Egypt. J. Exp. Biol. (Zool.), 6(1): 117 – 127 (2010)

RESEARCH ARTICLE

* Safia M. Hassan
* Ashour A. Abdel Mawla
**Sameh IH. Zaki
*Atef AA. Abd El-All

EFFECT OF GREEN TEA EXTRACT ON THE APOPTOTIC AND ANTI-ANGIOGENIC PHENOMENA IN PERIPHERAL BLOOD OF BREAST CANCER PATIENTS

ABSTRACT:
Green tea is natural dried leaves of the tea plant, *Camellia sinensis*. Green tea extract is bioflavonoid-rich compound, with several phenol groups. The dominant polyphynol (catechin) in green tea is epigallocatechin gallate (EGCG) which is a potent antioxidant. In the case of breast cancer, catechins were in fact shown to interfere with the binding of estrogen to estrogen receptors. This study evaluated the green tea extract effect on breast cancer patients using morphological and immunocytochemical techniques. It included 3 groups, 2 treated groups and one control group. Chemotherapy for 6 cycles was administered plus 1000mg green tea extract /day or alone. Blood smears were applied from both control and treated groups either preradicalmastectomy, or post 3 and 6 cycles of treatment. Apoptotic and antiangiogenic effects were revealed through Leishman's stain and immunocytochemical technique of both vascular endothelial growth factor (VEGF) and nuclear factor kappa B (NF_kB). Morphological results using Leishman's stain illustrated that the apoptosis in blood smears of women administered with green tea extract was more prominent than that of their corresponding without green tea extract administration or of control women. Immunocytochemical findings showed that there was a decrease in VEGF & NF_kB in women administered green tea extract as compared with their partners of control group as well as those treated with chemotherapy alone. In conclusion, green tea played a dual role in decreasing the angiogenesis while it increased apoptosis. This revealed that green tea as adjuvant therapy showed a positive effect.

KEY WORDS:
Human breast cancer, Green tea, immunocytochemistry, VEGF, NF-$_k$B

CORRESPONDANCE:
* Safia M. Hassan
*Histochemistry & Cell Biology Department, Medical Research Institute, Alexandria University
E-mail: dr_safiahassan@yahoo.com

*Histochemistry & Cell Biology Department, Medical Research Institute, Alexandria University
**Oncology Research and Treatment Departments, Medical Research Institute, Alexandria University

ARTICLE CODE: 14.01.10

INTRODUCTION:

Green tea extract is bioflavonoid-rich compound, which is used primarily for fighting free radicals (Boehm *et al*., 2009). The common name for green tea extract is epigallocatechin gallate (EGCG) (Zhang *et al*., 2009). This polyphenols group increases antioxidant activities in the blood and is also associated with protection against atherosclerosis (Miura *et al*., 2001). It is worth mentioning that EGCG helps block the cancer-promoting actions of carcinogens, ultraviolet light, and metastasis of the malignant tissue from original site in the skin, stomach, small intestine, liver or lung (Suganuma and Okabe, 1999). It was reported that people who drink green tea had significantly lower risk of cancer and some studies have shown that green tea blocked the formation of tumour cells (Sazuka and Imazawa, 1997). In particular, EGCG alone was also found to inhibit tumour growth of human breast cancer transplanted into mice (Fassina *et al*., 2004). In case of breast cancer, catechins, the active phenol groups found in green tea were in fact shown to interfere with the binding of estrogen to estrogen receptors. Catechins were also found to inhibit the release of tumour necrosis factor alpha (TNF-alpha), a highly inflammatory cytokine block angiogenesis (Yang, 1998).

In normal tissues, VEGF expression has been found in activated macrophages, RBCs, keratinocytes, renal glomerular visceral epithelium and mesangial cells (Patan, 2004). It has been also found in hepatocytes, smooth muscle cells, Leydig cells, embryonic

fibroblasts, and bronchial and choroid plexus epithelium (Patan, 2004).

NF-κB regulates expression of genes that participate in immune apoptotic and oncogenic processes (Bhat-Nakshatri, 2002). In some normal cells, such as B cells, some T cells, Sertoli cells and some neurons, NF-κB is constitutively located in the nucleus or at the centre of the cells as in case of RBCs. In addition, in many cancer cells including breast cancer, multiple mylomas, colon cancer, prostate cancer, lymphoid cancers, and many others NF-κB is also located in the nuclei of their cells (Gilmore, 2006).

The goal of the present study is to determine the efficacy of green tea polyphenols extract (Epigalocatechin) in the management of patients with breast cancer through immunocytochemical investigations of its anti–angiogenesis and apoptotic effects.

MATERIAL AND METHODS:

This study included 40 women. 10 volunteers of apparently normal breast tissues with comparable characteristics served as control cases (group A), and 30 were breast cancer women subjected to modified radical mastectomy and equally divided into groups B and C. All patients were admitted to Clinical Oncology Department in Medical Research Institute, Alexandria University. .

Patients of group B received 6 cycles (21 days for each cycle) of adjuvant chemotherapy in the form of cyclophosphamide 500mg/m^2, adriamycin 50 mg/m^2 and 5-flurouracil 500 mg/m^2.

On the other hand, patients of group C received 6 cycles of the same adjuvant chemotherapy plus one tablet of green tea extract equivalent to 1000 mg of EGCG/ day.

Blood smears of all cases-both the control and the patients-were subjected to the following parameters:-

1- Morphological study (Leishman's method) (Henry, 2001).
2- Immunocytochemical study for both VEGF and NF_kB: by using Biotin and Trividine technique (Turley *et al*., 1998).

After fixation and washing of slides, the primary monoclonal antibody was applied either for VEGF or NF-κB, and then covered with secondary antibody. When DAB chromogen was added it appears as dark brown granules at the site of antigen antibody reaction.

Three samples of blood were taken to examine VEGF and NF-κB. The first sample of blood was taken at pre. Mastectomy, the second sample was after 3 cycles, and the third sample was after 6 cycles for all patients of both groups B and C.

The samples of the peripheral blood were taken from the ten normal women (group A) at the time of sampling concurrently with that of the patients in groups B and C.

The reaction intensity was graduated according to the following stainability grades:

*High concentration: +3

* Moderate concentration: +2

*Low concentration: +1

* Negative to stain: 0

According to Tumour size, lymph nodes, and metastases (TNM), the patients in both groups were classified as shown in the following table:

Table 1. The stages of groups B and C

Stages	Groups	
	Group B	Group C
Stage I	1	2
Stage II	5	8
Stage III	9	5

RESULTS:

Morphological results

Leishman stain demonstrated the apoptosis in the white and red blood cells. The three groups of the study gave the following results:

Group A:

Normal control blood smears showed the normal morphological characters of white and red cells (Fig. 1).

