Effectsof *Camellia sinensis* Ethanolic Extract on Histometric and Histopathological Healing Process of Burn Wound in Rat

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Abstract: Green tea (Camellia sinensis) that were used as an ancient herbal drug in Asia, with having anti-oxidant and anti-inflammatory properties, may can improve wound healing process, thereforethe present study were design to determinate the effect of green tea ethanolicextract on burn wound healing process in rat. For this experimental study, 36 healthy male Wistar rats were randomly selected into three groupsof A, B and Cwhich, respectively treated with, Vaseline + 0.6% green tea extract, Vaseline andnormal salinefor a 21 days period. Burn Wounds' areas were measured and calculated by caliper every other day and pathologic samples were taken at 3rd, 12th and 21st day for histopathological examinations. In the end, all data were analyzed by SPSS 16 using survival analysis (Breslow test), repeated measured ANOVA, one-way ANOVA and Mann-Whitney. $P<0.05$ was considered as statistically significant.Regarding Breslow test, the mean healing duration in group A with18 days was shorter in compared to group B with 20.33 days, ($P=0.023$). In according to Mann-Whitney test, comparing groups A and C during the whole study, indicated no statistically significant differences in histopathologicalscoresof epithelial regeneration and angiogenesis except inflammatory cells. Green tea extract could help wound healing process, probably effective on burn wounds healing.

Key words: Green Tea · Camellia Sinensis · Healing Process · Burn Wound

INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in every culture all over the world. Data from WHO show that 70-80% of the world's population use herbal medicines as alternative medicine specially in middle east countries that is rich in herbal plant drugs like, Pakistan and Iran [1, 2].

Recently, the traditional use of plants for wound healing has receivedattention by the scientific community [3, 4]. Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs [5, 6].

Skin is one of the body's most important organs, which could preventdehydration and influx of external factors [7].

Among all kinds of skin injuries, Burn wounds are very common skin injury in all communities, however, in developing countries burns consider as a major health problem because the incidence of severe complications is high and financial resources are limited. Normal wound healing response begins the moment the tissue is injured that comprises a complex pathophysiological process including several cellular and biochemical processes, e.g. inflammation, vessel formation and collagen deposition [8, 9]. Inflammation maintenance and inadequate vessel formation comprise the most noticeable causes of delayed wound healing [10, 11]. This delay may cause major wound infection that is responsible for 50-75% of hospital deaths. On the other hand wound fibrosis or abnormal accumulation of collagen around the wound could lead to an unpleasant scar that is very important in some parts of body like face and limbs [12, 8, 13].

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Green tea (*Camellia sinensis*) were used as Asian people for health promotion healing diseases since 3000 B.C. This plant contains polyphenolic, in particular, catechin compounds [7, 13-17] Epicatechin, epicatechingallate, epigallocatechin and epigallocatechingallate that are responsible for anti-oxidant, anti-cancer, anti-aging and anti-inflammatory effects for this herbal medicinal plant [18]. Alsoit could prevent collagen production and accumulation and induce changes in immune responses in the healing phases [4, 5, 6, 18].

These compounds (e.g. epigallocatechingallate) have also been used as an agent for keratinocytes reproduction and distinction [14]. Also, itsanti-fibrinogen effects have been confirmed in some animal models [15].

Due topreviousstudies, traditional experiences and easy accessibility, we investigated the effect of ethanolic extract of green tea on Burn wound healing process in wistar rat and determination of antioxidant Activity.

MATERIALS AND METHODS

After obtaining approval in Shahrekord University of Medical Sciences (SKUMS) Ethics Committee by the number of 16.5.90, this experimental study was conducted in Medicinal Plants Research Center of SKUMS and Researches Centre of Medicinal Plants and Ethno-veterinary, Islamic azad university of shahrekord branch.

Preparation of Extract: Maceration method was employed to prepare the extract. For this purpose, 100g green tea (Herbarium No. 304, Medical Plants Research Center, SKUMS) was transported into an Erlenmeyer, 1 liter ethanol 70% (Nasr Co. Iran) was added and the solution was left at laboratory temperature. Forty eight hours later, the extract was filtrated through a filter paper and the pulp was squeezed to discharge. Then, the extract was concentrated by a rotary evaporator [17], dried and mixed with pure Vaseline (Ehsan Chemi, Iran) to make a Vaseline-based 0.6% ointment [19].

Animals: 36 Healthy male young Wistar Albino rats, weighing about (200-250g) obtained from animal house of Shahrekorduniversity of medical sciences. They were maintained under standard laboratory conditions of food and water andkept for quarantine for 24 hours. They were randomly assigned to three groups of A, B and C, the animals were treated with Vaseline+0.6% green tea extract (group A), Vaseline (group B) and normal saline (group C) for 21 days.

