



Evaluation of effect of topical application of 3% citric acid on wound healing

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Abstract

Background- Wound healing is a complex process influenced by multiple factors. This study looks for the efficacy of 3% citric acid as pH modifying dressing when compared to conventional method. The pH within the wound milieu indirectly and directly affects the various biochemical reactions in the wound healing.

Materials and methods- An unicentric, prospective, randomised study with a parallel design was used to compare patients treated with conventional dressing and with 3% citric acid respectively. The wounds were examined for 14 days and parameters of healing were evaluated. The results were analysed using chi-square test and unpaired t-test.

Results- Use of citric acid made the wound surface acidic (4-6). Patients treated with citric acid (72.06%) showed more reduction in wound size than those who were treated by conventional method (67.40%). The increase in granulation area was more when citric acid (65.36%) was used than that in control group (60.60%). Wounds treated with citric acid showed early reduction in amount of discharge as compared to control group. The mean hospital stay of patients in citric acid group (7.74 days) was comparatively less than that of patients in control group (10.38 days).

Conclusion- 3% citric acid is safe and effective in all types of wound management. It acts by reducing pH of wound surface and inhibits bacterial proliferation. It aids in early healing by promoting formation of healthy granulation tissue.

Keywords: Wound healing, granulation tissue, 3% citric acid.

Introduction

Wound healing is an intricate process whereby the body attempts to restore integrity of the injured part. After initial haemostasis, inflammatory phase ensues which mainly consists of cellular cleaning of wound by macrophages. The proliferative phase involves fibroblast activity with production of collagen, angiogenesis and re-epithelialisation of wound surface. The remodelling phase is characterized by maturation of collagen with realignment of its fibres along the lines of lesion and wound contraction.^[1]

The process of wound healing is affected by multiple factors. The final outcome of healing process is usually determined by local wound conditions, oxygenation and vascularity of tissues. The systemic factors as old age, diabetes, smoking and alcoholism also have their own effect.^[2]

The management of infected wound involves the interventional drainage and debridement of necrotic tissue. Disinfection of the wound with the use of local antiseptics is equally important. The ideal wound dressing aims at protecting the wound, decreasing pain, promoting fibroblast proliferation and facilitating in formation of healthy granulation tissue.

With more detailed understanding about the process of wound healing, there have been continuous and significant advancements in the field of wound dressings. Different pharmaceutical compounds in the form of analgetics, growth factors, autologous platelet rich fibrin and protease inhibitors are being used as a more advanced approach.^[3]

The natural healing process is supported by acidic pH milieu. Such a pH milieu suppresses bacterial growth, decreases the activity of proteolytic enzymes, increases oxygen supply and promotes fibroblast growth.^[4]

Many chemicals like acetic acid, hydrocolloids, occlusive carbon dioxide, honey and citric acid have been used for application to wounds as pH modifying agents.^[5,6,7,8] Citric acid has been in use

for a very long time, first in dental care and then subsequently in surgical dressings.^[9,10]

Use of citric acid is primarily based on pH modulation. By changing the wound pH, it inhibits the bacterial growth, increases oxygen concentration and promotes migration and proliferation of fibroblasts.^[11]

This study was aimed to evaluate the effect of topical application of 3% citric acid on wound healing as compared to conventional dressings on similar types of wounds. Citric acid was preferred because it is easy to prepare and has minimal side effects and long shelf life. The literature mentions its use in chronic wounds but no study showed its efficacy over conventional dressings. The aim of this study was to determine the role of citric acid in pH modulation of wound and to study its effect on the process of wound healing.

Materials and Methods

This was a randomised, prospective comparative study conducted in 100 patients admitted at our hospital. This study was conducted after approval from thesis and ethical committee.

The present study was conducted in total of 100 patients divided into two groups of 50 each.

GROUP A: The skin around the wound site was painted with povidone iodine. Wound was then cleaned with normal saline. Dressing was done by using paraffin gauze.

