e-ISSN:2321-6182
p-ISSN:2347-2332

Research and Reviews: Journal of Pharmacognosy and Phytochemistry

Evaluation of Immunoregulatory Activities of Green Tea (*Camelia Sinensis*) In Freund's Adjuvant Induced Arthritis Model.

Chattopadhyay Chandan*

Department of Pharmacology, KPC Medical College, Kolkata 700032, West Bengal, India.

Research Article

Received: 17/08/2013
Revised: 20/09/2013
Accepted: 22/08/2013

***For Correspondence**

Department of Pharmacology, KPC Medical College, Kolkata 700032, West Bengal, India.
Mobile: +91 9830271671

Keywords: Green tea, Immunoregulatory, Anti-inflammatory

ABSTRACT

Green tea, a daily beverage for time immemorial has already attracted very wide interest for its ability to control inflammation, improve immune function, suppress autoimmune disease and prevent cancer. Therefore we had set forward this study to examine the potential immuno-regulatory and chronic anti-inflammatory activities of Green Tea in rat model. Evaluation of immunoregulatory activities of Green tea in Freund's adjuvant induced arthritis model. Green tea decoction (10%,and 20%,)was prepared by soaking 10 gms and 20 gms of Green tea in 100 ml boiled water separately, soaked for 2 mins and thereafter filtered. This filtrate was designated as Green tea decoction. This is an experimental study. In chronic anti-inflammatory model Green tea decoction (10% and 20%) had shown significant immunosuppressive effects on rat paw edema (26.92%, and 38.46 %,) observed on 21st day in comparison to standard drug dexamethasone showed 84.6% improvement in pedal edema. The results were expressed as mean ± S.D. and the significance was evaluated by Student's t-test versus control, with $p<0.05$ implying significance. Taken together, our data indicated that Green tea had shown immunosuppressive action in rat model.

INTRODUCTION

The immune system has evolved to discriminate itself from non self and is always on the alert to fight infectious invaders or deregulated self yet maintaining its sanity i.e. by keeping normal cells intact. These organisms have developed robust receptor mediated sensing devices and effector mechanisms broadly described as innate and adaptive. Innate, or natural, immunity is primitive, does not require priming, is of relatively low affinity and is mediated mainly by complement, granulocytes, natural killer cells, macrophages, mast cells and basophils. Adaptive or learned immunity is antigen specific, depends upon antigen exposure or priming and can be of very high affinity. This is mediated by T and B cells. B cells make antibodies while T cells function as helper, cytolytic and regulatory cells. These cells are important components of the normal immune 'orchestra' that harmoniously fights infection and tumors but also mediates transplant rejection and autoimmunity [1]. Immune responses can be pharmacologically manipulated in three ways namely immunosuppression, tolerance and immunostimulation.

Green tea has already attracted very wide interest for its ability to control inflammation, improve immune function, suppress autoimmune disease and prevent cancer.

In a new study, scientists found that a compound in green tea, a polyphenol called EGCG, was able to increase the production of regulatory T cells and, while its effects were not as potent as some of those produced by prescription drugs; there are no concerns about long term toxicity with the tea.

It is suggested that the health benefits of EGCG may be due to an epigenetic mechanism, whereby the expression of some genes is changed. This could have significant implications for the suppression of autoimmune diseases.

This study was undertaken to examine the potential immunoregulatory activity of Green Tea in arthritic rat model.

Objectives

Evaluation of immunoregulatory effects of Green tea in arthritic rat model

MATERIALS AND METHODS

Animals

The entire study was carried out in a teaching institute in eastern India, as a part of posr-graduate dissertation using Sprague Dawley adult rats of either sex, weighing between 150-200 g. The animals were maintained under standard laboratory conditions with free access to commercial pellet feed and water *ad libitum*. The animals were housed for a period of seven days for acclimatization prior to the commencement of experimental work at a room temperature of 27^0 C under fixed 12-hour alternate light and darkness cycle. The protocol was approved and carried out after the permission of Institutional Animal Ethics Committee [2].

Preparation of the Plant Product

Green tea leaves were commercially obtained from P&A Arse, Rajdhani Apt. BIK-I Rg Barua Road, Ganeshguri, Guwahati-781 006. For the preparation of tea extract, tea leaves (100 gm) were extracted with ethyl acetate using soxhlet assembly. The extract was concentrated in a rotary flash evaporator under reduced pressure to semisolid mass. Green tea decoction (10%, and 20%) was prepared by soaking 10 gms and 20 gms of Green tea in 100 ml boiled water separately, soaked for 2 mins and thereafter filtered. This filtrate was designated as Green tea decoction. The dose of this decoction orally administered to each rat was 0.1ml/10 gm of body weight. Initial Pilot study suggested that 20% of this preparation has given best result. Therefore we had decided to set forward our study with 10% and 20% Green tea decoction [2].