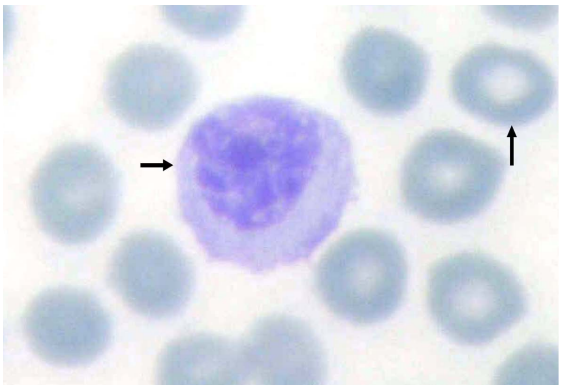

Fig. 1. Normal peripheral blood smear from group "A" showing characteristic appearance of normal RBCs (↑) and monocyte with characteristic large eccentric nucleus. Its cytoplasm contains numerous small purple granules (→). Note the pale staining of central region of RBCs indicating their bioconcave disc shape. Leishman's method-X 2000

Group B:

Less apoptotic changes could be detected in the three films which were prepared for the pre-treatment state and, after both three and six cycles of treatment. These changes included RBCs of abnormal shapes and WBCs of variable nuclear change (Fig. 2).

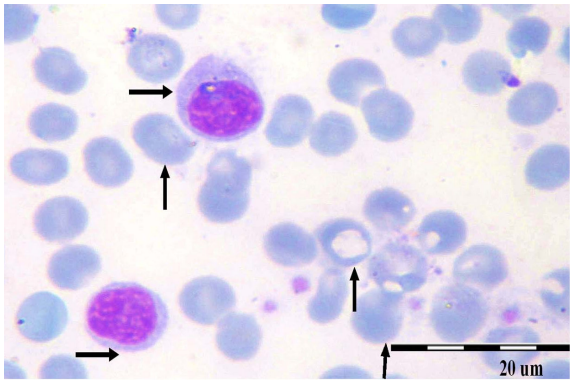

Fig. 2. Peripheral blood smear of a patient from group "B" after 6 cycles treatment showing abnormal shape of some RBCs (↑) and variable nuclear shape of lymphocytes (→) with delicate chromatin. Leishman's method

Group C:

The apoptotic features in this group were manifested after 3 cycles as multivacuole and granulation in RBCs and highly lobulated nucleus, as in case of neutrophils, and delicate chromatin, as in case of lymphocytes (Fig. 3). The degree of apoptotic changes was increased as compared with that of the pre-treated patients in the same group (Figs 3 & 4). After six cycles, the apoptosis became more prominent including the blebs of cell membrane and condensation of chromatin in neutrophil (Fig. 5).

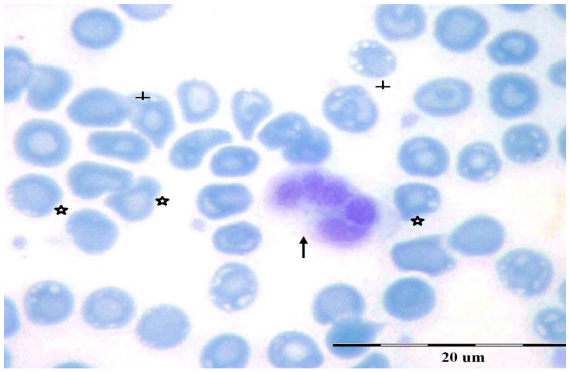

Fig. 3. Peripheral blood smear of a patient from group "C" at pre-treatment stage showing abnormal shape of some RBCs (*) with multivacuoles (+) and neutrophil with cell membrane changes and highly lobulated nucleus (Leishman's method

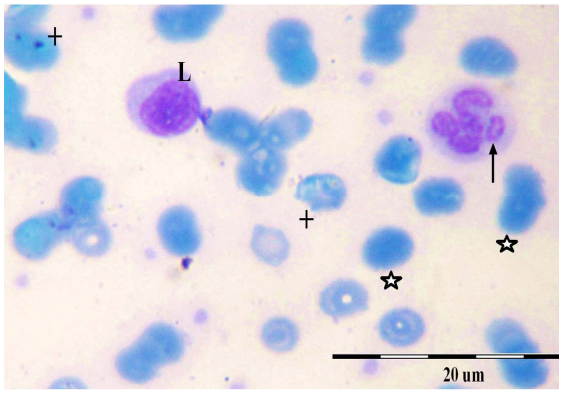

Fig. 4. Peripheral blood smear of a patient from group "C" after 3 cycles treatment showing abnormal shape of some RBCs (*) and granulation in others (+). Note the apoptotic changes in the highly segmented neutrophil which appears as chromatin aggregation around the nuclear membrane (↑). Also, notice the delicate chromatin in the lymphocyte (L) Leishman method

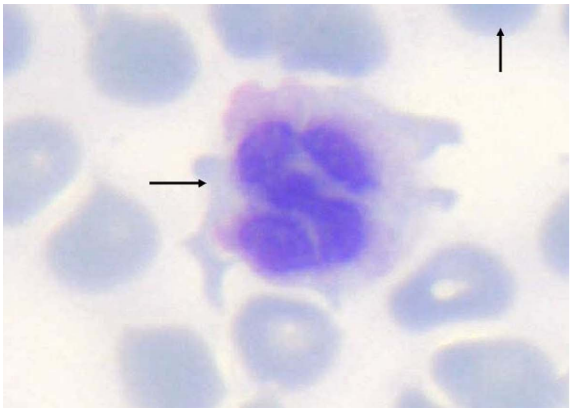

Fig. 5. Peripheral blood smear of a patient from group "C" in breast cancer after 6 cycles treatment showing the blebs of cell membrane and condensation of chromatin of the neutrophil (→). Leishman method-X 2000

Immunocytochemical results

Results of VEGF (Tables 2, 3, and 4):

VEGF was demonstrated as brown granules which were distributed around the cell membrane and in the extracellular matrix in red and white blood cells.

Table 2: VEGF Result of group B

Expression	Samples		
	Pre- mastectomy	After 3 cycles	After 6 cycles
High	15 (9 stage III + 5 stage II + 1 stage I)	6 (6 stage III)	0
Moderate	0	8 (3 stage III + 5 stage II)	10 (9 stage III + 1 stage II)
Low	0	1 (1 stage I)	5 (4 stage II+ 1 stage I)

Table 3. VEGF Results of group C

Expression	Samples		
	Pre- mastectomy	After 3 cycles	After 6 cycles
High	13 (5 stage III + 8 stage II)	3 (stage III)	1 (1 stage III)
Moderate	2 (stage I)	10 (2 stag III+8 stage II)	6 (4 stage III+2 stage II)
Low	0	2 (stage I)	8 (6 stage II + 2 stage I)

The overall percentages in VEGF expression were summarized in the following table.

Table 4. VEGF expression percentage in groups A, B & C

Expression	Groups		
	Group A (normal)	Group B (Chemotherapy)	Group C (Chemotherapy + green tea)
Pre-treatment			
High		Moderate 100%	86.6%
Moderate	Moderate 100%	0%	13.4%
Low		0%	0%
After 3 cycles			
High		40%	20%
Moderate	Moderate 100%	53.4%	66.6%
Low		6.6%	13.4%
After 6 cycles			
High		0%	6.6%
Moderate	Moderate 100%	66.6%	40%
Low		33.4%	53.4%

Group A:

In normal control blood films, the expression of VEGF was moderate in all cases studied (Fig. 6).

