

ANTI-INFLAMMATORY EFFECT OF AN ETHYL ACETATE EXTRACT OF *GARCINIA MANGOSTANA* L. BY DOWNREGULATION OF NITRIC OXIDE PRODUCTION IN RAW 264.7 CELL LINE

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ABSTRACT

This study identifies the efficacy of an ethyl acetate extract of *Garcinia mangostana* as a potent inhibitor of nitric oxide (NO) production. Crude extract in the range from 0.906 µg/ml to 15.625 µg/ml significantly decreased nitrite production in LPS-stimulated RAW 264.7 cells in vitro in a concentration dependent manner (11 µmol to 2.5 µmol) ($p < 0.01$). The findings also demonstrated that extract was not cytotoxic to cells by MTT assay at these concentrations (0.906 µg/ml to 15.625 µg/ml).

KEY WORDS

Anti-inflammation, RAW 264.7 cells, Nitric oxide, *Garcinia Mangostana*.

INTRODUCTION

Inflammation is physiological response to tissue injury and microbial invasion. These non-specific cellular and vascular responses are mediated by various immunocompetent cells including macrophages. Upon activation macrophages produces pro-inflammatory mediators such as prostaglandin E2 [PGE2], TNF-R, interleukins (IL)-1, 6, and 12 and free radical such as nitric oxide (NO) [1]. NO is produced by nitric oxide synthase (NOS) from L-arginine [2]. NOS can be categories into two forms, consecutive and inducible NOS. Expression of the inducible NOS (iNOS) is stimulated by inflammatory cytokines or bacterial products such as lipopolysaccharide (LPS) [3]. Prolonged exposure to NO can cause inflammatory diseases like inflammatory bowel disease, atherosclerosis, rheumatoid arthritis, glomerulonephritis, and septic shock [4-7]. Therefore, NO production, through iNOS induction

by LPS, may reflect the degree of inflammation in LPS stimulated RAW 264.7 cells and may provide possible ways to screen various anti-inflammatory compounds.

Garcinia mangostana L. (Clusiaceae), commonly known as mangosteen is well known for its medicinal properties and have been in used in Thai folk medicine for the treatment of skin infections, wounds and diarrhea [8, 9]. Earlier studies have shown anti-inflammatory effect of xanthenes from *G. mangostana* [10] and *in-vivo* anti-inflammatory has also been observed in hamster opisthorchiasis [11]. In view of this, present study undertaken to evaluate the anti-inflammatory activity *in vitro* by an ethyl acetate extract of *G. mangostana* on murine macrophages on RAW 264.7 cell line.

MATERIAL AND METHODS

Chemicals

RAW 264.7 cell line was procured from National Center for Cell Sciences (NCCS) Pune, India. DMEM (Dulbeccos Modified Eagles Medium), Fetal Bovine Serum (FBS) were purchased from JRH Biosciences, India. Streptomycin, Penicillin, Sodium pyruvate, Glutamine was purchased from Hyclone. DMSO, Nitric oxide, Orthophosphoric acid were purchased from Hi-Media, India. MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), N-[naphthyl]ethyl-ene-diamine dihydrochloride, Sulphanilamide, Sodium chloride, Potassium chloride, Di Sodium hydrogen phosphate, Potassium dihydrogen phosphate, EDTA, Tris Hcl, Ethanol was purchased from E-Merck. Lipopolysaccharide (LPS) was purchased from Difco, India.

Preparation of extract

The fruit was collected from the local market (voucher no. GM 1/USBT), the fruit was cut from the middle and the pericarp removed and was air-dried for 12-16 hours. It was then grounded in the electric grinder and the powdered material was weighed and then passed through a soxhlet apparatus for 6-8 hours. The solvent (ethyl acetate) so obtained from the extraction was subjected to evaporation by using the rota-evaporator at 160 rpm. It was continued till a thick paste was obtained. The solvent is discarded and the thick paste was taken out. For powdered extract, the thick paste was subjected to vacuum drying, by keeping it in the evacuated chamber for 4 hours. The extract so obtained was a dry powdered material (w/v) which was eventually weighed.

