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Enhancement of Wound Healing by Un-denatured Camel Whey Proteins in Protein Malnourished Mice

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ABSTRACT

According to the WHO, malnutrition is estimated to contribute to more than one third of all children deaths. This study aimed to investigate the impact of a diet containing whey protein (WP) on wound healing in malnourished mice. Diets comprised either 5 g/kg protein malnourished (MAL) or 150 g/kg protein (control) for 3 weeks. WP-supplemented animals received the MAL diet for 3 weeks, followed by a 1-week treatment with a WP supplemented diet. Thereafter, full thickness skin wounds were punched below the shoulder blades of each mouse. Results demonstrated that MAL-mice showed a very sharp increment in their malondialdehyde (MDA) level and a significant decrease in glutathione (GSH) level compared to the control during the first 24 h. In contrast, WP-supplemented MAL-mice showed a significant decrease in MDA level and displayed an improvement in GSH level compared to the MAL-mice. mRNA levels of IGF-1 and CCL22 (wound healing macrophages [WHM]) were significantly down-regulated in MAL-mice, while regulatory macrophage marker (RM) CCL-1 and the classically activated macrophages (CAM) marker, CCLX10, were higher than the control. WP was found to significantly restore the phagocytic activity in MAL mice closer to that of the control mice. Histological investigation of the skin revealed that epidermal cell proliferation and migration, and dermal reorganization was gradually improved in WP animals. Thus, the time required for wound healing was shorter in MAL-mice supplemented with a WP diet than in MAL-mice. Data of this study may recommend camel WP as a food additive for enhancing wound healing post-surgical operations.

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Authors' Contributions
BAS and HE designed the work. HE executed the experiments with the help of JAT. IMA provided the lab facilities and the fund support.

Key words
Protein-malnourished mice, wound healing, whey protein, macrophage chemokines.

INTRODUCTION

Despite great clinical efforts, malnutrition remains one of the most devastating problems worldwide, particularly in developing countries (Solis *et al*., 2002). Malnutrition affects multiple organs (Zanim *et al*., 2003) and its induced morphological alterations have been thoroughly investigated. Protein malnutrition leads to multiple detrimental alterations of host immune responses and cytokines (Dai and McMurray, 1998). Thus, prolonged wound healing resulting from the production of excessive reactive oxygen species (ROS) is a serious complication of protein malnutrition.

A multitude of cellular events, such as cell proliferation, cell migration, contraction, extracellular matrix degradation and synthesis, must occur to achieve wound closure and regeneration of the injured dermis (Singer and Clark, 1999). Macrophages play a crucial role in the inflammatory phase of wound healing, secreting cytokines and chemokines, and supporting innate immune responses to bacteria. Classically activated macrophages (CAMs) are induced by recognition of microbial patterns (Padgett *et al*., 1998) and cytokines, while wound-healing macrophages (WHMs) secrete components of the extracellular matrix and express numerous markers of tissue-remodelling (Rodero and Khosrotehrani, 2010). Regulatory macrophages (RMs), meanwhile, show anti-inflammatory properties (Sironi *et al*., 2006). Several chemokines have been identified and proved to be expressed constitutively on macrophages**,** function in the physiological traffic and targeting of leukocytes (Baggiolini and Loetscher, 2000).

Abbreviations: CAM, classically activated macrophages; CCL, chemokine; CXCL-10, chemokine interferon-γ inducible protein (10 kDa); C-C, motif ligand; GSH, glutathione; IGF-I, insulin-like growth factor; MAL, malnourished; MDA, malondialdehyde; RM, regulatory macrophages; WHM, wound healing macrophages; WP, whey protein.

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The 10 kDa chemokine interferon-γ inducible protein (CXCL-10) is a member of the CXC chemokine family which binds to the CXCR3 receptor to exert its biological effects (Liu *et al*., 2011). It has been proved that CAMs express CXCL-10 and efficiently kill pathogens (Martinez-Gonzalez *et al*., 2008). CXCL-10 is involved in chemotaxis, induction of apoptosis, regulation of cell growth and mediation of angio-static effects. In the central nervous system, CCL-5 is a key regulator involved in inducing rabies encephalomyelitis (Huang *et al*., 2014). The chemokine (C-C motif) ligand 1 (CCL-1), is expressed in T-helper type-2 lymphocytes and peritoneal macrophages and is involved in various pathological conditions, including peritoneal adhesions (Oshio *et al*., 2014). The insulin (IGF-1) signalling pathway plays a critical role in stress resistance and longevity (Warnhoff *et al*., 2014).

Many studies have confirmed the role of glutathione, which is increased by the dietary whey protein (WP), as a powerful antioxidant system (Bounous, 2000; Ebaid *et al*., 2012). WP has been found to significantly suppress hydroperoxide and ROS levels in leukocytes as well as liver and cutaneous tissues in mice by restoring the glutathione to its normal level (Ebaid *et al*., 2012). Therefore, WP is able to stimulate the epithelization and proliferation of fibroblasts and increase the secretion of pre-inflammatory and post-inflammatory cytokines. Furthermore, un-denatured WP is a good source of the amino acid proline, which helps to produce collagen (Belokrylov *et al*., 1992). Hence it is accepted that the un-denatured WP can enhance the wound healing process in diabetics. Thus, we hypothesized that protein malnutrition alters the recruitment and functions of macrophages during wound healing, thereby impairing bacterial clearance and the healing process. The effect of protein malnutrition stress and dietary supplementation with WP on macrophage recruitment and its chemokine gene expression during wound healing was investigated in this study herein.