GROUP B: The skin around the wound site was painted with povidone iodine. Wound was then cleaned with normal saline. Dressing was done by using autoclaved gauze. In addition, the 3% citric acid solution was applied.

A parallel study design was used. The patients included in the study were of different age groups ranging from 3years old to more than 70 years old. Mean age in group A was 36.53 years and that of group B was 38.66 years. Patients with different etiologies were included in this study [Acute abscesses(post incision and drainage); Traumatic wounds; Post laparotomy wound dehiscence; Burns; Chronic leg ulcer; Sinuses;

Bed sore; Fistula-in-ano (post fistulectomy); Carbuncle; Fournier's gangrene]. By permitted block randomisation, equal number of cases were taken in both the control and study group based on the etiopathogenesis.

Patients having fever, hypoproteinemia, signs of septicaemia and pre diagnosed cases of diabetes mellitus, tuberculosis, leprosy and vascular diseases were excluded.

The objective of the study was to evaluate the efficacy of 3% citric acid in wound healing. The wound was evaluated for healing using following parameters.

- 1) Percentage decrease in wound size.
- 2) Percentage increase in granulation area
- 3) Amount of discharge
- 4) Visual analogue scale score for pain
- 5) pH of the wound
- 6) Total hospital stay

At the time of admission, informed consent was taken. A printed proforma was filled recording age, sex, date of admission and chief complaint of the patient. Symptoms and physical findings of the wound were noted. The necrotic tissue was removed (debridement), wherever needed; wound irrigated with the solution keeping the wound environment moist, protecting against bacteria and other pathogenic microorganisms enabling the human body to perform wound healing process. The results of important routine investigations were also recorded wherever necessary. The antibiotics were given in all the cases after culture and sensitivity.

3% citric acid (w/v) was prepared in the laboratory. 15 gm of anhydrous citric acid was taken and added to the measuring beaker. Distilled water was added to the beaker to the volume of 500ml. The solution was stirred thoroughly and poured into the collecting bottles. The dressings were done using different methods such as washing, sprinkling, gauze dressing or immersion. Examination of the wound was done on day 1, 3, 5, 7, 9, 12, 14 and observations were noted in the written proforma. Different parameters were recorded to evaluate wound

healing like reduction in size, discharge, pain, and appearance of granulation tissue and epithelialisation of wound.

The wound size was measured by ruler method or bock-square method depending upon the shape of the wound. The wounds were assessed daily by two independent observers for degree of slough separation to minimize inter-observer error.

pH of the wound was monitored on 3rd, 7th, and 12th day. The pH was assessed by pH strips.

Reduction in pain was assessed by using VAS (visual analogue scale) for pain. Amount of discharge at wound bed was assessed depending on the soakage of the dressing pad scaled from none to copious as follows.

- 1) None—Wound tissues are dry.
- 2) Scanty—Wound tissues are moist, but there is no measurable drainage.
- 3) Small/minimal—Wound tissues are very moist or wet; the drainage covers less than 25% of the dressing.
- 4) Moderate—Wound tissues are wet; the drainage involves more than 25% to 75% of the dressing.
- 5) Large or copious—Wound tissues are filled with fluid that involves more than 75% of the dressing.

The findings were laid down for the evaluation of the wound and effect of antiseptic solutions. The collected data was analysed and interpreted using statistical tests.

Statistical study

Statistical analysis was done using mean, standard deviation, frequency and percentage as descriptive statistics. Unpaired *t*-test was used to check the significant difference between means of average decrease in wound size and average increase in granulation area. Karl Pearson's correlation coefficient was applied to check the relationship between decrease in percentage of patients with copious amount of discharge and use of citric acid as topical agent for dressing. Statistical analysis was done using SPSS 22, IBM, Bangalore.

Observations

The present study included 100 (n=100) patients divided into two groups of 50 each; Group A (control group) and Group B (citric acid or study group).

