

Comparing Effects of Silver sulfadiazine, Sucralfate and Brassica oleracea extract on Burn Wound Healing

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Abstract: The aim of this study was to investigate the effects of Silver sulfadiazine, Sucralfate and Brassica oleracea extract on second degree burns. 80 female Sprague dawley rats (220± 10g) were randomly divided into 5 groups (n=16/group). Deep second degree burns were induced by metal stamp and effects of Silver sulfadiazine, sucralfate, Brassica oleracea and base cream were evaluated. We studied the histological features of burn area and fibroblasts, macrophages, neutrophils and blood vessels were counted in each group by optika software. There was not any significant difference among study groups at the end of first week but a dramatic difference was seen after second week. Re epithelialization and vascularization were accelerated in sucralfate and Brassica oleracea groups. Welfare percentage was about 100 % in sucralfate and Brassica oleracea groups, whereas, the other groups were not completely healed. In conclusion, Sucralfate and Brassica oleracea have positive effects on burn wound healing.

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Introduction

Burns are dynamic injuries that can change into deeper injuries over time (1). Deep burns require prolonged healing often result in significant scarring and contractures (2). Incidence of burning in the developing countries is much more than developed countries and burning is one of the chief causes of mortality in the world (3,4).

The most common burn injuries result from exposure to heat and chemicals. Management of a burn induced wound has made remarkable progress over the last 20 years. Many studies indicate that burn survival markedly improved in recent years (5, 6, and 7). Healing is divided into four main components: formation of

granulation tissue, collagen deposition, re-epithelialization and contraction (8, 9).

Infection is one of the most common complications of burning (10, 11). As the majority of deaths occurred after burn injury are because of infection (12), most of the current topical antimicrobial agents retard wound healing (13). Silver sulfadiazine is a bactericidal agent, active against most germ-positive and germ-negative bacteria (14). Silver sulfadiazine at 1% has been used in the treatment of burn injuries (15) and it has a low toxicity and hyper sensitivity (14, 16). It is claimed that Silver sulfadiazine delays wound healing (17) because it impairs fibroblasts and epithelial proliferation (5).

Brassica oleracea family is rich in vitamins (A, B1, B6 and E), minerals (chloride, potassium, magnesium and sulfur), dietary fibers, glucosinolates, polyphenols and phenolic acids (18, 19). These plants are known to have anticancer, antioxidant, antihyperglycemic and antihypocholesterolemic properties (20). Brassica oleracea contains brassinin and indol-3-carbinol that are anticarcinogenic. It also contains vitamin E that is effective in wound healing process (19, 21, 22, and 23).

Sucralfate is a complex of the disaccharide sugar, sucrose, combined with sulfate and aluminum (13, 24). Sucralfate is used to induce healing of gastrointestinal tract ulcers (25, 26). Sucralfate binds to basic fibroblast growth factor (bFGF) and increases their concentration in the wound (27). It stimulate cell growth in rats (14) moreover, some researchers have tried it for various diseases (13). The objective of present study was to compare the effect of Silver sulfadiazine, Brassica oleracea and Sucralfate on burn wound healing.

Materials and methods

Animals

80 female Sprague dawley rats (220 ± 10 g) were purchased from Pasteur institute of Tehran. They were kept in standard laboratory conditions with a 12-hour light/dark cycle at room temperature. The rats were fed with pellet diet and water ad libitum.

Experimental design/wound creation

The animals were anesthetized by injection of 45 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally. Deep

Wound healing assessment method

Wound healing percentage was assessed by the following formula:

$$\text{Wound healing percentage} = \frac{\text{Wound area on the specified day}}{\text{Wound area on the day 1}} * 100$$

Wound healing percentage = 100-wound percentage

second-degree (partial thickness) burns were induced by applying a hot round metal stamp (2.5 cm in diameter, 100g in weight and 80°C) to the shaved skin of the dorsal surface of the neck. Pressure of application of metal stamp was its weight and duration of application was 10 seconds.

The animals were randomly divided into 5 groups (n= 16 /group). Control group rats were left untreated. Group 2 was treated by base cream (treated group only by base cream and without any effective agent), groups 3, 4 and 5 were treated by 1% Silver sulfadiazine, Sucralfate and Brassica oleracea, respectively. Treatment which should be provided once a day, started 24 hours after the burn injury.

Rats in each group after anesthesia with chloroform were sacrificed by neck dislocation at the end of first, second, third and fourth weeks.

Histological study

Skin tissue samples were taken for histological study with a small containing part of the wound area.

