



Contents lists available at ScienceDirect

## International Journal of Surgery

journal homepage: [www.journal-surgery.net](http://www.journal-surgery.net)

## Original research

Bioactive components and preventive effect of green tea (*Camellia sinensis*) extract on post-laparotomy intra-abdominal adhesion in ratsPouya Parsaei<sup>a</sup>, Mehrdad Karimi<sup>b</sup>, Sayyed Yazdan Asadi<sup>c</sup>, Mahmoud Rafeian-kopaei<sup>d,\*</sup><sup>a</sup> Member of Young Researchers Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran<sup>b</sup> Department of Surgery, Shahrekord University of Medical Sciences, Shahrekord, Iran<sup>c</sup> Medical Plants Research Center, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran<sup>d</sup> Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

## ARTICLE INFO

## Article history:

Received 12 June 2013

Accepted 18 August 2013

Available online 29 August 2013

## Keywords:

Intra-abdominal adhesions

Green tea

Laparotomy

Rat

## ABSTRACT

**Background:** Adhesion formation is an important complication of abdomino-pelvic surgery. Green tea (*Camellia sinensis*) has anti-oxidant and anti-inflammatory effects which prevent production and accumulation of collagen and, thus, may reduce adhesion formation. The present study examined the effect of green tea alcoholic extract on intra-abdominal adhesion formation. Total phenolic, flavonoid and flavanol contents as well as anti-oxidant activity were also evaluated.

**Methods:** Thirty healthy male Wistar rats were randomly assigned to two equal groups of green tea (A) and distilled water (B). After anesthesia, the abdominal wall was opened and three shallow longitudinal and transverse incisions of 2 cm in length were made on the right side of the peritoneum by scalpel blade. A 2 × 2 cm square of the left abdominal wall peritoneum was removed by surgical scissors. Green tea extract or distilled water was introduced into the abdominal cavity of each rat. The rats were sacrificed two weeks post-laparotomy and adhesion bands were scored according to severity, extent and appearance. Fibrosis and inflammation were also scored via histopathological examination.

**Results:** There was a significant difference in mean adhesion scores between the green tea and distilled water groups ( $3.2 \pm 3.503$  and  $7.33 \pm 0.51$ , respectively) ( $p = 0.001$ ). In terms of fibrosis ( $p = 0.002$ ) and inflammation ( $p = 0.003$ ) a statistically significant difference was also seen between the two groups following histopathological examination.

**Conclusion:** Green tea extract reduces intra-peritoneal adhesions in an animal model.

Crown Copyright © 2013 Published by Elsevier Ltd on behalf of Surgical Associates Ltd. All rights reserved.

## 1. Introduction

Adhesions develop following 50–97% of abdominal<sup>1</sup> and 60–90% of gynecological operations.<sup>2</sup> These adhesions develop due to incomplete lysis of fibrin and cellular exudates arising from peritoneal damage.<sup>2</sup> Adhesions are the leading cause of small bowel obstruction, accounting for 65–80% of cases.<sup>1</sup> Chronic pelvic pain, ureteral obstruction and voiding dysfunction are also associated with adhesions.<sup>2</sup> Intra-peritoneal adhesions can also prevent effective intra-peritoneal chemotherapy.<sup>2</sup> Adhesion prevention is a topic of ongoing interest.<sup>2,3</sup> Many potential preventive agents have been investigated, including glucocorticoids, heparin, dextran 70,

Ringer's lactate, antibiotics, promethazine, antihistamines, prostaglandin synthesis inhibitors, ringer lactate, calcium channel inhibitors,<sup>1</sup> rofecoxib,<sup>4</sup> methyl blue,<sup>5</sup> carboxy methyl chitosan and octreotide.<sup>6,7</sup>

Green tea has long been consumed by Eastern Asian people as a drink to promote health and increase longevity.<sup>8,9</sup> The plant, abundant in Asia, produces one of the most easily available drinks worldwide.<sup>8,10,11</sup> It has anti-bacterial, anti-inflammatory and anti-oxidant effects, mainly due to polyphenolic compounds, in particular, the catechin contents, in its leaves.<sup>12–16</sup> As a result of anti-oxidant activities, even low doses of the main green tea compounds, i.e., epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate are capable of increasing collagen volume and assisting wound healing.<sup>17</sup> Given its anti-oxidant and anti-inflammatory activities,<sup>18</sup> green tea could be useful as an intra-abdominal adhesion inhibitor. Among other green tea compounds are theophylline, L-theanine, tannins, gallic acid, oxalic acid, pectin, fluoride, minerals and vitamins such as B1, B2, C, and E,<sup>17</sup> which

\* Corresponding author.

