procedure (Odimegwu et al., 2008).

5-Rate of Wound Contraction

The rate of wound contraction was measured as percentage reduction of wound size until wound closure. Progressive decrease in the wound size was monitored periodically (every 3 days) using transparency graph paper and a marker. Wound closure was indicated by the formation of new epithelial tissue to cover the wound. The wound healing rate was calculated with a formula as following:

The Percentage (%) wound contraction = (wound area on day0 - wound area on day n) / wound area on day0 × 100. (Mukherjee and Suresh, 2000).

Experimental Design

Adult male rats were randomly allocated into four groups. Each group consisted of 12 rats. The tested plant extracts were topically applied after excision of the skin and continued daily for successive 15 days. The infected rats will be inoculated with 15ul of bacterial suspension of *staphylococcus aureus* and allowed to be infected for 24 h. The entire wound was left open.

Group1: Rats with excision wounds treated topically with Vaseline (ointment base) served as control group.

Group2: Rats with excision wounds infected by *Staphylococcus aureus* treated topically with Vaseline after 24 h (control infected group).

Group3: Rats with excision wounds treated topically with *Portulaca oleracea* extract (0.6gm /1gm Vaseline).

Group4: Rats with excision wounds infected by *Staphylococcus aureus* treated topically with *Portulaca oleracea* extract (0.6gm / 1gm Vaseline) applied after 24 h.

Total Leucocyte count:

This performed according to the methods of (Cheesbrough, 2000) , using the whole blood, hemacytometer and a special pipette of WBCs. Calculated by the number of total leucocytes counted in four squares of the hemocytometer counting slide then multiplied by 50 /Cmm.

Skin homogenate preparation

At day 8th and at the end of experiments, animals were anaesthetized using ether. Skin was excised, homogenized in 50 mM phosphate buffer saline (pH 7.4) using electronic homogenizer (Ezister Daihan Scientific Co., Ltd., Korea) to prepare 10 % w/v homogenate and stored at -80ºC. The skin homogenates were divided into aliquots after 3000 r.p.m. centrifugation for 15 min at 4°C using cooling centrifuge (Hermile Labortechnik, Wehingen, Germany). Skin homogenates were used for the determination of reduced glutathione and malondialdehyde.

Estimation of reduced glutathione (GSH) and malondialdehyde (MDA) content in skin homogenate

The level of glutathione was determined in skin homogenate using Biodiagnostic kit (Cairo, Egypt) according to the method described by Beutler *et al.* (1963).The absorbance of the yellow color was measured within 5 min at 405 nm against blank using a single beam spectrophotometer (UV-120 Shimadzu, kyoto, Japan).

The level of malondialdehyde was determined in skin homogenate using Biodiagnostic kit (Cairo, Egypt) according to the method described by Satoh K. *et al.* (1978).The absorbance of

sample (A_{Sample}) against blank and standard was read against distilled water at 534 nm was measured (UV-120 Shimadzu, kyoto, Japan).

Histopathological studies of skin

Portions of the skin of rats in different groups fixed in 10% neutral buffered formalin were embedded in paraffin and tissue blocks and were prepared for sectioning at 4 microns thickness. Skin sections were stained by hematoxylin & eosin (H&E) or Masson's trichome to assess collagen and maturation within dermis and were examined by light electric microscopy (Banchroft *et al*., 1996).

In vitro antibacterial sensitivity test :

The microorganisms used are: *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 19430; *Staphylococcus aureus* ATCC 25923, obtained from American type cultural collection washington, USA while listeria monocytogenes 1/2B were obtained from Institute FUR- Hygi AFe, Germany. The fungal strains were *Aspergillus Niger*, *Aspergillus flavus* and strains of yeast included *candida lipolytica* NRRLy 1095 , obtained from the Egyptian Microbial culture collection (EMCC), Faculty of Agriculture, Ain shams University. These organisms were checked for their purities and were reactivated monthly on the suitable medium as reported by Conner and Beauchat (1984). The media for *Escherichia coli* (Violet red bile lactose agar VRBLA), *Salmonella Typhiumrium* (S.S agar) ,*Staphylococcus aureus* (staph. medium 1.10) and *listeria monocytogenes* (Listeria selective agar Bafe) were prepared according to the method described in Difico – Manual (1998). *Aspergillus niger* and *Aspergillus flavus* were grown on PDA stant for 1-2 week at 25°C until well spotulated (Subba Rao 1977). Sabouraud's agar media was used for yeast growth. Diameter of inhibition zone was measured as the clear area centered on the agar well containing different concentration of the samples(0.75,0.3,0.5 u) .Well of no inhibition zone was recorded as 0.

