ORIGINAL ARTICLE

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 YAGHMAYEI4, 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

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

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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. Eleven mice were killed by smelling ether in air. Then, two samples of 50, 150 and 300 mL of 2% aqueous extract were injected into each group and two other samples were injected with 50, 150 and 300 mL of 2% alcoholic extract. 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 gentamicin and 0.2 mg penicillin were injected.

### Rating 1:

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

### Rating 2:

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

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

The development of wounds was assessed and the wound stages according to imaging digital camera and size measurement were recorded.

For microscopic evaluation, sampling and tissue study was carried out. On days 4, 7 and 15, 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;
- **Groups 5, 6 and 7:** the wound surface was treated with 50, 150 and 300 mL of 2% alcoholic extract respectively.

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**MATERIALS AND METHODS**

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**Rating 1:** The tissues with no repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.
Green tea effects on wounds healing

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 Aqueous extract</th>
<th>Control Alcoholic extract</th>
<th>Groups Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 µL. 150 µL. 300 µL.</td>
<td>50 µL. 150 µL. 300 µL.</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>4</td>
<td>4.50 ± 0.07 0.01 ± 4.10</td>
<td>0.02 ± 3.50 0.05 ± 3.52</td>
<td>0.02 ± 4.0 0.01 ± 3.70 0.001 ± 3.11</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.21 ± 0.05 0.2 ± 2.80</td>
<td>0.02 ± 2.50 0.09 ± 2.10</td>
<td>0.01 ± 2.70 0.01 ± 2.30 0.03 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.81 ± 0.01 0.03 ± 1.50</td>
<td>0.01 ± 1.2 0.001 ± 0.09</td>
<td>0.01 ± 1.40 1.0 ± 0.01 0.001 ± 0.07</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>4</td>
<td>4.81 ± 0.01 0.02 ± 4.51</td>
<td>0.01 ± 4.20 0.05 ± 3.91</td>
<td>0.02 ± 4.52 0.01 ± 4.52 0.0 ± 3.70</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.21 ± 0.01 0.001 ± 1.0</td>
<td>0.081 ± 0.02 0.01 ± 0.06</td>
<td>0.001 ± 0.06 0.0001 ± 0.07 0.50 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.31 ± 0.01 2.0 ± 0.02</td>
<td>0.01 ± 1.62 1.21 ± 0.02</td>
<td>0.05 ± 2.11 0.05 ± 0.70 0.001 ± 1.25</td>
</tr>
<tr>
<td>Epithelium</td>
<td>4</td>
<td>4.80 ± 0.01 0.01 ± 4.11</td>
<td>0.02 ± 3.80 0.05 ± 2.52</td>
<td>4.0 ± 0.01 3.20 ± 0.02 0.04 ± 2.32</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 ± 0.001 0.02 ± 1.42</td>
<td>0.01 ± 1.0 0.04 ± 0.51</td>
<td>0.02 ± 1.50 0.05 ± 1.0 0.03 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.01 ± 3.5 0.01 ± 3.25</td>
<td>1.10 ± 3.0 1.10 ± 3.01</td>
<td>0.01 ± 3.28 0.01 ± 3.01 1.10 ± 3.0</td>
</tr>
</tbody>
</table>

| Blood Vascular  | 4    | 5.0 ± 1.13 1.10 ± 4.92| 1.0 ± 4.90 1.0 ± 4.89   | 1.2 ± 4.93 1.0 ± 4.90 0.01 ± 4.88   |
|                 | 7    | 4.5 ± 1.10 1.12 ± 4.25| 1.12 ± 4.23 0.01 ± 4.210 | 1.12 ± 4.25 1.10 ± 4.21 1.02 ± 4.22 |
|                 | 15   | 0.01 ± 3.5 0.01 ± 3.25| 1.10 ± 3.0 1.10 ± 3.01   | 0.01 ± 3.28 0.01 ± 3.01 1.10 ± 3.0    |

125 **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 vessels148 has been illustrated in Fig 1. There is a meaningful extreme edema149 difference between groups (p < 0.001).

126 **Rating 3:** The tissues with epithelisation and150 The microscopic results show that edema, fibroblast127 fibroblast in small limit and also low number of vessels151 and epithelium amount in mice received aqueous or128 low edema.152 alcoholic extract did not have a meaningful difference.

