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Original Article

Anti-inflammatory Effects of *Vitex altissima* Leaf Extract in Lipopolysaccharide-induced RAW 264.7 Macrophages

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ABSTRACT

Vitex altissima has been conventionally utilized for its wide-ranging properties in the management of oxidative stress and inflammation. The present investigation was centered on the quantification of the anti-inflammatory efficacy of *V. altissima*. The samples were evaluated for their ability to impede the activity of proteinase, denature proteins, and stabilize the membrane of human red blood cells (HRBC). The present study investigated the inhibitory impact of extracts on the production of total cyclooxygenase, lipoxygenase-5 (5-LOX), myeloperoxidase (MPO), and nitric oxide (NO) using the RAW 264.7 cell line. Furthermore, the antioxidant properties were assessed through the employment of both DPPH assay and reducing power assay. According to the findings, the methanolic extract of *V. altissima* (VAME) was identified as the most efficacious fraction with anti-inflammatory and antioxidant properties. The findings indicate that the extracts exhibited dose-dependent inhibition of proteinase, protein denaturation, and hemolysis of HRBC membrane, which is beneficial. The extracts of *V. altissima*, when treated at concentrations that are not cytotoxic, were observed to have a significant effect in reducing the activity of COX, 5-LOX, and MPO in RAW 264.7 cell line treated with LPS. This resulted in a decrease in NO levels. The dose-dependent increase in in vitro anti-inflammatory activity of *V. altissima* suggests its potential use as a pharmacological agent for the management of diseases related to inflammation. Additional comprehensive phytochemical investigations, in conjunction with in vitro and in vivo analyses, are necessary to identify the active constituent within the extract.

INTRODUCTION

Inflammation is a physiological process involving immune cells and soluble factors. Uncontrolled inflammation is linked to tissue damage as well as the onset and progression of different diseases. A context-specific therapeutic selection is required because of the difficulties connected to conventional anti-inflammatory medications and inflammatory reactions. Medicinal plant extracts have significant pharmacological action, including anti-inflammatory activity, due to the potency of individual bioactive components or the synergistic effect of multiple powerful phytochemicals.[1] The pathogenesis of several diseases was believed to revolve around oxidative stress and inflammation as the primary mechanisms. Numerous scientific studies have presented evidence regarding the interconnected pathways that regulate inflammation induced by oxidative stress and the reciprocal relationship between the two.[2] Conducting a thorough assessment of these substances' phytochemical, pharmacological, and physiological properties is necessary to ensure their safe and efficacious application in the treatment of inflammatory disorders.

Vitex altissima belongs to the family of Verbenaceae. Leaves are used for wounds,[3] skin allergies, and rheumatism.[4] The leaves of this plant are rich in flavonoids. Preliminary findings indicated that *V. altissima*

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ethyl acetate extracts have anti-inflammatory action in a carrageenan-induced paw edema model.[5] The utilization of *V. altissima* in traditional medicine has been documented for treating various disorders, such as skin allergies, deep-cut wounds, poisonous bites, rheumatic swellings, and inflammatory conditions. Despite its various traditional and pharmacological uses, *V. altissima* has been unexplored, with only a few preliminary studies conducted. Nevertheless, it appears that the full extent of the anti-inflammatory properties of the extract derived from *V. altissima* has yet to be thoroughly investigated.

Natural compounds with antioxidant properties play a preventative role in mitigating the generation of free radicals. As such, natural antioxidants are a highly valuable therapeutic intervention for reducing the incidence of illnesses associated with oxidative stress.[6] COX and LOX enzymes start inflammatory reactions via the arachidonic acid pathway, which includes free radical reactions. Plant-derived compounds can obstruct and terminate COX and LOX-mediated processes. The aggregation of neutrophils and macrophages at the site of injury is another implication during inflammation. As a result, the myeloperoxidase (MPO) enzyme assay can be useful. Nitric oxide (NO), a second messenger produced by nitric oxide synthase (NOS), is required for appropriate cellular activity. One of the most essential inflammatory reactions is increased NO levels produced by NOS in activated macrophages.[7] Chronic inflammatory diseases are linked to inducible enzymes (COX and iNOS) and the products of their reactions.[8,9] Macrophages play a crucial role in the inflammatory response and are a fundamental component of the innate peripheral immune system. The utilization of the murine macrophage RAW 264.7 cell line, which has been stimulated with LPS, is a conventional approach for investigating inflammation.[10] In this context, to validate the ethnomedical anti-inflammatory potential of *V. altissima*, *in-vitro* assays on LPS-induced RAW 246.7 macrophage cells were conducted.

