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Role of platelet rich plasma gel in the wound healing of black Bengal goat

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Abstract: *The study was conducted to evaluate the efficiency of platelet rich plasma (PRP) gel in the healing of skin wound.This experiment was carried out to exploit the therapeutic effects of PRP gel on healing process. A total of 72 surgical wounds were madein 12 goatsbearing weight 15-20 kg with the age 1-3 years. Goats were divided into four groups with three animals in each group. Wounds of four groups were treated with homogenous PRP gel, heterogenousPRP gel, sulphonilamide powder and tincture benzoinCo. or benzoin seal (control). The wounds were 3 cm length and 0.5 cm depth sutured with cross mattress using nylon. Follow-up observation was recorded from day 1 to day 30 postoperatively. Some morphological characters such as swelling area of wound, elevation of sutured line from the skin surface, width of sutured area and contraction length between 7 to 15 days were recorded to determine the healing process of the wounds. Besides morphological study, histopathological studies were also performed on day 1, day 2, day 3, day 5 and day 15 to evaluate the healing process.Homogenous PRP gel showed admirable results in the healing of the wounds produced in goat. All wounds treated with homogenous PRP gel showed no inflammation with dry sutured area and complete healing with massive hair follicular growth within 15-21 days. On histopathological study, demonstrated epithelial tissues with a normal morphology. The dermis showed flabby connective tissue with organized interconnecting collagen fibers running parallel to each other and new hair follicular growth inhealed area within 15 days, when wounds were treated with homogenousPRP gel.This study could help to consider natural biomaterial product specially PRP gel homogenous for a good healing of skin wounds with minimum complications. Further studies are necessary for the molecular investigation of healing process and proper establishment of commercially available of PRP gel and easiest finding theway of it's application.*

Key words:*black Bengal goats, platelet rich plasma gel, skin wound, healing*

I. Introduction

Wound is a consequence of wound fatal if not treated timely and are aggravated by secondary bacterial infection. Improper development of epithelial tissues, angioblasts and fibroblasts also the facts of wound complications[1]. Sometimes the healing of wounds are delayed and the success of the wound healing added additional taxes to the owners. Various biomaterials, proteins, antibiotics, vitamins and minerals come to the scene as the angel to accelerate the repair of the wound by stimulating angiogenesis, fibroblastosis and epithelialization of wound[2],[3],[4],[5].Platelets have been demonstrated to be the natural source of several growth factors and cytokines that promote blood coagulation, tissue repair, and the process of bone mineralization [6],[7],[8],[9],[10]. Platelet-rich plasma (PRP) is the second cost effective source of growth factor that effectively hemostasizes and stimulates cellular regeneration[9], [11],[12],[13].Application of PRP is a new approach for tissue regeneration used as gel formulation containing different bioactive substances[10],[14].PRP gel exhibits rapid epithelial differentiation and enhance organization of dermal collagen in fresh wound [15]. Degranulation of platelets causes release of transforming growth factor-ß1 (TGF-ß1), platelet-derived growth factor (PDGF), fibrinogen, epidermal growth factor (EGF), histamine, and hydrolytic enzymes[9],[15], [16],[17].These are involved in the angiogenic cascade which assists in hard and soft tissue wound healing [7][9].PRP containing various growth factors, may speed up woundhealing in goats[18]. Moreover, there is limited number of researches of the use of PRP gel in goats. Therefore, the research theme was directed tofind out the easiest way of PRP gel formulation and exploit the therapeutic effects of PRP gel in skin wound of black Bengal goats.

II. Materials And Methods

A number of experiments were performed in black Bengal goat to find out the effect of platelets rich plasma (PRP) gel in healing of skin wounds. The proposed research works were conducted at the Department of

Surgery and Obstetrics, at the Bangladesh Agricultural University (BAU), Mymensingh. The duration of the study was from November, 2012 to July, 2013.

2.1Experimental animals

Twelve apparently healthy black Bengal goats were used for the experiment. The age of goats ranged from 1-3 years and body weight ranged from 15-20 kg. All the goats were maintained hygienically in animal shed of the Department of Surgery and Obstetrics. All of the experimental goats were dewormed with levamisol hydrochloride and triclabendazole (Bol. Lezoil-4®, FnF Pharmaceuticals) and vaccinated with Tetavex.

