Research Article

Therapeutic Effect of Heterologous Platelet-Rich Plasma on Third-Degree Burn Wound in Rabbits

Kazi Afsana Homayra Orchy[1], Mst. Antora Akter[1], Nelema Yesmin[1], Md. Moshiur Rahman Khan[1], Marzia Rahman[2], Md. Mahmudul Alam[1*]

[1]Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [2]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Kazi Afsana Homayra Orchy and Mst. Antora Akter contributed equally.

Abstract | The study was conducted on rabbits to evaluate the efficacy of heterologous platelet-rich plasma (hPRP) in the treatment of third-degree burn wounds. A total of 18 circular third-degree burn wounds measuring 10mm in diameter were made in six rabbits and studied under two groups: Group A and Group B. The wounds of Group A were treated topically with freshly prepared hPRP gel twice a week. The control rabbits received normal saline topically. The wounds were evaluated for 45 days post-burning. Morphological features of wounds such as pain sensation, the development of crusts, exudates and pus, and morphometric assessment like wound contraction were recorded. On the 7th, 14th and 21st days post-burning, biopsies were taken for histopathological assessment. In this study, the morphological and morphometric data revealed that in the animals of group A, wounds were healed up with improved morphological features significantly earlier than those of group B which was confirmed in the histological analysis of biopsy sample. Regarding the liver and kidney functions, the serum levels of liver enzymes (ALT, AST and ALP) and kidney function biomarkers (creatinine, BUN) showed a significant increase in burn patients of the saline-treated control animals, whereas, the animals treated with hPRP exhibited the normal range of these functional biomarkers. On the basis of morphology, histopathology, and biochemical analysis, it can be concluded that PRP, irrelevant of source improves and accelerates the burn wound healing with no adverse effects

Keywords | Burn, histopathology, epithelialization, PRP, rabbits.

Received | May 15, 2023; **Accepted** | June 05, 2023; **Published** | June 15, 2023
***Correspondence** | Md Mahmudul Alam, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; **Email:** mahmud.dso@bau.edu.bd
Citation | Orchy KAH, Akter MA, Yesmin N, Khan MMR, Rahman M, Alam MM (2023). Therapeutic effect of heterologous platelet-rich plasma on third-degree burn wound in rabbits. Adv. Anim. Vet. Sci. 11(8): 1280-1287.
DOI | https://doi.org/10.17582/journal.aavs/2023/11.8.1280.1287
ISSN (Online) | 2307-8316

INTRODUCTION

Burns are tissue injuries induced by a heat source, friction, electricity, ionizing radiation, or compounds that destroy the skin's structural integrity (Badis and Omar 2018). Burns predominantly affect the outermost layer of skin and membranes that surround it, but in extreme cases, they can also cause damage to tissues beneath the skin, internal structures, and even death. Mortality and morbidity resulting from burns are typically attributable to secondary infection and prolonged recovery time (Badade et al., 2016). The intensity of the injury determines the healing response of burns. First- and second-degree burns recover rapidly. However, intensed burn wounds are susceptible to infection and difficult to heal naturally (Sajjad et al., 2018). Burn healing is a complicated sequence of processes including coagulation, inflammation, the formation of granulation tissue, epithelium formation, collagen production, and tissue remodeling (Abegao et al., 2015) and a lack of blood flow, a compromised immune system, and an increased risk of developing a variety of illnesses all work together to impede the healing process (Sultana et al., 2020; Khalil et al., 2018).

One of the most significant challenges in regenerative medicine is the creation of bioactive surgical adjuvants that can sustain the inflammatory response while enhancing recovery. However, the need for tissue self-repair procedures across all medical specialties continues to be a problem and a topic of discussion in advanced studies (Adiga and Adiga, 2015). Based on recent studies, platelets are crucial to the healing of cutaneous wounds and their activations trigger the secretory synthesis of several growth factors that influence all steps of the healing cascade either directly or indirectly (Anandan, 2010). PRP can also release a number of antibacterial substances, minimize local inflammation, and prevent wound infection (Zhang et al., 2019). Platelet granules contain growth factors that promote wound healing and hemostasis, including PDGF, TGF/TGF, EGF, VEGF, platelet thromboplastin, and fibrinogen (Pietramaggiori et al., 2010). Animal studies have demonstrated that PRP is an active agent for cartilage regeneration, neovascularization, and tissue formation (Sultana et al., 2022). It has been reported that PRP has been used in a wide variety of fields, including but not limited to oral and maxillofacial, orthopedic, ophthalmology, dental, and plastic and reconstructive surgery. In this regard, PRP could be a viable alternative and the cornerstone of the future treatment of burns. However, there is very limited reports on its efficacy in the healing of burn wounds. Thus, the current research aimed to determine the efficacy of heterologous PRP (hPRP) in third-degree burn wound healing in rabbits.