Burn Wound Model: After anaesthetizing Rats, by a combination of 20 mg/kg ketamine 10% (Alfasan Co., Netherlands) and 2mg/kg xylazine 2% (Alfasan Co., Netherlands), administered intramuscularly. Then, type II burning was created by putting 120°C Circular metal for 5 second on the shaved and scraped back of the rats [20]. Then rats were maintained for recoveryin same situations and 12/12 hours light/dark cycle at 21±2°C.

Treatment: Performed every day at the same time by one person, treatment continued for 21 days, beginning from the day of making wound. For this, 1g Vaseline ointment containing green tea extract was topically applied on area of the burn wound in group A; the same was performed on group B and C using Vaseline ointment andnormal saline (Daru-Pakhsh, Iran), respectively [21].

Histometric Examinations: Since the 2nd day of the study, the length of the remaining Burn wound areas was measured by a using a caliper and photographed every other day; each case's data was separately recorded [22, 21].

Histopathological Examinations: For pathologic sampling, 3 rats were randomly selected from groups at 3rd, 12th and 21st days of study period. After sacrifice them through anesthesia over dosage, the wound tissue histological specimens in full thickness accompanied with neighboring healthy skin were taken and fixed into 10% Neutral Buffered Formalin. Tissue processing was done by paraffin and transverse section, 5 micrometers thick, were made by means of Microtome fixed blade. The sections were stained by hematoxylin and eosin [23, 24]. Histopathological examinations, in view of inflammatory cells, epithelial regeneration and angiogenesis,were separately done by a pathologist who was blinded in groupingby scoresthat mentionedon Table 1.

Table 1: System for the histological features scores

Score	Epithelial Regeneration	Inflammatory cell	Angiogenesis
0	Absent	<5	Altered angiogenesis (1-2 vessels per site)
1	Starting	5 to 10	Few newly formed capillary vessels (3-6 vessels per site)
2	Incomplete (partial thickness)	11 to 20	Newly formed capillary vessels (7-10 vessels per site)
3	Complete (full thickness)	>20	Newly formed and well-structured capillary vessels (>10 vessels per site)

Statistical Analysis: Data analysis weredone by SPSS 16 using survival analysis (Breslow test), repeated measures ANOVA, one-way ANOVA and Mann-Whitney. p<0.05 was significantly considered.

RESULTS

Morphological and Histometrical Findings: Totally, 5 of 6 (83%) rats in group A (Vaseline+0.6% green tea extract) and 2 of 6 (33%) rats in group B (Vaseline) recovered by 21st day and no inflammation, eczema or infection were observed.

Regarding Breslow test, the mean healing duration in group A [18 days (SE=0.624)] was shorter compared to group B [20.33 days (SE = 0.385)] and it was statistically significant (P=0.023).

The Burn wound area decrease process in group A, based on repeated measures ANOVA, was significantly faster than group B (p=0.002). Diagram 1 indicates Burn wound areas during the study and Table 2 indicates the mean decrease in burn area during 1st, 2nd and 3rd weeks.

Based on one-way ANOVA, the mean of increased burn area at the first week in group A were less than group B (P<0.001) on the other hand there was no significant difference in the mean decrease in burnarea between groups A and B during 2nd week (P=0.08) but during the 3rd week, the difference between the two groups was significant (P=0.013).

It is notable that the decrease in burn area in group C was significantly less than the two other groups during the 21 days andthere was no significant difference in the mean decrease in burn area between groups A and B during in all of the period (P=0.875).

Histopathological Findings: Table 3 shows Mean Scores±Standard deviation of histopathologicalfeatures of burn wound healing in different groups throughout the whole study.

Table 2: Comparison of mean decrease in burn area during the study (cm^2)

Groups	1st week	2nd week	3rd week	Total	Results of data analysis in 3 weeks
(Repeated measures) Vaseline+0.6% green tea extract(A)	-0.98	6.67	0.38	6.07	p<0.001
Vaseline (B)	-3.28	7.75	1.40	5.87	p<0.001
Normal salin(C)	-3.12	4.48	2.70	4.06	0.005
Results of data analysis (one-way ANOVA)	<0.001	0.005	<0.001	0.001	

Table 3: Histopathological evaluation ofwound tissue samples at whole study

Wound healing' Scaling	Groups	Epithelial Regeneration	Inflammatory cells	Angiogenesis
Green tea+Vaseline (A)	0.72±0.88		0.81±1	3.317*±4.88
Vaseline (B)	0.67±0.55	1.13±1.66	1.69±3	
Normal Saline (C)	0.72±0. 44	0.61±2.22	1.02±2.22	

-Values are mean ± SD for the histological features in each group

-Asterisk in superscript indicates significant difference
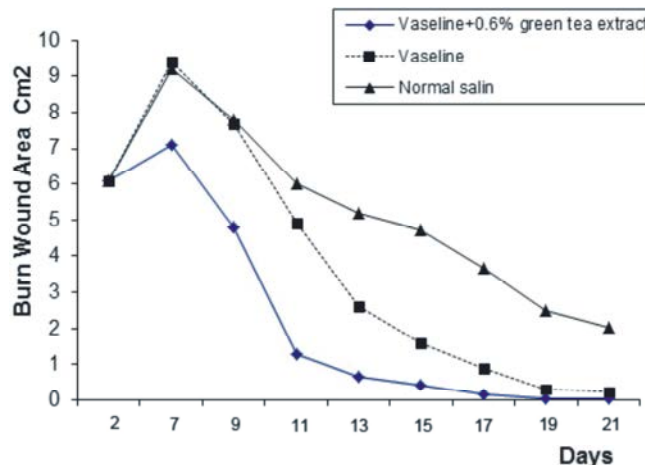
-All values are significant at P<0.05

