Study of Efficacy of Aqueous and Methanolic Extract of Green Tea on the Process of Opened Skin Wounds Healing in Male (NMRI) Mice Race

FAEZEH MOSHREFJAVADI¹, PARISA KADANEJADIAN², MOHAMMAD ALI NILFOROOSHZADE³, PARICHEHR YAGHMAEI⁴, and HOMEIRA MARDANI⁵

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 doses 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
first the mouse
20 grinded to a powder
|ific for their
|tary investigated
R
±
I
extract the wound surface was treated
s
C
the wound surface was treated
Aqueous extract

\[ \text{vol. 12} \]

10 | IJPT | January 2013 | vol. 12 | no. 1 Moshrefjavadi et al.

Published online: January 31, 2013

Fig 1. The macroscopic study of wound diameter average between control and treatment group on days 1, 3, 5, 7, 10, 13 and 15. \( p < 0.001 \)

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.

Greentea 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 Bormarry. 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:

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 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 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.
Green tea effects on wounds healing

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>50µL</th>
<th>150µL</th>
<th>300µL</th>
<th>50µL</th>
<th>150µL</th>
<th>300µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.321 ± 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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.181 ± 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>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td>4.41 ± 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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.121 ± 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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.231 ± 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>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td></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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.001 ± 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>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.2.001 ± 0.001</td>
<td>3.0 ± 0.05</td>
<td>1.10 ± 3.25</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>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

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).

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 and low edema. Alcoholic extract did not have a meaningful difference.

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 

Rating 3: The tissues with complete epithelisation, alcoholic extracts when compared with control group (p complete fibrotic tissue development, high number of not significantly different in groups received aqueous or vessels and no edema.

All the data were analyzed using one-way ANOVA by SPSS statistical software. The p values < 0.05 were considered significant.

RESULTS

There are 150 reports from in vitro and in vivo 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 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, epigallocatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as

Published online: January 31, 2013
apigallocatechin gallate (also referred to as EGCG). Healing [11]. The other researchers showed that polyphenols cause the infusion, contrast and anti-tumor, anti-edema, anti-virus, anti-ratification, anti-inflammatory processes like antioxidant and anti-GDNF has been shown [12]. The broad studies during past decades show that the alcoholic and aqueous extract of green tea [15]. In addition, increasing blood and oxygen availability to fibroblasts and influence the practical capacity of the 2% extract of green tea into mice wound caused the fibroblasts and increase the synthesis of fibro Collagen matrix components of primary outer cell of wound bed [16]. et al. also shows the prevention of collagen destruction including fibronectin and proteoglicans that provide a substrate for immigration and propagation of fibroblasts and influence the practical capacity of fibroblasts [15]. Many different effective in reducing wound surface and increase of oxidant power. Polyphenols and glycoprotein play the healing percent and also in reduction of required time. Role of scavenger in special conditions and thus it for complete healing. Reduction in edema resulted in implementing its preventing effects on bacteria and virus speeding the wound stage. In 2004, Bayer and colleagues' 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 interferon and have anti edema, anti oldness and wound has been shown [21]. It is possible that green tea...
Green tea effects on wounds healing

ARTICLE IN PRESS

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 in reconstruction of it [29]. In other hand, vitamin C is required for construction of veins, immigration of that preventing the wound infection for green tea macrophages and correct function of neutrophils [30], speeding the wound improvement. Bayat et al. explain that vitamin C is a rich resource of the ultrasound treatment effect and gel on healing the vitamin C includes 18 amino acids including lysine. It is observed that they believe that wet wound is the proline [9,12,20]. Lack of vitamin B1, (pyridoxine) speeding factor of wound healing process. In current damage to this vitamin, the wounds were daily wetted by the alcoholic B2 (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 of 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 results have shown that green tea includes vitamins B1, B2, B6 and B12, with relative thickness and burns. The usage activates the growth factors and their effects regarding the economic and hygiene. Higher the structure through autocrin or paracrin mechanisms have speed of wound healing, the less the wound infection is important role on them [23-25]. In 2003, Chung et al. showed an increased speed in all the process of wound showed that the green tea extract (EGCG) cause the healing. In all of current study for the first time it was epidemic creationists survival in human. In 2003, they shown that green tea extract can speed the wound Bollag et al. proposed cellular propagation and 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 mRNA [25]. It has been shown that the peptide growth factors increase significant proliferation of cells in wounds with relative wounds and also increase the influence on Mesenchyme cells [26]. In fact, the growth factors of exterior peptide will increase other production which is revealed from placettes and macrophages, indirectly activates the healing and improving the wound [27,28]. 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. 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 fibroplasia stage is stopped. In this study, even in the number of fibroblasts is natural, they do not produce sufficient collagen. Vitamin C is required for ion of (OH) with amino acid of proline and lysine and hydroxyl of them inside fibroblast cell. Without hydroxy-l-lysine, fibrils of collagens will not obtain width links. In extreme Scurvy, not only the new

REFERENCES

Published online: January 31, 2013


CURRENT AUTHOR ADDRESSES

Faezeh Moshrefjavadi, Department of physiology, College of Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail: fjavadifezeh.moshref@gmail.com (Corresponding author)

Parisa Kadanejadian, Department of Biophysics, College of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Mohammad Ali Nifarooshzade, Department of Dematology, College of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Parichehr Yaghmazian, Department of Animal Physiology, Tehran University, Tehran, Iran.

Homaiea Mardani, Department of Jaw and Face, Islamic Azad University, Khorasgan, Iran.