Tissues were fixed in formal saline and embedded in paraffin blocks. Sagittal sections (5µm thick) were prepared and stained with hemotoxylin and eosin. Ten fields from each sample were examined by optika light microscope and its morphometric software. In each microscope field, fibroblasts, macrophages, neutrophils and blood vessels were counted by optika software.

Preparation of Sucralfate cream

2 gram Sucralfate was mixed with 4 gram glycerin and 4 gram 70% D-sorbitol. The mixture was stirred to form a homogenous cream with suitable texture for topical application.

Preparation of Brassica oleracea extract

Brassica oleracea leaves were cut into small pieces and oven dried at 50 °C. Dried leaves were ground to a fine powder. The powder leaves (80 g) were macerated in distilled water (800 ml) at room temperature. After overnight maceration, the extract was freeze dried and dissolved in base cream (glycerin and 70% D-sorbitol) to concentration of 1 g/ml. The extraction procedure was performed at 15 °C.

Statistical analysis

The differences among groups were compared by Kruskal-wallis and Mann-Whitney U test. P values of less than 0.05 were considered significant.

Results

Histological studies indicated fibrinoleukocytic exudates and edema in all groups at the end of first week. Epidermis was presented in none of groups. Fibrinoleukocytic exudates consist of degenerated neutrophils, cellular debris and necrotic cells were seen. Predominant inflammatory cells were polymorphonuclears (fig.1). Granulation tissue including inflammatory background and proliferated new vessels were seen. There were no significant differences in the histological features among study groups (fig.2).

At the end of the second week, fibrinoleukocytic layer in Sucralfate and Brassica oleracea groups were decreased and granulation tissue increased in comparison with other groups (fig.3).

From histological point of view, the number of macrophages and neutrophils in Sucralfate and Brassica oleracea groups were significantly lower than control group ($p < 0.05$). The number of fibroblasts significantly increased in sucralfate group ($p < 0.05$) whereas there were no significant difference in control, Base cream, Silver sulfadiazine and Brassica oleracea groups (fig.4).

Vascular proliferation in Sucralfate group was more than the others. However, the number of vessels was not significant in all groups. At the end of the third week epidermis was not formed in control and base cream groups but sheet cells of wound were organized and the

number of fibroblasts was significantly increased in comparison with previous weeks ($p < 0.05$).

In Silver sulfadiazine group, epidermis was formed but stratum keratinosum was not present. The number of fibroblasts was increased similar to control group (fig. 5).

In Sucralfate group, epidermis was formed but stratum keratinosum was not as thick as normal rate. In this group newly formed small blood vessels were oriented toward the surface and were parallel to each other as same as the Silver sulfadiazine group. Stroma showed edema and a mixed inflammatory background including polymorphonuclear and mononuclear cells (fig.5).

In Brassica oleracea group, stratum keratinosum was in the state of formation and dermis was the same as Sucralfate group. The number of inflammatory cells particularly neutrophils was significantly decreased in Silver sulfadiazine, Sucralfate and Brassica oleracea groups comparing to base cream and control groups (fig.6).

There was a considerable increase in macrophages and fibroblasts in Sucralfate and Brassica oleracea groups (fig.6).

The increase of fibroblasts in Sucralfate and Brassica oleracea groups was significant ($p < 0.05$). At the end of the fourth week, in control and Base cream groups, epidermis was formed but yet there was an area without epidermis in the center of wound and stratum keratinosum was not formed.

In Silver sulfadiazine group, epidermis was completely formed and stratum keratinosum was in the forming state but hairs were not completely appeared. In Sucralfate and Brassica oleracea groups, epidermis was completely healed (fig.7).

Dermis has collagen deposition in Sucralfate group. The inflammatory cells were scattered in Sucralfate and Brassica oleracea groups and the majority of them were mononuclears. The number of vessels was decreased and the number of fibroblasts, neutrophils and macrophages were not significantly different among groups (fig.8).

The welfare percentage of all groups is shown in table 1. According to the table 1 and figure 9, welfare percentage was the most in sucralfate group at the end of the third week and Brassica oleracea group was the next most healed group. However, welfare percentage of both groups was the same at the end of the fourth week.

Discussion

Wound healing is essential for any organism to survive injury. There are several factors such as cytokines and growth factors, PH, oxygenation, temperature,

nutritional status and many other factors that influence tissue repair (28, 29).

In the present study, topical application of Silver sulfadiazine didn't accelerate burn healing but it started to reduce inflammation after second week. This finding is in accordance with the previous studies (17). This study showed the positive effect of sucralfate and Brassica oleracea on re-epithelialization and contraction of burn wound.

Extracellular and intracellular concentration of glutathione has an important role in cellular resistance against toxins and burns (30). Brassica oleracea activates natural antioxidants related to glutathione that these antioxidants are very important for skin protection(31). Vitamin E and Brassinin found in Brassica oleracea have significant role in burn healing(19,22).

