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 YAGHMAYEI4, and HOMEIRA MARDANI5

Received July 7, 2012; Revised October 23, 2012; Accepted November 8, 2012

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

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 people, and control of diabetes effect.
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°C. In this period, sufficient water and food were in hand of animals and they were randomly classified into 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.

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 recognized through quality method. The wound parameters as follows:

**Rating 1:** The tissues with no repeating epithelisation and fibrosis tissue but with the low numbers of vessels and extreme edema.
The tissues with no edema and the fibroblast in small limit and also low number of vessels and no edema.

## 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 photo carcinogens in animals. Generally, The polyphenols which are present in teas are categorized as catechins. Green tea leaves contain six primary catechins between 3 groups was observed (not significant). The compounds: catechin, gallocatechin, epicatechin, average of wound diameter among control and epigallocatechin, epicatechin gallate, as well as
Apigallocatechin gallate (also referred to as EGCG) is also the primary combination of green tea that is the beginning of antioxidant theory [11], bleeding and reducing thrombosis [9]. From seventh day on, the propagation stage indicator is considered as synthesis increase in collagen fibers and increase in the wound healing 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 in 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 metastasis [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 group control. 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. Hydrophobic with collagen prevent its activity and play 1. Fibroblasts are responsible for synthesis of a role in collagen's registration [18]. Research of Young matrix components of primary outer cell of wound bed also shows the prevention of collagen destruction 2016 including fibronectin and proteoglycans that provide a collagenase activity through setting reactions of proper substrate 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 3. Miofibroblasts that are exclusive fibroblasts: Neuron and hormonal like cell and vein factors or participate in wound shrinkage through providing motion and secretory activities influence the wound contraction force [14]. Location. In this relation, we can point out to study of During granulation, fibronectin develops a proper EGCG and the properties of antibacterial and antivirus substrate for immigration and growth of cells and EGCG of green tea in order to fasten the healing of wound therefore links with miofibroblasts 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 fibrillogenesis [16]. Cell division and anti apoptosis division. Also, it favorably decreases the Keratinocytes survival and influences on 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 antibacterial and antiviral observed in reduction of 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 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(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...
improve the healing speed of wound. It has been reported that antibiotic medicine speeds the healing of wounds due to infection control [21]. But in this study the amount of collagen synthesis will exceed the exterior symptom 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], 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 35 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 and aqueous extract. [29]. In other hand, B group vitamins are cofactors for study, the wounds are stopped. In this state, even when the number of fibroblasts is natural, they do not produce sufficient collagen. Vitamin C is required for formation polyphenols EGCG and ECG with iron (III). In 2003, Chung et al. noticed an increased speed in all process of wound showed that green tea extract (EGCG) cause the healing. In all of current study for the first time it was evident that the experimental animals are expressed in human also 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 edermal growth (EGF). This factor is a polypeptide of [25]. It has been shown that the peptide growth factors increase significant proliferation of cells in wounds with relative thickness and burnings. The usage of growth factors like transforming growth factor which is revealed from plackets and macrophages, indirectly activates the healing and improving the wound. Without considering the structure, immediate facing of exterior symptoms of infection act through autocrin or paracrin mechanisms have shown that green tea 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 effects regarding the economic and hygiene. Higher the 372 act through autocrin or paracrin mechanisms have shown that green tea 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 effects regarding the economic and hygiene. Higher the 372 act through autocrin or paracrin mechanisms have showed that green tea extract (EGCG) cause the healing. In all of current study for the first time it was evident that the experimental animals are expressed in human also 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.

REFERENCES


CURRENT AUTHOR ADDRESSES

Faezeh Moshrefjavadi, Department of physiology, College of Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail: fjavadi.faezeh.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 Dermatology, College of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

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

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