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Role of Platelet Rich Plasma Gel in Bone Healing of Black Bengal Goats

Raihana Nasrin Ferdousy, Abu Md. Mamun Tarif, Sattwikesh Paul, Nasrin Sultana Juyena, and Md. Mizanur Rahman

Department of Surgery and Obstetrics, Bangladesh Agricultural University,
Mymensingh-2202, Bangladesh

ABSTRACT: The study was conducted to evaluate homogenous platelets rich plasma (PRP) gel in the bone healing process. There are limited researches on the use of biomaterials to assist healing process in Bangladesh done earier. Therefore, this experiment was carried out to exploit the therapeutic effects of PRP gel on healing process. For bone healing study, total 6 oblique tibial fracture cases in goats were created. Goats were divided into three groups with two animals in each group bearing weight 15-20 kg and age 1-3 years. Among these two groups were treated with homogenous PRP gel and one group was untreated served as control and fracture cases were corrected with open reduction and internal fixation using bone plate with screw and surgical stainless steel wire (18 Gauze) suture. Goats showed a satisfactory result on bone healing after using PRP gel. Post operative radiography confirmed that bone fragments were well maintained until healing occurred and radiograph showed negligible callus at 30 days, complete healing occur. On the other hands, it takes 60 days for immobilization in untreated group. In treatment groups there was no periosteal reaction at the fracture site. No postoperative complication was observed at surgical site. This study could help veterinarians to consider natural biomaterial product specially homogenous PRP gel for a good healing of bone with minimum complications. Further studies are necessary for the molecular investigation of healing process and proper establishment of commercially available of PRP gel in our country and easyiest way of application.

KEYWORDS: Platelet rich plasma gel, therapeutic effect, fracture, immobilization, goat.

1 INTRODUCTION

Fracture is a common surgical affection of goats in Bangladesh. In our country the effective treatment of fracture is not developed properly. Here nearly limited facility to reach the modem treatment of fracture for their livestock resulting improper healing of bone and decrease the value of animals. Under the circumstances, platelet-rich plasma (PRP) gel is very much effective to bony reconstruction, it provides adhesion for the consolidation of cancellous bone and comminuted fracture segments [1]. Reference [2] shows that PRP gel is capable of improving soft tissue healing and positively influencing bone regeneration. Reference [3] shows that the application of PRP gel promoted new bone formation in goat. Animal and human studies have demonstrated that PRP enhances and accelerates soft tissue repair and bone regeneration [4], [5]. In the field of bone tissue surgery, a recent study, Reference [6] shows that the effect of autologous PRP on bone regeneration in mandibular fractures. He concluded that direct application of the PRP along the fracture lines may enhance bone regeneration. Platelets have been demonstrated to be the natural storage vessel for several growth factors and cytokines that promote blood coagulation, tissue repair, and the process of bone mineralization [7]. Degranulation of platelets by proteins such as thrombin causes them to release transforming growth factor-ß (TGF-ß1), platelet-derived growth factor (PDGF), fibrinogen, epidermal growth factor (EGF), histamine, and hydrolytic enzymes [8], [9], [5], [10]. Platelet-rich plasma (PRP), containing various growth factors, may speed up bone healing in goats [11]. Use of PRP enhanced the bone healing considerably in goats [12]. Moreover, there is limited number of researches of the use of PRP gel in black Bengal goats. Based in these reasons, we hypothesized that bone fractures in goats treated with PRP gel enhanced bone healing compared to control. Therefore, the present research theme has been directed to prepare a low-cost method of platelet-rich plasma (PRP) gel and to exploit the therapeutic effects of PRP gel on bone healing of black Bengal goats.

Corresponding Author: Raihana Nasrin Ferdousy (raihananasrin01@gmail.com)

2 MATERIALS AND METHOD

A series of experiments were performed in black Bengal goat to find out the effect of platelets rich plasma (PRP) gel in healing of bone. The proposed research works were conducted at Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The duration of the study was from January to June, 2013. Six apparently healthy black Bengal goats were used for the experiment. The age of those animals ranged from 1-3 years and body weight of the animals ranged from 15-20 kg. All the animals were maintained at the Surgery and Obstetrics departmental facilities throughout the experiment.