2.2 Preparation of Platelets Rich Plasma (PRP) Gel

Reference [15] shows the preparation procedure of the PRP gel. Fresh blood (10 ml) was collected from jugular vein of goat in each separation of plastic syringe before surgery.The blood was preliminary stored in Falcon tubes containing 3.8% sodium citrate anticoagulant. The tubes were centrifuged at 3000 rpm for 10 min promoting the separation of the plasma from red blood cells. After that, 2 ml of plasma was removed from the superior part of each tube and was taken in another tube called tube A. This part was used to obtain the autogenous thrombin. 600 µl of 10% calcium gluconate was added in tube A and incubated at at 37 ºC for 15 min. Total volume of plasma in tube A is thrombin rich substrate.The remaining plasma with white blood cells was transferred to another tube called tube B to obtain the platelet rich plasma (PRP). This tube was maintained at room temperature. PRP containing tube B was resuspended and homogenized by voltexing.Then contents of A and tube B mixed at the ratio of 1:2.After 40 min resting at room temperature the PRP gel was formed.

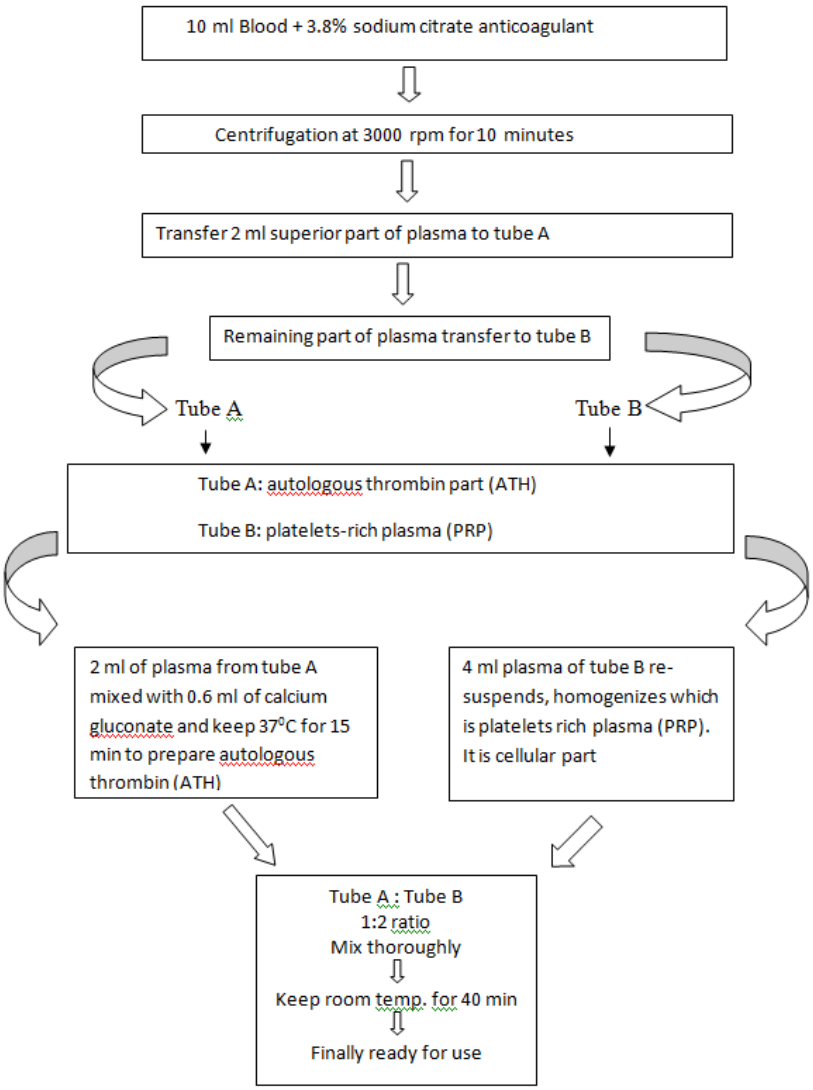

Fig.1 Schematic diagram of the preparation of the platelets-rich plasma (PRP) gel

2.3 Experimental design

A total of 72 skin wounds were created on the skin of twelve goats. Goats were divided into four groups with three animals in each group. All works have been repeated for three times in each group. Wounds created in each goat was 3cm length and 0.5cm depth.

