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ROLE OF PURSLANE (PORTULACA OLERACEA) IN THE WOUND HEALING AND ITS IMPACT ON BIOCHEMICAL PARAMETERS IN DIABETIC ALBINO RATS

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ABSTRACT

Portulaca oleracea L. has been used for treatment of various ailments like skin diseases and bleeding piles. This plant is reported to have versatile biological activities like antiulcerogenic, anti-inflammatory, antioxidant and antimicrobial properties. Delay wound healing in diabetics is mainly due to microbial infection, reduction in cell proliferation and survival, and decreasing in wound contraction. The present study has been designed to determine the possible antimicrobial role of *Portulaca oleracea* extract on wound healing, and to evaluate its impact on kidney functions and complete blood count in diabetic albino rats. Experimental diabetes was induced in albino rats by administration of Streptozotocin-Nicotinamide (60 and140 mg/Kg b.w respectively). Topical treatment with *Portulaca oleracea* extract (40 mg/250 mm^2 wound) has been applied to diabetic wounds 3 times per week for 3 weeks. The rate of wound contraction was observed at days 5, 10, 15 and 21. Microbial Samples were taken from the wound to investigate antimicrobial effect of *Portulaca oleracea* extract. At end of the experiment, rats were sacrificed and blood samples were taken for hematological and biochemical analysis.Our results showed that percentage of wound contraction has been significantly increased in the *Portulaca oleracea* extract-treated rats compared to positive control rats.The antimicrobial effect on the wound surface has also been elevated with the *Portulaca oleracea* extract treatment compared to untreated rats. Importantly, *Portulaca oleracea -extract* treatment reduced the oxidative stress of STZ on hematological and kidney function compared to positive control group. In conclusion, ethanolic extracts of *Portulaca oleracea* showed promising effects on wound healing in the diabetic rats that can increase our therapeutic avenue for treatment of diabetic wounds that are often slow to heal.

Keywords: Wound healing, *Portulaca oleracea*, Streptozotocin, Excision wound model

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia and abnormalities in proteinlipid and carbohydrate metabolism. Diabetic wounds are defined as chronic wounds or lesions that take long time to heal and may fail to heal [1]. Wound healing is a very orderly and highly controled process characterized by four distinct but overlapping phases: hemostasis, inflammation, proliferation and remodeling [2]. The repair process needs the coordination of various cells, growth factors and cytokines. Inflammation is the initial phase of wound healing in which the macrophages mainly take the role [3]. Many studies showed that high glucose concentration inhibits wound healing process which associated with prolonged inflammatory phase [4]that will lead to defected angiogenesis [5]. Improper wound healing control may result in diabetic ulcer or even amputation [6].These factors have encouraged studies on diabetes in recent years, particularly those trying to develop new drugs for the treatment of tissue injuries that affect diabetic patients. As the objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient. During the last two decades, there has been increased interest to assess the utility of plant extracts in wound healing and to gain more insight into the active constituents that promote or modulate the healing process [7].*P. oleracea* is listed in the WHO as one of the most used medicinal plants and it has been given the term 'Global Panacea [8].This plant is reported to have flavonoids, alkaloids, vitamins, minerals, terpenoids, organic acids and other compounds e.g.(chlorophyll and tannins) [9].The main effects of the active constituents of this plant extracts towards wound healing are summarized as phyto-chemical constituents contributing to antimicrobial activity plusphyto-chemical constituents which work as antioxidants and free radical scavengers. These active components have enhanced mitogenic activity (contributing to increased cell proliferation), angiogenesis, enhanced collagen production and increased DNA synthesis[7].Terpenoids of different structures including the monocyclic and the multicyclic ones have been identified to possess antimicrobial activity. It has versatile biological activities like anti-inflammatory [10].Also, a recent report indicated that an extract of *P. oleracea*is useful for treatment of indomethacine and phenylbutazone-induced ulcers [11].

2. MATERIAL AND METHODS:

2.1. Animals:-

Forty male albino rats, each weighed 150-200 gm.Were housed in special cages (10 - rats/cage) and maintained under controlled environmental conditions (12-hours light/dark cycle, temperature 27°C), and provided with standard laboratory food and water *adlibitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

2-2-Chemicals:

Streptozotocin (STZ) and Nictonamide were purchased from Sigma Co., St. Louis, USA. and all other chemicals were of analytical grade and were obtained from commercial sources.

2-3-Plant Extraction:

A-Plant Material

Fresh specimens of *P. oleraceae* were collected from the farm of zigzag – Egypt.