2.1 PREPARATION OF PLATELETS RICH PLASMA (PRP) GEL

Reference [10] shows the preparation procedure of the PRP gel. It has been followed. Fresh blood of goat was collected in tube containing 10 ml blood for platelet-rich plasma before the surgical procedures. The blood was preliminary stored in Falcon tube containing 3.8% sodium citrate anticoagulant. The tube was 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 tube and removed to another tube called tube A. This part was used to obtain the autogenous thrombin. To the tube A 600 µl of 10% calcium gluconate was added and incubated at 15 min at 37 ºC. The tube A contained thrombin rich substrate and all the volume was used

The remaining plasma with white blood cells was transferred to another tube called tube B to obtain the PRP. This tube was maintained at room temperature. Then the volume of tube B was resuspended and homogenized by voltexing. Then contents of tube A and tube B mixed at the ratio 1: 2 (1 ml of thrombin : 2 ml of PRP). After 40 min resting at room temperature the PRP gel was formed.

Fig. 1. (a) Blood collection from goat, (b) Centrifugation, (c) Plasma separation from RBC, (d) Collection of Platelet-rich plasma (PRP), (e) 300 µl of 10% calcium gluconate, (f) Platelet-rich plasma (PRP) gel

(a) In front of Surgery and Obstetrics Department, at garden, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh, (b+c+d+e+f) In Community-based Dairy Veterinary Foundation (CDVF) Field Fertility Clinic Laboratory at BAU Campus, Mymensingh, department of Surgery and Obstetrics, Bangladesh

2.2 EXPERIMENTAL DESIGN

Six apparently healthy black Bengal goats were used for the experiment and total six tibial fracture cases were made on the six goats. They were divided into three groups, each group containing two animals. Group A and B were treatment group and group C was untreated (control). **Group A** containing animals were treated with homogenous PRP gel prepared according

to a standardized protocol and fracture was immobilized using full cerclage by surgical stainless steel wire (18 Gauze) suture and bone plate with screw together. **Group B** containing animals were treated with PRP gel and fracture was immobilized using only bone plate and screw. **Group C** containing animals untreated, served as a control, no application of PPP gel and immobilization was performed using full cerclage by surgical stainless steel wire (18 Gauze) suture and bone plate with screw. Radiographic monitoring after closing immediately, at 5 days, 2 and 3 weeks and 4 weeks and after 2 months were done. To monitor fracture healing and bone formation overtime radiographic analysis was performed up to healing.

2.2.1 SURGICAL TECHNIQUE

The animal was sedated with Xylazine Hydrochloride 2% (Rompun® Bayer, Leverkusen) @ 0.1 mg/kg body weight. After clipping and shaving, the operation site was washed with soap water and then painted with antiseptic solution (Povin®, Opsonin Pharmaceuticals Ltd., Bangladesh). Local anaesthesia was performed with 2% Lignocaine Hydrochloride (Jasocaine®, Jayson Pharmaceuticals Ltd., Bangladesh). Then a tourniquet was tied over the operation site. Intravenous normal saline (DNS®, Opso Saline Ltd. Bangladesh) was given to avoid the hypovolumic shock due to excess hemorrhage during operation. After draping of operation site, a longitudinal incision was given on the lateral side of tibial part on hind leg to make fracture. After creation of artificial fracture, PRP gel was applied and further immobilized using different internal fixator (full cerclage by surgical stainless steel wire (18 Gauze) suture, bone plate with screw). Before final immobilization, again PRP gel was applied in fractured part. All types of process were placed according to reference [13]. Muscle and fascia were sutured using catgut 2-0. Sulphanilamide powder was applied locally (Sumid vet®, Square Pharmaceuticals, Bangladesh) then skin was closed by using cross mattress or simple interrupted pattern with nylon. A saline tube also inserted for drainage. Tr. bezoin seal was applied over the suture line and then modified Thomas Splint was applied in all cases for additional support for immobilization of the limb and of weight.

