

## Original Article

## Effect of green tea on the second degree burn wounds in rats

Mohammad Javad Fatemi, Babak Nikoomaram<sup>1</sup>, Amir Asadollah Khajeh Rahimi<sup>1</sup>, Donya Talayi<sup>1</sup>, Shahrzad Taghavi<sup>1</sup>, Yaser Ghavami

Departments of Plastic and Reconstructive Surgery, Hazrat Fateme Hospital and Burn Research Center, Iran University of Medical Sciences, <sup>1</sup>Department of Plastic and Reconstructive Surgery, Azad University of Medical Sciences, Tehran, Iran

**Address for correspondence:** Dr. Mohammad Javad Fatemi, Department of Plastic and Reconstructive Surgery, Hazrat Fateme Hospital and Burn Research Center, Iran University of Medical Science, Vali-asr Ave., Yasami St., Tehran 19967 14353, Iran. E-mail: mjavadfatem@gmail.com

## ABSTRACT

**Background:** Various studies indicate that the green tea has anti-inflammatory and anti-oxidative properties. Moreover, a few studies have been carried out that demonstrate beneficial effects of green tea on burned patients. **Materials and Methods:** In this study, green tea, Vaseline, and silver sulfadiazine dressings were used as first-aid treatment to deep dermal contact burns in rats, compared with a control of nothing. After creating second-degree burn on the dorsum of rats, the treatments were applied for 15 min in four groups. Wound dressing changes were daily. Macroscopic study was performed on days 1, 3, 7, and 14 by using a digital camera and software processing of photos. Microscopic examination was done by pathologic evaluation of skin specimens on day 14. **Results:** We observed that green tea usage significantly decreased burn size in comparison to the control group ( $P = 0.004$ ). **Conclusion:** Green tea is effective on healing process of second degree burn wounds.

## KEY WORDS

Green tea; second degree burn; silver sulfadiazine

## INTRODUCTION

Burn is one of the main reasons for referral to an emergency and surgery departments. About 1.5 out of 100 individuals will experience some kind of burn injury annually.<sup>[1,2]</sup> Wounds and scars are common after burn injuries which can cause numerous problems for patients.<sup>[3,4]</sup>

Burn injury treatment has a great burden on patients and community. Flame burn injury constitutes a large

proportion of burn injuries among adult population. There are different methods and drugs for treating this kind of injury. Furthermore, there have been various studies on the effect of herbal medicine for treatment of burn injuries. One of these herbs is green tea, which is derived from the plant *Camellia sinensis* and has beneficial characteristics such as antioxidant activity.<sup>[5,6]</sup> It has been used to treat diabetes in East Asia and the traditional Chinese medicine.<sup>[7]</sup> Therapeutic effects of green tea have been evaluated in different studies. It also can be used as an anticancer agent; or reducing blood lipids and glucose.<sup>[8-10]</sup> Some investigations indicate that green tea consumption protects against development of cardiovascular disease.<sup>[11]</sup> Moreover, there have been a few studies regarding usage of green tea in management of sun burn injury.<sup>[12,13]</sup> In this animal model study, we evaluated the possible beneficial effects of green tea on second degree burn

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wounds in comparison to silver sulfadiazine and Vaseline.

## MATERIALS AND METHODS

### Extract preparation

Green tea extract was obtained by maceration method. Leaves of green tea were converted to a powder by grinding. About 40 g of powder were mixed with 400 ml ethanol (70%) at room temperature for 48 h. Then, the mixture was filtered from paper-making machine to extract the liquid.

After preparing the liquid extract, it was placed in 10 plates in a water bath for 48 h with the 70°C temperature until it was completely dried. Then, 2 g of alcoholic extract of green tea was mixed with 100 ml saline; and finally 2% of the alcoholic extract of green tea was obtained. This extract was standardised.

### Animal preparation

In this experimental study, 40 Wistar albino rats weighing approximately 300-350 g were used. All the rats were healthy and they were screened for animal diseases by a veterinarian. The rats were kept in separate shelves in the animal laboratory with 12 h light-dark cycle and temperature of 22°C and *ad libitum*. All animals were housed in sterilised containers. They were randomly divided into four groups with 10 rats in each [Table 1].

To create a burn wound in animal, the rats were anaesthetised by intramuscular injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). After shaving animal's dorsum hair, a deep second degree burn wound was created by a metal cube with dimensions of 2 cm × 3 cm × 1 cm that was heated to 100°C for 15 s and an area of about 6 cm<sup>2</sup> was burned. Thereafter, medication was initiated for these four groups with different materials [Table 1].