B-Preparation of the Extracts:

2.5 Kg of fresh specimens of *Portulaca oleracea* were washed free of soil and debris. The roots were separated from the leaves and stems. The leaves and stems were air-dried for six weeks, and the dry specimens were pulverized using laboratory mortar and pestle. Briefly, 100 g of fine powder of shade dried leaves was macerated with 95% ethanol in dark and filtered. The filtrate was evaporated in rotary to harvest a viscous residue. The viscous residue was collected, weighed and kept at 4 °C until use according to [12].

2.4. *Experimental Induction of Type 2 Diabetes:*

Type 2 diabetes was induced in overnight fasted rats by intraperitoneal injection of freshly prepared STZ (60 mg kg-1, dissolved in 0.1 M cold citrate buffer, pH 4.5), 15 minutes after intraperitoneal administration of 140 mg kg-1 nicotinamide. Three days following STZ injection, animals with blood glucose level above 250 mg dL-1 were used for the study according to[13].

2.5. Blood Glucose Level:

Before surgery and 15 days following STZ injection, the blood glucose level of each rat in both treated and non-treated group was checked using a glucometer and test strips.

2.6. Wound Surgery:

After confirming the induction of diabetes, 10 animals per group were used to create wounds. Rats were anaesthetized by mild dosage of diethyl ether. About 250 mm^2 full thickness open excision wound was made on the back of the rat (Figure 1) as reported in earlier studies[14].Each rat was housed alone in a specific cage to prevent any risk on the wound. The control rats were left untreated and the treated rats were topically administered with 40 mg/250 mm^2 wound of ethanolic extract of *P. oleracea* 3times per week for 21 days.

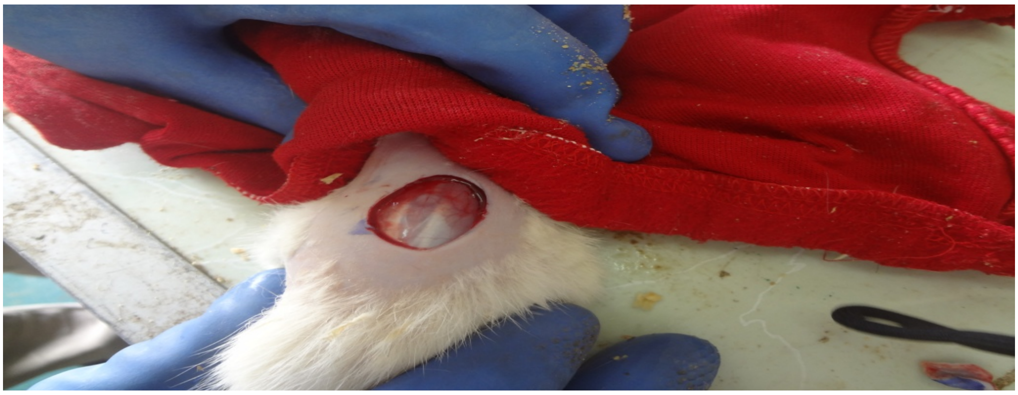


Fig 1: Open excision wound

2.7. Experimental Groups:

This work was designed to evaluate the wound healing in both treated and non-treated animal model. Therefore, the animals were classified into 4 main groups each one consisted of 10 rats.

Group I: Negative control group, healthy non-diabetic untreated rats.

Group II: Positive control group, diabetic rats with excision wound without *P. oleracea* treatment.

Group III: First experimental group, diabetic rats topically treated with ethanolic extract of *P.oleracea* (40 mg/250 mm^2 wound) for 21 days

Group IV: Second experimental group, diabetic rats with excision wound and topically treated with ordinary and broad antibiotic Fusidene (2 g/ 250 mm^2 wound) for 21 days.

2.5. Macroscopic evaluation:

For macroscopic evaluation of the wound area, digital images were captured with a Nikon Coolpix P100 digital camera (resolution of 10.6 Megapixels) mounted on a tripod at a constant distance of 30 cm from the surgical wound. All images were analyzed with the ImageJ® software, delimiting the wound periphery and comparing the initial and final wound area in all groups at the different time points studied. The percent reduction in wound size was calculated using the following formula [15]:

Percent reduction = **Initial wound area – final wound area) / initial wound area x 100**

2.6. Sample collection:

2.6.1. Microbiological sampling:

Cotton swap samples were taken from the wound surface to detect the ability of different treatments to prevent microbial growth on diabetic wound.

2.6.2. Blood sampling:

At the end of experiment (day 21) blood samples were obtained by decapitation of the rats. Two ml blood was collected into fresh vials containing anticoagulant and the rest of was left to coagulate and serum was separated by centrifugation at2000 rpm for 2 min.**2.7.Biochemical Analysis:**

Blood glucose level was measured immediately using blood glucose meter. Serum urea and creatinine was also estimated to evaluate impact on the kidney functions, Complete blood count and HbA1c were determined.**2.8. Statistical analysis:**

Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Dunckn s test using version 16 of SPSS software. The values were considered non-significant when p>0.05[16].