Studies shows that factors that cause increase in blood flow reduce inflammation and are anti-infectious, having a positive effect on wound healing (33). Many studies have shown that Sucralfate stimulates angiogenesis, which increase granulation tissue (33,34). These findings are in concurrence with our results.

Sucralfate is an effective agent for burn healing and it has no toxicity (35, 36, and 37). Fibroblast growth factor accelerates wound healing process (38). Sucralfate binds to basic fibroblasts growth factor (bFGF), prevents it from degradation and promotes healing (26).

Sucralfate stimulates epithelial cell protection by accumulation of epidermal growth factor in the ulcerated areas (39). Sucralfate enhances prostaglandin E₂ synthesis in keratinocytes and dermal fibroblasts which are responsible for the augmentation of the healing process (26). Synthesis of collagen in fibroblasts is controlled by Sucralfate; therefore, collagen deposits don't cause abnormal features (40). Reduction of wound size is the major outcome of healing (41). Our findings showed that both Sucralfate and Brassica oleracea extract have considerable effect on size of wound in second degree burns. Despite this study showed the positive effect of Sucralfate and Brassica oleracea extract on burn wound healing, we need further studies in micro biological and molecular fields.

Conclusion

The findings of this study confirm positive effect of Sucralfate and Brassica oleracea on burn wound healing. According to toxicity of Silver sulfadiazine and its negative effect on fibroblasts, we conclude that

Sucralfate and Brassica oleracea could be a better choice.

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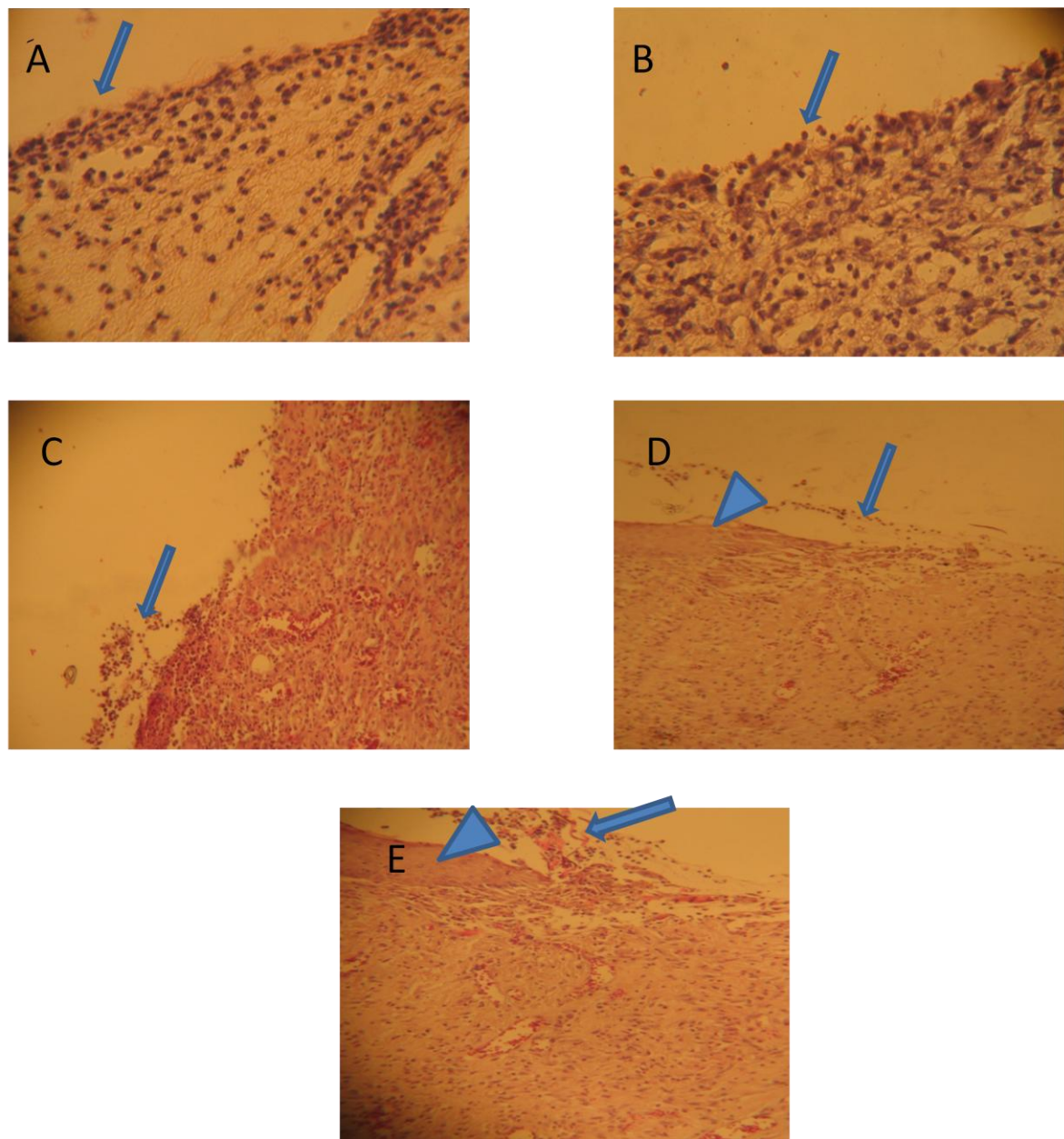