2.2.2 POST OPERATIVE CARE AND OBSERVATION

The animals were completely restricted and only limited movement was allowed. After 8 days, skin stitches were removed. Animals were allowed to move freely in an open enclosure after 15 days. During the postoperative period, the animals were closely monitored for the presence of fever, severity of pain, their tolerance to the fixators by their degree of weight bearing and lameness, and the range of movement of the adjoining joints. Periodical radiographic assessment was carried out to evaluate reduction and alignment of bone fragments, and callus formation and complications, if any. In all animals the wires, bone plates were kept *in situ.*

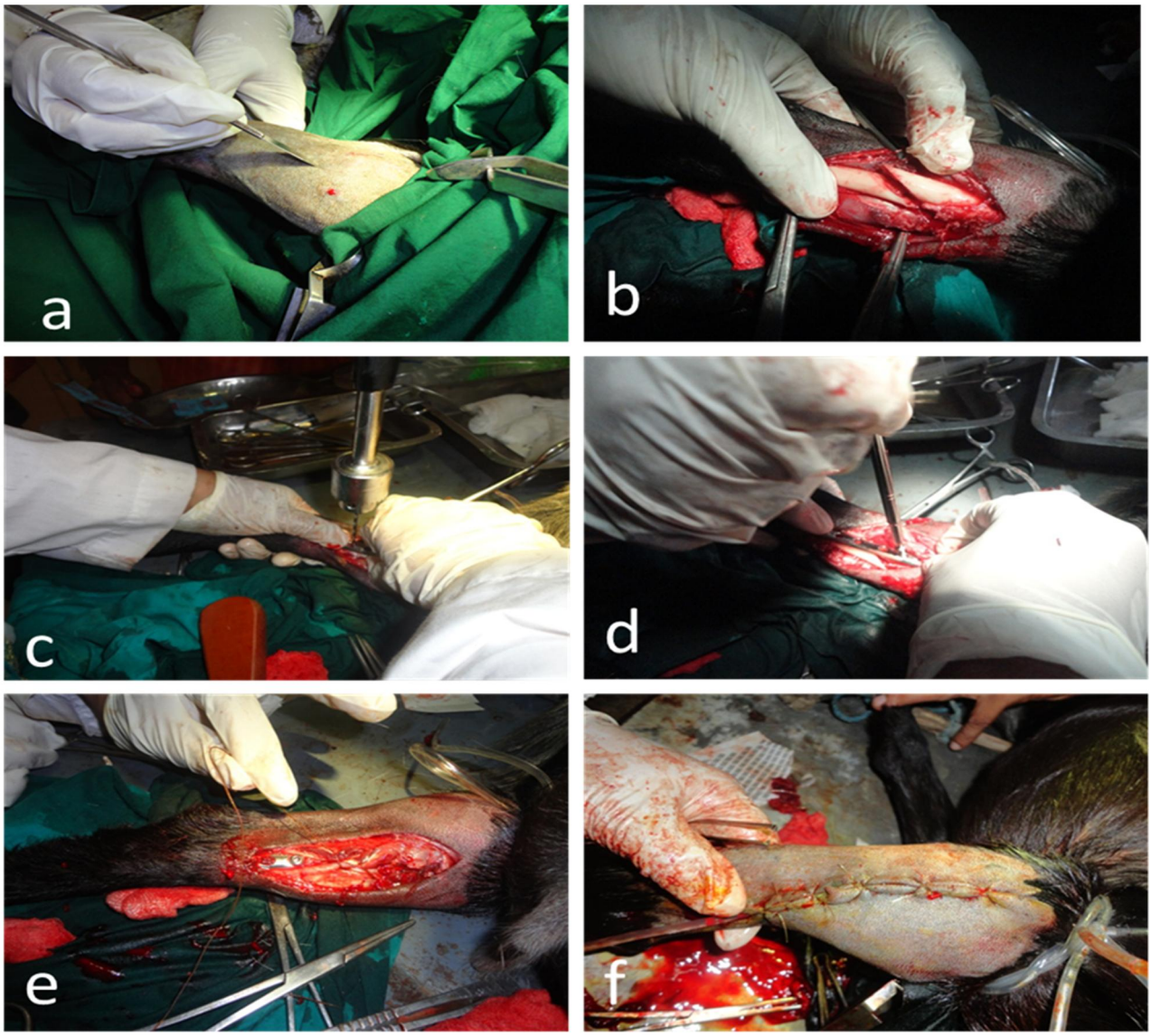

Fig. 2. Operation procedure of fracture (a) During incision on skin, (b)Fractured tibial bone, (c) Bone drilling for screw adjustment, (d) During screw setting, (e) Suture of muscles and soft tissue with simple continuous using catgut no. 2-0 and (f) skin suture using simple interrupted with nylon

