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 effects 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. 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.

Green tea extract was prepared using Soxhlet instrument. The green tea leaves were studied by 64. In 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 Bonmaray. 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:

**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 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 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>Aqueous extract 50 µL</th>
<th>Aqueous extract 150 µL</th>
<th>Aqueous extract 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>
<td>0.001 ± 3.11</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>
<td>0.01 ± 2.70</td>
<td>0.01 ± 2.30</td>
<td>0.03 ± 2.0</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 ± 1.2</td>
<td>0.001 ± 0.09</td>
<td>0.01 ± 1.40</td>
<td>1.0 ± 0.01</td>
<td>0.001 ± 0.07</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>
<td>0.0 ± 3.70</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.21 ± 0.001</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>
<td>0.50 ± 0.002</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>0.05 ± 0.70</td>
<td>0.001 ± 1.25</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>
<td>4.0 ± 0.01</td>
<td>3.20 ± 0.02</td>
<td>0.04 ± 2.32</td>
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<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>
<td>0.03 ± 0.51</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>
<td>1.10 ± 3.01</td>
<td>0.01 ± 3.28</td>
<td>0.01 ± 3.01</td>
<td>1.10 ± 3.0</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>
<td>1.2 ± 4.93</td>
<td>1.0 ± 4.90</td>
<td>0.01 ± 4.88</td>
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<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>
<td>1.12 ± 4.25</td>
<td>1.10 ± 4.21</td>
<td>1.02 ± 4.22</td>
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<td></td>
<td>15</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>
<td>1.10 ± 3.0</td>
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</tbody>
</table>

125 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).

128 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.

131 Rating 4: The tissues with no edema and The edema, fibroblast and epithelium amount were medium number of epithelisation and fibroblast significantly different in groups received aqueous or

133 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

136 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).

139 considered significant.

140 Results

141 There are 150 reports from in vitro and in vivo studies. The average wound diameter in control group was 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, galallocatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as

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are responsible for caused e i< | dehydr | Keratinocytes | faster. In add and properties like anti-polyphenol and show that in e c e important and i tissue et al. ARTICLE IN PRESS
12 | IJPT | January 2013 | vol. 12 | no. 1 Moshrefjavadi et al.
Published online: January 31, ... Sinensis) and black tea on the bacteria growth287 has been shown [21]. It is possible that green tea i
redu
n is through, |
11 January-aqueous survival and influences on that are exclusive fibroblasts e Moshrefjavadi Keratinocytes g c 1 | se
seventh day that these influences are d or 18 14 ructed and the width link using green tea e Miofibroblasts suface and increase of y e [18] and the prop

128 art 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-o2 between molecules also change [18]. The collagen ym 6-phosphate dehydrogenase in liver [11-12]. 245 causes the wound after healing to look like the tissue 16 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:46 wound location takes place through widening the veins reasons. Firstly, using green tea extract doesn’t have:47 [19]. Researches show that green tea reduces blood any relationship with aqueous or alcoholic treatment:48 sugar, blood lipids, blood pressure, heart disease 18 Secondly, in this study, the effect of aqueous and:49 reduction, heart bit and also vein widening [11,20]. This 40 alcoholic variables is excluded. In the current study, on:50 influences on the practical capacity of fibroblasts, fourth day, as the edema stage indicator is considered.31 synthesis increase in collagen fibers and increase in the wound treatment process [13], the excess of edema:52 wound insistence because of increase in collagen in treatment group is meaningfully less that of control:53 content and because fibroblasts are responsible for group (p < 0.001). This shows that the green tea makes:54 developing collagen. So we can conclude that green tea the edema stage of treatment process faster and:55 (polyphenol, catechin and EGCG) cause the propagation therefore the wounds heal faster. In addition, injecting:56 of fibroblasts and influence the practical capacity of 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:58 [20]. The higher the injection dose (300 mL), the higher 95 the edema in seventh day of study in comparison to the:59 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:61 EGCG prevent the collagenase activity against 202 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:63 action through linking with hydrogen and reaction with process. 264 hydrophobic with collagen prevent its activity and play 1. Fibroblasts are responsible for synthesis of:65 a role in collagens registration [18]. Research of Young 205 matrix components of primary outer cell of wound bed:66 et al. also shows the prevention of collagen destruction 207 including fibronec a proproteins that provide a67 and collagenase activity through setting reactions of proper substrate for immigration and propagation of:68 cellular signal by EGCG [19]. 209 cells [14]. 269 The broad studies during past decades show that the 210 2. The fibroblasts then synthesize the collagens that:70 healing process of wound through general and localized 211 develop tension power in wound substrate [15]. 271 different factors is under influence [19]. Many different 212 3. Miofibroblasts that are exclusive fibroblasts:72 Neuron and hormonic like cell and vein factors or 213 participate in wound shrinkage through providing:73 motion and secretary activities influence the wound 214 contraction force [14]. 274 location. In this relation, we can point out to study of 215 During granulation, fibronec develop a proper:75 EGCG and the properties of antibacterial and antivirus 216 substrate for immigration and growth of cells and:76 of green tea in order to fasten the healing of wound 217 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 fibronecin is a support for fibrilogenesis [16].279 cell division and anti apoptosis division. Also, it 220 Regarding the above-mentioned results, it was indicated:80 increases the Keratinocytes survival and influences on 221 that the green tea extract has improved the wound:81 the propagation and fixing of fibroblasts [20]. The 222 treatment at seventh day that these influences are:82 preventing effect of green tea is related to its anti- 223 observed in reduction of wound surface and increase of:83 oxidant power. Polyphenols and glycoprotein play the 224 healing percent and also in reduction of required time:84 role of scavenger in special conditions and thus it 225 for complete healing. Reduction in edema resulted in:85 implements its preventing effects on bacteria and virus 226 speeding the wound stage. In 2004, Bayer and colleges:86 growth. In this regard, preventing effect of green tea 227 show that polyphenols prevent the discharge of gamma-87 (Camellia Sinensis) and black tea on the bacteria growth 228 interferon and have anti edema, anti oldness and wound:88 has been shown [21]. It is possible that green tea
Green tea effects on wounds healing

