Assessment of Anti-oxidant and Anti-inflammatory Properties of Green Tea in Critically Ill Patients with Pneumonia

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Abstract

Background: Systemic inflammatory response syndrome is common in critically ill patients and negatively affects clinical outcomes. Preventing oxidative damage, may suppress this systemic response. Previous studies confirmed that polyphenols in green tea have both antioxidant and immuno-stimulant effect, so there could be a probability of green tea clinical application. Two groups of 8 patients enrolled in this randomized, controlled clinical trial. Treatment group received Green Tea Extract (GTE), and placebo group received extract's solvent for 7 days. Blood samples taken 3 times/week and oxidative, physiologic and inflammatory markers were measured.

Results: Differences between the baselines for each variable were not significant. FRAP value showed slower decrease in GTE group. Thiol level increased in GTE group and decreased in Placebo group. IL-6 and APACHE II score were lower in GTE group on day7. Leucocyte count showed overall increase in GTE group and decrease in placebo group. Differences between two groups in all aforementioned variables were statistically insignificant.

Conclusion: As a conclusion green tea had no significant effect on all measured variables; but in GTE group despite better oxidative status in the first 3 days, more inflammation was observed on day7. Considering small sample size further investigations are needed.

1. Background

Oxidative stress defines as ineffectiveness of anti-oxidant defence system. This system includes enzymatic and non-enzymatic molecules; enzymes includes Superoxide dismutase, Catalase, Peroxidases, Glutathione system, Thioredoxin, reductase system, Lipoamide system and nonenzymatic molecules includes Thiols, Ascorbic acid, Urates, Vitamin E, Vitamin A and carotenes, Ubiquinones and ubiquinol; which both scavenging reactive radical species. Any defect in this system either caused by over production of radicals or depletion of anti-oxidant molecules will lead to oxidative damage (lipid peroxidation, DNA damage, *etc.*) (1). Experience of oxidative damage is so common in critically ill patients (2).

Systemic inflammatory response syndrome (SIRS), septic shock, disseminated intravascular coagulation (DIC), multiple organ dysfunction (MOD), reperfusion injury and acute respiratory distress syndrome (ARDS) are the main critical care syndromes leading to oxidative injury (1).

SIRS is a common problem and according to reports SIRS affects 33% of all hospitalized patients, more than the half of all ICU patients and more than 80% of surgical ICU patients (3). These patients develop redox imbalance because of low intake of anti-oxidant vitamins (less than 66% of recommended daily allowance), alterations in endogenous levels of substances with anti-oxidant capacity and some kinds of nursing procedures (*e.g.* suctioning, turning, enteral or parenteral nutrition, *etc.*) (4–6).

There is no suitable drug to cover different pathological pathways of oxidative imbalance in SIRS, so combination therapy is preferred to monotherapy and in theory the best regimen could be a combination of anti-oxidants and immunostimulant (7).

The constituents in Tea (*Camellia sinensis* L. Theaceae) leaves consist of polyphenols (catechins and flavonoides), alkaloids (caffeine, theobromine, theophylline, *etc.*), volatile oils, polysaccharides, amino acids, lipids, vitamins (*e.g.*, vitamin C), chemical elements (*e.g.*, aluminium, fluorine and manganese), *etc.* Despite several active components, polyphenols are considered the most effective anti-oxidant compounds with major pharmacological health effects, such as local wide spectrum anti-microbial activity (8, 9), better blood sugar control, promotion of respiratory function and *etc* (10, 11). Green tea contains six polyphenolic structures including gallic catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG); EGCG is the most active one. Green tea contains more polyphenols than black tea (35% vs. 6%) (10).