E-mail addresses: [pouyaparsaei@yahoo.com](mailto:pouyaparsaei@yahoo.com) (P. Parsaei), [karimi.mehrdad89@yahoo.com](mailto:karimi.mehrdad89@yahoo.com) (M. Karimi), [yazdan.asadi@gmail.com](mailto:yazdan.asadi@gmail.com) (S.Y. Asadi), [rafeian@yahoo.com](mailto:rafeian@yahoo.com) (M. Rafeian-kopaei).

assist wound healing and inhibit intra-abdominal adhesion formation. Green tea may help in wound healing through production and accumulation of collagen<sup>10,16</sup> as well as prevention of complete fibrin lysis. However, these same properties may encourage adhesion formation. Therefore, the present study aimed to examine the effect of green tea extract on post-laparotomy adhesions. Total phenolic, flavonoid and flavonol contents as well as anti-oxidant activities of the extract were also evaluated.

## 2. Material and methods

After obtaining approval from Shahrekord University of Medical Sciences (SKUMS) Ethics Committee (Ethical code: 90-5-3), this preclinical study was conducted in the Medical Plants Research Center of SKUMS and Islamic Azad University, Shahrekord, Iran. Throughout the experiments, we tried to follow all ethical principles of working on laboratory animals to impose the lowest possible stress on them.

### 2.1. Extract preparation

The maceration method was employed to prepare the extract. For this purpose, 1 l ethanol (70%) was added to 100 g green tea (Herbarium No. 304, Medical Plants Research Center, SKUMS, Iran) and the solution was left at laboratory temperature for 48 h. Then, the extract was filtered through filter paper and the pulp was squeezed to discharge. The extract was concentrated by a rotary evaporator,<sup>19</sup> frozen and stored at  $-20^{\circ}\text{C}$  until use. A 4% solution was made from this extract when needed, using distilled water.<sup>20</sup>

### 2.2. Measurement of phenolic compounds

The phenolic compounds were evaluated equivalent to gallic acid using the Folin–Ciocalteu colorimetry method of Shirzad et al.<sup>21</sup> with some modifications. Different concentrations of standard gallic acid (12.5, 25, 50, 62.5, 100 and 125 ppm in methanol 60%) were prepared. Then, 0.1 ml from each sample was transferred into a test tube and 0.5 ml Folin–Ciocalteu 10% was added as a reactive agent. The solutions were left for 8 min at room temperature and then 0.4 ml sodium carbonate 7.5% was added. The tubes were maintained for 30 min at laboratory temperature and then assayed in three intervals by a spectrophotometer (Unico UV 2010) at 765 nm wavelength. To measure the total phenolic contents in the extracts, 0.01 g of the extract was dissolved in 60% methanol, reaching 10 ml and then, using the Folin–Ciocalteu method, the total phenolic content was measured. However, instead of using the standard solution, 0.1 ml extract was added. Finally, the total phenolic content was evaluated from the read optical density in mg/g extract in gallic acid equivalent.

### 2.3. Measurement of flavonoid compounds

Total flavonoids were evaluated equivalent to Rutin, using the chloride aluminum colorimetry method.<sup>22</sup> First, different concentrations of standard “Rutin” (25, 50, 100, 250 and 500 ppm) were prepared in methanol 60%. Then, from each solution, one ml was transferred into test tube and mixed with 1 ml of chloride aluminum 2%. Afterward, 6 ml potassium acetate 5% was added and the optical density level was read after 40 min at 415 nm wavelength. The concentration levels of the standard solutions were assayed in three intervals. In order to measure the total level of flavonoids in the extract, 0.01 g of the extract was dissolved in methanol 60%, reaching 10 ml. Then, using chloride aluminum colorimetry, the total level of flavonoids was measured. However, instead of using the standard solution, 1 ml of the extract was added. The total flavonoid level was calculated in mg per 1 g of the extract, equivalent to Rutin.

