Original Article

Comparative Study on the Effect of Injectable Platelet Rich Plasma versus its Topical Application in the Treatment of Thermal Burn in Adult Male Albino Rat: Histological and Immunohistochemical Study

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ABSTRACT

Background: Burn is one of the major health problems and several reports have focused on platelet rich plasma role in treatment of soft tissue lesions.

Aim: This work aimed to study the histological effect of injectable platelet rich plasma versus its topical application on skin layers (epidermis and dermis) after thermal burn induction in adult male albino rats.

Material and Methods: 60 adult male albino rats were divided into three groups; blood donors group, control and experimental group. The latter was subdivided into subgroups I and II. Subgroup II was subdivided into 3 subgroups. Subgroup IIA: animals were exposed to contact thermal burn without treatment. Subgroup IIB: animals were injected subcutaneously with 0.3 ml of autologous PRP. Subgroup IIC: autologous topical PRP was applied. Skin specimens were obtained at days 7, 14 and 28 post burn and involved in histological and immunohistochemical studies.

Results: The results of this study revealed that, treatment with PRP resulted in early and enhanced regeneration of skin burn injury. This was evidenced by light microscopic examination and morphometric studies. The PRP treated subgroups were manifested with early re-epithelialization, collagen fibers regeneration and enhanced neovascularization with restoration of normal histological features of skin tissue. Injectable PRP provides slightly better results regarding collagen deposition, fibroblasts infiltration with significant increase in angiogenesis than topical PRP causing high vascular density in wounds.

Conclusion: Early interference by PRP injection or topical application enhances wound healing in second degree burn rat model. More future researches are recommended to assess the efficacy of combined injectable and topical PRP in treating burns.

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Key Words: Burn; platelet rich plasma; skin; wound.

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INTRODUCTION

Burns are emergent serious conditions in medicine affecting all age groups of both genders in developed as well as developing countries causing physical and psychological impairments[1].

Burns are tissue lesions resulting after exposure to thermal origin as flames, hot surfaces, liquids, radiation, chemicals and friction. According to lesion severity, burns are classified into superficial (first degree), in which lesion is restricted to the epidermis. Partial thickness (second degree) which may be superficial if involving the epidermis and superficial dermis or deep when involving the deepest layer of the dermis. Full-thickness (third degree) when lesion extends to the subcutaneous layer[2].

Platelets are one of the essential components of the coagulative system with high level of growth factors found in their granules including platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF). They have important roles in the process of wound healing, this supporting the use of platelet-rich plasma PRP in different clinical issues[3,4].

Platelet rich plasma (PRP) is the portion of plasma with platelet concentration three to five times above the normal concentration. PRP is obtained from whole blood by a process of centrifugation[5,6]. This work aimed to study the histological effect of injectable platelet rich plasma versus its topical application on skin layers (epidermis and dermis) after thermal burn induction in adult male albino rats.

MATERIAL AND METHODS

In this study, sixty adult male albino rats (20 weeks) were used. Their weights ranged from 150 to 200 grams. Rats were maintained under specific clean conditions in the animal house of Faculty of Medicine, Tanta University. The rats were housed in plastic cages with free access to water and food. All the steps of the experiment were carried out according to the rules of ethical committee on animal's experiments of Tanta University.

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Rats were divided into three groups

1- Blood donors group (10 rats): from which blood was collected for platelet rich plasma preparation.

2- Control group (10 rats): from which skin specimens were obtained without any maneuvers.

3-Experimental Group (40 rats):

This group was subdivided into:

• Subgroup I (PRP group) (10 rats): this subgroup was subdivided into subgroup IA and IB:

- Subgroup IA (injectable PRP) (5 rats): five rats were injected with 0.3 ml of autologous PRP subcutaneously[7].
- Subgroup IB (topical PRP) (5 rats): which were exposed to autologous topical PRP application[8].

• Subgroup II (30 rats):

All rats in this subgroup were anesthetized and the hair over the proximal region of their dorsal surface was shaved then subjected to thermal burn using a solid aluminum bar (especially made for this experiment) 10mm in diameter, weighing 51 grams. The bar was previously heated in boiling water, (100°C). The bar was placed onto the skin perpendicularly for 15 seconds[2,9]. Subgroup II then was subdivided into:

- Subgroup IIA (Burn group) (10 rats): which were exposed to contact thermal burn without any treatment.

- Subgroup IIB (Burn + Injectable PRP group) (10 rats) were injected subcutaneously with 0.3 ml of autologous PRP once after induction of thermal burn into multiple points in wound area[7].

- Subgroup IIC (Burn + Topical PRP group) (10 rats) were exposed to autologous topical PRP application once daily at days 1, 3, 7, 10 after induction of thermal burn[8].

All rats in subgroup II were given analgesia; paracetamol 50 mg/kg/day via oro-gastric tube twice daily for a week, began on the day of burn induction[10].

Platelet rich plasma preparation: All the following procedures were done at central laboratory of Microbiology Department, Faculty of Medicine, Tanta University. Blood was collected from anesthetized donor rats by cardiac puncture in a tube containing sodium citrate as an anticoagulant[11].

The citrated blood was centrifuged two times to get PRP. The first centrifugation was at 2000 rpm for 10 minutes. The plasma above was collected in a separate sterile tube and RBCs at the bottom were discarded. Then the plasma was centrifuged for the second time at 4000 rpm for 10 minutes[12].

Then PRP was activated by adding calcium chloride at ratio 1:10 (0.1 ml CaCl2 for each 1 ml PRP)[13].

Rats in subgroup IA (injectable PRP) and subgroup IIB (burn + injectable PRP group), were subcutaneously injected by 0.3ml of activated PRP immediately to avoid jellification of the plasma[7].

Rats in subgroup IB (topical PRP) and sub group IIC (burn + topical PRP group), were exposed to application of activated PRP topically over wound area for four times at days 1, 4, 7, 10 after induction of thermal burn and covered with a gauze[8].

Skin lesions were evaluated for wound contraction using a ruler (Figure 1A). The wound contraction was estimated at days 7, 14, 28, after burn infliction. Wound contraction was stated as the decrease in percentage of original wound size. Wound contraction percent on day X = [(area on day 1 − open area on day X)/area on day 1] × 100 Kumar *et al*.[14]. Statistical analysis of the mean percentage of wound contraction was done.

