The Potential Co-treatment Effects of *Zataria multiflora*, *Camellia sinensis* Extracts and Bacterial Lipopolysaccharide on Skin Wound Model

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Abstract

Background: ***Camellia sinensis*** **and** ***Zataria multiflora*** **as the potent antioxidants and anti-inflammations are crucial in the management of wound therapy. Lipopolysaccharide (LPS) was used for the proliferative potential on fibroblast cells and induction of inflammatory mediators.**

Objective: **We examined the effects of LPS and herbal extracts combination in order to identify their mechanisms of action in fibroblast proliferation and tissue regeneration.**

Methods: **Human foreskin fibroblasts were treated by** ***Salmonella enterica*** **LPS (100µg) and extracts (5%w/w). Tissues of male Balb/c mice were harvested at 1, 3 and 7 days for biochemical and histopathological evaluations. Effects of LPS and extracts on cell viability, Nitric oxide (NO), Cyclooxygenase-2 (COX-2) and Hydrogen peroxide (H_2O_2) levels were examined respectively by XTT assay and related kits.**

Results: **Our pathological investigations for** ***Camellia sinensis*** **and LPS co-treated group indicated inflammatory cells on day 1 and fibroblast proliferation through wound area on day 3. After wound modelling the samples features were the same but with the difference in groups treated by LPS and** ***Camellia sinensis*** **extract which dermis and epidermis were seen. The** ***Camellia sinensis*** **extract and LPS co-treated wounds were showed low levels of H_2O_2 and higher levels of NO compared to extract treated group ($P<0.001$). Results illustrate a dose and time dependent significant difference in cell proliferation between groups.**

Conclusion: **These results suggest that** ***Camellia sinensis*** **extract in combination with LPS may have potential of accelerating inflammatory phase of wound healing process by regulation of COX-2, NO and H_2O_2 in skin fibroblast.**

Keywords: ***Camellia sinensis*****,** ***Zataria multiflora*****, LPS, wound model**

Introduction

The evolution in wound therapy is occurring with the progression in medicine but due to antibiotic resistance, scientists have revisited the ancient ideal dressing material containing traditional and alternative herbal medicines for wound healing. Clinical trials using herbal medicines in wound care holds bright prospect in the future [1-3]. Plant extracts share a common character; all of them comprised polyphenolic compounds [4]. The main bioactive constituents of *Camellia sinensis* that may contribute immunomodulatory effects include polyphenols and flavonoids [5]. Catechins such as epigallocatechin gallate (EGCG) are responsible for attenuating oxidative stress and inflammatory process. Theraputic potential of catechins for numerous clinical disorders has been proven repeatedly in studies [6 - 10]. Owing to *Zataria multiflora* components (thymol, carvacrol and ɣ-terpinene) antioxidative properties this herbal extract could be concluded as a natural radical scavenger agent in pharmaceutical industries [11 - 14]. Many herbal drugs are combinations of several substances which due to containing different mechanisms but synergistic induction effects on living organism are probably superior to their separated components [15].

Bacterial lipopolysaccharide (LPS) is one of the major inducers of inflammatory mediators [16, 17]. Production of mediators in low concentrations of endotoxin may lead to beneficial biological effects, but at higher concentrations, cytokines releasing have toxic effects and ultimately cause toxic shock syndrome [18 - 20]. LPS is one of the stimulus that induce COX-2 expression by mitogen activated protein kinase (MAPK) signal transduction pathway [21 - 23]. According to previous findings, the intracellular hydrogen peroxide as a signal for white blood cell increased accumulation after tissue damage to begin the repair process [24]. Since ROS at low concentrations provide signaling pathways for immunity against microorganisms, Low levels of these mediators can be beneficial in acute wound healing process. High levels of ROS exert oxidative damage which leads to impaired repairing; this abnormality is explained due to a deficiency in ROS detoxifying enzymes [25]. Nitric oxide acts as a vasodilator, antimicrobial compound, chemo-attractant, inhibitor of platelet accumulation, stimulator of vascular permeability in inflammation. NO is up regulator and down regulator of inflammatory phase of wound healing. High levels of NO may contribute as an anti-inflammation during the late phase of inflammation [26 - 28]. NO activates factors like VEGF, bFGF, TGF-β which are required for enhancing angiogenesis [29]. These growth factors stimulate migration, adhesion and proliferation of endothelial cells. NO produced by iNOS is essential for proliferation of keratinocytes. Attraction of IL-1 by NO indicates indirect effects of NO in re-epithelialization. Most in vitro investigations shows that NO enhances collagen synthesis in both wound model fibroblasts and normal skin fibroblasts. NO converts latent TGF-β_1 to the active form which is necessary for activation of fibroblasts [28, 30, 31].

