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Original Research Study

Anti-inflammatory Effect of the Aqueous Fruit Pulp Extract of *Tamarindus indica* Linn in Lipopolysaccharide-Stimulated Macrophages

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ABSTRACT

Aim: The aim of the present study was to evaluate the effect of the aqueous fruit pulp extract of *Tamarind indica Linn* on NO production and iNOS expression in LPS stimulated RAW 264.7 macrophages. **Material and Method**: The efficacy of tamarind extract on nitric oxide production was determined using RAW macrophages. RT - PCR was used to examine the expression of the iNOS gene in activated macrophages. The Statistical analysis for multiple comparisons was evaluated by one way ANOVA followed by the Dunnett's test when significant differences were detected. The data were considered to be statistically significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$. **Results**: LPS stimulated RAW macrophages strongly up regulated the iNOS gene expression levels. The iNOS levels were significantly suppressed in the presence of different concentrations of tamarind extract, compared to LPS treatment alone. The tamarind extract also exhibited dose – dependent decrease in the production of NO. The IC_{50} was found to be 35.69 µg/ml. LPS stimulated group showed 89.61 ± 0.47 % of NO. **Conclusion**: Nitric oxide production is found to be more in conditions such as periodontitis, oral squamous cell carcinoma and many other diseases. This study could prove the ability of tamarind fruit pulp extract to inhibit the production of nitric oxide and the iNOS gene expression. Hence, *Tamarind indica Linn* pulp extract may be used as a good anti-inflammatory agent in periodontitis as well as in conditions associated with over production of nitric oxide in different cancers such as oral squamous cell carcinoma.

Key words: *Tamarind indica,* Nitric oxide, iNOS expression, Oral squamous cell carcinoma, Periodontitis.

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INTRODUCTION

Inflammation is a protective response. It eliminates the pathogens and noxious stimuli and thereby restores the balance. However, prolonged dysregulated inflammation can cause tissue destruction through oxidative stress due to imbalance between the oxidants and antioxidants in the system which is common in conditions such as periodontitis and diabetesmellitus[1] Macrophages play important role in inflammation through the production of several pro-inflammatory molecules such as NO. Overproduction of NO has been associated with many inflammatory diseases such as arteriosclerosis, ischemic reperfusion, hypertension , septic shock , periodontitis, cancer.[2,3] (During inflammation, some cells especially macrophages promote the production of pro-inflammatory mediators such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α, reactive oxygen species (ROS), nitric oxide (NO) and prostaglandins.[4] Excess production of reactive oxygen and nitrogen species including nitric oxide is the characteristic feature of oxidative stress.[5] Lipopolysaccharide can induce, inducible nitric oxide synthase (iNOS) which is a pro-inflammatory enzyme. Under basal condition, the nitric oxide (NO) the product of iNOS, is involved in modulation of cellular functions and homeostasis which is regulated by the biosynthetic pathway. During inflammation, NO and PGs are released simultaneously in large quantities and NO can also directly increase PGE2 formation.[6]

Nitric oxide (NO) is a free radical produced by NO synthases, which is important for cardiovascular, neurological and immune functions. The NOS exits as endothelial NOS, brain NOS and inducible NOS. The inducible NOS (iNOS) expressed in response to pro-inflammatory stimuli such as endotoxin of Gram-negative bacteria alone or together with certain cytokines can produce a large amount of NO for sustained periods of time. iNOS is expressed in periodontitis, with localization mainly in inflammatory and epithelial cells. Selective inhibition of the inducible isoform of nitric oxide synthase and maintenance of constitutive NO production may be of therapeutic utility in periodontitis.[7,8]

Different cancer-related events such as angiogenesis, apoptosis, cell cycle, invasion and metastasis have been modulated by NO. Hence, it is also emerging as a potential anti-oncogenic agent.[9] NO is implicated in tumor progression of oral squamous cell carcinoma (OSCC). Studies report an increase in the clinical stage of the tumor in oral squamous cell carcinoma with increased tissue NO levels. The decrease in tumour differentiation indicates the association of NO with tumour growth. The role of NO in tumour

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growth represents a new dimension in cancer biology.[10] Increase in the level of NO and iNOS is also seen in breast cancer, cervical cancer, brain cancer, lung cancer, head and neck cancer and gastric cancer.[9,11]