Statistical analysis:

Results were expressed as mean $\pm$ SEM. Statistical analysis was performed using a prism computer program (GraphPad software Inc. V5, San Diego, CA, USA). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey-

Kramer Multiple Comparison Test. (Petrie, 1987; Po, 1998). Probability values of less than 0.05 were considered statistically significant.

RESULTS

Table 1 : Effect of Portulaca oleracea (Purslane) on the rate of wound contraction(%) in infected and non-infected wounds in rats

Groups	**Control vaseline**	***Staph.aureus* infected group**	***Purslane* group**	***Purslane* +*staph.aureus* group**
Day 3	11.78 ±1.239	6.4 ±1.002*	17.80 ± 1.93*	12.44 ±2.308*
Day 6	21.1±1.167	13.25±1.486*	37.83 ± 2.025*	29.18±2.845*
Day 9	36.9 ±2.709	22.79±2.174*	59.03±3.06*	52.12 ±3.875*
Day 12	49.14 ±2.71	39.87 ±2.75	83.52±1.25*	80.41 ±2.761*
Day15	71.95 ±3.621	65.59 ± 4.327	95.72±0.676*	87.11 ±1.893*

Each value indicates the mean SEM of 10-12 observations.* P 0.05 compared to control. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

Table 2: Effect of Portulaca oleracea on skin antioxidants in infected and non- infected wounds in rats

Groups	**Day**	**MDA (nmol/g)**	**GSH (mg/g.tissue)**
Control Vaseline	Day 0	27.65 ±2.28	47.4 ± 1.118
	Day 8	69.89 ± 1.121*	29.85 ± 1.84*
	Day 15	28.83 ± 2.007	53 ±2.969
***Staph.aureus* infected group**	Day 8	86.53 ±5.533*	20.98 ±1.593*
	Day 15	71.23 ±4.98	30.27 ±2.277
***Purslane* group**	Day 8	18.29 ± 1.781*	87.26 ± 3.39*
	Day 15	33.35 ± 1.069	67.55 ± 0.757*
***Purslane+staph.aureus* group**	Day 8	27.74 ± 1.789*	33.02 ± 1.333
	Day 15	66.58 ± 5.222*	79.11 ± 6.241*

Each value indicates the mean SEM of 10-12 observations.* P 0.05 compared to control. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

Table 3: Effect of portulaca oleracea on total leucocytic count in infected and non- infected wounds in rats

Groups	Day	Leucocytic count
Control vaseline	0	7.000 ±1.385
	Day 8	10.110 ±1.113*
	Day 15	6.965 ±1.468
***Staph.aureus* infected group**	Day 8	13.350 ±1.13*
	Day 15	18.611 ± 1.62*
***Purslane* group**	Day 8	10.978 ±1.277*
	Day 15	5.671 ±1.957
***Purslane+staph.aureus* group**	Day 8	11.788 ±1.309*
	Day 15	8.663 ±1.494*

Table 4: Effect of portulaca oleracea fresh whole plant, leaves and stem on antimicrobial Inhibition zone/ mm

Tested sample	Conc Well in μ	A. Niger	A. Flavus	C.lipolytic	Stap.oureus	E.coli	Salmonella.typh.	L.monocytogenes
FRESH LEAVES EXTRACT	0.75	-	-	-	1.5	1.1	-	-
	0.3	-	-	-	2.3.1.5	-	-	-
	0.5	-	10	-	2.5	-	-	-
FRESH STEM EXTRACT	0.75	-	-	-	1.9	1.1	1.4	-
	0.3	-	10	-	-	2.3-2.1	2	2.5-2
	0.5	1	10	-	-	2.5	2.5	1.1-1.9
WHOLE FRESH PLANT EXTRACT	0.75	-	-	-	-	-	-	-
	0.3	-	10	-	1.5	1.3-1.5	2.2	2.1
	0.5	-	-	-	2	2.5	2.4	2.5