Fig.1: Photomicrograph of skin on the 7th day after burning. (A) Control group, (B) Base cream group, (C) Silver sulfadiazine group, (D) Sucralfate group, (E) Brassica oleracea group. Fibrinoleukocytic exudates and cellular debris are seen in all groups (arrows). Normal epithelium besides the burning is indicated by arrow head. (A&B x100- C, D&E x40- H&E).

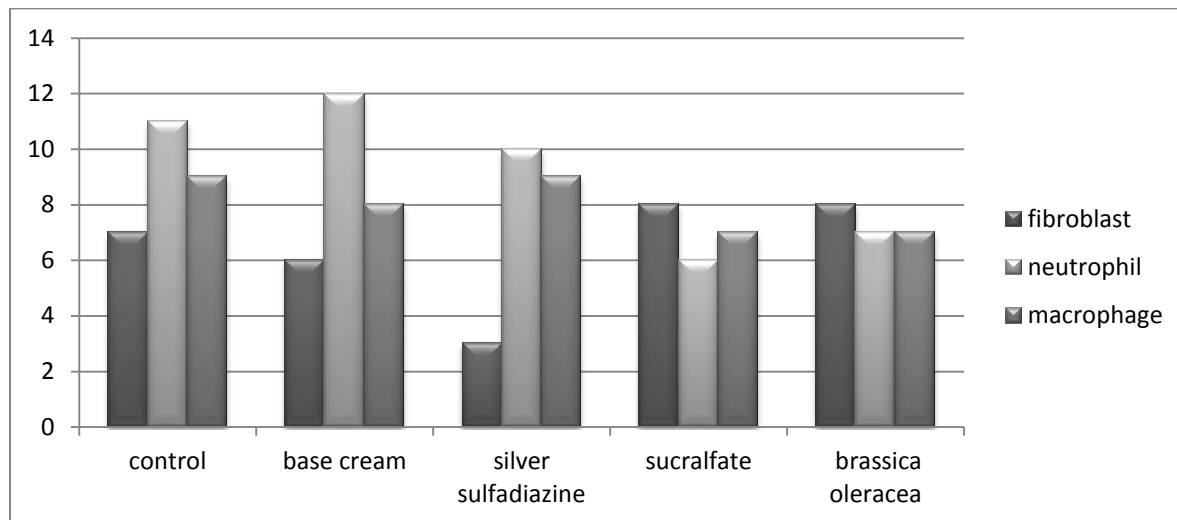


Fig.2: Mean number of macrophages, neutrophils and fibroblasts in all groups at the end of first week. Analysis of variance showed that there are not significant differences between groups

