ORIGINAL ARTICLE

Immunostimulatory effect of standardised alcoholic extract of green tea (*Camellia sinensis* L.) against cyclophosphamide-induced immunosuppression in murine model

Mohammad Rafiul Haque, Shahid Husain Ansari
Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, New Delhi, India

Backgrounds: Tea, *Camellia sinensis* (L.) O. Kuntze (Theaceae) is one of the most widely consumed beverages in the world. It can be classified into three major types, depending on the level of fermentation, that is, green and white (unfermented), oolong (partially fermented) and black (fermented) tea. Each type of tea has a distinct composition, dependent on how the leaves are processed, as well as maturation, geographical location and agricultural practices. Green tea (GT), the least processed tea, is one of the highest studied and is ascribed to have the highest content of phenolic compounds. **Aim:** The current study was intended to evaluate the immunostimulatory potential of alcoholic extract of green tea (GTEA or GTAE) in cyclophosphamide (CP)-induced immunosuppression in murine model. **Materials and Methods:** GTEA was standardised by high pressure thin liquid chromatography (HPTLC). CP (50 mg/kg, i.p.) was used to induce immunosuppression in mice. GTAE (50, 150 and 250 mg/kg) was administered orally for 14 day. **Results:** GTEA (150 and 250 mg/kg) treatment significantly ($P < 0.001$) elevated the thymus and spleen weight, haemagglutination titre and total leucocyte counts level and lowered the delayed type hypersensitivity response as compared with CP-induced immunosupressed mice. **Conclusions:** These observations suggest immunostimulatory effects of GTAE against CP-induced immunosuppression in murine model.

Key words: Cyclophosphamide, delayed type hypersensitivity, green tea, Haemagglutination titre

INTRODUCTION

The immune system plays an important role in our health. It is well recognised that suppressed immunological function can result in increased incidence and severity of infectious diseases as well as some types of cancer.[1,2] The role of immune function has become increasingly important in our understanding of the mechanisms that are involved in the body's ability to prevent diseases. The World Health Organization (WHO) estimated that 1500 people die each hour from infectious diseases including HIV/AIDS (WHO, 2008). The acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). It is one of the leading causes of death in the world.[3] The body's immunity has been shown to be suppressed in several diseases such as AIDS and cancer, the use of immunostimulatory agents can solve these problems to a great extent. Immunomodulation is a procedure that enhances the immune function of the organism by interfering with its function. Immunomodulatory are being used today in cancer therapy as well as infection diseases either in combination with chemotherapy or after chemotherapy and radiation therapy. They have a biphasic effect: Some stimulate termed as immunostimulation while others inhibit/suppressed host parameters that are normal or already activated called immunosupression. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence, both immunostimulating and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world.[4,5] Many plant used in traditional medicine were found to have immunomodulatory properties and it could be because of synergistic effects of variety of phytoconstituents such as phenolics, terpenoids, steroids and flavonoids.[6,7]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.126824

Tea is prepared as an infusion with the leaves of *Camellia sinensis* (L.), a plant cultivated in over 30 countries across the world that belongs to the Theaceae family.[8] There are two main varieties of tea plants: *C. sinensis* var. sinensis, a small-leaved, bushlike

Address for correspondence: Mr. Mohammad Rafiul Haque, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, Hamdard Nagar, New Delhi - 110 062, India. E-mail: hrafiul@gmail.com
Received: 13-09-2013; **Accepted:** 03-12-2013