MATERIALS AND METHODS

Ethical Approval

The Animal Welfare, Experimentation, and Ethics Committee (AWEEC) of the Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh approved and oversaw the animal experimentation [Permission number: AWEEC/BAU/2022(10)].

Animals

In this study, six New Zealand White rabbits with ages ranging from 90 to 120 days and average weights of 1.35 kg irrespective of sex were used. The rabbits were purchased from a large-scale commercial rabbit farm. They were maintained in standard conditions and under the supervision of a veterinarian in individual cages having unrestricted access to both food and water. Before beginning the experiment, a 7-day acclimatization period was observed to enable adaptation to the experimental conditions.

Experimental Design

A total of 18 third-degree burn wounds were generated on either side of the vertebral column of each rabbit. Experimental rabbits were separated into two distinct groups as follows: Group-A: Freshly prepared and activated hPRP was applied twice weekly to the nine burn wounds of three rabbits. To avoid contamination and disruption of the healing process, the animals received attentive care. Group-B: This group of rabbits was used as a control. Nine surgical burn wounds on three rabbits were treated with Normal Saline (NS) in this group.

Preoperative Patient Preparation

The animals were subjected to a clinical examination to ensure that they were physically fit and free of apparent infections or infestations. All the rabbits were kept in a state of fasting for 12 hours and water for two hours. The surgical sites (either side of the vertebral column) were clipped, shaved, scrubbed for the creation of burn wounds. Then, 70% ethanol and Povidone Iodine were applied for aseptic surgery.

Techniques of the Burn Wound

Before inflicting burn wounds, the animals were anaesthetized intramuscularly with 5mg/kg of xylazine hydrochloride (Xyla®, Interchemie Werken, Netherlands) and 35 mg/kg of ketamine hydrochloride (Ketalar®, Popular Pharmaceuticals, Bangladesh). The rabbits were positioned on lateral recumbency. On either side of the vertebral column, 10 mm-wide, sterilized third-degree burns were created using a red-hot iron bar heated with a burner. Immediately following the injury, intraperitoneal normal saline and intramuscular antihistamine were administered to prevent shock.

Preparation and Activation of hPRP Gel

hPRP was prepared from bovine peripheral blood and activated following the protocol as described in our previously published article (Sultana et al., 2022).

Treatment of Burn Wound

After wounding, the therapy began with the topical application of hPRP gel twice a week until the wound had completely healed up. Sterile vaseline gauze was used to cover the wounds to prevent sloughing of PRP gel from the wound. Antibiotics, and anti-inflammatory agents were not used after burning to avoid mitigation of the healing process.

Morpholgical Assessment of Wounds and Wound Morphometry

Clinical characteristics of the lesions such as pus formation, exudation, crust, pain sensation were recorded from the day of injury until 45 days post-wounding. Morphological parameters were scored as follows: Pain sensation: mild (0-1), moderate (1-3), severe (4 to above); Crust formation and itsquality: poor (0 -1), good (2-4), excellent (5-to above); Exudation: low (0-0.5), medium (0.5-1.5), high (1.5-to above); Pus formation: No (0-0.5), moderate (0.5 -1.5), high (2 to above).

The size of the wound was measured every other day using digital slide calipers and translated to a percentage of wound contraction using the following formula:

% wound contraction= {wound area at day 0 – wound area at day (n)}/ wound area at day 0×100

Here, n = 3rd, 6th, 9th, 12th, 15th and 18th … 45th day

Histopathological Examination

On the 3rd, 7th, and 14th post-burning days, full thickness of cutaneous tissue and underlying muscular structures were surgically collected from both groups. Tissues were formalin-fixed at 10% buffered formalin and then paraffin-embedded after a series of dehydration by graded alcohol and xylene clearance. Using a microtome (Histoline®, USA), 4 µm thick sections of tissue block were cut and stained with hematoxylin and eosin. To examine the variations in histopathological characteristics in the tissues of the control and hPRP treated wounds, the stained slides were examined using a photographic light microscope (Micros®, Austria).