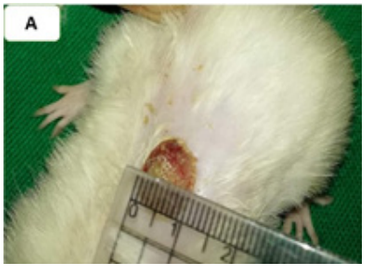

Fig. 1A: Evaluation of thermal burn wound contraction in rat using ruler.

All rats of all groups were sacrificed at the optimum time and skin specimens were subjected to the following:

1) Light microscopic study

Skin specimens were obtained at days 7, 14 and 28 post burn and fixed in 10% formal saline solution then subjected to histological examination using:

A. Hematoxylin and eosin stain to study the general histological structure[15].
B. Immuno-histochemical stain for detection of CD 34 antigen to assess angiogenesis[16].

2) Morphometric study

The image analysis was done by using the software (Image J) (National Instate of health, Bethesda, Maryland, USA). Ten different non-overlapping randomly selected fields from each slide were quantified for:

A. The mean number of new vessels in CD34 stained sections (at x400 magnification)
B. The mean area percentage of collagen fibers content in Masson's trichrome stained sections (at x400 magnification)

3) Statistical analysis

Data were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's test for

comparison between the groups using statistical package for the social sciences (SPSS).

All values were expressed as mean ± standard deviation (SD). Differences were considered significant if probability *p-value*<0.05 and highly significant if *p-value* <0.001[17].

RESULTS

1) Percentage of wound contraction

The healing rate was assessed by the wound contraction rate. Regarding subgroup II, it was clear that the percentage of wound contraction rate increased with time (Tables I,II).

2) Light microscopic study

A- Hematoxylin and eosin stained sections:

Control group

Light microscopic examination of skin sections of adult male albino rats stained with hematoxylin and eosin showed the normal histologic structure of the epidermis, dermis, hypodermis and panniculus carnosus which is a layer of striated muscle present beneath the hypodermis and it is absent in humans. The epidermis was formed of keratinized stratified squamous epithelium, mainly composed of keratinocytes. The epidermis was formed of four layers; the stratum basale was formed of low columnar cells with basal oval nuclei resting on dermo-epidermal junction. Stratum spinosum composed of polyhedral cells having central rounded nuclei. Then stratum granulosum with flattened cells having cytoplasmic basophilic keratohyaline granules. Lastly, the superficial stratum corneum was formed of non-cellular keratin lamellae. The dermo-epidermal junction was well demarcated between epidermis and dermis. The dermis appeared with superficial papillary layer which had connective tissue cells and blood capillaries. The dermis had inner reticular layer with dense connective tissue. Hair follicles and associated sebaceous gland were also found in dermis (Figures 1B, 1C).

Experimental group

Subgroup I

- Subgroup IA (injectable PRP) and Subgroup IB (topical PRP): Skin specimens showed the same histological findings as control group.

Subgroup II

- Subgroup IIA (Burn group)

At day 7, severe degenerative changes were seen in the form of loss of normal structure and arrangement of epidermis and dermis. Layers of skin were not demarcated with loss of the epidermis which was replaced by acidophilic material and cellular debris. The dermis appeared with extensive inflammatory cellular infiltration, nuclear pyknosis and dermal fibers separation (Figures 2A, 2B).

At day 14, focal areas of epidermal re-epithelialization were noticed with discontinuous dermo-epidermal junction. Eosinophilic homogenous material in the dermis was seen with apparent decrease in the dermal cellular infiltration. Also, areas of collagen separation of dermal fibers could be found (Figure 3A).

At day 28, regeneration was observed in the form of areas of disorganized epidermal epithelium with nuclear pyknosis and cytoplasmic vacuolations (Figure 3B).

-Subgroup IIB (Burn+ injectable PRP)

At day 7, the epidermis appeared with partial re-epithelialization, but degenerative changes were still present; stratum basale appeared with flat basal migratory nuclei from the periphery of the wound, ill-defined keratinocytes of stratum spinosum. Absence of keratin lamellae was also noticed. The dermis appeared with intense, eosinophilic material with separated collagen, hair follicles and few inflammatory cells (Figures 2C, 2D).

At day 14, some regenerative changes were obvious; with more developed epidermis The dermis showed intense cellular infiltration by flat fibroblast like cells (Figure 3C).

At day 28, almost regeneration was complete; well defined epidermis with its four layers; stratum basale, stratum spinosum, stratum granulosum and stratum corneum were observed. The dermis showed diffuse mild cellular infiltration by fibroblast like cells and inflammatory cells. Multiple blood vessels were noticed within the granulation tissue. (Figure 3D).

- Subgroup IIC (Burn+ topical PRP)

At day 7, thin epidermis appeared with rows of flat migratory cells beneath the epidermis. The dermis showed numerous hair follicles surrounded by flat cells represent migratory cells from stem cell niche in the follicles with few inflammatory cellular infiltration (Figures 2E, 2F).

At day 14, regenerative changes were noticed in the form of well-defined epidermis, dermis and dermo-epidermal junction. The epidermis was thickened and well defined (Figure 3E).

At day 28, well defined epidermis was found. The dermis appeared with few inflammatory cells and myofibroblasts like cells (Figure 3F).

B) CD34 Immuno-histochemical stain

**Control group*

Light microscopic examination of sections of the skin of adult male albino rats stained with CD34 showed brownish coloration indicating few CD34 positive cells (Figure 4A).

**Experimental group*

Subgroup I

- Subgroup IA (injectable PRP) and subgroup IB (topical PRP)

Light microscopic examination of sections of the skin of adult male albino rats stained with CD34 showed the same histological findings as control group.

Subgroup II

Very mild positive immunoreactivity in the endothelial cells of dermal blood vessels was observed at day 7 in untreated subgroup (Figure 4B). Unlike subgroups IIB and IIC that showed moderate CD34 immunoreactivity at day 7 (Figures 4C,4D) respectively. At day 14, mild to moderate positive immunoreactivity in the endothelial cells of dermal blood vessels was found in subgroup IIA (Figure 5A), but intense immunoreactivity was observed in subgroups IIB (Figure 5B) and IIC (Figure 5C). Then, very mild or absent positive immunoreactivity was demonstrated at day 28 post burn induction in subgroup IIA (Figure 5D) and mild positive immunoreactivity was found in subgroups IIB (Figure 5E) and and IIC (Figure 5F).

