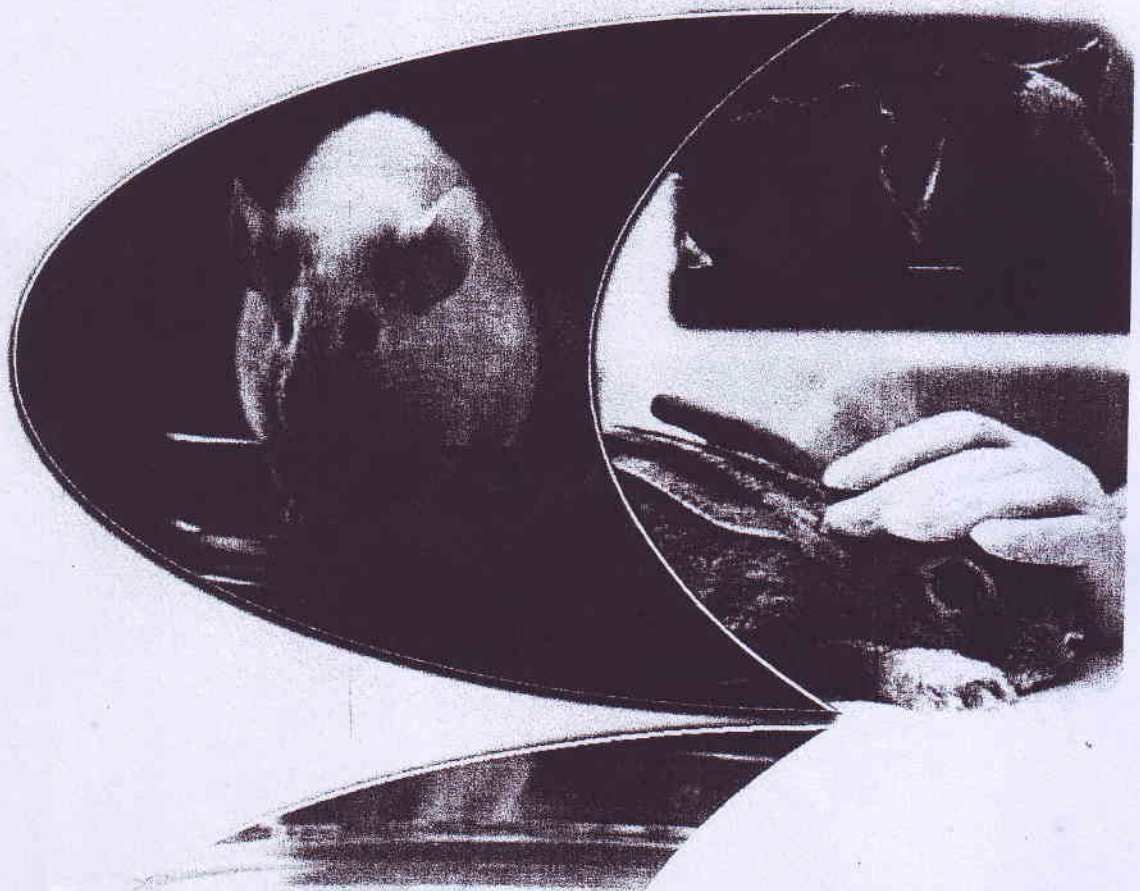


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NITRIC OXIDE SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF
Psychotria sarmentosa LEAVES

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Introduction: Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by different cells. Nitric oxide is a reactive nitrogen intermediate produced by macrophages and it plays a crucial role in both acute and chronic inflammatory processes. Our previous studies have confirmed for acute and chronic anti-inflammatory activities of the aqueous extract of *Psychotria sarmentosa* (Family: Rubiaceae, "Gonica" in Sinhala) leaves *in-vivo*. Hence, our next studies were focused to determine the mechanisms of its anti-inflammatory action.

Objectives: Present study has been aimed to evaluate the nitric oxide scavenging activity of this extract in *in-vitro*. This was adopted as a mean of reducing the number of animals that would be for *in-vivo* studies in the future.

Methodology: The nitric oxide (NO) radical scavenging activity was estimated by using Griess nitrite assay. The reaction mixture contained sodium nitropruside (SNP; 10 mM; 2 mL), phosphate buffered saline (PBS; 50 mM; pH 7.4; 500 μ L) and the test sample (500 μ L) at different concentrations (50 – 500 μ g / mL). The same reaction mixture without the test sample, with the phosphate buffered saline served as the negative control and quercetin (20 – 200 μ g / mL) used as a positive control. After incubation for 150 minutes at 25 °C, Griess reagent (500 μ L) was added to the mixture (500 μ L). The pink chromophore formed was measured at 540 nm after an incubation period of 30 minutes at 25 °C. All the tests were performed in triplicate. Absorbance values of the samples were corrected for interference with sample colour. The effective concentration of the sample required for scavenging nitric oxide radical by 50 % (EC₅₀) was obtained by linear regression analysis of the dose response curve plotted between percentage inhibition vs concentration.

Results and Discussion: The compound SNP is known to decompose in aqueous solution at physiological pH, producing NO. Under aerobic conditions, NO reacts with oxygen to produce the stable products nitrate and nitrite, the quantities of which can be determined using Griess reagent. When a solution of 10 mM SNP in PBS was incubated, time dependent nitrite production occurred. It was decreased by the presence of freeze dried aqueous extract of *P. sarmentosa* leaves (FDAP) or quercetin in a dose dependent manner. The EC₅₀ for FDAP was 275.7 ± 0.9 μ g / mL and 108.6 ± 0.6 μ g / mL for quercetin which was the standard anti-oxidant.

Conclusion: Based on the results obtained, it may be concluded FDAP showed nitric oxide radical scavenging activity which may contribute towards its ethno medically reputed anti-inflammatory effects.

Key words: Griess reagent, Inflammation, Nitric oxide, *Psychotria sarmentosa*