Serum Biochemistry

Peripheral blood samples were collected from the jugular vein of the experimental rabbits and taken into clot activator tubes on day 3, 7, 14, 21 post-burning and centrifuged at 3000rpm for 10min for serum separation. Blood Sera stored at -20°C until biochemical analysis. Following the manufacturer's instructions, the serum concentration of glucose, total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and blood urea nitrogen (BUN) were determined using commercial diagnostic kits (Linear Chemicals SL®, Spain). The determinations were carried out either by kinetic or endpoint methods. All the tests were performed on a semiautomatic biochemistry analyzer (Clindiag® SA-20, Belgium) at the determined wavelength.

Statistical Analysis

All the data were presented as Mean ± SEM (Standard Error of Mean). Statistical Package for the Social Science (SPSS) version 20.0 was used to conduct an independent sample t-test to compare data between the groups. The level of significance of the differences was assessed using the values of $P<0.05$ and $P<0.01$.

RESULTS

Macroscopic Follow-up

There were marked differences in exudation, pain sensation, crust development and pus formation between the hPRP and control groups at different observation time points. hPRP-treated rabbits exhibited less pain sensation compared to saline-treated control (Figure 1a) indicating that PRP may have an anti-inflammatory effect. The development of crust is a good sign of wound healing. In this study, hPRP exhibited rapid crust formation leading to accelerating burn wound healing which was remarkably delayed in the control group (Figure 1b). Discrete exudation was observed initially in both groups but it rapidly subsided when wounds received treatment of hPRP (Figure 1c). Initial examination revealed that both the control and the hPRP-treated lesions had a pinkish hue, however, no macroscopic signs of contamination or the presence of pus were evident in the hPRP-treated wounds. On the other hand, obvious infection and pus formation were recorded in the saline-treated control wounds (Figure 1d).

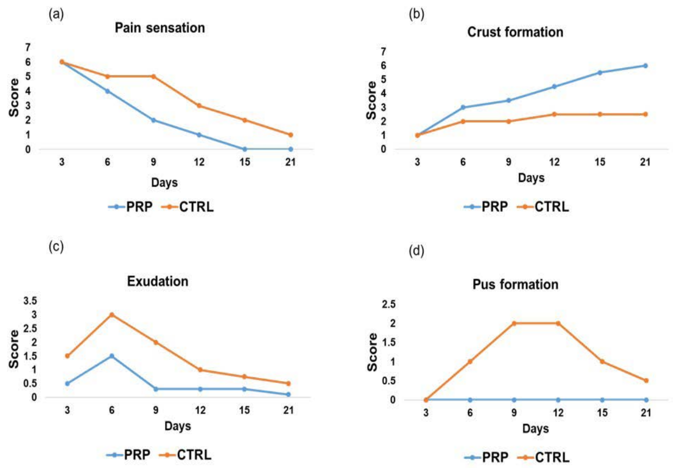

Figure 1: Morphological evaluation of burn wound over the observation days. (a) pain sensation, (b) crust formation, (c) exudation, (d) pus formation. Each of the macroscopic characteristics was arbitrarily assessed and scored by more than two observers, and the mean value is shown here.

Wound Morphometric Study

The evolution of wound contraction, along with healing time is presented in Figure 2. Grossly, figure 2a showed that when the burn wound received topical hPRP gel, the contraction of wounds was satisfactorily accelerated over time (Figure 2b) suggesting that PRP has an enhancing cutaneous wound regenerating capability. The complete contraction was achieved on day 24 in wounds treated with hPRP, whereas it required approximately 45 days in wounds of control animals those were received normal saline only. hPRP-treated burn wound healed up promptly whereas the healing time was significantly (P<0.01) delayed in the control group (Figure 2c).