Morphometric study and statistical analysis regarding mean number of blood vessels \ HPF using CD34 immune stain:

At day 7, highly significant increase in the mean number of blood vessels in subgroup IIB was detected when compared with untreated subgroup IIA (*P value* < 0.001). While, significant increase (*P value* < 0.05) was recorded when comparing subgroup IIC with subgroup IIA. No significant difference (*P value* > 0.05) between both treated subgroups IIB and IIC was detected (Figure 6A).

At day 14 and day 28, *P value* was < 0.001, indicating highly significant increase in the mean number of blood vessels in subgroup IIB when compared with untreated subgroup IIA. Significant increase (Figure < 0.05) was recorded when comparing subgroup IIC with subgroup IIA. Significant increase in subgroup IIB was detected in comparison with subgroup IIC (Figures 6 B,C).

Morphometric study and statistical analysis regarding mean area percentage of collagen fibers using Masson's trichrome stain:

At day 7, *P value* was < 0.001, indicating highly significant increase in the mean area percentage of collagen fibers in subgroup IIB when compared with untreated subgroup IIA, but significant increase was recorded when comparing subgroup IIC with subgroup IIA with highly significant difference between both treated subgroups IIB and IIC (Figure 6D).

At day 14 and 28, highly significant increase in the mean area percentage in both subgroups IIB and IIC was detected when compared with untreated subgroup IIA without significant difference between both treated subgroups (Figures 6 E,F).

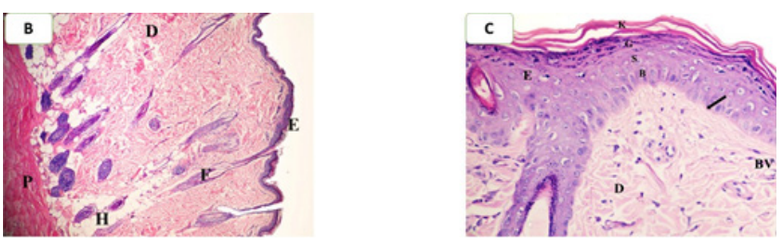

Fig. 1: (B): A photomicrograph of a section in the skin of control group (H. & E. x 100) showing epidermis (E), dermis (D), hypodermis (H), hair follicle (F), and panniculus carnosus muscle (P). **(C):** A photomicrograph of a section in the skin of control group (H. & E. x 400) showing the epidermis (E), dermis (D), stratum basale (B), dermo-epidermal junction (arrow), stratum spinosum (S), stratum granulosum (G), keratin lamellae (K) and dermal blood vessel (BV).

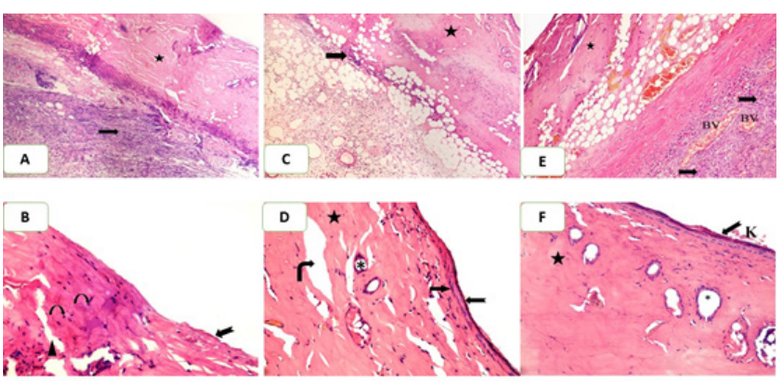

Fig. 2 (A): A photomicrograph of (untreated burn group) at day 7 (H. & E. x 100) acidophilic material (star), dermal extensive cellular infiltration (arrow). **(B):** A photomicrograph of (untreated burn group) at day 7 (H. & E. x 400) showing loss of the epithelial lining of the epidermis (bifid arrow), dermal pyknotic nuclei (curved arrows) and loss of continuity of dermal fibers (arrow head). **(C):** A photomicrograph of (burn + injectable PRP group) at day 7 (H. & E. x 100) showing eosinophilic material in the dermis (star) and moderate inflammatory cellular infiltration (arrow). **(D):** A photomicrograph of (burn + injectable PRP group) at day 7 (H. & E. x 400) showing areas of re-epithelialization (bifid arrow) with raw of migratory cells (arrow). The dermis shows separated collagen fibers (bent arrow) and hair follicles (asterisk). **(E):** A photomicrograph of (burn + topical PRP group) at day 7 (H. & E. x 100) showing intense eosinophilic material in the dermis (star), moderate inflammatory cellular infiltration (bifid arrows) and multiple dilated congested blood vessels (BV). **(F):** A photomicrograph of (burn + topical PRP group) at day 7 (H. &E. x 400) showing thin epidermis (bifid arrow), keratin layer (K), dermal homogenous material (star) and hair follicles (asterisk).

