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
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*)

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. 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.

The development of wounds was assessed and the wounding stages according to imaging with digital camera was used for all groups. 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 the German microtome with firm blade of Leitz to develop width cuts including skin, bed with the thickness of 4 microns. The cuts were stained by Haematoxylin and Eosin (H&E) coloring methods and reviewed by 120x magnifying power. In this period, epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema was improved. The wound surface of this group was named the day zero. After making the wound, in order to prevent potential putrefaction, 0.2 mg gentamicin and 0.2 mg penicillin were injected.

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 wounding stages according to imaging digital camera and size measurement were recorded.

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.

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 Soxhlet, it was dried and concentrated in rotary evaporator and then in 48-hour incubation in 70°C Borchmarry. In next stage, 2 g of each extract (alcoholic or aqueous) was solved in 100 mL normal saline and 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 Borchmarry. 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 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 Borchmarry. 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.

Materials and Methods

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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 Aqueous extract</th>
<th>Control Alcoholic extract</th>
<th>Treatment Aqueous extract</th>
<th>Treatment 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>
</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>
</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>
</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 ± 1.0</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>
</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>
</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>
</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>
</tr>
</tbody>
</table>

Rating 2: The tissues with repeating epithelisation
Rating 3: The tissues with fibroblast
Rating 4: The tissues with edema and the epithelium amount were
Rating 5: The tissues with complete epithelisation, alcoholic extracts when compared with control group (p = 0.001).

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 catechins: catechin, gallocatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as...
Apigalactocatechin gallate (also referred to as EGCG) healing [11]. The other researchers showed that glicoproteins have different biological activities like polyphenols cause the infusion, contrast and anti-tumor, anti-edema, anti-virus, anti-ratification, anti-oxidant propagation in epidermis Keratinocytes [9]. Catkins are oldness, and lowering the blood sugar [7-10]. Chemical also from polyphenol group that have anti-oxidant and anti-ratification property and have role in prevention of tea which is the beginner of antioxidant theory [11], bleeding and reducing thrombosis [9]. From seventh EGCG is the primary combination of green tea. Day 35 day on, is the propagation stage [17]. On seventh day, in polyphenolic that has properties like antioxidant, anti-oxidant treatment group, the wound surface is reducing in tumor, and anti-mutagenic [9]. The biological and contrast with control group that this shows the epidemiological studies in the past 10 years show that reconstruction stage commence [14] or in other EGCG can be the preventer of tumor growth in chest, word, the earlier stage 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-2 between molecules also change [18]. The collagen yarn 6-phosphate dehydrogenase in liver [11-12]. Causes the wound after healing to look like the tissue

In this study, there is not a meaningful difference before worsening 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. Wound location takes place through widening the veins reasons. Firstly, using green tea extract doesn’t have [19]. Researches show that green tea reduces blood any relationship with aqueous or alcoholic treatment. Sugar, blood lipids, blood pressure, heart disease. Secondly, in this study, the effect of aqueous and reduction, heart bit and also vein widening [11,20]. This non-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 EGCG 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 [20]. The higher the injection dose (300 mL), the higher the edema in seventh day of study in comparison to these the meaningful number of fibroblasts [9]. The research control group. This meaningful increase of treatment shows 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. Hydrophobic with collagen prevents its activity and play 1. Fibroblasts are responsible for synthesis of a role in collagens registration [18]. Research of Young matrix components of primary outer cell of wound bed. Also shows the prevention of collagen destruction 206 including fibronectin and proteoglicans that provide a and collagenase activity through setting reactions of 207 proper substrate for immigration and propagation of cellular signal by EGCG [19].

The broad studies during past decades show that the 1. Fibroblasts then synthesize the collagens that healing process of wound through general and localized 2. The fibroblasts then develop tension power in wound substrate [15]. 3. Mifibroblasts that are exclusive fibroblasts. Neuron and hormonic like cell and vein factors or participate in wound shrinkage through providing motion and secretary activities influence the wound contraction force [14].

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 [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 fibrilligenesis [16]. Cell division and anti apoptosis division. Also, it Regarding the above-mentioned results, it was indicated that increases the Keratinocytes survival and influences on the green tea extract has improved the wound's propagation and fixing of fibroblasts [20]. The treatment at seventh day that these influences are preventing effect of green tea is related to its antioxidant in reduced in wound surface and increase of oxidant power. Polyphenols and glycoprotein play the healing percent and also in reduction of required time's 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-97 (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
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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]. In other hand, vitamin C is control group. Therefore, it seems to be actions other required for construction of veins, immigration of that preventing the wound infection for macrophages and correct function of nutrofiles [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 wound is the wound and proline [9,12,20]. Lack of vitamin B6 (pyridoxine) speeding factor of wound healing process. In current damages this phenominal link process. Lack of vitamin study, the wounds were daily wetted by the alcoholic B1 (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 important influence on speed of epidermal healing. Other studies have shown that green tea includes vitamins B1, 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. epidermal healing is a complex phenomena from which. 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. The increased speed of healing has many been known, but it seems that the peptide growth factors that regulates regarding the economic and hygiene. Higher the act through autocrine or paracrine mechanisms have 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 anaplastic cell survivals 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. of wound through polyphenols of green tea. Many 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 proliferation of cells in wounds with relative wounds and increase traction [28]. influence on Mesenchyme cells [26]. In fact, the growth factors of exterior will increase other production [27]. growth factors like transforming growth factor which is revealed from plackets and macrophages, indirectly [28]. The proliferation of cells increase that is probably because [29]. Kwon et al. stated that EGCG motivates the growth of human hair through proliferation and has Anti-apoptosis effects on DPCs. The histology of wound showed that proliferation of cells increases that is probably because [29]. Without considering the structure, immediate facing of cells during healing with growth factors of epidermal, increases the epithelial [28]. 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