plant indigenous from China, which grows in several countries of Southeast Asia experiencing a cold climate, and *C. sinensis* var. assamica, a large-leaved tree discovered in the Assam region of India which grows in several countries with a semitropical climate. There are different types of tea depending on botanical varieties, geographical origin and processing. Concerning to the level of "fermentation", it can be categorised into three major types: Not fermented (green and white tea), partially fermented (oolong tea) and completely fermented (black tea). To produce GT, the leaves are rolled and steamed to minimise oxidation and inactivate polyphenol oxidase prior to drying.[9] It is reported that GT contain abundant amount of tea polyphones, known as catechins and caffeine, amino acid theanine (5-N-ethylglutamine), bisflavanols, theaflavins, epitheaflavic acids, and thearubigens, chlorogenic acid, coumarylquinic acid, etc., Green tea catechins (GTC) comprise four major epicatechin (EC) derivatives; namely, epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG); EGCG accounts for more than 40% of the total content.[10] It is investigated that GT extract has a wide spectrum of biological activity including antioxidant, antiobesity, anticancer and hepatoprotective activity.[11-13] In the present study, we have undertaken to explore the immunostimulatory effect of GT in cyclophosphamide (CP)-induced immunosuppression in murine model.

MATERIALS AND METHODS

Procurement of Plant Material

The fresh and dried *Camellia sinensis* L. leaves was obtained from local market of Assam, in the month of July 2008. The drug was identified on the basis of macroscopic and microscopic characters by expert botanist (Dr. H. B. Singh) from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. It was observed that tea leaves had dentate (margin), ovate (lamina structure), acuminate (apex) and imperipinate (venation). It was also examined that it had one single layer epidermis and spherical or oval parenchyma cell containing vascular bundles. Voucher specimen and identification certificate reference number NISCAIR/RHMD/Consult/2008-08/966/150 was obtained and kept in the department for future reference. Its purity was checked (authenticated) by the study of physico- chemical parameters and high pressure thin liquid chromatography (HPTLC) and found under limit when compared with standard scientific data.

Chemicals and Reagents

Folin–Ciocalteu reagent, 2,20-azino-bis-(3-ethylbenzo thiazoline-6-sulphonate) diammonium salts (ABTS), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), 2,20-azobis (2-amidinopropane) dihydrochloride (ABAP), hydrochloric acid (HCl), ferric chloride (FeCl3), acetate, quercetin, CP (purity 99.5%), caffeine (purity ≥ 95%), EGCG (purity ≥ 95%) and gallic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), methanol, potassium dihydrogen phosphate (KH2PO4), sodium chloride (NaCl), sodium hydroxide and hydrochloric acid were purchased from Chopra chemical centre (New Delhi, India). All reagents and solvents used were analytical or HPLC grade.

Preparation of Plant Extract

The dried leaves (1000 g) were powdered and passed through sieve number 80. Powder drugs were extracted with alcohol by the using of Soxhlet apparatus. The alcoholic extract was concentrated by evaporation of solvents with the help of rotary evaporator and found to be strength of 29.58%w/w.

HPTLC Quantification and Method Validation

Caffeine and EGCG contents of GTAE were assayed by HPTLC densitometry method using mobile phase, respectively; 2-butanol: Chloroform: Formic acid (3:2.5:0.5) and Water: Acetonitrile: Methanol: Ethyl acetate: Glacial acetic acid (89:6:1:3:1 v/v) and scanned at 254 nm. It is shown in Figures 1 and 2. For preparation of calibration curve for caffeine and EGCG, a stock solution of caffeine and EGCG (1 mg/mL) were prepared in methanol. 1, 2, 3, 4 and 5 µL from the stock solution were spotted on TLC plate in six (n = 6) to obtain final concentration range of 1000-5000 ng/spot. The data of peak area versus drug concentration were treated by linear least-square regression. Quality-control (QC) samples chosen for the study were 1000, 3000 and 5000 ng/spot. The developed method is validated as per the International Conference on Harmonization (ICH) guidelines.[14] The developed method was validated in terms of Linearity, recovery, limits of detection (LOD) and limits of quantification (LOQ).

UV Spectrophotometry Analysis

Total phenolic and flavonoid contents in the GTAE were investigated by using UV spectrophotometry. The total phenolic contents were determined by using of Folin Ciocalteu reagent method.[15] Aluminum chloride colorimetric method was used for estimation of flavonoid contents.[16] Gallic acid and quercetin were used as standard for estimation of total phenolic and flavonoid contents, respectively.