Many studies reports that the plant foods such as fruits, vegetables and medicinal herbs are excellent source of antioxidant molecules that effectively inhibit the inflammatory process by affecting different molecular targets.[12,13] *Tamarind indica* Linn (family *fabiaceae*) is a medicinal plant available in Indian subcontinents and many parts of the world. Most of the plant parts have medicinal value and is used in traditional medicine.[14] The plant contains many phenolic compounds such as catenin, procyanidin B_2, epicatechin, tartaric acid, mucilage, pectin, arabinose, xylose, galactose, glucose, uronic acid and triterpen.[15] The fruit pulp extract is reported to have moderate antioxidant effect and hypolipidemic effect and hence it has beneficial effect in cardiovascular conditions. It also reduced abundance of HepG2 mitochondrial, metabolic and regulatory proteins involved in oxidative phosphorylation, protein synthesis and cellular metabolism.[16,17] Fruit pulp is also known for its laxative, spasmolytic, weight reducing effect. It can reverse hepatosteatosis and can reduce plasma fluoride level and ameliorate fluoride induced liver and kidney toxicity.[14] As per WHO reports Tamarind fruit is an ideal fruit with most of the essential aminoacids except tryptophan. Hence, the anti-inflammatory effect of the aqueous fruit pulp extract of *Tamarindus indica Linn* in lipopolysaccharide stimulated macrophages was carried out by evaluating its ability to reduce nitric oxide production through inhibition of inducible nitric oxide synthase.

MATERIALS AND METHODS

Chemicals and plant extract

Lipopolysaccharide (LPS), Phenol free Dulbecco's modified Eagle medium (DMEM), MTT, Dimethyl sulphoxide (DMSO), phosphate buffer saline (PBS) and antibiotic-antimycotic solution (100U penicillin, 100 µg streptomycin and 0.25 µg amphotericin B per ml) were purchased from Sigma-Aldrich. Fetal bovineserum was purchased from GIBCO/BRL Invitrogen. The tamarind fruit pulp extract was obtained from Synthite Industries Pvt Ltd, Kerala as gratis.

Cell culture

Macrophage RAW 264.7 cells were obtained from the NCCS, Pune with Passage no 16. Cells were cultured in phenol red-free Dulbecco's modified Eagle medium (DMEM) supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal bovine serum and grown at 37°C with 5% CO_2 in humidified air. Exponentially the cells were used for experiment when they reached 80% confluency. Cells were washed with DMEM medium and detached with 0.25% trypsin-EDTA. The cells were seeded at a density of 5 x 105 cells/well in 24 well plate and incubated for 18 h at 37°C and 5% CO_2. Then media of each well were aspirated and fresh FBS-free DMEM media were replaced. Different concentrations of Tamarind extract (3.175 –150 µg/mL) were prepared in FBS-free DMEM to give a total volume of 500 µl in each well of a microtiter plate. The cells were co - incubated with 1 µg/ml of LPS for 24 hrs.

Estimation nitric oxide (NO)

The presence of nitrite, a stable oxidized product of nitric oxide (NO), was determined in cell culture media using Griess reagent as described by Padgett and Pruett in 1992. The Griess reagent consists of 1 part of 0.1% naphthyl ethylenediamine dihydrochloride in distilled water plus 1 part 1% sulphanilamide (or sulphanilic acid) in 5% concentrated H3P04, the 2 parts being mixed together within 12 h of use and kept chilled. From this, 50 µl of supernatant from the test culture was mixed with 50 µl of 1% (w/v) sulphanilic acid in 5% (v/v) phosphoric acid in a 96-well plate, followed by in cubation for 10 min at room temperature. After that 50 µl 0.1% (w/v) N-1-naphthylethylenediamine HCl in distilled water was added and incubated for 10 min at room temperature. The optical density at 540 nm was measured with a microplate reader. The NO concentration was calculated by comparison with a NaNO2 (0 – 100 µM) standard curve. The final concentration of DMSO was adjusted to less than 0.1% for all treatments. The results were expressed as inhibition of NO production compared to the control (LPS) using: ([nitrite] c - [nitrite] t)/ [nitrite] c, where [nitrite] c and [nitrite] t are the nitrite concentration in the control and test sample, respectively.[18]

RNA isolation and q - PCR analysis RAW macrophages were treated with 17.5 µg/ml, 35 µg/ml and 70 µg/ml of Tamarind extract with 1 µg/ml of LPS and incubated for 24 h. Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol and 2 µg of RNA was used for complementary DNA synthesis using M-MLV reverse transcriptase (Promega, Madison, WI, USA). Quantitative real-time polymerase chain reaction (q-PCR) was performed in an ABI 7500 Real-Time System with SYBR Green PCR Master Mix (Takara). Reactions were initiated with an initial incubation at 50°C for two min and 94°C for 10 min, followed by 40 cycles of 94°C for 5s, 60°C for 15s and 72°C for 10s.[15] The relative gene expression levels were calculated using the 2−ΔΔCt method. The specific primer sequences used were given below and β-Actin was used as an internal reference gene between different samples.