MATERIALS AND METHODS

Collection and Identification of *V. altissima*

The leaves of *V. altissima* were collected from Kollam district, identified by Department of Botany, TKM College of Arts and Science, Kollam, and deposited in the herbarium under voucher specimen number KOLIV2016AJ01. The plant material was thoroughly rinsed three to four times with flowing tap water and once with sterile distilled water. The leaves were then dried in the shade, roughly pulverized, and stored in tightly sealed vials for subsequent laboratory investigation.

Extraction of the Plant Material

Shade-dried *V. altissima* leaves were reduced to a coarse powder. Soxhlet extraction was used to produce *V. altissima* hexane extract (VAHE), *V. altissima* ethyl acetate extract (VAEA), and *V. altissima* methanol extract (VAME) in that order. The resulting extracts were then concentrated in a rotary evaporator and kept at 4°C until needed.

Antioxidant Assays

DPPH assay

The antioxidant activity of different extracts of *V. altissima* were estimated by using DPPH assay.[11]

Reducing Power Assay

The assay is based on the reduction of Fe^{3+} to Fe^{2+}.[12] Various concentrations of extracts (12.5–200 µg/mL) was combined with 2.5 mL phosphate buffer (0.2M. pH 6.6) and 2.5 mL 1% potassium ferricyanide, incubated at 50°C for 20 minute. Add 5 mL distilled water and 1-mL ferric chloride (0.1%) to the mixture. Absorbance was read at 700 nm. Quercetin was used as the standard.

In-vitro Anti-inflammatory Analysis

Protein-denaturation activity

This test was conducted according to the modified method of Djuichou-Nguemnang *et al.*[13] Different concentrations of extracts were added to a solution of BSA (5%) and incubated at 37°C for 25 minutes. The reaction mixture is deposited in a 70°C water bath for 15 minutes to initiate protein denaturation. The turbidity was measured by calculating the absorbance at 660 nm. Inhibition of protein denaturation was calculated.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

HRBC Membrane–Stabilization assay

This experiment was conducted in accordance with the protocol described by Tantary *et al.*[14] Blood collected from a healthy individual and centrifuge on an EDTA-coated. The packed RBC cells were rinsed with 0.9% NaCl, producing a 10% cell suspension. Take 5 mL of hyposaline (50 mM NaCl), 10 mL of phosphate buffer (pH 7.4), 0.5 mL of cell suspension with varying concentrations of extract, and 1-mL of standard drug diclofenac sodium for the assay. The reaction mixture was incubated for 10 minutes at room temperature, centrifuged, and the absorbance of the supernatant was measured at 560 nm. The inhibition of erythrocyte hemolysis was calculated.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Antiproteinase assay

The present study used the methodology of Gunathilake K *et al.*[15] The experimental solution consisted of 1-mL of plant extracts, 0.06 mg of trypsin, and 1-mL of Tris HCl buffer (20 mM, pH 7.4). Following incubation at a temperature of 37°C for a duration of 5 minutes, 1-mL of

0.8% casein was introduced and subsequently incubated at the same temperature for a period of 20 minutes. The reaction was halted by the addition of 2 mL of perchloric acid, followed by centrifugation. The absorbance of the resulting supernatant was measured at a wavelength of 210 nm. The experiment was replicated thrice, and the inhibition of proteinase was computed.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Cell culture

RAW 264.7 (macrophage) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing: Penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator.

Cytotoxicity assay

The MTT assay was conducted following the methodology outlined by Talarico *et al.*[16] The quantification of the absorbance of the formazan solution was performed using an ELISA microplate reader at a wavelength of 570 nm. Using an inverted phase contrast tissue culture microscope, cells were observed every 24 hours for a maximum of 72 hours.