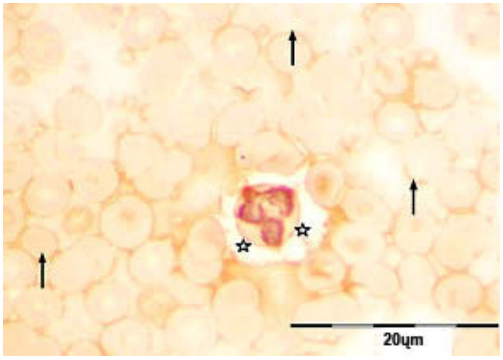

Fig. 6. Immunocytochemical staining of VEGF in blood smear of normal woman in group "A" showing moderate expression around the cell membrane either in RBCs (↑) or neutrophil (*). Biotin and Trividine technique

Group B

*Pre-chemotherapy: All patients of this group showed high expression of VEGF (Fig. 7).

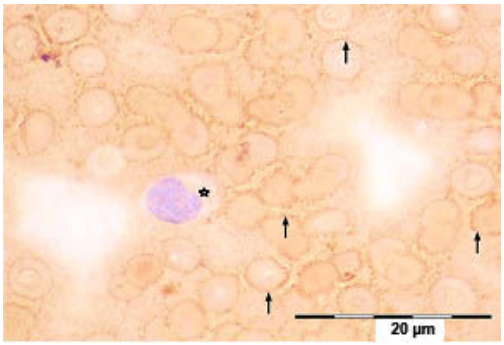

Fig. 7. Immunocytochemical staining of VEGF in blood smear of a patient in group B in stage III before radical mastectomy. Note the high intensity expression of VEGF in RBCs (arrows) and lymphocyte (*). Biotin and Trividine technique

*After 3 cycles of chemotherapy: These patients showed high expression of VEGF in 6 cases (Fig. 8), moderate expression in 8 cases (Fig. 9) and low intensity found in only one case (Fig. 10).

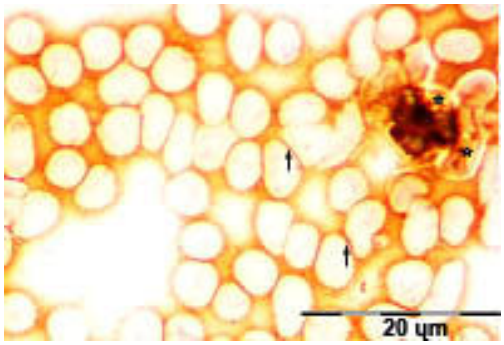

Fig. 8. Immunocytochemical staining of VEGF in blood smear of a patient in group "B" in stage III after radical mastectomy and three cycles chemotherapy, showing high expression in both RBCs (↑) and neutrophil (*). Biotin and Trividine technique

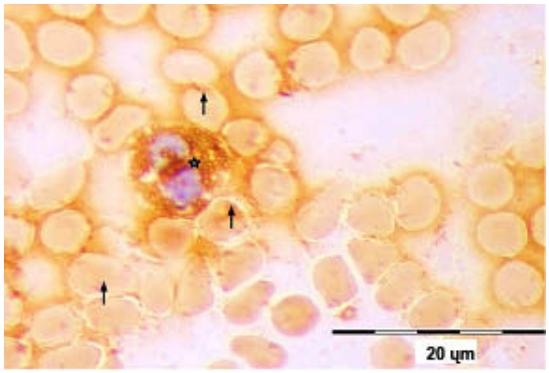

Fig. 9. Immunocytocochemical staining of VEGF in blood smear of a patient in group "B" in stage III after radical mastectomy and three cycles chemotherapy, showing moderate expression in RBCs (↑) and high expression in basophil (*). Note the karyolysis in one lobe of basophil nucleus. Biotin and Trividine technique

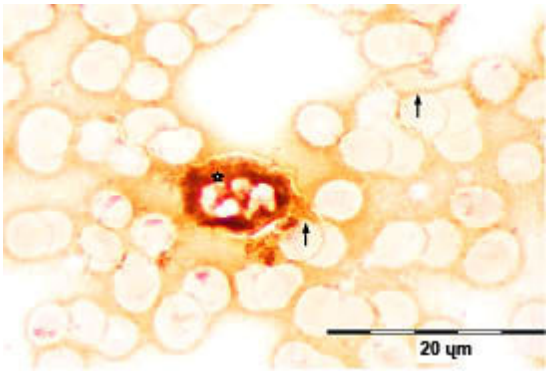

Fig. 10. Immunocytochemical staining of VEGF in blood smear of a patient in group "B" in stage I, after radical mastectomy and three cycles chemotherapy, showing low expression in RBCs (↑) and high expression in the neutrophil (*). Biotin and Trividine technique-X 1000

*After 6 cycles of chemotherapy: Moderate expression of VEGF was seen in 10

cases (9 patients in stage III and one patient in stage II) (Fig. 11) and low expression in 5 cases (Fig. 12).

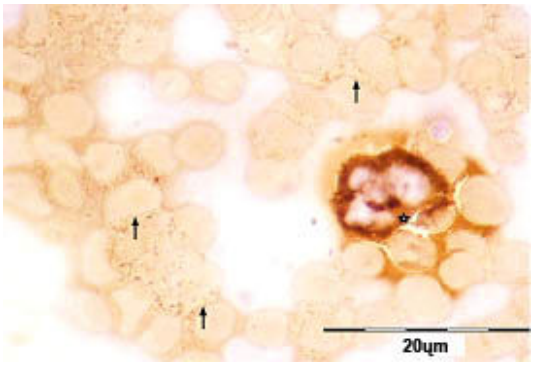

Fig. 11. Immunocytochemical staining of VEGF in blood smear of a patient in group "B" in stage III after radical mastectomy and six cycles chemotherapy showing moderate expression in the RBCs (arrows) and high in neutrophil (*). Biotin and Trividine technique

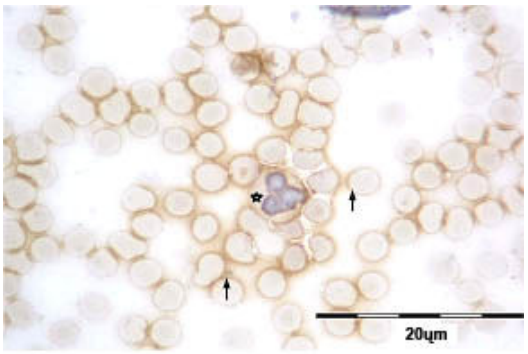

Fig. 12. Immunocytochemical staining of VEGF in blood smear of a patient in group "B" in stage I after radical mastectomy and six cycles chemotherapy, showing low expression in the RBCs (arrows) and neutrophil (*). Biotin and Trividine technique

Group C:

Pretreatment: The expression of VEGF was high in almost all blood samples of the 13 patients before any treatment; 8 in stage II and 5 in stage III (Fig. 13). While moderate expression was monitored in 2 cases (Fig. 14).