### 2.4. Measurement of flavonol compounds

The total flavonol was also measured using chloride aluminum colorimetry and Rutin procedure, however the optical density level reading, was obtained after 2.5 h at 440 nm wavelength.<sup>23</sup>

### 2.5. Measurement of anti-oxidant activity

A  $\beta$ -carotene model was employed to measure the anti-oxidant activity of the extract.<sup>24</sup> 0.5 ml chloroform, 5 ml  $\beta$ -carotene (0.2 mg), 20 ml linoleic acid (20 mg) and 0.2 ml Tween 40 were mixed in a suitable container and incubated at  $50^{\circ}\text{C}$  for 10 min in order to remove the solvent. The solution was diluted with distilled water and mixed in 4 ml aliquots in the following manner. The control solution was prepared including 0.2 ml ethanol and 0.2 ml of the extract sample with 0.15 ml ethanol. The optical density of the control group was recorded at  $t_0$  and  $t_{90}$  at 470 nm, similar to the standard group. The samples were incubated in a bain-marie at  $50^{\circ}\text{C}$ . The anti-oxidant activity was measured on the basis of the ability of the samples in

preventing the washing of  $\beta$ -carotene. The anti-oxidant activity was calculated using the following formula:

$$AA = 100[1 - (A_0 - A_t)/(A_0^0 - A_t^0)];$$

where,  $A_0$  is the optical density at  $t_0$ ,  $A_t$  is optical density of the sample at  $t_{90}$ ,  $A_0^0$  and  $A_t^0$  are optical density values in the control samples at  $t_0$  and  $t_{90}$ , respectively.

### 2.6. The animals and their maintenance

In this study, 30 healthy Wistar male rats, weighing 200–250 g, were randomly assigned into two equal groups of intervention (A) and control (B). The rats had no history of surgery and other medical interventions. They were kept on standard conditions, were fed by pelleted diet (Razi Co., Karaj, Iran) and water, at  $23 \pm 2^{\circ}\text{C}$  temperature.

### 2.7. Induction of adhesion lesions

Adhesion lesions were fashioned on anaesthetized rats. The operations were performed according to a standardized protocol.<sup>25</sup> Rats, in both groups, were anaesthetized by a combination of 2 mg/kg ketamine 10% (Alfasan Co., Netherlands) and xylazine 2% (Alfasan Co., Netherlands), administered intramuscularly. While anaesthetized, each rat was laid flat on the back in dorsal recumbency position, on a surgical table, the abdominal skin was disinfected with Betadine 10% (Tolid Daru Co., Iran), and the hair of the given area of the skin was completely shaved with a razor to make a 3 cm incision on the midline of the abdomen to access the peritoneal surfaces. After entering the abdominal cavity of the right wall of peritoneum, three shallow transverse incisions (2 cm in length) were made with a scalpel No. 24 longitudinally and transversely and a  $2 \times 2$  piece was taken from peritoneal surface on the left side of the abdominal wall with surgical scissors. Then, to prevent stimulation of peritoneal adhesions formation due to presence of surgical suture material, 4 sutures at 1 cm intervals were placed using absorbable catgut (Kamran Teb Co., Iran). Fascia and skin were closed with 4 sutures at 1 cm intervals using a non-absorbable silk (Kamran Teb Co., Iran). Finally, the given area of the skin was again disinfected and the rats were left at a suitable temperature to become conscious. External sutures were removed at 7th day of the treatment, under general anesthesia.<sup>25</sup>

### 2.8. Treatment

Four ml of the Green tea extract solution or distilled water, was administered, immediately after making lesions, into abdominal cavity of groups A or B, respectively and the abdominal cavity was sutured. The animals were left in normal conditions for fourteen days, when the second laparotomy was performed on the rats for evaluation of adhesion.

### 2.9. Macroscopic examinations

A second laparotomy was performed after 14 days. For this, the abdomen of each rat was opened and adhesions severity, extent and appearance were evaluated by a blinded observer (Table 1). The total degree of adhesions, ranging from 0 to 11, was calculated by adding the three measurements parameters (severity, extent and appearance of the adhesion bands) in each rat.<sup>26</sup>

### 2.10. Histopathological examinations

A sample of adhesion tissue from each animal was harvested at the second laparotomy and put into a fixator (10% Neutral Buffered Formalin). Tissue processing was done by paraffin and wax. Then, 5  $\mu\text{m}$  thick transverse incisions were made by means of a Microtome fixed blade. All incisions of samples were stained by hematoxylin and eosin. Histopathological evaluations of fibrosis and inflammation, were undertaken by a blinded pathologist (Table 2).