It seems that tea flavins are the better scavengers of NO than peroxynitrite. Due to the lack of tea flavins in green tea, peroxynitrite scavenging function is more than NO scavenging [32]. The present research investigated the protective wound healing effects of *Camellia sinensis* and *Zataria multiflora* extracts and lipopolysaccharide combination partly through the regulation of COX-2, NO and H_2O_2. The results indicated that the medicinal plant extracts cleveage radicals to exhibit antioxidative effects.

Material and method

Material:

Lipopolysaccharide (LPS; *Salmonella enterica* L6511-100mg. 109k4087) were purchased from Sigma, Nitric Oxide assay kit (Biovision, Ca, USA), H_2O_2 assay kit (Cayman Company), COX-2 assay kit (Assay designs & Stressgen Inc. Michigan, USA), ketamine (Alfasan, Worden, Holand), xylazine (Alfasan, Worden, Holand), PBS, formalin 10%, gentamicin, Human foreskin fibroblast cells were obtained from Tarbiyat Modarres University cell bank, FBS, PBS, XTT, tripan blue, lysis buffer, and all other cell culture regents were obtained from Gibco Life Technologies (Pasley UK) and Sigma.

Cell Culture:

Fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose glutamate supplemented with 5% penicillin-streptomycin solution, 10% fetal bovine serum (FBS) in standard culture conditions at 37°C, 95% humidity and 5% CO_2. When the confluence of cells reached to 80%, cells were trypsinized and transferred into another cultivation flask.

Treatment of the cells:

LPS from *Salmonella enterica* was employed for this study. For preparation of LPS stock solution (0.2 mg/ml), sterile distilled water was the solvent. For the evaluation of LPS effects on skin fibroblasts, the cells were cultured overnight and then treated with different concentrations of LPS (1, 3.16, 10, 31.6, 100 µg and 3.16, 10, 31.6, 100, 316 ng). Treated cells were incubated for 24, 48, 72 hours and prepared for cell viability assay and evaluation of NO, COX-2 and H_2O_2 levels.

Cell viability assay

24 hours before performing this procedure, cells were cultured overnight. All tests have a blank containing just cell culture media. For preparation of reactive solution for every plate, 120 µl of (PMS) and 6ml of XTT were required. After addition of reactive solution to all of the wells, microplates were incubates for 2 and 4 hours. The optical density of samples was read by ELISA-reader in 465 nm.

Experimental Animals

8-12 week old male Balb/c mice were obtained from animal laboratory of Pasteur Institute of Iran. For acclimatization of animals, they were maintained in a well-ventilated room, under standard condition of 19-21°C with 40-60% humidity and 12-h/12-h (light/dark) cycle for 1 week prior to experiment. The animals were housed one per cage and were fed with standard pellets and

water *ad libitum*. The animal care and housing were carried out with no significant levels of contaminants.

Preparation of Excision wound model

Mice weighting between 20-30 g were anaesthetized with a single injection of ketamine hydrochloride (i.p., 5 mg/kg), xylizine (i.p., 5 mg/kg) prior to and during the experiment. Subsequently, the hairs on the back of mice were shaved with electrical shaver and wiped with 70% ethanol. Four identical circular full-thickness wounds were generatedat the same distance on the dorsum of each mouse using a sterile biopsy punch (3 mm). Animals were inflicted according to excision wound model as described by Morton and Malone. The skin tissues were dissected out carefully and the wounds were left open during the study. The wounding day was considered as day 0. Two of the Wounds at left side of the body were selected as Control wounds and the other two wounds at right side were treated with LPS. This experimental study was carried out with male Balb/c mice, randomly divided in to four groups. Tissues were harvested at 1, 3 and 7 days for biochemical and histopathological evaluations of wounded skin tissues. Animals were divided into four groups of five mice in each group, *Camellia sinensis* treated group, *Zataria multiflora* treated group, *Camellia sinensis* and LPS co-treated group, *Zataria multiflora* and LPS co-treated group. All the surgical procedures were approved by the animals committee of Pasteur Institute of Iran and carried out under sterile conditions for animals which were housed one per cage [33-35].

Topical application of LPS

To examine the inflammatory potential of lipopolysaccharide in vivo, LPS solution was applied topically directly to the wound bed, 24 hours after wounding day, once daily. As explain previously, two of four open wounds created on each Balb/c mice were treated with equal dose of LPS (100 μg/ml). PBS was applied topically once in a day to the wounds selected as control group in the same manner [36 - 40].

Histopathological evaluation of wounded tissues

For histopathological analysis, 5 mice of each group at the days of 1, 2, 3 and 7 were sacrificed. The harvested skin wounds were included epidermis, dermis and subcutaneous connective tissue. Tissue samples were fixed in 10% formalin separately, processed by dehydrating through graded alcohol series, clearing in xylene and then embeding in paraffin wax. Thick slice were prepared (4-6 μm) and stained with hematoxylin eosin (H&E).