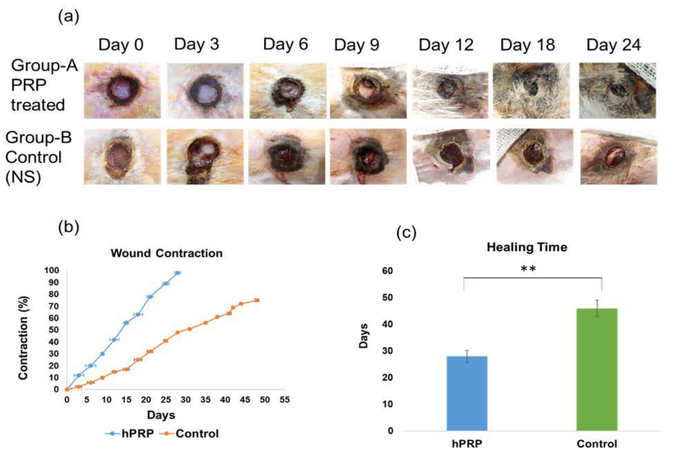

Figure 2: Wound morphometry. (a) Macroscopic examination of the wound edge contraction process in hPRP-treated wounds and NS-treated wounds at all time points, (b) the wound contraction study. The linear increase in the contraction percentage of the control group and the group treated with hPRP gel on the observation days. (c) Complete healing time in both groups (Mean value). T-test ** indicates *P*<0.01.

Histopathological Evaluation

There was massive inflammatory infiltration in the subdermal area of both the control and hPRP treated group on 3rd day post-wounding (DPW). The control section showed cellular debris in the scab consisting of neutrophils, tissue debris, and fibroblasts (Figure 3a). Hemorrhages in tissues were much higher in untreated groups. On the other hand, the reactive cells in hPRP-treated group following day 3 of the experiments were mostly lymphocytes, macrophages, monocytes, fibroblasts, keratinocytes, and other tissue debris (Figure 3b). On day 7 of burn wounding, inflammatory cells still gathered in the subdermal area along with other tissue debris in the control group (Figure 3c). On the other hand, keratinization over the injured skin was initiated in the hPRP group (Figure 3d). The tissue debris on the surface of wounds was scanty in this group. The reactive cells on the margin of injured skin were mostly macrophages and lymphocytes and reduced in number in the hPRP group (Figure 3d). Tissues of untreated control skin showed raw surface consisting of thin layer keratinization over the injured skin on 14 DPW (Figure 3e). Still, there were inflammatory cellular infiltration in the subdermal area and immature and disorganized collagen tissues in the untreated control. On the other hand, hPRP-treated wounds showed a state of satisfactory healing over the injured skin after 14 days of treatment. There was a thicker keratin layer above epidermis, and a massive amount of hair follicles beneath the dermis. In the subdermal area, the collagen tissues were organized and nearly mature (Figure 3f).

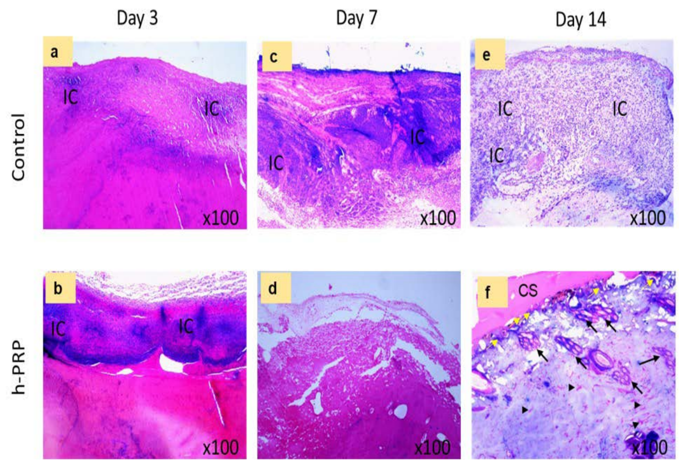

Figure 3: Histological sections of the healing of burn wound at 3, 7, and 14 DPB. The re-epithelialization process was completed in the hPRP-treated groups at 14 DPW. Moreover, rejuvenation of the skin appendages such as hair follicles was seen in the hPRP-treated groups at 14 DPB (Arrow). CS: crusty scab, IC: inflammatory cells, arrowheads: rejuvenation of hair follicles, yellow arrow: re-epithelialization. H&E stain, magnification x100.

Serum Biochemical Findings

Table 1 demonstrates that over the experimental period, the levels of total protein (TP) and serum albumin did not vary significantly between the control and hPRP groups. But there were remarkable changes in the liver and kidney function markers between the groups as shown in Table 2 and 3. ALT, AST, and ALP are the most reliable markers of the functional status of liver. In our study, we found that the serum level of all three markers of the liver were significantly (P<0.05) increased over the experimental period following burn wounding, especially at day 7 post-wounding. On the other hand, hPRP satisfactorily lowered the concentration within the normal ranges.