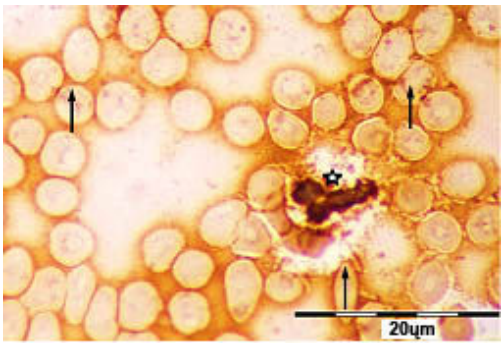

Fig. 13. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage III, before radical mastectomy, showing high expression in the cytoplasm and around the cell membrane of both RBCs (↑) and neutrophil (*). Biotin and Trividine technique

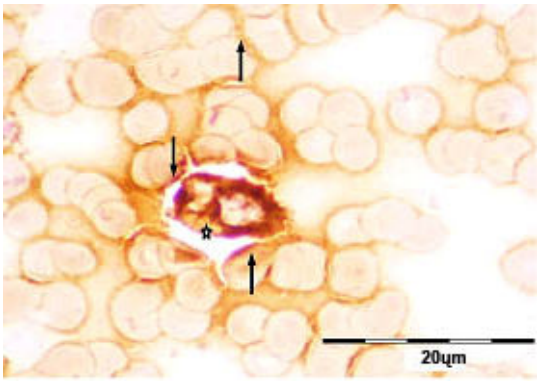

Fig. 14. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage I before radical mastectomy, showing moderate expression in RBCs (↑) and eosinophil (*). Biotin and Trividine technique

*After 3 cycles chemotherapy plus green tea. High expression in 3 cases; all in stage III (Fig. 15), moderate expression in 10 cases; 2 in stage III & 8 in stage II (Fig. 16) and low intensity in 2 cases were seen (Fig 17).

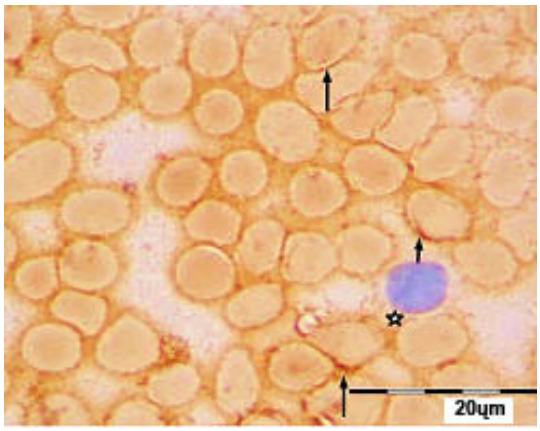

Fig. 15. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage III after radical mastectomy, three cycle chemotherapy and daily dose of green tea showing high expression in RBCs (arrows) and in lymphocyte (*). Biotin and Trividine technique

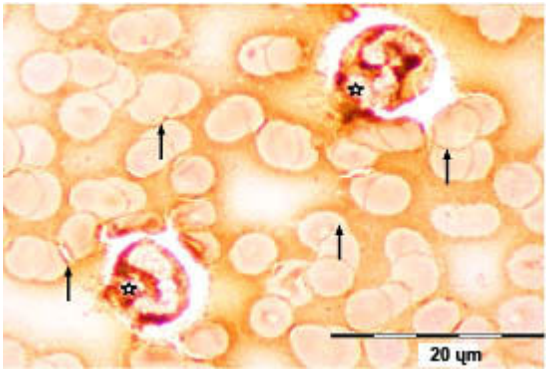

Fig. 16. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage II after radical mastectomy, three cycles chemotherapy and daily dose of green tea showing moderate expression in RBCs (arrows) and basophil (*). Note the aggregation of granules in the basophil. Biotin and Trividine technique

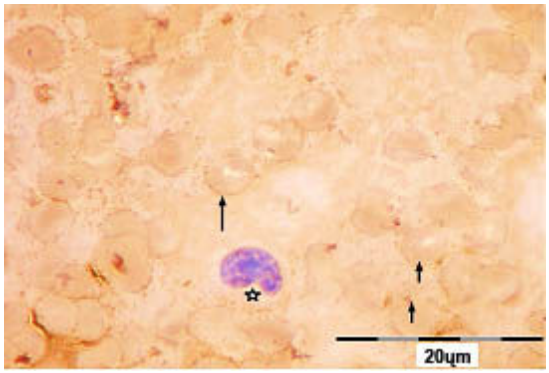

Fig. 17. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage I after radical mastectomy, three cycles chemotherapy and daily dose of green tea showing low expression in RBCs (arrows) and in basophil (*). Biotin and Trividine technique

After 6 cycles chemotherapy plus green tea. VEGF showed high expression in one case (Fig. 18), moderate intensity in 6 case (Fig. 19) and low expression in 8 cases (6 in stage II and 2 in stage I) (Fig. 20).

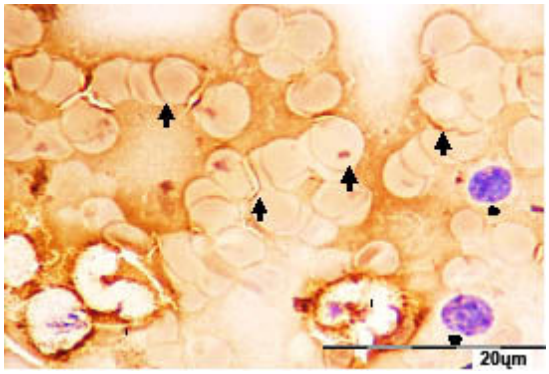

Fig. 18. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage III after radical mastectomy, six cycles chemotherapy and daily dose of green tea showing low expression in RBCs (↑) and in basophil (+) and low intensity in lymphocytes (*). Note the disintegration of basophil nuclei. (Biotin and Trividine technique

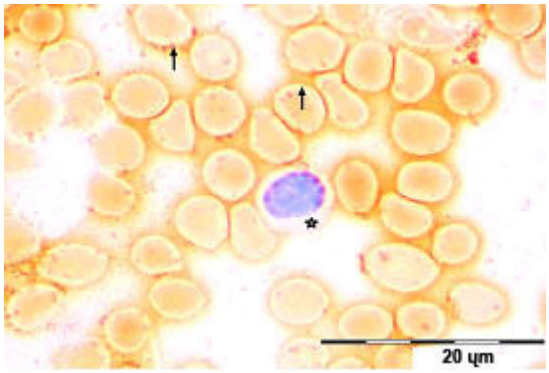

Fig. 19. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage II afterradical mastectomy, six cycles chemotherapy and daily dose of green tea showing moderate expression in RBCs (arrows) and low expression in the lymphocyte (*). The granules form a dot-like structure. Biotin and Trividine technique

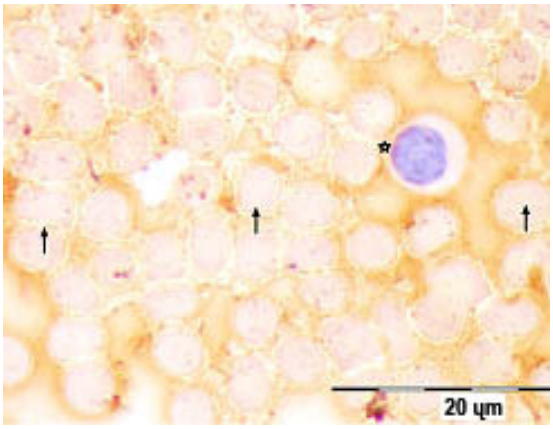

Fig. 20. Immunocytochemical staining of VEGF in blood smear of a patient in group "C" in stage II after radical mastectomy, six cycles chemotherapy and daily dose of green tea. Note the low VEGF expression in both RBCs (↑) and monocyte (*). Biotin and Trividine technique

Results of NF-κB (Tables 5, 6&7):

NF-κB was demonstrated as brown granules which were distributed in the centre of the cell, around the cell membrane and in the extracellular matrix of both RBCs and white blood cells, as well as around nuclear membrane of WBCs.

