The Effect of Topically Applied Boric Acid on Ephrin-Eph Pathway in Wound Treatment: An Experimental Study

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Abstract

Background: Wound healing has a vital importance for the organism and various agents are used to accelerate wound healing. Although the effect of boron on wound healing is known, its mechanisms are not completely clear yet. In this study, the effect of boron in the Ephrin /Eph pathway will be evaluated.

Methods and Results: Forty adult female rats were used in the study. A full-thickness excisional wound model was created in all groups divided as control, Fito cream, Boric acid and Pluronic gel groups. Histopathologically, inflammatory cell infiltration(ICI), edema and fibroblast proliferation density(FPD); immunohistochemically TNF-α(Tumor necrosis factor α), EphrinA1, EphrinB1, EphrinB2 and EphB4 antibodies were evaluated in the skin tissues obtained after the applications performed twice a day and lasting 7 days.

The ICI score was found to be higher in the Fito group compared to the boron group(p=0.018). FPD was higher in Plu group than boron group(p=0.012). While TNF-α was lower in boron group than Plu(p=0.027) and Fito(p=0.016) groups, EphrinA1 was higher in Boron group than plu group(p=0.005). EphrinB1 expression was higher in Boron group compared to Plu(p=0.015) and Fito(p=0.015) groups, and the same difference was also observed in EphrinB2 (p values 0.000). Similarly, EphB4 immunoreactivity was higher in the Boron group compared to the plu(p=0.000) and Fito(p=0.002).

Coclusion: One of the mechanisms of action of boron in wound healing is to increase EphrinB1, EphrinB2 and EphB4. Low TNF-α and histopathological findings indicate that boron limits extensive wound healing.

1. Introduction

Injuries occur on the skin as a result of the deterioration of the integrity of the skin for various reasons. After the breakdown of skin integrity, a complex process begins in the body, called wound healing, consisting of 5 main stages: homeostasis and inflammation, granulation tissue formation, neovascularization, re-epithelization, and remodeling. [1, 2]. In this process, which is regulated by many molecules, these events must occur sequentially in order for the wound to close effectively. Chemokines play important roles in regulating this sequence through the recruitment of inflammatory cells that secrete cytokines and growth factors to regulate angiogenesis and promote wound healing. [3].

Ephrins, which play an important role in tissue repair, and their receptors Ephs, constitute a large family of receptor tyrosine kinases. Ligands and receptors are all classified as A and B. This classification is determined by their binding affinity and structural similarity. This family consists of 9 Eph-A and 5 Eph-B receptors and 5 Ephrin-A and 3 Ephrin-B ligands. [4]. Eph / Ephrin proteins play an important role in wound healing such as angiogenesis and cell migration [5, 6].

It has been reported that Type A Ephrin-Eph proteins have angiogenetic effects on VEGF (Vascular endothelial growth factor) [4]. Ephrin-A1 expression is an important determinant of endothelial

proliferation. In addition, studies have emphasized that Ephrin A1 and Eph A2 receptors have angiogenetic effects through TNF-α (Tumor necrosis factor α) [7]. Ephrin A1, A3 and A4 and Eph A1, A2 and A4 are known to exist in human skin tissue. It is emphasized that Ephrin B2 and Eph B4, which are reported to be involved in both physiological and tumor angiogenesis, are associated with inflammation.

Boron is a nonmetallic element, always 3-valent and bonds with many other elements to form trigonal planar structures such as orthoboric acid or tetrahedral structures such as anionic borate. Boron is widely found in soil and water and its concentration in soil is approximately 3-100 ppm [8]. When Boron is taken orally into the body, it is absorbed by 100% from the intestines and is metabolized in the liver into boric acid form. Boron, whose distribution in tissues is in the form of boric acid, is excreted from the kidney at a rate of 88–93% within the days following intake [9].

Studies have shown that boron is effective in various areas such as prevention and treatment of various cancers, arthritis treatment, neurodegenerative diseases, bone growth and bone healing, and treatment of tendon ruptures [10]. There are studies in the literature showing that topically applied 3% boric acid is effective in wound healing [11–13]. It is stated that it especially accelerates the production of extracellular matrix during the wound healing process [14].