Tea catechins act better than popular anti-oxidants such as vitamin C, vitamin E and carotene in coronary artery disease. Tea polyphenols show anti-oxidant activity not only by scavenging superoxides but also act as immunostimulant by improving activity of detoxifying and anti-oxidant enzymes such (*e.g.* Glutathione peroxidase, Glutathione reductase, Glutathione-S-transferase, Catalase and Quinine reductase) (10). Because of unique anti-oxidant properties of green tea and the possibility of its effectiveness in SIRS, we conducted this study to examine green tea's effects on inflammatory and oxidative indices in critically ill patients.

2. Methods

2.1. Green tea extraction

Dried green tea was acquired from Lahijan, Iran, identified by a botanist affiliated to pharmacognosy department of the Islamic Azad University Pharmaceutical Science Branch (voucher No: 239-PMP/A).

The green tea leaves were grinded by MESH 16, extracted by hydro alcoholic solvent (ethanol 40%) for half an hour while shaking frequently and then dried, based on Rusak method (12).

2.2. Poly phenolic standardization

After every time of extraction, extract standardization based on poly phenolic content performed by Total phenolic assay with folin-ciocalteu reagent (Merck – 1.09001.0100) and based on Singleton study (13). The standardized dry extracts kept in glass bottles at 4 °C until administration.

2.3. Patients and methods

This randomized, controlled clinical trial approved by the committee of medical ethics of the Islamic Azad University, Pharmaceutical Science Branch (Code: IR.IAU.PS.REC.1395.6), registered in Iranian Registry of

Clinical Trials (IRCT2016092529327N2 approval of registration on 25 December 2016), and performed in Ziaieian and Naft hospitals.

We randomly selected 16 patients according to inclusion and exclusion criteria. Block randomisation process performed by randomisation webpage. Hospitalization less than 7 days in general ICU, having SIRS, APACHE II score more than 10, pneumonia diagnosis, consent, age more than 18 and rule out of pregnancy considered as inclusion criteria and *vice versa* as exclusion criteria. After a complete explanation about the process, written consent was taken from patients or their first-degree relatives.

Recommended daily amount of polyphenol intake is 300 to 400 mg (14). So in this study, we used 390 mg daily in three divided doses in GTE group.

Eight randomly selected patients were received standardized extract containing 130 milligrams of poly phenol dissolved in 10 milliliters of ethanol 10% (GTE group), three times a day at 8 AM, 2 PM and 8 PM for one week and the rest received 10 milliliters of ethanol 10% with the same regimen (placebo group). Physician and nurses were blinded from solution contents by using amber colored bottles. Blood samples withdrawn on day 0 before administration, day 3 and day 7 after five hours from the last administration. The Blood samples are collected in clot activating tubes, serum separated by centrifuge (4000 rpm, 10 minutes), total anti-oxidant capacity and total thiol of serum measured within 4.5 hours and the rest stored at -80 °C for IL-6 plasma level evaluation. Mean arterial pressure (MAP), respiratory rate (RR), leucocyte count, pulse rate (PR), APACHE II score were also recorded during the study.

2.4. Assessment of total antioxidant capacity

Ferric Reducing Ability of Plasma (FRAP) was considered as our major antioxidant assessing method because of lower cost and ease of use. In this method Fe^{3+} make a complex with reagent TPTZ (2,4,6-tripyridyl-s-triazine), when this complex become expose to antioxidants in serum, Fe^{3+} will reduce to Fe^{2+} and lead to blue color formation with maximum spectrophotometric absorption at 593 nm (15). The only limitation of this method is inability to measure SH- containing antioxidants, so there is need to use another test for detecting this group of antioxidants (16). We performed the tests as explained by Benzie et al. (17) study, while adjusting the process based on our equipment and limitations.