Cell viability

This is a non-radioactive detection method for the measurement of cell survival/proliferation. This assay is based on the cleavage of yellow tetrazolium dye (3-[4-5 dimethylthiazol-2-4] 2-5-diphenyl tetrazolium bromide MTT) into soluble

purple formazan, by succinate dehydrogenase in active mitochondria [12]. Dead cells are unable to perform this reaction. In this assay the amount of purple formazan generated is spectrophotometrically determined in a multiwell plate reader at 570 nm. The murine macrophage cell line, RAW 264.7, was cultivated in DMEM supplemented with 10% FBS at 37°C in a humidified atmosphere of 5% CO₂. Cells (0.5 x 10⁶ cells/mL) were plated in 96 well tissue culture plates at a final volume of 100 µl. Each test extract concentration ranging from 0.976µg/ml to 15.625µg/ml was tested in triplicates. Cultures plates were incubated at 37°C in a CO₂ incubator for appropriate duration of time. 20 µl of MTT stock solution was added to the cell culture. Plates were incubated at 37°C in a CO₂ incubator for 30 minutes, 75 µl/well of DMSO and ethanol was added to solubilize the formazan crystals. Optical density (O.D) was taken at 570nm wavelength using a multi well plate reader.

NO production by LPS-stimulated RAW 264.7 cells

The amount of NO formed was estimated from the accumulation of the stable NO metabolite, nitrite (NO₂⁻) by Griess assay [13]. In this assay, the culture supernatant from various treatment groups (100µl) and Griess reagent 100 µl are mixed together and the absorbance was measured at 550 nm. The amount of nitrite was calculated from a NaNO₂ standard curve. Murine macrophages RAW 264.7 cells were first incubated with LPS (5µg/ml) for 6 hours. The cells were then incubated with different concentrations of ethyl acetate (EA) extract of *Garcinia mangostana* ranging from 0.976 µg/ml to 15.625 µg/ml for 24 hours. The amount of nitric oxide produced by murine macrophages was assessed by collecting cell supernatant and treating it with equal amount of Griess reagent. Cell supernatant harvested from cells in which were stimulated with LPS only was taken as control. The nitrite

accumulated was measured spectrophotometrically at 550 nm, the amount of nitric oxide produced was calculated from a standard curve.

Statistical analysis

Statistical analysis was performed by unpaired t test using GraphPad software in which $p < 0.01$ considered as statistically significant.

RESULTS AND DISCUSSION

Pericarp of *G. mangostana* known to possess prenylated xanthenes, benzophenones, bioflavonoids and triterpenes [14-16]. Out of the 68 xanthenes present in mangosteen, the most explored for medicinal properties are α -, β -, and γ -mangostins, garcinone E, 8-deoxygartanin, gartanin [17-18] and most abundant xanthenes are α -, and γ -mangostins [19]. Previous studies have shown antibacterial, antimicrobial,

antiprotozoal, antifungal, antiviral, anti-parasitic, anti-tuberculosis, anthelmintic, anti-allergy, antioxidant and analgesic properties by isolated xanthone α -mangostin and anti-tumorigenic, anti-proliferative in both α - & γ -mangostin [11, 21-24]. In the present study, we showed concentration dependent downregulation of NO production by different concentrations of an EA extract of *G. mangostana* (Fig 1). The concentration ranging from 0.976 $\mu\text{g/ml}$ to 15.625 $\mu\text{g/ml}$ showed significant downregulations in NO production (11 μmol to 2.5 μmol). The $p < 0.01$ is highly significant compared to control (LPS only). At these very same concentrations the cytotoxicity of an extract was observed to be negligible as demonstrated by MTT assay in Fig. 2. Most of the concentration of test extract showed (90%) survival as comparable to control.