3. RESULTS:

3.1. Effect of STZ in different groups

Our data showed that there was a significant increase in both glucose level and HbA1c percent in positive control group "G2" compared with negative control group "G1".Thepercent change was 284.3% and 186 % for G2 and G1 respectively. On the other hand ,no significant difference could be detected in glucose level and HbA1c in day zero in either *Portulaca oleracea* "G3"or antibiotic group"G4" compared with G2.At the day 21,glucose level and HbA1c percent decreased significantly in G3 and G4 compared with G2(Table 1)

3.2. Effect of different treatments on wound contraction in STZ diabetic Rats:

There was highly significant decrease in the wound size in *P. oleracea* "G3" compared with positive control group "G2". In days5,10,15 and 21the percentage of wound contraction were 39.8%, 63.5%, 97%and99.44% respectiverly in *P. oleracea*, versus 9.1%,50.8 %,67.5 and 75% respectively in positive control group (Figure 2).

3.2. Macroscopic effects of different treatments on wound healing in STZ diabetic Rats:

As shown by representative images of wound healing at different time points (Figure 3), apparently the wound area was highly significantly reduced in treatment group (G3) compared to non treatment group(G2) and fusidne group(G4).

3.3. Antimicrobial effects of different treatments on wound surface in all groups.

No bacterial or fungal growth could be detected on the cultures prepared from samples taken from the wound surface of *Portulaca oleracea* group. In contrast, positive control group(G2) showed growth of both gram positive bacteria as well as fungal growth. Unexpectedly, fusidene group (G4) has also shown growth of both fungi and bacteria (Figure 4).

3.4. Effects of different treatments on (HB%, RBCs, WBC & PLT) in all groups.

As clearly shown in Figure 5, Hemoglobin concentration, Red blood cell count and platelets count were significantly reduced in positive control group compared with negative control group. On the other hand, these three parameters showed significant elevation in *Portulaca oleracea* compared with positive control group.

3.5. Effects of different treatments on kidney functions

Non-treated diabetic rats (positive controls),a significant increase in urea and creatinine levels was recorded compared with negative control group. In contrast, *Portulaca oleracea* extract treatment improved kidney functions as detected by the significant decrease in creatinine level in *Portulaca oleracea* group compared with the positive control group.

Table (1): Glucose level in all studied groups (mean ±SD)

Group		Glucose in mg/dl (Day 0)	Glucose in mg/dl (Day 21)	HbA1c (%)
control "G1"group	Mean ±SD	115±13.5	128.6±18	3.3±0.3
	%change	-----	------	-----
Diabetic group "G2"	Mean ±SD	442±84.4$^{\#\#\#}$	492±46.2$^{\#\#\#}$	9.44 ±0.2$^{\#\#\#}$
	%change	284.3%	284.4%	186%
Purslne +Diabetic Group "G3"	Mean ±SD	404±83.2	404±44***	7.7±0.8***
	%change	-8.59%	-17.88%	-18.4%
Fusidne +Diabetic Group "G4"	Mean ±SD	412±69.5	422±43**	8.5±1.0**
	%change	-6.78%	-14.22%	-9.95%

Positive control diabetic group (G2) was compared to negative Control group(G1) ($^{\#}$) and treatment groups (*).P value > 0.05 was considered non significant.,*P&$^{\#}$ P value <0.05 was considered significant. ** P&$^{\#\#}$ P value <0.01 was considered highly significant,* P&$^{\#\#\#}$ P value <0.001 was considered very highly significant**