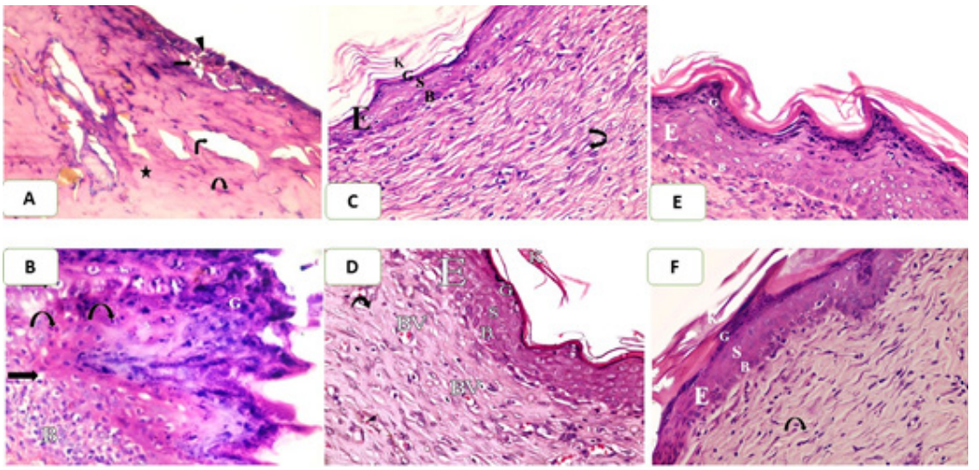

Fig. 3 (A): A photomicrograph of (untreated burn group) at day 14 showing focal re-epithelization of epidermis (arrow head), discontinuity of dermo-epidermal junction (arrow), dermal eosinophilic homogenous material (star). Cellular infiltration (curved arrow), with areas of collagen separation (bent arrow). **(B):** A photomicrograph of (burn + injectable PRP group) at day 14 showing more developed epidermis (E) with startum basale (B), stratum spinosum (S) appeared with vacuolated cytoplasm (thin arrow), stratum granulosum (G) and keratin lamellae of stratum corneum (K). Intense dermal infiltration by fibroblast like cells (curved arrow). **(C):** A photomicrograph of (burn + topical PRP group) at day 14 showing well defined epidermis (E) with stratum basale (B), stratum spinosum (S), stratum granulosum (G) and stratum corneum with keratin lamellae (K). **(D):** A photomicrograph of (untreated burn group) at day 28 showing disorganized epidermis with stratum basale cells (B), nuclear pyknosis (curved arrows), cytoplasmic vacuolations (arrow) and thickened stratum granulosum (G). **(E):** A photomicrograph of (burn + injectable PRP group) at day 28 showing well defined epidermis (E), st. basale (B), st. spinosum (S), st. granulosum (G) and keratin lamella (K). Dermal cellular infiltration (curved arrow) and multiple blood vessels (BV). **(F):** A photomicrograph of (burn + topical PRP group) at day 28 showing well defined epidermis (E). st. basale (B), st. spinosum (S), st. granulosum (G) and keratin lamella (K) The dermis appears with fibroblasts like cells (curved arrow).

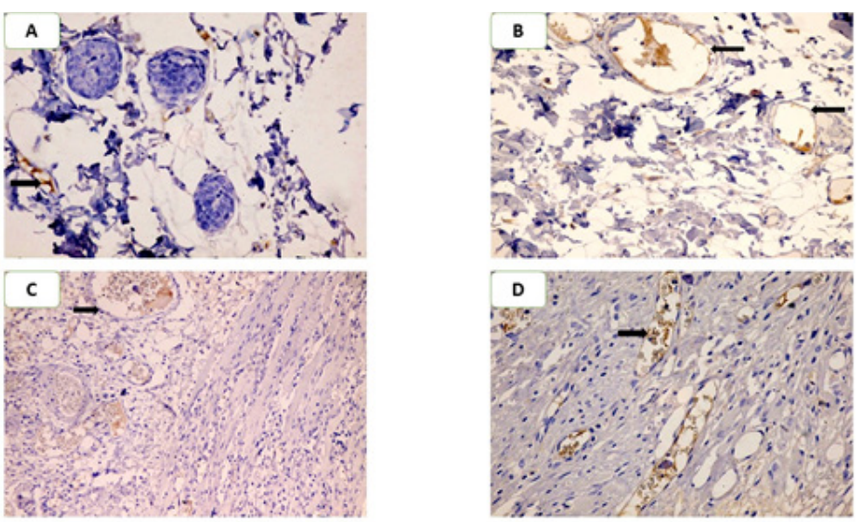

Fig. 4 (A): A photomicrograph of control group showing few CD34 positive cells (arrow). **(B):** A photomicrograph of untreated burn wound day 7 showing very mild positive CD34 immunoreactivity (arrow). **(C):** A photomicrograph of burn + injectable PRP wound at day 7 showing moderate positive CD34 immunoreactivity (arrow) (D): A photomicrograph of burn + topical PRP wound at day 7 showing moderate positive CD34 immunoreactivity (arrow)..

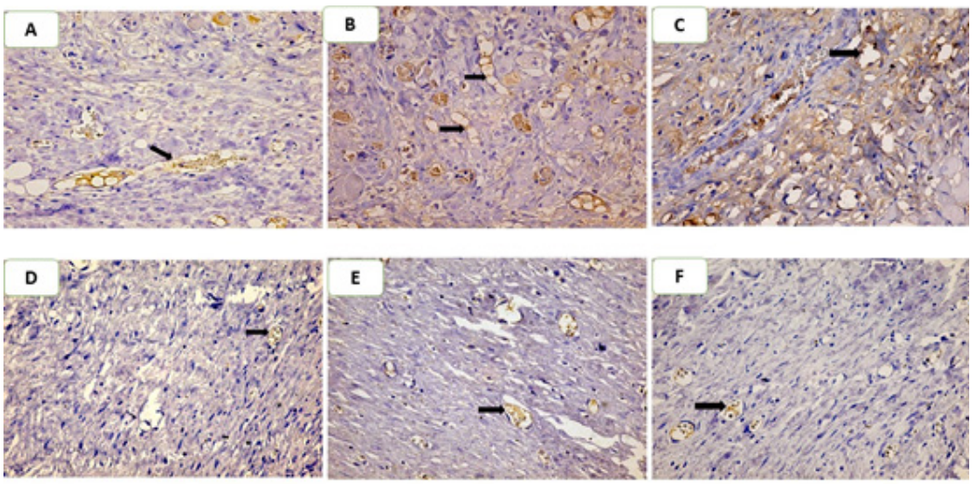

Fig. 5 (A): A photomicrograph of untreated burn wound at day 14 showing moderate positive CD34 immunoreactivity (arrow). **(B):** A photomicrograph of burn + injectable PRP wound at day 14 showing intense positive CD34 immunoreactivity (arrow). **(C):** A photomicrograph of burn + topical PRP wound at day 14 showing intense positive CD34 immunoreactivity (arrow). **(D):** A photomicrograph of untreated burn wound at day 28 showing very mild or absent positive CD34 immunoreactivity (arrow). **(E):** A photomicrograph of burn + injectable PRP wound at day 28 showing mild positive CD34 immunoreactivity (arrow). **(F):** A photomicrograph of burn + topical PRP wound at day 28 showing mild positive CD34 immunoreactivity (arrow).