We have screened the status of kidney function by evaluating serum creatinine and BUN following the burn and treatment (Table 3). Burn resulted in the elevation of serum creatinine and BUN particularly on day 7 post-burning. On the other hand, the animals that received hPRP as a burn remedy dramatically lowered creatinine and urea within the normal level.

We have evaluated the status of some electrolytes following burn wounding and post-therapy with hPRP. The blood Na^+ and K^+ levels in the saline-treated control group decreased significantly over the experimental period, but the pick decline was observed on day 7 post-burning (Table 4). The changes of Na^+ and K^+ in hPRP treated animals were within the basal level. All these data on electrolyte status indicated that burn may alter the electrolyte levels and hPRF may ameliorate the electrolyte abnormalities simultaneously during the healing cascade.

Links Researchers

Table 1: Status of serum proteins and albumin concentration in the rabbits of burn wound at different days of treatment.

SL. No.	Parameters	Groups	Day 3	Day 7	Day 14	Day21	Reference value
1	Total Protein (gm/dl)	PRP	6.41±0.24^{a}	5.49±0.23^{a}	7.31±0.12^{b}	7.74±0.14^{b}	5.4-7.5
		Control	5.59±0.14^{a}	5.40±0.11^{a}	6.48±0.26^{b}	5.50±0.15^{a}	
2	Albumin (gm/dl)	PRP	2.81±0.07^{a}	2.59±0.19^{a}	3.48±0.13^{a}	3.54±0.15^{a}	2.7-5
		Control	3.27±0.10^{a}	3.37±0.18^{a}	3.48±0.22^{a}	3.18±0.02^{a}	

Values with different superscript letters in the same column (a, b) differed significantly (P<0.05).
05)

Table 2: Changes in liver enzyme activity in response to burn and following treatment with hPRP and NS on day 3, 7, 14 and 21 post burning in rabbits.

SL. No.	Parameters	Groups	Day 3	Day 7	Day 14	Day21	Reference value
1	ALT (IU/L)	PRP	44.37±0.20^{a}	45.38±0.15^{a}	44.39±0.10^{a}	43.51±0.09^{a}	45-48
		Control	59.46±0.19^{b}	61.68±0.15^{b}	60.59±0.20^{b}	58.34±0.11^{b}	
2	AST (IU/L)	PRP	37.50±0.27^{a}	46.25±0.07^{a}	47.38±0.28^{a}	35.56±0.17^{a}	35-130
		Control	210.46±0.20^{b}	218.41±0.12^{b}	215.38±0.16^{b}	211.51±0.10^{b}	
3	ALP (IU/L)	PRP	30.39±0.20^{a}	32.22±0.68^{a}	37.59±1.08^{a}	35.64±0.46^{a}	12-96
		Control	51.6±0.82^{b}	65.99±0.21^{b}	61.13±0.33^{b}	45.22±0.38^{b}	

Values with different superscript letters in the same column (a, b) differed significantly (P<0.05).
05)

Table 3: Dynamics of kidney function in response to burn and post-burn treatment with hPRP and NS on day 3, 7, 14 and 21.

SL. No.	Parameters	Groups	Day 3	Day 7	Day 14	Day21	Reference value
1	Creatinine (mg/dl)	PRP	1.35±0.09^{a}	1.19±0.06^{a}	1.31±0.09^{a}	1.11±0.05^{a}	0.5-1.5
		Control	1.76±0.01^{a}	1.99±0.12^{a}	1.85±0.08^{a}	1.73±0.05^{a}	
2	BUN (mg/dl)	PRP	58.25±0.25^{a}	56.11±0.13^{a}	59.67±0.51^{a}	62.31±0.53^{a}	30-65
		Control	88.23±0.429^{a}	98.02±0.25^{a}	92.19±0.24^{a}	72.92±0.29^{b}	

Values with different superscript letters in the same column (a, b) differed significantly (P<0.05).
05)

Table 4: Changes in serum electrolytes level in response to burn and post-burn treatment with hPRP and NS on day 3, 7, 14 and 21.

