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Original Article

Anti-Inflammatory Potential of Arglabin in LPS-Activated RAW 264.7 Macrophages

Faiz Abdulaziz Alfaiz*

Department of Biology, College of Science, Majmaah University, Al-Majmaah, SAUDI ARABIA.

ABSTRACT

Background: Uncontrolled inflammation, however, adds to the pathophysiology of many chronic diseases even though it is a vital defense mechanism in health. Despite being necessary for reducing inflammation, anti-inflammatory medications have a number of negative effects. Thus, the purpose of this work is to assess the anti-inflammatory properties of Arglabin (AGN) that is a major bioactive compound of Artemisia sps. and belongs to guaianolide class. AGN, a renewable substance employed in the synthesis of novel chemicals, is one of the practically accessible sesquiterpene lactones. **Materials and Methods:** Using the murine macrophage RAW264.7 as a model, we examined the anti-inflammatory properties of AGN by preventing the generation of inflammatory markers that promote inflammation, as well as their ability to regulate oxidative stress. Using Lipopolysaccharide (LPS)-triggered RAW 264.7 cells, we evaluated AGN's anti-inflammatory effects using various techniques. **Results and Discussion:** AGN suppressed the generation of ROS and NO. It was shown that in RAW264.7 cells exposed to LPS, AGN diminished the inflammatory markers, such as IL-6 and TNF-α. Furthermore, AGN at various dosages up to 20 μM was not cytotoxic. Additionally, the data show that AGN reduces inflammation by suppressing Prostaglandin E2 (PGE2). **Conclusion:** In summary, these findings indicate that AGN may serve as a drug for inflammation to treat severe inflammatory conditions by inhibiting the generation of pro-inflammatory markers and successfully reducing macrophage activation.

Keywords: Anti-Inflammatory, Arglabin, LPS, RAW 264.7 Macrophage, ROS.

Correspondence:
Dr. Faiz Abdulaziz Alfaiz
Department of Biology, College of Science, Majmaah University, Al-Majmaah, 11952, SAUDI ARABIA.
Email: f.alfaiz@mu.edu.sa

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INTRODUCTION

To defend the body from harmful stimuli like the infectious invasion of viruses and poisons, the human immune system has the ability to initiate an inflammatory response.[1] An array of defense systems known as the inflammatory response work to remove dangers, encourage tissue healing and reestablish physiological balance.[2] The five primary indicators of inflammation that serve as the body's "natural defense system" against illness and injury, are temperature, redness, malfunction, edema and soreness. While some degree of inflammation is necessary for good health, too much inflammation can harm host cells and result in illnesses.[3] However, research has demonstrated a broad spectrum of diseases, including autoimmune disorders, cardiovascular disorders, chronic respiratory disorders, neurological disorders and cancers, are linked to the shift of inflammation into a chronic or dysregulated state.[2] Particularly, the onset of numerous metabolic illnesses, like obesity and insulin resistance, is directly linked to persistent inflammation. Macrophages, a major immune component, play a vital role in body's reaction to infection and injury.[4]

Macrophages have an imperative role in the development of inflammatory ailments and are essential in regulating immunological responses. Through Toll-Like Receptors (TLRs), macrophages are able to detect stimuli and initiate pathways, including NF-κB and MAPK.[2] Metamorphism is the first stage of every inflammation, followed by exudation defense and proliferative healing. The primary cause of chronic inflammation is hyperplasia, which typically involves lymphocytes and plasma. The primary pathogenic symptom is cell infiltration.[4] Activated macrophages produce a significant amount of inflammatory markers (NO), cytokines (TNF-α and IL-6), and chemokines (IL-8) during chronic inflammation.[1] TNF-α is an essential cytokine that controls the immune system's inflammation. It is capable of producing IL-6 and IL-1. Monocyte Chemoattractant Protein-1 (MCP-1) and Cyclooxygenase-2 (COX-2) create Prostaglandin-Endoperoxide synthase 2 (PGE2), which is a common pro-inflammatory markers that can control immunological responses.2 Remarkably, in chronic inflammation, activated macrophages start the inflammatory response by producing more NO, ROS and inflammatory cytokines.[5]

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The development of an inflammatory model is important practically to study the inflammatory medications and to treat inflammatory disorders because the features of these conditions are complex and challenging to address.[6] Around the world, established cell models are frequently employed for both *in vitro* and *in vivo* works. This is because there is an infinite supply of cells through successive passages that have the same genotype and phenotype. Authors now use greater caution when interpreting data from studies that are limited to using established cell lines, nevertheless. The most interesting topic is undoubtedly about the properties of the cells and how stable and comparable they are across different labs and even different stages. Macrophages are a fascinating class of cells. Their ability to polarize, differentiate and become osteoclasts, Kuppfer cells, or dendritic cells has made them renowned for their flexibility. As a result, macrophages are a widely varied kind of cell. While they occur in all organs and tissues of the body, their phenotype varies based on the physiological condition. Macrophages can swiftly adapt to novel stimuli and are highly sensitive to environmental changes.[5] RAW 264.7 cells are generated from BALB/c mouse-derived Abelson leukemia virus-transformed cell line and they resemble monocytes and macrophages. It is said that these cells are a suitable model for macrophages. They are able to carry out both phagocytosis and pinocytosis. RAW 264.7 cells generate more NO and improve phagocytosis after exposure to LPS. Furthermore, through antibody-dependent cytotoxicity, these cells can destroy target cells.[7]

Receptors located on the membranes of B cells, dendritic cells and macrophages detect and combine LPS to promote local inflammation, trigger the accumulation of inflammatory factors by immune cells and stimulate innate immunity.[6] Gram negative bacteria have a large distribution of Lipopolysaccharide (LPS) in their outer cell walls. As a result, LPS triggers a host inflammatory response, which causes the generation of more pro-inflammatory markers by the immune system. LPS exposure causes macrophages infected with microorganisms to release cytokines and chemokines, which trigger the immune system and cause inflammation. Therefore, the primary aim of therapeutic approaches to treat inflammatory illnesses is to prevent LPS-induced macrophage activation.[1]