Determination of NO production

Although NO is unstable but the content of its stable products namely nitrate and nitrite is a suitable index of NO production. NO levels in the above mentioned samples were determined using colorimetric assay kit (Nitric Oxide assay kit, Biovision, Ca, USA). The assay principle for measurement of NO in the supernatant was Griess reaction. An equal

volume of cell supernatants were mixed with Griess reagent and the absorbance was read at 540nm. The assay sensitivity was 0.2 nM and the intera assay coefficient of variation was 3.4% [41 - 47].

Determination of COX-2 level

For assessment of LPS effect on COX-2 level, different concentrations of LPS were added on cell culture media and incubate for 24, 48 and 72 hours. The activity of COX-2 was measured by radioimmunoassay kit (Assay design & Stressgen Inc. Michigan, USA) according to the recommendation of the manufacturer. The measured optical density in 450nm was proportional to the concentration of COX-2 in either standards or samples [47, 48].

Hydrogen Peroxide Measurement

Hydrogen peroxide levels in the supernatants of cell culture samples were determined using colorimetric assay kit (Hydrogen peroxide assay kit, Biovision, Ca, USA). The assay principle was peroxidase reaction, the assay sensitivity was 0.1nM and the intera assay coefficient of variation was 2.8% [49 - 52].

Statistical analysis

Statistical analysis was performed by using SPSS (version 20) software. Levels of NO, COX-2 and H_2O_2 between control and test samples were analyzed using ANOVA, followed by Student's *t* test. Values of $P<0.05$ were considered significant differences between groups. Data were expressed as means ± standard errors (SEM).

Results

Cell viability assay

When the effect of LPS on fibroblasts viability was evaluated immediately after treatment, it was unaffected by 0.1 μg/ml LPS challenge up to 24 and 48 hours, but at 72 hours, cell proliferation increased ($P=0.050$). In the groups that the treatment of LPS were after overnight incubation of fibroblasts, the significant effect was observed at 24 hours for the dose of 10 μg/ml ($P=0.001$), at 48 hours for the dose of 0.1 μg/ml ($P<0.001$) and at 72 hours for the dose of 0.1 μg/ml LPS ($P<0.001$).

LPS-induced skin inflammation and Determination of NO production

To investigate the effect of *Salmonella enterica* LPS and extracts on NO production in skin wound samples, Griees assay was performed. As shown in Figure 1. It seems that the effect of LPS and *Camellia sinensis* extract on production of NO in wound site was increased on day 2 ($P<0.001$) and then increased significantly on day 7. NO level was decreased in LPS and the *Zataria multiflora* extract treated group over the time (Figure 2).

Hydrogen Peroxide Measurement

The results obtained from H_2O_2 measurement of LPS and *Camellia sinensis* extract group indicated that the highest level for treated group was on day 2 and then decreased ($P<0.001$) (Figure 3). But it seems that the amount of H_2O_2 in the early phase was increased but in the late phase it was decreased after LPS treatment. Effect of *Salmonella enterica* LPS (100 μg) and *Zataria multiflora*

Number	Name of groups	No_µM1	No_µM2	No_µM3	No_µM7
1	Camellia Treated	35.12	70.21	45.44	37.34
2	LPS+Camellia Treated	27.12	73.4	40.88	90.5

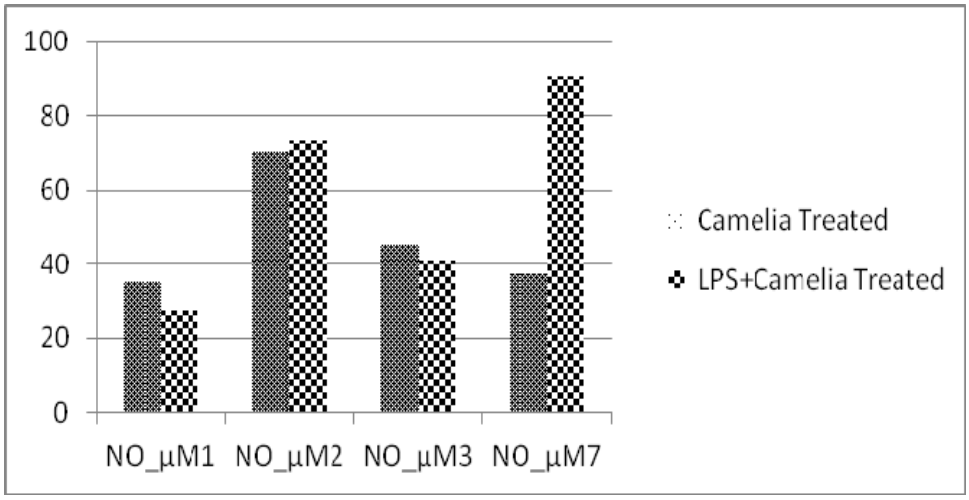

Figure 1- Effect of *Salmonella enterica* LPS (100 µg) and *Camellia sinensis* extract (5% w/w) on NO level in skin wound samples at 1, 2, 3 and 7 days after wounding day. In day 1 and 3 NO level was high in the *Camellia sinensis* treated group. In day 7, NO level was significantly high in LPS and *Camellia sinensis* extract group

