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# Determining serum globulin after consumption of green tea in rats

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## ABSTRACT

Nowadays uncontrolled administration of the antibiotics causes to high resistance in most of the pathogenic bacteria and it is necessary to use alternative methods. Using herbal plants and essences as alternative can be considered that aids in treatment process in addition to antimicrobial effects with positive impact on the body immune system. In this research the effect of green tea on some immunologic factors such as number of white cells and also serum globulin level were investigated by experiment on the rats. Finally it was concluded that in the studied group the blood white cells number, serum total protein and globulin level increased relatively to control group and albumin level is significantly higher in the treatment group relative to control group that indicates the effect of green tea on relative increase of the body immunology.

**Keywords:** serum globulin; green tea; rat

## INTRODUCTION

Antibiotics consumption is focus of treatment in infections, but due to the increasing rate of antibiotic resistance in bacteria, and medicines side effects, using secondary method to treat infections has gained special significance [1, 2]. However, over the years man has realized the effects of therapeutic herbal extracts. Known advantages of these extracts are absence of side effects and their extensive range. Nowadays, use of herbal extracts to treat bacterial infections and its effect on the immune system have been attracted the attention of many researchers [3,4].

According to this fact that tea is the most popular beverage in the world after water that has been associated with human daily life [3, 4], in this study we try to investigate the effect of green tea on the cellular immune and humoral mechanisms in rats.

Tea is product of leaves of *Camellia cinensis* that is consumed as fermented (black tea), nonferment (green tea) and semi- fermented (Oolong).

Flavonoids are products of plant -derived secondary metabolism that are found widely in the plant kingdom. Based on the structure and position of the oxygen heterocyclic ring, they can be divided into six groups of flavones, isoflavones, flavonoids, flavolones and anthocyanin. The main flavonoids in tea are flavonoids or more specifically

catechin [5] that includes more than 30 % of tea dry matter [6]. Catechins extracted from green tea and entered into the solution phase involving brewing[5].

More than three quarters of the material contained in tea leaves is flavonoles that 6 types of this class are known as catechin such as Epigallocatechin Gallate (EGCG) ,Epigallocatechin (EGC), epicatechin gallate, epicatechin, gallocatchin and catechin. Bitterness and astringency of tea is due to catechin and that these substances are colorless and water-soluble and usually comprise 25 to 40 percent of soluble solids in tea. Flavines are oxidized during the production of black tea and processed by enzymes and oxidant and converted into the flavines and thearubigins[7].

Green tea contains caffeine, glycoprotein, amino acids, carotenoids, chlorophyll, polysaccharides, lipids, vitamins E-CB, elements such as manganese, zinc, potassium and polyphenols [3, 8-10].

Glycoprotein of green tea composed of seven monosaccharides types: ribose, rhamnose, xylose, mannose, glucose, galactose and arabinose and 18 amino acids [11].

Green tea has anti-tumor effects such as induction of apoptosis, anti-angiogenesis, induction of cell differentiation, antioxidant, anti-inflammatory and carcinogen metabolism [3, 4, 12].

Today, the use of plant extracts to help in the treatment of microbial infections has attracted the attention of many researchers. In a study conducted in 1999 it was found that catechins include catechin, epicatechin gallate and epicatechin gallate in green tea inhibit release a toxin called Verotoxin from Enterohemorrhagic EC and thus inhibit the pathogenesis of these bacteria. This effect of polyphenols on plant has also been reported [1, 2].

In a study conducted in 2001, it was concluded that epigallocatechin gallate reduce transfer of the virulence plasmid Ecolic600 between R222 (donor) and Rc85 (receiver) of E. coli K12 [13].

## MATERIALS AND METHODS

Fifteen Wistar female rats were randomly divided into three groups of five rats, all groups were exposed to the same environmental and nutritional conditions. They were fed by with three meals by Dan pellet and water indefinitely.

Of three groups, a group was control group and the other groups were treatment groups.

One of the treatment groups, received green tea tablets twice daily for two weeks one tablet was administered in 2 mL of water to rats. The next group treatment received brewed green tea twice daily for two weeks, as 2 cc in each feed. Due to stress applied on treatment groups during gavages, the rat drank the same water amount twice a day by gavage and three groups were almost in identical conditions.

2 - The blood sampling from the animal tail vein blood sampling was chosen.