An anti-inflammatory assay using RAW 264.7 cells

The cells were maintained until it reached 60% confluency. Subsequently, the cells were subjected to a pre-treatment phase using varying concentrations (6.25, 12.5, 25 µg/mL) of VAEA and VAME for a duration of 1-hour. Following this, the cells were activated with 1-µL of lipopolysaccharide (LPS: 1-µg/mL) and incubated for a period of 24 hours. Following the incubation period, the anti-inflammatory assays were conducted utilizing the cellular lysate.

Assay of Cyclooxygenase

The COX activity was assayed by the method of Walker and Gierse.[17] COX activity was determined by reading absorbance at 632 nm. Total COX activity was expressed as µM MDA produced/mg protein.

Assay of 5-Lipoxygenase

The methodology employed for the determination of 5-LOX activity was in accordance with Axelrod *et al.*[18] The enzymatic activity of lipoxygenase was measured by monitoring the increase in absorbance at 234 nm using a spectrophotometer. This increase in absorbance is indicative of the formation of 5-hydroxyeicosa tetraenoic acid. The enzyme activity was quantified as the shift in optical density per minute per mg of protein.

Assay of myeloperoxidase

The method for assaying myeloperoxidase activity was implemented as per the description provided by Suzuki *et al.*[19] A single unit of MPO activity was defined as the degradation of 1-µM of peroxide per minute at a temperature of 25°C.

Estimation of cellular nitrite

The Greiss reaction method, as described by Lepoivre *et al.*, was utilized to estimate the nitrite level.[20] The standard solution used was sodium nitrite. The estimation of nitrite quantity in the samples was conducted through the utilization of standard curves.

Statistical Analysis

All the data were expressed as mean ± SEM (n = 3) and the results were analyzed by one-way ANOVA followed by Dunnet's post hoc analysis using GraphPad Prism (Version 5) software. Statistical significance was specified at $p<0.05$.

Results

Antioxidant Activity

DPPH assay

The assessment of antioxidant activity in the extracts was conducted using the DPPH method, which is a commonly utilized approach for this purpose. The methanol extract exhibited the greatest DPPH radical scavenging capacity, measuring 60.98% at a 20 µg/mL concentration. The standard ascorbic acid demonstrated 83.34% inhibition at the same concentration. The findings indicate that all of the extracts exhibited a significant DPPH radical scavenging activity, with a dose-dependent increase in percentage inhibition, as illustrated in Fig. 1.

Reducing power assay

The concentration-dependent increase in reducing power activity of *V. altissima* hexane, ethyl acetate, and methanol extracts was observed (see Fig. 2). The methanol extract

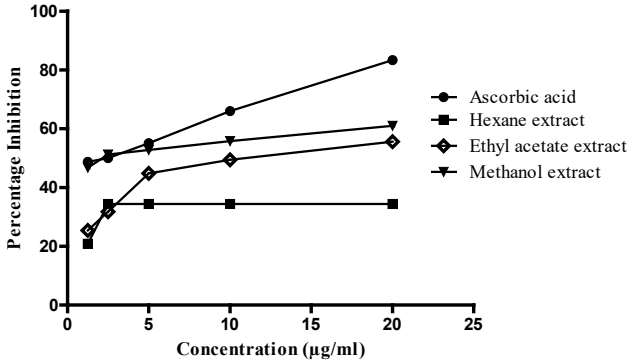

Fig. 1: Effect of methanol, ethyl acetate and hexane extracts of *V. altissima* and standard ascorbic acid on DDPH radical scavenging activity. Values are represented as means of three replicate determinations ± SEM.

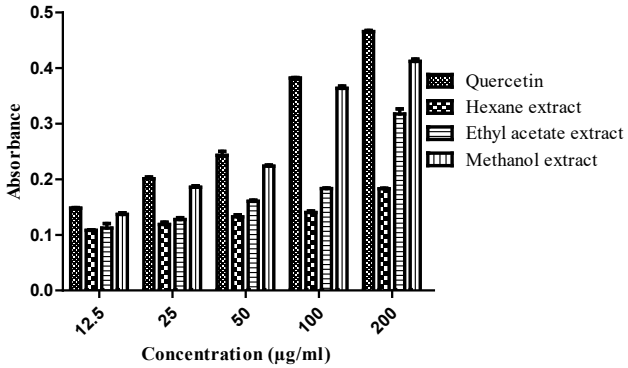

Fig. 2: Effect of methanol, ethyl acetate and hexane extracts of *V. altissima* and standard quercetin on reducing power assay. Values are represented as means of three replicate determinations ± SEM

exhibited a greater absorbance value, which suggests a potent reducing capacity. The absorbance values increased from 0.137 to 0.412 as the concentrations ranged from 12.5 to 200 µg/mL. The standard quercetin displayed an absorbance of 0.465 at 200 µg/mL concentration.