Experimental Animals

This study was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which is registered with Committee for the Purpose of

Figure 1: TLC profile of GTAE and caffeine

Figure 2: TLC profile of GTAE and EGCG

Control and Supervision of Experiments on of Experiments on Animals"(CPCSEA) in the use of animals for scientific research. Animals (CPCSEA), Government of India, India (letter No. 173/CPCSEA, 28 January 2009). Male Swiss albino mice (31-35 g) were obtained from the Central Animal House Facility, Hamdard University, New Delhi, and acclimatised under standard laboratory conditions at 25 ± 2°C, and relative humidity (50% ±15%) and normal. The animals were kept in polypropylene cages under standard laboratory conditions (12 h light and 12 h dark cycle) and had free access to tap water *ad libitum*.

Drugs

Alcoholic extract weighed accurately and suspended in alcohol to prepare a suitable dosage form 50, 150 and 250 mg/kg.

Induction of Immunosuppression

CP was used as a standard immunosuppressant agent for the induction of immunosuppressant. It was administered intraperitoneal rout at a dose of 50 mg/kg body weight, to induce immunosupression in mice.[17]

Antigen Agent

Sheep red blood cells (SRBCs) were used as an antigen at the concentration of 20% for immunisation and 1% for challenge. SRBCs were purchased from slaughter house, Pahar Ganj, New Delhi, India.

Grouping of Animals

After one week of acclimation, mice were randomly separated into five groups ($n = 6$): One normal control group, one CP control group and remaining GTAE (50 mg/kg) + CP, GTAE (150 mg/kg) + CP and GTAE (250 mg/kg) + CP as treatment groups.

Measurement of Relative Organ Weight of Lymphoid Organs

Normal control group received normal saline, CP group were injected with a single dose of CP on the 12th day of initiation of experiment, GTAE treatment groups were given GTAE at the dose of 50, 150 and 250 mg/kg body weight (b.w.), once per day orally for 14 days with a single intra peritoneal injection of CP on 12th day. The animals were sacrificed by cervical dislocation 24 h after the last dose. Relative organ weight of spleen and thymus were determined for each animal.

Total Leucocytes Count (White Blood Cell Count)

For estimation of white blood cell (WBC) count, we followed the procedure which was described by Manjarekar *et al*.[18] Normal control group and CP-treated control group received the normal saline for a period of 13 days. GTAE treatment groups were given GTAE at the dose of 50, 150 and 250 mg/kg b.w., once per day orally for 13 days and injected with CP (50 mg/kg, i.p.) on the 11th, 12th and 13th day and 1 h after the administration of the respective treatment. Blood samples were collected on the day before (day 0) and on the 14th day of the experiment and the total WBC count were determined by using a haemocytometer.

Measurement of Antibody Titre and Delayed Type Hypersensitivity Response

Haemagglutinin antibody titre (HT) and delayed type hypersensitivity (DTH) response estimation were done by using the procedure of Puri *et al*.,[17] with some modification. Normal control group and CP-treated control group received the normal saline for a period of 14 days. GTAE-treatment groups were given GTAE at the dose of 50, 150 and 250 mg/kg b.w., once per day orally for 13 days and injected with CP (50 mg/kg, i.p.) on the 11th, 12th and 13th day and 1 h after the administration of the respective treatment. The animals were immunised by injecting 0.1 ml of 20% of fresh SRBCs suspension intraperitoneally on day 0. Blood samples were collected in micro centrifuge tubes from individual animal by retro-orbital plexus on the 14th day and serum was separated. Antibody levels were determined by haemaglutination technique. Briefly, equal volumes

of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 µl volumes of normal saline in micro titration plate and to that were added 25 µl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemaglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre. The thickness of the right hind footpad was measured using Plethysmometer on the 14th day. The mice were then challenged by injecting 20 µl of 1% SRBCs in right hind footpad and after 24 h of this challenge the foot thickness was measured again. The pre- and post-challenge difference in the thickness of footpad was expressed in millimeter and taken as a measure of DTH.