Table 1: The experimental protocol of wound healing in goat

Group	No. of animals	No. of wounds
A.**Homogenous PRP gel**	3	(3X6)=18
B. **Heterogenous PRP gel**	3	(3X6)=18
C. **Sulphonilamide powder**	3	(3X6)=18
D.**Tincture benzoin solution**	3	(3X6)=18

All wound were closed with cross mattress pattern using nylon suture. Use of antibiotic, antihistaminic or anti-inflammatory drug was avoided to mitigate their effect on healing process. Follow-up information was recorded from the day of surgical operation up to day 30 after surgery. Morphological characters such as area of inflammation, swelling, elevation of suture line from skin surface, width of sutured area (cutting edge) to determine contraction length of suture line were recorded to determine the healing process. Swelling of wound was observed up to three days after operation. Elevation of suture line was recorded after 7 days during removal of stitches and that of characteristics of contraction was noted after 30 days of operation. To determine the length of wound contraction, width of sutured area were measured from day 1 (D_1), day 3 (D_3), day 5 (D_5), day 7 (D_7), day 15 (D_{15}), day 30 (D_{30}) of operation. Along with the physical characteristics, histopathological examinations were also conducted.

2.4 Preparation of surgical wound

All required instruments and appliances were Sterilized. Artificial fresh wounds, 3 cm length and 0.5 cm width were made following aseptic measures.One of four treatments was randomly assigned to each wound in each animal**:** (1) application of homogenous PRP gel, (2) application of heterogenous PRP gel, (3) application of sulphonilamide powder and (4) application of benzoin Co. in the subcutaneous tissue and the wound were closed in a cross mattress pattern with 2.0 nylon. Repeated wounds were made at least after 15 days in another animal.All sutures were placed 8 mm apart. Distance from the incision line to needle placement was 5 mm. Sutures were removed at day 7 of post operation. All wounds were closely monitored.

2.5 Observation of wound

Slide calipers were used to measure swelling area (mm), elevation of suture line (mm) and the contraction length of those surgical wound (mm). Healing score was categorized as:**Excellent**- no inflammation, no infection, no edema, no dehiscence, gradual decreasing of width of cutting edge and contraction length. **Good**- minimam inflammation with maximum exudation, no dehiscence, gradual decreasing of width of cutting edge and contraction length.**Fair**- marked inflammation, edema, presence of infection.All wounds were closely monitored daily to observe any complication.

2.6 Biopsy and Histopathology

For histopathological study, tissue samples were collected from treatment groups as well as control at day 1 (D_1), Day 2 (D_2), day 3 (D_3), day 5 (D_5) and day 15 (D_{15}) respectively. The biopsies (1.5cm X 1cm) were collected from wound area of each experimental animal. The biopsy sites were cleaned with saline solution and gauze. The sample were fixed in 10% PBS buffered formalin solution more than seven days for histopathology.

2.7 Statistical analysis

Statistical analysis were done by using utilized SPSS statistical software. All data were presented as mean±SEM. Total 18 data for each group, have been repeated for three times. To compare data among groups, one way ANOVA (Analysis of Variance) factor analysis was performed. For all statistical tests, p-value was 0.05.

III. Results And Discussion

Seventy two incised skin wounds were created and treated with homogenous platelet rich plasma (PRP) gel, heterogenous PRP gel, sulphonilamide powder and tincture benzoin to evaluate their effect on wound healing in goats.