In the light of existing data; It is aimed to examine the effect of boric acid, which has been proven to be effective in the wound healing process, on the Eph-Ephrin mechanism and to reveal the pathways that play a role in the wound healing process.

2. Materials And Methods

2.1. Animals and Ethical Procedure

This study was supported by the Scientific Research Projects Coordination Unit of Çanakkale Onsekiz Mart University (ÇOMÜ) with the project number TSA-2019-2831. Before starting the study, approval was obtained from the Animal Experiments Local Ethics Committee of Çanakkale Onsekiz Mart University (Ethical approval number: 2018 / 09 – 07). The current study was conducted in accordance with the recommendations of the World Medical Association's Declaration of Helsinki on animal studies. In the study, 40 adult female Wistar Albino rats weighing between 220 and 300 g were used and the rats were obtained from ÇOMÜ Experimental Research Center (ÇOMÜDAM). All steps of the study were carried out in the same center. Rats were housed in standard rat cages at standard humidity and temperature (45% -50% humidity and 22 ± 2 ° C), 12 hours dark, 12 hours light period. The rats were fed ad libitum with standard feed and tap water until 12 hours before the performance of the study procedure. 12 hours before the test procedure, the food was stopped and only water was given.

2.2. Preparation of Gel

The gel was prepared before the first use every morning and stored at + 4°C until the time of use. For each animal, 1 ml of Pluronic gel F127 (Sigma-Aldrich, Germany, Lot no: BCBW5376) per use is taken into a 30

sterile culture dish and 3% boric acid (Merck KGaA, Darmstadt-Germany Product No: B6768) was added and left at + 4°C for half an hour to dissolve.

2.3.Experimetal Protocol

Forty adult female Wistar Albino rats used in the experiment were randomly divided into 4 groups. The groups are given below;

Group 1: Control (C) group (n = 10)

Group 2: Fito cream (Fito) group (n = 10)

Group 3: Boric acid-Pluronic gel (Boron) group (n = 10)

Group 4: Pluronic gel (Plu) group (n = 10)

Intraperitoneally 50 mg / kg of Ketamine hydrochloride (Ketalar®, Pfizer Pharmaceuticals Ltd Sti, Istanbul, Turkey) and 15 mg / kg Xylazine (Alfazyn 2%, Aegean Vet Ind. Trade, Izmir, Turkey) were given to the rats in all groups and after administration of anesthesia, dorsal areas were shaved and cleaned with povidone iodine. The full-thickness excisional wound model was created as previously stated in the literature (6 mm diameter and 2 mm depth skin was excised) [15].

After the wound model was created, the rats were taken into separate cages as one animal in each cage. While no treatment was given to the animals in the control group, Fito cream (Ethyleneglycol ether monefenil + triticum vulgare aqueous extract, Tripharma Pharmaceutical Co., Turkey), which is a standart wound treatment, was applied topically twice a day for 7 days as covering the wound completely to the animals in Group 2. A 3% boric acid-pluronic gel mixture was applied to the animals in Group 3, and the Pluronic gel F127 standard form, which was prepared previously, was applied to the animals in Group 4, again for 7 days and twice a day, topically to cover the wound completely. After 7 days of wound treatment, wound biopsy was taken from the animals on the 8th day. The animals were prevented from accessing water and food 12 hours before the procedure. Then, 50 mg / kg of Ketamine hydrochloride (Ketalar®, Pfizer Pharmaceuticals Ltd Sti, Istanbul, Turkey) and 15 mg / kg Xylazine (Alfazyn 2%, Aegean Vet Ind. Trade, Izmir, Turkey) was given intraperitoneally and the wound area all were excised.

2.4. Histopathological Evaluation

At the end of the experiment, tissue samples taken from animals were placed in 10% neutral buffered formalin. Following the 48-hour fixation period, a manual routine tissue processing protocol was applied. After tissue processing, 5 micron thickness sections from the tissues embedded in paraffin blocks were taken with a Leica RM2125 RTS brand microtome. Sections were stained with the routine Hematoxylin-Eosin (H&E) staining protocol. H&E stained sections were evaluated under an Olympus CX43 camera attachment light microscope. Scoring was made in terms of inflammatory cell infiltration (ICI), edema and fibroblast proliferation density (FPD) criteria for microscopic evaluation of wound healing [15, 16].

