Reactive nitrogen and oxygen species are a ubiquitous part of human life having both beneficial and harmful effects. These are produced in cells under physiological conditions during oxygen metabolism and other redox processes that are of vital importance for the biological function. However, excess of these radicals react with cellular lipids, proteins and nucleic acids leading to local injury and eventual organ dysfunction. Oxidative stress has a probable role in the pathogenesis of a variety of human diseases.

Nitric oxide, a multi-faceted bioregulatory agent produced by many types of cells, is known to play a critical role in both regulatory processes and cell defense, yet it also participates in collateral reactions, leading to DNA damage and cell death in both nitric oxide generating and neighboring cells. Nitric oxide is an important mediator of the inflammatory response. It is a strong proinflammatory substance that may increase vascular permeability in inflamed tissues. There is extensive suggestive evidence that nitric oxide can participate as mediator of tissue damage in autoimmune diseases.

Reactive nitrogen and oxygen species are implicated in many disease conditions including chronic inflammation, arteriosclerosis, rheumatoid arthritis, some neurodegenerative diseases, systemic lupus erythematosus and respiratory conditions. The increased levels of nitrotyrosine, which have been detected in the tissues affected by these diseases, have been attributed to a higher formation of nitric oxide. Therefore, nitrotyrosine was proposed as a suitable chemical marker for assessing the nitration level in proteins, and it is generally believed that peroxynitrite is the main contributor to the formation of nitrotyrosine. However, it has been suggested that nitrotyrosine formation may be induced by other species such as nitrogen dioxide, nitrous acid or nitronium chloride. Nitration of tyrosine residues of proteins occurs, either on albumin or on lipoproteins. Nitric oxide may also interact with nucleic acids, causing DNA strand breakage, and react with guanine residues with the formation of 8-nitroguanine, thus contributing to a number of diseases.

Systemic lupus erythematosus is an autoimmune disease with a complex etiology and pathogenesis. It is a multisystem chronic, autoimmune disease encompassing a
spectrum of diseases defined by clinical criteria. Both cell mediated and humoral autoreactivity precipitates SLE. One of the characteristics of SLE is a long-term high affinity immune response to self-antigens. SLE is associated with the presence of autoantibodies against tissues and cellular components such as DNA, RNA, cytoplasmic elements found in serum, phospholipids, ribonucleoproteins and histones. However, the main autoantibodies found in SLE are antinuclear autoantibodies that react with nuclear antigens such as dsDNA, ssDNA, chromatin and histones. However, antibodies not only recognize nucleic acid antigens but also react with a number of cellular proteins.

In the present study, the possible role of native and nitrated poly L-tyrosine in SLE has been probed. Aqueous solution of poly L-tyrosine (pH 11) was exposed to nitric oxide generated by sodium nitrite in acidic medium. The generation of nitric oxide was confirmed using carboxy-PTIO, a quencher of nitric oxide radical. The nitric oxide induced modifications in poly L-tyrosine were analyzed by UV, fluorescence and circular dichroism spectroscopy.

The UV absorption spectra of nitrated poly L-tyrosine showed a peak shift of 4 nm towards shorter wavelength and hypochromicity at 280 nm as compared to native poly L-tyrosine. Another peak was observed at wavelength of 420 nm, which is characteristic of 3-nitrotyrosine at pH >9. The data reflects NO induced damage of the aromatic ring of tyrosine, as well as nitration of tyrosine forming 3-nitrotyrosine. Damage of the chromophoric group was further confirmed by fluorescence spectra and quantitation of tyrosine by Lowry’s method.

Formation of low molecular weight species, aggregation, peptide bond cleavage was further ascertained by DNA adduct formation, thermal denaturation studies, alkaline agarose gel electrophoresis and gel filtration.

The antigenicity of native and nitrated poly L-tyrosine was probed by inducing antibodies in rabbits. The repertoires of specificities of induced antibodies were evaluated by direct binding and competition ELISA. The induced antibodies against native and nitrated poly L-tyrosine exhibited polyspecificity, a property commonly associated with SLE anti-DNA autoantibodies. Anti-native and anti-nitrated poly L-tyrosine antibodies showed preferential recognition of respective immunogens in competitive inhibition.
assay as well as dot blot assay. The level of 3-nitrotyrosine was evaluated in the immune sera by HPLC.

A group of SLE sera with high antibody titre were investigated for their specificity towards native and nitrated poly L-tyrosine by ELISA. Direct binding ELISA showed preferential binding of SLE autoantibodies to nitrated poly L-tyrosine in comparison to native poly L-tyrosine and nDNA. Inhibition ELISA reiterated the direct binding results. Gel retardation assay further substantiated the binding of native and nitrated poly L-tyrosine with IgG isolated from SLE sera. The formation of immune complex between SLE IgG and native/nitrated antigens confirmed enhanced recognition of nitrated form over native poly L-tyrosine by circulating autoantibodies in SLE.

SLE sera were analyzed for the presence of 3-nitrotyrosine by Western blot analysis. The separation and measurement of 3-nitrotyrosine concentration in SLE sera was carried out by HPLC indicating that serum 3-nitrotyrosine level is elevated among patients with SLE.

In conclusion, the nitric oxide radical causes nitration as well as structural alteration and renders the nitrated form of poly L-tyrosine highly immunogenic. The induced antibodies against nitrated poly L-tyrosine showed polyspecificity towards various polymers, resembling the antigen binding characteristics of SLE anti-DNA autoantibodies. The possibility of nitrated poly L-tyrosine in the production of SLE autoantibodies has been probed in the present study.