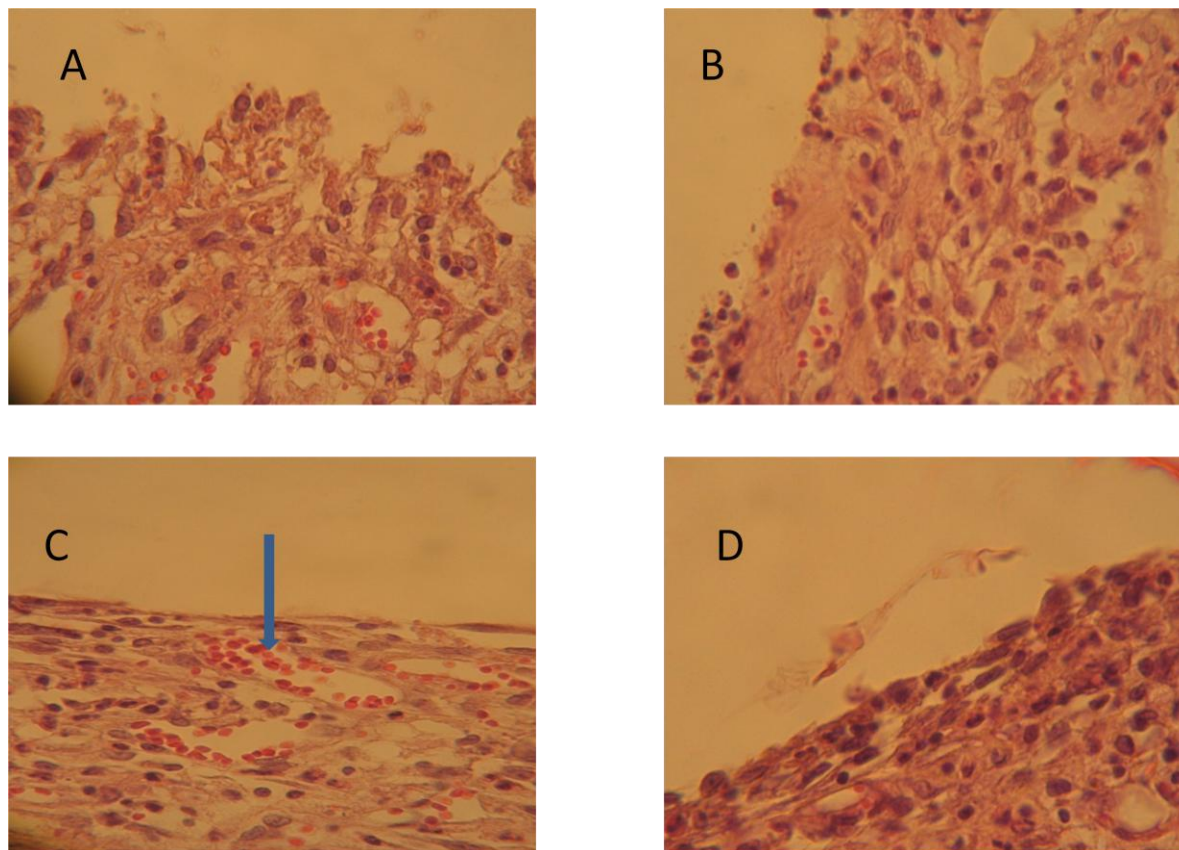


Fig.3: Photomicrograph of skin on the 14th day after burning. (A) Control group, (B) Silver sulfadiazine group, (C) Sucralfate group, granulation tissue is increased. Vascular proliferation is more than the other groups (arrow), (D) Brassica oleracea group, granulation tissue is increased as same as Sucralfate group. (X400, H&E).

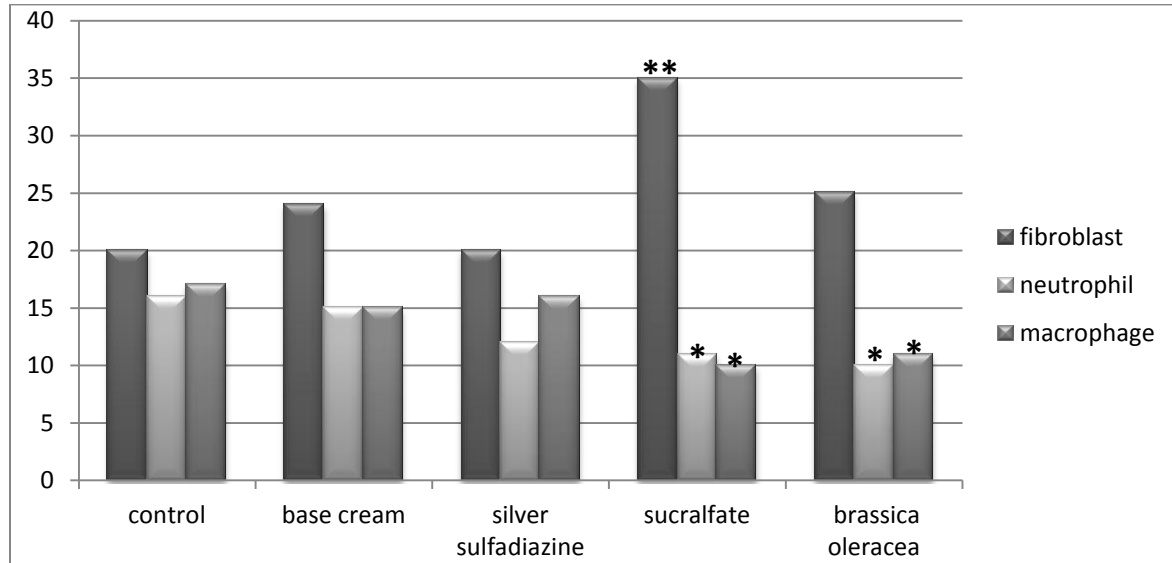


Fig.4: Mean number of macrophages, neutrophils and fibroblasts in all groups at the end of second week. The number of macrophages and neutrophils in Sucralfate and Brassica oleracea groups are significantly lower than control group (* $p < 0.05$). The number of fibroblasts is significantly higher in sucralfate group (** $p < 0.05$).

