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Aqueous Extract Of Camellia Sinuses Shows Immunological And Histological Changes In Induced Inflammatory Animal Models

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Abstract

The present study investigated the effect of green tea (Camellia sinuses) aqueous extract on the inflammatory response induced by Carrageenan (CGN) (1%) in Sprague dawaly rats. 48 rats were equally divided into 6 groups:control, green tea drinking, Carrageenan (1.2%) treated for 24 hours, green tea - Carrageenan treated for 24 hours, CGN treated for 72 hours, green tea - CGN treated for 72 hours. On the last day of drinking green tea aqueous extract, inflammation was induced to rats by Carrageenan . Twenty-four and seventy-two hours after CGN challenge, blood samples were withdrawn and animals were sacrificed. Animals which were injected with CGN had shown highly significant leucocytosis, monocytosis and eosinophilia. More reticuloendothelial organ damages like severe inflammation, cellular lymphocytic infilteration and congestion were distinguished in 72 hours animal group. Green tea-drinking and CGN treated groups showed a significant improvement in reticuloendothelial organs such as thymus gland, spleen and liver. A histopathological improvement of these organs was observed in green tea and CGN 72 hours treated group more than that group which treated for 24 hours. This group showed also a significant drop in total leucocyte count and peritoneal fluid neutrophils while a significant increase of bone marrow lymphocyte count was observed when compared with the CGN treated animal group. A significant modulation in differential leucocytic count especially the drop in lymphocytic and eosinophilic percentage occurred. This was associated with lower serum globulin and immunoglobulin G (IgG) in green tea-drinking-CGN treated animal group in comparison to CGN treated animal groups. This study explains the immunomodulatory role played by green tea in response to inflammatory immunostimulant agent.

Introduction

There is growing interest in the role of complementary and alternative medicine in health and disease. Of the various herbal and botanical agents used, tea (Camellia sinusis) has drawn a great deal of interest (Gary *et al*., 2001). Green tea is widely used in Asia and has also become popular in Western countries. (Hofbauer *et al*., 1999). It is cultivated in more than 30 countries (Mokhtar and Ahmed , 2000). Green tea is potent antioxidant, It has both anticancer and anti-inflammatory effects (Fajun *et al*., 1998).

Lau *et al*., (2002), evaluated the anti-inflammatory and hepato-protective activities of the green tea. The epidem - iologic observations and laboratory studies have indicated that polyphenolic compounds present in tea may reduce the risk of a variety of illnesses (Mokhtar and Ahmed, 2000). Zhu *et al.*

(1999), concluded that tea and its components ameliorate immune disfunction in mice bearing Lewis lung carcinoma since all immune functions were improved accompanied by inhibition of tumor growth, while in 1998, Zhu et al., concluded that green tea or its components showed a significant protection from early adverse changes in immune functions. Gary *et al*. (2001), postulated that green tea and its polyphenol fracture were useful dietary supplement in the treatement of some chronic inflammatory diseases.

Suganuma *et al*. (1996), stated that green tea anti-inflammatory effects may be possibly mediated through their antioxidant properties, while Chan *et al*. (1995), observed that green tea also inhibited production in peritoneal exudates (macrophage) cells. Similarly lin and lin (1997), showed that green tea inhibited lipopolysaccharide stimulated nitric oxide production and inducible nitric oxide synthesis gene expression in peritoneal macrophages by decreasing nuclear factor -B.

Its clear that green tea polyphenols have anti-inflammatory effects, antioxidant properties and inhibited tumor necrosis factor induction in macrophages by attenuating nuclear factor activation.

This study is a try to detect the anti-inflammatory effect of green tea in different reticuloendothelial organs in treated rats with carrageenan.

Material And Methods

Animals.

Fourty eight male Sprague dawely rats weighing between 160-210 gms, each were used in the present study. The animals were obtained from the animal house of NODCAR (National Organization For Drug Control And Research). The animals were divided into the following groups :

1. Control group : Untreated water drinking animals .
2. Green tea drinking group (1.2%): Rats were randomly assigned to receive green tea water extract as drinking for four weeks (Arteel *et al.*, 2002).
3. Carrageenan (CGN)treated group (1%): rats were injected intraperitonealy for 24 hours (Nacife, *et al*., 2000).
4. Carrageenan treated group (1%): rats were injected intraperitoneal for 72 hours (Ghosh *et al*., 2000).
5. Green tea-Carrageenan treated group for 24 hours: at the last day of drinking green tea, animals were injected with CGN interaperitonealy and then after 24 hours of injection , blood samples were withdrown and animals were sacrificed.
6. Green tea-Carrageenan treated group for 72 hours: at the last day of drinking green tea animals were injected with CGN and then after 72 hours of injection blood samples were withdrown before sacrificing.