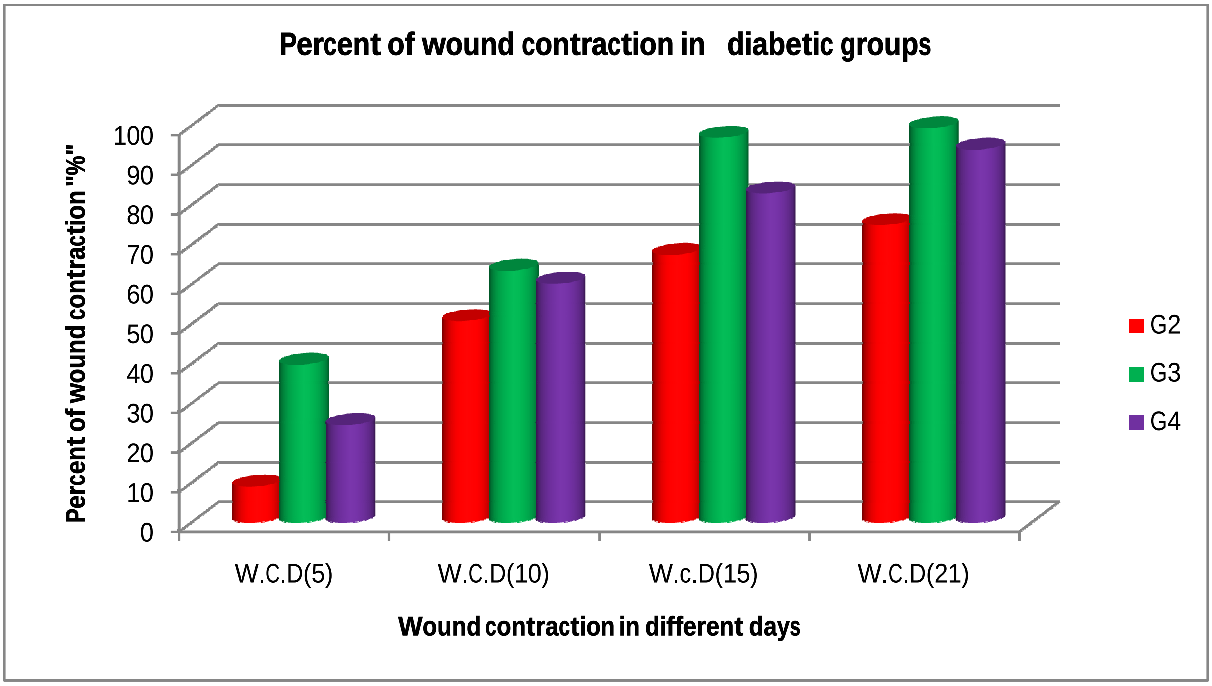
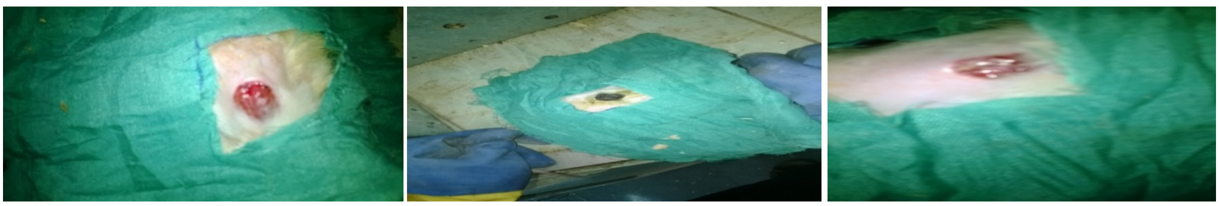


Fig. (2):Percent of wound contraction in (Day 5,10,15 &21) in adult male albino rats.

Day(0) G2 G3 G4

(A)Day(5) G2 G3 G4

(B)Day(10) G2 G3 G4

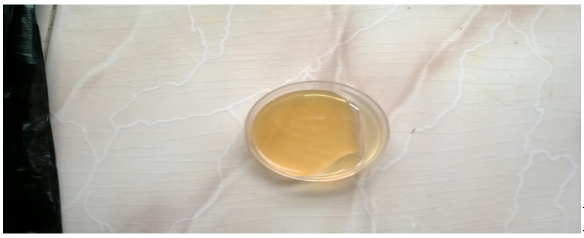


(C)Day(15) G2 G3 G4

(D)Day(21) G2 G3 G4

Fig. (3 A,B,C,D):Representative images of wound healing at different time(Day5,10,15&21) points. Apparently the wound area was highly significantly reduced in treatment groups (G3) compared to non treatment group (G2) and antibiotic group (G4)

A



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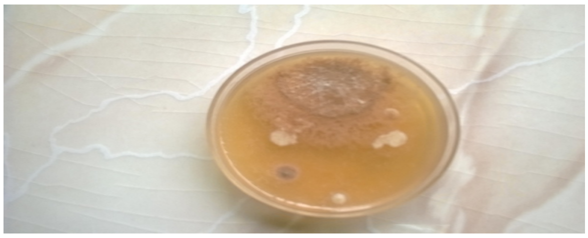

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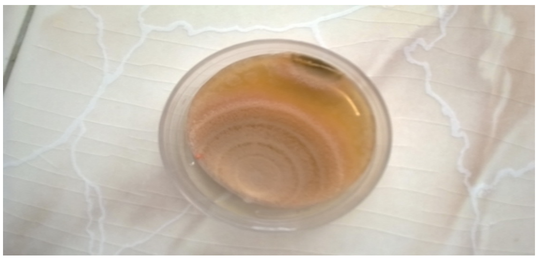

