ORIGINAL RESEARCH

International Journal of Surgery 11 (2013) 332–337

Contents lists available at SciVerse ScienceDirect

International Journal of Surgery

journal homepage: www.theijs.com

Original research

Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat

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ARTICLE INFO

Article history:
Received 26 October 2012
Received in revised form
20 January 2013
Accepted 18 February 2013
Available online 28 February 2013

Keywords:
Green tea
Camellia sinensis
Healing process
Surgical wound
Rat

ABSTRACT

Green tea (*Camellia sinensis*) has anti-oxidant and anti-inflammatory properties and may enhance wound healing process. The present study, therefore, was aimed to examine the effect of green tea ethanolic extract on wound healing process.

For this experimental study, 36 healthy male Wistar rats were randomly designated to three groups of A, B, and C which, respectively treated with, Vaseline + 0.6% green tea extract, Vaseline and normal saline for 21 days. Wounds' length and area were measured by caliper every other day and specimens were taken at 3rd, 12th, and 21st day for microscopical examinations. 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.

The mean healing duration of surgical wounds in groups A and B was 14.66 and 20.66 ($P = 0.018$), respectively. Decrease in healing duration in the group A was significantly higher within the first two weeks compared with control groups ($P = 0.05$). Microscopic examinations also indicated a significant difference in wound healing process between groups A and C throughout the whole study duration as well as groups A and B during the 3rd week of the study ($P < 0.05$).

Green tea extract could help wound healing process, probably effective on surgical wounds healing.

1. Introduction

Wound healing comprises a complex pathophysiological process including several cellular and biochemical sub processes, e.g. inflammation, angiogenesis, and collagen deposition.[1] Inflammation maintenance and inadequate vessel formation comprise the most noticeable causes of delayed wound healing.[2] On the other hand wound fibrosis or abnormal accumulation of collagen in the wound could lead to an unpleasant scar.[3] Recent research has shown that many of the compounds that are used for wound healing such as Acetic acid, Hydrogen peroxide, and etc., is toxic to cells needed for healing.[4]

The majority of plant extracts, e.g. Grape seed, Lemon, Rosemary, and Jojoba, have been employed for wound healing and longevity increase. All of these plants have a common property, i.e., producing compounds with phenolic structure.[5] These phytochemicals ordinarily react with some compounds such as Oxygen free radicals and other macromolecules in order to neutralize free radicals and/or initiate biological effects.[5]

Green tea (*Camellia sinensis*) which is a product of dried leaves has been consumed by East Asian people for health promotion since 3000 B.C.[6,7] Abundantly found in Asia, green tea is also one of the most prevalent drinks worldwide.[6,8,9]

Ample evidence indicates that this plant, with anti-oxidant, anti-cancer, anti-aging, and anti-inflammatory effects, could also prevent exaggerate collagen production and accumulation and induce changes in immune responses, as well[5,6,8]; the majority of

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http://dx.doi.org/10.1016/j.ijsu.2013.02.014

Table 1
System for scoring the histological features of wound tissue samples.

Score	Epithelial regeneration	Granulation tissue thickness	Angiogenesis
1	Little epithelial organization	Thin granular layer	Altered angiogenesis (1–2 vessels per site)
2	Moderate epithelial organization	Moderate granular layer	Few newly formed capillary vessels (3–6 vessels per site)
3	Complete epithelial organization	Thick granular layer	Newly formed capillary vessels (7–10 vessels per site)
4	Complete epithelial organization	Very thick granular layer	Newly formed and well-structured capillary vessels (>10 vessels per site)

these properties could be attributed to the plant's polyphenolic, in particular, catechin compounds.[10–14]

Epicatechin, Epicatechin gallate, Epigallocatechin, and Epigallocatechin gallate are among the key anti-oxidant compounds of green tea, little of which could increase collagen volume and hence heal the wounds.[15] These compounds (e.g. epigallocatechin gallate) have also been used as an agent for keratinocytes reproduction and distinction.[16] Also, its anti-fibrogen effects have been confirmed in some animal models.[17]

Regarding the above mentioned and easy accessibility, we investigated the effect of ethanolic extract of green tea on post-surgical wound healing process.

2. Materials and methods

After obtaining approval of Shahrekord University of Medical Sciences (SKUMS) Ethics Committee, this preclinical study was conducted in Medical Plants Research Center of SKUMS. Throughout the experiments, we tried to follow all ethical principles of working on laboratory animals so as to impose the lowest possible stress on them.

2.1. Extract preparation

Maceration method was employed to prepare the extract. For this purpose, 100 g green tea (Herbarium No. 304, Medical Plants Research Center, SKUMS) was transported into an Erlenmeyer, 1 L 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,[18] dried, and mixed with pure Vaseline (*Ehsan Chemi, Iran*) to make a Vaseline-based 0.6% ointment.[5]

2.2. Measurement of phenolic compounds

The phenolic compounds were evaluated equivalent to gallic acid using Folin-Ciocalteu colorimetry method.[19] Different concentrations of standard gallic acid, i.e., 12.5, 25, 50, 62.5, 100, and 125 ppm in methanol 60%, were prepared. Then, 0.1 ml from each sample was transferred into a test tube and 0.5 ml Folin-Ciocalteu 10% was added as reactive agent. The solutions were left for 8 min at room temperature and then 0.4 ml carbonate 7.5% was added. The tubes were maintained for 30 min at room temperature and then assayed in three intervals by a spectrophotometer (Unico UV 2010) at 765 nm wavelength. To measure the overall phenol in the extracts, 0.01–0.02 μg of the extracts was solved in methanol 60%, reaching 10 ml and then, using Folin-Ciocalteu method, the overall level of phenol was measured. However, instead of using the standard solution, 0.1 ml extract solution was added. Finally, the overall phenol level was obtained in mg/g extract in gallic acid equivalent.