In order to make reactant solution, 25 mL of acetate buffer 300 mmol/L (pH 3.6), 2.5 mL of TPTZ 10 mmol/L (Fluka – 93285) and 2.5 mL of Ferric chloride 20 mmol/L (Merck – 1.03943.0500) were mixed (10:1:1) and warmed to 37 °C. Calibration solution of ferrous sulfate (Fe^{2+}) (Merck – 103965) was prepared in the range of 100 to 2000 $\mu\text{mol/L}$. The absorption of reaction solution (dilution 1:34) containing 300 μL fresh warmed reactant, 10 μL of Calibration solution and 30 μL distilled was read at 593 nm wavelength after 4 minutes of mixing for 3 times with 15 seconds intervals. Average of three absorptions was recorded and calibration curve was established. FRAP assessment Process of serum was the same as calibration but calibration solution substitute with serum samples.

2.5. Assessment of Serum Total Thiol

SH-groups are protein dependent antioxidants and using Ellman reagent is the most common method for their assessment. Elman reagent is 2,2'-Dinitro-5,5'-dithiodibenzoic acid (DTNB) which can binds to sulfhydryl groups and forms yellow color with maximum absorption at 412 nm wavelength (18–20). This test adjunct to FRAP test would complete serum antioxidant capability coverage.

Fresh solution of Tris-EDTA buffer (pH 8.2, Merck – 648311, Merck – 324503) used for each day measurement. Calibration solutions were made by reduced glutathione (Sigma-Aldrich- G4251) in 0/01 to 0/3 mmol/L concentrations.

First, 50µL of calibration solution mixed with 1 ml of tris-EDTA solution and absorption at 412 recorded as A1. Second, 20µL of DTNB 10 mmol/L (Aldrich – D218200) added and absorption recorded after 15 minutes as A2. Third, absorption of only DTNB solution recorded as B.

Total thiol groups were calculated based on formula reported by Hu (18) :

For serum analysis, Process was the same as calibration but calibration solution substitute with serum samples.

2.6. IL-6 plasma level evaluation

Samples kept in -80 °c refrigerator until end of the study, then IL-6 assessed by Human IL-6 Platinum ELISA (e Bioscience, Affymetrix LOT# 106832061) based on manufacture instructions.

2.7. Statistical analysis

The results were analyzed by statistical program SPSS version 24. P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic and baseline characteristics of patients

Demographic and baseline characteristics of the patients are presented in Table1.

Table 1
Patients demographic and baseline characteristics

Patients demographic and baseline characteristics			
Variable	GTE	Placebo	p-value
No. of patients	8	8	> 0.05
Age, yrs			> 0.05
Apache II score			> 0.05
Heart rate, bpm			> 0.05
MAP, mmHg			> 0.05
WBC, cell/mm ³			> 0.05

3.2. Serum total anti-oxidant capacity analysis

Baseline anti-oxidant capacity was

mmol/L Fe²⁺ in GTE group and

mmol/L Fe²⁺ in placebo group. On day 3, GTE group shows slower decrease in anti-oxidant capacity (0.75±0.3 mmol/L) compare to placebo group (0.84±0.2 mmol/L) but on day 7 this value in GTE group (0.66±0.2 mmol/L) was lower than placebo group (0.88±0.2 mmol/L). However this difference was not statistically significant (P-value=0.252). (Fig. 1)

3.3. Serum total thiols analysis

Glutathione baseline was 0.055 ± 0.017 in GTE group and 0.053 ± 0.024 mg/L in placebo group (p = 0.623). On day 3 increase in serum glutathione levels in both groups (GTE 0.063 ± 0.028, placebo 0.075 ± 0.056 mg/L) was observed and on day 7 the level in GTE group was higher than placebo (GTE 0.073 ± 0.031, placebo 0.049 ± 0.008 mg/L), but no significant difference was seen (p = 0.20). (Fig. 2)

3.4. Serum Interleukin-6 level analysis

Baseline Serum interleukin-6 (IL-6) level was 38.5 ± 36.8 pg/mL in GTE group and 34.61 ± 43.18 pg/mL in placebo group (p = 0.695). On day 3 increase in serum value was seen in placebo group compare to GTE group (placebo 40.63 ± 28.55 pg/mL, GTE 21.08 ± 17.43 pg/mL) and in the final day GTE group had lower serum level compare to placebo group (GTE 25.47 ± 23.49 pg/mL, placebo 24.26 ± 23.99 pg/mL), but this difference was not statistically significant (P-value = 0.70). (Fig. 3)

