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COMPARISON OF PHOTOSENSITIZED TISSUE BONDING AND VET GLUE IN CLOSURE OF INCISIONAL WOUNDS

Muhammad Arslan Aslam *[1], Abdul Saboor [2], Azhar Shabbir Ather [1], Muhammad Bilal [3], Nauman Rafique [4], Saba Mehnaz *[4], Shahbaz Ul Haq [5] and Anum Ashraf [1]

[1]Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
[2]Department of Veterinary Surgery, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
[4]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
[5]Key Laboratory of New Animal Drug Project, Gansu Province, Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agriculture Sciences, Lanzhou, China

**Corresponding author*: drmarslan3@gmail.com (MAA), sabamehnaz2012@gmail.com (SM)

ABSTRACT

Photochemical tissue bonding (PTB) is considered to be used in surgery to enhance healing of cutaneous wounds and to minimize the complications i.e., wound dehiscence, inflammatory reactions, and infections affecting animal health. The aim of this study is to analyze the wound healing process by using PTB and cyanoacrylate-based tissue adhesive. The results showed that PTB group had quicker healing time, good healing score and stronger tensile strength for incisional wounds of rabbits. However, Vetbond group showed delayed healing, poor healing score, and weaker tensile strength. The 14-days post-surgery PTB treated rabbits cutaneous layers were stained with hematoxylin and eosin stain. The histological examinations of these cutaneous specimens revealed higher thicknesses (µm) of regenerated Epidermal, Dermal and Hypodermal layers, collagen fibers, and epithelial ridges. Whereas, for 14 days Vetbond treated rabbits cutaneous layers showed less thickness of these cutaneous layers, degenerated collagen fibers and no ridges. In future, large-scale analysis of combined suture less technique is required to enhance wound healing processes.

***Keywords*:** Skin Wounds, Cyanoacrylate Tissue Glue, Photochemical Tissue Bonding.

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1. INTRODUCTION

Damage to normal anatomical structure and function of tissue is known as wound. Wounds occur due to pathological processes in the affected organs internally or externally (Robson et al. 2001; Guo and DiPietro 2010; Childs and Murthy 2017). They are resulted into discontinuation in the epithelium of the skin, (Velnar et al. 2009; Han and Ceilley 2017; Hussain et al. 2016) cutaneous tissues, such as nerve tissues, muscle tissues, tendons, blood and lymphatic vessels, parenchymal organs, and bones (George et al. 2006; Menke et al. 2007; Gonzalez et al. 2016). Wounds can aggravate the pathology of a disease (Li et al. 2007; Velnar et al. 2009; Schreml et al. 2010; Rodrigues et al. 2019). Ultimately, they cause serious complications like morbidity and death (Young and McNaugh 2011; Pastar et al. 2014; Singh et al. 2017). Its healing process is constant and is categorized into different stages (Delavary et al. 2011; Dreifke et al. 2015; Han and Ceilley 2017; Qing 2017).

The regenerative process of an injury depends on coordination of four stages (Fung et al. 1999; Islam et al. 2014; Ruprai 2019) i.e. hemostasis and coagulation stage (Gomes et al. 2019) in which clotting of blood occurs to minimize blood loss (Wang et al. 2019; Unsihuay et al. 2021), inflammatory stage starts inflammatory reactions and removes the waste of dead cells (Feng et al. 2020; Wang et al. 2020; Lee et al. 2022), proliferatory stage initiates within few days of wound and involves reconstruction of connective tissues, starts production of denovo network of circulatory system and construction of epithelium (Redmond and Kochevar 2019; Fuentes-Lemus et al. 2020; Narayanan et al. 2020; Noori-Dokht et al. 2022; Yelkuvan et al. 2023) and maturation phase (Yan et al. 2022) which causes refurbishing of collagen form III to form I (Basov et al. 2019; Fuentes-Lemus and Lopez-Alarcon

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2020; Li et al. 2021; Liu et al. 2022; Liang et al. 2023). Maturation phase involves the formation of scar and may last up to a year or more (Sarabi et al. 2021; Wanasingha et al. 2021; Klelemen et al. 2022; Lee et al. 2023).