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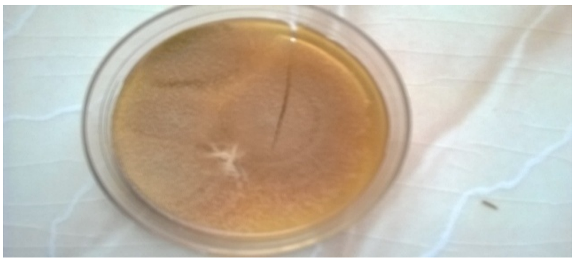

Fig.(4 A,B,C,D) : Photomicrograph of Microbial samples from diabetic wound (A) showing no growth of bacteria and fungus in G3 , (B) showing growth of both bacteria and fungus of positive control group (G2) ,(C) growth of only fungus from skin of negative control group (G1), (D) growth of fungus and one spot of bacteria in antibiotic treatment group(G4)

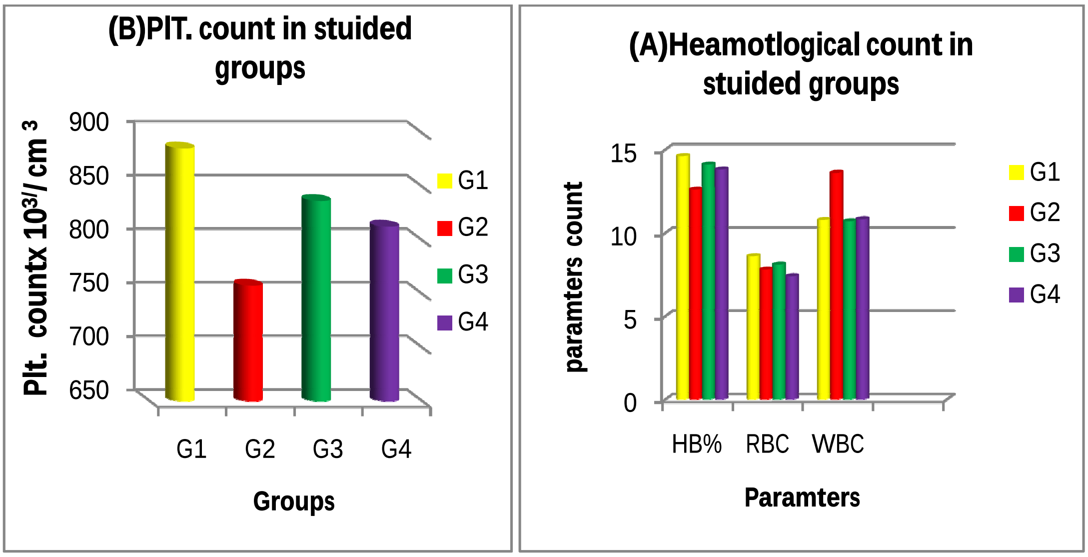

Fig.(5 A&B): Showed effect of different treatments on Hematological parameters

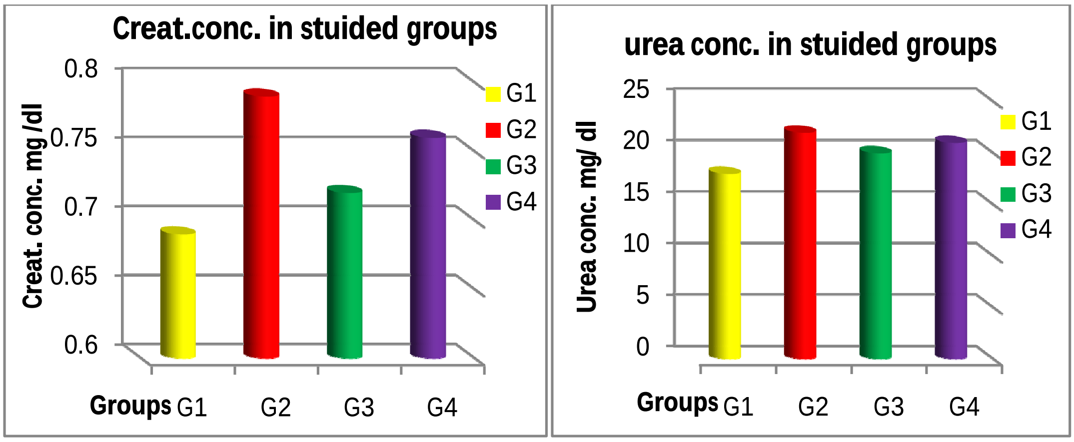

Fig.(6 A&B): Effect of different treatments on kidney function in all groups

4. DISCUSSION

Wound repair must occur in a physiologic environment conducive to tissue repair and regeneration. However, several clinically significant factors are known to impede wound healing, including hypoxia, infection and metabolic disorders such as diabetes mellitus [17]. Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. These abnormalities contribute to the impaired wound healing observed in diabetes [18].In the present study, we demonstrated high elevation in the glucose level in positive control group compared with negative control. In contrast, the glucose level in positive control group, *Portulaca oleracea*treated group and fusidne treated group was indistinguishable at the day 0 of the experiment. This is due to the fact that STZ induces diabetes by the rapid depletion of β-cells and thereby brings about a reduction in insulin release [13] and this effect was not detectable at day(0).Plant extracts and medicinal herbs have been shown to possess variety of potent antioxidant principals. For example, presence of large amount of bioactive principals like alkaloids, flavonoids in extract of *Portulaca oleracea*. Also, *P. oleracea* isa rich source of omega-3- fatty acids, which isimportant in promoting the immune system [19]. So, several biological properties have been attributed to *P .oleracea*:antiseptic, as antibacterial and wound-healing[20]. For example, there was highly significant increase in wound contraction of *P.oleracea* group compared with positive control group. This effect could be explained by the ability of *P. oleracea* extract to accelerate wound healing process[21] due to its content of biologically active substances. Indeed, presence of alkaloids, carbohydrates, glycosides, terpenoids, and tannins, proteins, flavonoids, minerals (Ca&Zinc), omega 3 faty acid, Caffeic acid and vitamins A, C and Niacin in *P. oleracea* which known to initiate modulation of the wound healing process[9]. For instance,flavonoids are expected to provide enabling support to the healing process initially by the moderation of superoxide anions,as well as via enhancing angiogenesis and blood flow as the repair process advances. Also, flavonoids are strong scavengers of reactive oxygen species. It is well documented that in wounds there is a tendency for sharp rise in the concentration of reactive oxygen species due to the activation of platelets, neutrophils, macrophages, lymphocytes and fibroblasts at different time points of the healing process. Infection from microbes also adds to the woes. In such situations, plant flavonoids would benefit the healing process by modulating the concentrations of reactive oxygen species[7].Also, caffeic acid is documented to have wound healing activities [22&23]by playing a role as free radical scavengers. Alkaloids from a diverse range of plants have been found to possess antioxidant properties and many of these have also antimicrobial characteristics [24].Additional role of *P.oleracea*extract in wound healing process could be attributed to its content of polysaccharides. Polysaccharides was known to accelerate the phases of re-epithelialization and remodeling of healing process by influencing interactions in the cell matrix and by moderating the deposition of lamin in [25].Polysaccharides are also believed to exhibit immune modulatory action on the cells around the wound site which stimulate cell proliferation [26].