A lot of work has gone into finding compounds or herbs that can cure a number of ailments and natural goods are crucial in the identification of innovative newly created chemical entities and lead compounds.[6] Natural compounds generated from plants have been utilized in various healthcare systems and have been scientifically assessed for a range of bioactivities. Natural medications are recommended since they have less side effects than synthetic drugs because of their associated negative effects. One such molecule is arglabin, a sesquiterpene γ-lactone belonging to the guaianolides class that was extracted from the Artemisia glabella plant, which is native to Central Kazakhstan.[8] Early in the 1980s, arglabin was separated from the plant's aerial portion.[9] Arglabin has numerous pharmacological properties. Several applications exist for this chemical, such as antibacterial, neuroprotective and anticancer properties.[8,9]

Sesquiterpene γ-lactones are an imperative compounds that has gained a lot of interest due to their wide range of pharmacological properties, which include antimicrobial, growth-regulating, anthelmintic, immunomodulatory, and antitumor properties.[10] Arglabin has a wide range of pharmacological applications. Numerous applications exist for this chemical, such as antibacterial, neuroprotective and anticancer properties.[8] Arglabin is a white crystalline substance with a chemical structure based on the bicyclo decane skeleton (molecular formula: $C_{15}H_{18}O_3$).[11] It has been observed that arglabin and its derivatives are cytotoxic to a number of cancer cell lines.[12]

The purpose of this research is to study the anti-inflammatory actions of AGN in LPS-challenged RAW 264.7 macrophage cells. To date, anti-inflammatory action and ROS modulation of AGN have not been extensively studied. In LPS-induced RAW 264.7 macrophages, we examined the impact of AGN on TNF-α, IL-6, NO and ROS production, cytotoxicity and PGE2 expression.

MATERIALS AND METHODS

Materials

Prior to the start of the study, all chemicals, reagents, kits and equipment were purchased commercially and made available.

Cell Culture

The authorized center provided the RAW264.7 murine macrophage cell line. The growth medium used for the cells was DMEM enhanced with 1% penicillin/streptomycin and 10% FBS. Every two days, cells were sub-cultured and kept in an incubator with 5% CO_2 and 37°C temperature. The RAW264.7 cells was employed in all tests and it was in passages 9-12.[2] Different concentrations of AGN dissolved in DMSO were used to treat the cells, whereas vehicle alone (<0.2% DMSO) was used as the control.[8]

Arglabin's effects on Raw264.7 macrophage viability

Following treatment with LPS and different doses of AGN, the 3-(4,5-dimethylthizaol-2-yl)-2,5-diphenyl Tetrazolium bromide (MTT) was utilized to assess the growth of AGN on the RAW264.7 cells. In a 48-well plate, macrophages (3×104 cells/well) were planted. Following a 24 hr period, the cells were subjected to a 30 min pretreatment with 0, 2.5, 5, 10, 15 and 20 µM AGN, followed a 24 hr incubation time in 5% CO_2 condition at 37°C. 1 mg/mL of MTT reagent was used to treat cells for 2 hr after which the culture media was removed. Following the removal of the MTT solution, to liquefy the formazan crystals, 200 µL of DMSO

was applied. The microplate reader was utilized to determine absorbance at 570 nm.[3]

Analysis of the generation of Nitric Oxide (NO)

Using the Griess test, the NO content in the cell supernatant was determined.[3] In a 96-well plate, 100 µL of RAW264.7 cells were loaded at 2 × 105 cells/mL population. After that, the cells were cultivated for 24 hr at 37ºC. Cells were pretreated with AGN and Positive control Dexamethasone (Dex) for 1 hour and then cultured for an extra 24 hr before exposed to LPS (1 µg/mL). Later, supernatant (100 µL) and Griess reagent (100 µL) was combined in wells. An absorbance at 540 nm wavelength was obtained following a 15 min incubation period.[2]

Analysis of reactive oxygen species (ROS) level

The DCFH-DA was utilized to quantify the levels of ROS.[1] A 24-well plate containing Raw264.7 macrophages (6×104 cells/well) was planted and cultured for 24 hr. Subsequently, the cells underwent a 30 min pretreatment with AGN (10, 15 µM and Dex), followed by an LPS treatment (0.1 µg/mL) and a 24 hr incubation period at 37ºC in an incubator with 5% CO2. After 30 min of DCF-DA treatment at 37ºC, the cells were given three HBSS washes. Using a fluorescent microscope, ROS levels were found and FACS analysis was performed.[3]

Determination of Prostaglandin-Endoperoxide synthase 2 (PGE2) utilizing ELISA Kit

A 60 mm cell culture was seeded with 4 mL per well and 2×105 cells/mL. The cells was cultivated in an incubator for 24 hr at 37ºC and 5% CO2. Supernatants were obtained after these cells were cultured for a further 20 hr with LPS (1 µg/mL) stimulation after being pre-exposed with AGN (10, 15 µM and Dex) for one hour. As directed by the manufacturer, ELISA kits were utilized to quantify the PGE2 content in the supernatants.[2]

Evaluation of impact of AGN on pro-inflammatory cytokines

Using an ELISA assay kit, the IL-6, IL-1β and TNF-α was evaluated. A 24-well plate was seeded with cells ($1.5x10^5$ cells/well) and left to incubate for 24 hr. The cells underwent a 1 hr pretreatment with AGN at different doses (10, 15 µM and Dex) followed by a 24 hr treatment with LPS (1 µg/mL). The supernatant was obtained as per the instructions of the manufacturer, and ELISA equipment was used to study the IL-6, IL-1β and TNF-α concentrations.[12,13]

In silico Study's

Preparation of Ligand

The ligand's 3D structure and calculated descriptors were acquired in SDF format from the library of PubChem (Table 1). Utilizing Open Babel GUI, an open-source toolkit for the transformation of chemical structures, atomic coordination was translated to a pdbqt format.[14] The energy was optimized utilizing the Universal Force Field.[15] employing Avogadro software, until a stable atomic conformation is achieved. Energy minimization is a mechanism that seeks to identify a minimum of potential energy surface, commencing from an initial structure of higher energy. Avogadro facilitates the chemical structure construction, visualization and analysis of molecules, structure optimization, quantum mechanical computations, and electron density assessments.