In-vitro Anti-inflammatory activity

Protein denaturation assay

Autoantigens are exhibited in disease states such as rheumatoid arthritis, cancer, diabetes, and other inflammatory conditions as a result of protein denaturation. Therefore, the inhibition of protein denaturation can lead to a reduction in inflammatory activity.[21] Table 1 displays the results of protein denaturation inhibition by three extracts, wherein VAME demonstrated the highest inhibition rate of 73.96 ± 0.07% at a concentration of 1000 µg/mL. VAEA followed with an inhibition rate of 68.89 ± 5.15%. The positive control, namely diclofenac sodium, exhibited the highest level of inhibition at 80.23 ± 4.08%. The results indicate that VAME exhibited an IC_{50} value of 477.534 ± 0.64 µg/mL, while the positive control demonstrated the lowest IC_{50} value of 438.718 ± 0.43 µg/mL.

Stabilization of HRBC membrane

Stabilizing the membranes avoids the passage of components into the tissues, which would otherwise occur due to the increased permeability that occurs during the inflammation process. The ability of the extracts to reduce inflammation was evaluated based on whether or not they could prevent hypotonicity in the membranes of red blood cells. As shown in Table 2, the different extracts of *V. altissima* provided a high level of protection against the erythrocyte membrane lysis brought on by the hypotonic solution. At a 1000 µg/mL concentration, VAME demonstrated the most significant suppression of RBC hemolysis, with a value of 73.25 ± 2.21%. At 1000 µg/mL, the positive control, diclofenac sodium, had an inhibition level of 75.14 ± 0.34%. The IC_{50} value shows that the extract is effective; it was 590.527 ± 3.32 g/mL in VAME and 560.05 ± 2.12 g/mL in the positive control. These values compare favorably to one another.

Inhibition of proteinase activity

Proteinases cause tissue injury in leukocytes during inflammation. Studies have indicated that the proteinase inhibitory properties of the polyphenols found in plants are responsible for the anti-inflammatory effects. A considerable dose-dependent reduction in proteinase

Table 1: Inhibition of protein denaturation activity of *V. altissima* extracts at different concentrations and their IC_{50} values

Treatment	% Inhibition at different concentrations				IC_{50} µg/mL
	100 µg/mL	200 µg/mL	500 µg/mL	1000 µg/mL	
VAHE	4.7 ± 0.76*	23.73 ± 0.08*	35.99 ± 0.18*	63.57 ± 0.87*	751.77 ± 0.32*
VAEA	18.15 ± 2.26*	31.24 ± 0.32^{ns}	47.306 ± 0.09*	68.89 ± 5.15*	612.086 ± 0.04*
VAME	20.88 ± 1.05^{ns}	34.246 ± 0.05^{ns}	64.663 ± 0.43^{ns}	73.96 ± 0.07^{ns}	477.534 ± 0.64*
Diclofenac Sodium	22.046 ± 0.12	35.98 ± 4.2	65.93 ± 0.21	80.23 ± 4.08	438.718 ± 0.43

Values are represented as means of three replicate determinations ± SEM. * Significantly different from Diclofenac sodium standard group ($p<0.05$).

Table 2: Inhibition of HRBC membrane hemolysis at different concentrations of *V. altissima* extracts

Treatment	% Inhibition at different concentrations				IC_{50} µg/mL
	100 µg/mL	200 µg/mL	500 µg/mL	1000 µg/mL	
VAHE	10.93 ± 1.23*	18.6 ± 0.38*	30.23 ± 3.76*	61.63 ± 0.56*	807.025 ± 7.45*
VAEA	13.42 ± 3.15*	22.77 ± 2.43^{ns}	37.6 ± 0.87*	62.63 ± 0.26*	750.756 ± 1.98*
VAME	15.7 ± 1.09^{ns}	29.6 ± 4.23^{ns}	47.4 ± 2.98^{ns}	73.25 ± 2.21^{ns}	590.527 ± 3.32*
Diclofenac sodium	20.5 ± 0.98	30.21 ± 5.34	48.07 ± 1.43	75.14 ± 0.34	560.05 ± 2.12