Statistical Analysis

Statistical analysis was carried out using Graph pad Prism 3.0 (Graph pad software, San Diego, California, USA). All of the data were expressed as mean ± SEM. Statistical analysis was performed by Dennett's *t*- test and *P* value less than 0.05 were considered significant.

RESULTS

HPTLC Analysis and Validation

HPTLC analysis results demonstrated that GT contain caffeine (2.5 ± 0.54%) and EGCG (7.4 ± 0.65%). The results of validation parameters of thymol and EGCG were shown in Table 1.

UV Analysis

The total phenolic and flavonoid contents were found to be 32.5 ± 2.32% and 3 ± 0.21%, respectively, in the GT.

Effect of GTAE on Lymphoid Organ Weight

Relative weight of spleen and thymus were found significant ($P < 0.001$) diminution in CP group as compared with normal control group. GTAE (150 and 250 mg/kg) treatment group showed significant ($P < 0.001$) augmentation in relative weight of spleen and thymus as compared with CP group. GTAE (50 mg/kg) treatment group did not show significant augmentation in relative weight of spleen and thymus as compared with CP group [Table 2].

Effect of GTAE on Total Leucocytes Counts

A significant ($P < 0.001$) diminution in WBC count was observed in mice treated with CP alone (toxic group) as compared with mice of the normal control group. GTAE (150 and 250 mg/kg) treatment group showed significantly ($P < 0.001$) augmented the levels of WBC count as compared with the toxic group. GTAE (50 mg/kg) treatment group did not show significant amplification in the levels of WBC count as compared with CP group [Table 3].

Table 1: Results of validation of caffeine and EGCG

Validated parameters	Caffeine	EGCG
Linearity		
Range (ng/spot)	1000-5000	1000-5000
Regression equation	Y=3926.880 X+4114.523	Y=23.40+125.18 X
Correlation coefficient (r2)	0.99	0.984
Slope	3926.880	125.18
Intercept	4114.523	23.40
Recovery (%)	98.79-101.12	99.43-100.32
LOD (µg/spot)	0.036	0.012
LOQ (µg/spot)	0.116	0.042

EGCG – Epigallocatechin gallate; LOD – Limits of detection; LOQ – Limits of quantification

Table 2: Effect of GTAE on relative organ weight

Groups	Relative organ weight (g/25 g mice) means±S.E.M	
	Spleen	Thymus
Normal	0.84±0.04	0.16±0.12
CP	0.22±0.81**[a]	0.87±0.06**,[a]
GTAE (50 mg/kg)+CP	0.29±0.82	0.093±0.08
GTAE (150 mg/kg)+CP	0.65±0.69***[b]	0.10±0.07***[b]
GTAE (250 mg/kg)+CP	0.75±0.29***[b]	0.16±0.09***[b]

CP – Cyclophosphamide; GTAE – Green tea alcoholic extract; SEM – Standard error of the mean; All values were expressed as mean±SEM for six rats in each group. **P<0.01, ***P<0.001. [a]Group CP were compared with normal control group. [b]GTAE treated groups were compared with CP group

Table 3: Effect of GTAE on total leucocytes counts

Groups	Total leucocytes counts (10^3/mm^3/25 g mice) means±S.E	
	Day 0	Day 14
Normal	5.90±0.078	11.04±0.210
CP	6.01±0.064	4.01±0.198***,[a]
GTAE (50 mg/kg)+CP	5.49±0.066	4.88±0.108
GTAE (150 mg/kg)+CP	5.97±0.084	8.15±0.048***,[b]
GTAE (250 mg/kg)+CP	5.90±0.076	12.05±0.043***,[b]