FIGURES

FIG 1 :Control normal wound (day 3,6,9,12,15)

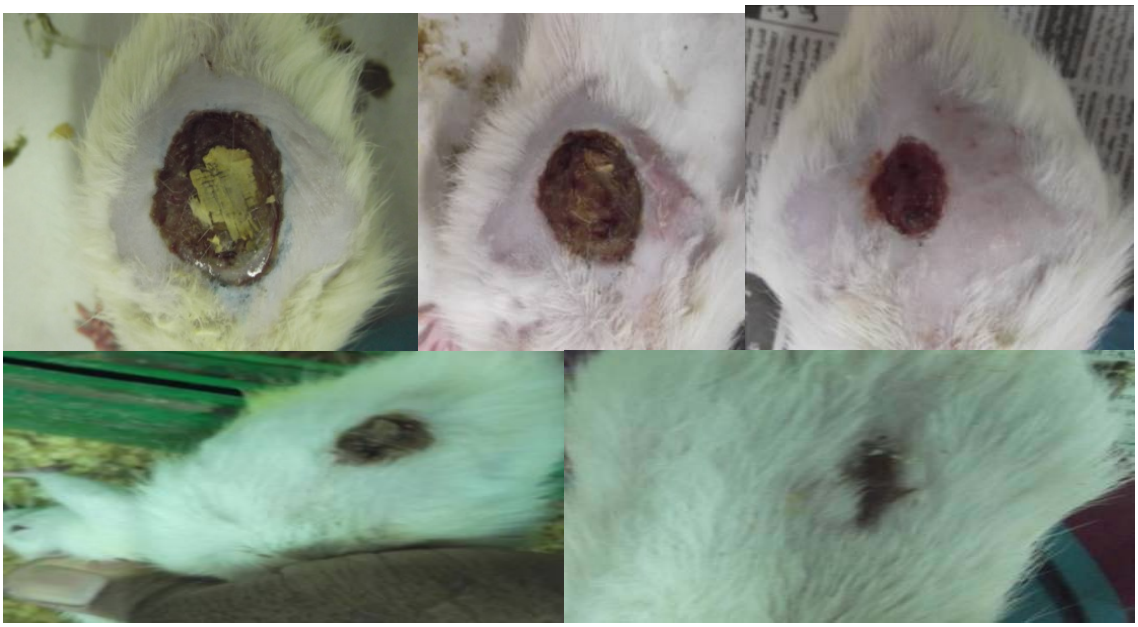


Fig2 : *Staph.aureus* Infected wound (day 3,6,9,12,15):