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3 RESULTS AND DISCUSSION

The study was undertaken to evaluate the feasibility of a simple, inexpensive platelets-rich plasma (PRP) gel application on fracture heals. Table 1 shows the characteristics of fractures cases in goats treated homogenous PRP gel by open reduction and internal fixation methods in this study.

Table 1. Results of the effect of PRP gel

No. of goats	Treatment group	Loaction, Type of fracture	Technique	Post operative observation		
				Cure period	Repeat Intervention	Observation
4	PRP gel	Mid shaft of the tibia (oblique)	(a) Full cerclage wire suture and bone plate (2 cases) (b) bone plate and screw (2 cases)	(a) 4 weeks both Cases union (b) 4 weeks 1 Case union, 1 case non union	amputation	Myiasis occur in one case, no support by muscle, excess callus and false bone formation finally amputation
2	Untreated (control)	Mid shaft of The tibia (oblique)	Full cerclage wire suture and bone plate (1 case) bone plate and screw (1 case)	2 month union		

During the study period, fracture site were observed regularly. In treatment group, three cases were cured within near about one month. Successfully fracture immobilization with bone plating and full cerclage wire suture were performed.

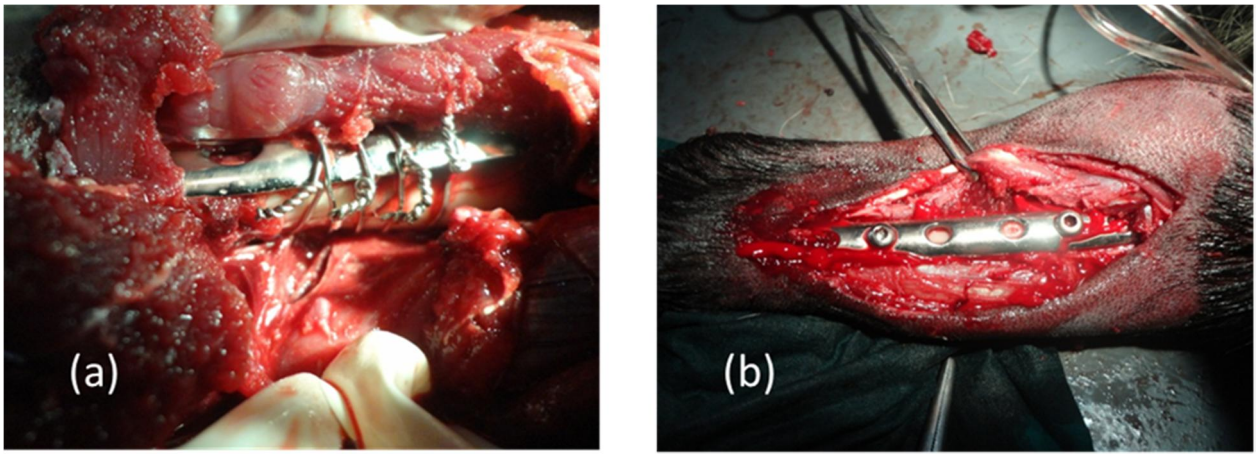

Fig. 3. (a) Full cerclage wire suture with bone plate and screw (b), Bone plate and screw

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Three goats showed a satisfactory result after using PRP gel. All goats showed good weight bearing in the immediate post-operative period (Figure 4a). Severe pain was noticed for first 3 postoperative days in all goats, which gradually lessened and subsided by 14^{th} postoperative day in almost all goats. The fixators applied to different bones were well-tolerated, and the animals could lie down, stand and walk freely with the fixator without any problems after 4 weeks (Figure 4b). On the other hands, in control group, it takes near about 2 months for fracture immobilization.

