IJMPR 2020, 4(5), 186-192

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

MECHANISTIC EVALUATION OF IMMUNOSTIMULANT PROPERTIES OF TEA ON MURINE MACROPHAGE (RAW264.7) CELL LINE

Vishal Sharma, Piyali Das, Shila Elizabeth Besra*

Central Instrumentation Facility and Cancer Biology & Inflammatory Disorder Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata-700032, West Bengal, India.

Received on: 18/09/2020
Revised on: 08/10/2020
Accepted on: 29/10/2020

***Corresponding Author**
Shila Elizabeth Besra
Central Instrumentation Facility and Cancer Biology & Inflammatory Disorder Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata-700032, West Bengal, India.

ABSTRACT

NO plays many more roles in the immune system as well as in other organ systems**.** Macrophages are key modulator and effectors cells in the immune response, their activation influences and respond to other arms of the immune system. Generation of NO is a feature of genuine immune-system cells (dendritic cells, NK cells, mast cells and phagocytic cells including monocytes, macrophages, microglia, Kupffer cells, eosinophils, and neutrophils). The present study elaborated the immunomodulatory effects of Tea on RAW264.7 cell line. Tea treated cells showed enhancement in NO production whereas it showed marked enhancement when cells were stimulated with rIFN-ϒ. Pretreatment with PDTC and N^{G}MMA to the rIFNϒ plus Tea treated RAW264.7 cells ameliorated NO production as compared to the primed cells. It was found that Tea acted as an accelerator of activation of RAW264.7 cells by rIFNϒ via a process involving L-arginine-dependent NO production and that Tea elevated NO production via activation of NF-Kb signaling pathway. These findings suggest that Tea can be a potential immunostimulant beverage.

KEYWORD: Beverage, Tea, Nitric Oxide, Macrophages, Immunomodulation.

INTRODUCTION

Nitric Oxide (NO) a short lived gaseous radical is a potent multifunctional reactive metabolite that is a major effector molecule of immune cells against tumor cells and pathogens.[1] Following its benchmark discovery, nitric oxide (NO) is now known to play important functional roles in a variety of physiological systems within the vasculature. NO induces vasodilation, inhibit its platelet aggregation, prevents neutrophil/platelet adhesion into endothelial cells, inhibits smooth muscle cell proliferation and migration and regulates programmed cell death (apoptosis) and maintains endothelial cell barrier function. NO generated by neurons acts as a neurotransmitter, whereas NO generated by macrophages in response to invading microbes acts as an antimicrobial agent.[2] In 1985, Stuehr and Marietta,[3] reported that activated macrophages synthesize nitrite/nitrate. In 1987, Hibbs *et al*.[4] suggested that L-arginine was the substrate for murine-derived nitrite/nitrate. Palmer et al. in 1988.[5] reported that NO is synthesized from L-arginine, and Marlette *et al*., 1988[6] reported that macrophages generate nitrite and nitrate from L-arginine.NO is a free radical having both cytoprotective as well as tumor promoting agent is formed from L-arginine by converting it to L-citrulline via nitric oxide synthase enzyme. Nitric oxide, is produced in a variety of tissues by nitric oxide synthase (NOS). NO, nitrogen oxide species, and NOS are readily detectable in macrophages obtained from individuals with a wide variety of conditions associated with infection and inflammation, including tuberculosis, malaria, AIDS, and rheumatoid arthritis. *In vitro*, LPS can stimulate macrophages to produce NO. Based on the location and the mechanism of regulation, three isoforms of NOS have been identified. They are neuronal NOS (nNOS, also termed NOS I), inducible NOS (iNOS, also termed NOS II), and endothelial NOS (eNOS, also termed NOS III).[1] nNOS and eNOS constitutively produce low levels of NO in neurons and endothelium, providing for neurosignaling and helping to maintain vascular homeostasis and tissue perfusion. Since nNOS and eNOS are constitutively expressed, they are also collectively called constitutive NOS (eNOS). iNOS is a 130-kDa protein and is expressed in macrophages in both cytosolic and membrane as forms.[7-8] NO is generated in to cytokines, such as interferon-y macrophages by iNOS following exposure to cytokines such as interferon gamma. TNF-α, and interlukin-1 or microbial products such as LPS. We studied the immunostimulant activity of different variety of Tea.

