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 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, epidermis 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
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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)

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 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 Edentulous 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 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 ± 1.20</td>
<td>0.001 ± 0.09</td>
<td>0.01 ± 1.40</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td></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 ± 1.00</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>0.05 ± 0.70</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>4</td>
<td>4.50 ± 0.07</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>1.21 ± 0.01</td>
<td>0.001 ± 1.00</td>
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<td>0.01 ± 1.62</td>
<td>1.21 ± 0.02</td>
<td>0.05 ± 2.11</td>
<td>0.05 ± 0.70</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>
<td>1.0 ± 4.89</td>
<td>1.2 ± 4.93</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>
<td>0.01 ± 4.21</td>
<td>1.12 ± 4.25</td>
<td>1.10 ± 4.21</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>
</tr>
</tbody>
</table>

Rating 2: The tissues with repeating epithelisation, treatment group on the days 1, 3, 5, 7, 10, 13, and 15 have been illustrated in Fig 1. There is a meaningful difference between groups (p < 0.001).

Rating 3: The tissues with edema, fibroblast, and low number of vessels and low edema.

Rating 4: The tissues with no edema and epithelisation and fibroblast medium number of vessels.

Rating 5: The tissues with complete epithelisation, fibroblast high number of vessels and low edema.

All the data were analyzed using one-way ANOVA 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 is the chemical carcinogens or theophylline compounds: catechin, gallicatechin, epicatechin, and gallicatechin of which are present in teas are categorized as catechins. Green tea leaves contain six primary catechins: catechin, gallocatechin, epicatechin, and gallicatechin of which is observed (not significant).
Fibroblasts are cells that are important for two main roles: participation in wound shrinkage through providing contraction force and developing collagen fibers and increase in synthesis of glycosaminoglycans [12]. The synthesis increase in collagen fibers and increase in glycosaminoglycans are the predictor of the wound healing process [13]. The edema after wounding and prevents the white and ugly scar. EGCG is the primary combination of green tea. During granulation, fibronectin develops a proper matrix for migration and growth of cells and also green tea in order to fasten the healing of wound. In addition, increasing blood and oxygen availability to wound location takes place through widening the veins and arteries like arteries. Polypeptides and glycoprotein play the role in prevention of fibroblasts and increase the synthesis of the polypeptides and glycoprotein of green tea which is the beginner of antioxidant theory [11]. Interferon and have anti edema, anti oldness, and lowe anti-tumor, anti-edema, anti-virus, anti-ratification, anti-structure of these molecules is the polypephol of green tea is the preventer of tumor growth in chest, the earlier start of revival phase 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 with more diameter are constructed and the width link chymotrypsin, tumor necrosis factor alpha and glucose-6-phosphate dehydrogenase in liver [11-12].

In this study, there is not a meaningful difference before wounding and prevents the white and ugly scar. The alcoholic and aqueous extract of green tea in addition, increasing blood and oxygen availability to studied groups. This finding is important for two main reasons. Firstly, using green tea extract doesn’t have side effects [19]. Researches show that green tea reduces blood sugar, blood lipids, blood pressure, heart disease and aging. Secondly, in this study, the effect of aqueous and alcoholic reduction, heart bit and also vein widening [11,20]. This alcoholic variables is excluded. In the current study, on influences on the practical capacity of fibroblasts, fourth day, as the edema stage indicator is considered as synthesis increase in collagen fibers and increase in the wound treatment process [13], the excess of edema wound insistence because of increase in collagen in treatment group is meaningfully less that of control content and because fibroblasts are responsible for group (p < 0.001). This shows that the green tea makes developing collagen. So we can conclude that green tea the edema stage of treatment process faster and (polyphenol, catechin and EGCG) cause the propagation therefore the wounds heal faster. In addition, injecting the extract of fibroblasts 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 edema [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 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 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.

1. Fibroblasts are responsible for synthesis of the role in collagens registration [18]. Research of Young matrix components of primary outer cell of wound bed: also shows the prevention of collagen destruction including fibronectin and proteoglycans that provide a scaffold and collagens activity through setting reactions of proper substance 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]. Many different factors is under influence [19]. Many different locations. In this relation, we can point out to study of During granulation, fibronectin develops a proper EGCG and the properties of antibacterial and antiviral 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 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 fibrillogenesis [16]. Cell division and anti apoptosis division. Also, it Regarding the above-mentioned results, it was indicated. Increases the Keratinocytes survival and influences on that the green tea extract has improved the wound the propagation and fixing of fibroblasts [20]. The treatment at seventh day that these influences are preventing effect of green tea is related to its observed in reduction of wound surface and increase of oxidant power. Polypehols 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 colleges growth. In this regard, preventing effect of green tea show that polypehols prevent the discharge of gamma- Amyris ag Laboratory) 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...
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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 [22]. Reconstruction of it [29]. In other hand, vitamin C is control group. Therefore, it seems to be actions other [30] required for construction of veins, immigration of that preventing the wound infection for green tea [31] macrophages and correct function of nutrofiles [30], fastening the wound improvement. Bayat et al. explain [32] 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 wound is the 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 B1 (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 an effect 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 burnsings. 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.

epidermal healing is a complex phenomena from which 367. It seems that one of the functions of green tea that the rest epidermal cells are propagated so there will help 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 371 affect the stochastic physiology and the hygiene. Higher the act through autocrin or paracrin mechanisms have 372 speed of wound healing, the less the wound infection important role on them [23-25]. In 2003, Chung et al. showed that the green tea extract (EGCG) cause the healing. In all of current study for the first time it was 375 epidermic reconstruction survival in human. In 2003, it was shown that green tea extract can speed the wound healing process of male mice NMRI skin.

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 the 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 exterior peptide will increase other production of growth factors like transforming growth factor which is revealed from placket and macrophages, indirectly activates the healing and improving the wound [27]. 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 proliferation and has Anti-apoptosis effects on DPCs [29]. The histology of wound showed that proliferation of cells increase that is probably because of chemical combination of green tea and epidermal growth factors.

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 if 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 hydroxyl of them inside fibroblast cell. Without hydroxy-lsine, fibrils of collagens will not obtain width links. In extreme Scvery, not only the new

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