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ORIGINAL ARTICLE

²Study 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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10 ABSTRACT

11 Green tea used for year has a popular cancer preventive activity. Researchers have showed green tea 12 inhibited growth of cancer in the animals. This research has been done with awareness of positives effect 13 of green tea, which is approved by researchers and the importance of treatment of opened skin wound. 14 This work has been done experimentally. There were 56 male mice in 7 different groups. Different dose 15 of water and alcohol such as 50, 150 and 300 µL were injected. After anaesthetizing the mice, skin 16 wound was created on the back of the mice by a 6-mm punch. While the mice in control group were 17 treated by normal saline, water and alcohol extract of green tea was injected around the wound on the 18 back of each mouse. The dimensions of ulcers and the recovery percent of the wound in the 1st , 3rd, 5th 197th, 10th, 13th and 15th day of study were measured. Furthermore, the needful time for recovery was 20 evaluated. Some histological studies were done as well. Two Specimen of wounds were supplied at 4th 217th and 15th day of the study. In this way, fibroblasts, inflammation, epitheleum and endothelial cell of 22 blood vessels from the wounds were studied. The results show that there are no significant differences ²³ among control, water and alcohol groups in recovery processes (p > 0.05.) Evaluation of recovery ²⁴ processes showed there were significant differences among these groups on 7th day of study (p < 0.01). 25 Evaluation of recovery processes showed there were significant differences among three injected doses $_{26}$ of study (p < 0.001). The degree of differences in fibroblasts, inflammation and epithelium distortion in 27 different days for 6 groups (p < 0.05) was meaningful. According to these findings, although both water 28 and alcohol extracts of green tea speed up the wound healing, there isn't any difference between the 29 uses of water or alcohol extracts.

30 Keywords: Green tea, Wound healing, Water and Alcohol extract, Race NMRI

Wound healing, or wound repair, is an intricate 40 32 process in which the skin (or another organ-tissue) 41 Leaves of this plant are processed with minimal 33 repairs itself after injury. The classic model of wound 42 oxidation. It is mainly used in Asia specifically in China ³⁴healing is divided into three or four sequential, yet ⁴³[3-4]. There have been extensive researches on the 35 overlapping phases: hemostasis (not considered a phase 36 by some authors), inflammatory, proliferative and 46 tea include; anti-Cancer properties, increases in 37 remodeling. Upon injury to the skin, a set of complex 47 metabolic rate, anti-diabetes effect, enhancement of 38 biochemical events takes place in a closely orchestrated 48 mental alertness, improvement of immune system, 39 cascade to repair the damage [1].

Green tea is made from Camellia Sinensis [2]. 44 effects of green tea and results have been surprisingly 45 pleasing. Some of the major potential benefits of green 49 improvement of quality of life for HIV-infected

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



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)

50 patients, cardioprotective effects [5-8]. In this study, 87 51 green tea extracts has been investigated for their effects 88 extract for 7 days, once a day and at 9 am. The amount 52 on the opened skin wound healing.

MATERIALS AND METHODS

In this experimental research, 56 male mice of 55 NMRI race with average weight of 25-35 grams were 56 studied. The mice were held in 7 cages in Professor 57 Torabi Nejad Research Center in Isfahan with light 58 cycle of 12 hours darkness and 12 hours light in 22 \pm 592°C. In this period, sufficient water and food were in 60 hand of animals and they were randomly classified to 99 61 control and experimental groups.

Green tea extract was prepared using Soxhlet.01 respectively. 63 instrument. The green tea leaves were studied by102 64 Isfahan University and were transferred into laboratory.10315, the length measurement method of wound and 65 Then using electric mill, they were grinded to a powder.104 imaging with digital camera was used for all groups. 66 Forty grams of green tea powder was placed into105 The development of wounds was assessed and the 67 filtration paper and were transmitted to a specific106 wound stages according to imaging digital camera and 68 container. In order to produce water extract, 400107 size measurement were recorded. 69 milliliters of purified water was added and in order to108 For microscopic evaluation, sampling and tissue 70 produce alcoholic extract, 400 milliliters of 85% 109 study was carried out. On days 4, 7 and 15, the mice 71 methanol was added. After producing the extract by_{110} were killed by smelling ether in air. Then, two samples 72 Soxhlet, it was dried and concentrated in rotary 111 were taken from wound tissue and surrounding skin 73 evaporator and then in 48-hour incubation in 70°C Bon 112 which were placed inside 10% Formalin solution. The 74 marry. In next stage, 2 g of each extract (alcoholic or ¹¹² which were placed histor 1070 romanni solution. The ⁷⁵ aqueous) was solved in 100 mL normal saline and ¹¹⁴ way and the German microtome with firm blade of 76 therefore, 2% aqueous or alcoholic extract was 114 wax and the German microtome with firm blade of 77 achieved.