SL. No.	Parameters	Groups	Day 3	Day 7	Day 14	Day21	Reference value
1	Na (mmol/L)	PRP	142.18±0.57^{a}	141.76±0.93^{a}	146.09±0.15^{a}	144.61±0.54^{a}	147-159
		Control	120.24±0.54^{b}	101.92±0.36^{b}	116.84±0.65^{b}	122.03±0.40^{a}	
2	K (mmol/L)	PRP	3.76±0.12^{a}	3.80±0.15^{a}	3.70±0.13^{a}	3.86±0.11^{b}	3.7-5.7
		Control	2.21±0.10^{a}	2.15±0.05^{a}	2.57±0.15^{a}	2.25±0.17^{b}	

Values with different superscript letters in the same column (a, b) differed significantly (P<0.05).

DISCUSSION

Burns are widely recognized as one of the most serious types of injuries, and infection is a frequently observed complication involving burn tissues. Antibiotics are extensively employed in the treatment of burns in order to avoid subsequent infections (Jull et al., 2008). However, antibiotics can provoke allergic responses in some people, which can prolong the healing process (Sultana et al., 2021; Tamanna et al., 2020). To treat chronic wounds, modern therapy has widely accepted the application of PRP as a substitute of antibiotics or other hazardous drugs (Li et al., 2023). Given that PRP contains a wealth of useful growth factors for wound healing, it may be a viable alternative to antibiotics and other natural medicines for treating burn wounds (Heidari et al., 2019; Kulac et al., 2013). In this study, experimental third-degree burns were generated in rabbits, and the therapeutic effectiveness of topically administered hPRP was assessed. After wounding, it has been noticed that there were significant differences between the hPRP and control group based on the morphological changes, wound contraction, histopathological changes, and clinical chemistry of vital organs.

In this experiment, morphological changes were observed in, pain sensation, pus, exudation, inflammation, crust in the animals of hPRP group and control group up to day 24. All these features were remarkably improved compared to the control wounds. Our results were similar to previous researchers who worked on wound healing with natural products (Sultana et al., 2020; Kar et al., 2022; Barrionuevo et al., 2015; Zheng et al., 2022).

Third-degree burn requires meticulous management and excellent therapeutic agents capable of accelerating tissue regeneration. In this study, we found that hPRP enhanced burn wound healing and skin contraction in around 24 days, whereas control group took around 45 days. Our finding is similar to the results of Zheng et al. (2022) who documented an ameliorative and accelerating burn wound healing by PRP.

A number of healing parameters revealed significant disparities upon histopathological examination. The topical application of hPRP resulted in re-epithelialization, keratinization, and an abundance of hair follicles beneath the dermis in our study. Marques et al. (2017) reported that PRP gel was effective in the treatment of acute burn wounds, and that autologous platelet gel influences wound-healing by inducing a vigorous inflammatory response. There was a considerable increase in the production of extracellular matrix and granulation tissue, as well as an acceleration in vascular in-growth, fibroblastic proliferation, and collagen production.

Considering the restoration of hair follicles, our result shows that dermal papilla cells are partially reconstituted at the site of healing for lesions treated with hPRP. Our findings are consistent with those of Badis and Omar (2018) and Sultana et al. (2022). In a similar study on sheep, Badis and Omar (2018) found that applying PRP topically to wounds accelerated healing and stimulated the growth of new hair follicles.

The liver and kidney are the most susceptible organs to burn damage, and the biomarkers can assist to determine the burn severity. The most common and accurate markers of hepatocellular activity are the AST, ALT, and ALP. We found significant increases in these biomarkers following burn in the rabbits. Multiple pathways, including hypoperfusion, pro-inflammatory cytokines, cellular edema, and lipid alterations contribute to the destruction of the liver caused by thermal traumas (Handoo et al., 2018; Adiga and Adiga, 2015). On the other hand, the serum concentration of these hepatocellular enzyme was within the normal range indicating that PRP may assist the normal hepatic function by limiting the excessive secretion of those enzyme.

Urea and creatinine are the two primary nitrogenous compounds that are ultimately removed by the kidneys, therefore changes in their blood levels would signify changes in renal function (Samira et al., 2018). We have recorded high level of serum creatinine and BUN in the control animals which were satisfactorily within the normal level when the animals were treated with hPRP. Anandani (2010) revealed that the high level of urea and creatinine, lead to renal failure in burn injury. During burn, there is fluid loss leading to hypovolemia, vasoconstriction and low cardiac output which may lead to destruction of kidney tubule. Moreover, there may be release of some myoglobinurea which has serious detrimental effects on kidney tubule (Stewart et al., 2013).