Receptors preparation

The XRD structure of EGFR signalling pathway are identified as critical targets for AGN anti-inflammatory anti-cancer action. The complex of EGFR (PDB code: 4URO) with novobiocin (resolution: 2.59 A; R-value free: 0.246; was discovered.[16-18] Target protein was acquired from the PDB database. The selection criteria for PDBs included (a) minimum resolution and (b) the conformation of docked ligand matching that of the crystalline structure post-redocking.[19] The selected PDB file for virtual screening investigation were modified by eliminating water, including hydrogen atoms, and ultimately generated using Biovia Discovery Studio.

Molecular docking

The AGN and its target protein, the EGFR (4URO) were input into PyRx AutoDock VINA.[20] The target protein was transformed into macromolecules, altering the atomic coordination to pdbqt format. The grid box for molecular docking was centered on a crystal structures, with other parameters set to default. The docking outcomes were evaluated for binding affinity, and subsequently, all potential docked conformations for the chemical was produced. After investigation using PyMOL and Discovery Studio, only the conformations that directly connect with active-site of the EGFR-targeted protein were chosen. Biovia Discovery Studio were employed utilized to study the intricate connections and their classifications, like hydrogen bonds, halogen interactions, alkyl interactions, and van der Waals forces between Arglabin and the target EGFR gene in human cancer therapy.

Pharmacodynamic works

Drug likeliness and bioactivity score

The physicochemical parameters of the AGN were obtained via the SwissADME internet server (http://www.swissadme.ch/index.php) to comply with Lipinski's rule of five, which is crucial for rational drug design. The compound arglabin adhered to all five criteria: a maximum of 5 hydrogen bond donors, a maximum of 10 hydrogen bond acceptors, a molecular weight under 500, a partition coefficient (log P) below 5, fewer than 10 rotatable bonds, and a Topological Polar Surface Area (TPSA) not exceeding 140 (Table 2).[21] The bioactivity of arglabin were assessed utilizing Molinspiration Cheminformatics website, and

the ADMET analysis was conducted with the admetSAR tool. The bioactivity of each substructure of fragment will be computed, and the overall bioactivity of the molecule will be determined as the aggregate of the contributions from all fragments inside the molecule. This yields a "molecule activity score," a numerical value generally ranging from -3 to 3. Molinspiration recommends that molecules with the highest activity scores has the greatest likelihood of being active.

ADMET predictions

Molecular descriptors are the determining factors for the pharmacokinetic characteristics and toxicity of a sample. In silico ADMET characteristics forecast the potential of compounds as therapeutic agents.[22] The admetSAR online server was employed to calculate the ADMET characteristics of the chemical arglabin. The chemical and the target were listed in Table 4. A chemical must possess a favorable ADMET profile. The BBB, HIA, water solubility, Caco-2 cell permeability, CYP450 inhibition, and Ames toxicity was also assessed.[23-28]

Statistical Analysis

The information is based on three distinct experiments, was presented as mean±SD. GraphPad Prism was used to statistically analyze the results. A noticeable difference between the groups was indicated by $p<0.05$. Software called SPSS was used to perform statistical analysis. Turkey's test is used after a one-way ANOVA to scrutinize significances among the groups.

RESULTS

Arglabin's effects on Raw264.7 macrophage viability

Following treatment with LPS and different doses of AGN, the MTT test was employed to measure the cytotoxicity of AGN on RAW264.7 cells. Over the course of 48 hr, the live cells was reduced in a dosage-dependent manner by AGN at various doses (0, 2.5, 5, 10, 15 and 20 µM) (Figure 1). The outcomes demonstrated no changes between the 10, 15 and 20 µM AGN treated groups, suggesting that up to 20 µM AGN didn't significantly affect the cytotoxicity (>100% cell viability). Therefore, in the next tests, AGN was utilized at 10 and 15 µM doses.

AGN reduces NO levels

The primary mediators of the inflammatory response include NO. In addition to controlling leukocyte movement and tissue toxicity, resulting in vasodilation and the development of edema, NO also performs other roles in inflammatory responses. To study the anti-inflammatory properties of AGN Raw264.7 macrophages' NO level was assessed. Upon administering AGN to the LPS-induced increase in NO production, it was shown that NO production was inhibited beginning at 10 µM (Figure 2). When compared to the LPS-only group, the AGN therapy decreased NO generation in a concentration-dependent way. The cells exposed to AGN exhibit a similar pattern to the Dex 10 µM treated cells (positive control).

Analysis of ROS generation

Tissue damage and endothelial dysfunction are due to excessive production of ROS. We investigated into how arglabin affected the accumulation of ROS in LPS administered RAW264.7 cells. The ROS generation by AGN was investigated using DCFH-DA probe technique. After 24 hr, LPS treatment increased the amounts of ROS within macrophages. On the other hand, AGN administration dramatically and dose-dependently decreased the formation of ROS generated by LPS. When compared to LPS-stimulated control cells, treatment with 10 and 15 µM AGN dramatically reduced ROS formation by 320%±10% and 800%±10%, respectively (Figure 3). When AGN was compared with control, AGN increased intracellular ROS levels by around