3.5. APACHE II score evaluation

Effects of treatment on APACHE II score on day 7 were assessed, results have shown more score increase in placebo group compare to GTE group (GTE 14.75 ± 6.90 points, placebo 17.50 ± 5.29 points), however no statistically significant difference was seen ($p = 0.49$). (Fig. 4)

3.6. Mean Arterial Pressure and pulse rate

Map and PR in both group decreased during the study and no significant differences were seen (P -value > 0.05). (Fig. 5- Fig. 6)

3.7. Leucocyte count evaluation

Baselines of leucocyte count between two groups (GTE 15.21 ± 4.74 , placebo 11.98 ± 6.76 (10^3 cells/mm³)) did not show significant difference (P -value = 0.623). Slower increase in white blood cell counts seen in GTE group on first three days of treatment compare to placebo group (GTE + 0.29, Placebo + 2.78 (10^3 cells/mm³)) but leukocyte count increased in GTE group and decreased in placebo group on day 7 (GTE 17.08 ± 6.03 , Placebo 9.79 ± 1.97 (10^3 cells/mm³)). Overall difference between two groups was not significant (P -value = 0.117). (Fig. 7)

4. Discussion

Oxygen is a necessary agent for cell respiratory and energy production. As a result of reduction and oxidation reactions, active radical species will produced, which have some benefits such as foreign objects confrontation and some disadvantages such as oxidative stress-related syndromes. Systemic Inflammatory Response Syndrome (SIRS) is one of the most common syndromes seen in critically ill patients, in which a strong systemic inflammation is happened in the whole body (1). Anti-oxidant therapy can be beneficial in prevention of SIRS. A study showed lower period of mechanical ventilation, length of stay in surgical ICU and lower risk of mortality and multi-organ failure in patients who received combination of α tocopherol and ascorbic acid (21). In a review article by Berger, et al., three nutritional anti-oxidants, proved their clinical benefit and reached level A evidence; selenium improve organ failure and infection, glutamine decreased infection's complications in large population clinical trials, Eicosapentaenoic acid (EPA) and anti-inflammatory micronutrients combination caused significant reduction in inflammatory response (22).

In a study performed by Gardner et al., anti-oxidant capacity after anti-oxidant intake from 6 nutritional regimens (cereals, fruits, vegetables, coffee, tea and wine) was assessed. Serum FRAP values for coffee 11.1, tea 1.4, wine 0.8, fruits 1.8, cereals 0.8 and vegetables 0.4 mmol were reported. So coffee showed the strongest effect on in-vivo anti-oxidant defense (23). A clinical trial showed beta carotene-rich beverage such as tomato juice (500 ml/day) is as powerful anti-oxidant as high dose vitamin E (800U/day) in promotion of LDL resistance to oxidation (14). So there is a potential of clinical application of natural anti-oxidants.

The best combination for anti-oxidant therapy would be a radical scavenger with immunostimulant properties (7, 10), so in this study we used green tea which is immunostimulant and powerful anti-oxidant at the same time. Health effects of anti-oxidant consumption for a short time remains even for months while single dose consumption only have effect for a few hours (24), thus it's better to use them in long term to see their effects. In this study we administered polyphenolic supplement for 7days.

Many studies evaluated maximum tolerable dose (MTD) of green tea (25), but expression of MTD base on weight of green tea leaves, milligram of extract achieved by different solvents or milligram of polyphenols, makes it hard to compare the results. We consider regular recommended dose of green tea which there was no report of complications by this dose (14).