**Table 1**  
Macroscopic criteria for adhesions' scaling.

Score	Appearance	Extent	Strength
0	No	No	No
1	Filmy, avascular	<25%	Separated easily
2	Dense, avascular	25–50%	Separated by traction
3	Dense, capillary vascularization	50–75%	Sharp dissection needed for separation
4	Dense, vascular	>75%	Sharp dissection needed for separation

**Table 2**  
Histopathological criteria for adhesions' scaling.

Score	Degree of inflammation	Degree of fibrosis
0	No inflammation	No fibrosis
1	Giant cells, lymphocytes, and plasma cells	Mild
2	Giant cells, plasma cells, eosinophils, and neutrophils	Moderate
3	Inflammatory cell infiltration and microabscess formation	Severe

#### 2.11. Data analysis

Data analysis was performed through SPSS v16 using Mann–Whitney test and  $p < 0.05$  was considered statistically significant.

### 3. Results

The total phenolic content of *green tea* was 76.3 mg/g Gallic acid equivalent, its total flavonoid content was 17.4 mg/g Rutin equivalent/g, and the total flavonol content was 45 mg/g Rutin equivalent (based on dry extract). The anti-oxidant activity of the green tea extract was 47% of  $\beta$ -carotene.

No sign of ascites, intra-abdominal viscous fluid or mortality was observed. Histopathological adhesion scaling results are summarized in [Table 2](#). The frequency of adhesion bands in group A were considerably less (5 cases of zero [without adhesion band]) ([Photograph 1](#)) than group B (all cases had adhesion bands) ([Table 3](#)). Adhesion bands in group B cases also encroached into bladder and some parts of bowel ([Photograph 2](#)) while no encroachment was detected in group A cases. The mean adhesion degree score in the green tea group was 3.2 versus 7.33 in the distilled water group ( $p = 0.001$ , [Table 3](#)). Histopathological examinations demonstrated statistically significant differences in fibrosis ( $p = 0.002$ ) and inflammation ( $p = 0.003$ ) severity favoring the green tea group ([Table 4](#)). [Photographs 3 and 4](#) illustrate sample histological pictures of histopathological criteria, i.e., fibrosis and inflammation, in both groups.



**Photograph 1.** No adhesion in intervention group.

**Table 3**  
The comparison of adhesion degrees' frequencies between the two groups.

Adhesion degree	Intervention group number (frequency percent)	Control group number (frequency percent)
0	5 (33.33%)	0 (0%)
1	0 (0%)	0 (0%)
2	0 (0%)	0 (0%)
3	6 (40%)	2 (13.33%)
4	0 (0%)	0 (0%)
5	0 (0%)	2 (13.33%)
6	0 (0%)	3 (20%)
7	2 (13.33%)	0 (0%)
8	2 (13.33%)	2 (13.33%)
9	0 (0%)	2 (13.33%)
10	0 (0%)	2 (13.33%)
12	0 (0%)	2 (13.33%)
Mean score	3.2	7.33
Total	15 (100%)	15 (100%)

### 4. Discussion

The present study was conducted to examine the preventive effect of green tea alcoholic extract on intra-abdominal adhesion formation. The results suggest the efficacy of this extract on intra-abdominal adhesion prevention. Intra-abdominal adhesions are common following abdominal and pelvic surgery, causing potentially fatal complications such as ileus, intestinal obstruction and infertility.<sup>27,28</sup> In a study on 2295 patients with small bowel obstruction, 64% cases had intra-abdominal adhesions and 84% of them had history of intra-abdominal surgery.<sup>29</sup> It should be noted that post-operative adhesion formation is favored by any factor which causes decreased tissue oxygenation, leading to ischemia<sup>30</sup> and free oxygen radical formation.<sup>31</sup> The source of superoxide and other free radicals can be mitochondrial cytochrome oxidase, in endothelial cells of different tissues, causing tissue damage.<sup>31</sup> Hydrogen peroxide and superoxide anion are both toxic for some cells such as endothelial cells, platelets, and fibroblasts because of extra-cellular cytolysis derived from cell membrane fat cytolysis and peroxidation, increase in vascular permeability, and exudate formation, which may initiate adhesion formation.<sup>18,32,33</sup>

No pharmacologic intervention has been so far able to completely prevent intra-abdominal fibrosis. Possibly, reduction of free radicals has been achieved through green tea's polyphenolic