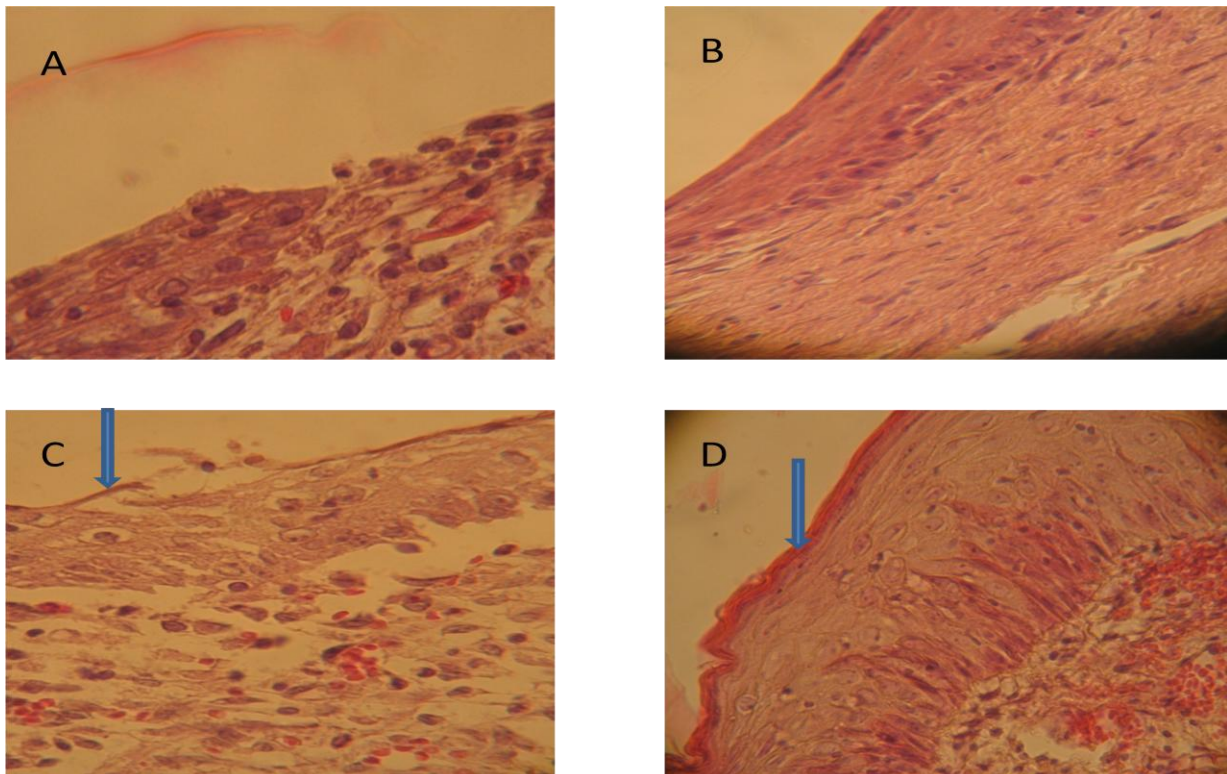


Fig.5: Photomicrograph of skin on the 21th day after burning. (A) Control group, epidermis is not formed. (B) Silver sulfadiazine group, epidermis is formed but stratum keratinosum is not present. (C) Sucralfate group, epidermis with stratum keratinosum is seen (arrow). Vascular proliferation is more than the other groups. (D) Brassica oleracea group, epidermis with stratum keratinosum is formed. (X400, H&E).