INOS: Forward:5′-ATGTCCGAAGCAAACATCAC-3′

Reverse:5′-TAATGTCCAGGAAGTAGGTG-3′

Statistical analysis

Statistical analysis was performed using Graph pad Prism 5.0 software version (Graph Pad Software Inc., San Diego, CA). Data obtained from the experiments were expressed as Mean ± SEM. The Statistical analysis for multiple comparisons was evaluated by one-way ANOVA followed by the Dunnett's test when significant differences were detected. The data were considered to be statistically significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$.

RESULTS

Effect of tamarind extract on NO production

The efficacy of tamarind extract on nitric oxide production in RAW macrophages was determined. Nitrite production was dependent on the activating state of the cells. LPS unstimulated macrophages (Control) for 24 h produced lowest levels of NO, whereas LPS stimulated group showed 89.61 ± 0.47 % of NO production. Tamarind extract at its tested concentrations exhibited dose – dependent decrease in the production of NO. The IC_{50} was found to be 35.69 µg/ml (Figure 1).

Gene Expression of iNOS

To determine whether tamarind extract inhibits NO production at the level of transcription, RT - PCR was used to examine the expression of the iNOS gene in activated macrophages. LPS stimulated RAW macrophages strongly up regulated the iNOS gene expression levels. In the presence of tamarind extract at three different doses of 17.5 µg/ml, 35 µg/ml and 70 µg/ml, the iNOS levels was significantly suppressed, compared to that of LPS treatment alone (Figure 2).

DISCUSSION

As observed in the study, the results showed the amount of nitric oxide production tend to decrease in macrophages in the presence of tamarind extract. At 1 µg/ml of tamarind extract, the percentage of nitric oxide observed was 89.61 ± 0.47, while nitric oxide production levels decreased with increase in amount of tamarind concentration. At

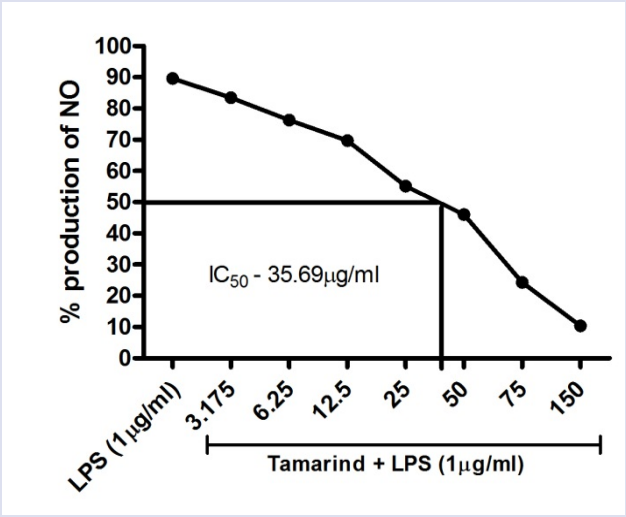

Figure 1: Graphical representation of Tamarind extract effect on production of NO production in LPS stimulated RAW 264.7 macrophages [Values are expressed Mean ± SEM (n = 3)].

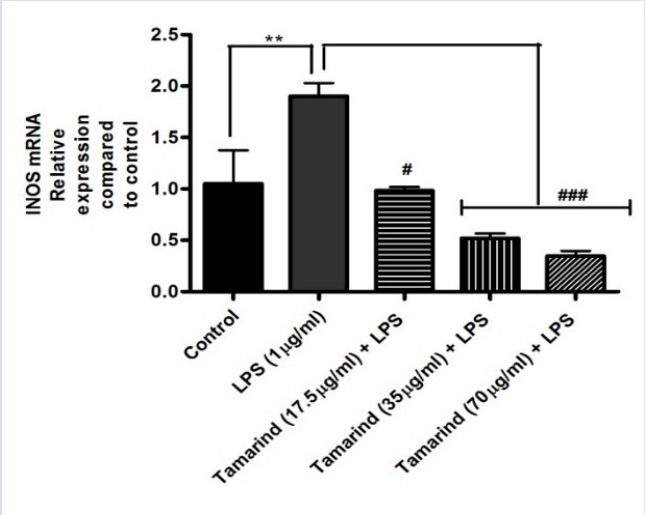

Figure 2: Effect of Tamarind on LPS – stimulated iNOS expression in RAW 264.7 macrophages [Values are expressed Mean ± SEM (n = 3)].