Closing of the surgically incised and excised wounds through a simple, quick and scar less procedure remains an aim in dermatology surgeries (Demartis et al. 2021; Kim et al. 2021; Liu et al. 2021; Ma et al. 2021; Lee et al. 2022). However, obtaining positive results in wound healing has been a difficult task **(**Ryou and Thompson 2002; Neto et al. 2008; Sangwan et al. 2014; Li et al. 2019; Vanerio et al. 2019; Hamedi et al. 2022**).** Negative effects of suturing occur due to more tension in stitches and placement of stitches for a long time upon wound area (Chan et al. 2002; Campomanes et al. 2009; Barton et al. 2013; Daykan et al. 2017; Rodrigues et al. 2022; Huang et al. 2023). Tight stitches produce imprints along with stitch line and lead to inside growth of epidermis (Gabay et al. 2011; Hussain et al. 2016; Lee et al. 2022; Zhao et al. 2022). Suture-less techniques to close surgically incised and excised wounds involve surgical staples, strips and surgical adhesives which produce reliable tissue connectivity and showed excellent blood clotting results (Bernard et al. 2001; Chan et al. 2002; Bao et al. 2020; Gummerer et al. 2020; Tarafder et al. 2020, Ortiz et al. 2021). However, these products are costly and lead to the spread of infection in the respective tissues (Currie et al. 2001; Hodges et al. 2001; Bal-Ozturk et al. 2021; Ceylan et al. 2021; Yuk et al. 2021). Cyanoacrylate based surgical glues are not recommended for patients having allergy to formaldehyde or cyanoacrylate (Judy et al. 1993; Marks et al. 2000; Simhon et al. 2007; Singh et al. 2021; Ma et al. 2021).

Surgical tissue adhesive is an emerging technique which is extensively used in the cosmetic surgeries of face and other body parts (Lambert and Kochevar 1997; Maw et al. 1997; Mandley et al. 2000; Morrissey and Swekla 2023). An ideal surgical tissue adhesive has excellent binding strength, good tissue bio adaptable properties, can produce biodegradable side products, easy to apply and can exhibit minimum tissue reaction (Mulroy et al. 2000; Singer and Thode 2004; Verter et al. 2011; Tsao et al. 2012).

This study was designed to compare the wound healing efficacy of PTB and cyanoacrylate-based tissue glue on rabbits' incisional cutaneous wounds through healing score, healing time, tensile strength, and histological examination.

2. MATERIALS AND METHODS

All the techniques were performed according to the ARRIVE guidelines. Vet Bond™ (cyanoacrylates-based tissue glue, manufactured by 3M Animal Care Products, U.S.A.), Rose Bengal dye, A light-sensitive dye, mixed with phosphate buffer saline (PBS) solution was used to make a fresh 0.1% solution.

2.1. Study Design and Sample Collection

The present study was conducted after approval from the guidelines of "WHO-2011" made by Research Ethics Committee of the faculty of Veterinary Medicine, University of Agriculture, Faisalabad, Pakistan. The research was conducted on 20 healthy adult rabbits of either gender having an average weight of 1.5kg/animal. These rabbits were placed in clean metal cages under standardized conditions, controlled light and ambient temperature (25°C) with standardized laboratory water and feed.

2.2. Samples Grouping and Study Procedures

The Rabbits were anesthetized by injecting ketamine hydrochloride (Ketarol®, Global Pharmaceuticals, Pakistan) @13-30mg/kg body weight intramuscularly in combination with acepromazin (Sedastress®, Farvet Laboratories, Holland) @0.02mg/kg body weight and were placed in dorsal recumbent position. Cutaneous incisions were made through ventral midline approach. Surgical operations were performed on all the animals by the induction of cutaneous incisions.

A total of 20 rabbits were selected and 2 groups (PTB and Vetbond) were designed containing equal numbers of Rabbits (10 animals in each) with a fortnightly monitoring period. In PTB group the cutaneous incisions were exposed to green light of monochromic origin (532nm) for 200 seconds after pasting the Rose Bengal dye (RB) on the incision surface (Yao et al. 2010). However, in Vet Bond Group, Vet Bond® (Cyanoacrylate-based Glue) was applied only on the edges of the cutaneous incisions.