**Table 1: Four different treatment modalities in four groups of rats**

Groups	Treatment method	Amount of extract, cream or ointment	Duration (days)
Group I	Green tea (1 g)+dressing	0.5 cc	14
Group II	Silver sulfadiazine cream 1% (Sobhan Darou Inc., Iran)+dressing	1 cc	14
Group III	Vaseline+dressing	1 cc	14
Group IV	Simple dressing (normal saline+bandage+plastic wrap)	—	14

Dressings were performed on days 1, 3, 7, 10, and 13. The rats were given anaesthesia while taking photos and also during dressing change. The macroscopic examination was performed by taking photographs of burned areas using a digital camera (Canon powershot D10) on days 1, 3, 7, and 14; also sizes and areas of burn surface were measured by the software ImageJ. Furthermore, the wounds were measured by a ruler on each rat and recorded.

Three rats were died within hours after creating burn on their dorsum which may be due to the stress or shock induced by burning. Green tea extract was partially standardised and it was not analysed for its active ingredients, which may contribute in healing of burn wounds.

### Microscopic examination

On day 14, rats were anaesthetised and a sample from burned scar tissue and skin adjacent were put in 10% formalin solution. Processing and moulding was done by using paraffin; thereafter transverse incision, including skin and wound bed thickness of 4 µm were prepared. Slices were coloured with haematoxylin and eosin staining method. Then, the colour slides were evaluated using pathological parameters including epithelisation, inflammatory cells, and vascularity.

All data were analysed using statistical software SPSS (version 14; Chicago, Illinois, USA). One-way ANOVA test was used to compare means.  $P < 0.05$  was considered as significant. This study was confirmed by animal Ethic Committee of Iran University of Medical Sciences, Tehran, Iran.

## RESULTS

Burned areas were evaluated and compared in days 1, 3, 7 and 14. The evident difference of healing process was observed after 14<sup>th</sup> day [Table 2]. Average burned area was reduced significantly in the group who was treated with green tea. The average burned area was statistically significant between green tea treated (GTT) group and control group ( $P = 0.003$ ); however there was no statistically significant difference between GTT and Vaseline treated or silver sulfadiazine treated (SST) groups [Figure 1]. Improvement of burn wounds using green tea is shown in Figure 2.

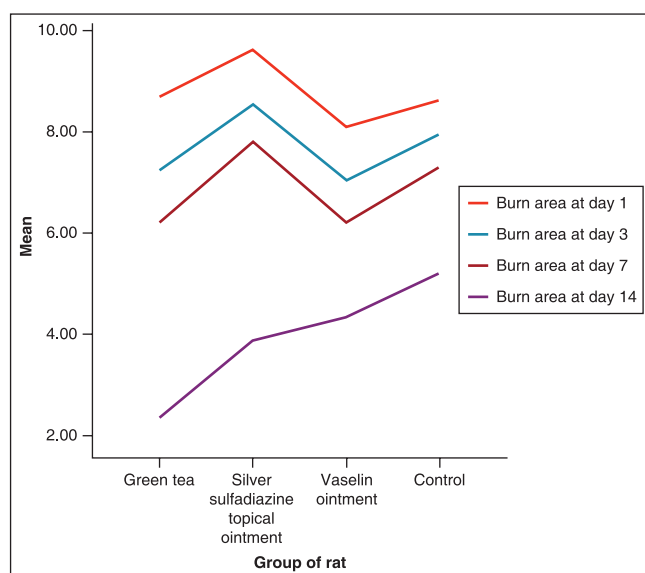
Pathologic results indicated that however burned area healing was quantitatively better in GTT group, but there was not statistically significant difference between GTT and SST groups [Table 3].