became comatose with ether and then its back hair was¹¹⁷ Haematoxylin and Eosin (H&E) coloring methods and 80 shaved. After immersing the skin with betiding, with 6-118 edematous cell, fibroblasts and sweating sections were 81 millimeter punch and in accordance to surgery¹¹⁹ recognized through quality method. The wound 82 principles, a 6-millimeter wound was developed. The¹²⁰ improving was determined through rating the pathology 83 wound depth was full skin thickness and the surgery day¹²¹ parameters as follows: 84 was named the day zero. After making the wound, in122 Rating 1: The tissues with no repeating

86 and 0.2 mg gentamicin were injected.

The mice were injected 2% aqueous or alcoholic 89 of 50, 150 or 300 mL of extract were injected in four 90 direction surrounding the wound. All injection were 91 performed by one person. After developing the wound, 92 the mice were classified into 7 groups each 8, as 93 follows:

Group 1 (control): the wound surface of this group 5 was treate d by normal saline;

Groups 2, 3 and 4: the wound surface was treated by 50, 150 and 300 mL of 2% aqueous extract 98 respectively;

Group 5, 6 and 7: the wound surface was treated 100 with 50, 150 and 300 mL of 2% alcoholic extract

For macroscopic study, on days 1, 3, 5, 7, 10, 13 and

115 LEItz to develop width cuts including skin, bed with the In order to make a wound in animal, first the mouse¹¹⁶ thickness of 4 microns. The cuts were painted by

85 order to prevent potential putrefaction, 0.2 mg penicillin123 epithelisation and fibrosis tissue but with the low 124 numbers of vessels and extreme edema.

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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.

	_	Groups						
		Control	Aqueous extract			Alcoholic extract		
Parameter	Days		50 µL	150 µL	300 μL	50 μL	150 μL	300 μL
Inflammation	4	4.50 ± 0.07	0.01 ± 4.10	0.02 ± 3.50	0.05 ± 3.52	0.02 ± 4.0	0.01 ± 3.70	0.001 ± 3.11
	7	3.21 ± 0.05	0.2 ± 2.80	0.02 ± 2.50	0.09 ± 2.10	0.01 ± 2.70	0.01 ± 2.30	0.03 ± 2.0
	15	1.81 ± 0.01	0.03 ± 1.50	0.01 ± 1.2	0.001 ± 0.09	0.01 ± 1.40	1.0 ± 0.01	0.001 ± 0.07
Fibrosis	4	4.81 ± 0.01	0.02 ± 4.51	0.01 ± 4.20	0.05 ± 3.91	0.02 ± 4.52	0.01 ± 4.52	0.0 ± 3.70
	7	1.21 ± 0.01	0.001 ± 1.0	0.081 ± 0.02	0.01 ± 0.06	0.90 ± 0.06	0.001 ± 0.70	0.50 ± 0.002
	15	2.31 ± 0.01	2.0 ± 0.02	0.01 ± 1.62	1.21 ± 0.02	0.05 ± 2.11	0.05 ± 0.70	0.001 ± 1.25
Epithelium	4	5	5	5	5	5	5	5
	7	4.80 ± 0.01	0.01 ± 4.11	0.02 ± 3.80	0.05 ± 2.52	4.0 ± 0.01	3.20 ± 0.02	0.04 ± 2.32
	15	2.0 ± 0.001	0.02± 1.42	0.01 ± 1.0	0.04 ± 0.51	0.02 ± 1.50	0.05 ± 1.0	0.03 ± 0.51
Blood Vascular	4	5.0 ± 1.13	1.10 ±4.92	1.0 ± 4.90	1.0 ± 4.89	1.2 ± 4.93	1.0 ± 4.90	0.01 ± 4.88
	7	4.5 ± 1.10	1.12 ± 4.25	1.12 ± 4.23	0.01 ± 4.210	1.12 ± 4.25	1.10 ± 4.21	1.02 ± 4.22
	15	0.01 ± 3.5	0.01 ± 3.25	1.10 ± 3.0	1.10± 3.01	0.01 ±3.28	0.01 ± 3.01	1.10 ± 3.0

Rating 2: The tissues with repeating epithelisation,147 treatment group on the days 1, 3, 5, 7, 10, 13, and 15 to quantity fibrotic tissue, low number of vessels and 148 has been illustrated in Fig 1. There is a meaningful 127 extreme edema 149 difference between groups (p < 0.001).