2.3. Measurement of flavonoid compounds

Total flavonoids were evaluated equivalent to Rutin, using chloride aluminum colorimetry.[20] First, different concentrations of standard Rutin (25, 50, 100, 250 and 500 ppm) were prepared in methanol 60%. Then, from each solution, 1 ml was transferred into test tubes and mixed with 1 ml of chloride aluminum 2%. Afterwards, 6 ml potassium acetate 5% was added and the optical density level was read after 40 min at 415 nm wavelength. The concentration levels of the standard solutions were assayed in three intervals. In order to measure the overall level of flavonoids in the extracts, 0.01–0.02 g of the extracts was dissolved in methanol 60%, reaching 10 ml. Then, the total level of flavonoids was measured using chloride aluminum colorimetry. However, instead of using the standard solution, 1 ml the extract was added. The total flavonoid level was calculated in mg/g extract, equivalent to Rutin.

2.4. Measurement of flavonol compounds

The total flavonol was measured using chloride aluminum colorimetry and Rutin procedure, however the optical density level reading was obtained after 2.5 h at 440 nm wavelength.[21]

2.5. Measurement of anti-oxidant activity

β-carotene model was employed to measure the anti-oxidant activity of the extract.[22] Half ml chloroform, 5 ml β-carotene (0.2 mg), 20 ml linoleic acid (20 mg), and 0.2 ml Tween 40 were mixed in a suitable container and incubated at 50 °C for 10 min in order to remove the solvent. The solution was diluted with distilled water and mixed with 4 ml aliquots in the following manner. The control solution was prepared including 0.2 ml ethanol, 0.2 ml the extract sample, and 0.15 ml ethanol. The optical density of the control group was recorded at t_0 and t_{90} at 470 nm, in a manner similar to the standard. The samples were incubated in a bain-marie at 50 °C. The anti-oxidant activity was measured on the basis of the ability of the samples in preventing β-carotene washing, calculated through $AA = 100[1-(A_o-A_t)/(Ao_o-Ao_t)]$; where, A_o is the optical density at t_0, A_t is optical density of the sample at t_{90}, and Ao_o and Ao_t are optical density values in the control samples at t_0 and t_{90} respectively.

2.6. Animals and study design

36 healthy Wistar male rats weighing 200–250 g were randomly assigned to three groups of A, B, and C and treated respectively, with Vaseline + 0.6% green tea extract, Vaseline and normal saline for 21 days. The rats had no history of surgery and other medical interventions, were kept in at most 3-member cages, at 23 ± 2 °C temperature, and on nutritionally similar and standard pelleted diet (*Razi* Co. Karaj, Iran).

2.7. Surgical wounds

Incisions were made by one person when the rats were anaesthetized. Rats, in *all* groups, were anaesthetized by a combination of 20 mg/kg Ketamine 10% (Alfasan Co., Netherlands) and 2 mg/kg Xylazine 2% (Alfasan Co., Netherlands), administered intramuscularly. Then, the cases while anaesthetized were positioned prone on a surgical table, their back skin was disinfected with Betadine 10% (*Tolid Daru* Co., Iran), and the hairs of an area of the skin preselected using a caliper were completely shaved with a razor to make a 4 cm incision by means of a No. 24 scalpel; the depth of incision included both dermis and hypodermis, i.e., the thickness was full. Then, 4 stitches at 1 cm intervals were made by means of 3.0 nylon string (*Kamran Teb* Co.,

Table 2
The mean decrease in wound length (cm) during 1st, 2nd, 3rd week and total.

Treatment	1st Week	2nd Week	3rd Week	Total weeks	Result of repeated measures test in total weeks
Vaseline + 0.6% green tea extract (A)	2.07 ± 0.72	1.83 ± 0.69	0.05 ± 0.12	3.95 ± 0.12	<0.001
Vaseline (B)	−1.50 ± 0.35	2.13 ± 0.39	0.30 ± 0.09	3.90 ± 0.08	<0.001
Normal saline (C)	1.12 ± 0.22	2.07 ± 0.21	0.65 ± 0.13	3.85 ± 0.06	0.009
Result of one-way ANOVA test	<0.001	0.001	0.003	0.274	

Tukey test showed statistically significant differences in these cases: between groups A and B in the third week ($P = 0.005$) between groups A and C in the first week ($P < 0.001$), second week ($P = 0.001$) and third week ($P < 0.001$); between Group B and C in the first week ($P = 0.001$), second week ($P = 0.004$) and third week ($P = 0.001$).