Name of groups	No_µM1	No_µM2	No_µM3	No_µM7
Zataria Treated	30. 2	26.03	19.24	17.76
LPS+ Zataria Treated	23.34	20.98	15.4	90.13

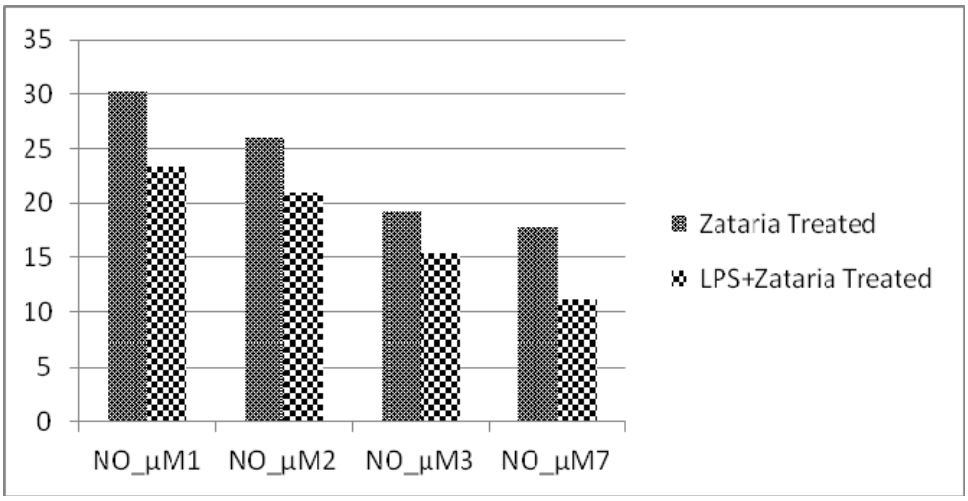

Figure 2- Effect of *Salmonella enterica* LPS (100 µg) and *Zataria multiflora* extract (5% w/w) on NO level in skin wound samples at 1, 2, 3 and 7 days after wounding day. NO level was decreased in LPS and the *Zataria multiflora* extract treated group over the time

Number	Name of groups	H_2O_2_mM1	H_2O_2_mM2	H_2O_2_mM3	H_2O_2_mM7
1	Camellia Treated	12.23	10.14	10.14	13.04
2	LPS+Camellia Treated	12.64	18.31	9.7	13.58

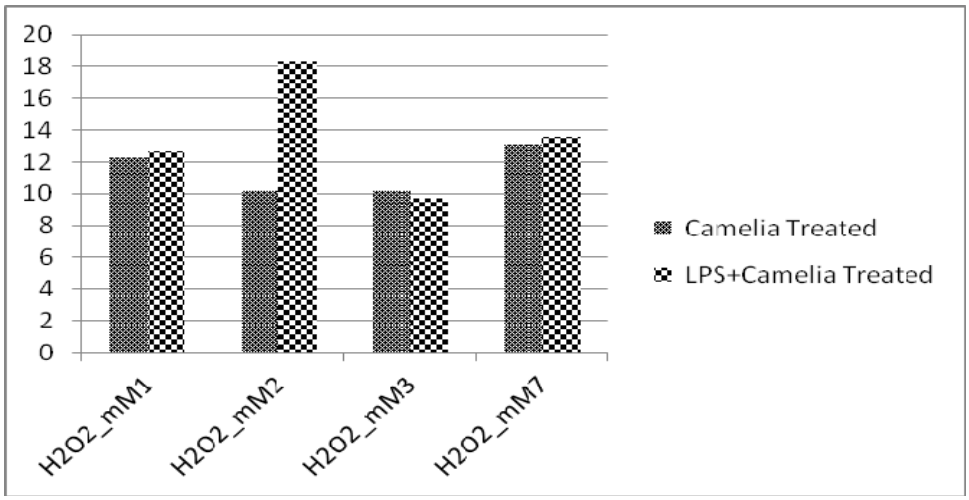

Figure 3- Effect of *Salmonella enterica* LPS (100 µg) and *Camellia sinensis* extract (5% w/w) on H_2O_2 level in skin wound samples at 1, 2, 3 and 7 days after wounding day. In day 2 H_2O_2 level was high in LPS and *Camellia sinensis* extract group. In day 3, H_2O_2 level was significantly low in LPS and *Camellia sinensis* extract group

extract (5 %w/w) on H_2O_2 level in skin wound samples was detected. H_2O_2 level in LPS and *Zataria multiflora* extract group was decreased over the time (Figure 4).