MATERIALS AND METHODS

Preparation of whey proteins

Camel milk from the Najd region (Alazeria farm; GPS: 300 02 47/300 02 27) in Saudi Arabia, was skimmed by centrifugation at 5000 g for 20 min using an IEC Model K centrifuge (Boston, USA) and un-denatured whey protein was extracted as previously performed (Ebaid *et al*., 2005).

Experimental animals and ethical approval

Regarding experimental animals, all procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The study protocol (care and handling of experimental animals) was approved by the Animal Ethics Committee of the Zoology Department in the College of Science at King Saud University.

Adult male mice were allocated into 3 groups of 15 animals each. Mice were housed in individual cages in a temperature- and humidity-controlled room (12-h light/dark cycle) with free access to tap water and diet. Mice were allowed for lab acclimatization for two days before initiation of dietary treatments.

Animal diet

Rodent powder diet was formulated to be isocaloric and contained either 5 g/kg protein diet (MAL) or 150 g/kg protein diet (control). The composition of the control and MAL diet has been published previously (Lim *et al*., 2006). The amount of WP added to the MAL diet was calculated so that the supplemented diet WP had the same amount of sulfur-containing amino acids as the control diet. Mice were fed control diet for 3 weeks. WP-supplemented animals received the MAL diet for 2 weeks, followed by a 1-week treatment with WP diet (Fig. 1).

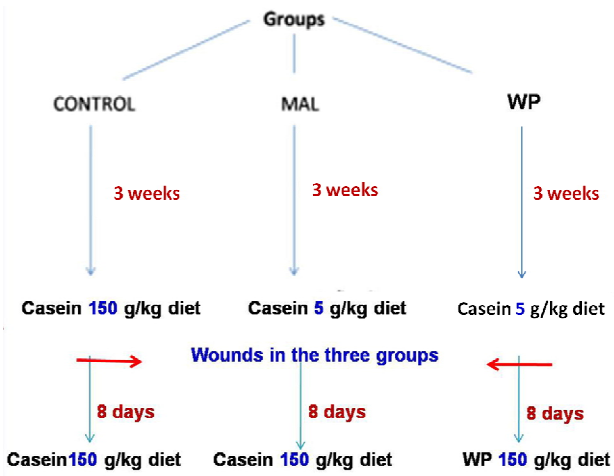

Fig. 1. Experimental design showing the three different mice groups according to the protein content in the experimental diet. MAL, malnourished; WP, MAL diet supplemented with why protein.

Excisional wound preparation

Mice were anesthetized, and the back was shaved and sterilized using an alcohol cotton swab. The wound biopsy model used in this experiment was performed as

previously described (Schwentker *et al.*, 2002) with slight modification. The shaved skin was pinched and folded, and the wound was punched through the full thickness of the folded skin to form a 5 mm diameter circle below the shoulder blades of each mouse.

Blood and tissue sampling

Animals were sacrificed, and blood samples were obtained from the carotid artery. Then, animals were decapitated and dissected, and their livers and skins were rapidly excised and kept at -20ºC for pending homogenization.

Estimation of glutathione and lipid peroxidation

Glutathione (GSH) assay was carried out on tissue as previously described (Clark, 1985). The peroxidation of the endogenous lipids in the liver homogenates was estimated spectrophotometrically following the method described by Okhawa *et al.* (1979) and expressed in nanomoles of malondialdehyde (MDA) per milligrams of homogenate (nmol/mg).

Histological analyses

Skin tissues were washed in PBS and immediately stored in a 10% formaldehyde solution in PBS, dehydrated in series of alcohol dilutions and embedded in paraffin. Microtome sections were cut vertically across the wound site and adhered to slides prior to staining with hematoxylin and eosin. Sections were examined under light microscopes and photographs of the sections in wound site were taken, and images were digitized using Adobe Photoshop (Adobe Systems, Mountain View, CA).

RNA extraction and reverse transcriptase PCR (RT-PCR)

RNA was extracted from the collected samples (RNAlater) using RNeasy Mini Kit (QIAGEN) according to the manufacturer instructions. RT-PCR was performed using

QIAGEN One Step RT-PCR kit as directed by the manufacturer's instruction manual. The targeted genes were amplified using specific primers (e-oligos, Hawthorne, USA) listed in Table I. For each sample, 25 µl reaction mixture was performed. PCR reaction was carried out using Gene-Amp 9700 thermal cycler. RT-PCR products were analyzed in 1.2 % agarose gel. The levels of the three mRNA and β-actin mRNA were quantified by gel electrophoresis and densitometry. mRNA levels were normalized versus β-actin and are expressed in arbitrary units.

Fluorescence microscopy

To detect the phagocytic activity in the inflammatory phase (4, 8, 24 h after wounding) of the skin tissues, fluorescence nano-particles were injected just one hour before sacrificing of mice. Skin pieces were embedded in OCT compound (Sakura, Zouterwede, The Netherlands) and snap frozen on dry ice before serial sections were mounted on superfrost plus slides (Menzel-Glaser, Braunschweig, Germany). Evaluation of fluorescence intensity was performed as previously described (Ebaid, 2014) using the Image J software.