After 24 and 72 hours of Carrageenan treated groups, blood samples were withdrawn for determination of total protein, albumin and globulin serum concentrations (using Randox chemicals). Also IgG level was determined using immunodiffusion plates (NANORID), The Binding site, Birmingham, UK. Then all rats were sacrificed , samples were withdrawn from the peritoneal fluid for both total and differential counting. Liver, spleen and thymus gland were obtained for recording their weights and histopathological studies . For light microscopy liver,spleen and thymus gland were fixed in Bouin's fluid, dehydrated in ascending grades of alcohol ,cleared in xylol and embeded in paraffin. Sections,5-6 micrometer thick were cut mounted and stained with haematoxy-

line and eosin. Bone marrow smears from femur were obtained for bone marrow lymphocytic count. All differential counts were carried out using leishman's stain. Results were evaluated using T-student test.

Table (1): Body and lymphoid and non-lymphoid organ weights from green tea drinking and Carrageenan-treated rats.

Animal group	Body weigh (g)	Thymus weight (% of body weight)	Spleen weight (%of body weight)	Liver weight (%of body weight)
Control	178 ± 1.4	0.16 ± 0.01	0.48 ± 0.02	3.8 ±0.20
Green tea drinking	197 ± 9.4	0.15 ± 0.01	0.46 ± 0.05	3.9 ±0.35
Carrageenan for 24 hours	152 ± 3.8 *	0.19 + 0.02 *	0.62 ± 0.06 o*	4.1 ±0.14
Green tea+Carrageenan for 24 hours	219 ± 7.7 * ... , oo	0.15 + 0.004	0.53 ± 0.02	4.1 ± 0.17
Carrageenan for 72 hours	159 ± 8.1 *	0.16 ± 0.12	0.82 ± 0.09 *** ooo	± 0.34 *
Green tea+Carrageenan For 72 hours	205 ± 7.4 *	0.15 ± 0.01	0.71 ± 0.02 *** ooo	± 0.12 * o

Table (2): Peripheral blood haematological findings from green tea drinking and Carrageenan treatment.

Animal group	Total Leucocyt. Count/cmm	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinopils %
Control	7725 ± 982	58 ± 1.1	39 ± 1.2	1.8 ± 0.3	1.2 ± 0.3
Green tea drinking	8631 ± 961	64 ± 4.0	33 ± 4.5	1.5 ± 0.4	0.7 ± 0.5
Carrageenan for24 hours	19330 ± 1699 *** ooo	58 ± 4.6	40 ± 4.7	± 0.3 ***	± 0.2 *
Green tea+Carrageenan for 24 hours	12850 ± 1645 *** …, ooo	59 ± 8.1	36 ± 7.6	2.1 ± 0.5 .	0.7 ± 0.4
Carrageenan for 72 hours	28742 ± 2603 *** ooo	68 ± 7.6 *	24 ± 7.7 *	5.0 ± 1.2 ***	3.0± 0.46 *** …,ooo
Green tea +Carrageenan for 72 hours	16090 ± 4849 *** …,ooo	65 ± 7.7	30 ± 6.5	0.6 ± 0.4 ** …,o	± 0.1 *** ..,ooo

*,**,*** Significance at P<0.05,0.01 and 0.001 respectively when compared with control group

o,oo,ooo Significance at P<0.05, 0.01 and 0.001 respectively when compared with green tea drinking group .

.,..,… Significance at P<0.05, 0.01 and 0.001 respectively when compared with Carrageenan treated groups

Table (3): Peritoneal fluid haematological findings from green tea drinking and Carrageenan treatment.