129 **Rating 4:** The tissues with no edema and the153 The edema, fibroblast and epithelium amount were130 medium number of epithelisation and fibroblast154 significantly different in groups received aqueous or131 **Rating 5:** The tissues with complete epithelisation,155 alcoholic extracts when compared with control group (p132 complete fibrotic tissue development, high number of156 < 0.001). In contrast, the blood vascular amount were133 vessels and no edema.157 not significantly different in groups received aqueous or134 All the data were analyzed using one-way ANOVA158 alcoholic extracts when compared with control group135 by SPSS statistical software. The p values < 0.05 were159 (Table 1).

136 considered significant.

160 **RESULTS**

161 There are 150 reports from in vitro and in vivo 162 The average wound diameter in control group was163 studies in the effects of green tea on skin. The primary 164 4.42 ± 1.66 mm, in the group which received the165 focuses of these studies are the chemical carcinogens or 166 alcoholic extract of green tea was 3.81 ± 1.74 mm, and167 photo carcinogens in animals [9]. Generally, The 168 in the group which received aqueous extract of green169 polyphenols which are present in teas are categorized as 163 tea, it was 3.93 ± 1.69 mm. No meaningful difference166 catechins. Green tea leaves contain six primary catechin 168 average of wound diameter among control and169 between 3 groups was observed (not significant). The167 compounds: catechin, gallaogatechin, epicatechin, 169 epigallocatechin, epicatechin gallate, as well as

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apigallocatechin gallate (also referred to as EGCG). Healing [11]. The other researchers showed that glicoproteins have different biological activities like: 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]. Chemicals also from polyphenol group that have anti-oxidant and anti-cancer 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 take place in this stage and collagen groups and prostate [11]. EGCG is the preventer of secretion of fibroblast with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-6phosphate between molecules also change [18]. The collagen yarn 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. between the alcoholic and aqueous extract of green tea [5]. In addition, increasing blood and oxygen availability to studied groups. This finding is important for two wound location takes place through widening the veins reasons. Firstly, using green tea extract doesn’t have [19]. Researchers show that green tea reduces blood any relationship with aqueous or alcoholic treatment [14]. sugar, blood lipids, blood pressure, heart disease Secondly, in this study, the effect of aqueous and fourth day, as the edema stage indicator is consider as: 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 the edema stage of treatment process faster and (polyphenol, catechin and EGCG) cause the propagation therefore the wounds heal faster. In addition, injecting fibroblasts and influence the practical capacity of the 2% extract of green tea into mice wound caused fibroblasts and increase the synthesis of fibroblast Collagen meaningful increases in fibrous tissue and reduction in [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 Madham 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. 1. Fibroblasts are responsible for synthesis of the a role in collagens registration [18]. Research of Young matrix components of primary outer cell of wound bed: 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 cellular signal by EGCG [19]. 2. The fibroblasts then synthesize the collagens that 3. Miofibroblasts that are exclusive fibroblasts. Neuron and hormone like cell and vein factors or participate in wound shrinkage through providing motion and secretary activities influence the wound contraction force. In the relation, we can point out to study of During granulation, fibronectin develops a proper: EGCG and the properties of antibacterial and antivirus 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 [20]. 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 fibrilogenesis [16]. Cell division and anti apoptosis division. Also, it Regarding the above-mentioned results, it was indicated increases the Keratinocytes survival and influences on that the green tea extract has improved the wound: The treatment at seventh day that these influences are: preventing effect of green tea is related to its anti- 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: 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 colleges: 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
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 the reconstruction of it [29]. In other hand, vitamin C is required for construction of veins, immigration of preventing the wound infection for 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 glyoxylate. Lack of vitamin B, (pyridoxine) speeding factor of wound healing process. In current damages this phenomenal link process. Lack of vitamin study, the wounds were daily wetted by the alcohol. Vitamin B, (riboflavin) disorders the wound healing process and aqueous extract. [29]. In other hand, B group vitamins are cofactors for 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 be 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 effect regarding the economic and hygiene. Higher the act through autocrin or paracrin mechanisms have sped of wound healing, the less the wound infection important role on them [23-25]. In 2003, Chung et al. showed that the green tea extract (EGCG) cause the healing. In all of current study for the first time it was showed that green tea extract can speed the wound healing process of male mice NMRI skin.

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