**Photograph 2.** Adhesion bands in control group.

**Table 4**  
The comparison of (mean  $\pm$  standard) deviation of adhesion degree between the two groups.

Group	Adhesions' scaling		
	Total scores of macroscopic criteria	Fibrosis	Inflammation
Green tea (A)	3.503 $\pm$ 3.2	0.941 $\pm$ 0.8	0.775 $\pm$ 0.8
Control (B)	0.51 $\pm$ 7.33	0.961 $\pm$ 2.067	0.961 $\pm$ 1.933

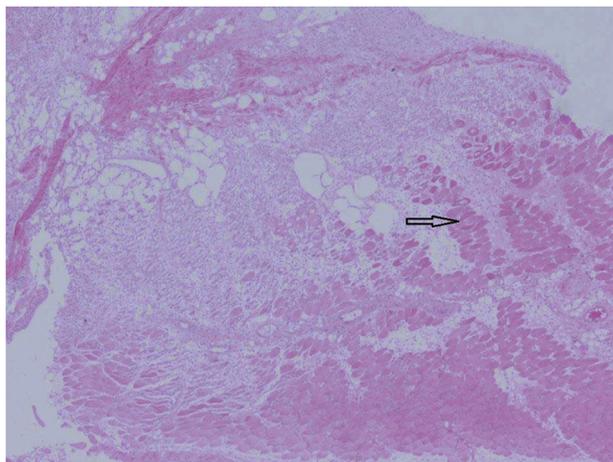
compounds (such as catechin), which are capable of absorbing free radicals. This is supported by the very low inflammation severity of pathological samples obtained from intervention cases in compare to control ones. In Saffari and Sadrzadeh's work, the anti-oxidant effects of these compounds have been demonstrated.<sup>34</sup> We used distilled water as control because no report has observed any relationship between distilled water exposure and adhesion formation.<sup>25</sup>

On the other hand, theophylline, L-theanine, tannin, oxalic acid, gallic acid, pectin, fluoride, minerals as well as vitamins B1, B2, C and E are among other green tea's compounds,<sup>34–36</sup> which might be helpful for intra-abdominal adhesion prevention via inhibition of collagen production and accumulation in addition to complete fibrin lysis.<sup>10,16</sup> The statistically significant difference in fibrosis severity between the two groups could be resulted from this advantage of green tea.

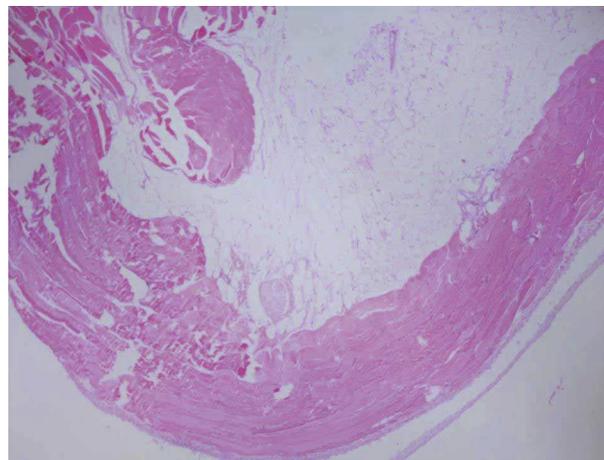
Green tea's wound healing effects have also been shown on cutaneous wounds and ulcers.<sup>22,37</sup> Although numerous studies targeted at treating these lesions have been conducted, the agents with anti-oxidant effects have not been satisfactorily investigated. However, the positive inhibitory effects of fat peroxidation on rabbit intra-abdominal adhesions and melatonin's anti-oxidant-associated effects on post-laparotomy adhesions in rats, consistent with the present study's findings, have been demonstrated.<sup>18,38</sup> Further experimental work to evaluate the potential of green tea as an adhesion prevention agent is warranted.

#### Ethical approval

All experiments were performed under supervision of Dr. Shahriyar Adibi and Prof. Mahmoud Rafeieian-kopaei, in accordance to the guidelines of the Animal Ethics Committee of Shahrekord University of Medical Sciences by the number of: 90-5-3.



**Photograph 3.** In the control group, the infiltration of inflammatory cells and areas of fibrosis consisting of fibroblasts are seen (marked with arrow) in the histopathological examination (H/E staining) ( $\times 100$ ).



**Photograph 4.** In the intervention group, fewer inflammatory cells and decreased fibrosis are seen (H/E staining) ( $\times 100$ ).