Comparing two groups of patients after treatment, shows no statistically significant difference for ferric reducing ability of serum as an anti-oxidant capacity marker. Moreover, measured Glutathione levels in GTE and placebo groups showed non-significant elevation in GTE group's serum level. Several in-vitro studies showed green tea polyphenols chemical structures have higher anti-oxidant activity compare to ascorbate and α -Tocopherol. Pharmacokinetic investigations on green tea polyphenols have shown that tea catechins rapidly and extensively metabolized by glucoronidation, sulfation and methylation process, thus these catechins have low bioavailability. Even high doses of green tea lead to low serum concentrations and low anti-oxidant activity in contrast to in-vitro studies (26).

In this study serum IL-6 level and APACHE II score were lower in GTE group but it did not reach statistical difference. According to studies hypoxia-induced reactive oxygen species are signaling agents for pro-inflammatory cytokines production and endothelial permeability alteration (27). Anti-oxidants are different in strength of ROS inhibition. They can decrease transcription of pro-inflammatory cytokines, so antioxidant therapy may prevent inflammatory-induced disease and septic shock (28). IL-6 and APACHE II score have a valid role in mortality prediction in sever sepsis patients (29, 30). One study assessed the effect of anti-oxidant therapy in surgical ICU patients by measuring α -Tocopherol and ascorbate serum levels and their relation with pro-inflammatory cytokines such as TNF α , IL-1, IL-6, and IL-8. Reductions in cytokines levels, organ failure incidence and length of ICU stay were observed in the anti-oxidant group at the end of therapy. These data demonstrate the benefits of early anti-oxidant therapy in these patients (21).

Both MAP and pulse rate decreased during the study in two groups, but they did not reach statistical significance. A clinical trial which enrolled 1507 individuals reported that regular tea consumption for at least 1 year, decrease the risk of developing hypertension in a dose dependent manner and intake of tea drink more than 600 ml/day reduce this risk by 65% (31), however in our study green tea administration in critically ill patients didn't have negative effect on patients tissue perfusion.

Slower increase in WBC counts and better response to antibiotic therapy was seen in GTE group on the first three days but unexpectedly, leukocyte count increased in GTE group and decreased in placebo group by continuing the therapy. Application of tea as an anti-microbial for topical treatment of surface infections is preferable, because systemic intake of tea can't reach Minimum Inhibitory Concentration

(MIC) as a consequence of low bioavailability (8, 26). Green tea leaves have immunostimulating effect depend on catechin content and this effect can be potentiated by formation of a complex of catechins and tea polysaccharides. High concentration of both catechins and polysaccharides can be detected in buds and first leaves (32). In this study we used first leaves thus high content of polysaccharides was expected. A pharmacological study reported an increase in white blood cells counts in dogs which received green tea, increase in neutrophil counts more than 1.7 fold, monocyte counts more than 2.7 fold and eosinophil counts more than 3 fold over vehicle control were seen (33). But in another experimental study on rats leukocytes counts reduced (34). Because of different results in each species, animal data cannot be extrapolated to human and no clinical trial specifically evaluated relation between green tea intake and leukocyte counts yet.

5. Conclusion

In this study regarding slower reduction in FRAP, increase in serum glutathione, lower serum IL-6, better anti-oxidant defense and lower inflammation trend in first three days were observed but worse oxidative stress and higher inflammation in later days of therapy in GTE group compare to placebo, which could possibly be explained that immuno-stimulant effects may overcome antioxidant effects by continuing intake of polyphenols after 3 days; Although this results didn't reach to the level of significance. Small sample size was the main limitation of this study, so further investigations with greater sample size is recommended and further evaluations to find green tea's MTD and effective dose and duration is needed.