Determination of NO production for fibroblast cells

Figure 5 shows the results of determination of NO production in the groups of *Camellia sinensis* extract treated group and LPS/*Camellia sinensis* treated cells. NO level in LPS/*Camellia sinensis* treated cells was increased over time ($P<0.001$). The results of determination of NO production in the groups of *Zataria multiflora* extract treated group and LPS/ *Zataria multiflora* treated cells showed increased NO level in LPS/ *Zataria multiflora* treated cells over time (Figure 6).

Determination of COX-2 level for fibroblast cells

COX-2 level in LPS/ *Camellia sinensis* treated cells was increased over time (Figure 7). Determination of COX-2 production in the groups of *Zataria multiflora* extract treated group and LPS/ *Zataria multiflora* treated cells showed increased COX-2 level in LPS/ *Zataria multiflora* treated cells over time (Figure 8).

Histopathological evaluation of wounded tissues

The pathological results indicated that all of the samples on day 1 showed inflammatory cells. On day 3 after wound modeling the samples features was the same but with the difference in the groups which was treated by LPS and *Camellia sinensis* extract. In this group, derm and epidermis were seen and this

result showed the better wound healing among the other groups. On day 7 after wound modelling all of the groups showed the healing at the same condition (Figure 9).

Number	Name of groups	H_2O_2_mM1	H_2O_2_mM2	H_2O_2_mM3	H_2O_2_mM7
1	Zataria Treated	12.23	10.14	10.14	13.04
2	Treated LPS + Zataria	12.64	18.31	9.7	13.58

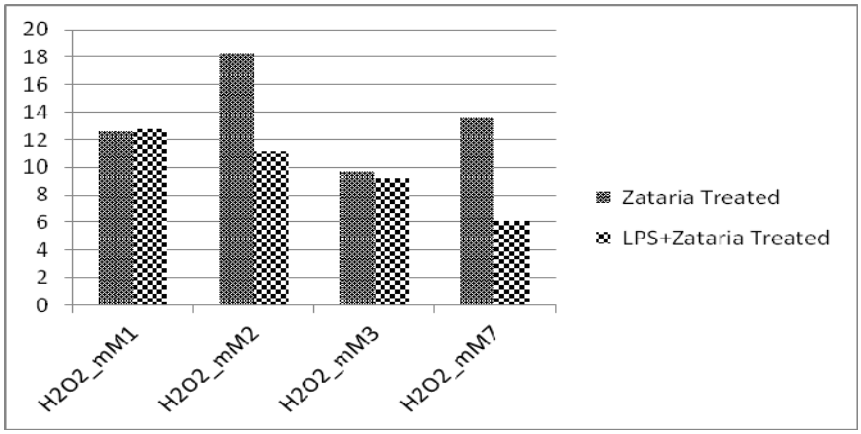

Figure 4 - Effect of *Salmonella enterica* LPS (100 µg) and *Zataria multiflora* extract (5 %w/w) on H_2O_2 level in skin wound samples at 1, 2, 3 and 7 days after wounding day. In day 2 H_2O_2 level was low in LPS and *Zataria multiflora* extract group. In day 7, H_2O_2 level was significantly low in LPS and *Zataria multiflora* extract group

Camellia	5%	100µg+5%
12h2	3.2	7.23
24h2	7.01	10.22
36h2	8.89	15.06
48h2	15.21	19.12

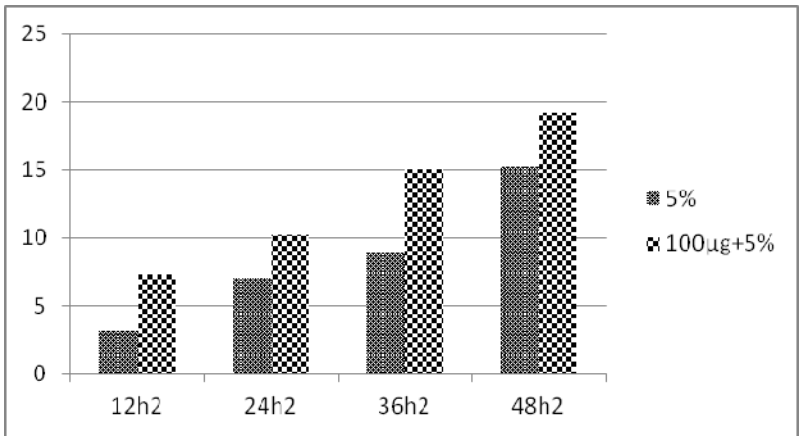

Figure 5 - Determination of NO production in the groups of *Camellia sinensis* extract treated group and LPS/*Camellia sinensis* treated cells. NO level in LPS/*Camellia sinensis* treated cells was increased over time