MATERIALS AND METHODS

Chemicals

RPM1 1640 was purchased from Gibco. USA. Fetal bovine serum. streptomycin and penicillin. L-glutamine,

HEPES, PDTC, N^GMMA, rIFN-ϒ were purchased from Sigma, USA. Griess reagent was purchased from MP Biomedical. All the other chemicals and reagents were of analytical grade and purchased locally.

Cell culture

The murine macrophage cell lines (Raw 264.7) are routinely maintained in RPMI 1640 medium supplemented with heat inactivated 10% FBS and were incubated at 37℃ in a humidified atmosphere containing 5% CO_2 inside a CO_2 incubator.

Tea samples

White Tea- Purchased from Amazon. in (Silver Leaf Tea Pvt. Ltd.)
Batch Code: 0917, Mfg. Date: 09/2017.
Green Tea – Purchased from local Supplier (TETLEY)
Batch Code: 20TT118, Mfg. Date:12/2018.
Black Tea- Purchased from local supplier (TATA TEA, CTC)
Batch code: 01TT117, Mfg. Date: 11/2018.

Preparation of sample

White Tea extract (WTE), Green Tea extract (GTE) & Black Tea extract (BTE) was prepared by dissolving 0.5g of tea in 25 ml of boiling water at boiling temperature (100℃) and brewing for 5 minutes according to **Fatimah *et al.* 2014.**[9] The samples are then filtered using a Millipore filter membrane (0.22µm) and aliquoted in small volumes in eppendroff. Different concentrations from tea samples (25, 50,100,200µg/m1) are taken and used to study immunostimulant properties on Raw264.7 cells.

Effect of WTE, GTE, BTE on NO production in non-primed and rIFN-y primed Raw 264.7 cells

Raw264.7 cells($1x10^6$) were seeded in 96 well plate for 24 hours. After 24 hours, to study the effect of WTE, GTE, BTE on non-primed (resting) Raw264.7, the cells were treated with 25,50,100,200 µg/ml of WTE, GTE and BTE separately for 24 hours. Further, in another set of experiment to observe the effect of the extracts on rIFN-y the Raw264.7 cells were activated with rIFN-y (10 U/ml) for 6 hours at 37 ℃ in an atmosphere of 5% CO_2. The cells were then incubated with various concentrations of sample separately for another 24 hours at 37℃ an atmosphere of 5% CO_2. NO synthesis was measured by a microtiter assay plate.[10] 100µl of each culture supernatant was allowed to react with 100 µl of Griess reagent at room temperature for 10 minutes. The absorbance was recorded at 540 nm.

Effect of PDTC on WTE, GTE, BTE induced NO production in rIFN-y -primed Raw264.7 cells

It is well known that PDTC, an anti-oxidant compound, inhibits activation of NF-KB.[11] As an approach to determine the signaling mechanism of the three extracts on NO production, the influence of PDTC, NF-KB inhibitor, on WTE, GTE and BTE treated rIFN-**y** primed Raw264.7 cells murine macrophages was examined. The cells ($1x10^6$) were incubated in the presence of rIFN-y (10 U/ml) with or without PDTC (100 µM) for 6 h inside a CO_2 incubator. The cells were then treated separately with WTE, GTE and BTE with various concentrations i.e., 25,50, 100 and 200 µg/ml for another 24 hours. NO assay was performed using Griess reagent as described previously. The O.D values were read at 540 nm.

Effect of N^GMMA on WTE, GTE, BTE induced NO production in rIFN-y primed Raw264.7 cells

N^GMMA is the specific inhibitor of Nitric Oxide production in the L-arginine-dependent pathway.[12] To define if the signaling mechanism in WTE, GTE and BTE induced NO production participates in the L-arginine-dependent pathway in Raw264.7 cell line, the cells ($1x10^6$) were incubated in the presence of rIFN-y with or without N^GMMA (10 mM) for 6 h inside a CO_2 incubator, the macrophages were then treated with 25, 50, 100 and 200 µg/ml of each of WTE, GTE and BTE for another 24 hours. NO assay was performed using Griess reagent as described previously. The O.D values were read at 540 nm.