The scores made subjectively; ICI was evaluated as 0: no infiltration, 1: mild infiltration, 2: moderate infiltration, 3: severe infiltration; edema was evaluated as 0: no edema, 1: mild edema, 2: moderate edema, 3: significant edema; and FPD was evaluated as, as 0: no fibroblast proliferation, 1: mild fibroblast proliferation, 2: moderate fibroblast proliferation, 3: significant fibroblast proliferation.

2.5. Immunohistochemical Evalution

4 µm thickness sections were taken from paraffin blocks and stained after antigen retrieval method by using the routine immunohistochemistry protocol according to the manufacturer's data sheets of Anti-rat TNF-α (Biorbyt Inc. Cambridgeshire, UK. Cat No: orb11495), Anti-rat Ephrin A1 (Boster Bio, Pleasanton, CA. Cat No: PA1573), Anti-rabbit Ephrin B1 (Biorbyt Inc. Cambridgeshire, UK. Cat No: orb101544), Anti-rabbit Ephrin B2 (Cell Signalling Danvers, USA. Cat No: #83029)and Anti-rabbit EphB4 (Cell Signalling Danvers, USA. Cat No: 84029) antibodies. Light microscopic (Olympus CX43) evaluation of immunohistochemically stained sections was Scored as described previously in the literature as 0, no staining; 1, weak but detectable staining; 2, moderate staining; and 3, strong staining [17].

2.6. Statistical Analysis

Data analysis was performed using SPSS Packet Program 20.0 version (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). In determining the statistical significance of the difference between the control variable in the study and the average values of other variables at 95% confidence interval, Independent Two-Samples -Test was used, and values below 0.05 were considered statistically significant.

3. Results

The mean scores and standard deviations of the histopathological and immunohistochemical examinations performed at the end of the study are given in Table 1.

Table 1
Means and standart deviations of histopatological and ve immunohistochemical evalution scores.

	ICI	Edema	FPD	TNF- α	EphrinA1	EphrinB1	EphrinB2	EphB4
Control	2.2 ± 0.6	2.0 ± 0.5	2.6 ± 0.5	2.5 ± 0.5	2.1 ± 0.7	0.5 ± 0.5	0.6 ± 0.5	0.5 ± 0.5
Plu	3.0 ± 0	2.0 ± 0.5	3.0 ± 0	2.5 ± 0.5	3.0 ± 0	1.3 ± 0.5	0.8 ± 0.4	0.5 ± 0.5
Fito	2.8 ± 0.4	2.4 ± 0.5	2.7 ± 0.5	2.6 ± 0.5	2.6 ± 0.5	1.3 ± 0.5	0.5 ± 0.5	0.8 ± 0.8
Boron	2.0 ± 0.8	2.0 ± 0.8	2.5 ± 0.5	1.7 ± 0.8	2.1 ± 0.9	2.0 ± 0.8	2.2 ± 0.6	2.2 ± 0.6

3.1. Histopatological findings

In the evaluation made in terms of ICI; It was noticed that the ICI values of Plu and Fito groups were significantly higher when compared to the control group (p values 0.002 and 0.025 respectively). The same significance was not observed between the Control and Boron groups (p = 0.564). Similarly, there was no significant difference between Plu and Fito groups in terms of ICI (p = 0.146), but when Plu and Boron groups were compared, it was found that the ICI score of the Plu group was statistically significantly higher (p = 0.002). When the Fito and Boron groups were compared, it was observed that the ICI score was statistically significantly higher in the Fito group (p = 0.018). Photographs of inflammatory cells and the presence of edema are presented in Fig. 1.

No statistically significant difference was found between the control and other groups in the evaluation in terms of edema (p = 1.000 for Plu; p = 0.090 for Fito; p = 1.000 for Boron). Similarly, there was no statistical significance in terms of edema between the Plu group and the Fito and Boron groups (p values of 0.900, 1.000 respectively) or between the Fito and Boron groups (p = 0.246).

In the evaluations made in terms of FPD; Plu group showed significantly higher FPD compared to the control group (p = 0.029). There was no statistically significant difference between the control group and the Fito and Boron groups (p values 0.648 and 0.661, respectively). Similarly, there was no significant difference between Plu and Fito groups (p = 0067), but when the Plu and Boron groups were examined, it was noted that FPD was statistically significantly higher in the Plu group compared to the Boron group (p = 0.012).