Statistical analysis

The one-way ANOVA statistical measure was used because data were normally distributed with homogeneous variances. The results were expressed as mean (M) ± standard deviation (SD). Only statistically significant differences with $P<0.05$ were found between the treatment group and the control, and between the treatment group and the malnourished group considered.

RESULTS

Effect of whey on protein concentration

Because this work was designed on the basis of a protein-malnourished mouse model, it was necessary to estimate the total protein in the sera. Data showed that total protein concentration was significantly reduced in the protein-malnourished mice in comparison with the control mice (One way ANOVA, $P<0.05$). On the contrary, the protein concentration was normal in the sera of the protein-malnourished mice supplemented with WP comparing to the control mice (Fig. 2).

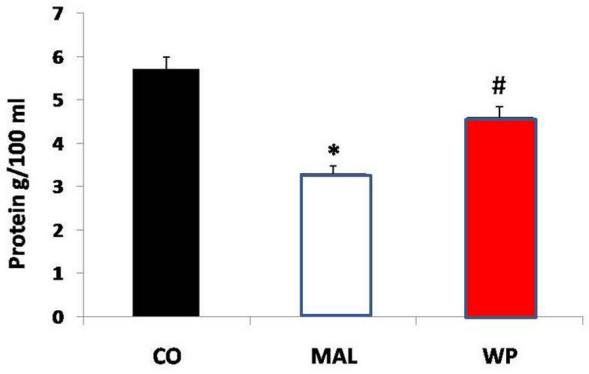

Fig. 2. Estimation of the total protein concentration in the sera of different mice groups. (CO, control; MAL malnourished; WP MAL diet supplemented with WP. * shows the significance in comparison to the control group.

Oxidative stress

Prolonged oxidative stress impaired the inflammatory phase of wound healing and thus delayed this process. Therefore, the oxidative stress in the

Table I.- List of primers used to amplify the desired genes.

Chemokine		Sequence	No. of nucleotide	Size of PCR band
CXCL-10	Forward	CCAAGTGCTGCCGTCATTTTC	21	157
	Reverse	GGCTCGCAGGGATGATTTCAA	21	
Ccl-5	Forward	GCTGCTTTGCCTACCTCTCC	20	104
	Reverse	TCGAGTGACAAACACGACTGC	21	
Ccl-22	Forward	AGGTCCCTATGGTGCCAATGT	21	11
	Reverse	CGGCAGGATTTTGAGGTCCA	20	
Ccl-1	Forward	GGCTGCCGTGTGGATACAG	19	219
	Reverse	AGGTGATTTTGAACCCACGTTT	22	
IGF-1	Forward	AAATCAGCAGCCTTCCAACTC	21	430
	Reverse	GCACTTCCTCTACTTGTGTTCTT	23	

malnourished mice and oxidative stability after supplementation with WP in the inflammatory phase (at 4, 12 and 24 h) and at the end of the experiment were estimated. MDA is the first stable product of lipid peroxidation and is therefore a good parameter to assess the oxidative stress in treated animals. MAL-mice showed very sharp increments in their MDA level of 8%, 38% and 17% with respect to the control at 4, 12 and 24 h, respectively. WP-supplemented MAL-mice showed a significant decrease in MDA level by 23%, 28% and 30% with respect to the MAL-mice at 4, 12 and 24 h, respectively (Fig. 3).

MAL-mice showed a significant decrease in GSH level by 44%, 25% and 19% with respect to the control at 4, 12 and 24 h, respectively. In contrast, WP-supplemented MAL-mice displayed improvement in GSH level by 56%, 34% and 30% compared to the MAL-mice at the same time periods (Fig. 3).

Wound closure rate

External changes in the wound morphology were monitored daily during the experimental period. The percentage of the protein-malnourished mice exhibiting wound closure was found to be significantly lower than that of the control mice at day 6. WP was found to be significantly normalizing the wound closure rate in the protein-malnourished mice to a level closer to that of the normal mice (Fig. 4).

Histological examination

Histological examination demonstrated that wounded tissues from the MAL mice appeared disturbed at two days post-wounding (Fig. 5), while those of the MAL mice supplemented with WP seemed similar to the control mice tissues. Four days after wounding, wound areas in the MAL mice exhibited an increased extent of wound margin neoepithelia, without obvious epidermal tongues. In contrast, the wound margin epithelia of MAL

