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 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...
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°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 Borrnarry. 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 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 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 endometrial section, fibroblasts and sweating sections were 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.
### Results

The average wound diameter in control group was 162 mm studies in the effects of green tea on skin. The primary 144 mm, in the group which received the polyphenols focuses of these studies are the chemical carcinogens or 146 mm, and 148 mm photo carcinogens in animals [9]. Generally, The 149 mm, No meaningful difference 150 mm between 3 groups was observed (not significant). The compounds: catechin, gallaogatechin, epicatechin, 152 mm, as well as

### Discussion

154 mm, and alcoholic extract did not have a meaningful difference. 155 mm, The edema, fibroblast and epithelium amount were 156 mm, and low edema. 157 mm, and 158 mm alcoholic extracts when compared with control group (p 159 mm, by SPSS statistical software. The p values < 0.05 were (Table 1).

### 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>0.01 ± 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 The microscopic results show that edema, fibroblast fibroblast in small limit and also low number of vessels and epithelium amount in mice received aqueous or low edema. alcoholic extract did not have a meaningful difference. Rating 4: The tissues with no edema and the The edema, fibroblast and epithelium amount were medium number of epithelisation and fibroblast significantly different in groups received aqueous or Rating 5: The tissues with complete epithelisation, alcoholic extracts when compared with control group (p complete fibrotic tissue development, high number of < 0.001). In contrast, the blood vascular amount were not significantly different in groups received aqueous or All the data were analyzed using one-way ANOVA alcoholic extracts when compared with control group by SPSS statistical software. The p values < 0.05 were (Table 1). considered significant.

There are 150 reports from in vitro and in vivo

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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-ratification, anti-angiogenesis propagation in epidermis Keratinocytes [9]. Catkins are
oldness, and lowering the blood pressure [7-10]. Chemical 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
polyphenol which is the beginner of antioxidant theory [11]. The bleeding and reducing thrombosis [9]. From seventh
EGCG is the primary combination of green tea day on, is the propagation stage [17]. On seventh day, in
polyphenolic that has properties like antioxidant, anti-cell 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, syntheses 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 woundning 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 controls. content and because fibroblasts are responsible for
49 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 of fibroblasts 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 [20]. The higher the injection dose (300 mL), the higher
the edema in seventh day of study in comparison to the meaningful number of fibroblasts [9]. The research
control group. This meaningful increase of treatment [20]. of Madham et al. show that catechin polyphenol and
fibroblasts in considering their role in following: EGCG prevent the collagenase activity against
issues are important and indicate the positive effect of EGCG on 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. 264. hydrophobic with collagens prevent its activity and play
1. Fibroblasts are responsible for synthesis of the in collagens registration [18]. Research of Young
matrix components of primary outer cell of wound bed: also shows the prevention of collagen destruction
207 208 including fibrinogen and proteoglicans that provide a and collagenase activity through setting reactions of
proper substrate for immigration and propagation of cellular signal by EGCG [19].
14 cells [14].
19 The broad studies during past decades show that the
210 2. The fibroblasts then synthesize the collagens that healing process of wound through general and localized
development tension power in wound substrate [15].
211 274. different factors is under influence [19]. Many different
212 3. Miofibroblasts that are exclusive fibroblasts: Neuron and hormonic like cell and vein factors or
participate in wound shrinkage trought providing: motion and secretary activities influence the wound
contraction force [14].
213 During granulation, fibrinogen develops a proper: EGCG and the properties of antibacterial and antivirus
substrate 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, diision, and
contraction is developed influentially. In addition, this motivation of natural cells growth and does this through
fibrinogen is a support for fibrillogenesis [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 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-
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
Green tea effects on wounds healing