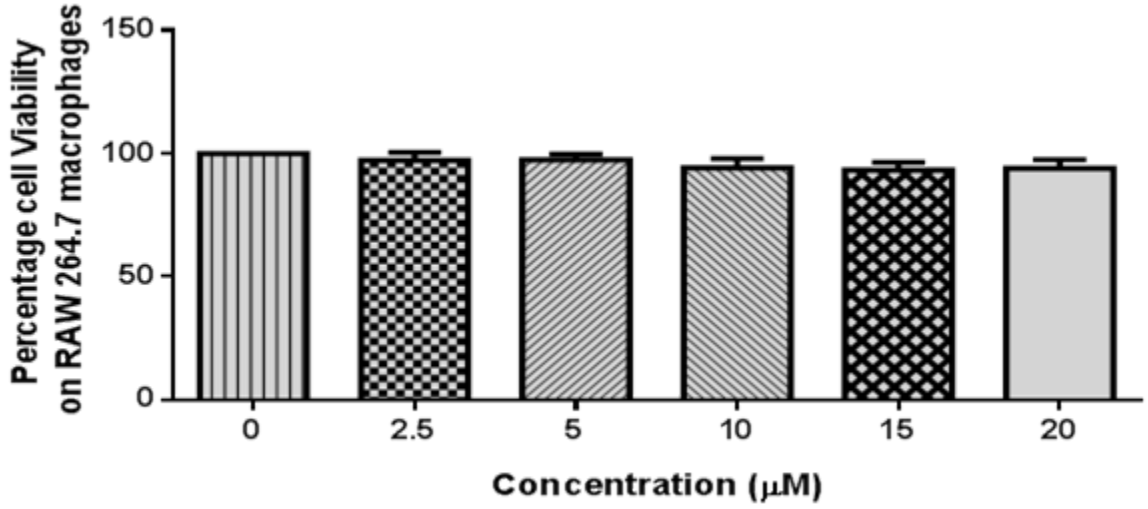

Figure 1: Effects of AGN on growth of Raw264.7 cells. The cells were pre-treated with AGN (0, 2.5, 5, 10, 15 and 20 µM) followed by treatment with LPS after which cell growth was studied using an MTT test. The values are illustrated as mean±SD of triplicates.

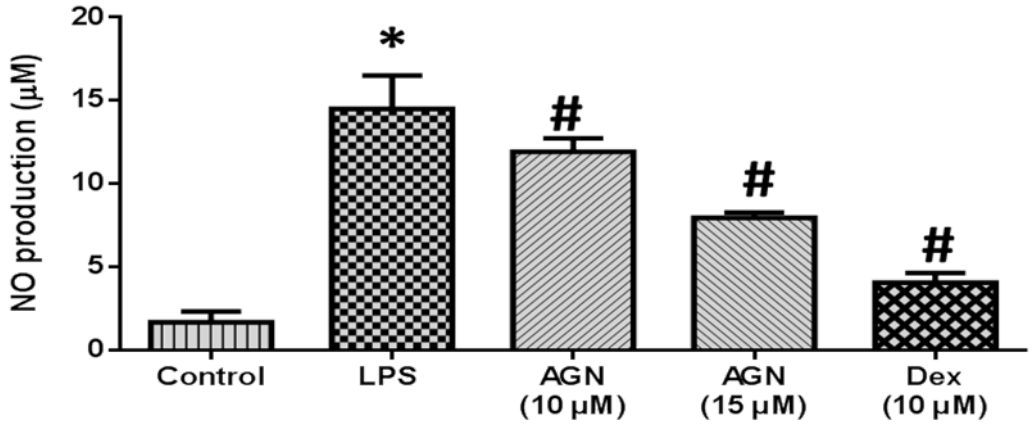

Figure 2: Measurement of Nitric Oxide production (NO). The data are illustrated as mean±SD of triplicate assays. * $p<0.05$, compared to control. ## $p<0.05$ compared to LPS-treated group.

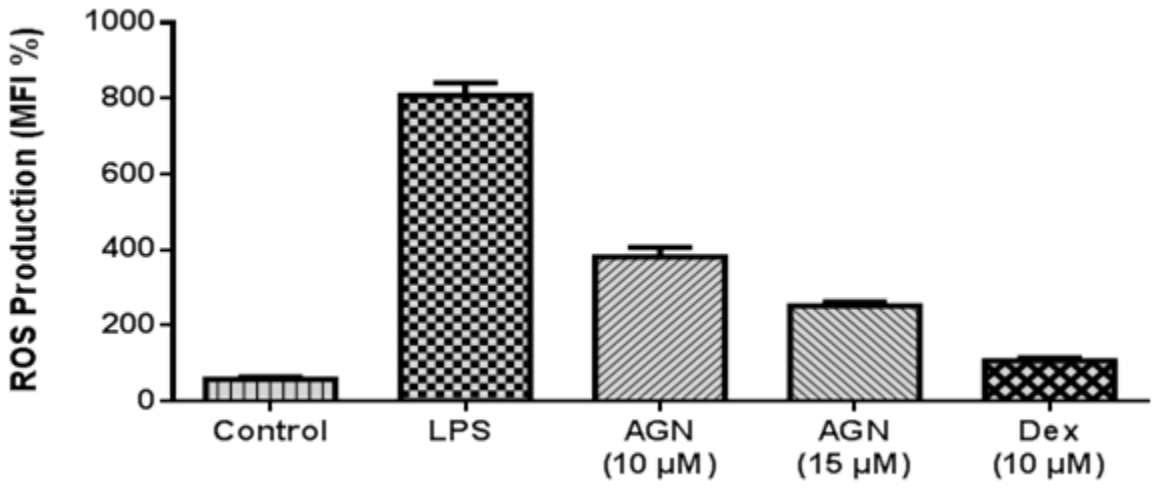

Figure 3: ROS accumulation in RAW264.7 cells. The data were illustrated as mean±SD of triplicates. * $p<0.05$, compared with control. ## $p<0.05$ compared with LPS group.

200%, suggesting that AGN created ROS in dose-dependently. The cells exposed to AGN exhibit a similar pattern to the Dex 10 μM treated cells (positive control). These outcomes imply that AGN antioxidant properties in RAW 264.7 cells are linked to its anti-inflammatory properties.