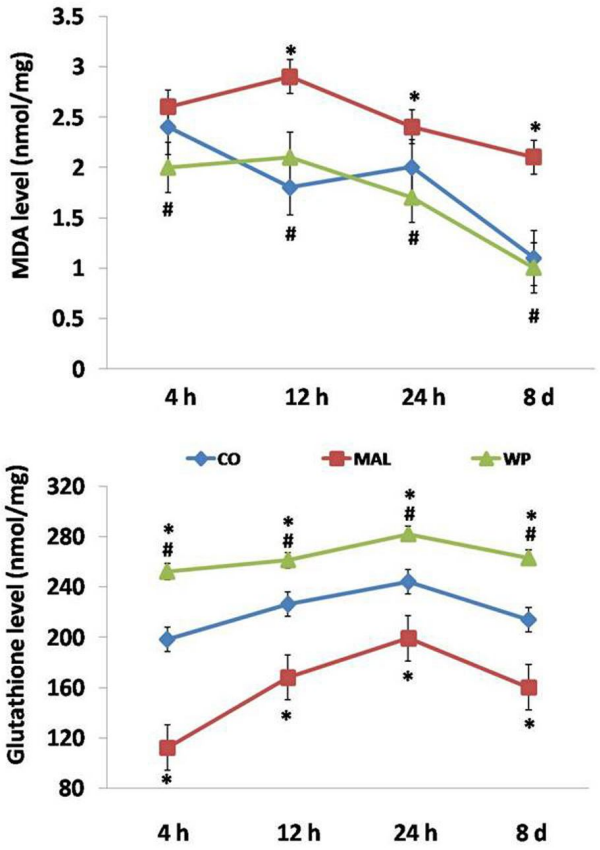

Fig. 3. Oxidative status in the early inflammatory stage of wound healing (4-24 hours) in malnourished and WP-supplemented malnourished mice. GSH and MDA were estimated in liver samples. D, days. * shows the significance in comparison to the control group. # shows the significance in comparison to the malnourished group.
For other abbreviations, see Figure 2.

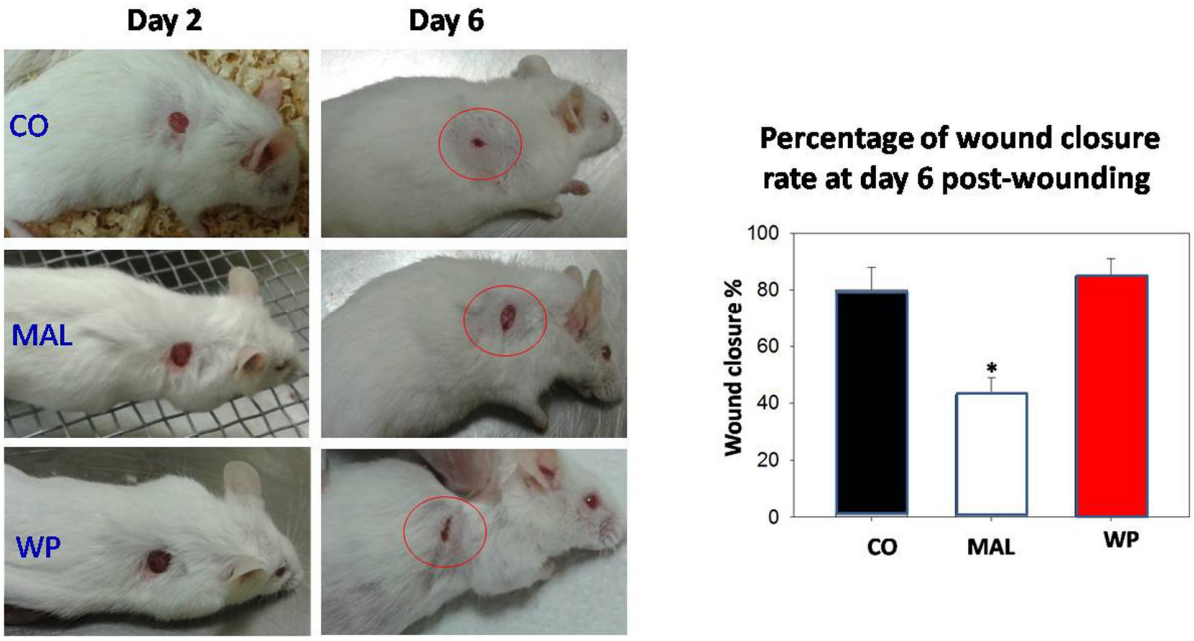

Fig. 4. The excisional wound preparation through the full thickness of the folded skin to form a 5 mm diameter circle below the shoulder blades of each experimental mouse. This figure shows wound closure rates from control (CO), malnourished (MAL) and mal-nourished mice supplemented with WP (WP) at days 2 and 6. * shows the significance in comparison to the control group.

supplemented with WP, showed an increase in both size and migration (Fig. 5) with two epidermal tongues, directed inwards, clearly visible on both sides of the wound. The migration of the epithelial cells at the edge of the wound in MAL mice supplemented with WP, therefore, showed a moderate degree of wound closure on the fourth day post-wounding while, by the eighth day, the wounds of MAL mice supplemented with WP were completely re-epithelialized, whereas epithelialization was still incomplete in untreated MAL mice.

Macrophage activity

To investigate the effect of stress on the phenotype of activated macrophages present at the wound site, gene expression of markers of wound healing macrophages (WHMs) (IGF-1 and CCL22), regulatory macrophages (RMs) (CCL1) and classically activated macrophages (CAMs) (CXCL-10 and CCL-5) were measured by RT–PCR in wounded tissue commencing from day 2 post-treatment. mRNA levels of IGF-1 and CCL22 were significantly lower in malnourished stressed mice at day 2 post-wounding (Fig. 6A). Both malnourished and WP-supplemented malnourished mice revealed higher CCl-1 gene expression than control (Fig. 6B). In mice supplemented with WP mRNA level of CCL-1 was 1.5 folds in comparison with the malnourished mice animals. mRNA levels of CCL-5 were not significantly changed in malnourished stressed mice at day 2 post-wounding in comparison with the control wounded mice. In malnourished mice, CCLX10 gene expression was higher (Fig. 6C) than the control, whereas in mice supplemented with WP mRNA levels of CCLX10 were higher in comparison with the control and malnourished mice (Fig. 6C).