CP – Cyclophosphamide; GTAE – Green tea alcoholic extract; SEM – Standard error of the mean; All values were expressed as mean±SEM for six rats in each group. ***P<0.001. [a]Group CP were compared with normal control group. [b]GTAE treated groups were compared with CP group

Effect of GTAE on Haemagglutination Antibody Titre

A significant ($P < 0.001$) diminution in antibody titre count was observed in mice treated with CP alone as compared with the mice of control group. Antibody titre response was found significant ($P < 0.001$) enhance in GTAE (150 and 250 mg/kg) treated mice as compared with mice treated with CP alone. GTAE (50 mg/kg) treatment group did not show significant amplification in the levels of antibody titre count as compared with CP group. It is shown in Figure 3.

Effect of GTAE on Delayed Type Hypersensitivity Response

The mice treated with CP alone showed a significant ($P < 0.001$) change in the DTH response as compared with mice of the normal control group. A significant ($P < 0.001$) response in DTH was observed in GTAE (150 and 250 mg/kg)

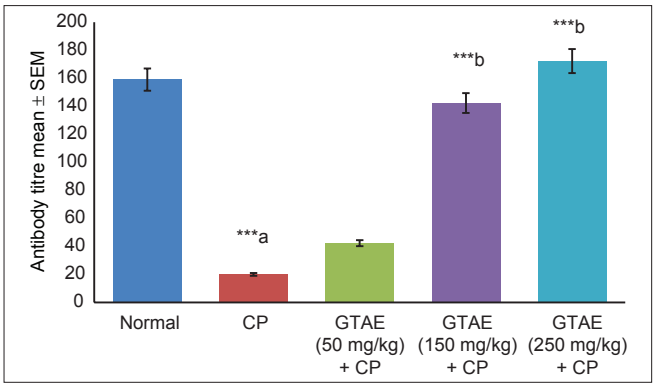

Figure 3: Effect of GTAE on haemagglutination antibody titre. All values were expressed as mean ± SEM for six rats in each group. ***P < 0.001. a Group CP were compared with normal control group. b GTAE treated groups were compared with CP group

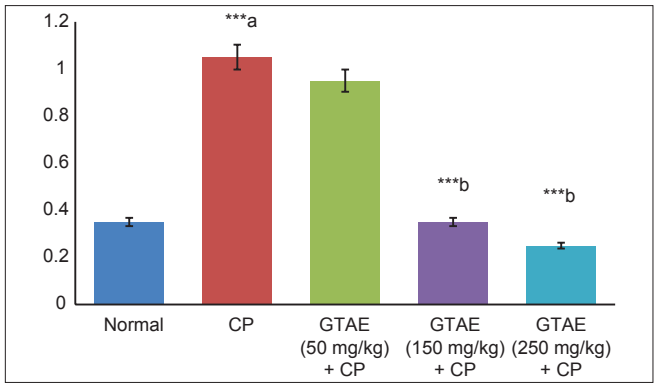

Figure 4: Effect of GTAE on delayed type hypersensitivity response. All values were expressed as mean ± SEM for six rats in each group. ***P < 0.001. a Group CP were compared with normal control group. b GTAE treated groups were compared with CP group

treated group as compared with CP group. GTAE (50 mg/kg) treatment group did not show significant response in DTH as compared with CP group. It is shown in Figure 4.