Statistical Analysis

This was done by Student's t-test $P < 0.05$ was considered as significant. The percentage cell inhibition was calculated by the following formula:

% Cell Inhibition: 10× (O. D of Control – O. D of Treated)/O.D of Control

Where O. D= Optical Density.

RESULTS

Effect of WTE, GTE, BTE on NO production in non-primed and rIFN-y primed Raw264.7 cells

Nitric Oxide production was slightly elevated in resting Raw264.7 cells treated alone with WTE, GTE, BTE. However, when the cells were activated with rIFN-y for 6 h and then treated separately with WTE, GTE, BTE the NO production was markedly enhanced as compared with that of non-primed cells (resting).

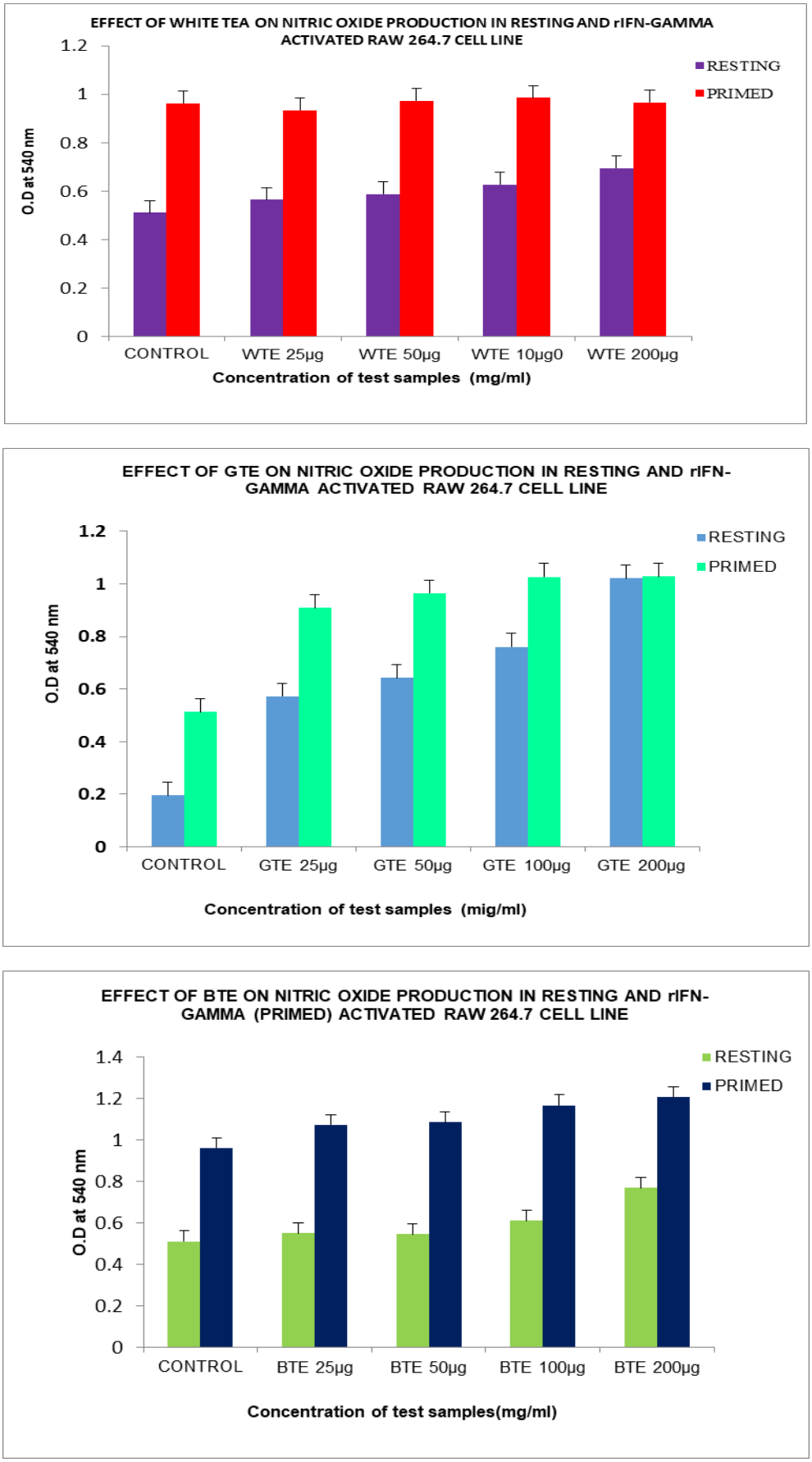
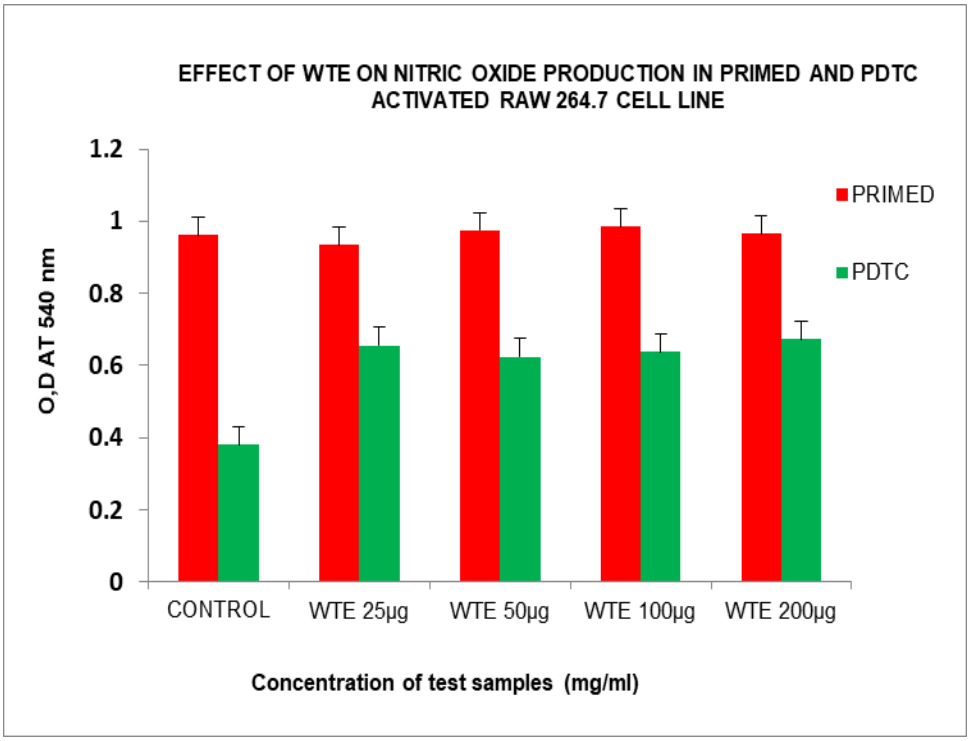
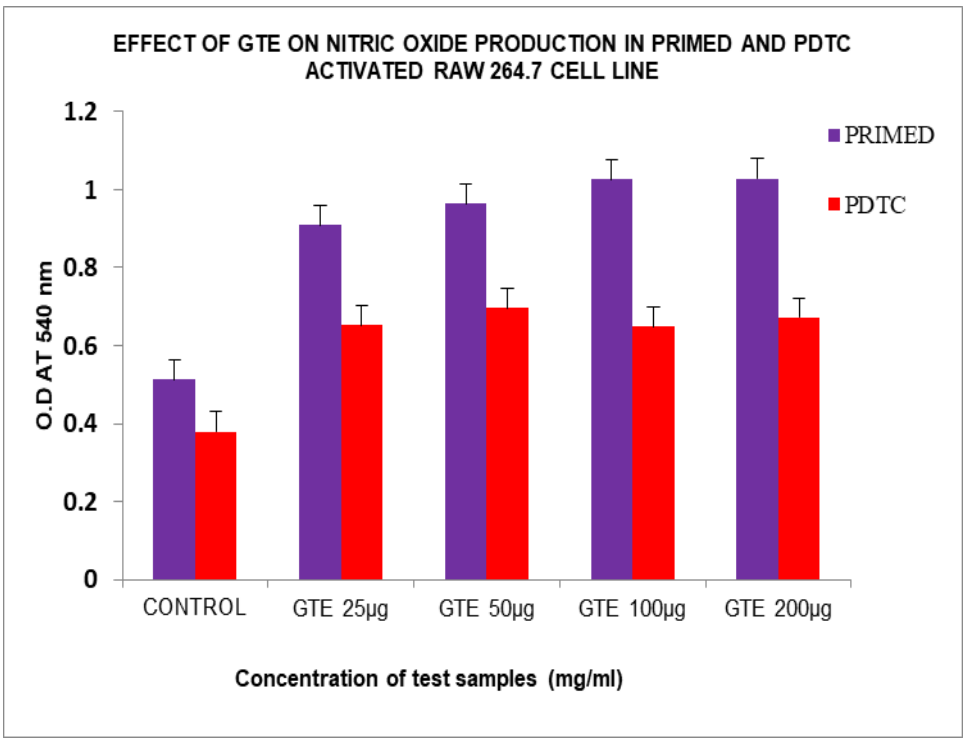