Fig. 4. (a) Goat immediately after surgical operation and (b) After 30 days of surgical operation

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Post operative radiography confirmed that bone fragments were well maintained until healing occurred and radiograph showed negligible callus and proper healing at 30 days (Figure 5a and 5b). There was no periosteal reaction around the wire suture at the fracture site. No exudation was observed at surgical site in any goats during removal of suture. There was no detectable lameness by 30 day in 3 goats. One case was amputated after 2 weeks due to myiasis, there was no support by muscles and skin. The leg was amputated, excess callus and false bone formation was found.

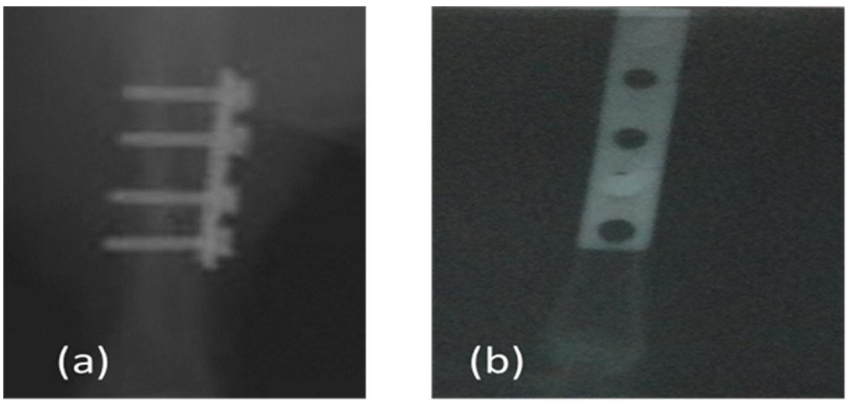

Fig. 5. (a) Radiograph around the bone plate at the fracture site at 28 days lateral view (b) and dorsal veiw after 28 days; 55 kV, 10 mA, 4s.

Radiograph was taken by portable X-ray machine at 55 kV, 10 mA, 4s in X- ray room and film processesing were performed at dark room.

In this study Platelets rich plasma (PRP) gel preparations (biomaterials) are used for bone healing process. Role of PRP gel on fracture healing was evaluated through clinical observation and radiographical findings. Fracture is one among the common orthopaedic affections encountered in domestic animals and pets. References [14], [15] show that most of the fractures are seen in tibia, metatarsal or metacarpal bones, which have less muscle covering. Therefore, in our study, we created oblique fracture in tibia planned with the objectives to evaluate bone fixation treated by PRP gel which are immobilized as internal fixation techniques for the correction of long bone fracture and to compare the outcomes with untreated (control) group. In this study, untreated group with delayed healing, in contrast PRP gel treated group was very quick healing within one month. Reference [2] shows that PRP gel is capable of improving soft tissue healing and positively influencing bone regeneration. Reference [3] shows that the application of PRP gel promoted new bone formation when autogenous bone used in a goat spinal transverse processes implant model. Reference [12] shows that shows use of PRP enhanced the bone healing considerably. When PRP gel is applied to bony reconstruction it provides adhesion for the

consolidation of cancellous bone and comminuted fracture segments [1]. Reference [16] shows that moderate periosteal reaction is present at healing site in most of the animals and have been due to the in stability at the fracture site leading to excess callus formation. In this experiment, PRP gel is a combination of biomaterial substance having no side effect showed excellent healing score without any postoperative complication and plays a beneficial role in bone healing. However, this study could help veterinarians to consider use of biomaterial substance in bone regenaration.

4 CONCLUSION

This study concludes that Platelets rich plasma (PRP) gel preparations (biomaterials) are inexpensive and effective for bone healing, PRP gel homogenous treated fracture provides good alignment and stabilization of fracture fragments, rapid union of fracture resulting in early functional usage of limb and further studies are necessary for the molecular investigation of healing process and proper establishment of commercially available of Platelets rich plasma gel in our country and easyiest way of application.

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