6. Abbreviations

APACHE Acute Physiology and Chronic Health Evaluation

ARDS Acute Respiratory Distress Syndrome

DIC Disseminated Intravascular Coagulation

DTNB 2,2'-Dinitro-5,5'-dithiodibenzoic acid

EGCG epigallocatechin gallate

EPA Eicosapentaenoic acid

FRAP Ferric Reducing Ability of Plasma

GTE Green Tea Extract

ICU Intensive Care Unit

MAP Mean arterial pressure

MIC Minimum Inhibitory Concentration

MOD Multiple Organ Dysfunction

MTD maximum tolerable dose

PR Pulse Rate

RR Respiratory Rate

SIRS Systemic inflammatory response syndrome

TNF α Tumor necrosis factor α

TPTZ 2,4,6-tripyridyl-s-triazine

7. Declarations

- **Ethics approval and consent to participate:** This study is approved by the committee of medical ethics of the Islamic Azad University Pharmaceutical Science Branch (Code: IR.IAU.PS.REC.1395.6)
- **Consent for publication:** Not applicable
- **Data availability:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** We have no conflicts of interest to disclose.
- **Funding:** This study is performed by authors personal funding.
- **Authors' contributions:** This article is based on Zohre Labbani-Motlagh, pharm.D thesis and performed under scientific supervision of by Ramin Abrishami and Saeed Mohammadi Motamed and Reza Shahrami. Herbal laboratory tests designed by Saeed Mohammadi Motamed, human trial designed by Ramin Abrishami and Clinical process directed by Reza Shahrami. Patient selection, monitoring and data collection were done by Zohre Labbani-Motlagh.
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8. References

1. Goodyear-Bruch C, Pierce JD. Oxidative stress in critically ill patients. American Journal of Critical Care. 2002;11:543-51.

2. Fuhrman MP, Herrmann VM, Smith GS. Reactive Oxygen Species and Glutathione: Potential for Parenteral Nutrition Supplementation? *Nutrition in clinical practice*. 1999;14(5):254-63.
3. Brun-Buisson C. The epidemiology of the systemic inflammatory response. *Intensive care medicine*. 2000;26(1):S064-S74.
4. Abilés J, de la Cruz AP, Castaño J, Rodríguez-Elvira M, Aguayo E, Moreno-Torres R, et al. Oxidative stress is increased in critically ill patients according to antioxidant vitamins intake, independent of severity: a cohort study. *Critical Care*. 2006;10(5):R146.
5. Oldham KM, Bowen PE. Oxidative stress in critical care: is antioxidant supplementation beneficial? *Journal of the American Dietetic Association*. 1998;98(9):1001-8.
6. Moosivand A, Abrishami R, Abdollahi M, Ahmadi A, Mojtahedzade M. The incidence of oxidative-stress occurrence following two metabolic support measures in critically ill patients. *Journal of Pharmaceutical Care*. 2013;1(1):3-7.
7. Crimi E, Sica V, Williams-Ignarro S, Zhang H, Slutsky AS, Ignarro LJ, et al. The role of oxidative stress in adult critical care. *Free Radical Biology & Medicine*. 2006;40(3):398-406.
8. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. *Food science and technology bulletin*. 2005;2:71.
9. Taguri T, Tanaka T, Kouno I. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biological and Pharmaceutical Bulletin*. 2004;27(12):1965-9.
10. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. *Food Research International*. 2009;42:529-35.
11. Mukhtar H, Ahmad N. Tea polyphenols: Prevention of cancer and optimizing health. *The American Journal of Clinical Nutrition*. 2000;164(14):1534-40.
12. Rusak G, Komes D, Likić S, Horžić D, Kovač M. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry*. 2008;110(4):852-8.
13. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*. 1999;299:152-78.
14. Upritchard JE, Sutherland WH, Mann JI. Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes care*. 2000;23(6):733-8.
15. Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest*. 2002;62:231-6.
16. Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical chemistry*. 1998;44(6):1309-15.
17. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*. 1996;239(1):70-6.