To confirm the above data from whole wounds, macrophages were generally tested for the presence of the phagocytosis process at the wound sites. To evaluate the phagocytic activity, fluorescent nano-particles were injected 1 h prior to animal scarifice. Results revealed that phagocytic activity was significantly reduced in Mal-mice in comparison to control mice (One way ANOVA: $P < 0.05$) (Fig. 6D). Interestingly, WP was found to significantly restore the phagocytic activity in MAL mice to be similar to that of the control mice.

DISCUSSION

Strategies to reduce the physiological and cytological consequences of malnutrition is important to be investigated. The current study designed a protein malnutrition mice-model to determine whether dietary supplementation with WP could enhance normal wound

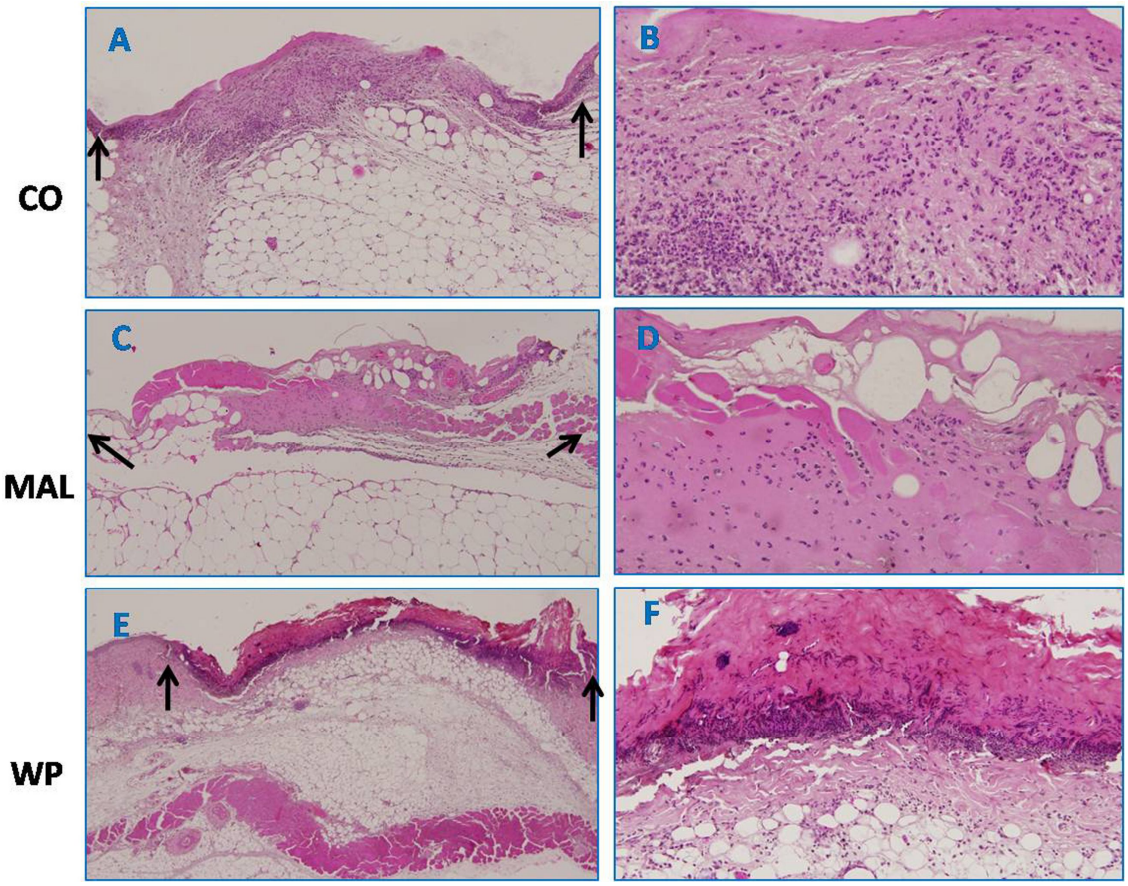

Fig. 5. Histological structure of wounds from different mice groups (A, C, E; H & EX100) and the neutrophil infiltration in the wound site (B,D,F; H& EX200) on day 2 post wounding.

healing. WP was found to reduce oxygen radicals and increase the levels of the antioxidant glutathione (Ebaid, 2014a,b; Ebaid *et al*., 2015). Our data indicated an increased capacity of the un-denatured WP-fed animals to normally heal wounds in the protein malnutrition mice-model.

Wound healing is initiated by an inflammatory phase (recruitment of leukocytes that produce growth factors), followed by a proliferation phase. The last phase involves the production and reorganization of the extracellular matrix leading to wound healing (Graves *et al*., 2001). This is stimulated by a number of mitogens and chemotactic factors (Altavilla *et al*., 2001). Here we found that MDA in malnourished mice was significantly higher than in normal mice. During the inflammatory phase, increased numbers of recruited leukocytes in the dermis of mice are able to cause tissue damage by prolonged release of ROS. This impairs keratinocyte endothelial cells, fibroblasts, and collagen metabolism (Silhi, 1998) which in turn, causes healing impairment (delayed granulation tissue formation, decreased collagen, and its organization) (Niki *et al*., 1991). This is probably what has occurred in protein malnourished mice. In contrast, WP was found to significantly elevate the concentration of glutathione which scavenges ROS. WP contains cysteine which is necessary for the synthesis of glutathione (Middleton *et al*., 2004) and this may be a potential factor controlling the healing process.