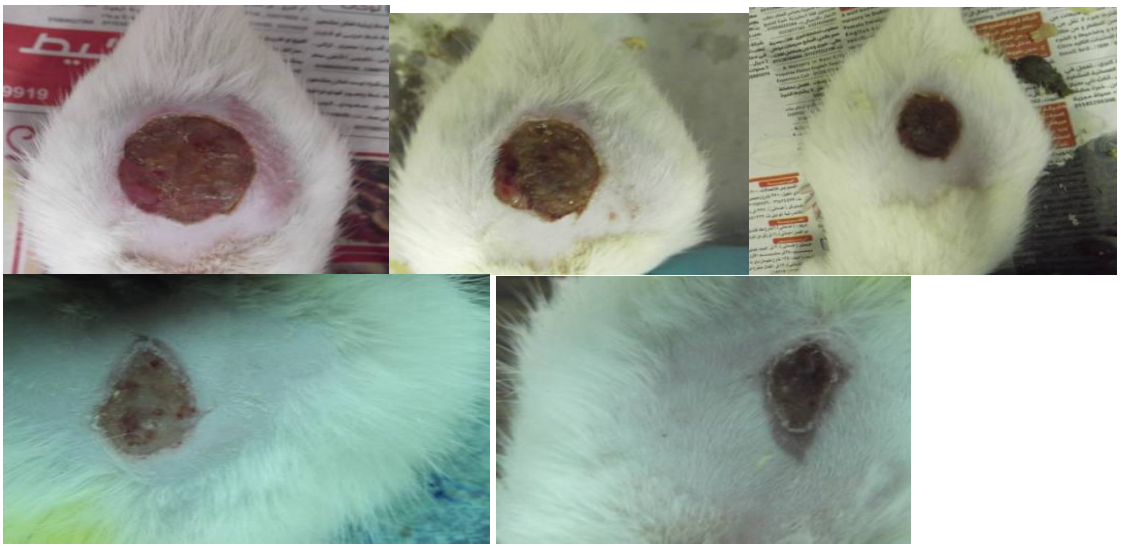


Fig 3 :*Purslane* treated group(day 3,6,9,12,15):

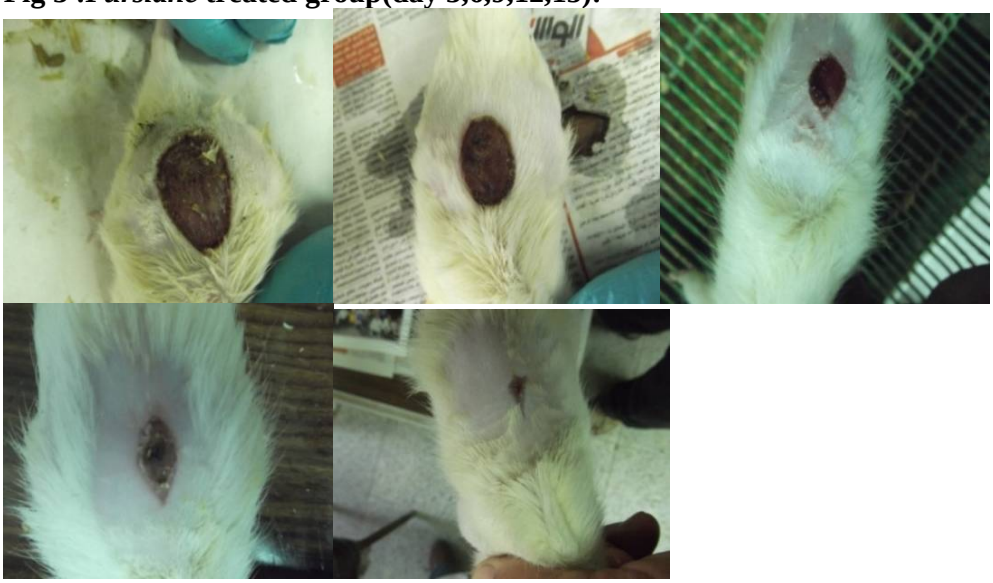
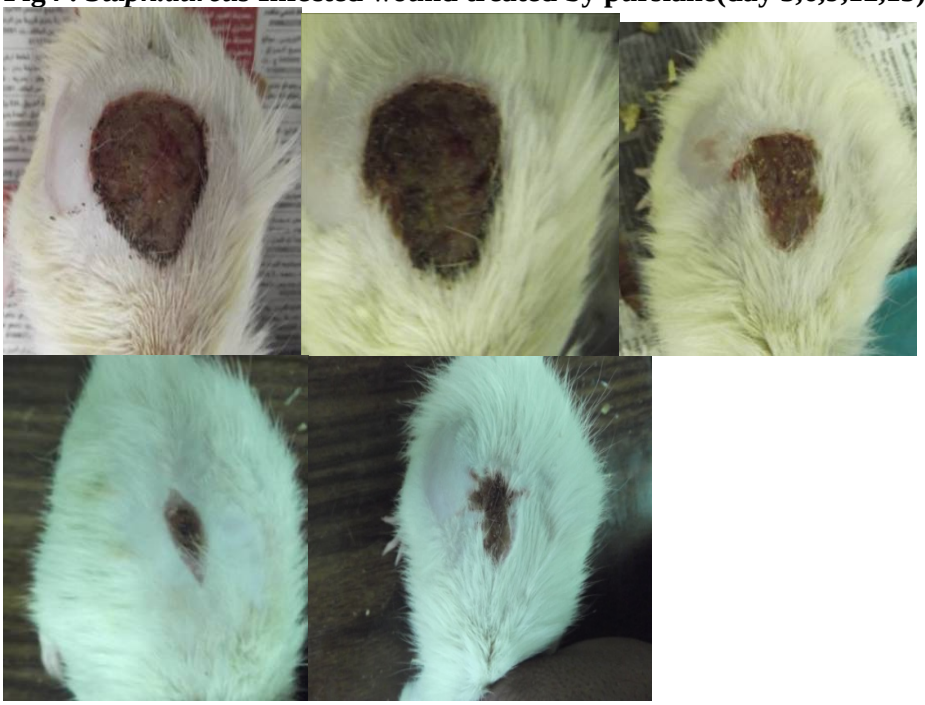