Fig. 1: All the histogram showed the effect of WTE, GTE & BTE on Nitric Oxide production in resting and rIFN-y activated Raw 264.7 cell line. Data are mean ± S.E.M.

Effect of PDTC on WTE, GTE & BTE induced NO production in rIFN-y stimulated Raw264.7 cells

Pre-treatment with PDTC to the rIFN-y plus WTE, GTE &BTE stimulated Raw264,7 cells caused significant block in the production of Nitric Oxide.





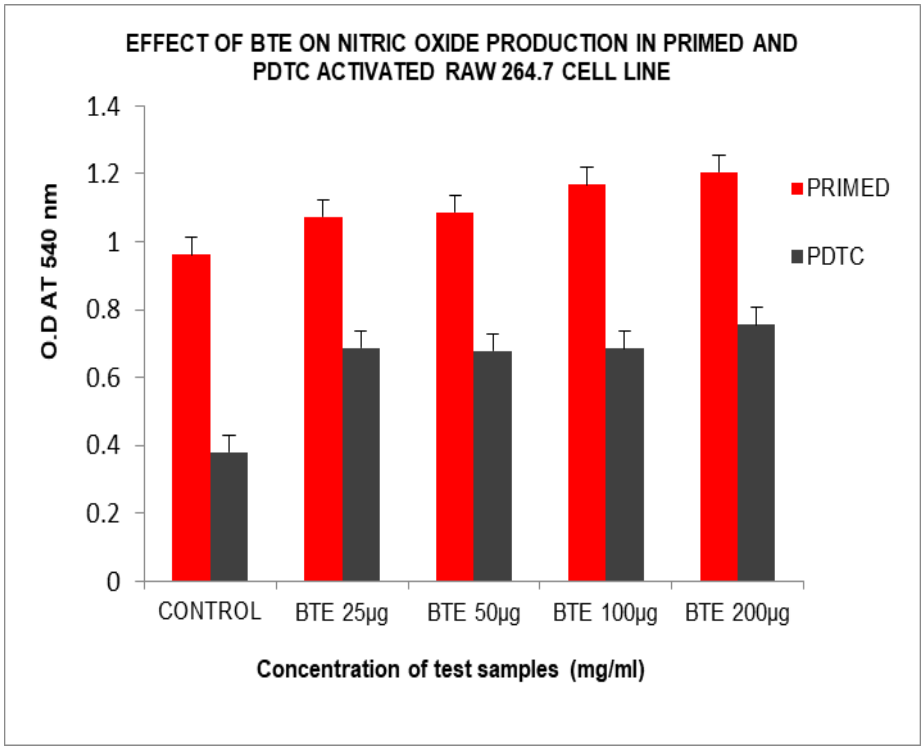
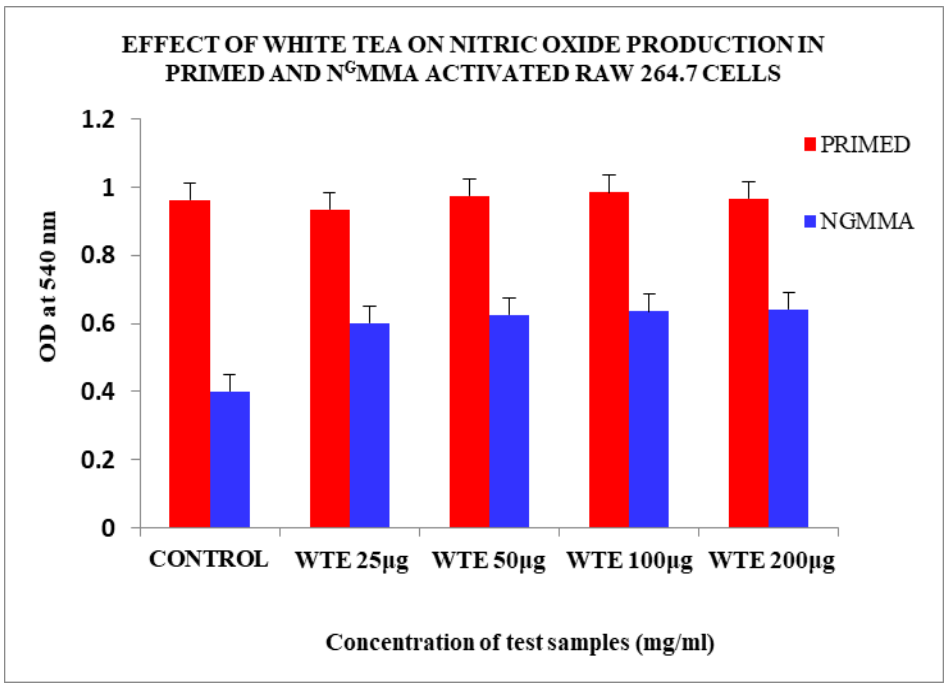
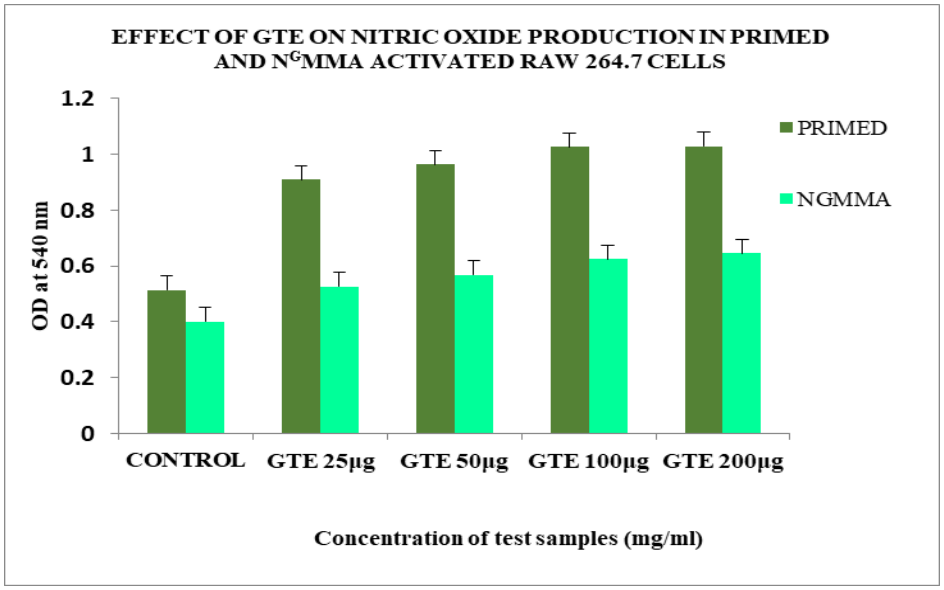


Fig. 2: All the histogram showed the effect of WTE, GTE & BTE on NO production in primed and PDTC activated Raw 264.7 cell line. Data are mean ± S.E.M.