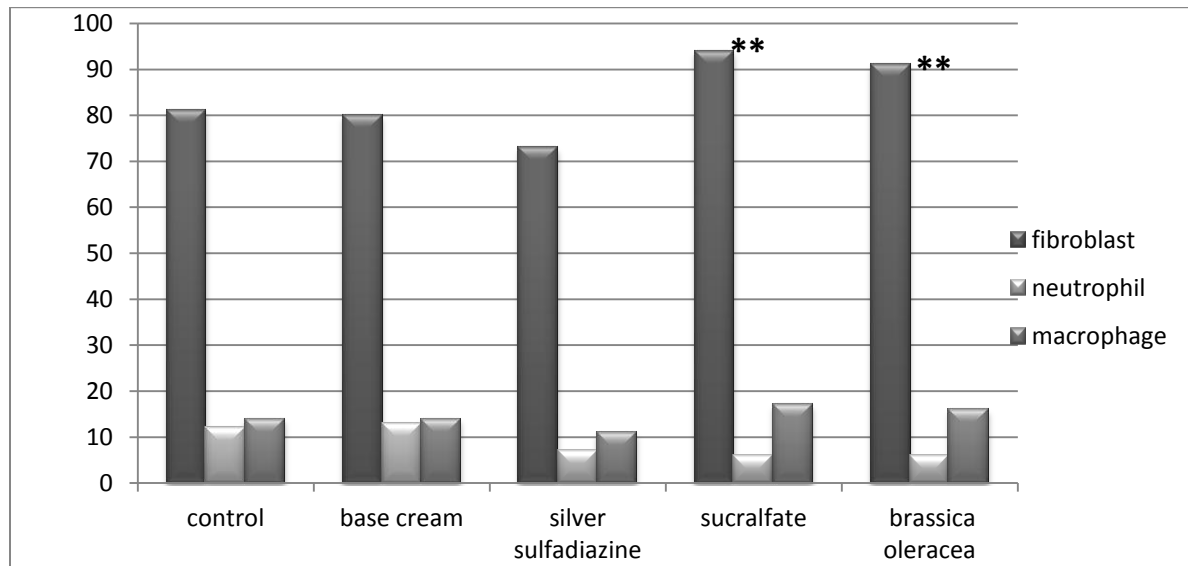


Fig.6: Mean number of macrophages, neutrophils and fibroblasts in all groups at the end of third week. The number of neutrophils in Silver sulfadiazine, Sucralfate and Brassica oleracea groups are significantly lower than control group (* $p < 0.05$). The number of fibroblasts is significantly higher in sucralfate and Brassica oleracea groups (** $p < 0.05$).

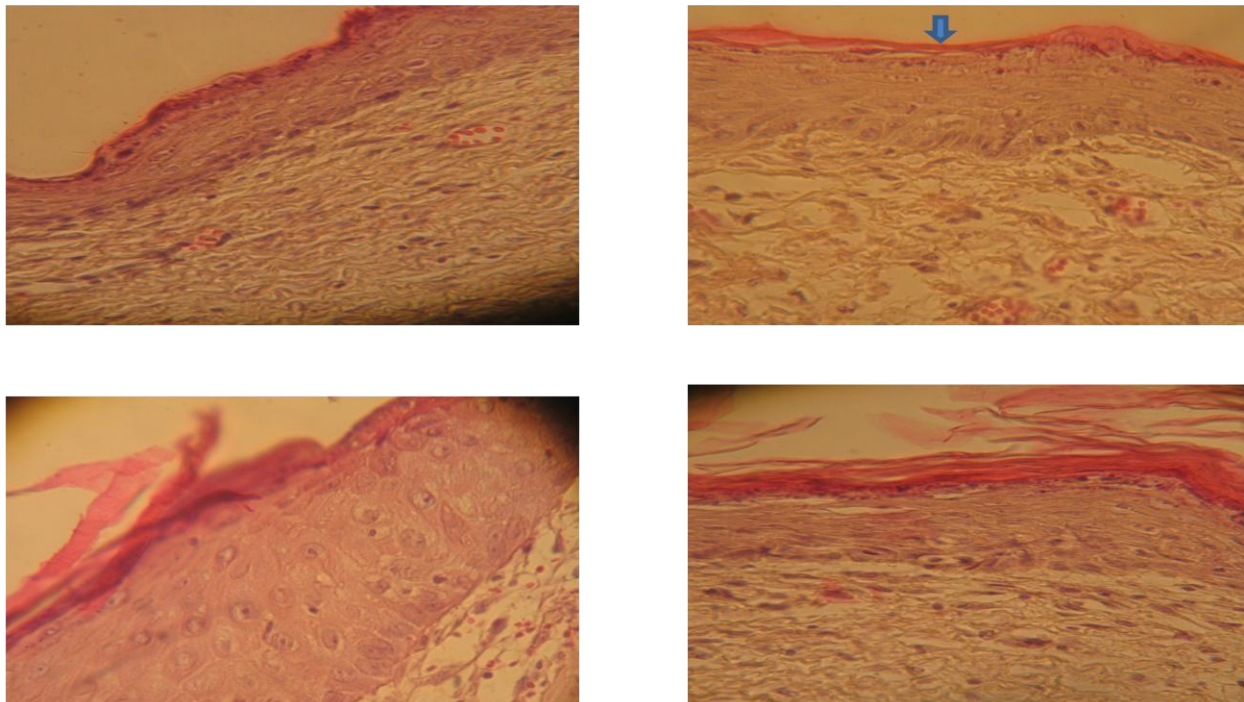


Fig.7: Photomicrograph of skin on the 28th day after burning. (A) Control group, epidermis is formed but stratum keratinosum is not present. (B) Silver sulfadiazine group, epidermis is completely formed and stratum keratinosum is in the forming state (arrow). (C) and (D) Sucralfate and Brassica oleracea groups, epidermis is completely healed. (X400, H&E).