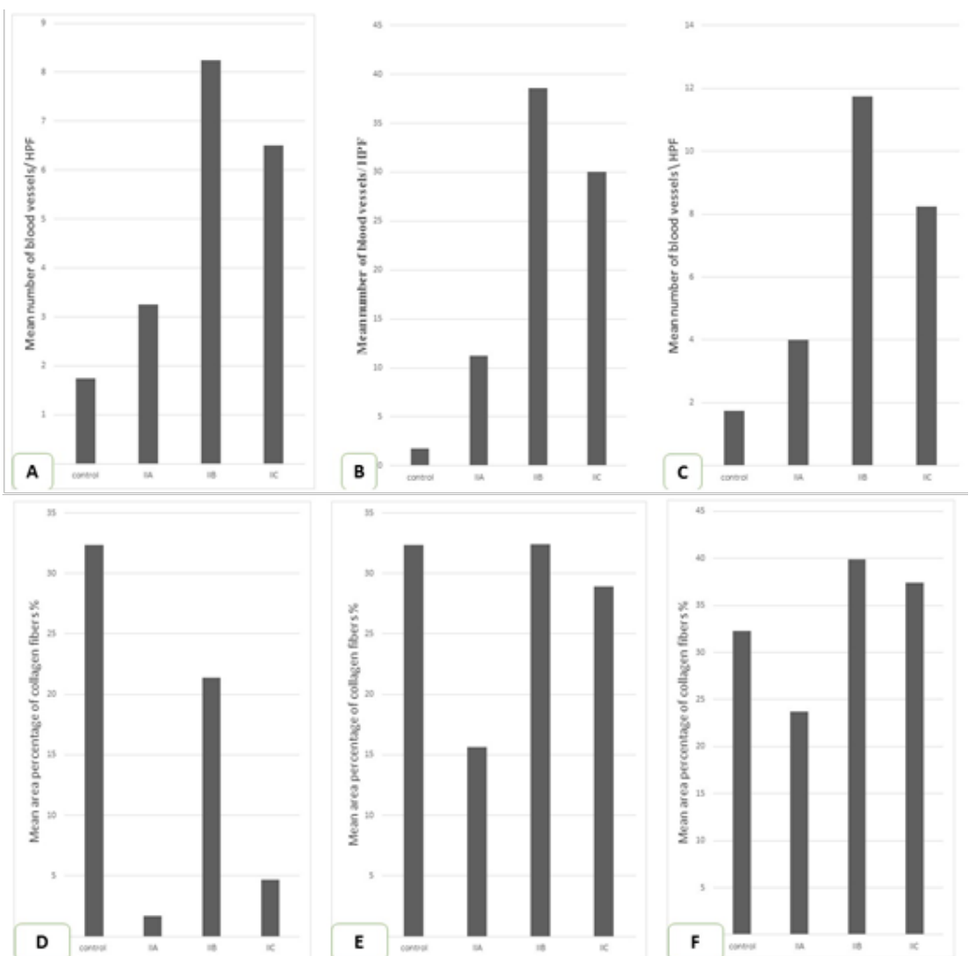

Fig. 6: Comparison between different groups as regard the mean number of blood vessels per HPF at day 7 **(A),** at day 14 **(B)** & at day 28 **(C)**. And comparison between different groups as regard the mean area percentage of collagen fibers at day 7 **(D),** at day 14 **(E)** & at day 28 **(F)**.

Table I: Wound contraction percentage in subgroup II at days 7, 14 and 28 post burn injury.

	Burn group (IIA)	Burn group + injectable PRP (IIB)	Burn group + topical PRP (IIC)
Day 7	10%± 0.02%	22%± 0.03%	24%± 0.05%
Day 14	40%± 0.04%	58%± 0.06%	60%± 0.04%
Day 28	72%± 0.06%	98%± 0.02%	98%± 0.02%

Table II: Statistical analysis regarding wound contraction percentage in subgroup II at days 7, 14 and 28 post burn injury.

P value at day 7		
IIA and IIB	IIA and IIC	IIB and IIC
<0.001**	<0.001**	>0.05
P value at day 14		
IIA and IIB	IIA and IIC	IIB and IIC
<0.001**	<0.001**	>0.05
P value at day 28		
IIA and IIB	IIA and IIC	IIB and IIC
<0.001**	<0.001**	>0.05

DISCUSSION

In the present work (subgroup IIA) burn at day 7, thick layer of acidophilic materials covering the surface of the wound was found and severe dermal inflammation was detected with few CD34 positive cells. The thick acidophilic material represented the scab which is composed of fibrin of blood clot[18].

The scab was essential for homeostasis, temporary closure of the wound and for control of bacterial contamination[19]. Also the initial inflammatory response was elicited by neutrophils recruitment to prevent bacterial infection[20].

In subgroup IIA, day 14, the present study revealed focal areas of re-epithelization of the dermis. Reepithelization was the major process for wound healing[21]. Also, the dermis showed coagulative necrosis and necrotic fat cysts with wide separation of collagen fibers by edema. Increase in CD34 positive cells were detected compared to day 7 with an obvious tendency to decrease towards day 28. These findings were coincided with previous study[22].

In subgroup IIA, day 28 of the present study, the epidermis was disorganized with nuclear pyknosis and cytoplasmic vacuolations. Defective epidermis in this subgroup might be due to cellular infiltration. Excessive inflammation of the wound could lead to impair cellular migration and extracellular matrix (ECM) collapsing[23].

Also proteases enzymes released from inflammatory cells could damage ECM and growth factors[24,25].

Furtherly, proteolytic destruction of ECM prevented the wound from moving forward into proliferative phase and attracted more inflammatory cells[26].

In subgroup IIB, at day 7 (burn + injectable PRP) showed migration of spindle shaped cells from periphery of the wound to cover the wounded area.

The dermis showed few cellular infiltrations with more or less thick homogenous collagen fibers that are still separated in some areas.

Other investigators revealed that some keratinocytes from basal layer of epidermis (stem cells) at wound edge underwent epithelial –mesenchymal transition process (EMT) and migrate to wound area. They recorded that this process is an early step in initiating wound healing process (18). Moreover, injection of PRP can activate stem cells via paracrine effect[27].

In subgroup IIB, day 14 (burn + injectable PRP), the epidermis appeared normal while the dermis showed intense fibroblast like cells infiltrations and multiple blood vessels. There is a correlation between fibroblastic proliferation and collagen content in wound area. Moreover, the period of epithelialization of skin wound is inversely proportional to fibroblasts and collagen content in wound bed[28].

In day 28, (burn + injectable PRP), the epidermis appeared normal while the dermis showed less cellular infiltration with numerous blood vessels.

In this study, statistical analysis revealed highly significant increase in CD34 positive cells was detected in subgroup (IIB) at both days 14 and 28 when compared with untreated subgroup, while significant increase was found in comparison with subgroup IIC.

Angiogenesis is very important in wound healing to maintain the newly formed granulation tissue and for keratinocytes' survival[29].

Furthermore, PRP had beneficial effects on epithelialization and neovascularization of skin graft donor sites[30]. Also, vascular endothelial growth factor (VEGF) found in platelet α-granules, acts as an endothelial cell stimulator resulting in activation, migration, and proliferation of endothelial cells in pathological situations[31].