**Table 2: Comparison of BSA in four groups in days 1, 3, 7 and 14**

BSA (cm <sup>2</sup> )	Green tea	Silver sulfadiazine topical cream	Vaseline ointment	Simple dressing
<b>BSA (cm<sup>2</sup>) day 1</b>				
Mean	8.7161	9.6186	8.1076	8.6275
<i>n</i>	10	10	10	10
Standard deviation	3.25432	1.03794	2.92733	0.94081
Minimum	4.80	7.47	4.81	7.28
Maximum	16.29	10.98	14.53	10.41
<b>BSA (cm<sup>2</sup>) day 3</b>				
Mean	7.2589	8.5530	8.1076	7.9580
<i>n</i>	10	10	10	10
Standard deviation	2.26236	1.12212	2.92733	0.74564
Minimum	4.37	5.73	4.81	6.85
Maximum	12.28	9.65	14.53	9.39
<b>BSA (cm<sup>2</sup>) day 7</b>				
Mean	6.2055	7.8116	6.2125	7.3010
<i>n</i>	10	10	10	10
Standard deviation	2.58080	1.25814	2.32445	1.16247
Minimum	2.91	5.19	2.89	5.73
Maximum	11.83	9.1	10.65	9.20
<b>BSA (cm<sup>2</sup>) day 14</b>				
Mean	2.3533	3.8910	4.3456	5.2085
<i>n</i>	10	10	10	10
Standard deviation	1.88190	1.93275	1.20692	1.51206
Minimum	0.00	0.00	2.50	2.84
Maximum	16.29	6.67	6.78	8.36
<b>Total</b>				
Mean	6.1335	7.4686	6.6933	7.2737
<i>n</i>	40	40	40	40
Standard deviation	3.41758	2.56229	2.83485	1.68992
Minimum	0.00	0.00	2.50	2.84
Maximum	16.29	10.98	14.53	10.41

BSA: Burn surface area; *n*: Number**Table 3: Pathological comparison between green tea group and control group**

Groups	Vascularisation	Inflammatory cells	Epithelialisation
Green tea treated versus simple dressing	<i>P</i> =0.336	<i>P</i> =0.166	<i>P</i> =0.167

**Figure 1: Mean total body surface area in different treated groups**

## DISCUSSION

This study evaluated the effect of three materials (green tea, silver sulfadiazine, and Vaseline) on partial thickness burn injury in rats. Parameters such as burn area, re-epithelialisation, and histology were measured. Burn area decreased significantly in GTT group. Furthermore, re-epithelialisation has improved in GTT group in comparison to other groups. However, histology results indicate that vascularisation was better in GT group, but it was not statistically significant. Our results showed that topical application of green tea is efficacious in treatment of second degree burn wounds in comparison to simple dressing. However, treatment with green tea showed better results in comparison to dressing with silver sulfadiazine and dressing with Vaseline, but the difference was not statistically significant.



**Figure 2:** Improvement process of healing in green tea treated group

It has been clarified that green tea has multiple ingredients which have antioxidant activity and bioactive properties. One of these compounds is polyphenols (catechins, flavonols, theaflavins and thearubigins) which has been shown to be antioxidant and anti-inflammatory. Epigallocatechin-3-gallate (EGCG) is the main polyphenolic compound of green tea and may contribute in improvement of burn wounds and scars.<sup>[14,15]</sup>

In a study by Klass *et al.*, it was clarified that EGCG has potential effects on wound contraction and healing. EGCG inhibits nuclear factor- $\kappa$ B and activator protein 1 in dermal fibroblasts. Furthermore, EGCG modifies Transforming growth factor- $\beta$  (TGF- $\beta$ ) signalling which suppresses TGF- $\beta$  receptors. Furthermore EGCG can reduce expression of matrix metalloproteinase-2 (MMP-2) and MMP-1. MMPs are enzymes that degrade extracellular matrix and their elevation is associated with impaired wound healing. EGCG can down-regulate synthesis of collagen type-1. All of these mechanisms make EGCG a potential anti-scarring factor.<sup>[16]</sup>

In an experience by Elmetts *et al.*, they found that application of green tea extracts on the ultraviolet-A (UVA) exposed skins of normal volunteers will result in a dose-dependent inhibition of the erythema. Presence of EGCG and epicatechin-3-gallate were most efficacious in prohibiting erythema. Furthermore, GTT skins showed reduced number of sunburn cells and preservation of Langerhans cells after UV damage. They concluded that polyphenolic extracts of green tea can play a role as chemo preventive factors against detrimental effects of sunlight.<sup>[13]</sup>

Other studies show protective effects of EGCG on the skin. For example, a study by Sevin *et al.* was conducted on rats, which shows protective effect of EGCG while applied topically before UVA exposure; but after UVA exposure, topical application of EGCG was useless.<sup>[17]</sup> Hong *et al.* evaluated effect of a formula containing tannase-converted green tea extract in order to inhibit oxidative damage by ultraviolet-B (UVB). Their results indicate that this formula may be effective in preventing UVB induced skin damage.<sup>[18]</sup>

However, there are several studies indicating protective effects of green tea against sunlight, but according to our knowledge there was not any evaluation of green tea products on the healing process of second-degree burn injuries. More studies are required to find out the exact components of green tea that causes this wound healing in second-degree burn injury in rats.

## CONCLUSION

Our results reveal the efficacious topical application of green tea on the improvement of second-degree burns. However, further studies are needed to prove the efficacy of green tea on human.

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Mob.: +91-9811011008

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