128Rating 3: The tissues with epithelisation and 150The microscopic results show that edema, fibroblast129 fibroblast in small limit and also low number of vessels151 and epithelium amount in mice received aqueous or130 and low edema.152 alcoholic extract did not have a meaningful difference.131Rating 4: The tissues with no edema and the153 The edema, fibroblast and epithelium amount were132 medium number of epithelisation and fibroblast154 significantly different in groups received aqueous or133Rating 5: The tissues with complete epithelisation,155 alcoholic extracts when compared with control group (p)134 complete fibrotic tissue development, high number of156 < 0.001). In contrast, the blood vascular amount were</td>135 vessels and no edema.157 not significantly different in groups received aqueous or136All the data were analyzed using one-way ANOVA158 alcoholic extracts when compared with control group137 by SPSS statistical software. The p values < 0.05 were159 (Table 1).</td>

138 considered significant.

RESULTS

DISCUSSION

The average wound diameter in control group was162 studies in the effects of green tea on skin. The primary 1414.42 \pm 1.66 mm, in the group which received the163 focuses of these studies are the chemical carcinogens or 142 alcoholic extract of green tea was 3.81 ± 1.74 mm, and164 photo carcinogens in animals [9]. Generally, The 143 in the group which received aqueous extract of green165 polyphenols which are present in teas are categorized as 144 tea, it was 3.93 ± 1.69 mm. No meaningful difference166 catechins. Green tea leaves contain six primary catechin 145 between 3 groups was observed (not significant). The167 compounds: catechin, gallaogatechin, epicatechin, 146 average of wound diameter among control and168 epigallocatechin, epicatechin gallate, as well as

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12 | IJPT | January 2013 | vol. 12 | no. 1

169 apigallocatechin gallate (also referred to as EGCG).229 healing [11]. The other researchers showed that 170 glicoproteins have different biological activities like230 polyphenols cause the 171 anti-tumor, anti-edema, anti-virus, anti-ratification, anti-231 propagation in epidermis Keratinocytes [9]. Catkins are 172 oldness, and lowering the blood sugar [7-10]. Chemical232 also from polyphenol group that have anti-oxidant and 173 structure of these molecules is the polyphenol of green233 anti-ratification property and have role in prevention of 174 tea which is the beginner of antioxidant theory [11].234 bleeding and reducing thrombosis [9]. From seventh 175 EGCG is the primary combination of green tea235 day on, is the propagation stage [17]. On seventh day, in 176 polyphenolitic that has properties like antioxidant, anti-236 treatment group, the wound surface is reducing in 177 tumor, and anti-mutagenic [9]. The biological and 237 contrast with control group that this shows the 178 epidemiological studies in the past 10 years show that 238 reconstruction stage commencement [14] or in other 179 EGCG can be the preventer of tumor growth in chest,239 word, the earlier start of revival phase of collagen 180 lung, liver, sweetbread, stomach, pancreas, skin, cyst,240 synthesis take place in this stage and collagen groups 181 and prostate [11]. EGCG is the preventer of secretion of 241 with more diameter are constructed and the width link 182 chymotrypsin, tumor necrosis factor alpha and glucose-242 between molecules also change [18]. The collagen yarn 183 6-phosphate dehydrogenase in liver [11-12].