PRP release growth factors like platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF) that stimulate cellular proliferation and differentiation[32].

In the present work, mean area percentage of collagen fibers at day 7 showed highly significant increase in collagen fibers in subgroup IIB and significant increase in subgroup IIC when compared with subgroup IIA. At day 14 and 28, highly significant increase was detected in both subgroups IIB and IIC when compared with subgroup IIA without significant difference between both treated subgroups. This is in match with other study that reported statistical increase in collagen fibers in PRP treated groups[33].

Collagen is a key factor for keeping skin strength and elasticity. The increase in wound tensile strength that took place during the fibroblastic phase corresponds to the increasing levels of collagen within the wound[34].

The role of PRP in collagen remodeling is still vague and collagen remodeling mostly affected by matrix metalloproteinases, especially MMP-1 and MMP-3 proteins. Furthermore, the induction of MMP-1 in the skin can assess the removal of damaged collagen fragments in dermis, thus providing a good base for the deposition of new collagen that is very important for wound healing[35].

Furthermore, PRP increases the expression of type I collagen, MMP-1 matrix metalloproteinase proteins, and mRNA in human dermal fibroblasts[36].

TGFB from platelets improve dermal regeneration, collagen and protein synthesis, endothelial migration and angiogenesis[37].

Topical application of PRP at day 7 revealed cellular migration from hair follicles. PRP causes activation of stem cell niche[38].

Also at day 14, complete regeneration of the epidermis occurred while dermis showed typical granulation tissues with numerous vasculatures. This was supported by significant increase in CD34 positive cells in comparison with untreated subgroup.

At day 28, the dermis revealed many spindle shaped elongated cells resembling fibroblasts with apparent decrease in vasculature The decrease in vasculature was supported by decrease in CD34 positive cells.

In this study, both injectable (IIB) and topical PRP (IIC) had positive effects on the wound healing process. Regarding wound contraction and collagen deposition, injectable PRP recorded better results, but without significant difference when compared with topical PRP. On the other hand, subcutaneous injection of PRP into multiple points in wound area recorded significant increase than topical PRP in accordance to new vessel formation at both days 14 and 28.

Skin needling which involve a series of skin punctures with sharp needles, can increase the activity of fibroblast in the skin and consequently increase the content of soluble collagen[39].

Moreover, the elevated levels of VEGF with skin needling are based on the mechanical stimulation of the wound healing process[40].

Moreover, the anti-inflammatory effect of PRP is due to its suppressive actions on cyclooxygenase (COX) expression and prostaglandin production. COX is involved in conversion of arachidonic acid (AA) to prostaglandins, which causes vasodilation and increased vascular permeability[41]. Additionally, PRP can significantly increase the intracellular expression of the cytokines which are known to have a key role in prohibiting and controlling inflammation[42].

PRP therapy is a preferred option in treating patients with different kinds of wounds regardless their cause or location, especially when other more traditional therapies are not effective or when surgical treatment is contraindicated. So PRP is indicated in free connective tissue graft procedures, manipulations with mucoperiosteal flaps and soft tissue augmentation for cosmetic purposes in medicine and dentistry[43].

Being autologous, PRP is a safe and optimal therapy with decreased probability of adverse reactions[44]. In the present study, blood donor group was used for blood collection and PRP preparation. The blood of an animal can be considered autologous to other animals due to the syngeneic nature of the sprague-dawley inbred rats[45,46].

CONCLUSION

The application of platelet rich plasma in different forms (injectable and topical) in this experimental study on second degree burn rat model revealed that PRP enhances healing of wounds through the granulation tissue formation acceleration, re-epithelialization process, and wound contraction. Injectable PRP provides better results regarding collagen deposition, fibroblasts infiltration with significant increase in angiogenesis causing high vascular density in wounds. All these expand the potential of therapeutic applications of platelet rich plasma in the treatment of thermal burns.

RECOMMENDATIONS

- Further investigations to guide the optimal use of platelet rich plasma to treat burn injuries in humans.
- More future studies to assess the efficacy of combined injectable and topical PRP in treating burns.
- The effects of PRP on other organs should be assessed on future research.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Mehrabani D, Farjam M, Geramizadeh B, Tanideh N, Amini M, Panjehshahin M. The healing effect of curcumin on burn wounds in rat. World Journal of Plastic Surgery. 2015; 4(1): 29–35.
2. Pereira T, Dos Santos D, Lima-Rebeiro MHM, Pontes-Filho NT, Carneiro-Leão AMA, Correia MTS. Development of animal model for studying deep second-degree thermal burns. Journal of Biomedicine and Biotechnology. 2012; Article ID 460841, 7 pages. https://doi.org/10.1155/2012/460841
3. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plastic and Reconstructive Surgery. 2004; 114(6): 1502-1508.