Fig4 : *Staph.aureus* Infected wound treated by purslane(day 3,6,9,12,15):



Histopathological study of skin of different groups:

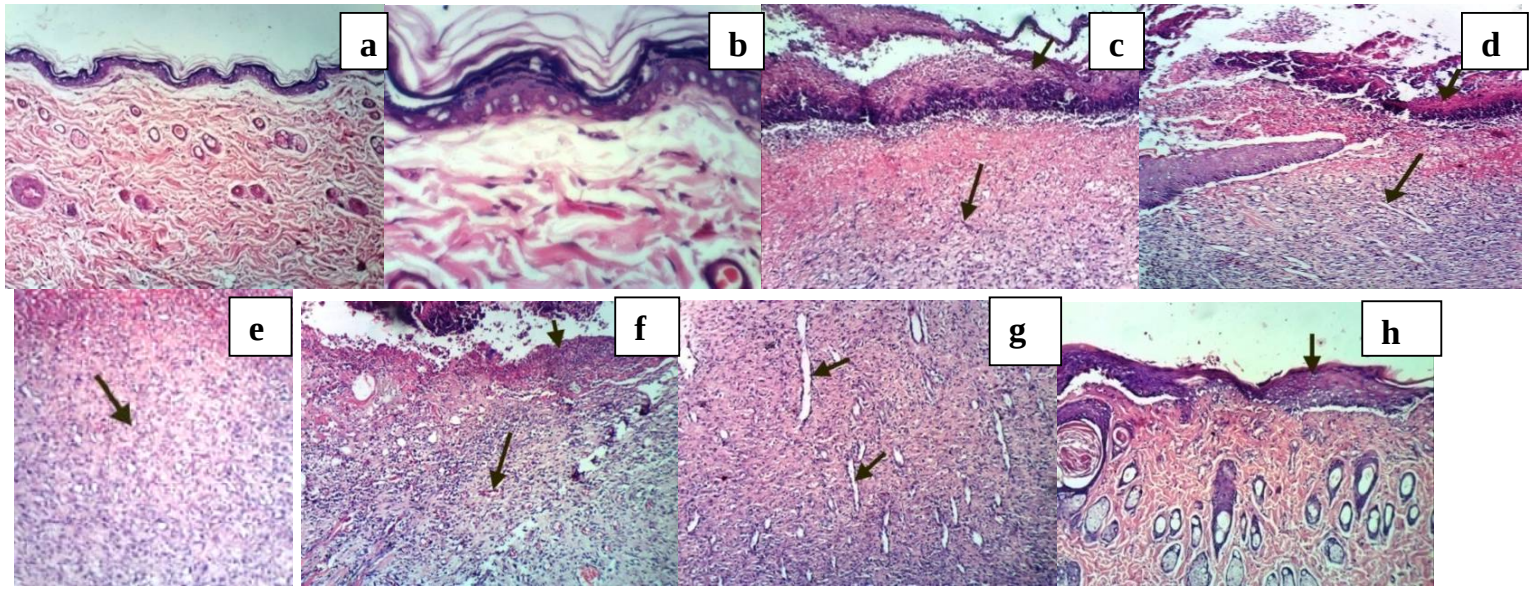


Fig. (a,b): skin of control rat showing normal skin layers from epidermis and dermis .**Fig. (c):** Skin of rat from group 2) showing necrosis, inflammatory cells infiltration and granulation tissue formation **Fig. (d):** Skin of rat from group 3) showing necrosis inflammatory cells infiltration , granulation tissue formation ,Less collagen fibers . **Fig. (e):** Skin of rat from group 3) showing necrosis, more and less oriented granulation tissue formation. **Fig. (f):** Skin of rat from group 4) showing necrosis, inflammatory cells infiltration and highly vascularized granulation tissue formation. **Fig. (g):** Skin of rat from group 4) showing well oriented granulation tissue formation and collagen fibers deposition. **Fig. (h):** Skin of rat from group 4) showing re-epithelization of wound.