The following test were performed:

- Counting of white blood cells
- Assessment of the total serum protein level
- Calculate the total amount of blood protein (formula: total protein gr/dl =  $A_{\text{test}}/A_{\text{standard}} \times 6$ )
- Assessment of serum albumin
- Calculate the amount of blood albumin (formula: Albumin gr/dl =  $A_{\text{test}}/A_{\text{standard}} \times 4$ )
- Methods of Statistical Analysis

Information obtained from this testing method was analyzed by CRD, and PCAANOVA and Duncan analysis was used to compare means among the groups.

## RESULTS

After blood sample drawing and serum separation according to protocol described in the materials and methods and

counts of white blood cells test performed on blood samples, the total protein albumin level was assessed according to BCG that was done on the serum collected and the values were calculated. The results are as follows.

*- White blood cells*

After counting the amount of white blood cells, which was described in detail in the Materials and Methods, the average white blood cells was achieved 7760 mm<sup>3</sup> in the group that had used pill of green tea and in the group consumed brewed green tea it was 7780 mm<sup>3</sup> and in the control group it was 7720 mm<sup>3</sup> which was not statistically significant with a relative increase in the treatment groups.

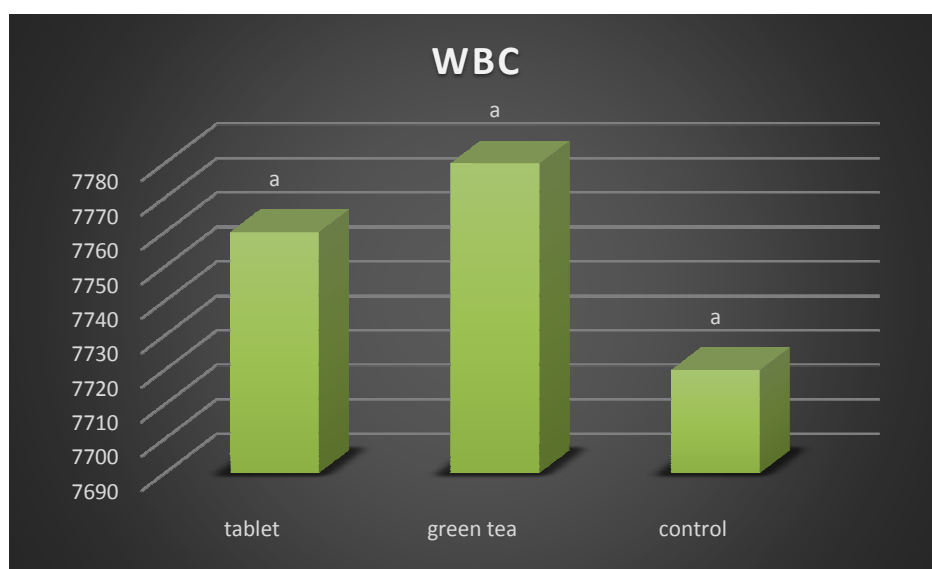
*- Total blood serum protein*

After testing and calculation of total serum protein, total serum protein mean was 6.872 gr/dl in the group that consumed green tea pills, 6.560 gr/dl in the group that had consumed brewed green tea and 6.110 gr/dl in the control group which was statistically significant, with a relative increase in the treatment groups and significant differences were not observed.

*- Blood serum albumin*

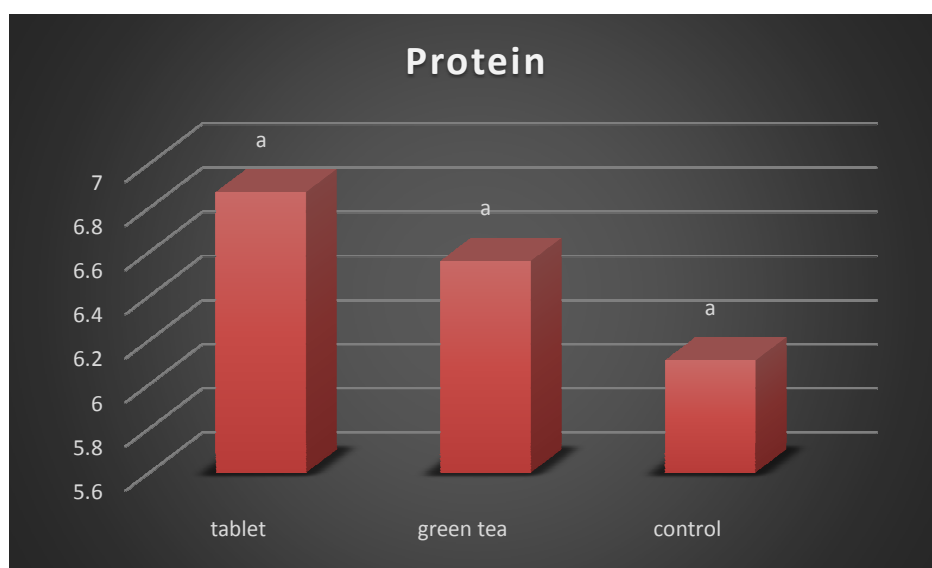
after testing and calculation of serum albumin, serum albumin average in the group that has been taking green tea pills was 3.904 gr/dl and in the group consumed brewed green tea was 3.584 gr /dl and in the control group it was 3.440 gr /dl. A statistically significant difference is seen among the group that has been taking green tea pills and two other groups.