AGN suppresses the levels of PEG2 when estimated by ELISA assay

One significant inflammatory cytokine that plays a role in mediating inflammation is PGE2. The PGE2 ELISA kit was utilized to identify their presence in the supernatant of RAW264.7 cell culture. As demonstrated, LPS markedly increased PGE2 production. On the other hand, it was evident that AGN could decrease LPS-induced PGE2 release and that this suppression of PGE2 was dose-dependent (Figure 4).

AGN inhibits inflammatory cytokines in LPS-administered RAW 264.7 Cells

Cytokines are mostly produced by mitogens, immunogens, or other promoters stimulating transcriptional and translational processes. Here, the kit was employed to assess the protein contents of the cytokines after arglabin was applied to RAW264.7 cells for 24 hrs. Using ELISA method, we assessed the impact of arglabin on the TNF-α and IL-6 amounts in the supernatant of RAW 264.7 cells treated with Lipopolysaccharide (LPS). The quantities of IL-6 and TNF-α were notably augmented in cell cultures exposed to LPS. Treatment with arglabin reversed the LPS-induced elevations in an approach dependent on dosage. The accumulation of IL-6 and TNF-α were greatly diminished by arglabin at 10 and 15 μM, as illustrated in Figure 5. The cells treated with AGN exhibit a similar pattern to the Dex 10 μM treated cells (positive control). Overall, these findings demonstrate that in

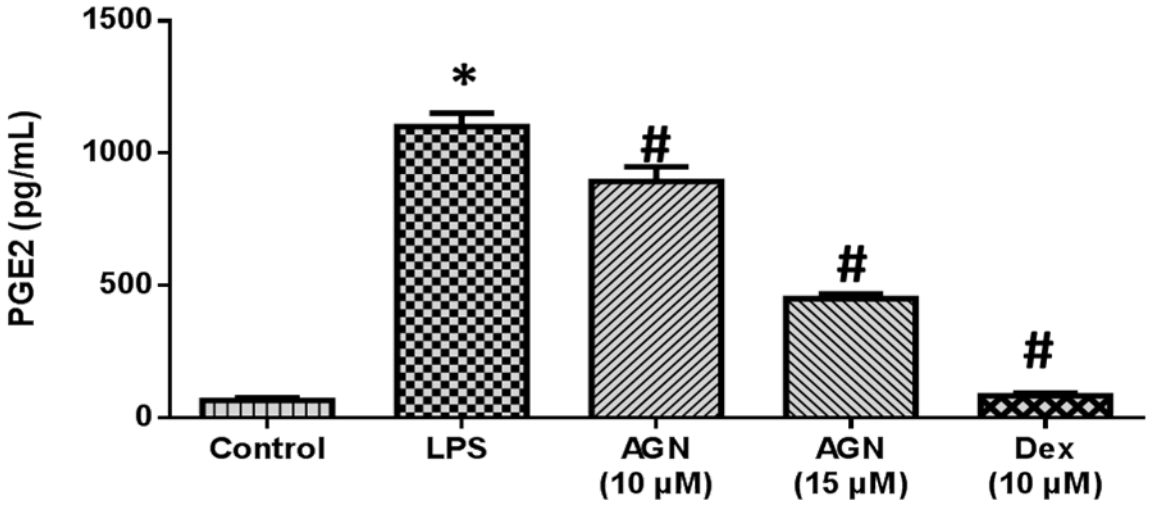

Figure 4: PGE2 level in the RAW264.7 cells. The data were illustrated as mean±SD of triplicates. * $p<0.05$, compared with control. ## $p<0.05$ compared with LPS group.

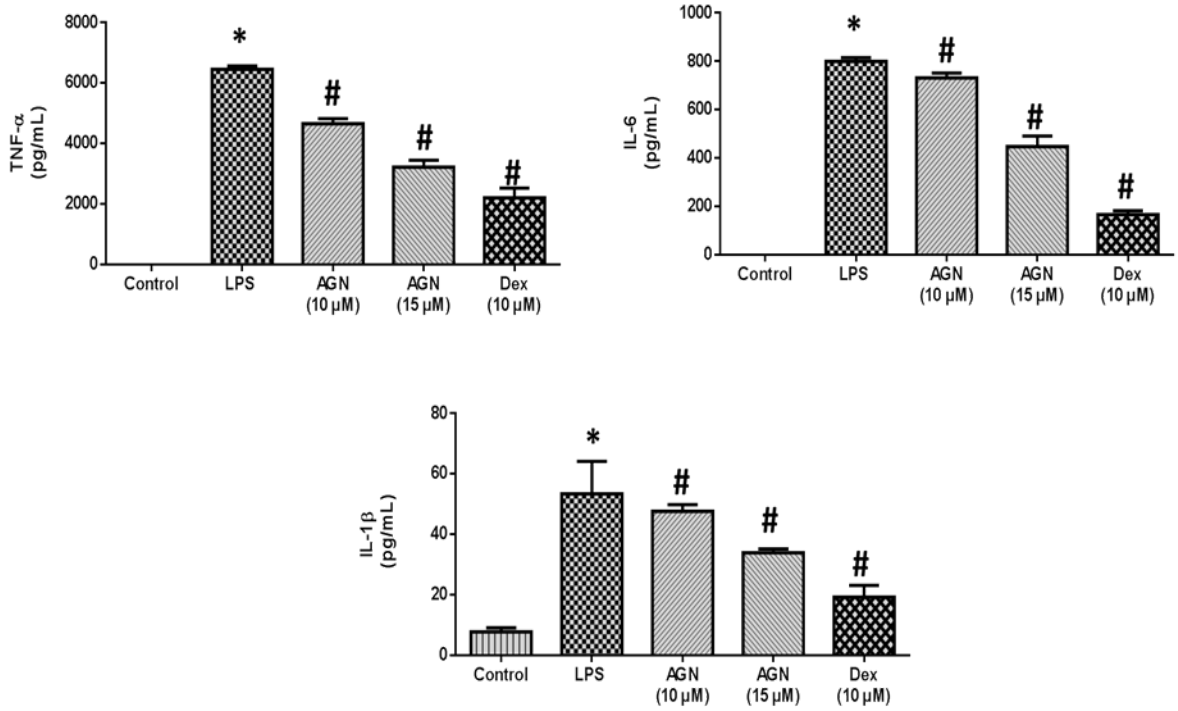

Figure 5: Inhibition of LPS-induced pro-inflammatory by AGN in LPS exposed RAW 264.7 cells. The data are depicted as mean±SD of triplicate assays. * $p<0.05$, compared to control. ## $p<0.05$ compared to LPS-treated group.

