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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Received July 7, 2012; Revised October 23, 2012; Accepted November 8, 2012

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, and even protection against cardiovascular disease [5].
The macroscopic study of wound diameter average between control and treatment group on days 1, 3, 5, 7, 10, 13 and 15. ($p < 0.001$)

### 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 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 Bombay. 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 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.
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 50 µL</th>
<th>Control 150 µL</th>
<th>Control 300 µL</th>
<th>Alcoholic extract 50 µL</th>
<th>Alcoholic extract 150 µL</th>
<th>Alcoholic extract 300 µL</th>
</tr>
</thead>
<tbody>
<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>
<td>0.02 ± 4.0</td>
<td>0.01 ± 3.70</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>
<td>0.09 ± 2.10</td>
<td>0.01 ± 2.70</td>
<td>0.01 ± 2.30</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>
<td>0.001 ± 0.09</td>
<td>0.01 ± 1.40</td>
<td>1.0 ± 0.01</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>
<td>0.02 ± 4.52</td>
<td>0.01 ± 4.52</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>
<td>0.01 ± 0.06</td>
<td>0.90 ± 0.06</td>
<td>0.001 ± 0.70</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>
<td>0.05 ± 2.11</td>
<td>0.001 ± 0.70</td>
</tr>
<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>
<td>4.0 ± 0.01</td>
<td>3.20 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 ± 0.001</td>
<td>0.02 ± 1.42</td>
<td>0.01 ± 1.0</td>
<td>0.04 ± 0.51</td>
<td>0.02 ± 1.50</td>
<td>0.05 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>15</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>
<td>1.2 ± 4.93</td>
<td>1.0 ± 4.90</td>
</tr>
<tr>
<td>Blood Vascular</td>
<td>4</td>
<td>4.5 ± 1.10</td>
<td>1.12 ± 4.25</td>
<td>1.12 ± 4.23</td>
<td>0.01 ± 4.21</td>
<td>1.12 ± 4.25</td>
<td>1.10 ± 4.21</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.01 ± 3.5</td>
<td>0.01 ± 3.25</td>
<td>1.10 ± 3.0</td>
<td>1.10 ± 3.01</td>
<td>0.01 ± 3.28</td>
<td>0.01 ± 3.01</td>
</tr>
</tbody>
</table>

**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 3.81 ± 1.74 mm, and photo carcinogens in animals [9]. Generally, The 3.93 ± 1.69 mm. No meaningful difference 166 catechins. Green tea leaves contain six primary catechin 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
The effect of green tea (also referred to as EGCG), healing [11]. The other researchers showed that polyphenols have different biological activities like: anti-tumor, anti-edema, anti-virus, anti- ratification, anti-propagation in epidermis Keratinocytes [9]. Catkins are also polyphenol group that have anti-oxidant and anti-inflammatory activity. The structure of these molecules is the polyphenol of green tea and have a role in prevention of cell division and anti-apoptosis cell. Also, it was shown that the green tea makes developing collagen. So we can conclude that green tea in order to fasten the healing of wound treatment process fastening effect of green tea [35].

In this study, there is not a meaningful difference before wounding and prevents the white and ugly scar. Therefore, the wounds heal faster. In addition, injecting polyphenols 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 Pain [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 [66] 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 EGCG [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 as 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 and collagenase activity through setting reactions of proper substrate for immigration and propagation of cellular signal by EGCG [19].

The broad studies during past decades show that the healing process of wound through general and localized development tension power in wound substrate [15]. Different factors is under influence [19]. Many different factors in wound contraction force [14]. Location. In this relation, we can point out to study of During granulation, fibronectin 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 fibroblasts 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 fibriligens [16]. Cell division and anti apoptosis division. Also, it was indicated that the green tea extract has improved the wound healing percent and also in reduction of required time [20]. The treatment at seventh day that these influences are preventing effect of green tea is related to its anti-oxidant power. Polyphenols and glycoprotein play the healing percent and also in reduction of required time [20]. The 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 interference and have anti edema, anti oldness and wound has been shown [21]. It is possible that green tea
The functions of green tea that long healing with growth factors of epidermal, glandular, and vascular tissue in wound healing have been reported. Antibiotic medicine speeds the healing of wounds, but antibiotics also have side effects on the healing process. Therefore, it seems to be actions other than antibiotics required for construction of veins, immigration of blood cells and construction of antibodies. The molecular actions that polyphenols, Catechin, Glycoproteins, EGCG and flavonoids influence on mesenchyme cells [26]. In fact, the growth of other epidermal factors like peptide growth factors increases the speed of wound healing [27,28]. It has been shown that the peptide growth factors increase proliferation of cells in wounds with relative thickness and burnings. The usage of polyphenols is important role on them [23-25]. In 2003, Chung et al. showed an increased speed in all the process of wound healing that green tea extract (EGCG) cause the wound healing. In all of current study for the first time it was shown that green tea extract can speed the wound healing process of male mice NMRI skin.

In addition, role of vitamins on wound healing process and the relationship of green tea contents with RNA, protein and vitamin has been studied. Paul R. Michale H. The kinetics and mechanism of the complex formation polyphenols EGCG and ECG with iron (III). J Inorgan Biochem 2007; 101:585-93. Therefore probably we can conclude that mentioned issue is one of the factors affecting the healing rate of wounds [26-28].

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