Because of their capacity to produce inflammatory cytokines and growth factors, macrophages play a central role in wound repair. WP was also found to significantly enhance phagocytosis in malnourished mice. Similarly, Michee *et al*. (2013) found that macrophages exposed to LPS and TNF-α had increased phagocytosis. Chemokine expressions, which are necessary for normal migration and chemotaxis of macrophages, declined in the first two days post-wounding in malnourished mice. In contrast, WP was found to up-regulate these chemokines in malnourished mice in the inflammatory phase of wound healing. Peritoneal macrophages isolated from mice

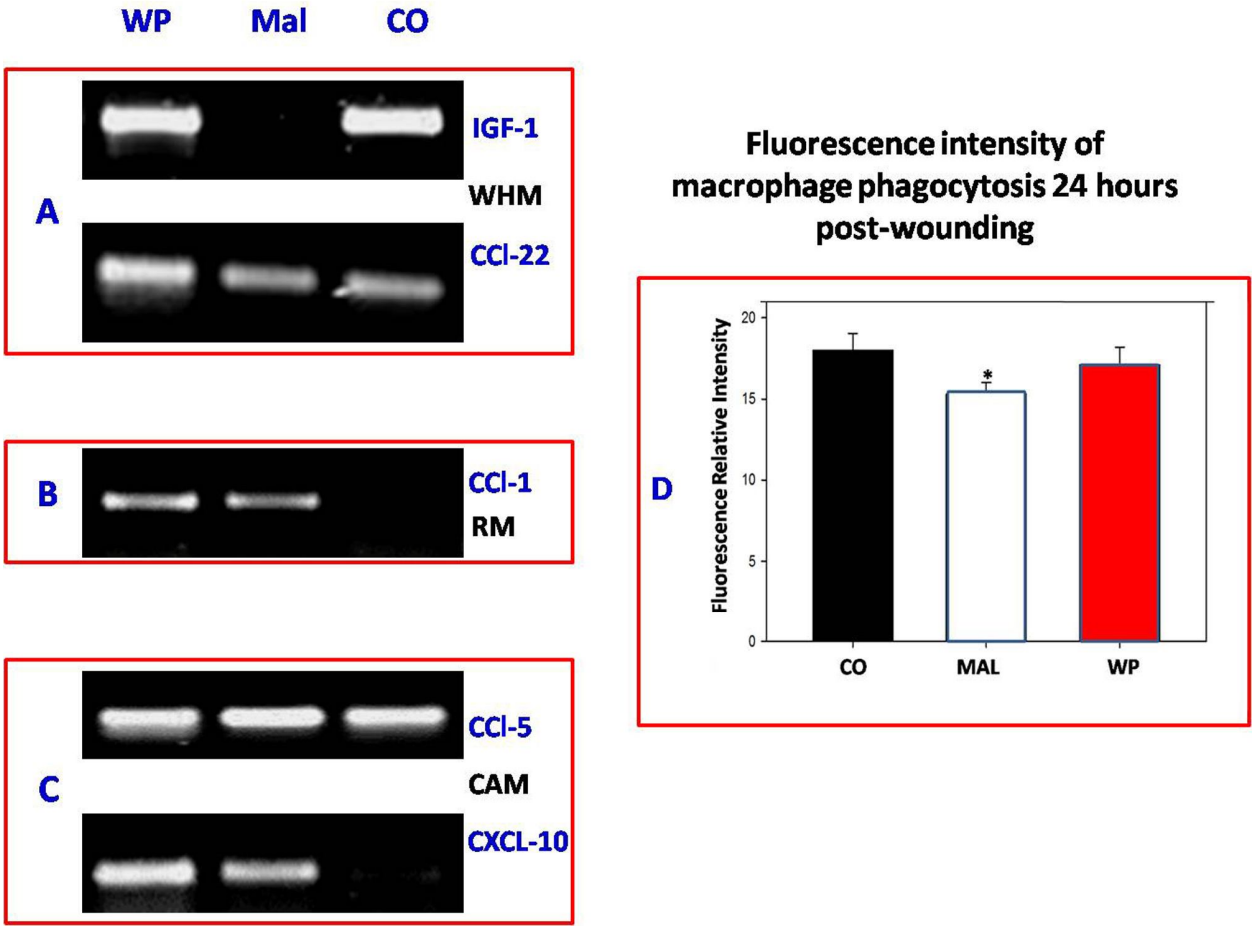

Fig. 6. Phenotype of activated macrophages present at the wound site. Gene expression of markers of wound healing macrophages (WHMs) (IGF-1 and CCL-22) (A), regulatory macrophages (RMs) (CCL-1) (B) and classically activated macrophages (CAMs) (CCL-5 and CXCL-10) (C), were measured by RT–PCR in wounded tissue from day two. D: Intensity of fluorescent nano-particles phagocytized by macrophages in the wounded dermis from different mice groups. * shows the significance in comparison to the control group.

showed increased mRNA levels of several inflammatory mediators, including, CCL-5 (Manoharan *et al*., 2014).

