Study of Efficacy of Aqueous and Methanolic Extract of Green Tea on the Process of Opened Skin Wounds Healing in Male (NMRI) Mice Race

FAEZEH MOSHREFJAVADI¹, PARISA KADANEJADIAN², MOHAMMAD ALI NILFOROOSHZADE³, PARICHEHR YAGHMAYEI³, and HOMEIRA MARDANI⁵

ABSTRACT

Green tea used for year has a popular cancer preventive activity. Researchers have showed green tea inhibited growth of cancer in the animals. This research has been done with awareness of positives effects of green tea, which is approved by researchers and the importance of treatment of opened skin wound. This work has been done experimentally. There were 56 male mice in 7 different groups. Different dose of water and alcohol such as 50, 150 and 300 µL were injected. After anaesthetizing the mice, skin wound was created on the back of the mice by a 6-mm punch. While the mice in control group were treated by normal saline, water and alcohol extract of green tea was injected around the wound on the back of each mouse. The dimensions of ulcers and the recovery percent of the wound in the 1st, 3rd, 5th, 7th, 10th, 13th and 15th day of study were measured. Furthermore, the needful time for recovery was evaluated. Some histological studies were done as well. Two Specimen of wounds were supplied at 4th, 7th and 15th day of the study. In this way, fibroblasts, inflammation, epithelium and endothelial cell of blood vessels from the wounds were studied. The results show that there are no significant differences among control, water and alcohol groups in recovery processes (p > 0.05.) Evaluation of recovery processes showed there were significant differences among these groups on 7th day of study (p < 0.01). Evaluation of recovery processes showed there were significant differences among three injected doses of study (p < 0.001). The degree of differences in fibroblasts, inflammation and epithelium distortion in different days for 6 groups (p < 0.05) was meaningful. According to these findings, although both water and alcohol extracts of green tea speed up the wound healing, there isn’t any difference between the uses of water or alcohol extracts.

Keywords: Green tea, Wound healing, Water and Alcohol extract, Race NMRI

Wound healing, or wound repair, is an intricate process in which the skin (or another organ-tissue) repairs itself after injury. The classic model of wound healing is divided into three or four sequential, yet overlapping phases: hemostasis (not considered a phase by some authors), inflammatory, proliferative and remodeling. Upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage [1]. Green tea is made from Camellia Sinensis [2]. Leaves of this plant are processed with minimal oxidation. It is mainly used in Asia specifically in China [3-4]. There have been extensive researches on the effects of green tea and results have been surprisingly pleasing. Some of the major potential benefits of green tea include; anti-Cancer properties, increases in metabolic rate, anti-diabetes effect, enhancement of mental alertness, improvement of immune system, improvement of quality of life for HIV-infected...
patients, cardioprotective effects [5-8]. In this study, green tea extracts has been investigated for their effects on the opened skin wound healing.

**MATERIALS AND METHODS**

In this experimental research, 56 male mice of NMRI race with average weight of 25-35 grams were studied. The mice were held in 7 cages in Professor Torabi Nejad Research Center in Isfahan with light cycle of 12 hours darkness and 12 hours light in 22 ± 2°C. In this period, sufficient water and food were in hand of animals and they were randomly classified to control and experimental groups.

Green tea extract was prepared using Soxhlet instrument. The green tea leaves were studied by a 6 millimeter punch and in accordance to surgery principles, a 6-millimeter wound was developed. The wound depth was full skin thickness and the surgery day was named the day zero. After making the wound, in order to prevent potential putrefaction, 0.2 mg gentamicin and 0.2 mg penicillin were injected.

The mice were injected 2% aqueous or alcoholic extract for 7 days, once a day and at 9 am. The amount of 50, 150 or 300 mL of extract were injected in four direction surrounding the wound. All injection were performed by one person. After developing the wound, the mice were classified into 7 groups each 8, as follows:

**Group 1 (control):** the wound surface of this group was treated by normal saline;

**Groups 2, 3 and 4:** the wound surface was treated by 50, 150 and 300 mL of 2% aqueous extract respectively;

**Group 5, 6 and 7:** the wound surface was treated with 50, 150 and 300 mL of 2% alcoholic extract respectively.

For microscopic study, on days 1, 3, 5, 7, 10, 13 and 15, the length measurement method of wound and imaging with digital camera was used for all groups. The development of wounds was assessed and the wound stages according to imaging and size measurement were recorded.