Effect of N^{G}MMA on WTE, GTE AND BTE extracts induced NO production in rIFN-gamma stimulated Raw264.7 cell line

The production of NO by rIFN-y plus WTE, GTE and BTE in Raw264.7 cells was significantly decreases due to pre-treatment of N^{G}MMA.





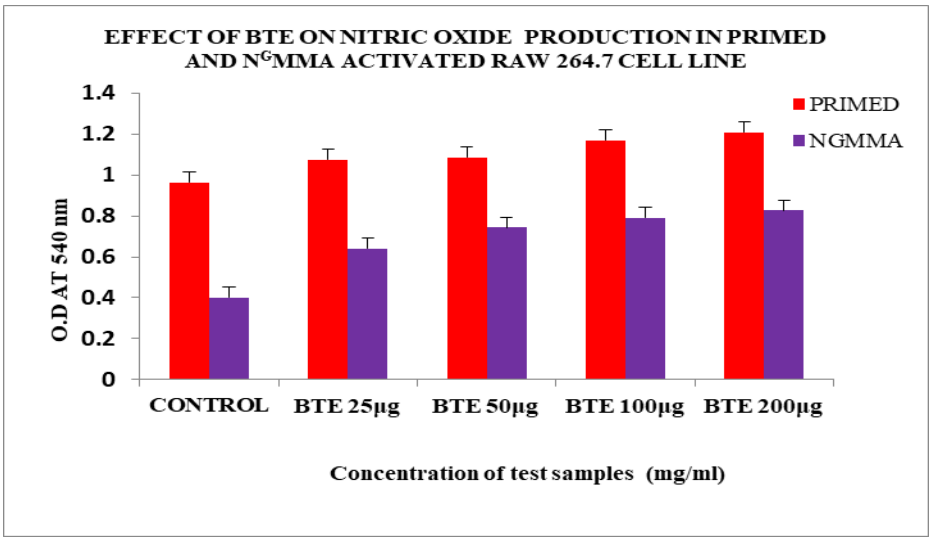


Fig. 3: All the histogram showed the effect of WTE, GTE & BTE on NO production in primed and N^{G}MMA activated Raw 264.7 cells. Data are mean ± S.E.M.

DISCUSSION

In this study, it was demonstrated that all the Tea themselves are able to induce NO production in resting and rIFN-y stimulated Raw264.7 cell line. However, NO production by the lowest concentration of WTE, GTE and BTE i.e., 25 µg/ml in rIFN-y activated Raw264.7 cells was lower compared with control rIFN-y-primed plus WTE, GTE and BTE activated cells. The elevated production of NO by rIFN-y primed plus WTE, GTE and BTE activated cells were markedly inhibited by pre-treatment with PDTC, an inhibitor of NF-KB. Nuclear factor Kappa-B), a transcription factor, plays an important role in the regulation of immune responses. The inducible form of nitric oxide synthase (iNOS) is also under the control of NF-KB.[13-16] Signal transduction pathway of NO production has been previously reported as LPS stimulation of primed macrophages induces NF-KB activation.[17] Since observations showed that pre-treatment with PDTC blocked NO production by resting rIFN-y stimulated, WTE, GTE and BTE treated Raw264.7 cells, these findings might explain that WTE, GTE and BTE extracts influence NO production via the NF-KB signaling pathway.

The results of this study suggest that WTE, GTE and BTE may provide a second signal for induction of NO production in Raw264.7 cells. N^{G}MMA, analogue of L-arginine inhibited WTE, GTE and BTE induced NO production by resting as well as rIFN-y activated Raw264.7 cells. The strong inhibition of NO production by N^{G}MMA indicates that the signaling mechanism in WTE, GTE and BTE induced NO production participates in the L-arginine dependent pathway in Raw264.7 cells.