*P.oleracea*extract has antimicrobial effect according to the data of the present study. The presence of terpenoids in *P.oleracea*extract which has been identified to possess antimicrobial activity [7] could explain the antimicrobial activity of *P.oleracea*. Tannins has also shown antimicrobial as well as antioxidant properties. Vitamins have been reported for their beneficial effect on wound management [26]. First of all, vitamin A is often required for epithelial cellular differentiation, and immune system function. Such substantial evidence supports the use of this vitamin as a preoperative nutritional supplement. Secondly, vitamin C (ascorbic acid) enhances neutrophil function [27].Also, vitamin C and vitamin A are effective for the treatment of inflammatory dermatoses and wound healing [28].Also ascorbic acid is an important modulator of collagen production and acts as a cofactor for the hydroxylation of proline and lysine residues in procollagen [30]. Niacin causes the release of leptin, and downstream signaling of leptin has profound effects on epidermal renewal, wound healing and hair follicle biology in skin [31&32].The current data revealed that Hb concentration, red blood cells count and platelets count were substantially reduced in, in the positive control group compared with negative control group. This reduction could be attributed to the oxidative stress of STZ. In addition, the increased glycosylation of number of proteins including hemoglobin during diabetes could be another explanation [33].On the other hand, *P. oleracea* treatment improved the hematological parameters including hemoglobin, red blood cells count and platelets count which were very close to the normal subjects. This improvement may be due to antioxidant activity of *P. oleracea* extract. Also administration of STZ caused significant increase in the levels urea and creatinine in serum of the positive control group compared with untreated subjects which was consistent with the previous studies [34,35].For example, a study by Nogueira and colleagues (2005) reported reduced renal blood flow along with 2-3fold increase in plasma creatinine levels in day 14 and day 28diabetic rats administered STZ [35].Fortunately, constituents of *P. oleracea* extract were able to mitigate the adverse effect of STZ on kidney functions according our data. Indeed, flavonoids (quercetin), omega-3, ascorbic acid β-carotene and glutathione have antioxidant activitiy[36].which decrease the oxidative stress of STZ. Also, the presence of tannins, may play a role in preventing and protecting cells from oxidative damage, thereby augmenting the body's natural resistance to oxidative damage [37].It is worth mentioning that *P. oleracea* extract demonstrated as a protector against cisplatin-induced renal toxicity [12] which supports our findings, who found that

CONCLUSION

Ethanolic extracts of *Portulaca oleracea* showed promising effects on wound healing in the diabetic rats that can increase our therapeutic avenue for treatment of diabetic wounds that are often slow to heal.. *Portulaca oleracea* extract not only accelerates diabetic wound healing process but also supersedes fusidine antibiotic in this aspect .with adverse effects. More research is needed to better understand the beneficial role of *P. oleracea* as a rich source of antioxidants and other compounds that potentially benefits the human health.