150 µg/ml, the nitric oxide production was observed to be only 10.39 ± 0.80. The effect on iNOS, tamarind extract could significantly inhibit NO production at the level of transcription.

Studies report the anti-inflammatory activity of *Chrysopogon aciculatis* by inhibiting LPS-stimulated RAW264.7 macrophages. Acicula tin present in the plant was responsible for the anti-inflammatory activity by inhibiting the expression of LPS-stimulated iNOS and COX-2 inflammation-associated genes *via* suppression of transcription factor NF-κB activation and JNK/p38 MAPKs pathway.[6] *S. melongena* and *S. macrocarpon* also reported to inhibit NO production and iNOS protein expression in RAW 264.7 cells.[19] Plants such as *C. ternatea* L. (Leguminosae) *Smallanthus sonchifolius* oleoresins such as capsicum and thyme, icariside E4from *U. Pumila* also possess anti-inflammatory effect.[20-24]

The ROS-generating enzymes such as iNOS, cyclooxygenase and lipoxygenase can be inhibited by flavonoids and thus can inhibit their pro-inflammatory activity. The plant, *M. longifolia* has a good anti-inflammatory activity. The hexane extract of *M. longifolia* with high levels of phenolic and flavonoid compounds significantly reduced NO production in LPS-stimulated cells. The possible mechanism reported was its ability to reduce NO secretion in macrophages by scavenging NO and inhibiting iNOS mRNA expression and TNFα pro-inflammatory cytokine expression.[25]

In general, the total phenolic content of the plant extracts is highly correlated with their free radical scavenging activities.[26] Phenolic compounds possess a remarkable anti-inflammatory capacity due to their multiple inhibitory activities of pro-inflammatory mediators. They are able to inhibit either the production or the action of pro-inflammatory mediators, resulting in anti-inflammatory capacity.[4] The tamarind fruit is rich in polyphenols and flavonoids which is associated with anti-inflammatory and anti-nociceptive properties.[15] Hence, the same flavonoids will be responsible for the inhibition of iNOS and in turn the production of NO.

Nitric oxide and antioxidant enzymes play an important role in etiology of oral cancer.[27] NO has been reported to modulate different cancer-related events including angiogenesis, apoptosis, cell cycle, invasion

and metastasis.[28] Expression of NOS has been detected in various human cancers. Presence of NO influence in various tumours such as breast cancer, cervical carcinogenesis, lung cancer and head and neck cancer. NO can initiate genetic alterations of gastric cells leading to gastric malignancy. NO seems to have an important part in the initiation, growth and metastasis of various cancers.[9] It has definite role in inflammatory conditions like periodontitis too. Hence, the herbs with inhibitory potential on NO and iNOS will be of great value in the management of different cancers such as oral squamous cell carcinoma and periodontitis where there is overproduction of NO.

CONCLUSION

Dietary phenolic compounds have potential for the treatment of inflammation and related diseases with fewer side effects. Tamarind fruit pulp is a culinary herb with nutritional as well as medicinal value. It has many traditional uses. The inhibitory effect on NO production and iNOS gene expression, make the aqueous fruit pulp extract a great anti-inflammatory agent in conditions with over production of NO including periodontitis and oral carcinoma.

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CONFLICTS OF INTEREST

Nil.

FUNDING SOURCE

Nil.