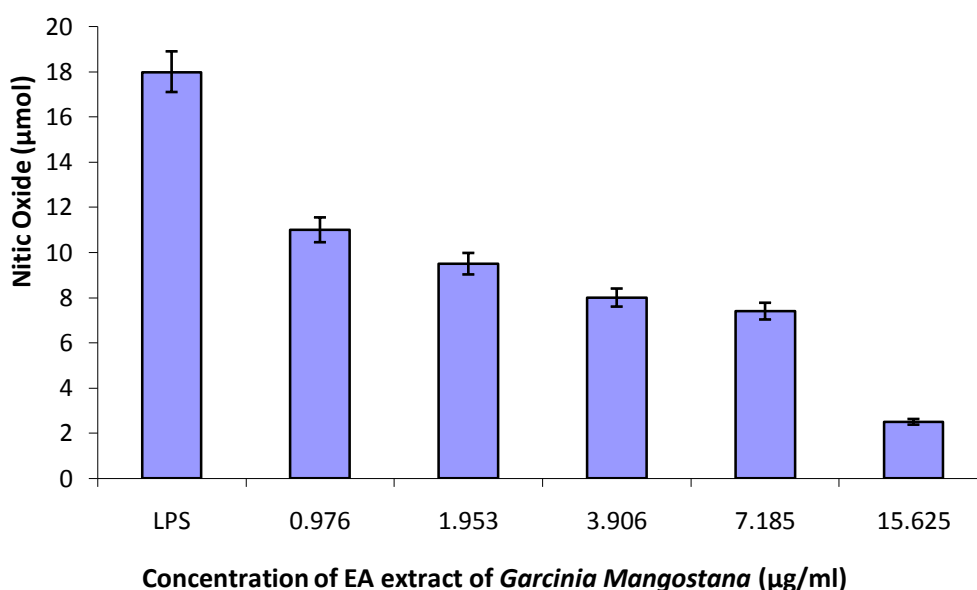


Fig 1. Effect of different concentrations of EA extract of *G. Mangostana* on LPS induced NO generation in murine macrophages RAW 264.7 cell line

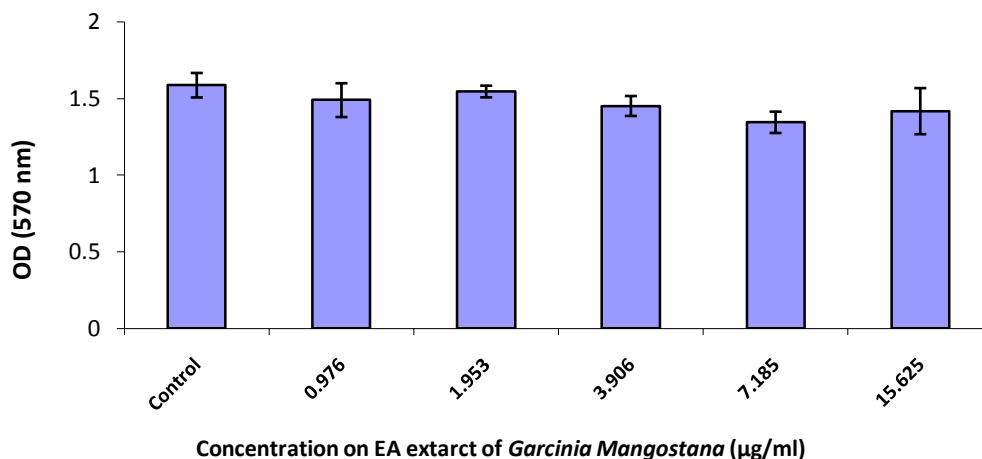


Fig 2. MTT Assay showing the effect of EA extract of *G. Mangostana* on survival of RAW264.7 cell line

CONCLUSION

NO is a potent inflammatory mediator and its concentration is greatly enhanced in inflammation conditions. High concentration of NO has been implicated in a number of diseases such as multiple sclerosis, diabetes, hypertension and Alzheimer's diseases. The present study demonstrated the downregulation of NO production in LPS induced *in-vitro* experimental model with varying concentration of an EA extract *Garcinia mangostana*. This shows the anti-inflammatory potential of the plant extract in different condition of inflammation but further studies are required to isolate, purify and characterize the active constituents which may give new lead in drug discovery for inflammatory conditions.

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