All the experimental animals were euthanized after the completion of trials. The cutaneous tissues were removed and fixed in 10% buffered formaldehyde. Histological processing was performed after embedding in paraffin and applying 4mm cuts for staining in hematoxylin and eosin stain.

2.3. Evaluation Methods

2.3.1. Healing Score: It was classified as excellent (minimum swelling, no exudate production, no dehiscence, gradual decrease in width of incisional edges), good (minimum swelling with little exudates production, no dehiscence, gradual decrease in thickness of cut edges) and fair (notable swelling, presence of infection and exudates production) (Yao et al. 2010).

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2.3.2. Healing Time: It represents the time (in days) between the infliction of an incision and the completion of regenerative process, and formation of epithelium. It is calculated by the total of everyday examinations until the scar is sloughed off (Kumar et al. 2008).

2.3.3. Tensile Strength: It describes the extent of reconstruction of a healed injury. After the completion of the healing process, it is calculated by Tensometer. The breaking strength was estimated by the following equation.

$$\text{Breaking strength (\%)} = \frac{\text{Mean tensile strength of the strips from treated wound.}}{\text{Mean tensile strength of the control wound.}} \times 100$$

2.4. Histological Examination

Tissue samples of abdominal incisional wounds of rabbits were collected and fixed in 10% neutral buffered formalin. Dehydration was done in alcohols. After clearing in chloroform and xylene, samples were impregnated with paraffin wax. The staining of the specimens was done with hematoxylin and eosin. Light microscope was used to observe the prepared histological slides (Gabay et al. 2011).

2.5. Statistical Analysis

The analysis of the obtained data was performed by using SPSS software for windows version 26.0 at significant level 0.05 ($P \leq 0.05$). Estimation of descriptive statistics was done in the form of Mean±SE. Comparison between 2 groups for each variable under trial was performed by using independent samples T-test. $P \leq 0.05$ is considered to be statistically significant.

3. RESULTS

3.1. Evaluation Criteria

Healing efficiency of the incisional cutaneous wounds was assessed by Healing score, Healing time, Tensile strength and Histological examination.

3.2. Healing Score

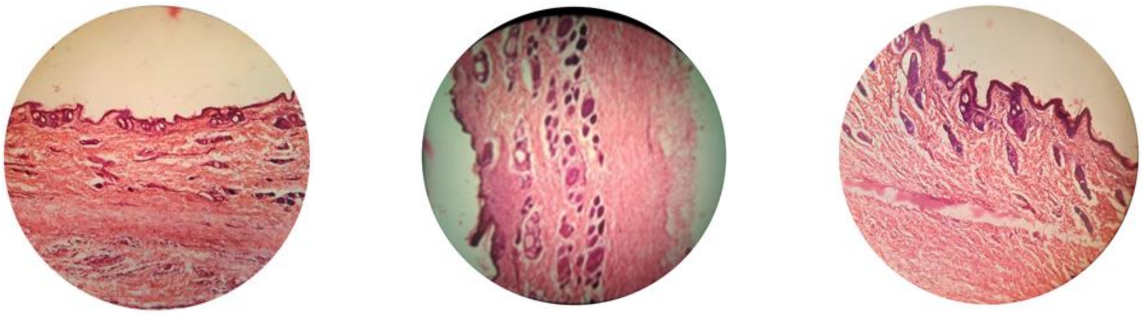
PTB treated rabbits showed a better healing score (2.6±0.39) with a highly significant association ($P \leq 0.05$) as compared to Vetbond treated rabbits which showed 2.1±0.44 healing score with a non-significant association as shown in Fig. 2.

3.3. Healing Time

Rabbits exposed to PTB showed significantly higher healing time (3±0.66 days) and had significant results ($P \leq 0.05$), in contrast with Vetbond (6±1.56 days), which can be due to decreased inflammatory response as shown in Fig. 3.

3.4. Histopathological Examination

There were considerable differences noticed in the thicknesses (in micrometers) of Epidermal, Dermal and Hypodermal layers of PTB treated rabbits and those treated with Vetbond as shown in Fig. 1. PTB treated rabbits



A. The photomicrograph is showing higher amount of dense irregular connective tissue with relatively lesser number of Mononuclear cells.