DISCUSSION

The present study was proposed to assess the immunostimulatory potential of GTEA in CP- induced immunosuppression in mice model by studying various parameters such as thymus and spleen weight, HT and total leukocyte counts level and DTH response. CP is one of the most popular alkylating anti-cancer drugs which produced toxic side effects including immunotoxicity, hematotoxicity and mutagenicity. It is also reported that CP has a potent immunosuppressive action, capable of inhibiting humoral and cell mediated immune function.[19] The assessment of humoral and cell mediated immune function were carried by the measurement of HT and DTH response. Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation.[20]

The results from the present study indicated that GTAE (50 mg/kg) treatment group did not show remarkable significant changes in thymus and spleen weight, HT and total leukocyte counts level and DTH response as comparison to the CP group. GTAE (150 and 250 mg/kg) treatment group showed the remarkable enhancement of weight of spleen and thymus. The augmentation in thymus and spleen weight was accompanied by augment in its cell counts. In the case of thymus, this may be partly due to stimulatory effect of plant extract on the lymphocytes and bone marrow hematopoietic cells, which ultimately home in the thymus.

The high degree of cell proliferation alters the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone marrow is the highly affected during any immunosuppression therapy with this class of drugs. Damaged or loss of stem cells of the bone marrow unable to regenerate new blood cells results in thrombocytopenia and leucopoenia.[21] Administration of the GT extracts (150 and 250 mg/kg) were found to enhance the total WBC count, which was lowered by CP, a cytotoxic drug indicate its stimulatory effect on hematopoietic stem cell of the bone marrow.

In DTH test, the DTH response, which directly correlates with cell mediated immunity (CMI), was obtained to be significantly after treatment of GTAE (150 and 250 mg/kg). The mechanism of action behind this raise DTH during the CMI response could be due to sensitised T-lymphocytes. When challenged by the antigen, they converted to lymphoblast and secrete a variety of molecules including pro-inflammatory lymphocytes, attracting more scavenger cells to the site of reaction. Mice treated with CP showed potentiating of DTH response as CP damaged the short-lived suppressor T-cells in immune regulatory systems.[22] Increase in the DTH response indicates that GT possesses stimulatory effect on lymphocytes and on other necessary cell types required for the expression of the reaction. Antibody production to T-dependent antigen SRBC requires co-operation of T- and B- lymphocytes and macrophages.[23]

CP has a particularly intense effect on short-lived lymphocytes known to include a great proportion of B-cells. In HT test, the GTAE (150 and 250 mg/kg) showed significantly increase in response. The high values of HT obtained in case of GT have indicated that immnostimulating was achieved through humoral immunity. This activity could be due to presence of flavonoids which augment the humoral response, by stimulating the macrophage and B-lymphocytes subsets involved in antibody synthesis.[24] Many scientific validated research data revealed that GT possess potential antioxidant capacity because of their presence of phenolic and flavonoid contents.[11] UV spectrophotmetry analysis study showed

that the phenolic and flavonoid contents were found to be 32.5% and 3%, respectively, in the leaf of GT. GT may induce the immunostimulant effect as several antioxidants have been reported to possess immunomodulatory properties.[25] Horrigan *et al*., reported that caffeine has strong immunomodulatory action.[26] It is reported that epigallocatechin-3-gallate has strong immunostimulatory action against cigarette smoke condensate-induced immunosuppression.[27] These chemical components may be responsible for immunostimulatory action of GT. The finding from our results suggested that GT has potential immune stimulatory action on CP-induced immunosuppression.

CONCLUSIONS

The immunostimulatory effect produced by GT in CP-induced immunosuppression is possibly through stimulation of cellular and humoral immune function and increased total leucocytes counts. Currently available data strongly suggest that GT may be a promising candidate as an immune modifier that maintains the homeostasis of immune function.