Blood White Cells				
(standard deviation)SD	standard error	mean	number	WBC
444/522	993/982	7760	5	tablet
815/721	1824/007	7780	5	Green tea
639/844	1430/734	7720	5	control



Mean comparison of the studied groups by measured variables

Serum Protein				
(Standard deviation)SD	Standard error	mean	number	Protein
0/235	0/526	6/872	5	tablet
0/250	0/559	6/560	5	Green tea
0/264	0/590	6/110	5	control



*Mean comparison of the studied groups by measured variables*

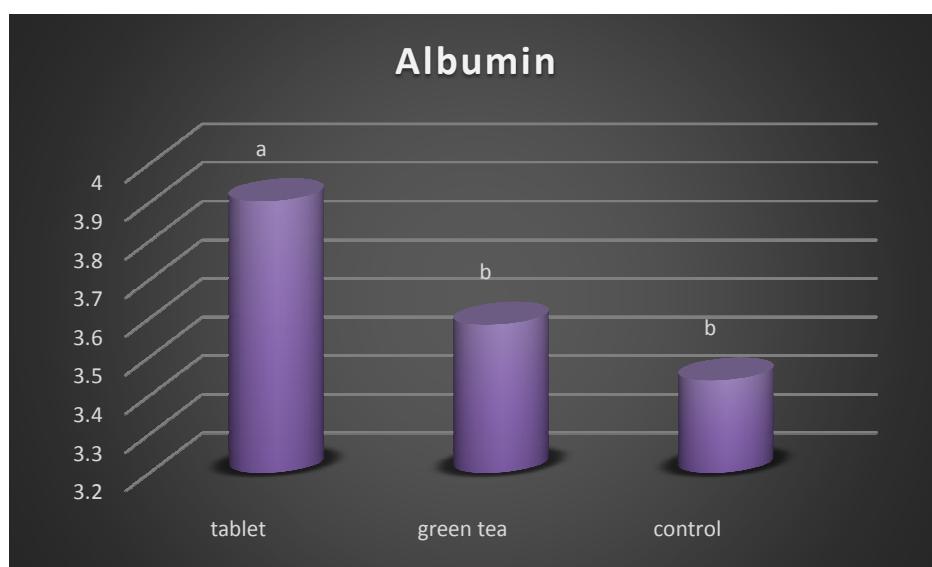
**Serum Albumin Descriptive Statistics**

(Standard deviation)SD	standard error	mean	number	Albumin
0/039	0/088	3/904	5	tablet
0/091	0/223	3/584	5	Green tea
0/070	0/156	3/440	5	control