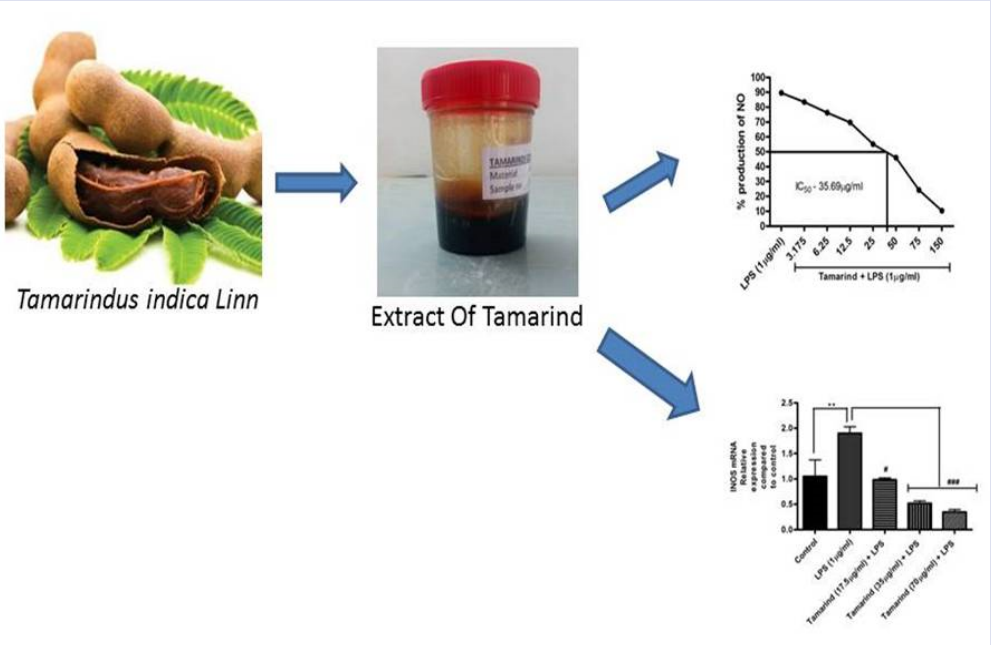
ABBREVIATIONS

LPS: Lipopolysaccharide; DMEM: Dulbecco's Modified Eagle Medium; DMSO: Dimethyl Sulphoxide; iNOS: Inducible Nitric Oxide Synthase; PBS: Phosphate Buffer Saline.