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REFERENCES

[1] Thangavel Ponrasu, Muthaiya Kannappan Subamekala, Moorthy Ganeshkumar Lonchin Suguna: Role of Annona squamosa on antioxidants during wound healing in streptozotocinnicotinamide induced diabetic rats. Journal of Pharmacognosy and Phytochemistry; 2 (4) ,2013, : 77-84 .

[2] Diegelmann, R.F., Evans, M.C., . Wound healing: an overview of acute, fibrotic and delayed healing. Frontiers in Bioscience 9,2004. 283–289.

[3] Jacqueline Chor Wing Tama,b, Kit Man Laua,b, Cheuk Lun Liua,b,c, Ming Ho Toa,b,Hin Fai Kwoka,b, Kwok Kin Lai a,b, Ching Po Laua,b, Chun Hay,The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action., Journal of Ethnopharmacology 134 ,2011,831–838

[4] Naguib, G., Al-Mashat, H., Desta, T., Graves, D.T., Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. Journal of Investigative Dermatology 123,2004. 87–92.

[5] Goren, I., Muller, E., Pfeilschifter, J., Frank, S., Severely impaired insulin signaling in chronic wounds of diabetic ob/ob mice: a potential role of tumor necrosis factor-alpha. The American Journal of Pathology 168, 2006.765–777.

[6] Levin, M.E., Management of the diabetic foot: preventing amputation. Southern Medical Journal 95,2002, 10–20

[7] Prasanta K Ghosh1 and Anjali Gaba. Phyto-Extracts in Wound Healing. Pharm Pharm Sci (www.cspsCanada.org) 16(5) 2013,760 – 820

[8] Dweck AC., Purslane – Portulaca oleracea The global panacea. , 2001.

[9] Yan-Xi Zhou,1,2 Hai-Liang Xin,3,4 Khalid Rahman,5 Su-JuanWang,6 Cheng Peng,1 and Hong Zhang.Portulaca oleracea L.: A Review of Phytochemistry and Pharmacological Effects.BioMed Research International (2015) 2014, 1:11

[10] Xiang L., Xing D., Wang W., Wang R., Ding Y.and Du L. , Alkaloids from *Portulaca oleracea* L, *Phytochem.* 66, pp. 2005, 2595–2601.

[11] Kumar Arun , Alok Sharma, M. Vijayakumar, Ch.V. Rao ., Antiulcerogenic effect of ethanolic extract of portulaca oleracea experimental study . *Pharmacologyonline* 1: 2010, 417-432

[12] Gholamreza Karimi, Alireza Khoei, Abbas Omidi, Mahmudreza Kalantari, Javad Babaei, Elahe Taghiabadi, 3Bibi Marjan Razavi. Protective Effect of Aqueous and Ethanolic Extracts of Portulaca Oleracea Against Cisplatin Induced Nephrotoxicity. Iranian Journal of Basic Medical Sciences Vol. 13, No. 2, Spring 2010, 31-35

[13] Issac Sam Raj Punitha1, Arun Shirwaikar2, Annie Shirwaikar1, Antidiabetic activity of benzyl tetra isoquinoline alkaloid berberine in streptozotocin – nicotyinamide induced type 2 diabetic rats. Scientific Paper.journal(34) ,2006,117-128

[14] Ponrasu T, Suguna L., Efficacy of Annona Squamosa on wound healing in streptozotocin induced diabetic rats. Int Wound J 2012; 9: 613-623.

[15] Nayana Pinheiro Machado de Freitas Coelho, Veruska Cronemberger Nogueira, Maria Angélica Gargione Cardoso, Luciano da Silva Lopes, Paulo., Cenostigma macrophyllum Tul. on the healing of skin wounds in rats with Diabetes mellitus1., 594 - Acta Cirúrgica Brasileira - Vol. 2013, 28 (8).