185 between the alcoholic and aqueous extract of green tea245 In addition, increasing blood and oxygen availability to 186 in studied groups. This finding is important for two246 wound location takes place through widening the veins 187 reasons. Firstly, using green tea extract doesn't have247 [19]. Researches show that green tea reduces blood 188 any relationship with aqueous or alcoholic treatment.248 sugar, blood lipids, blood pressure, heart disease 189 Secondly, in this study, the effect of aqueous and 249 reduction, heart bit and also vein widening [11,20]. This 190 alcoholic variables is excluded. In the current study, on250 influences on the practical capacity of fibroblasts, 191 fourth day, as the edema stage indicator is considered as251 synthesis increase in collagen fibers and increase in 192 the wound treatment process [13], the excess of edema252 wound insistence because of increase in collagen 193 in treatment group is meaningfully less that of control253 content and because fibroblasts are responsible for 194 group ($p \le 0.001$). This shows that the green tea makes 254 developing collagen. So we can conclude that green tea 195 the edema stage of treatment process faster and 255 (polyphenol, catechin and EGCG) cause the propagation 196 therefore the wounds heal faster. In addition, injecting256 of fibroblasts and influence the practical capacity of 197 the 2% extract of green tea into mice wound caused257 fibroblasts and increase the synthesis of fibro Collagen 198 meaningful increases in fibrous tissue and reduction in 58 [20]. The higher the injection dose (300 mL), the higher 199 the edema in seventh day of study in comparison to the 250 the meaningful number of fibroblasts [9]. The research 200 control group. This meaningful increase of treatment 260 of Madham et el. show that catechin polyphenol and 201 group fibrous in considering their role in following261 EGCG prevent the collagenase activity against 202 issues are important and indicate the positive effect of 202 Collagens [18]. In fact, Catkin and EGCG prevent the 203 green tea on distribution phase of wound treatment 53 action through linking with hydrogen and reaction with 204 process.

206 matrix components of primary outer cell of wound bed266 et al. also shows the prevention of collagen destruction 207 including fibronectin and proteoglicans that provide a267 and collagenase activity through setting reactions of 208 proper substrate for immigration and propagation of 268 cellular signal by EGCG [19]. 209 cells [14].

211 develop tension power in wound substrate [15].

213 participate in wound shrinkage through providing273 motion and secretary activities influence the wound 214 contraction force [14].

216 substrate for immigration and growth of cells and 276 of green tea in order to fasten the healing of wound 217 therefore links with miofibroblasts so that wound277 [20]. EGCG causes the propagation, division, and 218 contraction is developed influentially. In addition, this 278 motivation of natural cells growth and does this through 219 fibronectin is a support for fibrilligenesis [16].279 cell division and anti apoptosis division. Also, it 200 Regarding the above-mentioned results, it was indicated 280 increases the Keratinocytes survival and influences on 221 that the green tea extract has improved the wound281 the propagation and fixing of fibroblasts [20]. The 222 treatment at seventh day that these influences are 282 preventing effect of green tea is related to its anti-223 observed in reduction of wound surface and increase of 283 oxidant power. Polyphenols and glycoprotein play the 224 healing percent and also in reduction of required time284 role of scavenger in special conditions and thus it 225 for complete healing. Reduction in edema resulted in285 implements its preventing effects on bacteria and virus 226 speeding the wound stage. In 2004, Bayer and colleges286 growth. In this regard, preventing effect of green tea 227 show that polyphenols prevent the discharge of gamma-287 (Camellia Sinensis) and black tea on the bacteria growth 228 interferon and have anti edema, anti oldness and wound288 has been shown [21]. It is possible that green tea

Moshrefjavadi et al.

infusion, contrast and 243 causes the wound after healing to look like the tissue In this study, there is not a meaningful difference244 before wounding and prevents the white and ugly scar. 264 hydrophobic with collagens prevent its activity and play 1. Fibroblasts are responsible for synthesis of the265 a role in collagens registration [18]. Research of Young

The broad studies during past decades show that the 2. The fibroblasts then synthesize the collagens that 270 healing process of wound through general and localized 271 different factors is under influence [19]. Many different 3. Miofibroblasts that are exclusive fibroblasts272 Neuron and hormonic like cell and vein factors or 274 location. In this relation, we can point out to study of During granulation, fibronectin develops a proper275 EGCG and the properties of antibacterial and antivirus

Green tea effects on wounds healing

290 reported that antibiotic medicine speeds the healing of 350 will lose their integrity and will open, Because the 291 wound by infection control [21]. But in this study the351 amount of collagen synthesis will exceed the 292 exterior symptoms of infections are not observed in 352 reconstruction of it [29]. In other hand, vitamin C is 293 control group. Therefore, it seems to be actions other353 required for construction of veins, immigration of 294 that preventing the wound infection for green tea for 354 macrophages and correct function of nutrofiles [30]. 295 fastening the wound improvement. Bayat et el. explain355 Some studies show that green tea is a rich resource of 296 the ultrasound treatment effect and gel on healing the 356 vitamin C and includes 18 amino acids including lysine 297 wound section and they believe that wet wound is the 357 and proline [9,12,20]. Lack of vitamin B_6 (pyridoxine) 298 speeding factor of wound healing process. In current358 damages this phenomenal link process, Lack of vitamin 299 study, the wounds were daily wetted by the alcoholic 359 B₂ (riboflavin) disorders the wound healing process 300 and aqueous extract.