3.2.Immunohistochemical findings

In the statistical analyzes regarding the evaluations made with the TNF-α primary antibody, no significance was found between the control group and the Plu and Fito groups (p values 1.000 and 0.661 respectively). In the comparison made with the control group and the Boron group, it was observed that TNF-α showed more immunoreactivity in the control group (p = 0.027). It was noted that TNF-α showed a statistically significant positivity in the Plu group in the analyzes performed with the Plu and Boron groups (p = 0.027), while there was no statistically significant difference between Plu and Fito groups (p = 0.661). Similarly, in the analyzes between the Fito and Boron groups, it was observed that the Boron group showed less immunoreactivity than Fito group (p = 0.016). TNF-α immunohistochemical staining patterns are shown in Fig. 2.

In the statistical analysis of the scores of the staining for the Ephrin A1 antibody demonstrated in Fig. 3, there was no significant difference between the control group and the Fito and Boron groups (p values were 0.112 and 0.968 respectively). A statistically significant increase in positivity was found in the control group compared to the Plu group (p = 0.002). When the Plu and Boron groups were compared, a statistically significant increase in immunreactivity was noticeable in the Boron group (p = 0.005), while the same significance was not observed between the Fito and Boron groups (p = 0.185).

Ephrin B1 immunohistochemical staining images are presented in Fig. 4. When the Ephrin B1 immunexpression was evaluated, it is observed that the expression in the control group was statistically significantly lower than the Plu, Fito and Boron groups (p values 0.005, 0.005 and 0.001 respectively). While the same significance was not observed in the analyzes performed between Plu and Fito groups (p = 1.000), it was found that Ephrin B1 expression in Boron group was statistically significantly higher than Plu and Fito groups (p values 0.015 and 0.015 respectively).

In the evaluation results of Ephrin B2 stained immunohistochemical sections, when the control group and Plu (p = 0.342) and Fito (p = 0.661) groups were compared, no statistically significant difference was observed, while statistically significantly lower positivity was found compared to the Boron group (p = 0.000). When boron group and Plu (p = 0.000) and Fito (p = 0.000) groups were compared, it was observed that the immunoreactivity observed in Boron group was higher than both groups. Immunohistochemical staining patterns are shown in Fig. 5.

In the comparison of Eph B4 primary antibody scores between the groups, no significant difference was observed between the control group and Plu (p = 1.000) and Fito (p = 0.403) groups, while a statistically significantly increased positivity was found in the Boron group compared to the control group (p = 0.000). In the analyzes performed with the boron group, it was noted that Eph B4 immunoreactivity was higher compared to Plu and Fito groups (p value 0.000 and 0.002, respectively). Eph B4 immun positivity is demonstrated in Fig. 6.

4. Discussion

The disruption of the integrity of the skin, which is the most important barrier between higher organisms and the outside world, is called a wound. The response of the organism to wound formation, which can occur due to many reasons such as abrasion, incision, and burns, is through a process called wound healing. Since the skin protects the important tissues of the body from damage caused by mechanical, infection, ultraviolet radiation and excessive heat; it is quite important to repair the wounds that may occur on the skin for any reason [2]. Wound healing, which occurs after skin disintegration, is a highly dynamic complex event involving inflammation, keratinocyte proliferation, epithelial cell and fibroblast activation and cell differentiation [18]. Wound healing, which starts with inflammation, continues with proliferation and ends with regeneration, is a regular and programmed sequence of events in itself. Factors such as oxygenation, infection, age and sex hormones, stress, diabetes, obesity, drugs, alcoholism, smoking and nutrition can affect one or more phases of this process and cause inaccurate or impaired wound healing [19]. The effects of the wound healing process and the therapeutics used in the wound healing process should be well documented in order to have smooth and / or rapid wound healing and to reduce / eliminate the effects of the factors that adversely affect wound healing. Formulas containing boric acid have been previously used in the literature to accelerate the wound healing process and its effectiveness has been demonstrated [11,15,19].