[16] Levesque R. SpSS ., Programming and Data Management: A Guide for SPSS and SAS Users , Fourth Edition , 2007, SPSS Inc., Chicago

[17] Douglas MacKay, ND, and Alan L. Miller, ND: Nutritional support for Wound Healing. Alternative Medicine Review Volume 8, Number. 2003 ,359-377

[18] Thangavel Ponrasu, Muthaiya Kannappan Subamekala, Moorthy Ganeshkumar Lonchin Suguna., Role of Annona squamosa on antioxidants during wound healing in streptozotocinnicotinamide induced diabetic rats. Journal of Pharmacognosy and Phytochemistry; 2 (4): 2013, 77-84

[19] Simopoulos, A.P., Omega-3 fatty acids and antioxidants in edible wild plants, Biological Research 37, 2004, pp. 263–277.

[20] Lim Y.Y. and Quah E.P.L., Antioxidant properties of different cultivars of *Portulaca oleracea*, *Food Chem.* 103, pp. 2007, 734–740.

[21] Rashed AN, Afifi FU, & Disi AM., Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. Journal of Ethnopharmacology, 88: 2003, 131–136.

[22] Ghaisas MM, Kshirsagar SB and Sahane RS., Evaluation of wound healing activity of ferulic acid in diabetic rats, Int. Wound J, PMID: 23236955.2012

[23] Song HS, Park TW, Sohn UD, Shin YK, Choi BC, Kim CJ and Sim SS, The effect of Caffeic acid onWound Healing in Skin-Incised mice, Korean J. Physiol. Pharmacol, , 12(6), 2008, 343-347

[24] Vinothapooshan G. and Sundar K., Wound healing effect of various extracts of Adhatoda vasica, Int J of Pharm and Bio Sci, 2010; 1,530-536

[25] Trombetta D, Puglia C, Perri D, Licata A, Pergolizzi S, Lauriano E.R., De Pasquale A, Sajja A, Bonina F.P., Effect of polysaccharides from Opuntia ficus-indica (L.) cladodes on the healing of dermal wounds in the rat,Phytomedicine,2006; 13,352-358

[26] Agyare C, Lechtenberg M and Hensel A.,Composition of polysaccharides from aqueous extracts of some wound healing plants, Planta Med, DOI: 10.1055/s- 2011, 0031-1282204

[27] MacKay D, Miller AL., Nutritional Support for Wound Healing. Alternative Medicine Review 8(4), 2003,359-377

[28] Burgess C ., Topical vitamins. Journal of Drugs in Dermatology 7(7 Suppl):S, 2008, 2-6.

[29] Savini I, Catani MV, Rossi A, Duranti G, Melino G and Avigliano L. , Characterization of keratinocyte differentiation induced by ascorbic acid: protein kinase C involvement and vitamin C homeostasis. Journal of Investigative Dermatology 118(2), 2002, 372-379

[30] Kamer Erdinc , MD;1 Haluk Recai Unalp, MD; Omer Gundogan, MD; Gulden Diniz, Effect of Ascorbic Acid on Incisional Wound Healing in Streptozotocin-Induced Diabetic Rats. WOUNDS;22(2): ,2010, 27–31

[31] Jacobson EL, Kim H, Kim M, Williams JD, Coyle DL, Coyle WR, Grove G, Rizer RL, Stratton MS, Jacobson MK. , A topical lipophilic niacin derivative increases NAD, epidermal differentiation and barrier function in photodamaged skin. Experimental Dermatology 16(6), 2007, 490 -499

[32] Benavente CA, Jacobson MK, Jacobson EL. , NAD in Skin: Therapeutic Approaches for Niacin. Current Pharmaceutical Design 15(1), 2009, 29-38.

[33] Chandramohan G, Ignacimuthu S, Pugalendi KV., A novel compound from Casearia esculenta (Roxb.) root and its effect on carbohydrate metabolism in streptozotocin-diabetic rats. Eur J Pharmacol ; 590: 2008, 437-443

[34] Chen H, Brahmbhatt S, Gupta A, Sharma AC., Duration of streptozotocin-induced diabetes differentially affects p38-mitogen-activated protein kinase (MAPK) phosphorylation in renal and vascular dysfunction. Cardiovasc Diabetol; 4:2005, 3-15.

[35] Nogueira Junior FC, Coelho DA, Almeida MM, Silva TC, Ferreira EC, Macedo UB., Effect of tamoxifen in lipids of diabetic rats induced by streptozotocin. Acta Cir Bras 2005; 20: 114-120

[36] Cai Y, Luo Q, Sun M, Corke H (2004)., Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004; 74:2157-2184

[37] Christian Agyare, Eunice Baiden, John Antwi Apenteng, Yaw Duah Boakye, Louis Adu-Amoah , Anti-infective and Anti-inflammatory Properties of *Portulaca oleracea* L. Donnish Journal of Medicinal Plant Research Vol 2(1)2015, pp. 001-006