Chronic or Immunological induced inflammation

Adjuvant induced arthritis

The method of adjuvant arthritis in rats as described by Pearson et al. (1959) exhibits many similarities to human rheumatoid arthritis and was accordingly followed as a model of chronic or immunologically induced inflammation [3]. Male Sprague Dawley rats with an initial body weight of 130- 150 g were used. Sixty rats were taken and divided into five groups of twelve rats each. The groups were treated as follows:

Group (n=12)	Treatment
Group I	Control
Group II	Standard drug, Indomethacin (10mg/kg)
Group III	Standard drug, Dexamethasone (0.1mg/kg)
Group III	10% Green tea decoction
Group IV	20% Green tea decoction

On day 1, 0.1 ml of complete Freund's adjuvant was injected into the subplantar region of the left hind paw of each rat. (Each ml. of complete Freund's adjuvant contains 1 mg Mycobacterium tuberculosis (H37RA, ATCC 25177) heat killed and dried 0.85 ml. mineral oil and 0.15 ml Mannide mono oleate). Dosing with a test compound or the standard was started on the same day and continued daily orally for 12 days. Paw volumes of both legs and body weight were recorded on the day of injection. On day 5, the volume of the injected paw was measured again, indicating the primary lesions and the influence of therapeutic agents on this paw. The severity of the adjuvant induced disease was followed by measurement of the non-injected paw (secondary lesions) with a plethysmometer. On day 21, the body weight was determined again and the severity of the secondary lesions was evaluated visually and graded according to the following scheme. The results were expressed as mean $\pm$ S.D. and the significance was evaluated by Student's t-test versus control, with $p<0.05$ implying significance.

Evaluation

a) For primary lesions: the percent inhibition of paw volume of the injected left paw over control was measured at day 5.

e-ISSN:2321-6182
p-ISSN:2347-2332

b) For secondary lesions: The percent inhibition of paw volume of the non-injected right paw over control was measured at day 21.

c) An arthritic index was calculated as the sum of the scores as indicated above for each animal. The average of the treated animals was compared with the control group.

Arthritic Index [4,5]

Site of lesion	Nature of lesion	Score
Ears	absence of nodules and redness	0
	Presence of nodules and redness	1
Nose	no swelling of connective tissue	0
	Intense swelling of connective tissue	1
Tail	absence of nodules	0
	Presence of nodules	1
Forepaw	absence of inflammation	0
	Inflammation of at least one joint	1
Hind paw	absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

OBSERVATIONS AND RESULTS

In chronic anti-inflammatory model Green tea decoction (10% and 20%) had shown significant suppression of rat paw edema on 21st day (Table1 and Table2). Dexamethasone showed most significant inhibition on 5th and 21st day (Table1, 2). Green Tea (10%, 20%) decoction showed a maximum inhibition of 29.62%, and 38.46%, respectively on 21 st day and was statistically significant (Table 1).

Table 1: Effects of Green tea decoction on Freund's adjuvant induced arthritis in rats

Gr (n=12)	Dose	Edema Volume(ml) (% inhibition)		
		5th day	13th day	21st day
Control	-	1.37 ±0.40	1.36±0.3	1.3±0.1
Indomethacin	1 mg/kg	0.70±0.16 (48.9%)#	0.8±0.04 (27.65%)	0.9±0.13 (29%)
Dexamethasone	0.1mg/kg	0.37±0.1 (73%)	0.37±0.12 (73%)	0.2±0.1 (84.6%)©
Green tea (10%)	1ml(p.o)	1.07±0.3 (21.90%)	0.8±0.08 (27.27%)	0.95 ±0.12 (26.92%)©
Green tea(20%)	1 ml(p.o)	0.75±0.19 (45.26%)#	0.68±0.12 (38.18%)	0.8±0.08 (38.46%)©

p.o per orally © P<0.001 #P<0.05

Table 2: Effect of Green tea on arthritic index in a model of Freund's adjuvant induced arthritis

Treatment groups(n=12)	Dose	Arthritic index on 21st day
Control	-	4±0
Dexamethasone	0.7 mg/kg	1.25± 0.5ƀ
Green tea(10%)	1ml(p.o)	3.25±0.5¥
Green tea(20%)	1ml(p.o)	2.75 ±0.2 ƀ

ƀ P<0.001 ¥ P<0.05

DISCUSSION

In the immunologically mediated chronic inflammatory model of Freund's adjuvant induced arthritis, considered as the best available model of rheumatoid arthritis ,green tea showed a profound degree of anti-inflammatory activity in both the primary and secondary phase.5 This procedure has been proposed by several

authors to differentiate between anti-inflammatory and immunosuppressive activity .6 It has been proposed that those anti-inflammatory compounds capable of inhibiting secondary lesions can be considered as immunosuppressive agents .7 In this model of immunologically mediated chronic synovial inflammation and arthritis, macrophages play a central role. After activation they are capable of synthesizing mediators such as PGE2 and cytokines such as TNF-α and IL-1. In turn, these synthetic products induce the production of variety of enzymes which initiate cartilage and bone destruction.8 Indomethacin and Green tea 20% produced a significant inhibition of 48.9% and 45.26%, respectively on 5th day, which indicates its action in primary phase (Table 1). Dexamethasone showed most significant inhibition on 5th and 21st day (Table1). Green Tea (10%, 20%) decoction at above mentioned doses showed a maximum inhibition of 26.92%, and 69.53%, respectively and was statistically significant (Table 1). Therefore tea definitely has some effect on the immunological and systemic secondary phase of adjuvant arthritis [6,7,8,9].