Raw264.7 cells challenged with LPS, AGN inhibits the cytokine expressions.

DISCUSSION

A common innate immune response that shields the body from outside stressors is an inflammatory reaction.[2] Numerous medical disorders, such as atopy, multiple sclerosis, metabolic condition, headache, multiple sclerosis, Alzheimer's and Parkinson's diseases are primarily caused by the advancement of inflammation.[1] Numerous illnesses, including arthritis, arthrophlogosis and asthma, can cause inflammation.[29] Anti-inflammatory medication development and research have made considerable use of inflammation models created by exposing macrophages to Lipopolysaccharide (LPS) exposure.[13]

In silico Study

Table 1: Computed descriptors of the compound AGN.

Compound Name	Canonical SMILES	Molecular Formula	2D Structure
Arglabin	CC1=CCC23C1C4C(CCC2(O3)C)C(=C)C(=O)O4	$C_{15}H_{18}O_3$	

Table 2: Physico-chemical characteristics of the compound AGN by SwissADME

Compound Name	RB	HBA	HBD	TPSA	Log P	Log S	Solubility (mg/ml)	M.wt (g/mol)	violations
Arglabin	0	3	0	38.83 Å	2.68	-2.45	1.74	246.3	0

Table 3: Bioactivity detection of selected inhibitors by Molinspiration.

Compound Name	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease Inhibitor	Enzyme Inhibitor
Arglabin	0.06	-0.17	-0.68	0.84	-0.02	0.77

Table 4: Pharmacodynamics parameters of selected inhibitors admetSAR.

Compound Name	Log S (>-4)	Blood Brain Barrier (BBB)	Human Intestinal Absorption (HIA)	Caco2 permeability	CYP substrate /Inhibitor	Ames toxicity	Carcinogenicity	LD_{50} (rat acute toxicity) (mol/kg)
Arglabin	-3.4109	0.9323	0.9960	0.6309	0.9311	0.8426	0.9200	2.3621

Table 5: Binding interactions of AGN with 4URO.

Sl. No.	Target gene	Binding energy (Kcal/mol)	Amino acids of interaction	Types of Interaction
1.	EGFR(4URO)	-5.3	ASP A:81	Hydrogen bond
			SER A:128	Corban Hydrogen bond
			ASN A:54	Hydrogen bond
			ARG A:144	Hydrogen bond
			ARG A: 200	Pi Anion
			ARG A: 84	Pi Anion
			GLU A: 58	Pi Anion
			PRO A: 87	Pi Alkyl
			ALA A :98	Pi Alkyl
			ILE A: 102	Pi Alkyl
			ASP A: 89	Hydrogen bond

LPS in Gram-negative bacteria's outer membrane stimulates macrophages, which in turn causes them to generate large amounts of inflammatory mediators and pro-inflammatory cytokines when Gram-negative bacteria infect cells. Thus, it is common practice to test anti-inflammatory drugs using the inflammatory effects of Lipopolysaccharide (LPS) on macrophages.[1] An immunological response is primarily brought on by the activation of macrophages and Lipopolysaccharides (LPS) are a common agent that triggers a macrophage-mediated inflammatory response.[2] Therefore, RAW264.7 macrophage cells were used for the examination in the current study. Numerous studies have demonstrated that when LPS is added to RAW264.7 cells, which are macrophages generated from rodents, the levels of NO, PGE2 and other components related to inflammation rise. Arachidonic acid is converted by LPS-producing macrophages into PGE2, an inflammatory mediator.[2]

It may be possible to shield host tissues from the damaging impact of chronic or extreme inflammation by employing strategies for inflammation mitigation. The negative effects