4. Chicharro-Alcántara D, Rubio-Zaragoza M, Damiá-Giménez E, Carrillo-Poveda JM, Cuervo-Serrato B, Peláez-Gorrea P, Sopena-Juncosa JJ. Platelet rich plasma: new insights for cutaneous wound healing management. Journal of Functional Biomaterials. 2018; 9(1): 10.
5. Woodell-May, J. E.; Ridderman, D. N.; Swift, J. and Higgins, J. Producing accurate platelet counts for platelet rich plasma: validation of a hematology analyzer and preparation techniques for counting. Journal of Craniofacial Surgery. 2005. 16 (5): 749-756.
6. Dhurat R, Sukesh MS. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. Journal of Cutaneous and Aesthetic Surgery. 2014; 7(4): 189–197.
7. Mansoub NH, Gürdal M, Karadadas E, Kabadayi H, Vatansever S, Ercan G. The role of PRP and adipose tissue-derived keratinocytes on burn wound healing in diabetic rats. Bioimpacts .2018; 8(1): 5–12.
8. Duymus ME, Temel S, Ozer H, Urhan MK, Kaya F, Aslan F, Celepi P, Senes M, Dogan K, Kuru S, Barlas AM, Kismet K. Comparison of the effects of platelet-rich plasma prepared in various forms on the healing of dermal wounds in rats. Wounds: A Compendium of Clinical Research and Practice. 2016; 28(3): 99-108.
9. Cai EZ, Ang CH, Raju A, Tan K B, Hing ECH, Loo Y, Wong YC, Lee H, Lim J, Moochhala SM, Hauser CA. Creation of consistent burn wounds: a rat model. Archives of Plastic Surgery. 2014; 41(4): 317.
10. Van der Vijver RJ, Van Laarhoven CJ, Lomme RM, Hendriks T. Paracetamol does not compromise early wound repair in the intestine or abdominal wall in the rat. Anesthesia and Analgesia. 2012; 115(6): 1451-1456.
11. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics. 2010; 1(2): 87.
12. Liao JC. Positive effect on spinal fusion by the combination of platelet-rich plasma and collagen-mineral scaffold using lumbar posterolateral fusion model in rats. Journal of Orthopaedic Surgery and Research. 2019; 14(1): 39.
13. Cavallo C, Roffi A, Grigolo B, Mariani E, Pratelli L, Merli G, Kon E, Marcacci M, Filardo G. Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules. BioMed Research International. 2016; (1): 1-7.
14. Kumar MS, Sripriya R, Raghavan HV, Sehgal PK. Wound healing potential of Cassia fistula on infected albino rat model. Journal of Surgical Research. 2006; 131(2): 283-289.
15. Bancroft JD, Gamble M. The hematoxylins and eosin (9) in Theory and practice of histological techniques. By Bancroft, J.D. and Gamble, M. (eds): Sixth Edition. Chuchill Livengstone; London; Sydney; Newyork; Toronto. 2008; 121-134.
16. Jackson P, Blythe D. Immunohistochemical techniques. In: Theory and Practice of Histological Techniques. By: Bancroft JD and Gamble M (eds): Sixth Edition. Chuchill Livengstone. London, Sydney, New York, Toronto. 2008, 433-472.
17. Dawson, Trapp. Research questions about relationships among variables (8) in basic and clinical biostatistics. The McGraw-Hill companies. USA. 2004, 190-220.
18. Braiman-Wiksman L, Solomonik I, Spira R, Tennenbaum T. Novel insights into wound healing sequence of events. Toxicologic pathology. 2007; 35(6), 767-779.
19. Marinho PC, Neto-Ferreira R, José de Carvalho J. Evaluation of therapeutic intervention with a natural product in Cutaneous wound healing: The use of capybara oil. Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 217198, 10 pages, 2013.
20. Jones HR, Robb CT, Perretti M, Rossi AG. The role of neutrophils in inflammation resolution. In Seminars in immunology. 2016; 28(2), 137-145. Academic Press.
21. Nagata H, Akiba Y, Suzuki H, Okano H, Hibi T. Expression of Musashi-1 in the rat stomach and changes during mucosal injury and restitution. FEBS letters. 2006; 580(1), 27-33.
22. Ojeda JE, Cardenas G, Klassen R, von Plessing C, Grez P, Sepulveda J. Nitric oxide synthase activity and angiogenesis measured by expression of CD34 in burns treated with chitosan films. Wounds. 2011; 23(5): 135.
23. Diegelmann RF, Evans, MC. Wound healing: an overview of acute, fibrotic and delayed healing. Frontiers in bioscience: a journal and virtual library. 2004; 9, 283–289.
24. Pierce GF. Inflammation in nonhealing diabetic wounds: the space-time continuum does matter. The American journal of pathology. 2001; 159(2), 399–403.
25. Schultz G, Mozingo D, Romanelli M, Claxton K. Wound healing and TIME; new concepts and scientific applications. Wound repair and regeneration. 2005; 13(4), S1-S11.
26. McCarty SM, Percival SL. Proteases and Delayed Wound Healing. Advances in wound care. 2013; 2(8), 438–447.

27. Hersant B, Sid-Ahmed M, Braud L, Jourdan M, Baba-Amer Y, Meningaud JP, Rodriguez AM. Platelet-Rich Plasma Improves the Wound Healing Potential of Mesenchymal Stem Cells through Paracrine and Metabolism Alterations. Stem cells international. 2019; 1234263.
28. Kwansang J, Itthipanichpong C, Limpanasithikul W. Evaluation of wound healing activity of Thunbergia laurifolia supercritical carbon dioxide extract in rats with second-degree burn wounds. Journal of Advanced Pharmaceutical Technology & Research. 2015; 6(3): 103–107.
29. Koo MA, Hong S H, Lee MH, Kwon BJ, Seon GM, Kim MS, Nam KC, Park JC. Effective stacking and transplantation of stem cell sheets using exogenous ROS-producing film for accelerated wound healing. Acta Biomaterialia. 2019; 95:418-426.
30. Kakudo N, Kushida S, Minakata T, Suzuki K, Kusumoto K. Platelet-rich plasma promotes epithelialization and angiogenesis in a splitthickness skin graft donor site. Medical Molecular Morphology. 2011; 44(4): 233-236.
31. Zhang J, Nie D, Williamson K, Rocha JL, Hogan MV, Wang JH. Selectively activated PRP exerts differential effects on tendon stem/progenitor cells and tendon healing. Journal of Tissue Engineering. 2019; 10: 1-14.
32. Xu Z, Yin W, Zhang Y, Qi X, Chen Y, Xie X, Zhang C. Comparative evaluation of leukocyte- and platelet-rich plasma and pure platelet-rich plasma for cartilage regeneration. Scientific reports. 2017; 7, 43301.
33. Abuaf O.K, Yildiz H, Baloglu H, Bilgili ME, Simsek HA, Dogan B. Histologic Evidence of New Collagen Formulation Using Platelet Rich Plasma in Skin Rejuvenation: A Prospective Controlled Clinical Study. Annals of Dermatology. 2016; 28(6):718–724.
34. Farghali HA, AbdElKader NA, Khattab MS, AbuBakr HO. Evaluation of subcutaneous infiltration of autologous platelet-rich plasma on skin-wound healing in dogs. Bioscience Reports. 2017; 37(2): BSR20160503.
35. Ibrahim ZA, El-Tatawy RA, El-Samongy MA, Ali D. Comparison between the efficacy and safety of platelet-rich plasma vs. microdermabrasion in the treatment of striae distensae: clinical and histopathological study. Journal of Cosmetic Dermatology. 2015; 14(4): 336-346.
36. Kim D. H, Je YJ, Kim CD, Lee YH, Seo YJ, Lee JH, Lee Y. Can platelet-rich plasma be used for skin rejuvenation? Evaluation of effects of platelet-rich plasma on human dermal fibroblast. Annals of Dermatology. 2011; 23(4): 424-431.
37. Liarte S, Bernabé-García Á, Nicolás FJ. Role of TGF-in Skin Chronic Wounds: A Keratinocyte Perspective. Cells. 2020; 9(2), 306.
38. Ramaswamy Reddy SH, Reddy R, Babu NC, Ashok GN. Stem-cell therapy and platelet-rich plasma in regenerative medicines: A review on pros and cons of the technologies. Journal of oral and maxillofacial pathology: JOMFP. 2018; 22(3), 367–374.
39. Lu Y. Effects of" surrounding needling" on hydroxyproline content and ultrastructures in the dermis of aged rats. Chinese acupuncture and moxibustion. 2008; 28(1): 61-64.
40. Konicke K, Knabel M, Olasz E. Microneedling: A Primer for Dermatologists. J Dermatol Plast Surg. 2017; 2 (2), 1014.
41. Zhang J, Middleton K, Fu FH, Im HJ, Wang JH. HGF mediates the anti-inflammatory effects of PRP on injured tendons. PloS One. 2013; 8(6): e67303.
42. Moussa M, Lajeunesse D, Hilal G, El Atat O, Haykal G, Serhal R, Chalhoub A, Khalil C, Alaaeddine N. Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. Experimental Cell Research. 2017; 352(1): 146-156.
43. Obolenskiy VN, Ermolova DA, Laberko LA, Semenova TV. Efficacy of platelet-rich plasma for the treatment of chronic wounds. EWMA journal. 2014; 14(1):37-41.
44. Merchán WH, Gómez LA, Chasoy ME, Alfonso-Rodríguez CA, Muñoz AL. Platelet-rich plasma, a powerful tool in dermatology. Journal of tissue engineering and regenerative medicine. 2019; 13(5), 892–901.
45. Hammond JW, Hinton RY, Curl LA, Muriel JM, Lovering RM. Use of autologous platelet-rich plasma to treat muscle strain injuries. The American Journal of Sports Medicine. 2009; 37(6): 1135-1142.
46. Wright-Carpenter T, Klein P, Schäferhoff P, Appell HJ, Mir LM, Wehling P. Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. Int J Sports Med. 2004 Nov;25(8):588-93.