Table 5. NF-κB result of Group B

Expression	Samples		
	Pre- mastectomy	After 3 cycles therapy	After 6 cycles therapy
High	15 (9 stage III + 5 stage II + 1 stage I)	11 (9 stage III + 2 stage II)	1 (stage III)
Moderate	0	4 (3 stage II + 1 stage I)	9 (8 stage III + 1 stage II)
Low	0	0	5 (4 stage II + 1 stage I)

Table 6. NF-κB results of group C

Expression	Samples		
	Pre- mastectomy	After 3 cycles therapy	After 6 cycles therapy
High	12 (5 stage III + 7 stage II)	0	0
Moderate	3 (1 stage II + 2 stage I)	13 (5 stage III + 8 stage II)	5 (5 stage III)
Low	0	2 (2 stage I)	10 (8 stage II + 2 stage I)

The overall percentages in NF-$_{\kappa}$B expression are illustrated in table 7.

Table 7. NF-κB expression percentage in groups A, B & C

Expression	Groups		
	Group A (normal)	Group B (Chemotherapy)	Group C (Chemotherapy + green tea)
Pre-treatment			
High		100%	80%
Moderate	Moderate 100%	0%	20%
Low		0%	0%
After 3 cycles			
High		73.4%	0%
Moderate	Moderate 100%	26.6%	86.6%
Low		0%	13.4%
After 6 cycles			
High		6.6%	0%
Moderate	Moderate 100%	60%	33.4%
Low		33.4%	66.6%

Group A:

In normal control women the blood smears showed moderate intensity of NF-κB in all cases (Fig. 21).

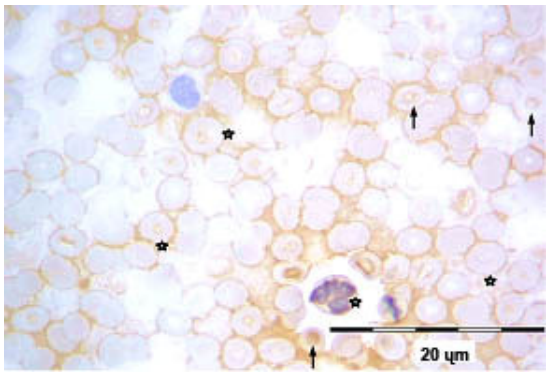

Fig. 21. Immunocytochemical staining of NF-κB in normal blood smear of normal woman in group "A" showing moderate expression in both RBCs and neutrophil. Note reaction at the centre of RBCs (arrows) and at the cell membrane (*). Biotin and Trividine technique

Group B:

Before chemotherapy: All cases of this group showed high intensity of NF-κB reaction (Fig. 22).

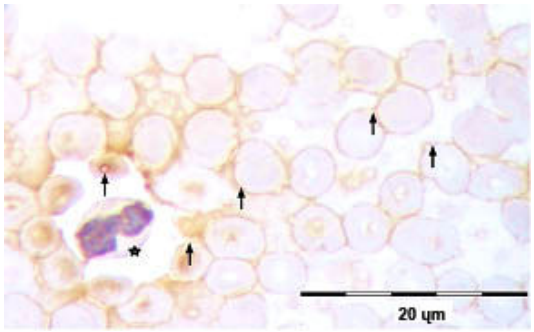

Fig. 22. Immunocytochemical staining of NF-κB in blood smear of a patient in group B in stage III, before radical mastectomy and chemotherapy, showing high expression in RBCs (arrows) and neutrophil (*). Biotin and Trividine technique

After 3 cycles of chemotherapy: NF-κB reactions showed high intensity in 11 cases (Fig. 23) and moderate intensity in 4 cases, 3 in stage II and one case in stage I (Fig. 24).

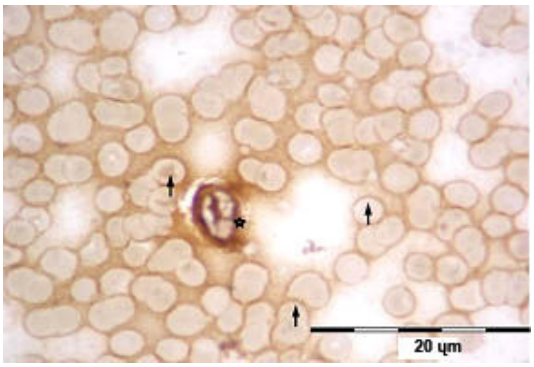

Fig. 23. Immunocytochemical staining of NF-κB in blood smear of a patient in group "B" in stage III, after radical mastectomy and three cycles chemotherapy, showing high expression in RBCs (arrows) and neutrophil (*). Biotin and Trvidine technique

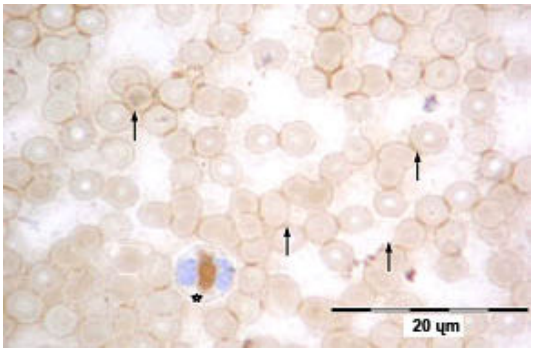

Fig. 24. Immunocytochemical staining of NF-κB in blood smear of a patient in group "B" in stage II, after radical mastectomy and three cycles chemotherapy, showing moderate expression in RBCs (arrows) and eosinophil (*). Biotin and Trividine technique

After 6 cycles of chemotherapy: High intensity in one case (Fig. 25), moderate intensity of the reaction was found in 9 cases, 8 in stage III and 1 in stage II (Fig. 26) and 5 case is low intensity (Fig. 27).