Moreover, results indicated that wound healing macrophages (IGF-1 and CCL22) were inactivated under the protein malnutrition conditions, while both regulatory macrophages (CCL-1) and classically activated macrophages (CXCL-10 and CCL-5) were alternatively activated under this stress in comparison with the control mice. WP was found to activate all macrophage phenotypes even under malnutrition stress, while WP moderated levels of IGF-1 and CCL22 closer to the control, significantly upregulated CCL1 and CCLX10 levels. However, to explain why CCL1 and CCLX10 were higher than that of the MAL mice after WP administration, may be due to increasing defence mechanism under malnutrition stress. In disease situations, however, prolonged expression of inflammatory chemokines and cytokines induce chronic complications. We previously found that although WP encourages inflammatory mediators at the beginning of wound healing, it finally suppresses these mediators in order to permit the ensuing normal recovery phases (Ebaid *et al*., 2011, 2013). Thus, it is expected that high levels of inflammatory chemokines after 4 days were suppressed by WP treatment. This suggestion can be proved by normalizing wound healing in WP mice.

Wound contraction is a significant component of dermal excisional wound healing in mice and in humans (Singer and Clark, 1999). WP is a good donor of the amino acid proline which helps in production of collagen

(Belokrylov *et al*., 1992). This might assist the normal development of the collagen fibrils in the wounded area of the mice. WP was found to significantly normalize the wound closure rate in the protein-malnourished mice to a similar level to that of the normal mice

In conclusion, the current study proved that camel un-denatured WP enhanced the wound healing process in malnourished mice. This could be probably because the efficiency of cysteine in increasing the glutathione level is greater when it is delivered in the WP (Bounous *et al*., 1989), and that the glutathione influenced the reduction of the oxidative stress and increased the macrophage inflammatory chemokines, which in turn enhanced wound healing.

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REFERENCES

Altavilla, D., Saitta, A., Cucinotta, D., Galeano, M., Deodato, B., Colonna, M., Torre, V., Russo, G., Sardella, A., Urna, G., Campo, G.M., Cavallari, V., Squadrito, G. and Squadrito, F., 2001. Inhibition of lipid peroxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. *Diabetes*, **50**:667-674.

Baggiolini, M. and Loetscher, P., 2000. Chemokines in inflammation and immunity. *Immunol. Today*, **21**:418-420.

Belokrylov, G.A., Popova, O.Y., Molchanova, I.V., Sorochinskaya, E.I. and Anokhina, V.V., 1992. Peptides and their constituent amino acids influence the immune response and phagocytosis in different ways. *Int. J. Immunopharmacol*, **14**:1285-1292.

Bounous, G., Batist, G. and Gold, P., 1989. Immunoenhancing property of dietary whey protein in mice: role of glutathione. *Clin. Invest. Med*., **12**:154-161.

Bounous, G., 2000. Whey protein concentrate (WPC) and glutathione modulation in cancer treatment. *Antican. Res*., **20**:4785-4792.

Clark, R.F., 1985. Cutaneous tissue repair: Basic biological considerations. *J. Am. Acad. Dermatol.*, **13**: 701-725.

Dai, G. and McMurray, D.N., 1998. Altered cytokine production and impaired antimycobacterial immunity in protein-malnourished guinea pigs. *Infect. Immunol.*, **66**:3562-3568.

Ebaid, H., Al-Tamimi, J., Metwalli, A., Allam, A., Zohir, K., Ajarem, J., Rady, A., Alhazza, I. and Ibrahim, K., 2015. Effect of STZ-induced diabetes on spleen of rats: Improvement by camel whey proteins. *Pakistan J. Zool*., **47**: 1109-1116.

Ebaid, H., Ahmed, O.M., Mahmoud, A.M. and Ahmed, R.R., 2013. Limiting prolonged inflammation during proliferation and remodeling phases of wound healing in streptozotocin-induced diabetic rats supplemented with camel undenatured whey protein. *BMC Immunol.*, **14**:31.

Ebaid, H., Hassanein, K.A. and El-Feki, M.A., 2005. The undenatured whey protein enhanced wound healing in mice. *J. Egyp. Germ. Soc. Zool.*, **47C**: 267-287.

Ebaid, H., Salem, A., Sayed, A. and Metwalli, A., 2011. Whey protein enhances normal inflammatory responses during cutaneous wound healing in diabetic rats. *Lipids Hlth. Dis*., **10**: 235.

Ebaid, H., 2014a. Neutrophil depletion in the early inflammatory phase delayed cutaneous wound healing in older rats: improvements due to the use of un-denatured camel whey protein. *Diagn. Pathol.*, **9**:46.

Ebaid, H., 2014b. Promotion of immune and glycaemic functions in streptozotocin-induced diabetic rats treated with un-denatured camel milk whey proteins. *Nutr. Metab. (Lond).*, **11**:31

Ebaid, H., Badr, G. and Metwalli, A., 2012. Immunoenhancing property of dietary un-denatured whey protein derived from three camel breeds. *Biologia*, **67**: 425-433

Graves, D., Nooh, N., Gillen, T., Davey, M., Patel, S., Cottrell, D. and Amar, S., 2001. IL-1 plays a critical role in oral, but not dermal, wound healing. *J. Immunol*., **167**:5316-5320.