Boron is an important non metallic element in so called sugars world which may have played an essential role in the prebiotic origins of genetic material [20] Boron with high affinity for oxygen; forms compounds containing adjacent hydroxyl groups in cis position, such as sugars, their derivatives and riboflavin in organisms, and these complexes bind calcium ions [10]. In higher organisms, boron plays a role in the metabolism of many nutrients, especially vitamin D, calcium and many hormones [21]. Boron, which has an effect on the production of inflammatory cells as well as enzymes such as elastase and collagenase; It accelerates extracellular matrix production and affects TNF-α release from fibroblasts. [14,20,21]. Boron participates in hydroxylation reactions and in addition to its use in musculoskeletal diseases and cardiovascular diseases, it acts as an anti-inflammatory and antioxidant agent in cancer, reducing genotoxicity and modulating mitochondrial membrane activity [1,20,22,23] When wound healing is examined, topically applied boric acid is effective in wound healing; It has even been reported that 3% boric acid solution reduces the duration of stay in intensive care units by 2/3 in deep wounds [12,19,24]. In this study, boron did not show a statistically significant difference with the control group in terms of ICI ($p = 0.564$), while it was found that the ICI score in the boron group was statistically significantly lower than the Plu and Fito groups (p values, respectively, 0.002, 0.018). While no statistically significant difference was observed between the groups in terms of edema ($p > 0.05$), it was noted that FPD was statistically significantly lower in the Boron group compared to the Plu group ($p = 0.012$). Considering these histopathological findings, it is thought that the positive effects of boron on wound healing may be related to the limitation of inflammation and extensive fibrosis.

It has been noted in in vitro studies that the effects of boron on wound healing are versatile. The production of extracellular matrix via fibroblasts and its effects on elastase, collagenase, trypsin-like enzymes in fibroblasts indicate that boron regulates collagen and extracellular matrix relations [14, 25]. In addition, a study published in 2010 found that boron regulates many extracellular matrix proteins by messenger RNA regulation, suggesting that it may play a role in many events other than wound healing [26]. One of the important factors in wound healing is keratinocyte proliferation and migration. In vitro studies have reported that wound closure in keratinocytes incubated with boron salts is faster than in control medium [27]. In the same study, it was suggested that this effect of boron salts is related to keratinocyte migration rather than keratinocyte proliferation [27]. In another study performed in vitro in wound healing, two molecules related to keratinocyte migration and granulation tissue formation were examined. In this study, which examined the expression of Matrix metalloproteinase (MMP) -2 and MMP-9 in human keratinocyte culture by immunohistochemical and western blot method, it was noted that boron increased MMP-9 expression [28]. In an in vitro + experimental study on burns, one of the most serious wound types, it was reported that boron contributes not only to fibroblastic activity but also to wound healing with increased vascularization [12]. In addition, migration, angiogenesis, and contraction-related protein expressions including collagen, α-smooth muscle actin, transforming growth factor-β1, vimentin, and vascular endothelial growth factor have also been reported to increase in the boron application group [12]. However, there is no information about the effects of boric acid on Ephrins, which are a type of tyrosine kinase family, and their receptors Ephs.

Eph receptors were first identified in carcinomas in which they overexpress [29]. The Eph receptor family gets its name from its expression in an erythropoietin-producing hepatocellular (EPH) carcinoma cell line, and Ephrins are ligands of the Eph receptor family (Eph family Receptor INteracting proteins) [30]. Ephrins are members of the tyrosine kinase family that play a role in developmental processes, cell adhesion, motility, proliferation and differentiation. As a result of studies related to Eph / Ephrin, it was understood that Eph proteins allow short-range cell-cell communication by binding ephrin ligands attached to the membrane [31]. The activation of signaling pathways affecting the cytoskeleton first causes cell separation and sometimes cell adhesion. So Ephrin-Eph signals are required for rapid changes in cellular mobility and / or morphology [31]. In other words, while the ligands of other receptor tyrosine kinase families are generally soluble, the binding of ephrins -which are ligands of Eph receptors- to the cell surface causes the signaling through Eph receptors to be dependent on cell-cell contact. With this feature that makes Eph receptors unique, cells become aware of their own microenvironment. Thus, they play an important role in normal physiological processes such as the formation of embryonic tissue borders and the orientation of developing axons [32]. In adult tissues, they help wound healing and preservation of intestinal cell populations in certain parts [32, 33].