Patients with different age groups were included in our study. Most of the patients in both the groups were in the age group of 31 to 60 years followed by the age group of 11 to 30 years. In both the groups, only 10% of patients had age more than 60 years. Group A had 70% of males and 30% of females while Group B had 68% of males and 32% of females.

Wounds with different sizes were included. Mean wound size before treatment in group A and B was 27.91 ± 26.18 and 30.44 ± 25.75 respectively.

The p-value of 1.000 indicated the comparability of the wounds in both the groups. 70% of patients had wound size less than 40cm^2 in both the groups. Four patients with superficial burns (25% to 50%) were also included in both the groups.

Examination of the wound was done on day 1, 3, 5, 7, 9, 12, and 14. The wound was evaluated for healing. Group B showed more reduction in average wound size on 5th, 9th and 12th day as compared to group A. But, the difference seen in average reduction in wound size in both groups was not statistically significant (p-value >0.05) on respective days [Table 1]. However, significant (p-value=0.042) difference is seen in mean reduction of wound size in group A and B on 14th day with mean values of 67.40 ± 16.82 and 72.06 ± 14.05 respectively.

TABLE 1- Comparison of average (percentage) reduction in wound size (cm^2) on different days.

	Group A		Group B		t-value	p-value
	Mean	SD	Mean	SD		
Day 5	24.68	14.97	27.98	15.60	-1.356	0.179
Day 9	44.22	21.89	45.93	20.57	-1.695	0.094
Day 12	58.57	23.52	60.21	18.21	-1.893	0.062
Day 14	67.40	16.82	72.06	14.05	-2.245	0.029

(Unpaired t test)

Patients treated with citric acid showed comparatively more reduction in wound size than those who were treated by conventional method. And, this difference between the average reduction of wound size came out to be statistically significant by 14th day.

Group B showed comparatively more increase in average granulation area on 5th and 9th day than that of group A. But p-value of 0.219 and 0.110 on respective days suggested statistically non-

significant difference. However, on 12th and 14th day (p-value 0.044 and 0.039 respectively) significant difference was noted in increase in average granulation area in group B than that of group A [Table 2]. The increase in granulation area was more when citric acid was used for dressing than that in control group. The difference of average increase in granulation area of both groups became statistically significant by 12th day.

TABLE 2- Comparison of average (percentage) increase in granulation area (cm^2) on different days.

	Group A		Group B		t-value	p-value
	Mean	SD	Mean	SD		
Day 5	21.38	13.02	25.52	14.33	-2.470	0.219
Day 9	40.65	18.62	43.88	19.19	-2.654	0.110
Day 12	51.86	20.32	55.57	14.66	-2.524	0.044
Day 14	60.60	12.27	65.36	9.19	-3.155	0.039

(Unpaired t test)

Percentage of patients differed in both groups when distributed according to the amount of discharge scaled from none to copious on different days. On 1st day, 54% of patients had moderate to copious amount of discharge in group A while in group B there were 50% patients with same amount of discharge. However, by 5th day, there were only 5 patients with copious amount of discharge in group B. On the other hand, in

control group, there were 15 patients with copious amount of discharge (p-value-0.01). A significant reduction in number of patients with the moderate to copious amount of discharge has been seen in patients of group B (16% from 50%) as compared to that in group A (44% from 54%) on 9th day (p-value 0.016). On day 12th and 14th most of the patients had scanty to minimal amount of discharge in both the groups [Figure 1 and 2].