B. This photomicrograph is showing all the three layers of skin namely epidermis, dermis and hypodermis. Reticular layer of the dermis is showing remnants of granulation tissue while epidermis and hypodermis are completely healed.

C. This photomicrograph is showing higher degree of Keratinization and showing all the three layers of skin. The part of dermis where incision was made can still be appreciated with partial healing. Sebaceous glands are also visible.

Fig. 1: Microscopic view of tissue slides stained with hematoxylin and eosin stain at 100X.

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showed higher epidermal thickness 51.47±12.76µm with a highly significant association (P≤0.05) as shown in Fig 4. Whereas Vetbond treated rabbits showed less (34.35±5.3µm) epidermal thickness with a non-significant association. The overall thickness of dermal layer observed in rabbits of PTB treated group was 1480.12±453.89µm (P≤0.05) as shown in Fig. 5. Whereas the dermal thickness of rabbits of Vetbond group was 1201±255.125µm. Similar thickness was recorded for Hypodermis. The overall thickness of hypodermis noticed in PTB exposed rabbits was 433.39±277.15µm (P≤0.05), whereas the values for rabbits of Vetbond group was 365.37±209.58µm (Fig. 6).

3.5. Tensile Strength

A statistically significant difference was observed among the experimental groups. PTB treated incisional wounds showed higher tensile strength (86.4±14.18µm) with a highly significant association (P≤0.05), as compared to those treated with tissue glue (74±13.91µm) as shown in Fig. 7.

4. DISCUSSION

A wound is a break in the continuity of the epidermis which leads to infection and septicemia (Li et al. 2007; Velnar et al. 2009; Schreml et al. 2010; Rodrigues et al. 2019). Wounds occur due the injury or surgical operations following a wound restoration phenomenon that mainly includes 4 principal stages (coagulation, inflammation, cell-proliferation and Matrix repair, epithelialization and remodeling of the scar tissue). Wound management is an essential part of emergency medicine (Kamegaya et al. 2005; Ananda et al. 2019; Chen et al. 2023; Chudek et al. 2020).

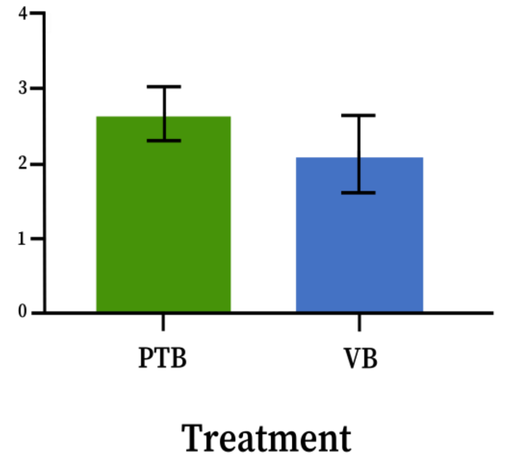

Fig. 2: Comparison of healing score (Mean±SE) in two groups treated with PTB and tissue glue in rabbits (n=20).

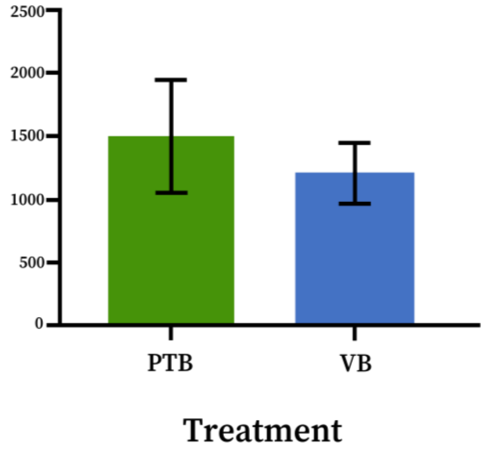

Fig. 3: Comparison of healing time (Days; Mean±SE) in two groups treated with PTB and tissue glue in rabbits (n=20).

