INTRODUCTION

A wound is defined as damage or disruption to the normal anatomical structure and function of tissues [1]. Chronic wounds are defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner. Often, chronic wounds stay in the inflammation phase of healing [2]. Wound healing, a natural restorative response to tissue injury, is governed by an elaborate response driven by resident and circulating cells, homing to the injury site that releases soluble mediators or signals generated from the extracellular matrix [3]. The wound healing process consists of four highly integrated and overlapping phases: Hemostasis, inflammation, proliferation, and tissue remodeling. These phases and their biophysiological functions must occur in the proper sequence, at a specific time and continue for a specific duration at an optimal intensity [4].

Platelets have important secretory functions, express internal membrane proteins, and release adhesive proteins, coagulation, and growth factors; platelets play an important role in inflammatory and proliferative events and play a critical role for tissue remodeling and wound healing [5]. Platelet-rich plasma (PRP) is a portion of the plasma fraction that has a platelet concentration above baseline values [6]. PRP works by secretion of growth factors following platelet activation and released growth factors bind to the external surface of cell membranes in applied tissue [5,6]. Uses of PRP in animals are steadily increasing, and its effects on tissue healing have been reported in many studies, particularly in bone regeneration and ligament or tendon reconstruction. Some studies have also described its healing effects in cutaneous wounds in horses, dogs, goats, and others animals species [8-13].

Platelet-rich fibrin (PRF) is classified as the second generation platelet derivative, unlike PRP; PRF is a strictly autologous fibrin matrix containing a large quantity of platelets and leukocytes. Its preparation technique does not require artificial or exogenous biochemical modifications such as the use of anticoagulants or bovine thrombin or any other jellifying agent [14]. PRF clot is obtained by inducing a natural polymerization process during centrifugation without the addition of anticoagulants, and due to this, the obtained fibrin clot has a very dense fibrin network in which platelets and leukocytes are entrapped and activated in a natural mechanism, thus releasing growth factors and cytokines in a slow rate during a period of 7 days or more [15].

METHODS

Animals

A total of 24 adult apparently healthy local breed bucks were used. Weighted 25–35 kg and aged 2–3 years, they were examined physically and clinically, all animals were housed under similar management conditions and feeding.

Preparation of PRP

The blood was drawn from the jugular vein carefully into syringe (18 mL) containing 2 mL 3.8% sodium citrate as anticoagulant [16] divided into two tubes of 10 mL and centrifuged (Electric centrifuge, China) at 2800 rpm for 15 min. This procedure divides the blood into three basic components: Red blood cells (RBCs) at the bottom, PRP in the middle, and platelet poor plasma (PPP) at the top. RBCs were isolated from the overlying buffy coat and plasma by the gel-like plug within the tubes.
From each tube (9 mL of blood) yielded approximately 4–5 mL of PPP. The buffy coat of each tube, contained mononuclear cells and platelets. The final solution, that obtained by mixing different buffy coats in a sterile 10 ml tube, was second centrifuged at 2800 rpm for 15 min for good separation of platelets in two layers with platelet in the supernatant layer. The platelet accumulates at the bottom of the tube, the PPP on top. The PPP is drawn off so that the PRP remains in the tube; the final PRP was obtained approximately 1 mL drawn up with an insulin syringe into Petri dish and platelets were activated by adding 0.2 mL of 10% calcium chloride to form PRP gel which was used in the wounded area [17].

**Preparation of PRF matrix**

The protocol for PRF preparation includes collection of whole venous blood (10 mL) in each of the two sterile tubes (10 mL) without anticoagulant and the tubes immediately were placed in a centrifugal machine at 3000 rpm for 10 min, after which it settles into the following three layers: Upper straw-colored a cellular plasma, red-colored lower fraction containing RBCs, and the middle fraction containing the fibrin clot. The upper straw-colored layer was removed and middle fraction was collected which was PRF. A fibrin clot is then formed in the middle between the RBCs at bottom and a cellular plasma at the top. The middle part is platelets trapped massively in fibrin meshes. The fibrin clot then withdrawn up with thumbs' forceps from tube and cutting off the RBC that adhered to it; the clot was squeezed between two sterile gauge pieces to obtain autologous fibrin membrane [18,19].