For microscopic evaluation, sampling and tissue study was carried out. On days 4, 7 and 15, the mice were killed by smelling ether in air. Then, two samples were taken from wound tissue and surrounding skin which were placed inside 10% Formalin solution. The tissue processing and molding was done by paraffin and wax and the German microtome with firm blade of LEItz to develop width cuts including skin, bed with the thickness of 4 microns. The cuts were painted by Haematoxylin and Eosin (H&E) coloring methods and recognized through quality method. The wound improving was determined through rating the pathology parameters as follows:

**Rating 1:** The tissues with no repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.
**Table 1.** The microscopic study of aqueous and alcoholic extract of green tea on days 4, 7 and 15 based on the inflammation, fibrosis, epithelium and blood vessels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Control</th>
<th>Aqueous extract</th>
<th>Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 μL</td>
<td>150 μL</td>
<td>300 μL</td>
</tr>
<tr>
<td>Inflammation</td>
<td>4</td>
<td>4.50 ± 0.07</td>
<td>0.01 ± 4.10</td>
<td>0.02 ± 3.50</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.21 ± 0.05</td>
<td>0.2 ± 2.80</td>
<td>0.02 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.81 ± 0.01</td>
<td>0.03 ± 1.50</td>
<td>0.01 ± 1.2</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>4</td>
<td>4.81 ± 0.01</td>
<td>0.02 ± 4.51</td>
<td>0.01 ± 4.20</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.21 ± 0.01</td>
<td>0.001 ± 1.0</td>
<td>0.081 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.31 ± 0.01</td>
<td>2.0 ± 0.02</td>
<td>0.01 ± 1.62</td>
</tr>
<tr>
<td>Epithelium</td>
<td>4</td>
<td>4.80 ± 0.01</td>
<td>0.01 ± 4.11</td>
<td>0.02 ± 3.80</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 ± 0.001</td>
<td>0.02 ± 1.42</td>
<td>0.01 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.0 ± 1.13</td>
<td>1.10 ± 4.92</td>
<td>1.0 ± 4.90</td>
</tr>
<tr>
<td>Blood Vascular</td>
<td>7</td>
<td>4.5 ± 1.10</td>
<td>1.12 ± 4.25</td>
<td>1.12 ± 4.23</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.01 ± 3.5</td>
<td>0.01 ± 3.25</td>
<td>1.10 ± 3.0</td>
</tr>
</tbody>
</table>

**Rating 2:** The tissues with repeating epithelisation, treatment group on the days 1, 3, 5, 7, 10, 13, and 15 low quantity fibrotic tissue, low number of vessels and has been illustrated in Fig 1. There is a meaningful extreme edema difference between groups (p < 0.001).

**Rating 3:** The tissues with epithelisation and alcoholic extract of green tea was 3.81 ± 1.74 mm, in animals [9]. Generally, The fibroblast in small limit and also low number of vessels and epithelium amount in mice received aqueous or low edema.

**Rating 4:** The tissues with no edema and the medium number of epithelisation and fibroblast significantly different in groups received aqueous or alcoholic extract did not have a meaningful difference.

**Rating 5:** The tissues with complete epithelisation, alcoholic extracts when compared with control group (p complete fibrotic tissue development, high number of alcoholic extract when compared with control group (p not significantly different in groups received aqueous or by SPSS statistical software. The p values < 0.05 were (Table 1).

**DISCUSSION**

The average wound diameter in control group was studies in the effects of green tea on skin. The primary 4.42 ± 1.66 mm, in the group which received the focuses of these studies are the chemical carcinogens or alcoholic extract of green tea was 3.81 ± 1.74 mm, and photo carcinogens in animals [9]. Generally, The in the group which received aqueous extract of green polyphenols which are present in teas are categorized as tea, it was 3.93 ± 1.69 mm. No meaningful difference catechins. Green tea leaves contain six primary catechins between 3 groups was observed (not significant). The compounds: catechin, gallaocatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as