302 localized usages of epidermal growth factors have an 362 of blood cells and construction of antibodies [30]. The $_{303}$ important influence on speed of epidermal healing in $_{363}$ results have shown that green tea includes vitamins B₁, 304 wounds with relative thickness and burnings. The usage364 B₂ and B₆ [9,12,20]. Therefore probably we can 305 of this material on human wounds also has similar365 conclude that mentioned issue is one of the factors 306 effects and its usefulness has been proved [22]. The 366 speeding the healing process in treatment group. 307 epidermal healing is a complex phenomena from which 367 308 the rest epidermal cells are propagated so there will be368 helps the healing of wound is the positive effect of 309 another healthy epidermis. The molecular actions that 369 polyphenols, Catechin, Glycoproteins, EGCG and 310 set the natural epidermal healing are not completely370 vitamins. The increased speed of healing has many 311 known, but it seems that the peptide growth factors that371 effects regarding the economic and hygiene. Higher the 312 act through autocrin or paracrin mechanisms have 372 speed of wound healing, the less the wound infection 313 important role on them [23-25]. In 2003, Chung et el.373 and an increased speed in all the process of wound 314 showed that the green tea extract (EGCG) cause the374 healing. In all of current study for the first time it was 315 epidemic creationists survival in human. In 2003,375 shown that green tea extract can speed the wound 316 Bollag et el. proposed cellular propagation and healing376 healing process of male mice NMRI skin. 317 of wound through polyphenols of green tea. Many

318 numbers of growth factors are known including the 319 epidermal growth (EGF). This factor is a polypeptide of 81. 320 53 amino acids that DNA and protein is activated by the 321 mRNA [25]. It has been shown that the peptide growth 80.2 322 factors increase significant proliferation of cells in 381 323 wounds with relative wounds and also increase traction382 324 influence on Mesenchyme cells [26]. In fact, the growth_{383 3}, 325 factors of exterior peptide will increase other production384 326 of growth factors like transforming growth factor which³⁸⁵ 327 is revealed from plackets and macrophages, indirectly^{3864.} 328 activates the healing and improving the wound [27]. $\frac{397}{388}$ 329 Without considering the structure, immediate facing of 3895. 330 cells during healing with growth factors of epidermal, 390 331 increases the epithelial [28]. Kwon et al. stated that 391 6 332 EGCG motivates the growth of human hair through 392 333 proliferation and has Anti-apoptosis effects on DPCs393 334 cells [28]. The histology of wound showed that 3947. 335 proliferation of cells increase that is probably because³⁹⁵ 336 of chemical combination of green tea and epidermal^{396 8}. 337 growth factors.

In addition, role of vitamins on wound healing₃₉₉ 339 process and the relationship of green tea contents with 400.9340 them can be considered. Lack of vitamin C is important401 ³⁴¹ in delay of wound healing. In such patients, wound₄₀₂₁₀, 342 healing in fibroplasis stage is stopped. In this state, even403 343 when the number of fibroblasts is natural, they do not40411. 344 produce sufficient collagen. Vitamin C is required for⁴⁰⁵ 345 ion link of (OH) with amino acid of proline and lysine⁴⁰⁶ 346 and hydroxyl of them inside fibroblast cell. Without⁴⁰⁷12. 347 hydroxy-lysine, fibrils of collagens will not obtain $\frac{100}{409}$ 348 width links. In extreme Scurvy, not only the new410

ijpt.iums.ac.ir 13

289 improve the healing speed of wound. It has been 349 wounds will not heal, but also the previous healed scars 360 [29]. In other hand, B group vitamins are cofactors for

The experimental studies on animals show that the361 enzyme reactions and are required for correct function

It seems that one of the functions of green tea that

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14 | IJPT | January 2013 | vol. 12 | no. 1

Moshrefiavadi et al.

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