Animal group	Total Leucocyt. Count/cmm	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinopils %
Control	6840 ± 1166	58 ± 2.5	43 ± 2.5	1.3 ± 0.3	0.0 ± 0.00
Green tea drinking	5704 ± 972	41 ± 4.5 **	57 ± 4.9 *	1.0 ± 0.2	0.1 ± 0.3 ***
Carrageenan for24 hours	16233 ± 2932 *** ooo	58 ±4.6	± 4.7 o	± 0.3 ***	± 0.3 ***
Green tea+Carrageenan for 24 hours	5050 ± 746 …	55 ± 7.5	43 ± 7. 0	1.4 ± 02 .	± 0.2 ***
Carrageenan for 72 hours	10186 ± 1269 ooo	62 ± 5.6 *	37 ± 5.8 o	0.4 ± 0.2 oo*	1.0 ± 0.2 ***
Green tea +Carrageenan for 72 hours	4750 ± 1062 …	65 ± 5.9 o	43 ± 5.9 o	0.8 ± 0.3 *	+ 0.2 .***

Table (4): Bone marrow lymphocytic count, peripheral blood lympohocytic count and peritoneal fluid lymphocytic count from green tea drinking and Carrageenan-treated rats.

Animal group	Bone marrow Lmphocytes %	Peripheral blood Lymphocytes %	Peritoneal fluid Lymphocytes %
Control	56 ± 3.3	58 ± 2.5	58 ± 1.1
Green tea drinking	54 ± 5.1	64 ± 4.0	41 ± 4.4 **
Carrageenan for24 hours	64 ± 2.6	58 ±4.5	± 4.4
Green tea+Carrageenan for 24 hours	116 ± 7.3 *** …,ooo	59 ± 8.1	55 ± 7. 5
Carrageenan for 72 hours	76 ± 5.2 ** oo	68 ± 7.6 *	62 ± 5. 6
Green tea +Carrageenan for 72 hours	100 ± 11.1 *** …,ooo	65 ± 7.6	56 ± 59 o

*,**,*** Significance at P<0.05,0.01 and 0.001 respectively when compared with control group

o,oo,ooo Significance at P<0.05, 0.01 and 0.001 respectively when compared with green tea drinking group .

.,..,… Significance at P<0.05, 0.01 and 0.001 respectively when compared with Carrageenan treated groups

Table (5): Serum total protein albumin and globulin concentrations from green tea drinking and Carrageenan treated rats.

Animal group	Total protein conc. Gm/dL	Total Albumin Con. Gm/dL	Total globulin Conc. Gm/dL
Control	8.0 ± 0.4	5.0 ± 0.5	2.6 ± 0.39
Green tea drinking	6.8 ± 0.24	4.0 ± 4.0	2.8 ± 0.26
Carrageenan for24 hours	8.2 ± 0.28	6.6 ±0.89 o	1.9 ± 0.68 o
Green tea+Carrageenan for 24 hours	7.8 ± 0.57	6.7 ± 0.59 ** ooo	± 0.07 * ooo
Carrageenan for 72 hours	6.8 ± 0.30	5.3 ± 0.25 oo	± 0.31 *ooo
Green tea +Carrageenan for 72 hours	6.2 ± 0.94 **	5.5 ± 0.66 o	0.76 ± 0.47 *** ooo

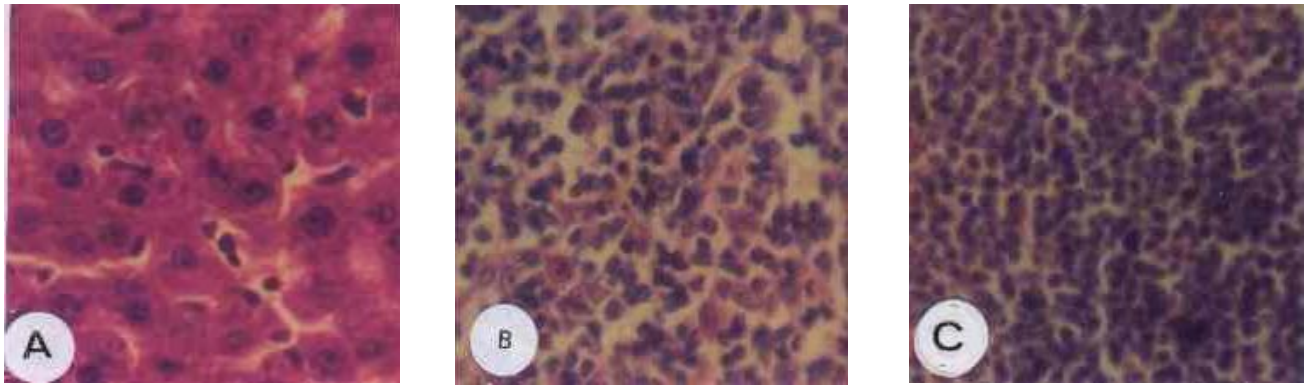
Table (6):Serum total Immunoglobuline(G) concentrations from green tea drinking and Carrageenan treated rats.