FIGURE 1

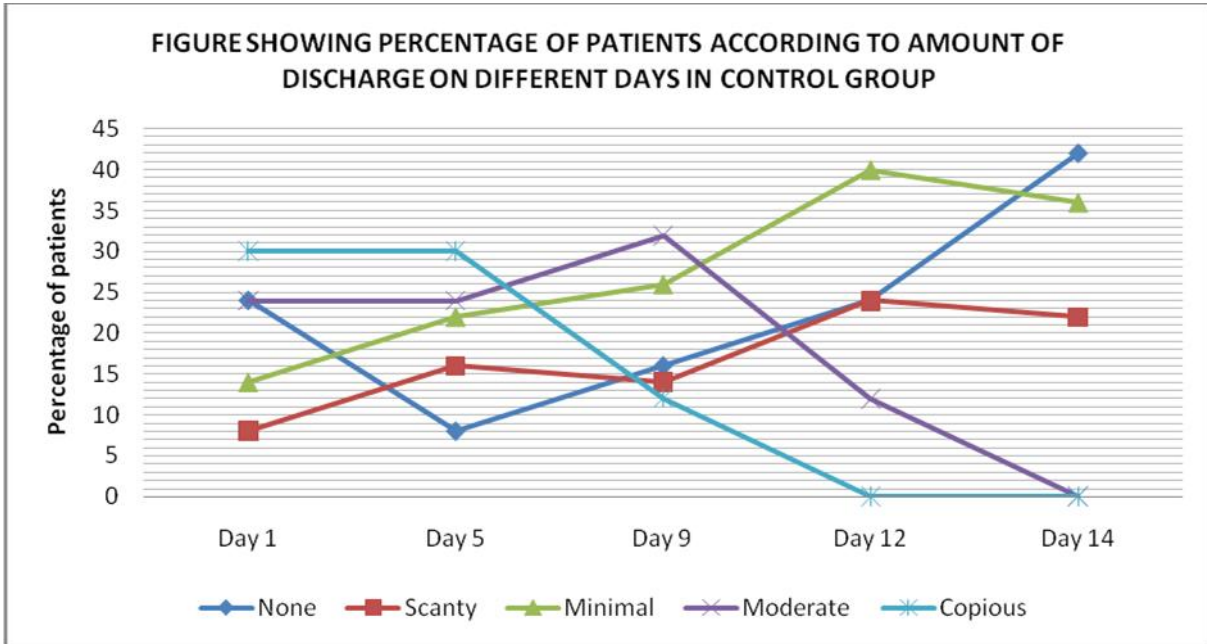
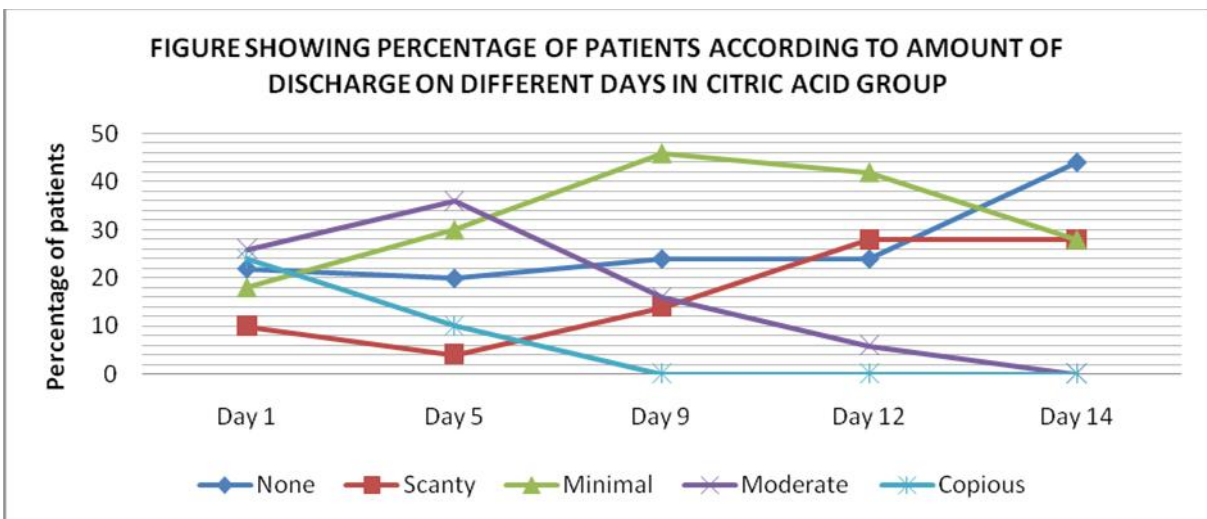


FIGURE 2



The wounds where citric acid was used for dressing showed early reduction in amount of discharge as compared to control group which was statistically evident by 5th day. However, by 12th day most of the patients had scanty to minimal amount of discharge in both the groups.