3.1 Morphological changes in skin wound

In this experiment, four parameters of wound healing swelled area (mm), elevation of suture line (mm),

wound contraction and width of sutured area (mm) of wounds were evaluated postoperatively. Swelling was observed up to three days of operation because swelling started decreasing gradually from day 3 (D_3). Among four groups, negligible swelled area (6.84±0.20 mm) was observed in wounds treated with homogenous PRP gel. On the other hand, more swelled area (11.19±0.19 mm) was observed in wounds of control group which was significantly ($P<0.01$) higher (table 2). The homogenous PRP gel treated group showed admirable result. Elevation of suture line was recorded at day 7 (D_7) after removal of sutures.. Similar trend was observed in case of elevation of suture line. In this study, suture bite was 0.5 mm followed in all wounds. The sutured line were highly elevated 4.07±0.16 mm in sulphonilamide powder group, 3.92±0.11 mm elevation found in tincture benzoin group at day 7. The elevation of suture line in homogenous PRP gel treated group was 2.53±0.13 mm (Table 2).The differences in swelled area in the wound are there is lower elevation in homogenous PRP group among the groups. Swelled area, elevation of suture line and contraction length recorded in different groups are shown in Table 2. Contraction length was recorded for 8 days starting from day 7 (D_7) to days 15 (D_{15}) and it was observed that highest contraction length 3.02±0.19 mm in control group and lowest contraction length 2.30±0.16 mm in homogenous PRP group. The variation in contraction length among the groups is significantly significant ($P<0.05$). The width of sutured area was recorded from day of operation to 30th day of postoperation to understand the contraction of healing process. Width of sutured area of four groups increased up to day 3 and then decreased gradually (Table 3). Wounds treated with tincture benzoin had higher diameter of sutured area (10.84±0.25 mm) in comparison to that of wound treated with homogenous PRP gel which was lower diameter (9.23±0.29 mm) up to day 3 and the variation was statistically significant ($p<0.05$). Later width of sutured line decreased gradually up to day 30.

Table 2: Effects of PRP gel on wound healing in goats.

Groups	**Swelling suturing area** (Mean±SEM)	**Elevation of sutured line (mm)** (Mean±SEM)	**Average contraction Length** (Mean±SEM)	**Observation**	**Healing score**
Homogenous PRP gel	$6.84±0.20^{c}$	$2.53±0.13^{b}$	$2.30±0.16^{b}$	Dry suture surface, no inflammation, high contraction of wound, early healing	Excellent
Heterogenous PRP gel	$10.61±0.11^{b}$	$3.77±0.19^{a}$	$2.43±0.19^{b}$	Moist suture surface,more inflammation, moderate healing	Fair and Good
Sulphonilamide Powder	$10.11±0.34^{b}$	$4.07±0.16^{a}$	$2.31±0.16^{b}$	Moist suture surface,more inflammation, and moderate healing	Good
Benzoin seal	$11.19±0.19^{a}$	$3.92±0.11^{a}$	$3.02±0.19^{a}$	Moist suture surface,more inflammation, infection and delayed healing	Good

Values bearing different superscripts within a column differ significantly ($p<0.05$).

Table 3: Effects of PRP gel on width (mm) (Mean±SEM) of surgical wounds in goats.

Groups	**D_1**	**D_3**	**D_5**	**D_7**	**D_{15}**	**D_{30}**
Homogenous PRP gel	$8.61±0.30^{b}$	$9.23±0.29^{c}$	$7.83±0.22^{c}$	$6.27±0.19^{c}$	$3.91±0.16^{c}$	$1.63±0.11^{c}$
Heterogenous PRP gel	$10.27±0.19^{a}$	$10.47±0.17^{b}$	$8.93±0.15^{b}$	$7.88±0.21^{b}$	$5.45±0.19^{b}$	$3.27±0.17^{b}$
Sulphonilamide Powder	$9.77±0.19^{a}$	$9.97±0.13^{b}$	$8.58±0.14^{b}$	$7.52±0.17^{b}$	$5.21±0.24^{b}$	$3.02±0.18^{b}$
Benzoin seal	$10.05±0.31^{a}$	10.84±0.25a	$10.10±0.27^{a}$	$9.16±0.33^{a}$	$6.19±0.20^{a}$	$3.75±0.14^{a}$

Here, Day 1=D_1, Day 2=D_2, Day 3=D_3, Day 5=D_5, Day 7=D_7, Day 15=D_{15} andDay 30=D_{30}
Values bearing different superscripts within a column differ significantly ($p<0.05$).