Animal group	Total protein conc. Gm/dL
Control	18288 ± 2528
Green tea drinking	22200 ± 2528
Carrageenan for24 hours	15870 ± 1027 ooo
Green tea+Carrageenan for 24 hours	15870 ± 1340 ooo
Carrageenan for 72 hours	22450 ± 1043
Green tea +Carrageenan for 72 hours	17300 ± 2719 ..ooo

*,**,*** Significance at P<0.05,0.01 and 0.001 respectively when compared with control group

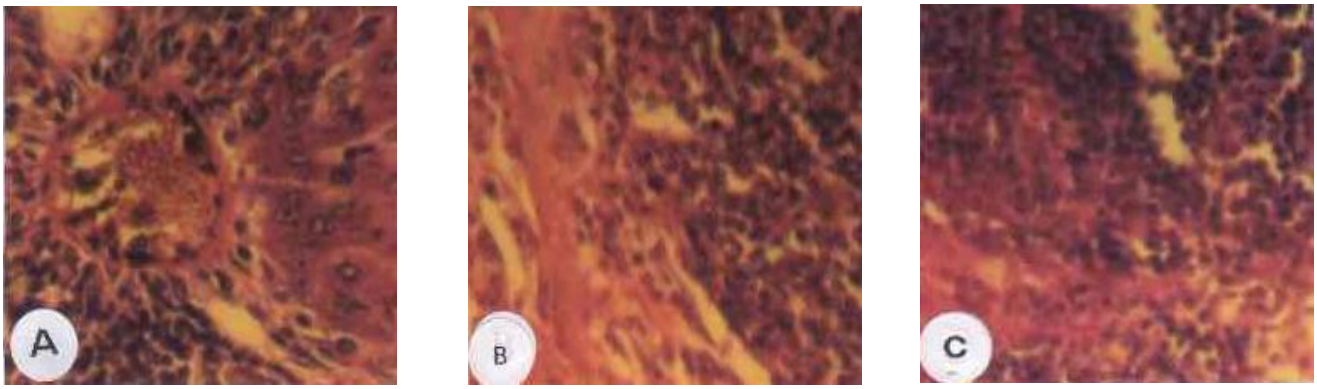
o,oo,ooo Significance at P<0.05, 0.01 and 0.001 respectively when compared with green tea drinking group .

.,..,… Significance at P<0.05, 0.01 and 0.001 respectively when compared with Carrageenan treated groups



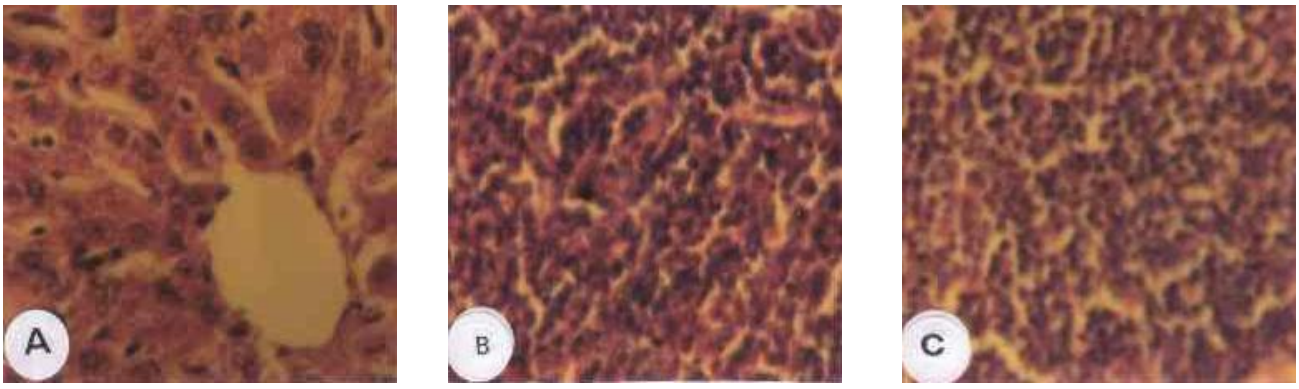
(H&E X 400) (H&E X 200) (H&E X 200)

Fig (1) : Histological sections in the liver (A), spleen (B) and thymus gland (C) green tea drinking group (H&E) Note normal appearance of the different cells.



