Original Article

Anti-inflammatory activity of Caralluma Fimbrata- raw 264 macrophage cell lines

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ABSTRACT

Introduction: The aim of the study was to estimate the anti-inflammatory activity of Caralluma Fimbrata on Raw 264 Macrophage cell lines enhanced with LPS which stands for lipo polysaccharide. Materials and Methods: The ethanolic extract of Caralluma Fimbrata was obtained from Green Chem Herbal Extract, Bangalore as a gift sample. Raw 264 macrophage cell lines were obtained from NCSS, Pune for the study. The sample was then studied along with the obtained cell lines to estimate the expression of nitrous oxide. Nitrous oxide being a known substance involved in inflammation. The gene expression of iNOS was also studied. Results: The effects of different concentrations of Caralluma Fimbrata was assessed and the nitrous oxide levels was estimated. It was observed that with increased concentration of the ethanolic extract of Caralluma Fimbrata the nitrous oxide Level was lowered and thus it is proven that Caralluma Fimbrata possesses anti-inflammatory properties. Conclusion: Caralluma Fimbrata extract possesses anti-inflammatory activity showed by significantly decrease in production of pro-inflammatory mediator NO and also by the suppression by the iNOS gene expression even when enhanced with LPS.

Keywords: Anti-inflammatory, anti inflammation, caralluma fimbrata, anti arthritic, nitrous oxide.

Introduction

Nature has provided a complete store-house of remedies to cure all aliments of mankind [1]. This is where, nature provides us drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effect [2]. Research on medicinal plants is an important fact of biochemical research in India because of several reasons. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules [3]. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation [4]. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease [5]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [6-8].

Macrophages after LPS stimulation produce NO by up-regulating iNOS expression through mitogen-activated protein kinases (MAPK) and NF-κB signaling pathways. In response to macrophage activation, LPS stimulates a Toll-like receptor 4 (TLR4)-mediated myeloid differentiation factor (MyD88)-dependent pathway, which in turn activates the transforming growth factor-β-activated protein kinase 1 (TAK1), which subsequently results in activation of nuclear factor-κB (NF-κB) and activating protein-1 (AP-1), and produces inflammatory cytokines including TNF-α, IL-6, and IL-1β [9,10]. Therefore, inhibition of these inflammatory mediators has been considered as an effective strategy for the development of anti-inflammatory drugs.

The aim of the study is to evaluate the anti-inflammatory activity of Caralluma Fimbrata on Raw 264 Macrophage cell lines.

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Materials and Methods

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Plant Material

Caralluma fimbrata bark ethanolic extract used for the study were obtained from Green Chem Herbal extract and Formulations, Bengaluru as a gift sample.

Chemicals

Lipopolysaccharide (LPS), Phenol free Dulbecco's modified Eagle medium (DMEM), MTT, Dimethyl sulphoxide (DMSO), phosphate buffer saline (PBS), and antibiotic-anti mycotic solution (100U penicillin, 100µg streptomycin, and 0.25µg amphotericin B per ml) were purchased from Sigma-Aldrich. Fetal bovineserum was purchased from GIBCO/BRL Invitrogen.

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 100units/ml penicillin, 100µg/ml streptomycin, and 10%heat-inactivated fetal bovine serum at 37°C with5% CO2. Cells were washed with DMEM medium and detached with 0.25% trypsin-EDTA. The cells were re-suspended in DMEM medium at a density of 2 x 106cells/ml.

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. Briefly, 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 incubation for 10 min at room temperature. After that 50 µl 0.1% (w/v) N-1-naphthylethylenediamineHCl in distilled water was added and incubated for 10 min at room temperature. The optical density at 540 nm was measured with a micro plate 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.

RNA Isolation and q - PCR Analysis

RAW macrophages were treated with 30µg/ml, 60µg/ml and 90µg/ml of Caralluma Fimbrata extract with 1µg/ml of LPS and incubated for 24h. Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and 2µg 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 minutes and 94◦C for 10 min, followed by40 cycles of 94◦C for 5s, 60◦C for 15s, and 72◦C for 10 s. The relative gene expression levels werec alculated using the 2−ΔΔCt method. The specific primer sequences used were given below:

INOS: Forward: 5′-ATGTCCGAAGCAAACATCAC-3′

Reverse: 5′-TAATGTCCAGGAAGTAGGTG-3′

β-actin was used as an internal reference gene between different samples.

Statistical analysis

Data obtained from the experiments were expressed as Mean ± SEM. The Statistical analysis of the difference between the groups was evaluated by Dunnett's following one-way ANOVA Post hoc comparisons in Graph pad Prism 5.0 software version. $p<0.001$, $p<0.01$ and $p<0.05$ were considered to be statistically significant.