Values are represented as means of three replicate determinations ± SEM. * Significantly different from Diclofenac sodium standard group ($p<0.05$)

Table 3: Inhibition of proteinase activity exhibited with different concentration of *V. altissima* extracts and their IC_{50} values

Treatment	% Inhibition at different concentrations			IC_{50} µg/mL
	50 µg/mL	100 µg/mL	200 µg/mL	
VAHE	8.36 ± 3.84*	19 ± 0.01*	45 ± 4.21*	221.66 ± 0.65*
VAEA	9.96 ± 1.05*	22.3 ± 2.51*	53 ± 3.4*	191.131 ± 0.87*
VAME	16.6 ± 2.48*	25 ± 2.01 *	61 ± 1.06*	168.46 ± 2.13*
Indomethacin	20 ± 1.1	32.3 ± 3.0	70.66 ± 0.3	142 ± 0.08

Values are represented as means of three replicate determinations ± SEM. * Significantly different from indomethacin standard group ($p<0.05$)

activity (Table 3), with maximum inhibition reaching 61 ± 1.06% at 200 µg/mL observe when treated with VAME. Both VAEA and VAHE demonstrated inhibition, with the former showing 53 ± 3.4% and the latter 45 ± 4.21%. The highest level of inhibition was observed with indomethacin (the positive control), which was 70.66 ± 0.3%. The IC_{50} for VAME was the lowest of all other extracts (168.46 ± 2.13 µg/mL). The IC_{50} value for indomethacin was found to be 142.84 ± 0.08 µg/mL.

Cell viability

The effects of VAEA and VAME on the viability of RAW 264.7 cells were tested using the MTT assay after incubating the cells for 24 hours without LPS, and the results are shown in Fig. 3. Microscopic images were shown in Fig. 4. There were no significant differences in cell viability compared to the control group.

Total COX activity

Increase in total COX activity was observed in RAW 264.7 murine macrophage cells when inducted with LPS

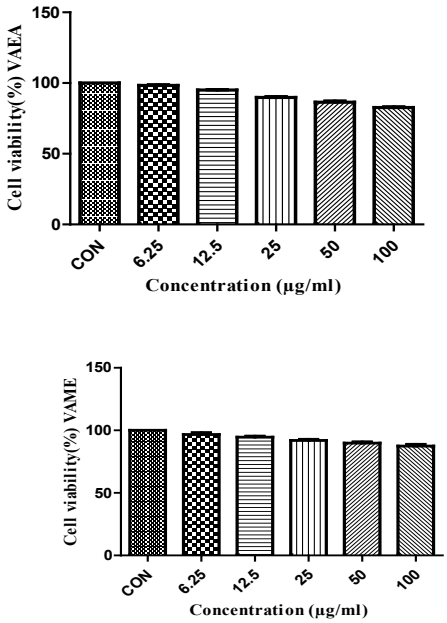

Fig. 3: Effect of *V. altissima* ethyl acetate (VAEA) and methanolic (VAME) extracts on cell viability in RAW 264.7 cells. Cells were treated with different concentration of extracts (6.25,12.5, 25, 50, 100 µg/mL) for 24 hours and cell viability quantified by MTT assay.