**Surgical procedure**

Food was withdrawn for 24 h and water restricted 12 h before surgery. The animals were controlled in standing position with light sedation using xylazine hydrochloride (Xyla–MD, Germany) in dose of 0.2 mg/kg b.w. IM and local anesthesia using inverted L technique in wound borders with lidocaine hydrochloride (lido 2%, India) [20], skin and subcutaneous tissues were removed to make four square full-thickness skin wounds (4 cm × 4 cm) on the dorsal sides of the back of each animal (two wounds on each side), 10 cm apart after preparation of the area in routine surgical manner one for treatment group and second as control group.

Under effect of local anesthetic using lidocaine hydrochloride spray 10% (lidocaine hydrochloride, Media, Syria), the wounds were daily scratched to interrupt healing process continuation to prolong inflammatory reaction to form chronic wound, this surgical procedure was continued for 8 weeks to ensure the chronicity [21], then the wounds treated using PRP in the first group (12 animals) and PRF for the second group (12 animals) which were prepared as previously described directly at time of treatment in 1-week interval.

**Clinical evaluation**

Physiological parameters exemplified body temperature, heart rate, respiration, and appetite were evaluation after inducing wounds and every day of treatment. Wounds were checked for evaluated such as swelling, inflammation, and infection during induction of chronic wounds and after treatment.

**Histopathological evaluation**

Biopsies were taken from edges and periphery of wounds and kept in 10% buffer formalin solution for histopathological study in the period
of 7, 14, 28, and 45 days postoperatively; then, these sections were processed for hematoxylin-eosin and Mallory’s trichrome stains to observe the healing prominence of wounded area.

RESULTS AND DISCUSSIONS

Clinical evaluation

In this study, both PRP gel and autologous of PRF matrix in the treatment of induced open chronic cutaneous wounds in bucks were used.

Clinical findings of the present study can be put into two categories as follows:

1. Period of inducing wounds: This period started from time of induced surgical wounds and left for 8 weeks with daily mechanical irritation to become chronic as referred by searchers which they refer to time of chronic wound such as Izadi and Ganchi and Reghini et al. [21,22] who revealed that the chronic wound take 6 weeks or more in repair process which reflects chronic state of healing.
Clinical follow-up in the period of induced chronic open wound showed, inflammatory signs in wounded area with systemic reaction, characterized by anorexia, depression and lethargy in the first three days post operation, disappeared gradually within first 3 days to become within normal values. While local inflammatory reaction persisted and were graded from slight to moderate inflammatory swelling with bloody clot formation, inflammatory exudate without signs of infection in all animals during this period except one case, which had scanty pus formation in one wound appeared in the first 3 days post operation, which was treated locally with povidone iodine 10% for 5 days and systemic antibiotic (20 mg/kg B.W. oxytetracycline).

2. Period of treatment: A complete clinical examination was performed on all animals daily during the treatment periods, the clinical finding showed no infection and all wounds continued on healing process without signs of complications in all animals. In both treated groups, the physiological parameters were in normal values (body temperature, appetite, heart, and respiratory rate) during study period, this result agrees with Kim et al. and Reghini et al.[9,23]. This result of two groups demonstrated that applying PRP and PRF matrix to chronic wound resulted in a significant healing effect, which may be related to platelets role in wound healing in hemostasis and initiation of wound healing, after platelet activation and clot formation, growth factors are released from α-granules including PDGF, bTGF-β, VEGF, FGF, IGF, and EGF and bioactive factors such as serotonin, histamine, dopamine, calcium, and adenosine are also stored in these dense granules these growth factors work as biologic mediators to promote cellular activity by binding to specific cell surface receptors [2,4,25]. DeRossi et al., 2009, who referred to the use of PRP gel in non-healing wounds in equine and in immunocompromised, diabetic or elderly individuals could provide quality healing of acute wounds [10], and Ferdousy et al., 2013, who revealed to the uses of PRP gel for the treatment of cutaneous wounds shown excellent results of healing in goat with skin wounds, and [26] Alisahi et al., 2013, demonstrated that PRF accelerated incisional wound healing in canine [27].