Results

Effect of Carulluma fimbriata on NO production

NO is a pluripotent signaling molecule produced by different isoforms of enzyme, Nitric enzyme synthase (NOS). Despite possessing various beneficial effects, overproduction of nitric oxide lead to various disorders including inflammatory diseases. Hence effect of different concentrations Carulluma extract was evaluated on LPS induced NO production. LPS significantly increased NO production in RAW macrophages. The level of NO increased by LPS induction was significantly decreased in a dose – dependent manner when treated with different concentrations of Carulluma extract.

Table 1: The effect of Carulluma fimbriata on NO production in LPS stimulated RAW 264.7 macrophages

S.No	Concentration (µg/ml)	% of NO production in LPS Stimulated RAW 264.7 macrophages
1	3.175	80.84±0.60
2	6.25	75.01±0.56
3	12.5	68.57±0.52
4	25	59.73±0.34
5	50	47.85±1.23
6	75	29.71±0.52
7	150	8.61±0.76

Values are expressed Mean±SEM (n = 3)

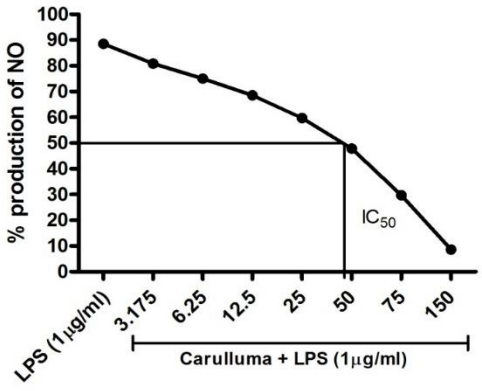
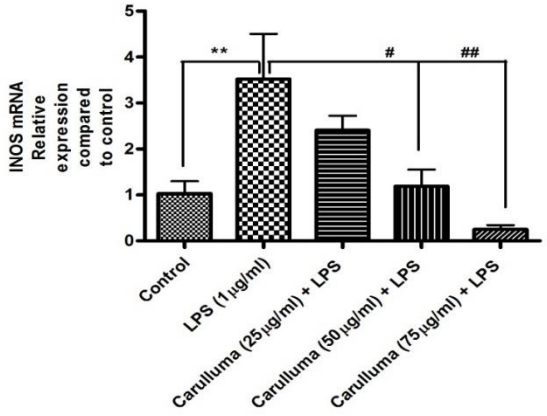


Figure 1: Graphical representation of the effect of Carulluma fimbriata on production of NO production in LPS stimulated RAW 264.7 macrophages

Gene Expression of iNOS

LPS stimulation of RAW macrophages strongly upregulated the iNOS gene expression levels. However, when Carulluma was added at three different doses of 25µg/ml, 50µg/ml and



75µg/ml, the iNOS levels was significantly suppressed, compared to that of LPS treatment only.

Figure 2: The effect of Carulluma fimbriata on LPS – stimulated iNOS expression in RAW 264.7 macrophages. Values are expressed Mean±SEM (n = 3)

Discussion

Several studies have demonstrated the properties of various compounds from plants tend to possess rich pharmacological properties that play beneficial roles in many different conditions, including inflammation-related diseases [11-13]. Inflammation is a dynamic process involving proinflammatory cytokines such as nitrous oxide and it acts as important biological response toward injury [14, 15]. In the present study, we have examined the anti-inflammatory activity of Caralluma fimbrata n Raw 264 macrophage cell lines enhanced with LPS.

NO is a free radical produced from l-arginine by nitric oxide synthases (NOSs), and an important cellular second messenger [16]. The modulation of iNOS-mediated NO release is one of the major contributing factors during the inflammatory process [17]. Thus suppression of the response of NO would in turn indicate the anti-inflammatory activity of the particular extract. NO has the property to modify or generate intercellular signals and thus it has an effect on Immune cells, tumour cells and the cells of different tissues or organs.

From the above results of the present study it is evident that Caralluma fimbrata as an anti-inflammatory activity from table 1. On increasing the concentration of the extract the nitrous oxide level continues to decrease and thereby inhibiting the effect of iNOS mediated inflammation. Thus the anti-inflammatory effect of Caralluma fimbrata has been confirmed.

The limitations of the study would include that only the NO pro-inflammatory messenger system has been analyzed in the current study. Other secondary messengers [18, 19] in case of inflammatory reactions can be analyzed with the same extract and a conclusive final analysis of the particular source can be obtained.

Conclusion

Caralluma fimbrata Extract possess anti-inflammatory activity showed by significantly decrease in production of pro-inflammatory mediator NO and also by the suppression by the iNOS gene expression even when enhanced with LPS.

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