The preparation of the PRP gel was simple without using sophisticated materials or instruments were used. PRP gel prepared from self blood homogenous group was very good medicine in case of wound healing without any post-operative complications.

3.2Histopathological changes in wound healing

Presence of reactive cells, accumulation of exudates, regeneration of epidermis, proliferation of fibrous

connective tissue were observed to understand the normal healing process. The infiltration of reactive cells including neutrophils, macrophages, and lymphocytes were present in all samples of four groups collected at D_1 (Figure 2), which indicated presence of inflammation. At D_1 massive amount of inflammatory cellswere accumulated at suture site of wounds treated with homogenous PRP gel in opposed to other three groups.Reactive cells decreased gradually in wounds of homogenous PRP group, exudates were accumulated and tissue reaction had occurred at D_2. Proliferation of fibrous connective tissue and migration regenerative tissue were observed markedly in wounds of homogenous PRP gel group at D_3. An interesting finding was observed in samples of homogenous PRP gel group at D_5, where newly formed keratinized layer of epidermis along with newly formed connective tissue was found under the scab infiltrated with reactive cells(Figure 3a). Histological findings of other three groups at D_5 postoperatively indicated comparatively delayed healing of wounds (Figure 3b, 3c and 3d). Homogenous PRP group at D_{15} demonstrated epithelial tissues with a normal morphology. The dermis showed flabby connective tissue with organized interconnecting collagen fibers running parallel to each other and new hair follicular growth (Figure 4a). In contrast disruption of epithelial covering, newly formed keratinized layer of epidermis and broken epithelial covering keratin layer in sample of heterogenous PRP gel, sulphonilamide powder and benzoin Co. treated respectively (Figure 4b,4c and 4d).