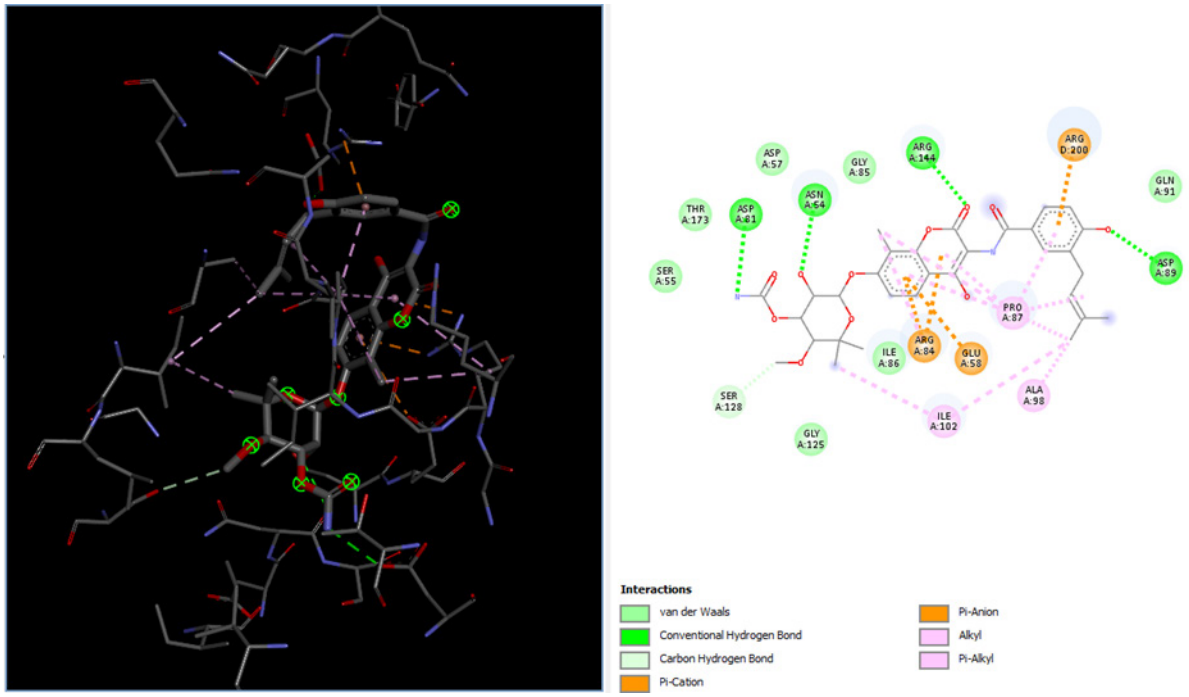

Figure 6: The docking pose for the AGN enzymes complex was selected based on the binding affinity.

of anti-inflammatory medications that are currently on the market vary according on the patient, the length of treatment and the dosage.[3] Plants have long been the source of a vast array of medicinally active ingredients that prolong human life. Many of these components belong to a unique class of natural chemicals known as Sesquiterpene Lactones (SLs) and have an α-methylene-γ-lactone structural motif. The Asteraceae family has a large number of these chemicals, which are recognized to have potential biological and medicinal properties. These properties include contraceptive, antihelminthic, antishistosomal and anticancer properties. Plant extracts high in SLs have drawn a lot of interest in the treatment of human illnesses. Due to their unique features, SLs in cancer clinical trials are able to selectively target cancer and tumor stem cells while avoiding normal ones. AGN and dimethyl amino-arglabin are utilized to treat lung, liver and ovarian malignancies in clinical tests. These are the SL medicines, or their derivatives, that are now undergoing clinical testing.[30]

The active ingredient of the novel anticancer medication created in Kazakhstan is arglabin, a sesquiterpene lactone of the guaian type. According to toxicological research, the medication in lyophilized dosage form does not alter the morphological makeup of bone marrow or peripheral blood at maximally tolerated levels. Neither does it impact the functional state of the kidneys, liver, heart, or lungs. Subcutaneous, intramuscular, intravenous and abdominal injections of the medication do not cause local irritation. There were no discernible pyrogenic or allergic properties. Furthermore, arglabin is neither teratogenic, mutagenic, or embryotoxic. The medication remains in the body for about 22 hr, which is a rather long retention time. Arglabin enters the peripheral tissues quickly after exiting the central compartment (blood). The lungs and spleen contain the highest quantities of the medication within the first hour, while the liver and skeletal muscles contain the largest concentrations within 3 hr. The medication is more heavily concentrated and held in the liver for a longer duration of period compared with the other tissues. The medication can break through Blood Brain Barrier (BBB).[31] The low water solubility of arglabin has a detrimental effect on the compound's bioavailability and, in turn, its pharmacological efficacy. Therefore, enhancing the compound's water solubility through structural change makes sense as a way to increase its bioavailability.[11]

LPS-induced macrophage activation increases excessive NO output by breaking down L-arginine.[1] Short-lived free radical NO is an endogenous messenger that plays a number of roles in host defense, neurotransmission and vascular homeostasis. Nitric Oxide Synthase (NOS) converts L-arginine into NO.[7] iNOS in macrophage cells controls the production of NO in response to immune system stimuli that cause inflammation in mammals.[1] Thus, in RAW264.7 cells, the current study showed that arglabin significantly downregulated NO, which was elevated as a result of LPS. NO, which is generated by the catalysis of L-arginine by iNOS, is a crucial signal molecule in the body. Overabundance of NO can cause inflammatory disorders to develop. Therefore, one of the key strategies to manage the inflammatory condition is to effectively restrict the NO generation.[6] First, we found that arglabin inhibited NO production.

Focusing on proteins linked to extracellular redox as a cancer treatment strategy has attracted increased and persistent interest

in recent years. In fact, crucial cellular processes, interactions between cells in the microenvironment and the operation of ion channels and cell surface receptors are all regulated by the extracellular as well as intracellular redox state. Crucially, thiol-based antioxidants were found to decrease arglabin's biological activity. Remarkably, ROS can also cause reversible alteration of redox-sensitive cysteines in physiological settings. Nevertheless, arglabin causes a delay in the generation of ROS in cancer cells, coinciding with the damage caused in lysosome and mitochondria.[12] One small but extremely reactive molecule is ROS. ROS buildup can trigger a variety of defense mechanisms in intracellular activities. Moreover, ROS are byproducts of regular metabolism in cells. Overabundance of ROS can upset the proportion between the pro- and anti-oxidative systems.[6] They are organic byproducts of oxygen metabolism and are crucial for maintaining homeostasis and cell signaling. However, in some pathological and infectious conditions, excessive ROS formation can lead to oxidation of nucleic acid and protein and can have detrimental impacts on the structure of cells. New studies have revealed that ROS can cause inflammation by inducing the accumulation of several cytokines through the MAPK pathway.[1] Inflammation is a clinical disorders that is closely linked to increased ROS generation. Intracellular ROS generation is accelerated by the entrance of macrophages, which is facilitated by inflammatory mediators and cytokines. Additionally, ROS take part in the inflammatory signaling cascade and function as secondary messengers. Hence, arglabin's capacity to scavenge free radicals may be the reason behind its potent suppression of ROS in RAW 264.7 cells induced with LPS. The substantial anti-inflammatory properties of arglabin may be explained by the inhibition of inflammatory markers that arglabin mediated ROS production inhibition may possibly potentially block. Mononuclear macrophages secrete TNF-α, which triggers the cytokine pathway in inflammatory reactions, which triggers macrophages to generate IL-6.[6] One of the most significant pro-inflammatory cytokines, TNF-α, has several cellular actions and is necessary for the initiation and maintenance of inflammation. IL-6 is another cytokine that stimulates inflammation. These cytokines are implicated in tissue damage and multiple organ failure and are in charge of the acute phase response.[29] Arglabin has a strong immunomodulatory potential and restores the manufacture process of cytokines that inhibit inflammation. It also supports research findings on the system of interferon and cytokines in breast cancer patients.[31] Immune regulation of inflammation can be achieved by inhibiting this, according to numerous prior research.[2] Our study findings showed that arglabin diminished TNF-α and IL-6 amounts.