DISCUSSION

In this study, The rate of wound contraction in infected wound was 65.59 %, by applying purslane the rate of wound contraction increased to 88.11 % .Wound healing was promoted by purslane meanwhile Infection by staph. aureus delayed wound healing .Bacterial infection, causing slow healing of wounds (Rojas et al., 2002). Research into the role of antioxidants from plant extracts in wound healing has been published widely (Joharapurkar et al., 2003).Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent, but independent to each other(Iba et al., 2004) .The preliminary wound healing activity of Portulaca oleracea has been appraised in Mus musculus showing that a fresh crude extract significantly accelerates the wound healing course by the stimulation of wound contraction and down regulation of the surface area of the excision wound (Rashed et al 2003).

In this study**,** Skin reduced glutathione (GSH) had decreased in infected group meanwhile malonaldehyde (MDA) had increased as compared to control group .Administration of purslane significantly increased GSH and decreased MDA. The antioxidant activities of Purslane extract are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids and tannins. These compounds, play a major role in the wound healing by preventing and protecting oxidative damage from free radicals (Okuda , 2005). The antioxidant activity of Purslane was reported by Hao et al. (2009) who used purslane as a medicinal plant for anti-aging, thereby increasing the level of SOD and decreasing the level of MDA in the brains of mice treated with D-galactosamine. The antioxidant property of Portulaca oleracea is attributed to its constituents, such as gallotannins, omega-3 fatty acids, ascorbic acid, α-tocopherols, kaempferol, quercetin, and apigenin (Zhu et al.2010)

In this study, the anti-staph. property of purslane in wounded rats could be confirmed by the in vitro results in which a concentration of fresh extract (0.5ml)have inhibition effect on the growth of staphylococcus aureus (2mm) **.**

The highest antimicrobial Inhibition zone to A.favus (10mm) coincide with the studies of Elkhayat(2008) on methyl alcohol extract of Portulaca oleracea and the high inhibition zone to fungi and bacteria.

Young et al., (2008**)** reported that purslane showed high antimicrobial activities against Helicobacter Pylori, staphylococcus aureus, Escherichia coli and streptococcus mutans.

Recent study by Peng et al. (2014) showed the efficiency of Aqueous and Ethanolic Extracts of Portulaca Oleracea against staph. aureus isolated from Cow Mastitis.

According to Fabry et al. (1998), the antimicrobial activity of the plant may be due to the presence of tannins. Different mechanisms have been proposed to explain tannin antimicrobial activity including; inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Terpenoids also may be involved in the antimicrobial activity of the extract (Nayak et al., 2010). A recent study by Hanumantappa et al.(2014) showed that the flavonoid apigenin isolated from ethanolic extracts of Portulaca oleracea L has antibacterial property and could be used to develop antibacterial drugs.

The total leucocytic count in this study increased with infection and decreased by purslane .The process of inflammation leads to the release of chemical mediators which attracts neutrophils, leucocytes and monocytes, to an inflamed or wounded area and these attack foreign debris and micro-organisms through phagocytosis (Guo and DiPietro, 2010).Since purslane has anti-nflammatory effects (lee et al. 2012) so it could reduced leucocytosis induced by infection.

The histopathological study of infected wound showing necrosis, inflammatory cells infiltration and granulation tissue formation and by applying purslane in infected wound well oriented granulation tissue formation and collagen fibers deposition and re-epithelization of wound had occured .Tannins ,in purslane extract ,lead to increasing formation of capillary vessels and fibroblasts and this promote cicatrisation of wounds (Atiyeh et al. 2007).

CONCLUSION

This study shows that Portulaca oleracea has antibacterial and wound healing effects when applied locally in excisional wound in rats. The Presence of high content of antioxidants in the plant extract, making it one of the potentially important foods for the future and a highly likely candidate useful cosmetic ingredient.

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