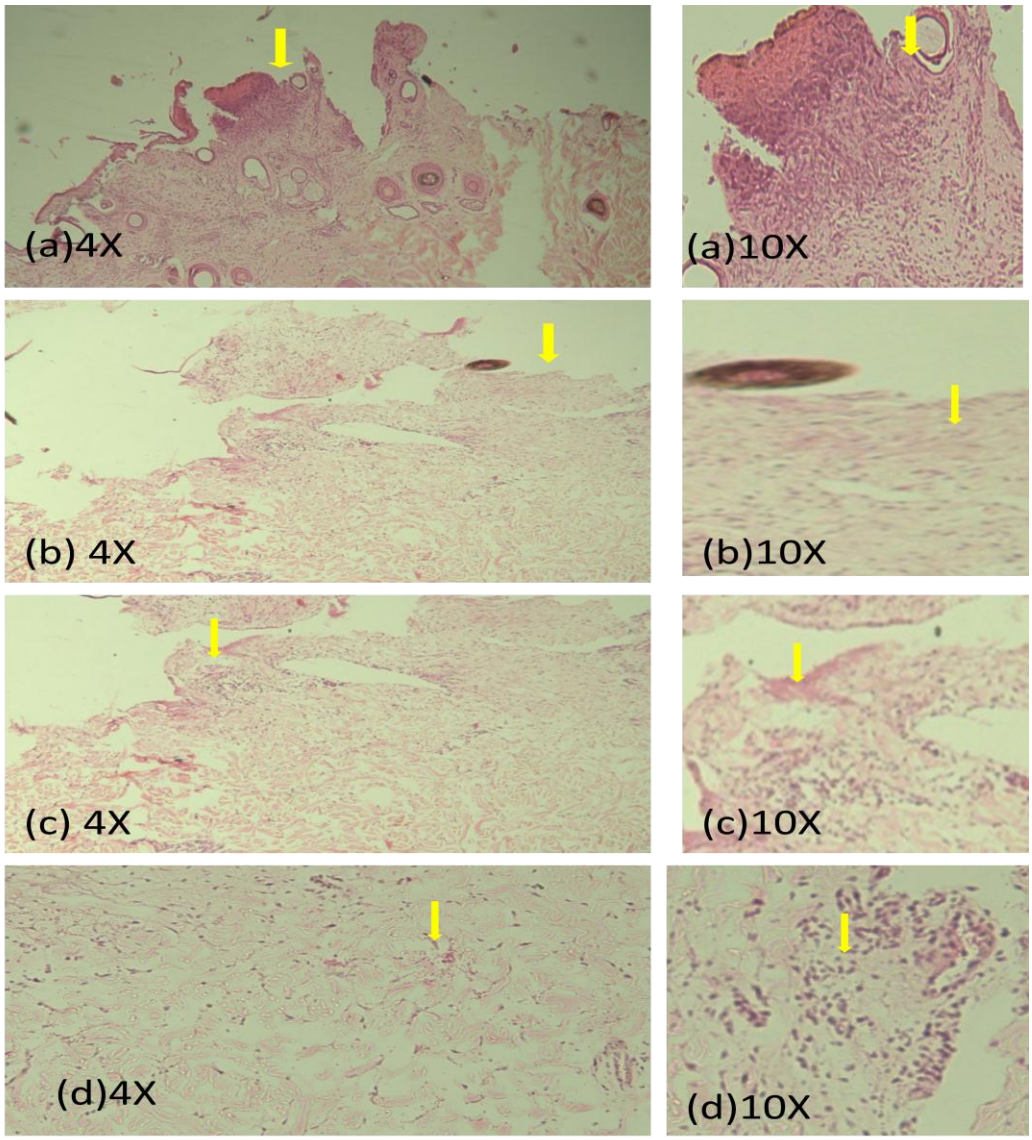

Fig.2(a) Presence of massive reactive cells (yellow arrows) beneath the keratinizes tissues of epidermis of wounds of homogenous PRP gel, (b) presence of reactive cells (arrows) beneath the keratinizes tissues of epidermis of wounds of heterogenous PRP gel (c) sulphonilamide powder and (d) control groups at **day 1** (D_1) after treatment. (magnification 4X, 10X).

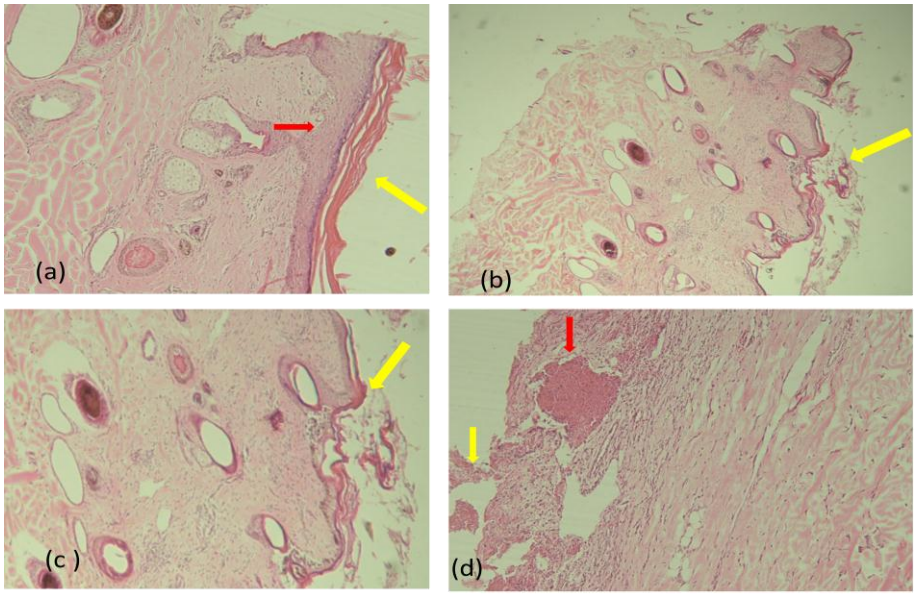

Fig.3(a) Newly formed keratinized layer of epidermis (yellow arrow) along with newly formed connective tissue under the scab infiltrated with reactive cells (red arrow) in wounds of homogenous PRP gel, (b) reduced number of reactive cells and tissue reaction (yellow arrow) in wounds of heterogenous PRP gel, (c) tissue reaction (yellow arrow) in wounds of sulphonilamide powder and (d) huge number of reactive cells and hemorrhage in wounds of control groups collected at **day 5** (D_5) after treatment.

