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

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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

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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 °C. In this period, sufficient water and food were in hand of animals and they were randomly classified into control and experimental groups. The green tea leaves were studied by Isfahan University and were transferred into laboratory. After using electric mill, they were grinded to a powder. Forti 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 Bormar. 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 betioding, 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 edematous cell, 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.
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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</th>
<th>Aqueous extract</th>
<th>Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 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 and 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 vessels and no edema.

**Rating 5:** not significantly different in groups received aqueous or.

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

## RESULTS

The average wound diameter in control group was 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 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, gallaogatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as

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Polyphenols cause the infusion, contrast and anti-tumor, anti-edema, anti-virus, anti-ratification, 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 33 anti-ratification property and have role in prevention of
tea which is the beginner of antioxidant theory [11].
From seventh
EGCG is the primary combination of green tea. 35 day on, is the propagation stage [17]. On seventh day, in
eighty polyphenolic that has properties like antioxidant, anti-
36 treatment group, the wound surface is reducing in
tumor, and anti-mutagenic [9]. The biological and contrast with control group that this shows the this
epidemiological studies in the past 10 years show that 14 reconstruction stage commencement [14] or in other
EGCG can be the preventer of tumor growth in chest, 39 word, the earlier start of revival phase of collagen
lung, liver, sweetbread, stomach, pancreas, skin, cyst, 40 synthesis take place in this stage and collagen groups
and prostate [11]. EGCG is the preventer of secretion of 41 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].

2. Fibroblasts are responsible for synthesis of the 65 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
70 including fibronection and proteoglicans that provide a71 collagenase activity through setting reactions of
proper substrate for immigration and propagation of68 cellular signal by EGCG [19].

2. The fibroblasts then synthesize the collagens that70 healing process of wound through general and localized
develop tension power in wound substrate [15]. 271 different factors is under influence [19]. Many different
3. Miofibroblasts that are exclusive fibroblasts:72 Neuron and hormonal like cell and vein factors or
participate in wound shrinkage through providing:73 motion and secretary activities influence the wound
contraction force [14].

During granulation, fibronection develops a proper:75 EGCG and the properties of antibacterial and antivirus
substrate for immigration and growth of cells and76 of green tea in order to fasten the healing of wound
therefore links with miofibroblasts so that wound:77 [20]. EGCG causes the propagation, division, and
contraction is developed influentially. In addition, this:78 motivation of natural cells growth and does this through
fibronection is a support for fibrillogenesis [16]. 279 cell division and anti apoptosis division. Also, it
Regarding the above-mentioned results, it was indicated:80 increases the Keratinocytes survival and influences on
the green tea extract has improved the wound:81 the propagation and fixing of fibroblasts [20]. The
treatment at seventh day that these influences are:82 preventing effect of green tea is related to its anti-
observed in reduction of wound surface and increase of:83 oxidant power. Polyphenols and glycoprotein play the
healing percent and also in reduction of required time:84 role of scavenger in special conditions and thus it
for complete healing. Reduction in edema resulted in:85 implements its preventing effects on bacteria and virus
speeding the wound stage. In 2004, Bayer and colleges:86 growth. In this regard, preventing effect of green tea
show that polyphenols prevent the discharge of gamma-87 (Camellia Sinensis) and black tea on the bacteria growth
interferon and have anti edema, anti oldness and wound:88 has been shown [21]. It is possible that green tea
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... 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 reconstruction of it [29]. In other hand, vitamin C is control group. Therefore, it seems to be actions other required for construction of veins, immigration of preventing the wound infection for green tea macrophages and correct function of nutrofiles [30], fastening the wound improvement. Bayat et al. explain that preventing the wound infection for green tea, speeding factor of wound healing process. In current study, the wounds were daily wetted by the alcoholic B2 (riboflavin) disorders the wound healing process and aqueous extract... 

The experimental studies on animals show that the enzyme reactions and are required for correct function localized usages of epidermal growth factors have an62 of blood cells and construction of antibodies [30]. The important influence on speed of epidermal healing in results have shown that green tea includes vitamins B1, wounds with relative thickness and burnings. The usage B2 and B6 [9,12,20]. Therefore probably we can of this material on human wounds also has similarly conclude that mentioned issue is one of the factors effects and its usefulness has been proved [22]. The results regarding the economic and hygiene. Higher the... 

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, EGC and set the natural epidermal healing are not completely76 vitamins. The increased speed of healing has many known, but it seems that the peptide growth factors that71 effects regarding the economic and hygiene. Higher the... 

ACT through autocrin or paracrin mechanisms have72 speed of wound healing, the less the wound infection important role on them [23-25]. In 2003, Chung et al. showed an increased speed in all the process of wound effects regarding the economic and hygiene. Higher the... 

Bollag et al. proposed cellular propagation and healing the healing process of male mice NMRI skin... 

Many numbers of growth factors are known including the epidermal growth (EGF). This factor is a polypeptide of mRNA [25]. It has been shown that the peptide growth factors increase significant proliferation of cells in wounds with relative wounds and also increase traction influence on Mesenchyme cells [26]. In fact, the growth factors of epidermal peptide will increase other production of growth factors like transforming growth factor which is revealed from plackets and macrophages, indirectly activates the healing and improving the wound [27,28]. Without considering the structure, immediate facing of cells during healing with growth factors of epidermal, increases the epithelial [28]. Kwon et al. stated that EGCG motivates the growth of human hair through pro-proliferation and has Anti-apoptosis effects on DPCs... 

In addition, role of vitamins on wound healing process and the relationship of green tea contents with them can be considered. Lack of vitamin C is important in delay of wound healing. In such patients, wound... 

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