#### Funding

This research was supported by Deputy of Research of Shahrekord University of Medical Sciences and Deputy of Research of Islamic Azad University, Shahrekord Branch.

#### Author contribution

**Pouya Parsaei:** Experimental works (Animal study and medical plants extraction), data collecting and article writing.

**Mehrdad Karimi:** Determination of surgical and anesthesia Procedure and its methods.

**Sayyed Yazdan Asadi:** Experimental works (Extract Preparation, Determination of components and animal study), Data analysing, Article writing.

**Mahmoud Rafeieian-kopaei:** Supervisor of research plan, Study design, Determination of Antioxidant Activity and article writing.

#### Conflict of interest

None declared.

#### Acknowledgments

This work was financially supported by Research Deputy of SKUMS, Shahrekord, Iran (grant No. 960) and Young researchers club, Islamic azad university, Shahrekod, Iran. We would like to thank Dr Shahriyar Adibi (DVM) and all people who helped us throughout the study.

#### References

- Schindler AE. Gonadotropin-releasing hormone agonists for prevention of postoperative adhesion: an overview. *Gynecol Endocrinol* 2004;**19**(1):51–5.
- Monk BJ, Bertram ML, Montz FJ. Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention. *Am J Obstet Gynecol* 1994;**170**(5):1396–403.
- Gonenci R, Altug ME, Koc A, Yalcin A. Effects of bovine amniotic fluid on acute corneal alkali burns in the rat. *J Anim Vet Adv* 2009;**8**(4):617–23.
- Aldemir M, Ozturk H, Erten C, Buyukbayram H. The preventive effect of rofecoxib in postoperative intraperitoneal adhesions. *Acta Chir Belg* 2004;**104**(1):97–100.
- Cetin M, Ak D, Duran B, Cetin A, Guvenal T, Yanar O. Use of methylene blue and N, O-carboxymethylchitosan to prevent postoperative adhesions in a rat uterine horn model. *Fertil Steril* 2003;**80**(2):698–701.
- Alatas E, Gunal O, Alatas O, Colak O. Octreotide prevents postoperative adhesion formation by suppressing peritoneal myeloperoxidase activity. *Hepato-gastroenterology* 2000;**47**(34):1034–6.
- Okamoto Y, Takai S, Miuzaki M. Chymase inhibitor suppresses adhesion formation in a hamster experimental model. *Eur J Pharmacol* 2002;**435**(2–3):265–7.