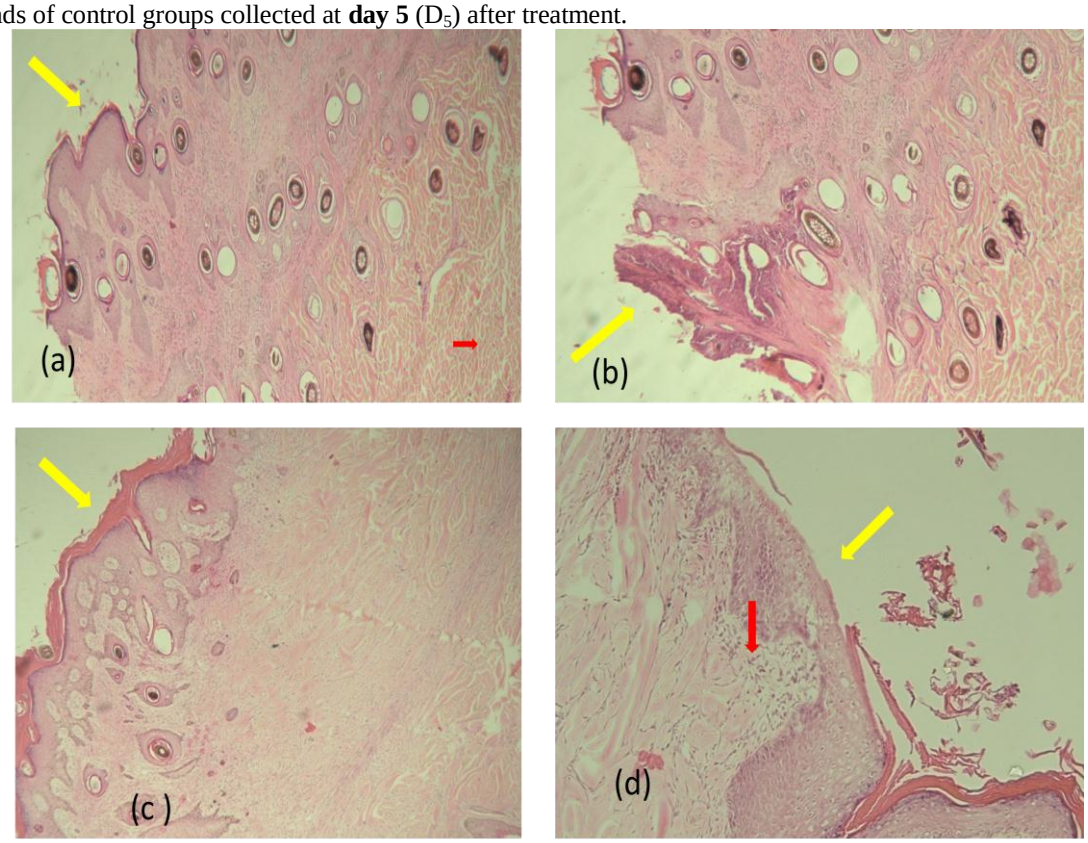

Fig.4(a) Epithelial tissues with a normal morphology and complete healing (yellow arrow), the dermis shows flabby connective tissue with organized interconnecting collagen fibers running parallel to each other and with thin collagen fibrils with discrete inflammatory infiltrate (red arrow) and new hair follicular growth (black arrow) in wounds of homogenous PRP gel, (b) disruption of epithelial covering in wounds of heterogenous PRP gel, (c) sulphonilamide powder treated group newly formed keratinized layer of epidermis along with newly formed connective tissue and (d) broken epithelial covering keratin layer is (yellow arrow) and dermis with flabby connective tissue disorganized with healing area, intermixing of fibrous connective tissue and collagen fibre (red arrow) in wound of control group at **day15** (D_{15}) after treatment.

4.3. Other observations of wound healing

In our study, according to healing score scale, all sutured wounds treated with homogenous PRP gel maintained excellent healing score,contraction of cutting edges and reddish area have dry sutured area (Figure 5a) and almost complete healing at day 7 (Table 2) with massive hair follicular growth (Figure 5b) in wounded area at day 15-21.While, prominent scar formation on the wound surface (Figure 5c) was observed in tincture benzoin treated group at day 15-21 postoperatively.