CONCLUSION

Our results demonstrated that the WTE, GTE and BTE acted as an accelerator of activation of Raw264.7 cells by rIFN-y via a process involving L-arginine dependent

NO production and that WTE, GTE, BTE elevated NO production via activation of NF kappa- B signaling pathway. So, in conclusion it can be said that tea has immunomodulatory activity and that of white tea is far better as compared to that of Green and Black tea.

ACKNOWLEDGEMENT

We gratefully acknowledge the Director, CSIR-IICB, Indian Institute of Chemical Biology, Kolkata for providing the platform to perform the work without any obstacle. The authors are also thankful to the Council of Scientific and Industrial Research, Govt. of India for giving the fundamental assets to lead the investigation.

CONFLICT OF INTEREST

The authors proclaim that they have no conflict of interest.

REFERENCES

1. David S, Min Qui C, Guha K, Chuanfru L, Stone W. Regulation of nitric David, oxide production from macrophages by lipopolysaccharide and catecholamine's. *Nitric Oxide*, 2003; 8: 27- 32.
2. Rosseli M, Keller R, Dubey RK, Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Human Reproduction Update*, 1998; 4: 3-24.
3. Stuehr D, Marietta M. Mammalian nitrate biosynthesis mouse macrophages produce nitrate and nitrite in response to Escherichia coli lipopolysaccharide. *Proc. Natl. Acad. Sci.*, 1985; 82: 773 8-7742.
4. Hibbs J, Taintov R, Varzin Z. Macrophage cytotoxicity: role for L-arginine deaminase and imino nitrogen oxidation to nitrite. *Science*, 1987; 235: 473-476.
5. Palmer R, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, 1988; 33: 664-668.
6. Marietta M, Yoon P. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric acid is an intermediate. *Biochemistry*, 1988; 27: 8706-8711.
7. Stuehr J, Cho NS, Purification and Characterization of the cytokine-induced macrophage nitric oxide synthase: An FAD- and FMN-containing flavo-protein. *Proc. Natl. Acad. Sci.* USA, 1999; 88: 7773-7777.
8. Yui Y, Hattori R, Kosuga K, Eizawa H, Hiki K, Kawai C. Purification of Nitric oxide synthase from rat macrophages. *J. Biol. Chem*, 1991; 226: 12544-12547.
9. Fatemeh H, Kanthimathi MS., White tea (*Camellia sinensis*) inhibits proliferation of the colon cancer cell line, HT-29, activates caspases and protects DNA of normal cells against oxidative damage. *Food Chemistry*, 2014; 169: 401-410.
10. Xie W, Cho JJH, Mumford AR, Swiderek MK, Lee Ding A, Troso T, Nathan C. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages". *Science*, 1992; 256: 225-228.
11. Schreck R, Meier B, Manna ND, Droge W, Baueuerle AP. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *Journal of Experimental medicine*, 1992; 175: 1181-1194.
12. Takao Y, Tetsuro U, Akemi A, Fujiro S. Nitric oxide is an effector molecule in inhibition of tumour cell growth by r1FN-y-activated rat neutrophils. *International journal of cancer*, 1997; 71: 223-230.
13. Barnes PJ. Nuclear factor- kappa B., *Int J Biochem Cell Bio1*, 1997; 29(6): 867-870.
14. Muller JM, Ziegler-Heitbrock H., Baeuerle PA. Nuclear factor kappa B: a mediator of lipopolysaccharide effects. *Innnunobiology*, 1993; 187(3- 5): 233-256.
15. Williams DL, Ha TLC, Ferguson DA. Early hepatic NF kappa B and IL-6 in poly microbial sepsis. *Ann Surg*, 1999; 230(1): 95-104.
16. Kim HS, Inhibitory effects of *Ixeris dentata* on the mutagenicity of aflatoxin Bi, N-methyl-N'-nitro-N-nitrosoguanidine and the growth of MG-63 human Osteosarcoma cells. *Journal of the Korea society of food science and nutrition*, 1995; 24: 304-305.
17. Dirksen-Hughes J, Day D, Laster M, Zachariass A, Aquino L, Gooding R. Both tumour necrosis factor and nitric oxide participate in lysis of Simian virus 40-transformed cells by activated macrophages. *Journal of immunology*, 1992; 149: 2114-2122.