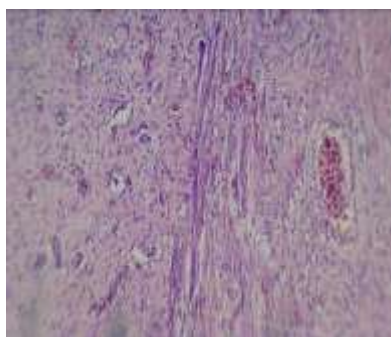Diagram 1: Burn wound area (cm^2) in all groups during the study

Fig. 1: Histopathologic sample Group A, appropriate angiogenesis and arranged collagen bands with low inflammatory cells. (H and E, X100, 400)
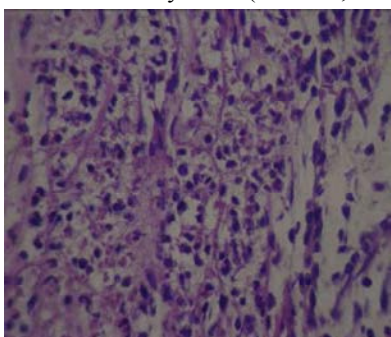

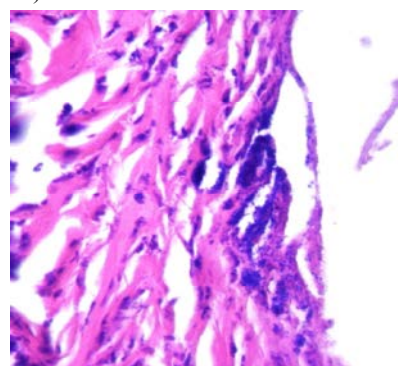Fig. 2: Remaining some inflamatory cells (H and E, X100, 400)

Fig. 2: Incomplete epithelial in group Cregeneration in group C (H and E, X100, 400)

In according to Mann-Whitney test, comparing groups A and C during the whole study, indicated no statistically significant differences in histopathological scores of epithelial regeneration (P=0.192) and angiogenesis (P=0.106) except inflammatory cellsthat was statistically significant (P=0.008).

In addition, this comparison at 21st day of the study indicated statistically significant differences in no histopathologicalscores of epithelial regeneration, inflammatory cells except angiogenesis (P=0.361, P=0.068 and P=0.046, respectively.

Following microscopic photographs shows some difference between groups during the study.

DISCUSSION

This study carried out to determine beneficial effects of green tea extract on healing of type II burn injuries in a rat model.

Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes [25]. It is depends on the type and extent of damage, the general state of health and the tissue recovery ability.

Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage [25, 26]. But some of problems that occurred may cause todelay in wound healing that lead to increases the possibility of infection, inappropriate recovery and unpleasant scar [27, 26].

There are many studies in green tea healing aspects in diseases, like: anti-oxidant, anti-cancer, anti-aging, anti-inflammatory effects and preventive effects of collagen production and accumulation [28, 19]; the majority of these properties could be attributed to the plant's polyphenolic compound, i.e., catechin in the leaves [29, 30].

Consistent with our findings, Safari and Sadrzade's study indicated anti-oxidant effects of epigallocatechin, one of the green tea's compounds [31].

In addition, the beneficial effect of epicatechingallate and other phenolic components on wound healing quality and hence leaving more pleasant scar has been shown in Asadietal researches [20], that which confirm its effect on increased level of vascular endothelial growth factor and increasing angiogenesis in Group A in comparison to C in 3rd week by accelerated vessel formation and enhanced nitric oxide and cyclooxygenase [32]. Also Significant reduce, in number of inflammatory cells specially notrophilsin all duration of study in compare to control group C is the other results of the anti- oxidant components and phenolic substances and their activities [19, 30].

In morphometric examination, although the burn wound area were increased in all groups due to increasing the inflammation and skin tensionon the dorsal region of rats at 1st week but it was significantly less than group B in A group (P<0.001). In the other hand Analyzing the burn wounds areas during the study

demonstrated the significant effect of Vaseline+0.6% green tea extract in compare to control in decreasing the burn area in all period of study that is confirm the previous studies.

In according the properties mentioned for components of the green tea, it could help wound healing process. therefore, Further studies need to be done to identify and separate the group of active constituents responsible for anti-inflammatory activity and wound healing activity, Thus, after clinical trials in human, it can be considered as a natural, low cost easily available, treat to cure type II burns.

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