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

ABSTRACT

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. 2 grams of each extract (alcoholic or aqueous) was solved in 100 mL normal saline and evaporator and then in 48 hour incubation in 70°C Bonna and 2% 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; 2% alcoholic 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 development of wounds was assessed and the wound stages according to imaging with digital camera was used for all groups. The length measurement method of wound 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.

**Rating 2:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and moderate edema.

**Rating 3:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and moderate edema.

**Rating 4:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and extreme edema.

**Rating 5:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

**Rating 6:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and extreme edema.

**Rating 7:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

**Rating 8:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and extreme edema.

**Rating 9:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

**Rating 10:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and extreme edema.

**Rating 11:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

**Rating 12:** The tissues with some repeating epithelisation and fibrosis tissue but with the high numbers of vessels and extreme edema.

**Rating 13:** The tissues with some repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.

**Rating 14:** The tissues with some repeating epithelisation and fibrosis tissue but with the high 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>
</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>
<td>0.03 ± 2.0</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>
<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 ± 0.01</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>
<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>
</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>
<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>0.10 ± 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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<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>
<td>1.12 ± 4.25</td>
<td>1.10 ± 4.21</td>
<td>1.02 ± 4.22</td>
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<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>0.10 ± 3.01</td>
<td>0.01 ± 3.28</td>
<td>0.01 ± 3.01</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 difference between groups (p < 0.001).

**Rating 3:** The tissues with epithelisation and fibroblast in small limit and also low number of vessels and epithelium amount in mice received aqueous or alcoholic extract did not have a meaningful difference.

**Rating 4:** The tissues with no edema and the edema, fibroblast and epithelium amount were significantly different in groups received aqueous or alcoholic extract when compared with control group (p < 0.001). In contrast, the blood vascular amount were not significantly different in groups received aqueous or alcoholic extracts when compared with control group by SPSS statistical software. The p values < 0.05 were considered significant.

**RESULTS**

There are 150 reports from in vitro and in vivo studies in the effects of green tea on skin. The primary focus of these studies are the chemical carcinogens or polyphenols which are present in teas are categorized as catechins. Green tea leaves contain six primary catechin between 3 groups was observed (not significant). The compounds: catechin, epigallocatechin, epicatechin, and average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as...
The synthesis of green tea (Camellia Sinensis) and black tea on the bacteria growth has been shown. It is possible that green tea has anti-oxidant and anti-inflammatory properties. The fibroblasts then synthesize the structure of these molecules is the polyphenol of green tea which is the precursor of antioxidant theory. EGCG is the primary combination of green tea polyphenols. EGCG and the properties of antibacterial and antivirus activity against mammalian and viral infections like cell and vein factors or anti-oxidant and anti-inflammatory effect on the blood sugar, blood lipids, blood pressure, heart disease, and oldness, and lowering the blood sugar. Chemical also from polyphenol group that have anti-oxidant and anti-tumor, anti-edema, anti-virus, anti-oxidation, anti-angiogenesis, anti-inflammation, and anti-allergy activity. The other researchers showed that polyphenols have different biological activities like polyphenols cause the infusion, contrast and anti-oxidant and anti-inflammatory effect on the blood sugar, blood lipids, blood pressure, heart disease, and oldness, and lowering the blood sugar. Chemical also from polyphenol group that have anti-oxidant and anti-tumor, anti-edema, anti-virus, anti-oxidation, anti-angiogenesis, anti-inflammation, and anti-allergy activity. The other researchers showed that polyphenols have different biological activities like polyphenols cause the infusion, contrast and anti-oxidant and anti-inflammatory effect on the blood sugar, blood lipids, blood pressure, heart disease, and oldness, and lowering the blood sugar. Chemical also from polyphenol group that have anti-oxidant and anti-tumor, anti-edema, anti-virus, anti-oxidation, anti-angiogenesis, anti-inflammation, and anti-allergy activity. 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Green tea effects on wounds healing


The experimental studies on animals show that the intense enzyme reactions and are required for correct function.

Localized usages of epidermal growth factors have an 262 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 B6, [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.

Epidermal healing is a complex phenomena from which 267. It seems that one of the functions of green tea that the rest epidermal cells are propagated so there will 268 helps the healing of wound is the positive 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 269 effects regarding the economic and hygiene. Higher the act through autocrin or paracrin mechanisms have 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 the 266 healing. In all of current study for the first time it was reported that antibiotic medicine speeds 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 253 amino acids that DNA and protein is activated by the 262 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 [26]. The influence on Mesenchyme cells [26]. In fact, the growth factors of exterior peptide will increase other production of growth factors like transforming growth factor which is revealed from plackets and macrophages, indirectly 266, activates the healing and improving the wound [27]. Without considering the structure, immediate facing of cells during healing with growth factors of epidermal, 269 increases the epithelial [28]. Kwon et al. stated that 269. EGCG motivates the growth of human hair through 262 proliferation and has Anti-apoptosis effects on DPCs 263. The histology of wound showed that 267. Proliferation of cells increase that is probably because 269 of chemical combination of green tea and epidermal growth factors.

In addition, role of vitamins on wound healing 269 process and the relationship of green tea contents with 260 can be considered. Lack of vitamin C is important in delay of wound healing. In such patients, wounds 262, healing in fibroblasts stage is stopped. In this state, even 260, when the number of fibroblasts is natural, they do not 262 produce sufficient collagen. Vitamin C is required for 262 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

REFERENCES


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