(H&E X 400) (H&E X 200) (H&E X 200)

Fig (2) : Histological sections in the liver (A), spleen (B) and thymus gland (C) of Carrageenan treated group. Note many histopathological changes in liver, spleen and thymus gland.



(H&E X 400) (H&E X 200) (H&E X 200)

Fig (3) : Histological sections in the liver (A), spleen (B) and thymus gland (C) of green tea-Carrageenan treated group. Note: signs of improvement in the different tissues.

Result And Discussion

Green tea is a popular beverage consumed world wide. The epicatechin derivatives, which are commonly called polyphenols which are the active ingr - edients in green tea and possess antiox - idant, anti-inflammatory and anti-carci - nogenic properties. It seems that the green tea affects the immune system through immunomodulatory properties especially in peripheral blood mononuc- lear cells (Zvetkova *et al*., 2001). The green tea intake was associated with increased total leucocytic and lymphocytic counts associated with elevated level of serum globulin and serum IgG (Tables, 2,5 & 6). Many studies have proved that green tea polyphenols inhibit inflammatory responses. Polyphenols block the acti - vation of the transcription factor, NF_kB, which plays a central role in numerous immunologic processes. $NF\text{-}_kB$ controls the expression of a wide variety of genes active in inflammation including cytokines, enzymes, adhesion molecules and acute phase proteins (Varilek *et al*., 2001). Inhibitors of NF_kB have been shown to decrease inflammation in animal model (Neurath *et al*., 1996). These observations suggest that $NF\text{-}_kB$ is a suitable target to prevent or reduce an inflammatory response. The ability of green tea to inhibit $NF\text{-}_kB$ activation and to decrease the level IL-2 product- ion may be responsible in part for its anti-inflammatory effects (Varilek *et al.*, 2001 and Wilasrumee, *et al.*, 2002) . In this study an inflammatory model was induced by Carrageenan which is considered as a standard irritant for examining acute inflammation and anti-inflammatory drugs (Di Rosa, 1972). Animals injected intraperiton- ealy with Carrageenan have shown highly significant leucocytosis, monoc- ytosis and eosinophilia. Also peritoneal fluid leucocytic counts and bone marrow lymphocytes were increased . Besides increased liver, spleen and thymus gland weights were recorded. The reticuloendothelial organs changes were more distinguished in the 72 hours animal group.

Polyphenols have been reported to exhibit anti-inflammatory properties. Therefore the effects of drinking green tea on the inflammatory reaction induc- ed by Carrageenan after 24 and 72 hours were studied. A highly signific- ant reduction in the total leucocytic count in the peripheral blood and peritoneal fluid was recorded .

Both monocytosis and eosinop- hilia were significantly corrected in peripheral blood and similar observati- ons were recorded in the peritoneal fluid in green tea drinking-CGN group of both 24 and 72 hours groups .Also spleen and thymus weight percentages have shown relative modulation, besid- es a very highly significant increase in the bone marrow lymphocytic infiltra- tion was recorded in the same previous groups. While a significant drop in the levels of serum globulin and IgG was reported in the green tea drinking- Carrageenan treated groups when compared with its corresponding Carrageenan treated groups and green tea drinking animal group. These changes may be explained by the immunosuppressive immunomodulatory action of green tea recorded by Wilasrusmee *et al*. (2000). Haggi *et al*. (1999), has mentioned that green tea- fed mice had lower levels of total and CII-specific IgG antibody, because the Th 1-type response (IFN-γ producing) associated with the production of complement -fixing Ig G2a antibodies which are thought to bind with the cartilage and cause initial damage. They added that the level of total IgG antibodies in the arthritic joints of non-

green tea-fed mice was markedly higher in comparison to the levels detected in the joints of green tea-fed mice. Similar results were obtained in the serum (Das *et al*., 2002) .