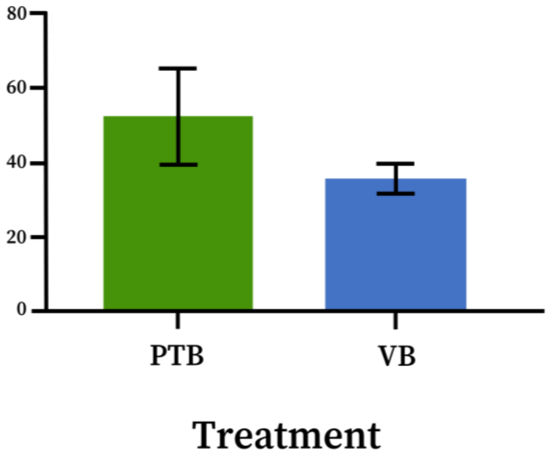

Fig. 4: Comparison of epidermis thickness (µm; Mean±SE) in two groups treated with PTB and tissue glue in rabbit (n=20).

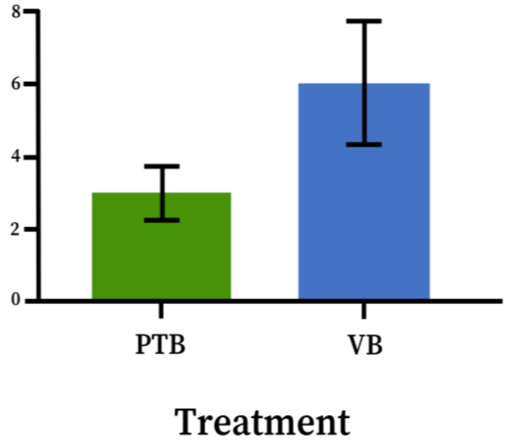

Fig. 5: Comparison of dermis thickness (µm; Mean±SE) in two groups treated with PTB and tissue glue in rabbits (n=20).

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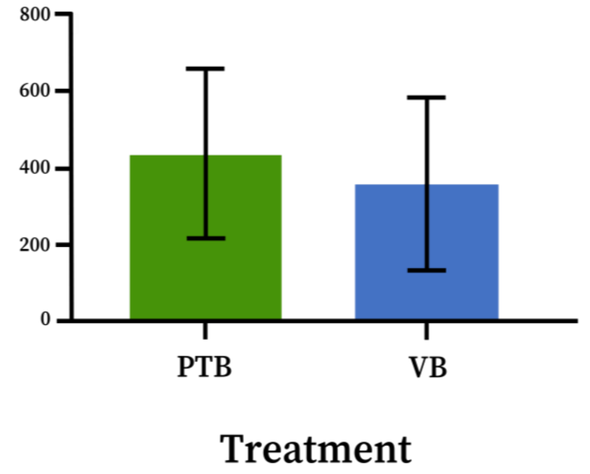

Fig. 6: Comparison of hypodermis thickness (µm; Mean±SE) in two groups treated with PTB and tissue glue in rabbits (n=20)

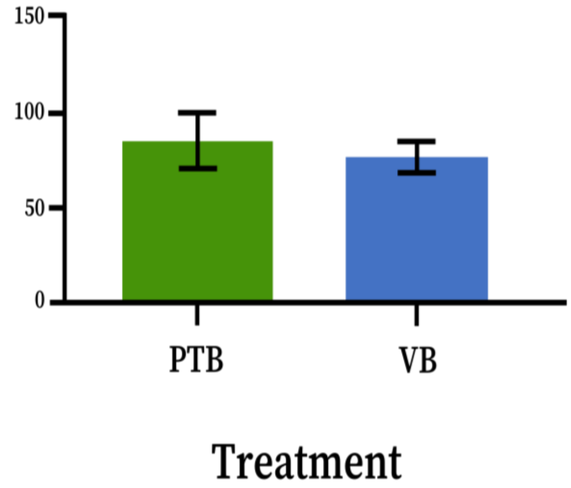

Fig. 7: Comparison of breaking strength (%; Mean±SE) in two groups treated with PTB and tissue glue in rabbits (n=20)