Eph receptors are divided into EphA or EphB subfamilies according to their binding status to Ephrin ligands called membrane-anchored ephrin-As and transmembrane ephrin-Bs [34]. In the light of the data obtained as a result of the studies conducted to define the functions of Eph / ephrin family members, it is thought that the Eph-Ephrin mechanism affects wound healing, especially through EphB2 and ephrin-B1 / 2. Upregulation of EphB2 and ephrin-B1 / 2 and the resulting interactions cause actin stress fibers to break up and their junction to rupture when the wound is formed. As a result, the epithelial layer loosens and the wound is closed by providing the cells with the environment it needs to migrate collectively [35]. In addition, the in vitro coupling of EphB1 and Ephrin-B1 leads to increased cell adhesion through α1β5 integrin activation, an effect that is dependent on the surface density of Ephrin-B1 expression [35]. Increased cell adhesion is also a process that accelerates wound healing by increasing connection complexes between keratinocytes. Ephrin B2 acts through its receptor, EphB4, and cell-cell and cell-extracellular matrix adhesion increases as a result of EphrinB2 / EphB4 interaction. Cell migration and proliferation are also induced [36]. In this way, it is thought to contribute to the acceleration of wound healing, as well as to strengthen the scar tissue by increasing cell-cell and cell-extracellular matrix adhesion. In the current study, it was observed that Ephrin B1 increased in all three experimental groups when compared with the control group. However, when Boron group was compared with Plu and Fito groups, a significant increase was observed in Boron group (p values 0.015, 0.015 respectively). This indicates that Boric acid accelerates cell adhesion by increasing the expression of Ephrin B1, thus contributing to the wound healing process. In addition, a significant increase was observed in Ephrin B2 immunoexpression in the Boron group compared to the control group ($p = 0.000$), while the positivity in the Boron group was also significantly higher in the analysis between the Boron group and the Plu and Fito groups (p values were 0.000, 0.000 respectively). This result reveals that the increase in Ephrin B2 expression also plays a role in the positive effects of boric acid on wound healing. In addition, Eph B4, which is the receptor of Ephrin B2, showed a statistically significant increase in the Boron group

compared to the Control (p = 0.000), Plu (p = 0.000) and Fito (p = 0.002) groups. It also supports that the receptor EphB4, which is necessary for the Ephrin B2, is increased by Boric acid, thus revealing the effect of accelerating wound healing.

Ephrin A's are known to suppress cell adhesion genes, especially integrins, while inducing epidermal differentiation markers. In addition, Ephrin A1 has been shown to increase collagen production in the skin [37]. Ephrin A1 becomes upregulate in endothelial cells and skin with TNF-α stimulation [7, 37]. The angiogenetic effects of Type A Ephrin-Eph group on VEGF are reported [4]. Ephrin-A1 expression is a critical determinant of endothelial proliferation. According to current knowledge, Ephrin-A1 is notably involved in basic processes of endothelial migration such as cellular polarization, migration direction and velocity. These data support the idea that Ephrin-A1 plays an important role in the basal mechanisms of re-endothelialization [4]. In addition, it has been emphasized in various publications that Ephrin A1 and Eph A2 receptors have angiogenetic effects on TNF-α [7]. Ephrin A1 has been shown to play an important role in the adhesion of TNF-α mediated monocytes to endothelial cells [7]. In the present study, TNF-α expression was found to be statistically significantly lower in the Boron group compared to the Control (p = 0.027), Plu (p = 0.027) and Fito (p = 0.016) groups. According to previous studies in the literature, the increase in TNF-α is expected to induce Ephrin A1. However, in the study, it was noted that although TNF-α value in Boron group showed lower immunoreactivity compared to all other groups, Ephrin A1 had higher activity in Boron group than Plu group (p = 0.005). When these results are analyzed together; It suggests that TNF-α values and Ephrin A1 values may have increased in the first days of wound healing when inflammation was high, but their expression tended to decrease in the 7-day healing period to limit wound healing. Similarly, in an in vitro study, boric acid was reported to reduce TNF-α secretion by a thiol-dependent mechanism [38]. When evaluated together with the histopathological findings in the current study, the low ICI and FPD scores observed in the Boron group compared to the other groups indicate that boron prevents extensive wound healing in addition to its accelerating effect on wound healing.