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In this study, there is not a meaningful difference in the edema stage indicator is constructed and the width link is important for two have role in proliferation of Young Keratinocytes is exclusive fibroblasts. The fibroblasts then synthesize the collagen of green Collagens 13,23 anti-ratification property and have role in prevention of wound which is the beginner of antioxidant theory [11]. EGCG is the preventer of tumor growth in chest, word, the earlier start of revival phase of collagen lung, liver, sweetbread, stomach, pancreas, skin, cyst, 40 synthesis take place in this stage and collagen groups and prostate [11]. EGCG is the preventer of secretion of 41 with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-6 between molecules also change [18]. The collagen yarn 6-phosphate dehydrogenase in liver [11-12]. EGCG causes the wound after healing to look like the tissue in treatment group, the wound surface is reducing in 2%, the excess of edema is wound insistence because of increase in collagen in treatment group is meaningfully less that of control 53 content and because fibroblasts are responsible for group (p < 0.001). This shows that the green tea makes developing collagen. So we can conclude that green tea 14 during granulation, fibronectin develops a proper EGCG and the properties of antibacterial and antivirus 20 substrate for immigration and growth of cells and 76 of green tea in order to fasten the healing of wound therefore links with fibroblasts so that wound 77 [20]. EGCG causes the propagation, division, and contraction force [14]. Location. In this relation, we can point out to study of During granulation, fibronectin develops a proper [75] EGCG and the properties of antibacterial and antivirus 21 2. The fibroblasts then synthesize the collagens that 70 healing process of wound through general and localized 26 development tension power in wound substrate [15]. 271 different factors is under influence [19]. Many different 21 3. Miofibroblasts that are exclusive fibroblasts [72]. Neuron and hormonal like cell and vein factors or participate in wound shrinkage through providing motion and secretory activities influence the wound 24 contraction force [14]. Location. In this relation, we can point out to study of 25 225 Regarding the above-mentioned results, it was indicated 880 increases the Keratinocytes survival and influences on 227 that the green tea extract has improved the wound 81 the propagation and fixing of fibroblasts [20]. The 222 treatment at seventh day that these influences are preventing effect of green tea is related to its anti- 223 observed in reduction of wound surface and increase of oxidant power. Polyphenols and glycoprotein play the healing percent and also in reduction of required time. 849 role of scavenger in special conditions and thus it 225 for complete healing. Reduction in edema resulted in implementing its preventing effects on bacteria and virus speeding the wound stage. In 2004, Bayer and colleagues growth. In this regard, preventing effect of green tea 227 show that polyphenols prevent the discharge of gamma-874 (Camellia Sinensis) and black tea on the bacteria growth interferon and have anti edema, anti oldness and wound 886 has been shown [21]. It is possible that green tea
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improve the healing speed of wound. It has been reported that antibiotic medicine speeds the healing of wounds will lose their integrity and will open. Because the wound by infection control [21]. But in this study the amount of collagen synthesis will exceed the exterior symptoms of infections are not observed in reconstruction of it [29]. In other hand, vitamin C is control group. Therefore, it seems to be actions other required for construction of veins, immigration of that preventing the wound infection for green tea macrophages and correct function of neutrophils [30].

fastening the wound improvement. Bayat et al. explain Some studies show that green tea is a rich resource of the ultrasound treatment effect and gel on healing the vitamin C and includes 18 amino acids including lysine wound section and they believe that wet wound is the 357 and proline [9,12,20]. Lack of vitamin B, (pyridoxine) speeding factor of wound healing process. In current damages this phenomena link process. Lack of vitamin study, the wounds were daily dented by the alcoholic B, (riboflavin) disorders the wound healing process and aqueous extract. [29]. In other hand, B group vitamins are cofactors for The experimental studies on animals show that the enzyme reactions and are required for correct function localized usages of epidermal growth factors have an influence on epidermal healing [36]. Results have shown that green tea includes vitamins B, wounds with relative thickness and burnings. The usage and B, [9,12,20]. Therefore probably we can of this material on human wounds also has similar conclude that mentioned issue is one of the factors effects and its usefulness has been proved [22]. The speeding the healing process in treatment group.

epidermal healing is a complex phenomena from which. It seems that one of the functions of green tea that the rest epidermal cells are propagated so there will helps the healing of wound is the positive effect of another healthy epidermis. The molecular actions that polyphenols, Catechin, Glycoproteins, EGCG and set the natural epidermal healing are not completely vitamins. The increased speed of healing has many known, but it seems that the peptide growth factors that regarding the economic and hygiene. Higher the exhibit through autocrin or paracrin mechanisms have speed of wound healing, the less the wound infection important role on them [23-25]. In 2003, Chung et al. and an increased speed in all the process of wound showed that the green tea extract (EGCG) cause the healing. In all of current study for the first time it was epidemic creationists survival in human. In 2003, showed that green tea extract can speed the wound Bollag et al. proposed cellular propagation and healing the healing process of male mice NMRI skin. of wound through polyphenols of green tea. Many numbers of growth factors are known including the epidermal growth (EGF). This factor is a polypeptide of 53 amino acids that DNA and protein is activated by the mRNA [25]. It has been shown that the peptide growth factors increase significant proliferation of cells in wounds with relative wounds and also increase traction influence on Mesenchyme cells [26]. In fact, the growth factors of exterior epidermal will increase other production of growth factors like transforming growth factor which is revealed from plaquettes and macrophages, indirectly activates the healing and improving the wound [27]. Without considering the structure, immediate facing of cells during healing with growth factors of epidermal, increases the epithelial [28]. Kwon et al. stated that EGCG motivates the growth of human hair through proliferation and has Anti-apoptosis effects on DPCs [29]. The histology of wound showed that proliferation of cells increase that is probably because of chemical combination of green tea and epidermal growth factors. [29].

In addition, role of vitamins on wound healing process and the relationship of green tea contents with them can be considered. Lack of vitamin C is important in delay of wound healing. In such patients, wound healing in fibroblasts stage is stopped. In this state, even when the number of fibroblasts is natural, they do not produce sufficient collagen. Vitamin C is required for ion link of (OH) with amino acid of proline and lysine and hydroxyl of them inside fibroblast cell. Without 10 hydroxy-l-sine, fibrils of collagens will not obtain width links. In extreme Scurvy, not only the new...


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