Our results illustrated that PTB treated incisional wounds of rabbits showed quicker healing time, good healing score, and more tensile strength. However, Vetbond treated incisional wounds that showed delayed healing, bad healing score, and weaker tensile strength. Our results revealed that the healing score of 2.6±0.39µm of PTB treated group was better than Vetbond group 2.1±0.44µm. Our results were in line with the study of Judy and his colleagues who reported the similar results in rabbits treated with PTB to improve standards of microsurgical re-anastomoses of blood vessels in the *in vitro* and *in vivo* models (Judy et al. 1994; Lee et al. 2022). On the basis of clinical studies, scientists reported that cosmetic results of PTB were excellent as compared to conventional suturing technique. No edema was found after PTB treatment which was in agreement to our study (Mobley et al. 2002; Kamegaya et al. 2005; Jan et al. 2020).

The healing-time of PTB treated Rabbits was significantly faster (3±0.66 days) in contrast to Vetbond treated rabbits (6±1.56 days). This can be due to decreased inflammatory response. Our results are in line with Tariq et al. (2018) who reported similar findings in animals treated with PTB technique. The animal's skin healed with minimal scar width of 6 weeks post-treatment. The PTB technique was comparatively faster, simple and pain free. Based on the clinical observations the healing time of the PTB treated incisions was 3 to 4 days whereas, it was 7 days in case of sutures. Histopathological examinations revealed that PTB showed significantly dense collagen fiber in contrast to the conventional suturing-technique to close the cutaneous incisions (Tariq et al. 2018; Ding et al. 2019).

The general epidermal thickness of PTB treated rabbits was 51.47±12.76µm. Whereas, the epidermal thickness of Vetbond treated rabbits was 34.35±5.3µm. The overall thickness of dermal layer in rabbits of PTB group was 1480.12±453.89µm. Whereas, the dermal thickness for rabbits treated with tissue Vetbond was 1201±255.125µm.

Similar thicknesses were also recorded for Hypodermis. The overall thickness of hypodermis observed in PTB treated rabbits was 433.39±277.15µm. The hypodermal thickness of Vetbond treated group was 365.37±209.58µm. These results are in agreement with the findings of Simhon et al. (2004) who reported epidermal thickness of PTB group considerably more due to infiltration of granulation tissue which produces a coagulum for healing of tissue. PTB treated rabbits showed epidermal thickness 125.1±6.5µm whereas in conventional suturing the epidermis thickness was 116.2±3.1µm. PTB treated rabbits dermis thickness was 120.9±7.7µm whereas the thickness of dermis in conventional suturing was 113.0±5.6µm (Talmor et al. 2001; Tariq et al. 2018; Balomenos et al. 2023).

5. Conclusion

It is concluded that wounds treated with PTB have reduced healing time, excellent healing-score, thickened dermal and epidermal layers and higher tensile strength of the repaired tissue in comparison with tissue glue, and is more efficient. However, PTB did not cause wound complications such as wound dehiscence and inflammatory reaction PTB provides better results in comparison with tissue glue. In future, more trials should be done for tissue intoxication of the dyes, thermal changes in the tissue and surrounding tissue destruction before applying the procedure clinically.

Conflict of Interest

The authors have no conflict of interest.

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Authors Contributions

Muhammad Arslan Aslam conducted the research while Abdul Saboor, Azhar Shabir and Muhammad Bilal helped in writing the research paper. Muhammad Nauman Rafique and Saba Mehnaz helped in proofreading this research paper. Shahbaz Ul Haq made the graphs of the paper. While Anum Ashraf helped in final revision of paper.

ORCID

Muhammad Arslan Aslam	https://orcid.org/0009-0003-9456-7330
Abdul Saboor	https://orcid.org/0009-0003-6136-043X
Azhar Shabbir Ather	https://orcid.org/0009-0003-8067-0898
Muhammad Bilal	https://orcid.org/0000-0003-1525-6562
Nauman Rafique	https://orcid.org/0000-0001-8442-9784
Saba Mehnaz	https://orcid.org/0000-0002-3557-9492
Shahbaz Ul Haq	https://orcid.org/0000-0002-2380-7419
Anum Ashraf	https://orcid.org/0009-0005-5773-718X

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