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fastening the wound improvement. Bayat el. explain Some studies show that green tea is a rich resource of

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vitamin C and includes 18 amino acids including lysine

wound section and they believe that wet wound is the

53 and proline [9,12,20]. Lack of vitamin B6 (pyridoxine)

speeding factor of wound healing process. In current study, the wounds were daily wetted by the alcoholic B2 (riboflavin) disorders the wound healing process anand aqueous extract.

[29]. In other hand, B group vitamins are cofactors for

The experimental studies on animals show that the61 enzyme reactions and are required for correct function

tionalized usages of epidermal growth factors have an362 of blood cells and construction of antibodies [30]. The

important influence on speed of epidermal healing in636 results have shown that green tea includes vitamins B1

wounds with relative thickness and burnings. The usage643 B2 and B6 [9,12,20]. Therefore probably we can

of this material on human wounds also has similar655 conclude that mentioned issue is one of the factors

effects and its usefulness has been proved [22]. The666 speeding the healing process in treatment group.

epidermal healing is a complex phenomena from which677 It seems that one of the functions of green tea that

the rest epidermal cells are propagated so there will be688 helps the healing of wound is the positive effect of

another healthy epidermis. The molecular actions that699 polyphenols, Catechin, Glycoproteins, EGCG and

set the natural epidermal healing are not completely701 vitamions. The increased speed of healing has many

known, but it seems that the peptide growth factors that712 effects regarding the economic and hygiene. Higher the

act through autocrine or paracrin mechanisms have723 speed 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 the730 healing. In all of current study for the first time it was

epidermis creationists survival in human. In 2003,731 shown that green tea extract can speed the wound

Bollag et al. proposed cellular propagation and healing the735 healing process of male mice NMRI skin.

of wound through polyphenols of green tea. Many740

numbers 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 the752 mRNA [25]. It has been showed that the peptide growth

factors increase significant proliferation of cells in760 wounds with relative wounds and also increase traction

influence on Mesenchyme cells [26]. In fact, the growth773 factors of exterior peptide will increase other production

of growth factors like transforming growth factor which782 is revealed from plackets and macrophages, indirectly

activates the healing and improving the wound [27].

Without considering the structure, immediate facing of

39 cells during healing with growth factors of epidermal,1370

increases the epithelial [28]. Kwon et al. stated that

EGCG motivates the growth of human hair through3962 proliferation and has Anti-apoptosis effects on DPCs933 cells [28]. The histology of wound showed that3974 proliferation of cells increase that is probably because3995 of chemical combination of green tea and epidermal

growth factors.

In addition, role of vitamins on wound healing3999 process and the relationship of green tea contents with3000 them can be considered. Lack of vitamin C is important

in delay of wound healing. In such patients, wound3009 healing in fibroblasts stage is stopped. In this state, even3010 when the number of fibroblasts is natural, they do not3011 produce sufficient collagen. Vitamin C is required for3012 ion link of (OH) with amino acid of proline and lysine

and hydroxyl of them inside fibroblast cell. Without3013 hydroxy-lysine, fibrils of collagens will not obtain3014 width links. In extreme Scurry, not only the new

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