5. Conclusion

In many studies on Boron, which has been used in wound healing since the 1990s; Boron has been shown to act in different pathways. The Ephrin / Eph system is not included among the known effect pathways in wound healing. In the present study, it has been determined that boric acid may increase wound healing by Ephrin / Eph system leading an increase in Ephrin B1 resulting with increased cell adhesion, increasing the expression of Ephrin B2 and its receptor EphB4 and accelerating wound healing while histologically limiting wound healing by reducing TNF-α expression as well as inflammation and fibroblastic activity (Graphical abstract). In the light of this information, it is thought that boric acid prevents extensive wound healing in addition to its wound healing accelerating properties.

Declarations

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Author's contributions:

	BB	CA	YA	HAE
Study design	+	-	+	+
Data collection and interpretation	+	+	-	+
Analysis	+	+	+	-
Writing	+	+	+	-
Revision	+	-	+	-
Final approval	+	+	+	+

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figures

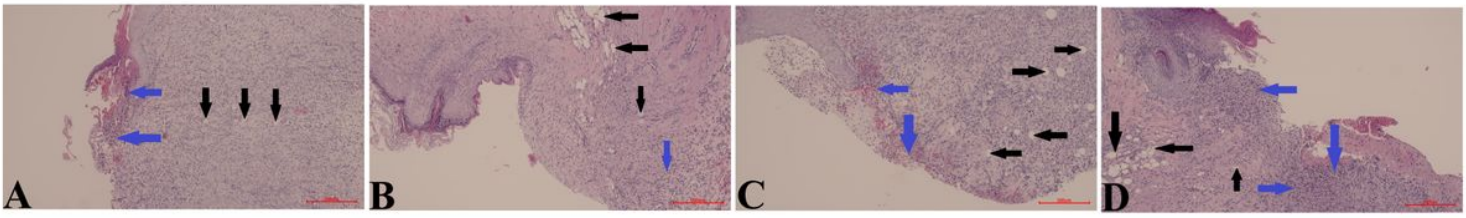

Figure 1

Light microscopic images of H&E stained sections of experimental groups. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D, respectively. Blue arrows in the sections indicate areas of inflammatory cell infiltration, while black arrows indicate areas of edema (Magnification x100).

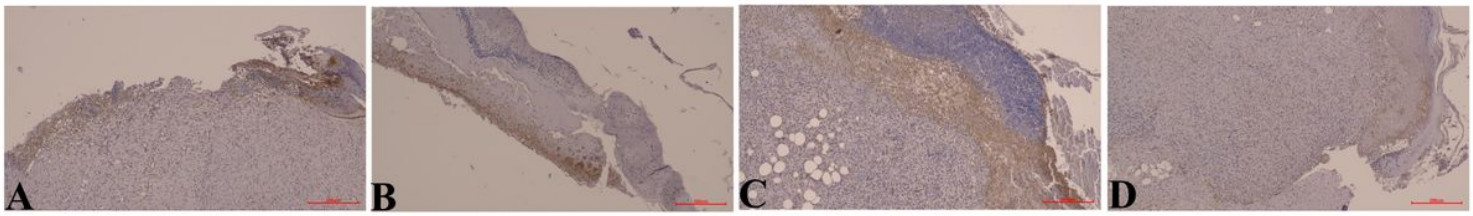

Figure 2

Light microscopic images of histological sections of experimental groups and sections stained immunohistochemically for TNF-α. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D respectively (Magnification x100).

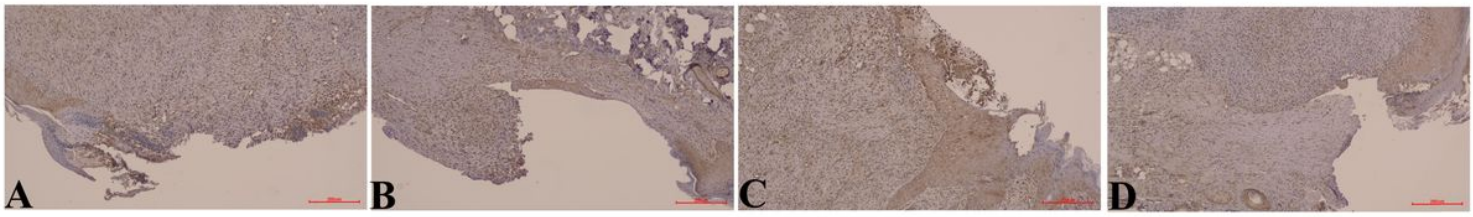

Figure 3

Light microscopic images of the histological sections belonging to the experimental groups and sections stained immunohistochemically for Ephrin A1. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D respectively (Magnification x100).