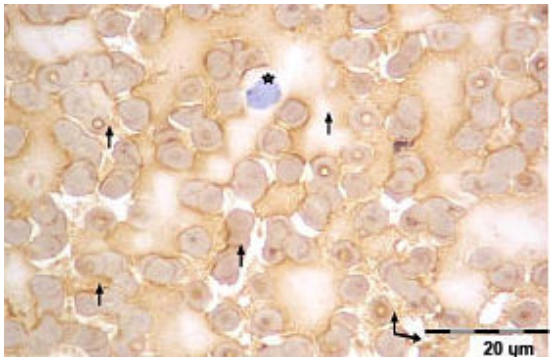

Fig. 25. Immunocytochemical staining of NF-κB in blood smear of a patient in group "B" in stage III, after radical mastectomy and six cycles chemotherapy, showing high expression in RBCs (arrows) and monocyte (*). Biotin and Trividine technique

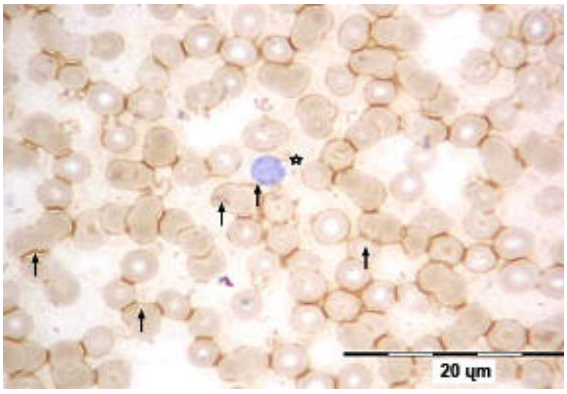

Fig. 26. Immunocytochemical staining of NF-κB in blood smear of a patient in group "B" in stage III, after radical mastectomy and six cycles chemotherapy, showing moderate expression in RBCs (arrows) and lymphocyte (*). Biotin and Trividine technique

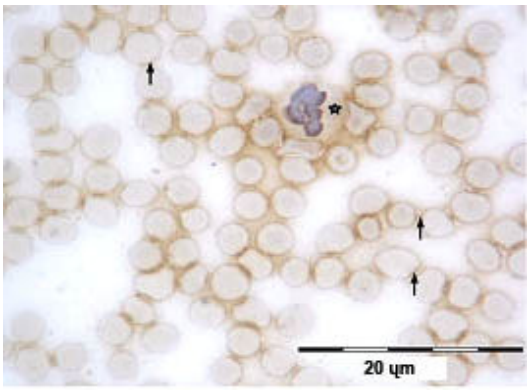

Fig. 27. Immunocytochemical staining of NF-κB in blood smear of a patient in group "B" in stage II, after radical mastectomy, six cycles chemotherapy, showing low expression in RBCs (arrows) and neutrophil (*). Biotin and Trividine technique

Group C:

Before chemotherapy and green tea: High intensity of the reaction of NF-κB was found in 12 cases, 5 cases in stage III, and 7 cases in stage II (Fig. 28) and moderate intensity in 3 cases (Fig. 29).

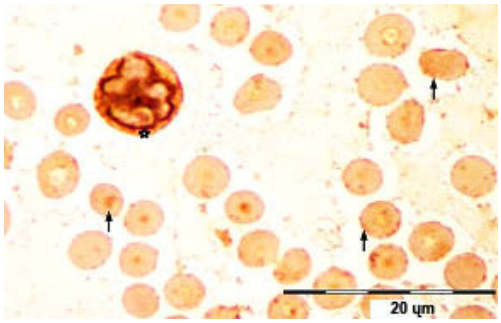

Fig. 28. Immunocytochemical staining of NF-κB in blood smear of a patient in group "C" in stage III before radical mastectomy, showing high expression in both RBCs (arrows) and neutrophil (*). Biotin and Trividine technique

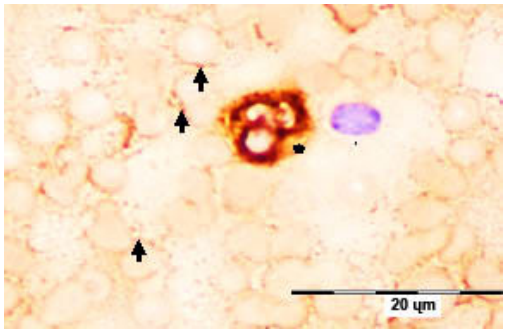

Fig. 29. Immunocytochemical staining of NF-κB in blood smear of a patient in group "C" in stage I before radical mastectomy, showing moderate expression in both RBCs (arrows) and neutrophil (*). The monocyte shows low expression (+). Biotin and Trividine technique

After 3 cycles of chemotherapy and green tea: NF-κB reaction showed the moderate intensity in 13 cases, 5 in stage III and 8 in stage II (Fig. 30) and low intensity in 2 cases (Fig. 31).

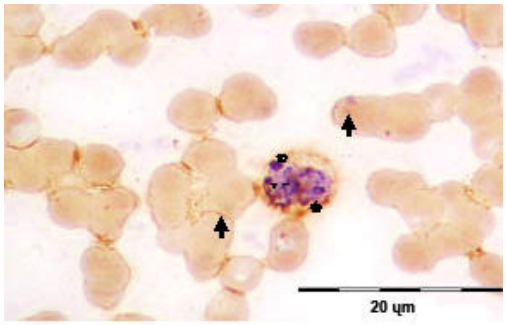

Fig. 30. Immunocytochemical staining of NF-κB in blood smear of a patient in group "C" in stage II after radical mastectomy, three cycles chemotherapy and daily dose of green tea showing moderate expression in both RBCs (↑) and lymphocyte (*). Note the karyorrhexis of lymphocyte (K). Biotin and Trividine technique

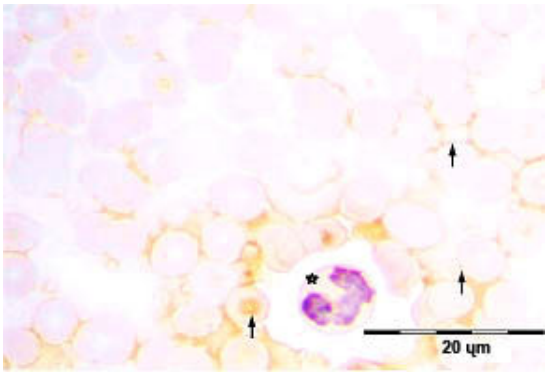

Fig. 31. Immuncytochemical staining of NF-κB in blood smear of a patient in group "C" in stage I, after radical mastectomy, three cycles chemotherapy and daily, dose of green tea showing low expression in RBCs (arrows) and neutrophil (*). Biotin and Trividine technique

After 6 cycles of chemotherapy and green tea: the patients showed moderate intensity of NF-κB in 5 cases, all in stage III (Fig. 32) and low intensity in 10 cases (Fig. 33).