Intraperitoneal injection of Carrageenan also caused many histopa - thological changes. The liver has shown severe inflammation, cellular lymphoc-ytic infiltration, severly congested liver sinuses, Both fatty hydropic degenerat-ion hepatocytes and necrosis in the liver was noticed. The spleen showed many necrotic areas, congested sinusoidal spaces filled with erythrocytes. Many degenerated cells with pyknotic nuclei were observed. Similar findings were observed in the thymus gland which showed severly congested area with erythrocytic infiltration mainly in the medulla, intralobular adipose tissue and intralober hyaline degeneration. Besides cortical region with degener-ative changes in the cortex was obse-rved. Fig. (2). These necrotic and degenerative changes of (CGN) injec-tion animal groups were markedly improved in green tea-drinking animal groups. The green tea drinking-Carrageenan treated animals revealed normal hepatic lobules and most of the hepatocytes appeared normal and the inflammatory reaction was markedly reduced. Green tea acts as chemoprev-entive agent that can modulate apopto-sis and thereby affected the steady state cell population(Das *et al*., 2002) Histopathological examination reveald effective protection against induction of hepatic degenerative changes. Fig.(3).

Green tea has been found to provide protection to the liver against a variety of toxic substances. (Sano *et al*., 1995 and Lau *et al*., 2002) .

On the other hand similar anti-inflammatory histological response was observed in the spleen and thymus gland of green tea drinking-Carrageenan treated animal groups. Splenic red pulps were enriched with lymphoid cells. The thymus gland degenerative changes were much reduced under the effect of green tea and thymocytic cell counts were much preserved. This can be explained by the immuno protective-immuno modulatory effects of green tea (Zhu *et al*., 1997) and were also observed in this study in (Fig. 3)

The usefulness of tea polyphenols may be extended by combining them with other consumer products such as food items and vitamin supplements. It is concluded that green tea can play a role in adverse changes in immune function and acts as an anti-inflammtory agent.

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التغيرات المناعية والهيستولوجيه للمستخرج المائى كاميليا ساينا سايس فى الجرذان المحدث بها الإلتهاب

مها غازى سليمان
قسم الفارماكولوجى (وحده الميكرو بيولوجى والمناعه)
الهيئة القومية للرقابة والبحوث الدولية

يهدف هذا البحث الى دراسه تأثير المستخرج المائى للشاى للاخضر (كاميليا ساينا سايس) على رد الفعل للالتهاب المحدث بواسطه الكاراجينان فى الجرذان البيضاء وقد إستخدم فى هذا البحث 48 جرذ قسموا الى 6مجموعات : المجموعة الضابطه , مجموعة اعطيت الشاى الاخضر , مجموعة عولجت بالكاراجينان لمدة 24 ساعة , مجموعة اعطيت الشاى الاخضر وعولجت بالكاراجينان لمده 24 ساعة , مجموعة عولجت بالكاراجينان لمده 72 ساعة ومجموعة اعطيت الشاى وعولجت بالكاراجينان لمده 72 ساعه. وقد لاحظ ان الحيوانات التى تم حقنها بالبطن بالكاراجينان اظهرت ارتفاعاً فى نسبة خلايا الدم البيضاء والخلايا المونوسايت وايضا الخلايا المحبه للصبغة الحامضية . كما نتج عنه ايضاً تغيرات باعضاء الجهاز المناعى والتى كانت فى المجموعة التى عولجت بالكاراجينان لمده 72 ساعة اكثر منها فى المجموعة التى عولجت بالكاراجينان لمده 24 ساعة . كما انه وجد تحسن كبير فى وظائف أعضاء الجهاز المناعى مثل الغدة الثيموسيه والطحال والكبد حيث لوحظ التحسن النسيجى بالمجموعة والتى تم سقيها بالشاى الاخضر ومعالجتها بالكاراجينان لمده 72 ساعة اكثر منها فى التى تم معالجتها لمده 24 ساعه . كما لوحظ انخفاض هائل فى العدد الكلى لخلايا الدم البيضاء وخلايا الكرات المتعادله بالسائل البريتونى فى حين وجود زياده ملحوظة فى عدد خلايا النخاع الليمفاوية بالمقارنه بمجموعة الحيوانات المعالجه بالكاراجينان . هناك ايضاً تحسن ملحوظ مصحوب بانخفاض نسبه تركيز الجلوبيولين والأجسام المضاده (G) فى المجموعة التى تم سقيها بالشاى الاخضر ومعالجتها بالكارجينان بالمقارنه بالمجموعة المعالجة بالكارجينان فقط وقد أوضحت هذه الدراسه الدور الوقائى المناعى للشاى الاخضر لتثبيط التغيرات المناعية للكاراجينان كماده مسببه للالتهاب.