Visual analogue scale (VAS) score was used to assess the severity of pain. The average VAS score decreased from 6 to 1 in both the groups. No significant change has been noted in both the groups [Table 3]

TABLE 3- Comparison of average visual analogue scale (VAS) score on different days.

	Group A		Group B		t-value	p-value
	Mean	SD	Mean	SD		
Day 1	6.50	0.931	6.42	0.673	0.492	0.067
Day 5	5.48	0.839	5.14	0.670	2.239	0.087
Day 9	4.40	0.606	4.18	0.720	1.653	0.856
Day 12	3.18	0.661	3.30	0.814	-0.809	0.095
Day 14	1.52	0.580	1.38	0.490	1.304	0.083

(Unpaired t test)

The pH of wounds was monitored using pH strips on 3rd, 7th and 12th day. The wound surface of citric acid group showed a pH ranging from 4 to 6, while the control group showed a pH of 6-8, indicating that the wound environment was acidic in Citric acid group. None of the patients of both the groups complained of any local complaints such as skin irritation, or burning sensation on application.

The mean hospital stay in the patients of citric acid group (7.74±5.21) was comparatively less than that of patients in control group (10.38±7.50). There were 13 patients in group A, who stayed for more than 15 days. In group B, however only 4 patients stayed for more than 15 days.

Discussion

Since ages, it has been a challenge to treat the wounds. Attempts have been made worldwide to identify an appropriate and economical treatment for wounds. Although, multimodal therapy is the key but topical wound dressings have a paramount role. Our study aimed at evaluating the effect of topical application of 3% citric acid on wound healing as compared to conventional dressings. Citric acid is a weak organic acid and is referred to as E330 within the European Union. It

has multiple uses owing to its antimicrobial activity and as pH adjusting agent.

Over time, different agents have been developed by authors to hasten the process of wound healing such as cold argon plasma, protease inhibitors, growth factors and platelet rich fibrin.^[12,13,14,15] However, in developing countries like ours where there is voluminous patient load; application of these advancements is not practical. We therefore evaluated for efficacy of citric acid as it is cheap, easy to prepare, has long shelf life and is stable at room temperature.

Citric acid mainly does its action by modulating the surface pH. Chronic non healing wounds generally show alkaline pH. Pus and necrotic tissue are clinical indicators of ongoing healing process and a lower pH milieu. For healing to occur, the activity of proteolytic enzymes (with pH optimum of 8.0) should be diminished.^[16] Monitoring the pH of wound interior is challenging and needs advanced instrumentation. Hence, we monitored surface pH using pH strips. Most patients in the citric acid group showed pH of surface in the range of 4-6 as compared to 6-8 in control group. Use of citric acid prompts fibroblast proliferation and increases oxygen concentration.^[11]

Citric acid has its antimicrobial action. Most of the pathogens thrive at pH more than 6. *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most common pathogens in our study followed by *Pseudomonas* in burn patients. Nagoba et al determined the susceptibility of different microorganisms to citric acid and showed MIC in the range of 500-2500ug/ml.^[17] Citric acid has also been found effective against Methicillin Resistant *Staphylococcus Aureus* (MRSA) and Vancomycin Intermediate *Staphylococcus Aureus* (VISA).^[18] In our study, significant (p-value 0.016) reduction in number of patients with the moderate to copious amount of discharge was noted in patients treated with citric acid (16% from 50%) as compared to control group (44% from 54%) by 9th day. The wounds where citric acid was used for dressing showed early reduction in amount of discharge as compared to control group which was statistically evident by 5th day (p value-0.01). However, by 12th day most of the patients had scanty to minimal amount of discharge in both the groups owing to the use of systemic antibiotics.

