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 IC₅₀ 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.**

**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 diabetes mellitus. 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. 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 isofrom of nitric oxide synthase and maintenance of constitutive NO production may be of therapeutic utility in periodontitis. Nitric oxide (NO) is a free radical produced by NO synthases, which is important for cardiovascular, neurological and immune functions. The NO 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 isofrom of nitric oxide synthase and maintenance of constitutive NO production may be of therapeutic utility in periodontitis.

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. 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 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.

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growth represents a new dimension in cancer biology.\textsuperscript{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.\textsuperscript{11,12}

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.\textsuperscript{13,14} \textit{Tamarindus indica} Linn (family \textit{fabaceae}) 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.\textsuperscript{15} The plant contains many phenolic compounds such as catechin, procyanidin \textit{B1}, epicatechin, tartaric acid, mucilage, pectin, arabinose, xylose, galactose, glucose, uronic acid and triterpen.\textsuperscript{17} 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.\textsuperscript{18,19} Fruit pulp is also known for its laxative, spasmytic, weight reducing effect. It can reverse hepatosteatosis and can reduce plasma fluoride level and ameliorate fluoride induced liver and kidney toxicity.\textsuperscript{20} 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 \textit{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. Bovine serum was purchased from GIBCO/BRL Invitrogen. The tamarind fruit pulp extract was given below and β-Actin was used as an internal reference gene between different samples.

**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\textsubscript{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 10\textsuperscript{5} cells/well in 24 well plate and incubated for 18 h at 37°C and 5% CO\textsubscript{2}. To determine whether tamarind extract inhibits NO production at 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\textsubscript{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
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 E4 from *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.\(^{17}\) 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.

**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)].
and metastasis. Expression of NO 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. 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 value 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.

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Nil.

ABBREVIATIONS

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

REFERENCES

**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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