18. Hu M-L. [41] Measurement of protein thiol groups and glutathione in plasma. *Methods in Enzymology*. Volume 233: Academic Press; 1994. p. 380-5.
19. Ellman GE. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*. 1959;82:70-7.
20. Soltan-Sharifi MS, Mojtahedzadeh M, Najafi A, Khajavi MR, Rouini MR, Moradi M, et al. Improvement by N-acetylcysteine of acute respiratory distress syndrome through increasing intracellular glutathione, and extracellular thiol molecules and anti-oxidant power: evidence for underlying toxicological mechanisms. *Human & experimental toxicology*. 2007;26(9):697-703.
21. Nathens AB, Neff MJ, Jurkovich GJ, Klotz P, Farver K, Ruzinski JT, et al. Randomized, Prospective Trial of Antioxidant Supplementation in Critically Ill Surgical Patients. *Annals of Surgery*. 2002;236(6):814-22.
22. Berger MM, Chiolerio RL. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit Care Med*. 2007;35[Suppl.]:S584-S90.
23. Gardner PT, White TA, McPhail DB, Duthie GG. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food chemistry*. 2000;68(4):471-4.
24. Møller P, Loft S. Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. *The American journal of clinical nutrition*. 2002;76(2):303-10.
25. Schonthal AH. Adverse effects of concentrated green tea extracts. *Mol Nutr Res*. 2011;55:874-85.
26. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *The Journal of nutrition*. 2003;133(10):3275S-84S.
27. Ali MH, Schlidt SA, Chandel NS, Hynes KL, Schumacker PT, Gewertz BL. Endothelial permeability and IL-6 production during hypoxia: role of ROS in signal transduction. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 1999;277(5):L1057-L65.
28. Yi A-K, Klinman DM, Martin TL, Matson S, Krieg AM. Rapid immune activation by CpG motifs in bacterial DNA. Systemic induction of IL-6 transcription through an antioxidant-sensitive pathway. *The Journal of Immunology*. 1996;157(12):5394-402.
29. Hamishehkar H, Beigmohammadi M, Abdollahi M, Ahmadi A, Mahmoodpour A, Mirjalili M, et al. Identification of enhanced cytokine generation following sepsis. Dream of magic bullet for mortality prediction and therapeutic evaluation. *DARU Journal of Pharmaceutical Sciences*. 2010;18(3):155.
30. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Critical care medicine*. 1985;13(10):818-29.
31. Yang Y-C, Lu F-H, Wu J-S, Wu C-H, Chang C-J. The Protective Effect of Habitual Tea Consumption on Hypertension. *Archive of Internal Medicine*. *Archive of Internal Medicine*. 2003;164(14):1534-40.
32. Monobe M, Ema K, Kato F, Maeda-Yamamoto M. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *Journal of agricultural and food chemistry*. 2008;56(4):1423-7.
33. Kapetanovic I, Crowell J, Krishnaraj R, Zakharov A, Lindeblad M, Lyubimov A. Exposure and toxicity of green tea polyphenols in fasted and non-fasted dogs. *Toxicology*. 2009;260(1):28-36.

34. Takami S, Imai T, Hasumura M, Cho Y-M, Onose J, Hirose M. Evaluation of toxicity of green tea catechins with 90-day dietary administration to F344 rats. Food and chemical toxicology. 2008;46(6):2224-9.

Figures

Figure 1

Comparison the effect of therapy between two groups shows statistically non-significance difference (P-value>0.05).

Figure 2

Level of serum thiol at the end of treatment duration in GTE group was higher than placebo group, but this difference was not significant (P-value>0.05).

Figure 3

Serum IL-6 fluctuations during 7 days in two groups. Serum IL-6 level in GTE group was insignificantly lower than placebo group (P-value>0.05)

Figure 4

Comparison of APACHE II score between groups. After 7 days treatment, insignificant lower APACHE II score was observed in GTE group (P-value>0.05).

Figure 5

MAP comparison between two groups. No significant difference was seen (P-value>0.05)

Figure 6

Effect of treatment on Pulse Rate have shown non-significant difference between two groups (P-value>0.05).

Figure 7

Leukocyte count measured 3 times. After 7 days treatment, GTE group showed higher level than placebo group (P-value>0.05)