الملخص العربى

دراسة مقارنة لتأثير حقن البلازما الغنية بالصفائح الدموية مقابل دهنها موضعيا في علاج الحروق الحرارية المستحثة في ذكور الجرذان البيضاء البالغة:دراسة نسيجية ومناعية يذاكر

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الخلفية: إصابات الحروق هي مشكلة صحية رئيسية وأحد الأسباب الرئيسية المتسببة في حدوث الأمراض والوفيات. ركزت العديد من التقارير على البلازما الغنية بالصفائح الدموية ودورها في علاج جروح الأنسجة الرخوة. تعمل البلازما الغنية بالصفائح الدموية من خلال تسلل تركيز عالي من الصفائح الدموية في موقع الجرح.
الهدف من الدراسة: كان الهدف من هذا العمل هو دراسة التأثير النسيجي للبلازما الغنية بالصفائح الدموية على الحروق الحرارية المستحثة في ذكور الجرذان البيضاء البالغة.
مواد وطرق البحث: تم تقسيم ٦٠ من ذكور الجرذان البيضاء البالغة إلى ثلاث مجموعات. مجموعة المتبرعين بالدم (١٠ جرذان)، المجموعة الضابطة (١٠ جرذان) والمجموعة التجريبية (٤٠ جرذا) والتي تم تقسيمها إلى مجموعات فرعية I & II. تم تقسيم المجموعة الفرعية الأولى إلى مجموعتين فرعيتين أخريين ;المجموعة الفرعية IA (٥ جرذان): تم حقن خمسة فئران بـ ٠,٣ مل من البلازما الغنية بالصفائح الدموية الذاتية تحت الجلد والمجموعة الفرعية IB (٥ جرذان): تم وضع البلازما الغنية بالصفائح الدموية موضعيا بدون إصابة. المجموعة الفرعية الثانية (٣٠ جرذا): تم تخدير جميع الفئران في هذه المجموعة الفرعية وتعرضها للحرق الحراري ثم تم تقسيمها إلى؛ المجموعة الفرعية IIA (١٠ جرذان) حيث تعرضت للحرق الحراري دون أي علاج. المجموعة الفرعية IIB (١٠ جرذان) تم حقنها تحت الجلد بـ ٠,٣ مل من البلازما الغنية بالصفائح الدموية مرة واحدة بعد التعرض للحرق الحراري. المجموعة الفرعية IIC (١٠ جرذان): تم وضع جل البلازما الغنية بالصفائح الدموية موضعيا في الأيام ١ و٣ و٧و ١٠ بعد استحداث الحرق. تم الحصول على عينات الجلد في ال أيام٧ و١٤ و٢٨ بعد الحرق والمشاركة في الدراسات النسيجية والكيميائية المناعية. تم إجراء تقييمات لمتوسط عدد الأوعية الدموية ونسبة المساحة المتوسطة لألياف الكولاجين ثم تعرضوا للتحليل الإحصائي في المجموعات الفرعية المختلفة.
النتائج: أوضحت نتائج هذه الدراسة أن العلاج بـالبلازما الغنية بالصفائح الدموية أدى إلى التجدد المحسن لإصابة حروق الجلد. وقد ثبت ذلك من خلال الفحص المجهري الضوئى والدراسات المورفومترية. تتجلى المجموعات الفرعية المعالجة البلازما الغنية بالصفائح الدموية من حيث إعادة تكون البشرة المبكر، وتجديد ألياف الكولاجين وتعزيز الأوعية الدموية المحسنة في اليوم السابع بعد الإصابة بالحرق مع استعادة السمات النسيجية الطبيعية لأنسجة الجلد. يوفر البلازما الغنية بالصفائح الدموية عن طريق الحقن نتائج أفضل فيما يتعلق بترسب الكولاجين، وتكوُّن الأوعية مما يسبب كثافة عالية في الأوعية الدموية في الجروح.
الاستنتاج: تمكنت البلازما الغنية بالصفائح الدموية بمختلف أشكالها (عن طريق الحقن والموضعية) من تعزيز شفاء الجروح من الدرجة الثانية من خلال تسريع تشكيل الأنسجة الحبيبية، وعملية إعادة تكوين البشرة، وتقلص الجروح. كما يوصى بإجراء المزيد من الأبحاث المستقبلية لتقييم فعالية استخدام البلازما الغنية بالصفائح الدموية عن طريق الحقن والموضعية معا في علاج الحروق