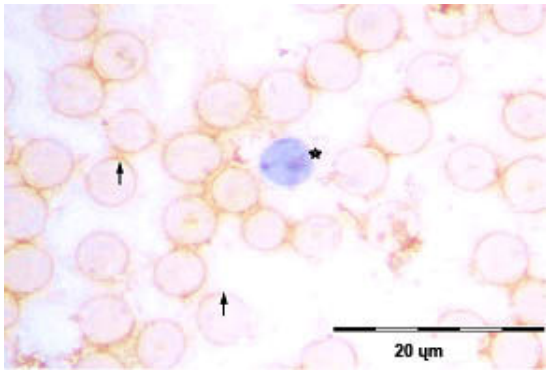

Fig. 32. Immunocytochemical staining of NF-κB in blood smear of a patient in group "C" in stage III, after radical mastectomy, six cycles chemotherapy and daily dose of green tea showing moderate expression in RBCs (arrows) and lymphocyte (*). Biotin and Trividine technique

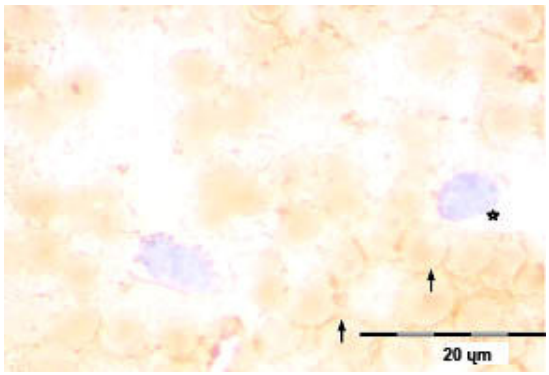

Fig. 33. Immuncytochemical staining of NF-κB in blood smear of a patient in group "C" in stage II, after radical mastectomy, six cycles chemotherapy and daily dose of green tea showing low expression in RBCs (arrows) and lymphocyte (*). Biotin and Trividine technique

DISCUSSION:

Some studies have shown that populations which drink green tea have low rates of breast cancer (Kavanagh and Helin, 2001). An approximate 70% decrease in tumour weight, and a slower tumour development were found in rats drinking tea. Also, the tumours in the tea-drinking rats were less malignant (Kavanagh and Helin, 2001).

It was found that premenopausal women who consumed more green tea had a lower number of lymph node metastases. In postmenopausal women, the greater consumption of green tea was correlated with increased expression of the estrogen and progesterone receptors, which implied more differentiated tumour cells and better prognosis. Finally, in the seven-years follow up, it was found that women with cancer of stage I or II who consumed five or more cups of green tea /day had approximately half the recurrence rate of those women who consumed four cups or less (Rahman and Sarkar, 2005).

It must be recognized that the cancer-protective effect of green tea is only apparent at the relatively high intake of more than 10 cups per day (Rahman and Sarkar, 2006).

The main tumor-inhibitory mechanism of green tea was suggested through many ways. First, its ability to interfere with the tumour form of the enzyme quinol oxidase called t-NOX, or tumor-associated NOX (Jung *et al*., 2001).

Second, is by enhancing glucuronization of estrogens in the liver, a process through which estrogens are rendered inactive by being conjugated with glucuronic acid, a form in which they are excreted from the body. Perhaps, this is found in Japanese women who consume a significant amount of green tea (Ahmed, 1997).

Third, is by inhibition of gelatinase and proteolytic enzymes as urokinase (uPA) that makes tumour cells able to invade cells and form metastases (Jankun, 1997; Sartippour *et al*., 2002; Gupta *et al*., 2004)

Fourth, its inhibition to special enzymes called collagenases which are secreted by cancer cells in order to penetrate and colonize various tissues (Templeton *et al*., 1990).

Fifth, its responsibility for the suppression of angiogenesis (Yang *et al*., 2001).

The present study showed that green tea was highly effective to suppress angiogenesis and increasing the apoptosis process. In this study the angiogenesis and apoptosis were measured by the expression of VEGF receptors and NF-KB receptors. Decreasing the intensity of VEGF on the cell membrane and extracellular-matrix of the blood cells indicated a decrease in the angiogenesis process while decreasing the intensity of NF-κB in the centre of the cell, on the cell membrane and extracellular matrix of blood cells indicates an increase of the apoptosis process. This result means that green tea in therapeutic doses, when given daily in combination with chemotherapy showed a positive effect on decreasing angiogenesis process and increasing apoptosis.

In an accordance with our results, Amin *et al*. (2009) showed low expression of VEGF following the administration of green tea extract after and before the induction of mammary tumour cells of mice. Also Kondo *et al*. (2002) showed that VEGF receptor binding was inhibited by EGCG only while all the other polyphenols were ineffective. Yuasa *et al*. (2003) found that green tea extract inhibited the expression of VEGF receptors on the endothelial cells in human umbilical vein.

With respect to NF- $_{k}$B, EGCG may block its activation at two potential steps. First, as an antioxidant, it may inhibit signalling events upstream to I-κB kinase

enzyme (IKK) that results in decreased IKK activation. Secondly, its unique structure inhibits IKK activity. Both mechanisms would lead to inhibition of NF- $_k$B activation (Lin and Lin, 1997).

The current study measured NF-κB, which was found to be decreased in patients who consumed green tea with the chemotherapy.

The present results coincide with those of Zhao *et al*. (2006) which suggested that EGCG might be useful in treatment and prevention of breast cancer by inducing apoptosis. Also, Thamgapazham *et al*. (2007) suggested that green tea polyphones and EGCG treatment inhibit proliferation and induce apoptosis of MDA-MB-231 cells in-vitro and in-vivo.

EGCG induces apoptosis and promotes cell growth arrest by altering the expression of cell cycle regulatory proteins, activating killer caspases, and suppression NF-$_k$B activation Butt and Sultan (2009).

These results highlighted the role of green tea in the treatment via increasing the process of apoptosis in the breast cancer cells.

REFERENCES:

Ahmad N. 1997. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. Cancer, 89: 1881-1886.

Amin AM, Abdel-Mawla AA, Hassan SM, Sheta MI, Matar NA. 2009. Histopathological, immunohistochemical and ultra-structure studies of in vivo-cultured mammary tumour cells under the effect of green tea. Egypt. J. Exp. Biol. Zool., 5: 123-132.

Bhat-Nakshatri P, Sweeney CJ, Nakshatri H. 2002. Identification of signal transduction pathways involved in constitutive NF-KappaB activation in breast cancer cells. Oncogene, 21: 2066-2078.

Boehm K, Borrelli F, Ernst E, Habacher G, Hung SK, Milazzo S, Horneber M. 2009. Green tea (*Camellia sinensis*) for the prevention of cancer. Cochrane database Syst. Rev., 8: 5004.

Butt MS, Sultan MT. 2009. Green tea: Nature's defense against malignancies. Crit. Rev. Food Sci., 49: 463-473.

Fassina G, Vene R, Morini M, Minghelli S, Benelli R. 2004. Mechanisms of inhibition of tumour angiogenesis and vascular tumour growth by epigallocatechin-3-gallate. Clin. Cancer Res., 10: 4865-4873.

Gilmore TD. 2006. NF-KB: From basic research to human disease. Crit. Rev. Oncogenes, 51: 6679-6899.

Gupta S, Hastak K, Afag F, Ahmed N, Muktar H. 2004. Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappa B and induction of apoptosis. Oncogene, 23, 2507–2522.

Henry, JB. 2001. Clinical Diagnosis and Management by Laboratory Methods. 20th Edn. Philadelphia, PA: Saunders, 100: 220-225.

Jankun J. 1997. Why drinking green tea could prevent cancer. Nature, 387: 561.

Jung YD, Kim M, Shin BA. 2001. EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br. J. Cancer, 84: 844-850.