Taken together, our data indicates that Green tea has potential chronic anti-inflammatory action. Our results support the idea that tea has a beneficial effect. Significant anti arthritic activity was observed with regular administration of Green tea 10% and 20% in the Freund's adjuvant induced model of arthritis. Roy DK et al stated that chronic treatment with Green tea (in arthritic rats) resulted in a decrease of paw diameter and tissue lipid peroxidation, along with a restoration of GSH, catalase and superoxide dismutase levels.9 Novel immunoregulatory properties of EGCG on reducing inflammation in EAE had been depicted by Sun Q et al in 2013 which was another aspect of different work observed with Green tea (Camelia sinensis) suppresses B cell production of IgE without inducing apoptosis [10,11]. Ratnasooriya et al showed Green tea possessed strong, oral gastric ulcer healing activity which is mediated via multiple mechanisms [12].

Green tea epigallocatechin-3-gallate mediates T cellular NF-kappa B inhibition and exerts neuro-protection in autoimmune encephalomyelitis. 2004 which reflects its immunosuppressive activity [13].

However, such a study would be difficult to undertake in humans as the majority of the population are 'tea consumers'. Hence, we propose that after a 'wash out' period of 2 weeks, preliminary studies can be undertaken with normal healthy volunteers.

REFERENCES

1. Janeway CA, Travers P, Walport M and Capra JD. Immunobiology: The Immune System in Health and Disease, 4th ed. Current Biology Publications, London, 1999.
2. Chattopadhyay C, Chakrabarti N, Chatterjee M, Chatterjee S, Bhattacharyay D, Ghosh D. Evaluation of acute anti-inflammatory and analgesic activities of green tea decoction on experimental animal models. Int J Nutr Pharmacol Neurol Dis. 2012; 2: 20-5.
3. Pearson CM, Wood FD. Studies on polyarthritis and other lessions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. Arthr Rheum. 1959; 2: 440.
4. Schleyerbach R. Antiarthrotic and Immunmodulatory Activity. Vogel, G H, eds. Drug Discovery and Evaluation. 2nd edition. Berlin, Germany. Springer – 2002: 802.
5. Waltz DT, DiMartino MJ, Kuch JH, Zuccarello W. Adjuvant-induced arthritis in rats – Temporal relationship of drug effects on physiological, biochemical and haematological parameters. Pharmacologist. 1969; 11: 266.
6. Perper RJ, Alvarej B, Colombo C, Schroder H. The use of a standardized adjuvant arthritis assay to differentiate between anti-inflammatory and immunosuppressive agents. Proc Soc Exp Biol Med. 1971; 137: 506.
7. Middleton E Jr. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol. 1998; 439: 175.
8. Hopkins SJ. Cytokines and eicosanoids in rheumatic diseases. Ann Rheum Dis. 1991; 49: 207.
9. Roy DK, Kumar KT; Zothanpuia, Karmakar S, Pal S, Samanta SK, Adhikari D, Sen T. Pharmacological studies on Indian Green tea (leaf variety) in acute and chronic inflammatory conditions. Phytother Res. 2008; 22: 814-9.
10. Hassanain E, Silverberg JI, Norowitz KB, Chice S, Bluth MH, Brody N, Joks R, Durkin HG, Smith-Norowitz TA. Green tea (Camelia sinensis) suppresses B cell production of IgE without inducing apoptosis. Ann Clin Lab Sci. 2010; 40(2): 135-43.
11. Sun Q, Zheng Y, Zhang X, Hu X, Wang Y, Zhang S, Zhang D, Nie H. J Immunol. Novel immunoregulatory properties of EGCG on reducing inflammation in EAE. Front Biosci 2013; 18: 332-42.
12. WD Ratnasooriya, TSP Fernando. Gastric ulcer healing activity of Sri Lankan Green tea (*Camellia sinensis* L.) in rats. Pharmacog Mag. 2009; 5: 260-265.
13. Aktas O, Prozorovski T, Smorodchenko A, Savaskan NE, Lauster R, Kloetzel PM, Infante-Duarte C, Brocke S, Zipp F. Green tea epigallocatechin-3-gallate mediates T cellular NF-kappa B inhibition and exerts neuroprotection in autoimmune encephalomyelitis. 2004; 173(9): 5794-800.