Histopathological evaluation

Inducing open wound

The histopathological examination of these group at 8 weeks showed hemorrhage in the epidermis, irregular few cellular collagen fibers, mononuclear cells around blood vessels in the wound, it also showed immature granulation tissue in the incision (H&E stain ×100X).

PRP group

The microscopical study at 7 days postincision treated with PRP showed thickened layer of epithelial cells extended over granulation tissue and under cellular debris with mature granulation tissue in the incision, deep blue color mature granulation tissue in the incision site with Mallory’s trichrome stain, while the histological examination of control group of PRP at 7 days of treatment showed thin layer of epithelial cells extended under cellular debris and over immature granulation tissue characterized by irregular cellular collagen fiber with numerous blood vessels as well as mononuclear cells infiltration.

On day 14, post-treatment of PRP showed complete epidermal layer over mature granulation layer and under cellular debris, dense thickness mature granulation tissue in the incision site (H and E stain), and deep blue color of mature granulation tissue in other section (Mallory’s trichrome stain ×400). In control group at 14 days after treatment with PRP showed mature granulation with moderate mononuclear cells aggregation around blood vessels (H and E stain ×100), other section showed neutrophils and mononuclear cells infiltration in the mature granulation tissue in the incision (H and E stain).

On day 28th of treatment, histopathological evaluation was characterized by complete layer of epidermis over mature granulation tissue (H and E stain), and very dense blue color collagen fibers (Mallory’s trichrome stain), other section showed complete layer of epidermis with rete ridge over mature granulation tissue (H and E stain). The control group at this time showed mature granulation tissue in the wound site (H and E stain ×400). Few mononuclear cells infiltration in the wall of blood vessels.

On day 45, the histopathological section showed complete thickness of epidermal layer over dense collagen fiber (H and E stain) and blue color of dense collagen fiber on special stain. In control group, there was dense collagen fibers in the wound site with mononuclear cells aggregation around blood vessels in the dermal layer.

PRF matrix

The histopathological evaluation of PRF group on 7th day of treatment showed thick layer of epithelial cells extended over granulation tissue and under cellular debris infiltrated by few mononuclear cells (H and E stain), another section showed granulation tissue in the dermis with complete epidermal layer and showed deep blue color collagen fiber with Mallory’s trichrome stain. In control group of PRF on day seven showed moderate mononuclear cells aggregation around blood vessels, and another section showed mononuclear cells infiltration in the immature granulation tissue in the wound.

On day 14 post-treatment showed thickened layer of epithelial cells extended over granulation tissue and under cellular debris infiltrated by few mononuclear (H&E stain) (Fig. 4.25). Another section also showed dense thick mature granulation tissue (H&E stain) and on the special stain appeared as deep blue stained collagen fibers in the wound site.

On day 28 post-treatment, there was complete layer of epidermis over mature granulation tissue and in the other section showed complete layer of epidermis with rete ridge over mature granulation tissue (H and E stain ×400) and deep blue color collagen fiber (Mallory’s trichrome stain ×400). Control group showed mononuclear cells aggregation around blood vessels in the dermal layer (H and E stain ×400).

On day 45 postincision, there was showed complete thickness of epidermal layer over dense collagen fiber (H and E stain ×400) and in special stain appeared blue color of collagen fiber and in control group showed dense collagen fiber with mononuclear cells aggregation around blood vessels in the dermal layer (H and E stain ×400).

The histopathological evaluation of this study of PRP and PRF matrix in both control and treatment groups revealed that chronic wound healing process was superior with clear of inflammatory reaction in treatment group. This may be due to both PRP and PRF matrix ability of enhancing wound healing process and high contains of growth factors and activation of these factors such as release of VEGF a mediator of angiogenesis that stimulates endothelial cell proliferation. PDGF also stimulates the production of fibrotin, a cell adhesion molecule used in cellular proliferation and migration during wound healing these results are supported by other studies revealed the effect of PRP and
PRF on acute and chronic wound healing and tissue repair in different animals’ species and human medical studies [28-31].

CONCLUSION
The use of PRP gel and PRF matrix as improved therapy for open chronic or non-healing wounds accelerates epithelialization and scar formation; this study demonstrates the beneficial effect of both PRP and PRF as a biological wound healing enhancer.

REFERENCES