Zataria	5%	100µg+5%
12h2	3.2	7.23
24h2	6.69	7.45
36h2	5.12	10.22
48h2	8.67	13.65

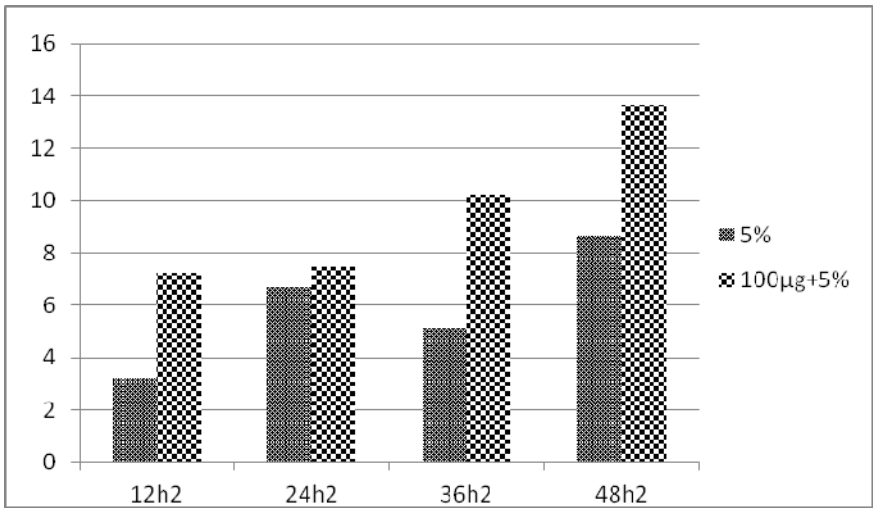

Figure 6- Determination of NO production in the groups of *Zataria multiflora* extract treated group and LPS/ *Zataria multiflora* treated cells. NO level in LPS/ *Zataria multiflora* treated cells was increased over time

Camellia sinensis	5%	100µg+5%
24h2	7.01	10.22
48h2	15.21	19.12

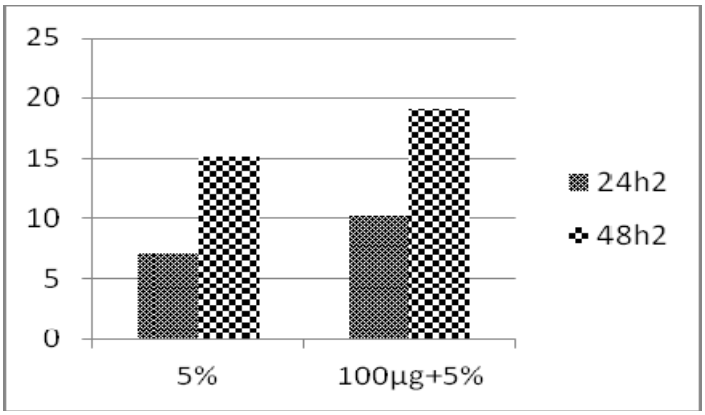

Figure 7- Determination of COX-2 production in the groups of *Camellia sinensis* extract treated group and LPS/ *Camellia sinensis* treated cells. COX-2 level in LPS/ *Camellia sinensis* treated cells was increased over time

Zataria multiflora	5%	100µg+5%
24h2	6.69	7.45
48h2	8.67	13.65

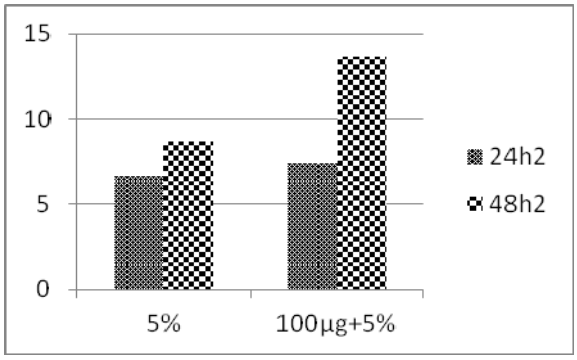

Figure 8- Determination of COX-2 production in the groups of *Zataria multiflora* extract treated group and LPS/ *Zataria multiflora* treated cells. COX-2 level in LPS/ *Zataria multiflora* treated cells was increased over time