The current research demonstrated that the blood Na+ level dropped significantly during the course of the experiment, notably on the seventh day after the burn in the control group as compared to the animals that had been treated with hPRP. Kaddoura et al. (2017) and Samira et al. (2018) reported that a substantial hyponatremia started in the first day of burn before initiating resuscitation therapy and grew after resuscitation. This contradicts our data, which showed that hyponatremia began on the second day after burn. According to a retrospective research by Stewart et al. (2013), 6.8% of burn patients had hyponatremia, while 9.9% had hypernatremia.

CONCLUSIONS

The results of this study revealed that PRP, regardless of the source, improves and speeds up the healing of third-degree burn wounds. It also showed that PRP has therapeutic potential for treating cutaneous lesions, as evidenced by superior morphological and morphometric features and histological architecture.

ACKNOWLEDGEMENTS

We thank the Ministry of Science and Technology (MoST), Government of People's Republic of Bangladesh for the financial support in this research (Project title: The role of Platelet Rich Plasma (PRP) on induced third-degree burn wound healing in rabbits, project number: 2021/63/MoST). We also thank Bangladesh Agricultural University Research System (BAURES) for their great support to conduct the research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

NOVELTY STATEMENT

The study discussed the therapeutic capabilities of heterologous platelet-rich plasma (hPRP) in the treatment of burn wounds in rabbits. We have shown that hPRP was very much potent in wound closure and accelerating wound contraction. The histological architecture of the wound was very much satisfying after treatment with hPRP. Thus, hPRP could be an excellent biological agent to treat third-degree burn wounds in both humans and animals.

AUTHORS CONTRIBUTION

Kazi Afsana Homayra Orchy, Mst. Antora Akter: Conducting research, acquisition of data, writing manuscript; Nelema Yesmin, Md. Moshiur Rahman Khan: conducting research, Marzia Rahman: editing manuscript; Md. Mahmudul Alam: Designing and overall supervision of research, writing and finalizing manuscript.

REFERENCES

Abegão K. G., Bracale B., Delfim I., Santos E., Laposy C., Nai G., Giuffrida R., Nogueira R (2015). Effects of heterologous platelet-rich plasma gel on standardized dermal wound healing in rabbits. Acta Cirurgica Brasileira., 30: 209–215. https://doi.org/10.1590/S0102-865020150030000008

Adiga U., Adiga S (2015). Biochemical changes in burns. Int. J. Res. Stud. Biosci., 3(7): 88-91.

Anandani J.H. (2010). Impact of thermal injury on hematological and biochemical parameters in burnt patients. Biosci. Biotechnol. Res. Commun., 3: 97-100.

Badade P. S., Mahale S. A., Panjwani A. A., Vaidya P. D., Warang A. D. (2016). Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin. Indian J. Dental Res., 27(3): 300–304. https://doi.org/10.4103/0970-9290.186231

Badis D., Omar B (2018). The effectiveness of platelet-rich plasma on the skin wound healing process: A comparative experimental study in sheep. Vet. World, 11(6): 800–808. https://doi.org/10.14202/vetworld.2018.800-808.

Barrionuevo D., Laposy C., Abegão K., Nogueira R., Nai G., Bracale B., Delfim I. (2015). Comparison of experimentally-induced wounds in rabbits treated with different sources of platelet-rich plasma. Laborat. Anim., 49(3): 209–214. https://doi.org/10.1177/0023677214567747

Handoo N., Parrah J., Gayas M., Athar H., Shah S., Mir M., Amin U., Kashani B., Ashraf I (2018). Effect of different extracts of R. emodi on hemato-biochemical parameters in rabbit wound healing model. J. Pharmacog. Phytochem., 7(6): 204–210.

Heidari M., Bahramsoltani R., Abdolghaffari A.H., Rahimi R., Esfandyari M., Baeeri M., Hassanzadeh G., Abdollahi M., Farzaei M.H. (2019). Efficacy of topical application of standardized extract of *Tragopogon graminifolius* in the healing process of experimental burn wounds. J. Trad. Complement. Med., 9(1): 54-59.

Jull A.B., Rodgers A., Walker N. (2008). Honey as a topical treatment for wounds. Cochrane Database Syst. Rev. 8(4): CD005083. https://doi.org/10.1002/14651858.CD005083.pub2

Kaddoura I., Sittah G.A., Karamanoukian R., Papazian N. (2017). Burn injury: review of pathophysiology and therapeutic modalities in major burns. Ann. Burns Fire Disasters., 30(2): 95–102.