REFERENCES

1. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effect of Ashwagandha. J Ethnopaharmacol 1996;50:69-76.
2. Nishiguri C, Yorosh DB, Donawho C, Kripke ML. The immune system in ultraviolet carcinogenesis. J Invest Dermatol Symp Proc 1996;1:143-6.
3. Lakhashe S, Thakar M, Godbole S, Tripathy S, Paranjape R. HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis. J Biosci 2008;33:515-25.
4. Ramnath V, Girija K, Kuttan R. Immunomodulatory effect of Abrin in normal mice. Amala Cancer Bul 2001;21:76-81.
5. Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA. Search of immunomodulatory agents: A review. Indian Drugs 1990;28:348-58.
6. Premanathan M, Rajendran S, Ramanathan T, Kathiresan K, Nakashima H, Yamamoto N. A survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) activity. Indian J Med Res 2000;112:73-7.
7. Mullurwar VR, Pathak, AK. Studies on immunomodulatory activity of *Ficus reigiosa*. Indian J pharm Edu Res 2008;42:341-3.
8. López V, Calvo MI. White tea (*Camellia sinensis* Kuntze) exerts neuroprotection against hydrogen peroxide- induced toxicity in PC12 cells. Plant Foods Hum Nutr 2011;66:22-6.
9. De Mejia EG, Ramirez-Mares MV, Puangpraphant S. Bioactive components of tea: Cancer, inflammation and behavior. Brain behav Immun 2009;23:721-31.
10. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21:334-50.
11. Leung LK, Su Y, Chen R, Zang Z, Huang Y, Chen ZY. Theaflavins in black and catechins in green tea are equally effective antioxidants. J Nutr 2001;131:2248-51.
12. Kim HJ, Jeon SM, Lee MK, Jung UJ, Shin SK, Choi MS. Antilipogenic effect of green tea extract in C57BL/6J-Lep ob/ob mice. Phytother Res 2009;23:467-71.
13. Salminen WF, Yang X, Shi Q, Greenhaw J, Davis K, Ali AA. Green tea extract can potentiate acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol 2012;50:1439-46.
14. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures, ICH-Q2A, Geneva; 1995.
15. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001;73:73-84.
16. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food drug analysis 2002;10:178-82.
17. Puri A, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbour-tristis* L. J Ethnopharmacol 1994;42:31-7.
18. Manjarekar PN, Jolly CI, Narayanan S. Comparative studies of the immunomodulatory activity of *Tinospora cordifolia* and *Tinospora sinensis*. Fitoterapia 2000;71:254-7.
19. Vigila AG, Baskaran X. Immunomodulatory effect of coconut protein on cyclophosphamide induced immune suppressed Swiss albino mice. Ethnobot Leaflets 2008;12:1206-12.
20. Agarwal R, Diwanay S, Patki P, Patwardhan B. Studies on immunomodulatory activity of *Withania somnifera* (Ashwagandha) extracts in experimental immune inflammation. J Ethnopharmacol 1999;67:27-35.
21. Mitsuoka A, Mitsuo B, Morikawa S. Enhancement of DTH by depletion of suppressor T-cells with cyclophosphamide in mice. Nature 1976;262:77-9.
22. Mitra SK, Gupta M, Sarma, DN. Immunomodulatory effect of VIM-133. Phytother Res 1999;13:341-3.
23. Benacerraf B. A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and borrower lymphocytes. J Immunol 1978;120:1809-32.
24. Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of *Magnifera indica* L.in mice. J Ethanopharmacol 2001;78:133-49.
25. De La Fuente M, Victor VM. Anti-oxidants as modulators of immune function. Immunol Cell Biol 2000;78:49-54.
26. Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: Friend or foe. Pharmacol Ther 2006;111:877-92.
27. Matsunaga K, Friedman H, Yamamoto Y. Epigallocatechin Gallate, a Potential Immunomodulatory Agent of Tea Components, Diminishes Cigarette Smoke Condensate-Induced Suppression of Anti-Legionella pneumophila Activity and Cytokine Responses of Alveolar Macrophages. Clin Diagn Lab Immunol 2002;9:864-71.

How to cite this article: Haque MR, Ansari SH. Immunostimulatory effect of standardised alcoholic extract of green tea (*Camellia sinensis* L.) against cyclophosphamide-induced immunosuppression in murine model. Int J Green Pharm 2014;8:52-7.

Source of Support: Nil, **Conflict of Interest:** None declared.