REFERENCES

1. Vincent RR, Appukuttan D, Victor DJ, Balasundaram A. Oxidative stress in chronic periodontitis patients with type II diabetes mellitus. Eur J Dent. 2018;12:225-31.
2. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 2007;87(1):315-424.
3. Terao J. Dietary flavonoids as antioxidants. In Food Factors for Health Promotion. 2009;61:87-94.
4. Ambriz-Pérez DL, Leyva-López N, Gutierrez-Grijalva EP, Heredia J B. Phenolic compounds: Natural alternative in inflammation treatment. A Review Cogent Food and Agriculture. 2016;2(1):1131412.
5. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems and apoptosis. Free Radical Biology and Medicine. 2010;48(6):749-62.
6. Hsieh I, Chang AS, Teng C, Chen C, Yang C. Aciculatin inhibits lipopolysaccharide-mediated inducible nitric oxide synthase and cyclooxygenase-2 expression via suppressing NF-_κB and JNK/p38 MAPK activation pathways. Journal of Biomedical Science. 2011;18(1):28.
7. Sundar NM, Krishnan V, Krishnaraj S, Hemalatha VT, AlamMN. Comparison of the salivary and the serum nitric oxide levels in chronic and aggressive periodontitis: A biochemical study. Journal of Clinical and Diagnostic Research. 2013;7(6):1223-7.
8. Reher VGS, Zenóbio EG, Costa FO, Reher P, Soares RV. Nitric oxide levels in saliva increase with severity of chronic periodontitis. Journal of Oral Science. 2007;49(4):271-6.
9. Choudhari S, Chaudhary M, Bagde S, GadbailAR, Joshi V. Nitric oxide and cancer: A review World Journal of Surgical Oncology. 2013;11(1):118.
10. Sangle VA, Chaware SJ, Kulkarni MA, Ingle YC, Singh P, Pooja VK. Elevated tissue nitric oxide in oral squamous cell carcinoma. Journal of Oral and Maxillofacial Pathology. 2018;22(1):35.
11. Bakan E, Taysi S, Polat MF, Dalga S, Umudum Z, Bakan N, *et al.* Nitric oxide levels and lipid peroxidation in plasma of patients with gastric cancer. Japanese Journal of Clinical Oncology 2002;32(5):162-6.
12. Tsai PJ, Tsai TH, Yu CH, Ho SC. Evaluation of NO suppressing activity of several Mediterranean culinary spices. Food and Chem Toxicology. 2007;45(3):440-7.
13. Taira J, Nanbu H, Ueda K. Nitric oxide-scavenging compounds in AgrimoniapilosaLedeb on LPS-induced RAW264.7 macrophages. Food Chem. 2009;115(4):1221-7.
14. Kuru P. *Tamarindus indica* and its health related effects. Asian Pacific Journal of Tropical Biomedicine 2014;4(9):676-81.
15. Bhadoriya SS, Mishra V, Raut S, Ganeshpurkar A, Jain SK. Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of *Tamarindus indica* leaves. Scientia Pharmaceutica. 2012;80(3):685-700.
16. Lim CY, Junit SM, Abdulla MA, Aziz AA. *In vivo* biochemical and gene expression analyses of the antioxidant activities and hypocholesterolaemic properties of Tamarindus indica fruit pulp extract. PloS One. 2013;8(7):e.0070058.
17. Chong URW, Abdul-Rahman PS, Abdul-Aziz A, Hashim OH, Mat-Junit S. Effects of *Tamarindus indica* Fruit pulp extract on abundance of HepG2 cell lysate proteins and their possible consequential impact on metabolism and inflammation. Bio Med Research International. 2013;2013.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS. Analysis of nitrate, nitrite and [15N]nitrate in biological fluids. Analytical Biochemistry. 1982;126(1):131-8.
19. Rebecca Fang Ling Ng, Nurhayati Zainal Abidin, Adawiyah Suriza Shuib, Daud Ahmad Israf Ali. Inhibition of nitric oxide production by *Solanum melongena* and *Solanum macrocarpon* on RAW264.7 cells. Frontiers in Life Science. 2015;8:(3):241-8.
20. Nair V, Bang WY, Schreckinger E, Andarwulan N, Cisneros-Zevallos L. Protective role of ternatinanthocyanins and quercetin glycosides from butterfly pea (Clitoriaternatea Leguminosae) blue flower petals against lipopolysaccharide (LPS)-induced inflammation in macrophage cells. Journal of Agricultural and Food Chemistry. 2015;63:6355-65.
21. Oliveira RB, Chagas-Paula DA, Secatto A, Gasparoto TH, Faccioli LH, Campanelli A P, *et al.* Topical anti-inflammatory activity of yacon leaf extracts. Revista Brasileira de Farmacognosia. 2013;23(3):497-505.
22. Prathoshini SM, Anitha R, Lakshmi T. The effect of Capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line. Pharmacognosy Research. 2018;10(4):343-6.
23. Mangal CSK, Anitha R, Lakshmi T. Inhibition of nitric oxide production and nitric oxide synthase gene expression in LPS activated RAW 264.7 macrophages by thyme oleoresin from *Thymus vulgaris*. Journal of Young Pharmsicists. 2018;10(4):481-3.
24. Joo T, Sowndhararajan K, Hong S, Lee J, Park S, Kim S, *et al.* Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmuspumila* L. Saudi Journal of Biological Sciences. 2014;21(5):427-35.
25. Karimian P, Kavoosi G, Amirghofran Z. Anti-inflammatory effect of *Menthalongifolia*in lipopolysaccharide-stimulated macrophages: Reduction of nitric oxide production through inhibition of inducible nitric oxide synthase. Journal of Immunotoxicology. 2013;10(4):393-400.
26. Cho SY, Ko HC, Ko SY, Hwang JH, Park JG, Kang SH, *et al.* Correlation between flavonoid content and the NO production inhibitory activity of peel extracts from various citrus fruits. Biological and Pharmceutical. Bulletin. 2007;30(4):772-8.
27. Patel JB, Patel PS, Shah FD, Shah PM, Shukla SN. Role of nitric oxide and antioxidant enzymes in the pathogenesis of oral cancer. Journal of Cancer Research and Therapeutics. 2009;5(4):247-53.
28. Ying L, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. Cancer Research. 2007;67(4):1407-10.

GRAPHICAL ABSTRACT



SUMMARY

Reactive nitrogen species and reactive oxygen derived from nitrogen play important roles in acute inflammation and their overproduction can cause tissue damage and vascular leakage. Plant extracts may help in such conditions by interfering with the nitric oxide production. The aqueous extract of Tamarind fruit pulp has showed good inhibitory effect on NO production and iNOS gene expression. Hence, *Tamarind indica L* pulp extract may be used as a good anti-inflammatory agent in conditions associated with over production of Nitric Oxide. The plant being available in most part of India, its culinary use also recommended as a medicinal plant.

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