Arglabin effectively prevents the production of prostaglandin PGE2, which is primarily generated by the enzyme Cyclooxygenase-2 (COX-2). PGE2 is implicated in numerous physiological processes and acts as an inflammatory mediator and immunological regulator.[6] In the previous investigation, it was demonstrated that suppression of growth and reduced viability by arglabin in different cancer cells in human, includes cells from advanced prostate cancer that were originating from several metastatic locations and had variable tolerance to androgens. Notably, this compound successfully suppressed both anchorage-dependent and anchorage-independent 2D and 3D prostate cancer cell formation and proliferation after long-term treatment.[31] In our present work, arglabin found to have significant effect on cell viability dose dependently.

Our findings demonstrated that arglabin strongly inhibits IL-6, NO, TNF-α, ROS and PGE2, pointing to the effective utilization of arglabin as an anti-inflammatory drug. Treatment with arglabin is anticipated to have an anti-inflammatory effect on LPS-induced cells, consistent with these results. Therefore, the pharmacological effect of arglabin will be studied in order to establish for its anticancer, antimicrobial, anti-inflammatory and immune regulatory characteristics. These principles will then be used to build highly effective medications.[31]

The current study's objective was to elucidate the compound arglabin's inhibitory effect on the gene involved in the EGFR signalling. Table 2 summarizes the physicochemical properties of AGN predicted using SwissADME. Lipinski's Rule of Five evaluate the chemical characteristics conducive to oral bioactivity in humans; the molecule arglabin exhibited significant binding affinity and drug-like qualities. The compounds have molecular weight below 500 Daltons and have <5 hydrogen bond donors and ten hydrogen bond acceptors. The goal of this *in silico* investigation is to find effective of anti-cancer compound. The bioactivity of Arglabin was evaluated using Molinspiration by assessing its efficacy against a GPCR ligand, a kinase inhibitor, an ion channel modulator, a protease inhibitor, and an enzyme inhibitor.

A compound exhibiting a bioactivity score beyond 0.00 is considered to possess remarkable pharmacological effects, whilst scores between -0.10 and 0.00 were regarded as slightly active, and scores below -0.10 are deemed inactive.[28] Table 3 displays the anticipated bioactivity as determined by Molinspiration. The compound arglabin selected for the present study has an acceptable range of kinase inhibitors, enzyme links, ion channel modulators and protease inhibitors.

The ADMET profile was evaluated using the admetSAR program to ascertain the pharmacodynamic properties of arglabin and to understand the drug's mechanism of action on a host organism. The ADMET test examined parameters that characterize absorption, distribution, metabolism, excretion, and toxicity. The chemical exhibited optimal solubility with a value of 2.006, above 4, along with other highly favorable attributes, including non-toxicity and non-carcinogenicity, as indicated in Table 4. The capacity to traverse the BBB and the intestinal absorption was also illustrated in the admetSAR forecasts.[31] Arglabin is identified

as a potentially active drug targeting the EGFR signaling pathway gene in anticancer applications using molecular docking. The inhibition was examined, revealing the critical residues in the binding site. Arglabin has been discovered to have a binding energy of -5.3 kcal/mol, (PDB code: 4URO) (Table 5 and Figure 6). A less binding energy denotes the increased affinity of the ligand for receptor. Thus, the ligand exhibiting the highest affinity may be utilized as a prospective medication for subsequent investigations.

After thorough assessment of all docked conformations with arglabin, specific connections with EGFR (PDB code: 4URO) were detected, as four hydrogen bonds with ASP 81, SER 128, ASN 54, ARG 144 ARG 200, ARG 84, GLU 58, PRO 87 ALA 98, ILE 102 & ASP 89 of the compounds is demonstrate in the active site of EGFR, as illustrated in Figure 1. The other interactions, such as Pi-Pi, Pi-Anion, Pi-Alkyl and Van der Waals connections of the compound arglabin were presented in Figure 1. These connections aid to lock the arglabin within the substrate-binding sites thereby potentially block the EGFR signalling pathway gene in anti-cancer activity. After being tested *in vitro*, the study concluded that the arglabin's binding and alutary characteristics make it a promising candidate for developing a potential anti-cancer inhibitor.

CONCLUSION

The findings showcase that arglabin exhibited strong anti-inflammatory properties on RAW 264.7 cells. By decreasing the relevant expression, arglabin treatment in RAW 264.7 cells induced with LPS drastically reduced the generation of inflammatory markers. There was a correlation found between arglabin's capacity to suppress the inflammatory response and a decrease in intracellular ROS generation. Arglabin had a major impact on the viability of cells. The study's findings are in favor of arglabin as a substitute medication that may be used to treat inflammatory illnesses in a way that is both secure and efficient. To confirm its role as a regulator of macrophage activation and to comprehend the exact molecular processes governing the anti-inflammatory effect using an animal model, more research is required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **BBB:** Blood Brain Barrier; **CO_2:** Carbon dioxide; **COX-2:** cyclooxygenase-2; **DCFH-DA:** Dichlorodihydrofluorescein diacetate; **DMSO:** Dimethyl Sulfoxide; **FACS:** Fluorescence-activated cell sorting; **IL:** Interleukin**; iNOS:** Inducible nitric oxide synthase; **MAPK:** Mitogen-Activated Protein Kinase; **NF-κB:** Nuclear Factor-κB; **NO:** Nitric oxide; **PGE2:** Prostaglandin E2; **ROS:** Reactive oxygen species; **TLRs:** Toll-like receptors; **TNF-α:** Tumor necrosis factor-alpha.

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