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In Vitro Anti-Inflammatory Effect of Andrographis Paniculata (Andropure) Against LPS-Induced TNF- α, IL-6, And Nitric Oxide (NO) Generation in Activated Macrophage RAW 264.7 Cell Lines

C A Anzar[1], Joseph M V[1], Dr. Sundaram R[1], Vadiraj G B*[1], Prasad C P [1], Bineesh Eranimose[1]

1. Olive Lifesciences Pvt Ltd No.165/5, Near NH-4, Nelamangala, Bangalore – 562123, Karnataka, India

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ABSTRACT

Plants in the genus Andrographis paniculata contain huge amounts of andrographolide (AG), which possesses several advantages, such as neuroprotective, anticancer, anti-inflammatory, and antidiabetic properties. Andrographolide has been shown in clinical research to be effective in treating a variety of illnesses, including osteoarthritis, upper respiratory conditions, and multiple sclerosis. The precise mechanism of A. paniculata is still unclear in spite of several pharmacological studies. As a result, andrographolide, the primary active ingredient isolated from A. paniculata (Burm. f.) Wall ex Nees, has anti-inflammatory properties; however, the key molecular pathways are yet unknown. The objective of this study was to evaluate the anti-inflammatory effect of A. paniculata (Andropure) against LPS-induced TNF-α, IL-6, and nitric oxide production in mouse macrophages (RAW 264.7). A. paniculata (Andropure) was tested for anti-inflammatory effects at various doses and exhibited dose-dependent TNF-α inhibition compared to LPS-induced cell controls in an anti-inflammatory investigation, A. paniculata (Andropure) demonstrated the highest percentage suppression of TNF-α and IL-6 at 500 µg/ml. A. paniculata (Andropure) showed the inhibition of nitric oxide also.

KEYWORDS:A. paniculata (Andropure), Cytotoxicity, RAW 264.7 cell line, TNF-α, IL-6 and LPS, Nitric Oxide, Anti-inflammatory

I. INTRODUCTION

In general, inflammatory reactions occur as a physiological reaction to harmful stimuli, such as the invasion of pathogens and toxic substances [1]. Although many diseases, including rheumatoid arthritis [2], atherosclerosis [3], asthma [4], diabetes [5], chronic hepatitis [6], septic shock [7], and inflammatory neurodegenerative disorders [8], are associated with uncontrolled and aberrant inflammation, TNF-α, IL-1, IL-6, GM-CSF, and pro-inflammatory mediators such as nitric oxide, inducible nitric oxide synthase, and cyclooxygenase-2 (COX2) are all secreted in high amounts by activated macrophages during chronic inflammation [9]. The outer cell wall of gram-negative bacteria contains a large amount of lipopolysaccharide (LPS). Therefore, LPS induces a host of inflammatory response that causes the immune system to produce more chemokines, cytokines, and pro-inflammatory mediators [10]. During bacterial infections, LPS-exposed macrophages activate the immune system to produce cytokines and chemokines, which then induce inflammation. Therefore, preventing macrophage activation by LPS is an essential objective of medicinal remedies for the management of inflammatory disorders [11–14].

In Asian traditional medicine, the Andrographis paniculata plant has been extensively utilized for many years [15]. The popular herb Andrographis is derived from A. paniculata. The entire plant or its aerial portions are used for purposes such as cooling properties and a bitter taste that helps reduce heat, detoxify, chill the blood, and minimize swelling [16]. The A. paniculata plant produces andrographolide, a labdane diterpenoid that has been shown to have a number of pharmacological properties, including antibacterial, antiviral, and antiplatelet properties; stimulation of cell differentiation; liver protection; cholagogue activity; antitumor properties; and immunoregulation [17]. In a rat model of egg white protein and carrageenan-induced hind paw edema, andrographolide clearly exhibited anti-inflammatory effects [18]. Inducible nitric oxide

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synthase (iNOS) expression and prostaglandin E2 synthesis by LPS-activated macrophages can be suppressed by andrographolide [19]. The expected pharmacodynamics function and methods by which andrographolide exerts such anti-inflammatory activities, however, are still unknown [20].