8. Kim HR, Rajaiah R, Wu QL, et al. Green tea protects rats against autoimmune arthritis by modulating disease-related immune events. *J Nutr* 2008;**138**(11): 2111–6.
9. Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E. Why drinking green tea could prevent cancer. *Nature* 1997;**387**(6633):567.
10. Park G, Yoon BS, Moon JH, et al. Green tea polyphenol epigallocatechin-3-gallate suppresses collagen production and proliferation in keloid fibroblasts via inhibition of the STAT3-signaling pathway. *J Invest Dermatol* 2008;**128**(10):2429–41.
11. Hamaishi K, Kojima R, Ito M. Anti-ulcer effect of tea catechin in rats. *Biol Pharm Bull* 2006;**29**(11):2206–13.
12. Katiyar SK, Afaq F, Perez A, Mukhtar H. Green tea polyphenol epigallocatechin 3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. *Carcinogenesis* 2001;**22**(2):287–94.
13. Rafieian-Kopaei M, Baradaran A, Rafieian M. Plants antioxidants: from laboratory to clinic. *J Nephropathol* 2013;**2**(2):152–3.
14. Chan MMY, Dunne F, Ho CT, Huang HI. Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. *Biochem Pharmacol* 1997;**54**(12):1281–6.
15. Yen GC, Chen HY. Scavenging effect of various tea extracts on superoxide derived from the metabolism of mutagens. *Biosci Biotechnol Biochem* 1998;**62**(9):1768–70.
16. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J Nutr* 2003;**133**(10):3275–84.
17. Asadi SY, Parsaei P, Karimi M, et al. Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat. *Int J Surg* 2013;**11**(4):332–7.
18. Movahedi M, Hajhashemi M, Adibi Sh. Effect of melatonin in the prevention of post-operative adhesion formation in an experimental study. *J Isfahan Med Sch* 2008;**25**(87):1–7.
19. Maghsoudi A, Baradaran-Ghahfarokhi M, Ghaed-Amini F, Nasri H, Dehghani-Mobarekeh M, Rafieian-Kopaei M. Renal failure and submental lymphadenopathy in a 68 years old woman. *J Nephropathol* 2012;**1**(3):198–201.
20. Zamanzad B, Karimi A, Nafisi MR, Shirzad H. Clinical and bacteriologic correlates of the PapG alleles among uropathogenic *Escherichia coli* strains isolated from cases of adult urinary tract infection. *Kuwait Med J (KMJ)* 2012;**44**(1):26–30.
21. Shirzad H, Shahrani M, Rafieian-Kopaei M. Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes in vivo. *Int Immunopharmacol* 2009;**9**(7–8):968–70.
22. Rafieian-Kopaei M, Nasri H, Nematbakhsh M, et al. Erythropoietin ameliorates gentamicin-induced renal toxicity: a biochemical and histopathological study. *J Nephropathol* 2012;**2**(1):109–16.
23. Sharafati R, Sherafati F, Rafieian-Kopaei M. Biological characterization of Iranian walnut (*Juglans regia*) leaves. *Turk J Biol* 2011;**35**:635–9.
24. Mirzaei MGh, Sewell RDE, Kheiri S, Rafieian-Kopaei M. A clinical trial of the effect of St. John's wort on migraine headaches in patients receiving sodium valproate. *J Med Plants Res* 2012;**6**(9):1519–23.
25. Abbasian B, Kazemini H, Esmaeili A, Adibi S. Effect of bovine amniotic fluid on intra-abdominal adhesion in diabetic male rats. *J Diabetes Complicat* 2011;**25**(1):39–43.
26. Celepli S, Kismet K, Kaptanoğlu B, et al. The effect of oral honey and pollen on postoperative intraabdominal adhesions. *Turk J Gastroenterol* 2011;**22**(1):65–72.
27. Evers BM. Small bowel. In: Townsend CM, Beauchamp RD, Evers BM, Mattox KL, editors. *Sabiston textbook of surgery*. 16th ed. Philadelphia: Saunders; 2001. p. 882–3.
28. Solomkin JS, Wittman DW, West MS, Barie PS. Intraabdominal infections. In: Schwartz SI, Shires GT, Spencer FC, Daly JM, Fischer IE, Galloway AC, editors. *Principles of surgery*. 7th ed. New York: McGraw-Hill; 1999. p. 1515–50.
29. Räf LE. Causes of small intestinal obstruction. A study covering the Stockholm area. *Acta Chir Scand* 1969;**135**(1):67–72.
30. Gutmann J, Penzias AS, Diamond MP. Adhesions in reproductive surgery. In: Wallach EE, Zacur HA, editors. *Reproductive medicine and surgery*. St. Louis: Mosby; 1995. p. 681–93.
31. Bertuglia S, Colantuoni A, Intaglietta M. Effect of leukocyte adhesion and microvascular permeability on capillary perfusion during ischemia-reperfusion injury in hamster cheek pouch. *Int J Microcirc Clin Exp* 1993;**13**(1):13–26.
32. Ley K, Arfors KE. Changes in macromolecular permeability by intravascular generation of oxygen-derived free radicals. *Microvasc Res* 1982;**24**(1):25–33.
33. Vipond MN, Whawell SA, Thompson JN, Dudley HA. Peritoneal fibrinolytic activity and intra-abdominal adhesions. *Lancet* 1990;**335**(8698):1120–2.
34. Saffari Y, Sadrzadeh SMH. Green tea metabolite EGCG protects membranes against oxidative damage in vitro. *Life Sci* 2004;**74**(12):1513–8.
35. Zhang MH, Luypaert J, Fernández Pierna JA, Xu QS, Massart DL. Determination of total antioxidant capacity in green tea by near-infrared spectroscopy and multivariate calibration. *Talanta* 2004;**62**(1):25–35.
36. Dona M, DellAice I, Calabrese F, et al. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. *J Immunol* 2003;**170**(8):4335–41.
37. Kapoor M, Howard R, Hall I, Appleton I. Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats. *Am J Pathol* 2004;**165**(1):299–307.
38. Rodgers KE, Girgis W, St Amand K, Campeau J, diZerega GS. Reduction of adhesion formation in rabbits by intraperitoneal administration of lazard formulations. *Hum Reprod* 1998;**13**(9):2443–51.