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

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ABSTRACT

Green tea used for year has a popular cancer preventive activity. Researchers have showed green tea inhibited growth of cancer in the animal. 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...
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.

For macroscopic 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 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 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 tissue sections were recognized through quality method. The wound healing was evaluated by microscopic analysis of tissue sections and the pathological parameters as follows:

**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</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>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>0.001 ± 3.5</td>
<td>0.01 ± 3.25</td>
<td>1.10 ± 3.0</td>
</tr>
<tr>
<td>Blood Vascular</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></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 the microscopic results show that edema, fibroblast
fibroblast in small limit and also low number of vessels, epithelium amount in mice received aqueous or
and low edema.
Alcoholic extract did not have a meaningful difference.
Rating 4: The tissues with no edema and the middle number of epithelisation and fibroblast are significantly different in groups received aqueous or
fibroblast and epithelium amount were vascular amount were not significantly different in groups received aqueous or
Blood Vascular vessels and no edema.

All the data were analyzed using one-way ANOVA and the controls were by SPSS statistical software. The p values < 0.05 were considered significant.

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 focuses of these studies are the chemical carcinogens or
alcoholic extract of green tea was 3.81 ± 1.74 mm, and 64 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, galloatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as

DISCUSSION

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Apigalocatechin 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-ratification, anti-sensitivity propagation in epidermis Keratinocytes [9]. Catskins are oldness, and lowering the blood sugar [7-10]. Chemicals also from polyphenol group that have anti-oxidant and structure of these molecules is the polyphenol of green [11] anti-ratification property and have role in prevention of tea which is the beginner of antioxidant theory [11]. EGCG is the primary combination of green tea. After 35 day on, is the propagation stage [17]. On seventh day, in polyphenolic that has properties like antioxidant, anti-tumor 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 stage 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 with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-62 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. In addition, increasing blood and oxygen availability to in 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]. Researches show that green tea reduces blood any relationship with aqueous or alcoholic treatment. Sugar, blood lipids, blood pressure, heart disease. Secondly, in this study, the effect of aqueous and reduction, heart bit and also vein widening [11,20]. This alcoholic variables is excluded. In the current study, on influences 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 (ρ < 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 is 56 of fibroblasts and influence the practical capacity of the 2% extract of green tea into mice wound caused 57 fibroblasts and increase the synthesis of fibro 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 [60] 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. Hydrophobic with collagens prevent its activity and play 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: [66] et al. also shows the prevention of collagen destruction 207 including fibrocinet and proteoglicans that provide a67 and collagenase activity through setting reactions of proper substrate for immigration and propagation of 68 cellular signal by EGCG [19].

cells [14]. The broad studies during past decades show that the 209 2. The fibroblasts then synthesize the collagens that healing process of wound through general and localized 210 develop tension power in wound substrate [15]. Different factors is under influence [19]. Many different 211 3. Miofibroblasts that are exclusive fibroblasts: Neuron and hormonic like cell and vein factors or participate in wound shrinkage through providing motion and secretary activities influence the wound 212 contraction force [14]. Location. In this relation, we can point out to study of 213 During granulation, fibrocinet develops a proper EGCG and the properties of antibacterial and antivirus 214 substrate for immigration and growth of cells and 215 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 216 contraction is developed influentially. In addition, this 217 motivation of natural cells growth and does this through fibrocinet is a support for fibriligenesis [16]. Cell division and anti apoptosis division. Also, it 218 Regarding the above-mentioned results, it was indicated: increases the Keratinocytes survival and influences on 219 that the green tea extract has improved the wound 220 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- 221 observed in reduction of wound surface and increase of oxidant power. Polyphenols and glycoprotein play the 222 healing percent and also in reduction of required time role of scavenger in special conditions and thus it 223 for complete healing. Reduction in edema resulted in implements its preventing effects on bacteria and virus 224 speeding the wound stage. In 2004, Bayer and colleges 225 growth. In this regard, preventing effect of green tea 226 show that polyphenols prevent the discharge of gamma-87 (Camellia Sinensis) and black tea on the bacteria growth 227 interferon and have anti edema, anti oldness and wound 228 has been shown [21]. It is possible that green tea
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