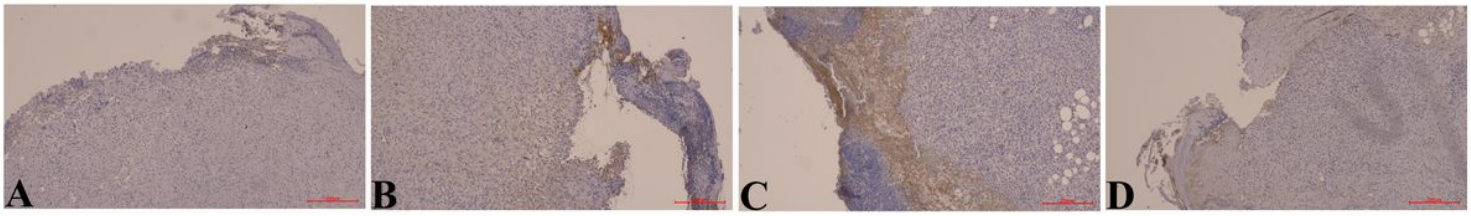

Figure 4

Light microscopic images of the histological sections belonging to the experimental groups, immunohistochemically stained for Ephrin B1. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D respectively (Magnification x100).

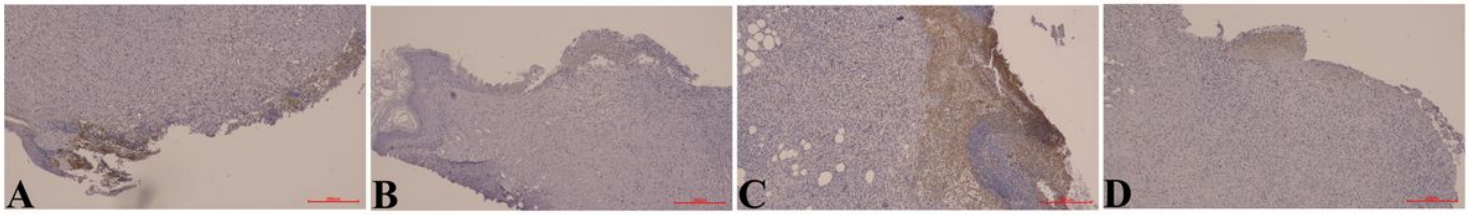

Figure 5

Light microscopic views of the histological sections belonging to the experimental groups and sections stained immunohistochemically for Ephrin B2. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D respectively (Magnification x100).

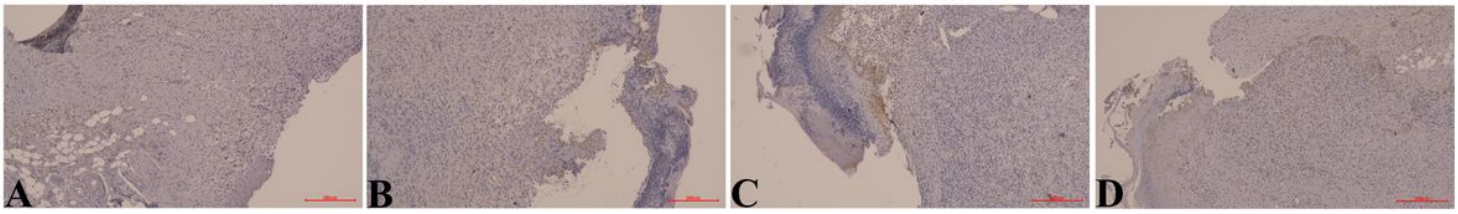

Figure 6

Light microscopic images of the histological sections belonging to the experimental groups and sections stained immunohistochemically for Eph B4. Microscopic photographs of Control, Fito, Boron and Plu groups are given in A, B, C and D respectively (Magnification x100).