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...
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.

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 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 betadine, 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 study, on days 1, 3, 5, 7, 10, 13 and 15, the length measurement method of wound and imaging with digital camera was used for all groups.

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 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 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.
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>Experimental Aqueous extract</th>
<th>Experimental Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 µL</td>
<td>150 µL</td>
<td>300 µL</td>
<td>50 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>
<td>0.05 ± 3.52</td>
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<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>
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<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>
</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>
</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>
</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>
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<tr>
<td>Epithelium</td>
<td>4</td>
<td>4.80 ± 0.01</td>
<td>0.01 ± 4.11</td>
<td>0.02 ± 3.80</td>
<td>0.05± 2.52</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 ± 0.001</td>
<td>0.02 ± 1.42</td>
<td>0.01 ± 1.00</td>
<td>0.04 ± 0.51</td>
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<tr>
<td></td>
<td>15</td>
<td>0.01 ± 3.50</td>
<td>0.01 ± 3.25</td>
<td>1.10 ± 3.00</td>
<td>1.10 ± 3.01</td>
</tr>
<tr>
<td>Blood Vascular</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>
</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>
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<tr>
<td></td>
<td>15</td>
<td>0.01 ± 3.50</td>
<td>0.01 ± 3.25</td>
<td>1.10 ± 3.00</td>
<td>1.10 ± 3.01</td>
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</table>

**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 163 focuses of these studies are the chemical carcinogens or 164 alcoholic extract of green tea was 3.81 ± 1.74 mm, and 166 photo carcinogens in animals [9]. Generally, The 168 in the group which received aqueous extract of green 169 polyphenols which are present in teas are categorized as 170 tea, it was 3.93 ± 1.69 mm. No meaningful difference 171 catechins. Green tea leaves contain six primary catechin 172 between 3 groups was observed (not significant). The 173 compounds: catechin, gallaogatechin, epicatechin, 174 average of wound diameter among control and 175 epigallocatechin, epicatechin gallate, as well as

**Discussion**

There is a meaningful difference between groups (*p* < 0.001). The microscopic results show that edema, fibroblast 285 small limit and also low number of vessels [15] and epithelium amount in mice received aqueous or 287 alcoholic extract did not have a meaningful difference. The edema, fibroblast and epithelium amount were 289 significantly different in groups received aqueous or 290 complete fibrotic tissue development, high number of 291 not significantly different in groups received aqueous or 292 by SPSS statistical software. The *p* values < 0.05 were considered significant.
apigallatechin 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-ratification, anti-propagation in eidermis Keratinocytes [9]. Catkins are anti-oxidant and lowering the blood sugar [7-10]. Chemicals also from polyphenol group that have anti-oxidant and anti-tumor activity. Also, the propagation and fixing of fibroblasts is the propagating stage of green tea which is the beginning of antioxidant theory [11]. EGCG: the propagating stage [17]. On seventh day, in polyphenolic that has properties like anti-oxidant, anti-protection, and the wound surface is reducing in tumor, and anti-mutagenic [9]. The biological and contrast with control group that shows this the wound healing 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 the regeneration process 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 growth factors with more diameter are constructed and the width link also from polyphenol group that have anti-oxidant and antioxidant property and prevents the white and ugly scar. Many different matrix components of primary outer cell of wound bed [6] also shows the prevention of collagen destruction and includes fibroblast and proteoglycans that provide a structural collagen and cell motion and secretary activities influence the wound healing. The higher the injection dose (300 mL), the higher healing effect of green tea [19]. The research 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. Hydrophilic with collagens prevent its activity and play a role in collagens registration [18]. Research of Young 1. Fibroblasts are responsible for the synthesis of collagen [14]. The broad studies during past decades show that the two different factors is under influence [19]. Many different 2. The fibroblasts then synthesize the collagens that healing process of wound through general and localized 3. Miofibroblasts that are exclusive fibroblasts. Neuron and hormonlic like cell and vein factors or participate in wound shrinkage through providing motion and secretary activities influence the wound 4. During granulation, fibroeblast develops a proper EGCG and the properties of antibacterial and antivirus 5. Effect of green tea is related to its anti-oxidant and reducing in 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 complete healing. Reduction in edema resulted in implementing 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-aminobutyric acid (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 reconstruction of it [29]. In other hand, vitamin C is in control group. Therefore, it seems to be actions other required for construction of veins, immigration of that preventing the wound infection for green tea macrophages and correct function of neutrophiles [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 the wound is the 357 and proline [9,12,20]. Lack of vitamin B6 (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 alcoholic B2 (riboflavin) disorders the wound healing process and aqueous extract. [29]. In other hand, B group vitamins are cofactors for The experimental studies on animals show that the enzyme reactions and are required for correct function localized usages of epidermal growth factors have an 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 371 effects regarding the economic and hygiene. Higher the act through autocrine or paracrine mechanisms have sped of wound healing, the less the wound infection important role on them [23-25]. In 2003, Chung et al. and an increased speed in all the process of wound showed that the green tea extract (EGCG) cause the healing. In all of current study for the first time it was epidemic creationists survival in human. In 2003,75 shown that green tea extract can speed the wound Bollag et al. proposed cellular propagation and healing the healing process of male mice NMRI skin.

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 healing in fibroblasts stage is stopped. In this state, even when the number of fibroblasts is natural, they do not produce sufficient collagen. Vitamin C is required for ion link of (OH) with amino acid of proline and lysine and hydroxy-l-lysine, fibrils of collagens will not obtain width links. In extreme Scurry, not only the new number of growth factors are known including the epidermal growth (EGF). This factor is a polypeptide of 53 amino acids that DNA and protein is activated by the mRNA [25]. It has been shown that the peptide growth factors increase significant proliferation of cells in wounds with relative thickness and burnings. The usage of B6 and B12 [9,12,20]. Therefore probably we can of this material on human wounds also has similar conclude that mentioned issue is one of the factors effects and its usefulness has been proved [22]. The speeding the healing process in treatment group.

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