The objective of this study was to investigate the anti-inflammatory activity of A. paniculata (Andropure) in macrophage RAW 264.7 cells because, as far as we are aware, the anti-inflammatory effects of A. paniculata have not been evaluated. In LPS-induced RAW 264.7 cells, we examined the effects of A. paniculata (Andropure) on cytokine (TNF-α and IL-6) production as well as its impact on the expression of NO (nitric oxide), TNF-α, and IL-6 genes. In addition, andrographolide found in A. paniculata (Andropure) inhibits the production of various pro-inflammatory genes, such as IL-6, nitric oxide (NO), and TNF-α. We feel that A. paniculata (Andropure) can be added to the list of important natural remedies to be explored as a possible anti-inflammatory agent because it demonstrated the ability to attenuate the LPS-induced inflammatory response in macrophages.

II. MATERIAL AND METHODS

Procurement of A. paniculata (Andropure)

A. paniculata (Andropure) is manufactured and registered by Olive Life Sciences Pvt Ltd Nelamangala, Bangalore, Karnataka, India.

Preparation of A. paniculata (Andropure)

To create a stock solution of 1 mg/ml concentration, 10mg of A. paniculata (Andropure) were individually dissolved, and a volume was built up with DMEM-supplemented media containing 2% inactivated FBS. This stock solution was then sterilized by 0.22-syringe filtration. From this, serial two-fold dilutions were made for the purpose of conducting research.

Cytotoxicity of A. paniculata (Andropure)

Cytotoxicity Studies:

Trypsinization of the cell culture and an increase in cell density to 1.0 x 10^5 cells/ml were accomplished using DMEM-HG/MEM with 10% FBS. 0.1 ml of the diluted cell suspension was added to each of the 96 wells of the microtiter plates. After 24 hours, when a partial monolayer formed, the supernatant was discarded, the monolayer was washed once with media, and 100µl of various A. paniculata (Andropure) doses were added on top of the partial monolayer in microtiter plates. The plates were then incubated for 3 days at 37°C in a 5% CO_2 environment, and microscopic examination and observations were carried out every 24 hours. The A. paniculata (Andropure) solutions in the wells were removed after 72 hours, and 50µl of MTT in PBS were added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in a 5% CO_2 environment.To dissolve the produced formazan, 100 µl of 2-propanol was added after the supernatant was taken out of the mixture. The plates were then gently agitated.At a wavelength of 540 nm, the absorbance was calculated using a microplate reader.

In vitroAnti- Inflammatory Activity

TNF-α and IL-6 inhibitory activity

RAW 264.7 cells were seeded at a density of 1.5 to 2 x 10^5 cells/ml in DMEM with 10% FBS in six-well culture dishes. The cells were treated with known and varied concentrations of test chemicals after 24 hours, along with 1 µg/ml of lipopolysaccharide (LPS), and then incubated for another 24 hours at 37°C with 5% CO_2. After incubation, the cell supernatant was collected, centrifuged, separated, and kept at -20°C until use. Elisa kits are used to estimate the levels of TNF-α and IL-6 in cell supernatants.

Nitric oxide inhibition

In order to measure the amount of nitrite, RAW 264.7 cells were treated with LPS and the test chemicals as previously mentioned, incubated for 24 hours, and then the conditioned media were collected. A biomarker for nitric oxide (NO) called nitrite was identified and measured. In a flat-bottomed 96-well plate, equal volumes (50 µL) of 0.1% N-1 napthylethylenediaminedihydrochloride made in water, 1% sulphanilamide prepared in 5% phosphoric acid, and cell culture medium were mixed and incubated for 10-15 minutes. 530 nm was used to measure the colour of the final product. In the LPS control, the percentage of nitric oxide inhibition was determined.

III. RESULT AND DISCUSSION

The A. paniculata (Andropure) showed dose-dependent inhibition of TNF-α, IL-6, and nitric oxide (NO) in mouse macrophages, as mentioned in Table 1.