Huang, Y., Jiao, S., Tao, X., Tang, Q., Jiao, W., Xiao, J., Xu, X., Zhang, Y., Liang, G. and Wang, H., 2014. Met-CCL5 represents an immunotherapy strategy to ameliorate rabies virus infection. *J. Neuroinflamm.*, **21**:146.

Lim, J., Hao, T., Shaw, C., Patel, A.J., Szabo, G., Rual, J.F., Fisk, C.J., Li, N., Smolyar, A., Hill, D.E., Barabasi, A.L., Vidal, M. and Zoghbi, H.Y., 2006. A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. *Cell*, **125**:801-814.

Liu, M., Guo, S. and Stiles, J.K., 2011. The emerging role of CXCL10 in cancer (Review). *Oncol. Lett*., **2**:583-589.

Manoharan, P., Basford, J.E., Pilcher-Roberts, R., Neumann, J., Hui, D.Y. and Lingrel, J.B., 2014. Reduced miR-124a and miR-150 levels are associated with increased pro-inflammatory mediator expression in KLF2-deficient macrophages. *J. biol. Chem*., **289**:31638-31646.

Martinez-Gonzalez, M.A., De, L.A., Fuente-Arrillaga, C., Nunez-Cordoba, J.M., Basterra-Gortari, F.J., Beunza, J.J., Vazquez, Z., BENITO, S., Tortosa, A. and Bes-Rastrollo, M., 2008. Adherence to Mediterranean diet and risk of developing diabetes: Prospective cohort study. *Br. med. J.*, **336**:1348.

Michee, S., Brignole-Baudouin, F., Riancho, L., Rostene, W., Baudouin, C. and Labbe, A., 2013. Effects of

benzalkonium chloride on THP-1 differentiated macrophages *in vitro*. *PLoS One*, **8**:e72459.

Middleton, N., Jelen, P. and Bell, G., 2004. Whole blood and mononuclear cell glutathione response to dietary whey protein supplementation in sedentary and trained male human subjects. *Int. J. Fd. Sci. Nutr*., **55**:131-41.

Niki, H.A., Jaffe, N., Imamura, T., Ogura, S. and Hiraga, S., 1991. The new gene mukB codes for a 177 kd protein with coiled-coil domains involved in chromosome partitioning of *E. coli*. *EMBO J.*, **10**:183-193.

Okhawa, H., Ohishi, N. and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*., **95**:351-358.

Oshio, T., Kawashima, R., Kawamura, Y., Hagiwara, T., Mizutani, N., Okada, T., Otsubo, T., Inagaki-Ohara, K., Matsukawa, A., Haga, T., Kakuta, S., Iwakura, Y., Hosokawa, S. and Dohi, T., 2014. Chemokine receptor CCR8 is required for lipopolysaccharide-triggered cytokine production in mouse peritoneal macrophages. *PLoS One*, **9**:e94445.

Padgett, D.A., Marucha, P.T. and Sheridan, J.F., 1998. Restraint stress slows cutaneous wound healing in mice. *Br. Behav. Immunol*., **12**: 64-73.

Rodero, M.P. and Khosrotehrani, K., 2010. Skin wound healing modulation by macrophages. *Int. J. clin. exp. Pathol*., **7**:643-653.

Schwentker, A., Vodovotz, Y., Weller, R. and Billiar, T.R., 2002. Nitric oxide and wound repair: role of cytokines. *Nitric Oxide*, **7**:1-10.

Silhi, N., 1998. Diabetes and wound healing. *J. Wound Care*, **7**:47-51.

Singer, A.J. and Clark, R.A., 1999. Cutaneous wound healing. *N. Engl. J. Med*., **341**:738-746.

Sironi, M., Martinez, F.O., D'Ambrosio, D., Gattorno, M., Polentarutti, N., LOCATI, M., Gregorio, A., Iellem, A., Cassatella, M.A., Van Damme, J., Sozzani, S., Martini, A., Sinigaglia, F., Vecchi, A. and Mantovani, A., 2006. Differential regulation of chemokine production by Fcgamma receptor engagement in human monocytes: association of CCL1 with a distinct form of M2 monocyte activation (M2b, Type 2). *J. Leukocy. Biol*., **80**:342-349.

Solis, B., Nova, E., Gomez, S., Samartin, S., Mouane, N., Lemtouni, A., Belaoui, H. and Marcos, A., 2002. The effect of fermented milk on interferon production in malnourished children and in anorexia nervosa patients undergoing nutritional care. *Eur. J. clin. Nutr*., **4**:S27-33.

Warnhoff, K., Murphy, J.T., Kumar, S., Schneider, D.L., Peterson, M., Hsu, S., Guthrie, J., Robertson, J.D. and Kornfeld, K., 2014. The DAF-16 FOXO Transcription factor regulates natc-1 to modulate stress resistance in *Caenorhabditis elegans*, linking insulin/IGF-1 signaling to protein n-terminal acetylation. *PLoS Genet*, **10**:e1004703.

Zanim, S.M., Molinari, S.L., Sant'ana, D.G. and Miranda, M.H., 2003. Neurônios NADH-diaforase po-sitivo do jejuno de ratos adultos (*Rattus norvegicus*) desnutridos. *Arq. Neuropsiquiatr*., **61**:650-653.