**Figure : Mean albumin levels among groups with separation of variables**

**- Serum globulin**

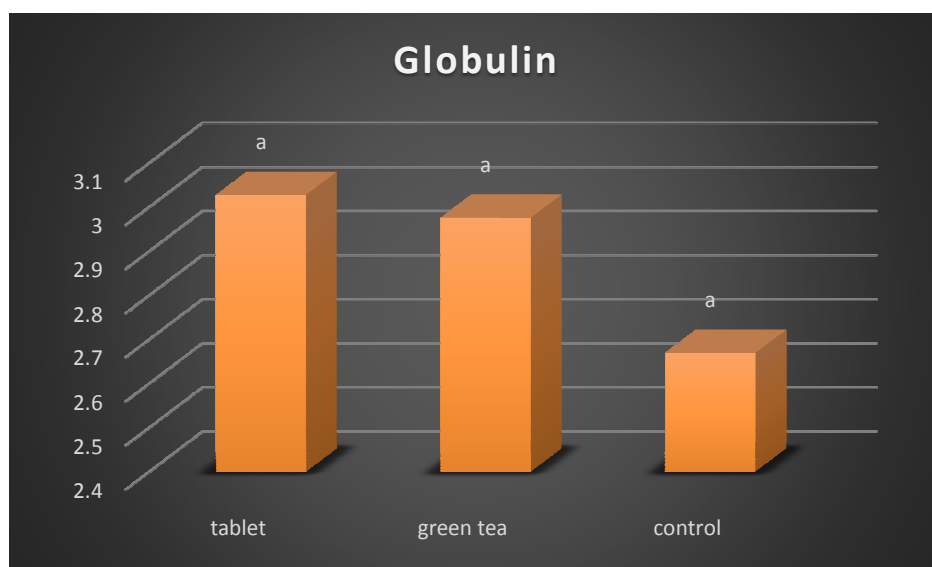
Blood proteins are divided into two general categories of albumin and globulin, if albumin level is deduced from the level of total protein, the globulin levels in blood is obtained. The average serum globulin in the group has used the green tea pills was 3.028 gr /dl and in the group that brewed green tea was consumed it was 2.976 gr/dl and in the control group was 2.670 gr/dl respectively. A statistically significant difference by the relative increase was not observed in the treatment groups.



*Mean comparison of the studied groups by measured variables*

Blood Globulin Descriptive Statistics

(standard deviation)SD	Standard error	mean	number	Globulin
0/219	0/490	3/028	5	tablet
0/190	0/425	2/976	5	Green tea
0/221	0/495	2/670	5	control



Mean comparison of the studied groups by measured variables

Globulin	Albumin	Mean square Protein	WBC	FD	variability
ns0/187	**0/282	ns0/734	ns4666/667	2	studied groups
0/222	0/027	0/313	2120666/667	12	Error

analysis of variance of the variables in three studied groups

Significant in probability level of 1% \*\*(*p* value = 0.01 ); Ns no significant

Figure : Mean globulin levels among groups with separation of variables

## DISCUSSION

The results of this study showed the relative increase of white cell in the treatment group represents the effect of green tea on white cell proliferation. The mechanism of this effect was examined in previous studies that depict high correlation with the results of this research. For example in the study of Ko and et al. in South Korea conducted on pigs the positive effect of green tea on splenialgia by concurrent increase of macrophage and B lymphocytes level indicating high effect of green tea [4].

Due to the direct effect of macrophages on specific immune activation through the presentation of antigens and the production of interleukin- 1 and the effect of lymphocyte T helper in activating lymphocytes B and antibody production by IL- 2 and 5 influences on increase in lymphocytes B production of anti-body [14].

Numerous studies prove this relationship.

Chae and colleagues (2004) studied the effect of green tea extracts in increasing numbers and capabilities of T lymphocytes and B lymphocytes and the stimulatory effect on increasing antibody [15].

In another study by Shin et al (2004) on some of the materials isolated from green tea extract the growth stimulatory effect of B lymphocytes and increase the production of antibodies was reported [16].

Barakat(2010) studied the effects of green tea on rats, accompanied by increased IgG, IgM levels compared to the control group [17].

The results of these reaches are consistent to our study so that relative increase in serum globulin is reported in treatment groups according to this fact that significant part of these proteins is immunoglobulin that depicts increase of serum antibody level in treatment groups and finally, B lymphocytes stimulated activity. This increase was also dependent on stimulation of T lymphocytes and ultimately represents a direct effect of green tea that can boost the immune system.

Numerous studies prove the effects of green tea and other immune mechanisms.

Devries et al (1999) suggested the effect of green tea that stimulates production of interleukin- 6 and B -cell activity, and an increase of this substance stimulates the production of antibodies [18].

In the study of Schall and et al. (1994) the effect of green tea on increase of splenalgia growth led to increase of production of TNF and this substance has increasing effect of receptors expression in white cells cytoplasm that leads to increase of neutrophils in the phagocyte microorganisms [19].

### CONCLUSION

The relative increase in serum protein and globulin in the present study also confirm the effectiveness of green tea that can increase the safety mechanisms.

Csanaky and et al (2005) studied the effect of green tea on increase of amcyt cells proliferation and increase of cell resistance on chemotropic side effect.

In other research conducted by Zhu and et al (1999) the effect of green tea in stopping of suppressing of some drugs and increase of lymphocytes number and other immunologic activities and finally control of tumors in rats were reported.

Finally, the results of this study and similar studies show the relative effect of green tea on reinforcing factors associated with the immune system.

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