Citric acid aids in early healing and thus helping patients to resume function at the earliest. In our study, the topical use of 3% citric acid significantly (p value-0.029) increased the average reduction of wound size by 14th day. Patients treated with citric acid showed average reduction of wound size by 72.06% in 14 days as compared to 67.40% in control group. Similarly, 50 out of 52 cases of snake bite ulcers (at MIMS Medical college, Latur) showed complete healing in 16 to 43 applications of 3% citric acid.^[17] In accordance with our study, Amol et al also found that ulcer size of a chronically deceased patient decreased to one-third of its original size in 3 weeks when citric acid was used for local management.^[19] In our study, the mean hospital stay in the patients of citric acid group (7.74days±5.21) was comparatively less than that of patients in control group (10.38days±7.50). In the control group, there were 7 patients who stayed in the hospital for more than 20 days. However, in the study group only 2 patients stayed for 20 days.

Early healing with citric acid is due to formation of healthy granulation tissue and proliferation of fibroblasts. The histopathological study of chronic infected wound following citric acid application showed that citric acid boosts fibroblast growth and neovascularisation.^[20] Prabhu et al used the formation of healthy granulation tissue ready for graft as end point of their study. They found this ulcer-granulation interval to be 10.56 days when 3% citric acid was used to treat the infected wounds. On histopathological examination, they also found that wounds treated with citric acid showed an increase in fibroblasts and granulation tissue on 7th day.^[21] In the current study, the average increase in percentage of granulation area was more when 3% citric acid was used for dressing (65.36%) than that in control group (60.60%). The difference seen in both the groups was statistically significant (p value-0.044) by 12th day. On average, by 12th day, more than 50% of wound area showed granulation tissue when 3% citric acid was used for topical dressing.

Although, literature suggest the correlation between the citric acid application and fibroblast proliferation but the exact molecular mechanism behind the same has not been studied. However, in their multianalysis, Nagoba et al proposed the possibility of the role of pluripotency following the application of 3% citric acid in chronic infected wounds based on the unexpected phenomenon of somatic cell reprogramming into pluripotent cells by exposure to sublethal stimuli such as low pH exposure, also known as stimulus triggered acquisition of pluripotency (STAP).^[22,23]

Conclusion

This study concludes that three per cent citric acid is safe and effective in all types of wound management and gives better efficacy and faster response as compared to conventional methods. Treatment with three per cent citric acid reduces the microbial flora, and is less painful during cleaning and debridement procedures. It can be used safely in various conditions such as chronic foot ulcers, bed sores, burns, traumatic wounds, post operative infective wounds, cellulites and abscesses.

Use of citric acid as topical agent reduces the pH of the wound surface and inhibits the bacterial proliferation. Use of three per cent citric acid aids in early wound healing and reduces hospital stay of patients by promoting formation of granulation tissue and fibroblast proliferation.

The result of this study therefore appear to show more favourable results for Citric acid group than for conventional dressing without citric acid. However, although the results are statistically significant, the strength of evidence depends upon the study design. The results of this study justify further research into the use of three per cent citric acid in treatment of various wounds and ulcers. It is important to ensure that possible source of bias in further studies are excluded e.g. by blinded assessment of outcomes.

Citric acid is a natural non toxic product with no apparent side effects. We used Visual analogue scale score (VAS) to assess the severity of pain in patients. The average VAS score decreased from 6 to 1 in both the groups by 14th day. This decrease might be credited to the use of systemic analgesics. No systemic or local allergic manifestations were seen in the study except mild irritation and pain during application of povidone iodine especially in burn patients.

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