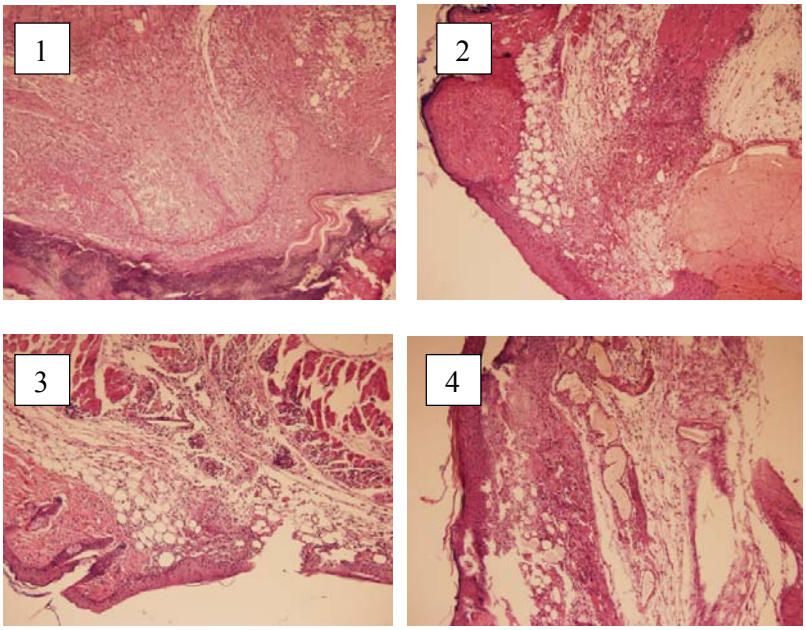

Figure 9- Histopathological evaluation of wounded tissues. Number 1 shows *Camellia sinensis* and LPS co-treated group, number 2 shows *Camellia sinensis* treated group, number 3 shows *Zataria multiflora* treated group and number 4 shows Zataria *multiflora* and LPS co-treated group

Discussion

Accordingly, it was concluded that essential oil from *Z. multiflora* has antioxidant properties that can reduce oxidative stress and can be used in the therapy of oxidative damage accompanying some inflammatory conditions. Although more studies are needed for understanding the major strategy for these

reductions, they are probably mediated by two mechanisms: [53] by the radical scavenging activity of phenolic antioxidants; [54] through inhibition of the related enzyme by phenolic antioxidants. The mechanism of this inhibitory activity is not clear; however, this inhibition may be due to a redox cycling property of the phenol antioxidant.

Studies indicate that *Z. multiflora* essential oil, carvacrol and thymol reduced NOS and NOX activities [55]. Extracts of *Eucalyptus globulus Labill* and *Thymus vulgaris* L. [56], *Saposhnikovia divaricata* [57], *Pinus sylvestris* L. and *Plantago lanceolata* L. [58], *Acanthopanax senticosus* [59], *Cimicifuga racemosa* (aqueous) [60], and *Actinodaphne lancifolia* (methanolic) [61] confirmed the inhibitory activity on NO production in LPS activated macrophage cells during translational and post-translational levels. In addition, the two main compounds in thyme (i.e., thymol and carvacrol) displayed concentration dependent antioxidant capacity and showed a protective effect against oxidative damage in human lymphocytes and V97 Chinese hamster lung fibroblast cells while c-terpinene, which lacks a phenolic group, did not show any antioxidant capacity [54, 62]. Furthermore, Vernonia cinerea, Cardiospermum halicacabum, *T. vulgaris*, Eucalyptus bridgesiana and Cymbopogon martini showed antioxidant and anti-inflammatory activity by modulating proinflammatory cytokines, NO synthase and cylooxygenase-2 [63 – 66].

The *Z. multiflora* essential oil has potent radical scavenging activity. The antioxidant activity of compounds is mainly due to their redox properties, which can play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides, and is related to phenolic hydroxyl groups [67]. Radical scavenging activity of the essential oil was analyzed. The essential oil from *Z. multiflora* analyzed in this research showed potent radical scavenging activity, which is mainly due to its oxidation-reduction potential, which is mainly due to its oxidation-reduction potential, which can play an important role in neutralizing free radicals.

The beneficial effect of epicatechin gallate on wound healing quality and hence leaving more pleasant scar has been shown, which probably confirm its effect on increased level of vascular endothelial growth factor, accelerated vessel formation, and enhanced nitric oxide and cyclooxygenase. The recovery acceleration could be due to the anti-oxidant effect of the present epigalocatechin on speeding up vessel formation of the skin as well as anti-inflammatory properties. Epicatechin, Epicatechin gallate, Epigallocatechin, and Epigallocatechin gallate are among the key anti-oxidant compounds of green tea, little of which could increase collagen volume and hence heal the wounds [53]. These compounds (e.g. epigallocatechin gallate) have also been used as an agent for keratinocytes reproduction and distinction. Also, its anti-fibrogen effects have been confirmed in some animal models [68] Regarding the above mentioned and easy accessibility, we investigated the effect of ethanolic extract of green tea on postsurgical wound healing process.