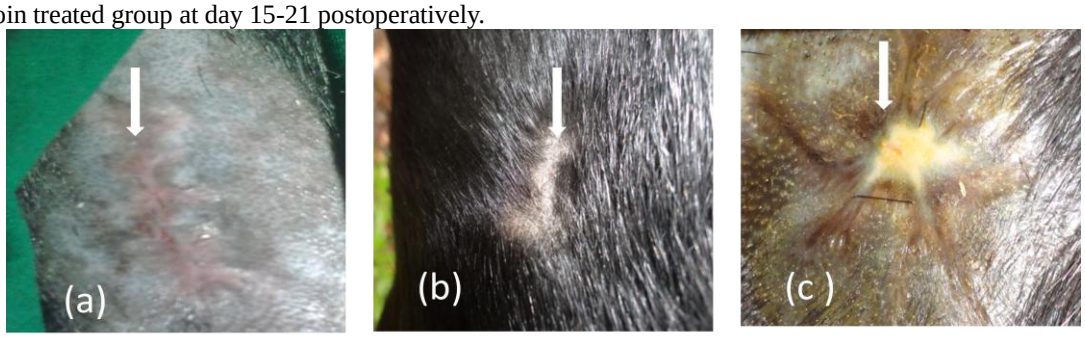

Fig.5(a) Homogenous PRP gel treated wound at day 7, (b) day 15 and (c) prominent scar formation in control group at day 21.

Platelet rich plasmagel preparations were used for traditional woundmanagement to promote wound healing process. There is no research on the use of PRP gel on wound healing to assist healing process in goats in Bangladesh. Both morphological parameters and histopathological studies showed significant ($P<0.05$) variationon in the action of wound healing in homogenous PRP gel treated group. At D_1 massive amount of inflammatory cells were accumulated at suture site in case of homogenous PRP gel treated wounds. Gradual decrease of reactive cells, accumulation of exudates and tissue reaction had occurred at D_2. Moreover, proliferation of fibrous connective tissue and migration of regenerative tissue was observed markedly in wounds of homogenous PRP gel group at D_3. These are happened due to presence of bioactive substances in PRP gel which enhance the healing process [10],[15]. PRP gel is thought to cause molecular and cellular induction of normal wound healing responses similar to that seen with platelet activation[19].Platelet rich plasma is the plasma fraction of autologous blood having a platelet concentration above baseline and topical application of this PRP as a gel could be considered an effective method for replacing essential growth factors [15].Homogenous PRP is a native concentration of fibrinogen, utilizing the patient's own blood is safe and no risk of infections [20],[21],[22].PRP gel is formed by mixing PRPwith thrombin and calcium gluconate. Reference [9]reported that adding of thrombin and calcium gluconate to PRP gel automatically activates the alpha granules to release different growgh factorssuch as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) 1 and 2, and vascular endothelial growth factor (VEGF) played of angiogeneic effects which assists wound healing [7]. The release of VEGF a mediator of angiogenesis that stimulates endothelial cell proliferation [17]. The major effects of PRP derived from PDGF, which has been identified as an important protein tissue healing. PDGF also stimulates the production of fibronectin, a cell adhesion molecule used in cellular proliferation and migration during healing of wound [23].Similarly the concentration TGF β- is significantly higher in the PRP gel (7481 pg/ml) [24]. TGF β - increases granulation tissue, collagen formation, and wound tensile strength when applied locally in animal models [25]. The results of these studies demonstrate that PRP is effective in soft tissue healing.In this experiment, homogenous PRP gel is a combination of biomaterial substance having no side effect showed excellent healing score in skin wound healing.

IV. Conclusions

This study concludes that homogenous PRP gel showed excellent results in the healings of skin wounds in goats.However, this study could help veterinarians to consider the use of biomaterial substance in soft tissue healing.Further studies are necessary for the molecular investigation of healing process and proper establishment of commercially available of platelet rich plasma gel in our country and easiest way of application.

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