Kavanagh KT, Helin HJ. 2001. Green tea extracts decrease carcinogen-induced mammary tumour burden in rats and rate of breast cancer cell proliferation in culture. J. Cell. Biochem., 82: 387-398.

Kondo T, Ohia T, Igura K, Hara Y, Kaji K. 2002. Tea catechins inhibit angiogenesis in vitro, measured by human endothelial cell growth, migration, and tube formation, through inhibition of VEGF receptor binding. Cancer Letts., 180: 139-144.

Lin YL, Lin JK. 1997. Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kB. Mol. Pharmacol., 52: 465-472.

Miura Y, Chiba T, Tomita I. 2001. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. J. Nutr., 131(1): 27-32.

Patan S. 2004. Vasculogenesis and Angiogenesis. Cancer Treat. Rev., 117: 3-32.

Rahman KW, Sarkar FH. 2005. Inhibition of nuclear translocation of nuclear factor-{n} B contributes to 3,3-diindolylmethane induced apoptosis in breast cancer cells. Cancer Res., 65: 364–371.

Rahman KW, Sarkar FH, Banerjee S. 2006. Therapeutic intervention of experimental breast cancer bone metastasis by indole-3-carbinol in SCID human mouse model. Mol. Cancer Ther., 5: 2747–2756.

Sartippour MR, Shao ZM, Heber D. 2002. Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J. Nutr., 132: 2307-2311.

Sazuka M, Imazawa H. 1997. Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. Biosci. Biotech. Bioch., 61: 1504-1506.

Suganuma M, Okabe S. 1999. Synergistic effects of epigallocatechin gallate with epicatechin, sunlindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. Cancer Res., 59: 44-47.

Templeton NS, Brown PD, Levi ET. 1990 Margulies IMK Cloning and characterization of human tumour cells interstitial collagenase. Cancer Res., 50: 5431-5437.

Thangapazham RL, Singh AK, Sharma A, Warren J, Gaddipati JP, Maheshwari RK. 2007. Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells *in vitro* and *in vivo*. Cancer Lett., 8: 232-241.

Turley H, Scott P, Watts VM, Bicknell R, Harris AL, Gotter KC.1998. Expression of VEGF in routinely fixed material using a new monoclonal antibody VG1. J. Pathol., 186: 313-318.

Yang F, Oz HS, Barve S, Villiers WJ, Varilek GW. 2001. The green tea polyphenol (-)-epigallocatechin-3-gallate blocks nuclear factor-κB activation by inhibiting 1kB kinase activity in the intestinal epithelial cell line IEC-6. Mol. Pharmacol., 60: 528–533.

Yang F. 1998. Green tea polyphenols block endotoxin-induced tumour necrosis factor alpha production and lethality in murine model. J Nutr., 128: 2334-2340.

Yuasa AK, Hua JJ, Kennedy DO, Yuasa IM. 2003. Endothelial cells through reduction of expression of VEGF receptors. Life Sci., 73: 1299-1313.

Zhang Y, Han G, Fan B, Zhou Y, Zhou X, Wei L, Zhang J. 2009. Green tea (-)-epigallocatechin-3-gallate down-regulates VASP expression and inhibits breast cancer cell migration and invasion by attenuating Racl activity. Eur. J. Pharmacol., 5: 172-179.

Zhao X, Tian H, Ma X, Li L. 2006. Epigallocatechin gallate, the main ingredient of green tea induces apoptosis in breast cancer cells. Front Biosci., 1: 2428-2433.

دراسة تأثير خلاصة الشاى الأخضر على موت الخلايا المبرمج وإعاقة نمو الأوعية الدموية الجديدة فى خلايا الدورة الدموية الطرفية لمرضى سرطان الثدى

***صفية محمد حسن، عاشور عبد السلام عبد المولى، **سامح إبراهيم زكى، عاطف أحمد عبد الرحيم**

*قسم كيمياء وبيولوجيا الخلايا والأنسجة، معهد البحوث الطبية ـ جامعة الإسكندرية

** قسم علاج وأبحاث الأورام ، معهد البحوث الطبية ـ جامعة الإسكندرية

ترجع أهمية استخدام الشاى الأخضر كمشروب أكثر استهلاكا فى العالم فى الآونة الأخيرة إلى احتوائه على العديد من المواد الفعالة مثل الكاتيكينات والمضادات البوليفينوليه. وتحتوى هذه المواد على خواص مقاومة للأكسدة وتعمل على الوقاية من أمراض القلب والأوعية الدموية والجلطات الدماغية ولوحظ أيضا أن الشاى الأخضر لديه القدرة فى المساعدة على منع كثير من السرطانات من الانتشار مثل سرطان الثدى والبروستاتا والمعدة والمرئى. تختص هذه الدراسة بالكشف عن تأثير الشاى الأخضر على ورم الثدى فى بعض السيدات بدرجاته المختلفة من خلال دراسات هيستوباثولوجيه وهيستوكيميائية مناعية. وقد قسمت السيدات إلى ثلاث مجموعات: مجموعة (أ) وهى المجموعة الضابطة واشتملت على عشر سيدات متبرعات سليمات من ورم الثدى. أما المجموعة (ب) فقد اشتملت على خمس عشر سيدة عولجت بعد استئصال الورم والتعرض للإشعاع بالجرعات الكيميائية لمدة ست دورات تكونت كل دورة من واحد وعشرين يوماً. وبالنسبة للمجموعة (ج) وعددها كما فى المجموعة (ب) فقد عولجت بالجرعات الكيماوية وخلاصة الشاى الأخضر (1000 مجم كجرعة يومية) ولنفس المدة. بعد ذلك أخذت عينات من الدم من جميع السيدات على ثلاثة مراحل: قبل استئصال الورم، بعد ثلاث دورات، بعد ست دورات من العلاج. أوضحت النتائج الهيستوباثولوجيه باستخدام صبغة ليشمان أن خلايا دم المرضى قد دخلت فى عمليات نخر وموت للخلايا السرطانية بدرجات مختلفة، وقد كان موت الخلايا ملحوظاً فى المرضى اللائى أخذن الشاى مع دورات العلاج الكيميائى. أما بالنسبة للدراسات السيتوكيميائية المناعية باستخدام صبغة مناعية خاصة بمعامل نمو خلايا الأوعية الدموية (VEGF) فقد بينت الدراسة انخفاض تركيزه فى خلايا الدم الحمراء والبيضاء مع استمرار استخدام الشاى مع العلاج الكيميائى وهذا مؤشر لقلة تكوين أوعية دموية جديدة مما يقلل انتشار المرض. وقد أظهرت الدراسة أيضا قلة تركيز NF-kB باستمرار استخدام الشاى الأخضر مع العلاج الكيمائى أيضاً مما زاد فرصة موت الخلايا السرطانية وقلة انتشار المرض. مما سبق يتبين لنا أن تناول الشاى الأخضر مع العلاج الكيميائى يقلل من انتشار الورم ويزيد من موت الخلايا السرطانية مما يساعد على تحسن حالة المرضى.

المحكمون:

أ.د. محمد سيد جبري قسم علم الحيوان، علوم حلوان

أ.د. منير عزالدين الجنزوري قسم علم الحيوان، علوم عين شمس