Cyclooxygenase is another classical enzyme involved in inflammation and wound

repair. COX catalyzes the conversion of arachidonic acid to prostaglandins (PGs) and thromboxanes (TXs). COX contributes to the process of inflammation, cellular proliferation and neovascularization through the production of prostanoids. The major prostaglandin formed during inflammation is PGE2. In this study, the ECG-treated group showed a significant increase in COX activity, which was almost exclusively attributable to the inducible isoform, COX-2. COX-2 has been reported to also have possible anti-inflammatory properties [17]. Recently, it was proposed that the COX-2 isoform may have pro-inflammatory action during the early inflammatory phase but it might help in the resolution of inflammation later on by generating anti-inflammatory prostaglandins [70]. Also COX and PGE2 have shown to be more important in the early stages of wound healing by promoting fibrosis and decreasing the accumulation of macrophages within the wound site. PGE2 has been reported to be involved in proliferation of fibroblasts and the promotion of collagen synthesis at earlier time points during wound healing. VEGF up-regulation is linked to COX-2 expression and activity. This is illustrated by the finding that COX-2 inhibition, inhibits the expression of VEGF and angiogenesis. Furthermore, PGE2 can stimulate VEGF expression in cultured osteoblasts and fibroblasts, Therefore, the observation that ECG treatment caused an increase in COX-2 and VEGF expression, in conjunction with up-regulation of iNOS, might be another contributing factor for the accelerated rate of angiogenesis in the ECG group at the earlier time points [7].

Wound healing is an angiogenesis dependent process, as oxygen and nutrients are required to promote the newly forming granulation tissue. During normal wound healing, new blood vessel formation begins within 3 days, peaks at day 7, and thereafter resolves, resulting in the characteristic avascular scar. Blind immunohistochemical analysis using the endothelial cell marker CD31, showed that in the ECG-treated group, a significant number of new blood vessels started to appear at day 1. After day 7, almost no blood vessels were discerned. ECG treatment, therefore, resulted in an acceleration of the normal angiogenic response and wound healing [7].

ECG treatment resulted in a significant increase in nitrite levels on days 1 and 3; an almost identical profile to iNOS activity. Recent studies have shown that NO derived from iNOS is vital for wound repair. NO derived from iNOS has also been closely linked to VEGF expression and angiogenesis. NO derived from iNOS increases the expression of VEGF during cutaneous wound healing. Furthermore, NO has been shown to increase VEGF mediated endothelial cell migration. It has been reported that fibroblasts derived from iNOS knockout mice have slower rates of proliferation and decreased collagen synthesis [27]. NO derived from iNOS is therefore critical for wound collagen synthesis and in acquiring mechanical strength. This is further confirmed by a study which reported that inhibition of iNOS significantly decreased wound fibroblast collagen synthesis. More recently, iNOS has been shown to also have an anti-fibrotic role.

Furthermore it was shown that, iNOS inhibition resulted in a decrease in the ratio of NO: ROS (reactive oxygen species) with a resultant increase in collagen deposition and fibrosis. Thus induction of iNOS may be one of the protective mechanisms against fibrosis and abnormal wound healing. NO derived from iNOS has also been shown to have cytostatic activity with a net increase in collagen synthesis [72]. Thus the finding that ECG caused an increase in iNOS activity, and hence NO, could have resulted in increased cytostatic activity, accelerated collagen synthesis and an up-regulation of VEGF. This might, in part, be one of the mechanisms through which ECG showed a significant improvement in wound healing and scar formation.

Catechins have varying effects on the three different isoforms of NOS. Evidence also exists that the inhibition of inducible NOS (iNOS) may also be a mechanism behind the anti-inflammatory effects of catechins. Epigallocatechin gallate and other catechins have inhibited the induction of iNOS mRNA and activity after treatment with LPS, IFN g [2, 74, 75] interleukin (IL)-1 and tumor necrosis factor a (TNF a) in vitro. Inhibition of iNOS by catechins appears not to be through a direct mechanism but by preventing inhibitor nB disappearance, which inhibits nuclear factor kB (NF-kB) from binding to the promoter of the iNOS gene thereby inactivating it. However, Tedeschi et al. showed that green tea extract did not inhibit iNOS by reducing NF-kB but down-regulated DNA binding activity of the transcription factor signal transducer and activator of transcription-1. In contrast, we have shown in a model of wound healing that ECG improved the quality of scarring by inducing iNOS and cyclooxygenase (COX)-2, which were originally thought to be exclusively proinflammatory enzymes. However, this can be explained by the fact that NO derived from iNOS is vital to the wound healing process (an angiogenic-dependent process) and can enhance angiogenesis by inducing vascular endothelial growth factor [76].

Conclusion

These results suggest that Camellia sinensis extract in the combination with LPS may have potential of accelerating inflammatory phase of wound healing process partly through the regulation of COX-2, NO and H2O2 in skin fibroblast cells. It might be thought Low levels of H2O2 and high levels of NO lead to some extant better regeneration in this investigation.

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