Kar M., Rahman A.M.E., Akter M.A., Islam M.S., Rima U.K. (2022). The Comparative Efficacy of Honey, Turmeric and Silver Sulfadiazine Dressing on the Healing of Burn Wounds in Rabbits. EC Vet. Sci., 7 (12): 04-12.

Khalil M.A., Shanab O.M., Zannoni G.F., Heygazy E.M. (2018). Assessment of hematological and biochemical markers in the early post-burn period for predicting recovery and mortality in animals with burn injuries. J. Clin. Exper. Pathol., 8(343): 2161-0681.

Kulac M., Aktas C., Tulubas F., Uygur R., Kanter M., Erboga M., Ceber M., Topcu B., Ozen O.A. (2013). The effects of topical treatment with curcumin on burn wound healing in rats. J. Molecul. Histol., 44: 83-90. https://doi.org/10.1007/s10735-012-9452-9.

Li S., Xing F., Yan T., Zhang S., Chen F. (2023). The Efficiency and Safety of Platelet-Rich Plasma Dressing in the Treatment of Chronic Wounds: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J. Personalized Med., 27;13(3):430. https://doi.org/10.3390/jpm13030430

Marques M.E.M., Laposy C.B., dos Santos Silva M.L., Breda M.R., Matsumoto M.M., Junior L.A.J., Nai G.A., Osaki G.A.T., Camargo Filho J.C., Nogueira R.M.B. (2017). Collagen quantification in rabbit dermal wounds treated with heterologous platelet-rich plasma gel. Semina: Ciências Agrárias., 38(1): 249-258.

Pietramaggiori G., Scherer S.S., Mathews J.C., Gennaoui T., Lancerotto L., Ragno G., Valeri C.R., Orgill D.P. (2010). Quiescent platelets stimulate angiogenesis and diabetic wound repair. J. Surg. Res., 160(1): 169-177.

Sajjad W., Khan T., Ul-Islam M., Khan R., Hussain Z., Khalid A., Wahid F. (2019). Development of modified montmorillonite-bacterial cellulose nanocomposites as a novel substitute for burn skin and tissue regeneration. Carbohydrate polymers, 206: 548-556.

Samira M., Saleh M., Hassan H., Ahmed M., Tohamy M. (2018). The effect of moderate and severe burn injuries on human liver, kidney & blood (biochemical study). J. Forensic Med. Toxicol., 16(1): 91-102.

Stewart I.J., Morrow B.D., Tilley M.A. (2013). Dysnatremias and survival in adult burn patients: a retrospective analysis. American J. Nephrol., 37:59–64

Sultana A., Hasan M., Rahman M., Alam M.M. (2021). Healing potentials of Marigold flower (*Tagetes erecta*) on full thickness dermal wound in caprine model. European Res. J., 7: 332-339.

Sultana A., Rahman M., Alam, M.M. (2020). Amelioration of External Surgical Wound by Topical Application of the Aqueous Formulation of Neem Leaves (*Azadirachta indica*) in Caprine Model. Curr. Perspect. Med. Aromatic Plants., 3: 87.

Sultana M.J., Akter M.A., Yesmin N., Haque M.A., Rahman M., Alam M.M. (2022). Wound healing effects and antibacterial properties of heterologous platelet-rich plasma on *Staphylococcus aureus* induced septic wounds in rabbits. J. Adv. Vet. Anim. Res., 9(3): 481.

Tamanna S.J., Shihab M.M., Akter M.A., Rahman M., Alam M.M. (2020). Therapeutic potentialities of green tea (*Camellia sinensis*) and aloe vera (*Aloe barbadensis*) on *Staphylococcus aureus* induced septic wound in goats. J. Bangladesh Agricult. University., 18(1): 105–110. https://doi.org/10.5455/JBAU.94746

Zhang W., Guo Y., Kuss M., Shi W., Aldrich A. L., Untrauer J., Kielian T., Duan B. (2019). Platelet-rich plasma for the treatment of tissue infection: Preparation and clinical evaluation. Tissue Engineering - Part B: Reviews., 25(3): 225–236. https://doi.org/10.1089/ten.teb.2018.0309.

Zheng W., Zhao D.L., Zhao Y.Q., Li Z.Y. (2022). Effectiveness of platelet rich plasma in burn wound healing: a systematic review and meta-analysis. J. Dermatolog. Treatment, 33(1): 131-137.