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 YAGHMAYEI2, and HOMEIRA MARDANI1

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 effect 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 Isfahan University and were transferred into laboratory. Then using electric mill, they were grinded to a powder. Forty grams of green tea powder was placed into filtration paper and were transmitted to a specific container. In order to produce water extract, 400 milliliters of purified water was added and in order to produce alcoholic extract, 400 milliliters of 85% methanol was added. After producing the extract by Soxhlet, it was dried and concentrated in rotary evaporator and then in 48-hour incubation in 70°C. In next stage, 2 g of each extract (alcoholic or aqueous) was solved in 100 mL normal saline and Evaporation was achieved.

In order to make a wound in animal, first the mouse became comatose with ether and then its back hair was shaved. After immersing the skin with betiding, with 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 penicillin and 0.2 mg gentamicin 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 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 the evaluated parameters as follows:

Rating 1: The tissues with no repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

Fig 1. The macroscopic study of wound diameter average between control and treatment group on days 1, 3, 5, 7, 10, 13 and 15. (p < 0.001)
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></td>
<td>50 µL</td>
<td>150 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>5.0 ± 1.13</td>
<td>1.10 ± 4.92</td>
<td>1.0 ± 4.90</td>
</tr>
<tr>
<td></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>

**RESULTS**

There are 150 reports from in vitro and in vivo studies in the effects of green tea on skin. The primary 4.42 ± 1.66 mm, in the group which received the 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 between 3 groups was observed (not significant). The compounds: catechin, epigallocatechin, and gallic acid, as well as the average of wound diameter among control and epicatechin gallate, as well as...
The fibroblasts then synthesize the catechin using green tea alcoholic treatment. They have a role in proliferation and contraction force of tissue [11]. EGCG is the primary combination of green tea, 35 day on, is the propagation stage [17]. On seventh day, in polyphenolicit that has properties like antioxidant, anti-ratification property and have role in prevention of tea which is the beginner of antioxidant theory [11]. EGCG is the preventer of collagen growth in chest, word, the earlier start of revival phase of collagen lung, liver, sweetbread, stomach, pancreas, skin, cyst, synthesis take place in this stage and collagen groups and prostate [11]. EGCG is the preventer of secretion of collagen with more diameter are constructed and the width chymotrypsin, tumor necrosis factor alpha and glucose-6-phosphate dehydrogenase in liver [11-12]. causes the wound after healing to look like the tissue.

In this study, there is not a meaningful difference before wounding and prevents the white and ugly scar. In addition, increasing blood and oxygen availability to the studied groups. This finding is important for the wound location takes place through widening the veins reasons. Firstly, using green tea extract doesn’t have any relationship with aqueous or alcoholic treatment [18]. Secondly, in this study, the effect of aqueous and alcohol reduction, heart bit and also vein widening [11,20]. This alcoholic variables is excluded. In the current study, on the practical capacity of fibroblasts, fourth day, as the edema stage indicator is considered: synthesis increase in collagen fibers and increase in the wound treatment process [13], the excess of edema wound insistence because of increase in collagen in treatment group is meaningfully less that of control 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 in the edema stage of treatment process faster and (polypheinol, catechin and EGCG) cause the propagation therefore the wounds heal faster. In addition, injecting (+) EGCG and influence the practical capacity of the 2% extract of green tea into mice wound caused 67 fibroblasts and increase the synthesis of fibro Collagen meaningful increases in fibrous tissue and reduction in edema [20]. The higher the injection dose (300 mL), the higher the edema in seventh day of study in comparison to these, the meaningful number of fibroblasts [9]. The research control group. This meaningful increase of treatment of Madam et al. show that catechin polyphenol and group fibrous in considering their role in following: EGCG prevent the collagenase activity against issues are important and indicate the positive effect of Collagens [18]. In fact, Catkin and EGCG prevent the green tea on distribution phase of wound treatment action through linking with hydrogen and reaction with process. hydrophobic with collagens prevent its activity and play 1. Fibroblasts are responsible for synthesis of the fibro collagen registration [18]. Research of Young matrix components of primary outer cell of wound bed: et al. also shows the prevention of collagen destruction 2. including fibroblast and proteoglycans that provide a6 collagen and collagenase activity through setting reactions of proper substrate for immigration and propagation of collagen by EGCG [19].

The broad studies during past decades show that the healing process of wound: 1. The fibroblasts then synthesize the collagens that healing process of wound through general and localized 2. develop tension power in wound substrate [15]. different factors is under influence [19]. Many different 3. Miofibroblasts that are exclusive fibroblasts: Neuron and hormonal like cell and vein factors or participate in wound shrinkage through providing motion and secretary activities influence the wound contraction force [14]. location. In this relation, we can point out to study of During granulation, fibroblast develops a proper: EGCG and the properties of antibacterial and antivirus substrate for immigration and growth of cells and of green tea in order to fasten the healing of wound therefore links with miofibroblasts so that wound develops [20]. EGCG causes the propagation, division, and contraction is developed influentially. In addition, this motivation of natural cells growth and does this through fibroblast is a support for fibriligenesis [16]. cell division and anti apoptosis division. Also, it Regarding the above-mentioned results, it was indicated: increases the Keratinocytes survival and influences on 1. that the green tea extract has improved the wound: the propagation and fixing of fibroblasts [20]. The treatment at seventh day that these influences are: preventing effect of green tea is related to its anti-oxidant activity in reduced wound surface and increase of: oxidant power. Polyphenols and glycoprotein play the healing percent and also in reduction of required time: role of scavenger in special conditions and thus it is for complete healing. Reduction in edema resulted in: implements 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 show that polyphenols prevent the discharge of gamma-(Camellia Sinensis) and black tea on the bacteria growth interferon and have anti edema, anti oldness and wound has been shown [21]. It is possible that green tea
Green tea effects on wounds healing

To improve the healing speed of wound. It has been reported that antibiotic medicine speeds the healing of wound by infection control [21]. But in this study the amount of collagen synthesis will exceed the wound with or without infection. The healing speed of wound is a complex phenomenon from which it can be seen that the speed of wound healing is improved by the presence of antibacterial activity. Some studies have shown that green tea polyphenols influence on the speed of wound healing. In this study, it was found that polyphenols of green tea enhance the speed of wound healing in a dose-dependent manner. However, in this study, the speed of wound healing was not significantly increased by the presence of green tea polyphenols. Therefore, it is concluded that the speed of wound healing is not significantly increased by the presence of green tea polyphenols. However, it is suggested that the speed of wound healing can be improved by the presence of antibacterial activity. It has been shown that the presence of antibacterial activity enhances the speed of wound healing. Therefore, it is suggested that the speed of wound healing can be improved by the presence of antibacterial activity.


Kwon OS, Han JH, Yoo HG, Chung JH, Eun HC, Kim KH. Human hair growth enhancement in vitro by green tea epigallocatechin-3-gallate (EGCG). *Phytomedicine* 2007; 14:551-5. 482


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