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 YAGHMAEI4, 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 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

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.

For microscopic evaluation, sampling and tissue processing and molding was done by paraffin. After producing 2 cm thick slices, they were stained by Hematoxylin and Eosin (H&E) coloring method and microscopically examined with 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.

**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)

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.

Green tea extract was prepared using Soxhlet instrument. The green tea leaves were studied by Professor 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 Bowl. In next stage, 2 g of each extract (alcoholic or aqueous) was solved in 100 mL normal saline and therefore, 2% aqueous or alcoholic extract 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 macroscopic study of wound diameter average between control and treatment group on days 1, 3, 5, 7, 10, 13 and 15. (p < 0.001)
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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 50 µL</th>
<th>150 µL</th>
<th>300 µL</th>
<th>Alcoholic extract 50 µL</th>
<th>150 µL</th>
<th>300 µL</th>
</tr>
</thead>
<tbody>
<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>
<td>0.05 ± 3.52</td>
<td>0.02 ± 4.0</td>
<td>0.01 ± 3.70</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>
<td>0.09 ± 2.10</td>
<td>0.01 ± 2.70</td>
<td>0.01 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.81 ± 0.01</td>
<td>0.03 ± 1.50</td>
<td>0.01 ± 1.20</td>
<td>0.001 ± 0.09</td>
<td>0.01 ± 1.40</td>
<td>1.0 ± 0.01</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>
<td>0.05 ± 3.91</td>
<td>0.02 ± 4.52</td>
<td>0.01 ± 4.52</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.21 ± 0.01</td>
<td>0.001 ± 1.00</td>
<td>0.081 ± 0.02</td>
<td>0.01 ± 0.06</td>
<td>0.90 ± 0.06</td>
<td>0.001 ± 0.70</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>
<td>1.21 ± 0.02</td>
<td>0.05 ± 2.11</td>
<td>0.05 ± 0.70</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>
<td>1.0 ± 4.89</td>
<td>1.2 ± 4.93</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>
<td>0.01 ± 4.210</td>
<td>1.12 ± 4.25</td>
<td>1.10 ± 4.21</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>
<td>1.10 ± 3.01</td>
<td>0.01 ± 3.28</td>
<td>0.01 ± 3.01</td>
</tr>
</tbody>
</table>

Rating 2: The tissues with repeating epithelisation,147 treatment group on the days 1, 3, 5, 7, 10, 13, and 15 low quantity fibrotic tissue, low number of vessels and148 has been illustrated in Fig 1. There is a meaningful149 extreme edema difference between groups (p < 0.001).

Rating 3: The tissues with epithelisation and150 The microscopic results show that edema, fibroblast fibroblast in small limit and also low number of vessels151 and epithelium amount in mice received aqueous or and low edema.152 alcoholic extract did not have a meaningful difference.

Rating 4: The tissues with no edema and the153 The edema, fibroblast and epithelium amount were medium number of epithelisation and fibroblast154 significantly different in groups received aqueous or and low edema.

Rating 5: The tissues with complete epithelisation,155 alcoholic extracts when compared with control group (p complete fibrotic tissue development, high number of156 < 0.001). In contrast, the blood vascular amount were vessels and no edema.

All the data were analyzed using one-way ANOVA158 alcoholic extracts when compared with control group by SPSS statistical software. The p values < 0.05 were159 (Table 1).

RESULTS

There are 150 reports from in vitro and in vivo162 studies in the effects of green tea on skin. The primary163 4.42 ± 1.66 mm, in the group which received the164 focuses of these studies are the chemical carcinogens or alcoholic extract of green tea was 3.81 ± 1.74 mm, and166 photo carcinogens in animals [9]. Generally, The167 in the group which received aqueous extract of green168 polyphenols which are present in teas are categorized as tea, it was 3.93 ± 1.69 mm. No meaningful difference169 catechins. Green tea leaves contain six primary catechin between 3 groups was observed (not significant). The170 compounds: catechin, gallocatechin, epicatechin,171 average of wound diameter among control and172 epigallocatechin, epicatechin gallate, as well as

DISCUSSION

Published online: January 31, 2013
apigallocatechin gallate (also referred to as EGCG), healing [11]. The other researchers showed that polyphenols cause the infusion, contrast and anti-tumor, anti-edema, anti-virus, anti-rationation, anti-propagation in epidermis Keratinocytes [9]. Catkins are oldness, and lowering the blood sugar [7-10]. Chemical also from polyphenol group that have anti-oxidant and structure of these molecules is the polyphenol of green EGCG anti-rationation property and have role in prevention of tea 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, synthesis place take in this stage and collagen groups and prostate [11]. EGCG is the preventer of secretion of with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-6-d 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 this study, there is not a meaningful difference before wounding and prevents the white and ugly scar. between the alcoholic and aqueous extract of green tea. In addition, increasing blood and oxygen availability to polyphenolic that has properties like antioxidant, anti-transfer treatment group, the wound surface is reducing in tumor, and anti-mutagenic [9]. The biological and contrast with control group that this shows the epidemiological studies in the past 10 years show that reconstruction stage commencement [14] or in other EGCG can be the preventer of tumor growth in chest, word, the earlier start of revival phase of collagen lung, liver, sweetbread, stomach, pancreas, skin, cyst, synthesis place take in this stage and collagen groups and prostate [11]. EGCG is the preventer of secretion of with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-6-d between molecules also change [18]. The collagen yarn in treatment group is meaningfully less that of control. EGCG 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 the edema stage of treatment process faster and polyphenol, catechin and EGCG cause the propagation therefore the wounds heal faster. In addition, injecting polyphenols and influence the practical capacity of the 2% extract of green tea into mice wound caused fibroblasts and increase the synthesis of fibro Collagen meaningful increases in fibrous tissue and reduction in [16] [20]. The higher the injection dose (300 mL), the higher the edema in seventh day of study in comparison to the 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 EGCG in collagens registration [18]. Research of Young matrix components of primary outer cell of wound bed. Also, et al. also shows the prevention of collagen destruction including fibronectin and proteoglycans that provide a and collagenase activity through setting reactions of proper substrate for immigration and propagation of EGCG [19]. cells [14]. The broad studies during past decades show that the 2. The fibroblasts then synthesize the collagens that healing process of wound through general and localized 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. During granulation, fibronectin develops a proper EGCG and the properties of antibacterial and antivirus substance for immigration and growth of cells and green tea in order to fasten the healing of wound therefore links with miofibroblasts so that wound EGCG causes the propagation, division, and contraction is developed influentially. In addition, this motivation of natural cells growth and does this through fibronectin is a support for fibrillogenesis [16]. cell division and anti apoptosis division. Also, it Regarding the above-mentioned results, it was indicated that green tea extract has improved the wound 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-oxidation in reduction of wound surface and increase of oxidant power. Polyphenols and glycoprotein play the role of scavenger in special conditions and thus it 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
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