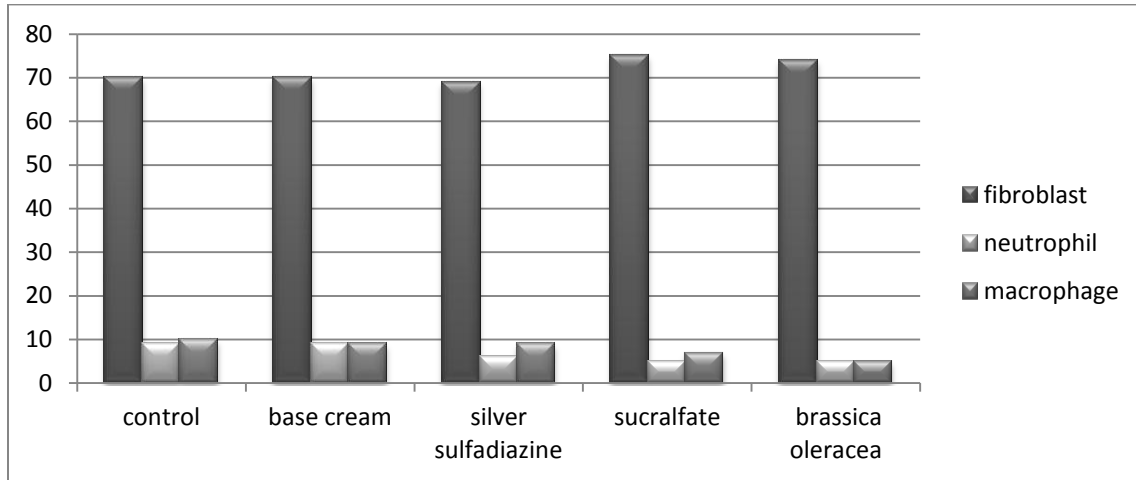


Fig.8: Mean number of macrophages, neutrophils and fibroblasts in all groups at the end of fourth week. There are not significant differences between groups.

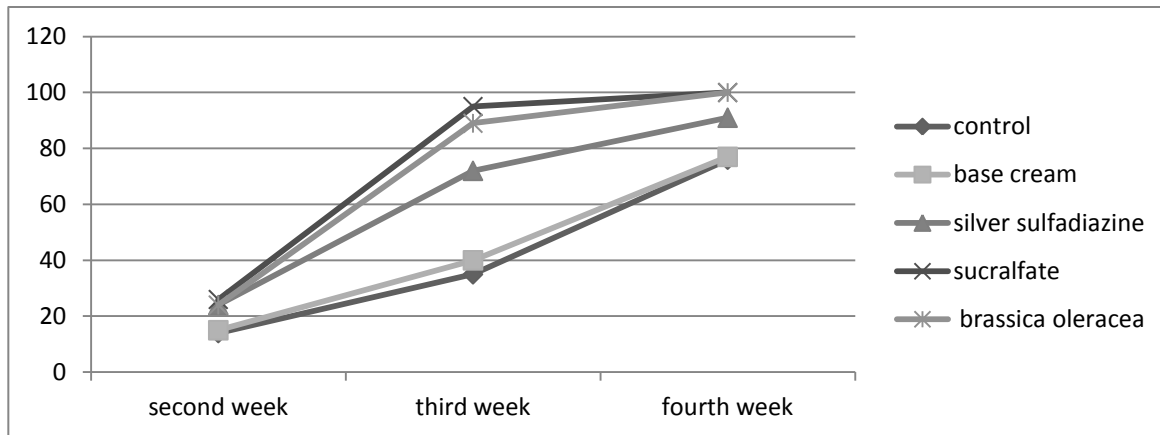


Fig.9: Welfare percentages at the end of second, third and fourth weeks. After one week, healing process is accelerated by Silver sulfadiazine, Sucralfate and Brassica oleracea but the rate of healing is faster in Sucralfate and Brassica oleracea groups.

Table 1: Welfare percentages at the end of second, third and fourth weeks.

weeks groups	2	3	4
control	14	35	76
Base cream	15	40	77
Silver sulfadiazine	24	72	91
Sucralfate	26	95	100
Brassica oleracea	24	89	100

1/16/2013