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Table 1: Effects of in vitro anti-inflammatory activity of A. paniculata (Andropure) in RAW cell lines

SL.NO.	Sample name	% of TNF-α inhibition	% of nitric oxide (NO) inhibition	% of IL-6 inhibition
1	Control	Control group		
2	Control + LPS 1µg/ml	Inflammation inducer group		
3	Control + LPS 1µg/ml + Andropure 100µg/ml	17.12±1.05	28.16±1.15	7.35±1.57
4	Control + LPS 1µg/ml + Andropure 250µg/ml	16.44±0.13	50.77±2.76	15.40±1.38
5	Control + LPS 1µg/ml + Andropure 500µg/ml	42.22±0.35	55.53±0.64	33.86±0.98
6	Control + LPS 1µg/ml + Dexamethasone (standard)10µM	71	73	70

Andrographis paniculata has long been known to have anti-inflammatory benefits in both in vitro and in vivo experimental inflammation models, according to earlier studies. A. paniculata and its derivatives reduce the levels of adhesion molecules, pro-inflammatory cytokines, chemokines, lipid mediators, and NO, primarily via reducing NF-B activation [21-23].Whether A. paniculata influences NF-B or other signalling pathways to reduce inflammation is still unclear. In this study, we investigated the underlying mechanism of A. paniculata's anti-inflammatory activities and verified their existence. We discovered that A. paniculata suppresses inflammatory reactions in macrophages caused by LPS by activating AMPK. A. paniculata's anti-inflammatory properties appear to be a result of its capacity to suppress a number of inflammatory mediators at the transcriptional level. Our findings showed a high correlation between the decrease in inflammatory gene mRNA expression, including those of iNOS, COX-2, and TNF-α, and A. paniculata-mediated inhibition. In addition to our data, other research groups have shown that A. paniculata can strongly suppress transcriptional upregulation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-4, IL-5, IL-6, and IL-13), cytokine receptors (IL-1βR and TNFR), adhesion molecules (E-selectin and VCAM-1), and chemokines (CCL8 and CXCL11) in macrophages, vascular endothelial cells, and eosinophils [24-26].

The primary active ingredient isolated from A. paniculata, andrographolide, has been shown to possess a wide range of pharmacological properties, including antibacterial, antiviral, and antiplatelet properties. It also stimulates cellular differentiation, protects the liver, acts as a cholagogue, has antitumor properties, and is immunoregulatory. In addition, earlier research has demonstrated that andrographolide can prevent the capillary permeability increases in mice carried out by xylene and acetic acid.

In the current investigation, LPS was used to create an in vitro model of inflammation in mouse RAW 264.7 cells, which were then treated with various concentrations of A. paniculata (Andropure). Our studies revealed that andrographolide dramatically reduces the levels of nitric oxide (NO), IL-6, and TNF-α in LPS-stimulated RAW 264.7 cells. Andrographolide has been shown to have in vitro anti-inflammatory properties, too. Andrographolide could protect neurons from inflammation-related damage by inhibiting NF-kB and activating Nrf2/HO-1, according to the current concept. This is due to a decrease in the release of TNF-α, nitric oxide (NO), and IL-6, as well as the generation of ROS.

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IV. CONCLUSION

We showed that A. paniculata (Andropure) significantly inhibits macrophage in vitro inflammatory responses, including nitric oxide (NO) generation and the expression of pro-inflammatory genes including TNF-α and IL-6. A. paniculata (Andropure), which contains the active ingredient andrographolide, has anti-inflammatory properties and significantly lowers the expression of TNF-α and IL-6 in RAW 264.7 cells that have been exposed to LPS. Its anti-inflammatory mechanism may be through the inhibition of the NF-*κ*B and MAPK signalling pathway. In conclusion, these findings offer a clear understanding of the anti-inflammatory mechanism of A. paniculata (Andropure), which suppresses inflammatory responses by activating AMPK. These findings suggest that A. paniculata (Andropure) produces anti-inflammatory actions in activated macrophages and may have benefits in the management of inflammatory diseases.

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