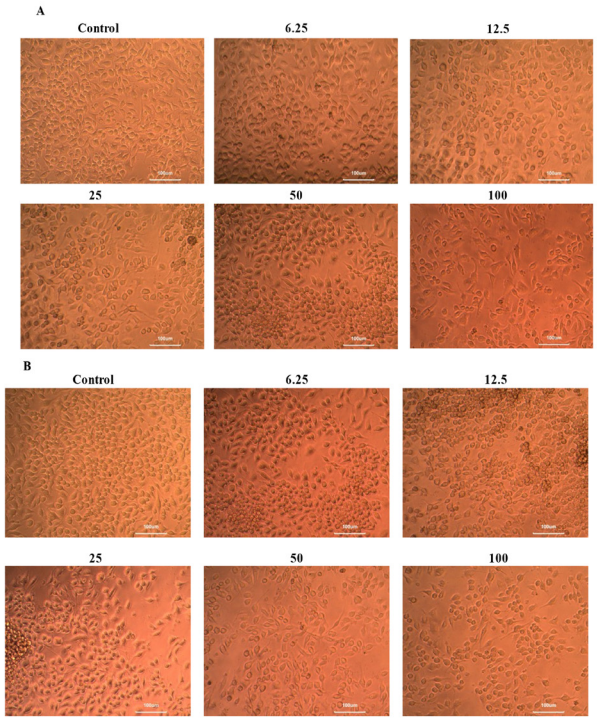

Fig. 4: Effect of (A) *V. altissima* ethyl acetate (VAEA) and (B) methanolic (VAME) extracts on cell viability in RAW 264.7 cells determined by direct microscopic examination.

(1-µg/mL). Administering VAME extract led to a significant reduction ($p< 0.05$) in COX activity, and the effect is found to be dose dependent (Fig. 5).

5-LOX activity

The extracts exhibited a dose-dependent inhibition of 5-LOX activity. The induction of RAW 264.7 murine macrophage cells with LPS resulted in an increase in 5-LOX activity. The 5-LOX activity exhibited a significant reduction ($p<0.05$) upon pre-treatment with various extracts. VAME exhibited the highest level of inhibition at a concentration of 25 µg/mL.

Myeloperoxidase activity

An elevation in MPO activity was detected in RAW 264.7 murine macrophage cell lines stimulated with LPS. The administration of plant extracts resulted in a noteworthy reduction ($p < 0.05$) in MPO activity when the sample concentration was 25 µg/mL.

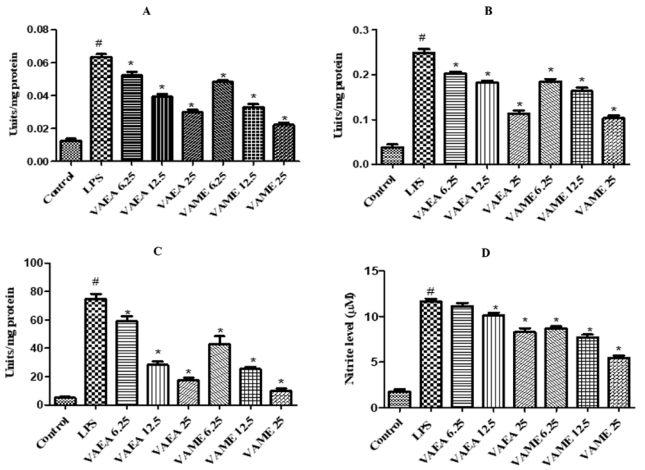

Fig. 5: Effect of *V. altissima* ethyl acetate (VAEA) and methanolic (VAME) extracts on (A) Total COX activity (B) 5-LOX activity (C) MPO activity (D) Nitrite levels. The RAW 264.7 cells were pretreated with the extracts of different concentration (6.25, 12.5, 25 µg/mL) for 1 hour. Following the pre-treatment, cells were stimulated with 1-µg/mL of LPS. Values are expressed as average of 3 samples ± SEM in each group.

#Significantly different from control group ($p<0.05$), * Significantly different from LPS group ($p<0.05$).

Nitrite level

Upon induction with LPS, the nitrite levels in RAW 264.7 murine macrophage cell lines exhibited a sudden increase in nitrite level. Various concentrations of extracts were administered to the cells induced with LPS. The nitrite concentration was then calculated as a measure of the NO level present in the culture medium. In comparison to the LPS group (untreated), the treated group exhibited a decreased level of nitrite release in the surrounding medium. The results indicate that VAME exhibited a concentration-dependent reduction ($p < 0.05$) in the production of nitrite.

Discussion

V. altissima, a plant species commonly employed in traditional medicine, contains high concentration of secondary metabolites. Antioxidants from plants exhibit the ability to scavenge free radicals and mitigate cellular damage caused by these radicles. Nutraceutical and ethnopharmacological benefits of polyphenols and flavonoids can be attributed to their natural sources. The solvents used in the extraction process of secondary metabolites, especially phenolic compounds have a significant role in the quantity and quality of isolated compound. The methanolic extract exhibited a relatively higher level of antioxidant activity. A direct relationship exists between polyphenols and antioxidant capacity.[22] Phenolic and flavonoid compounds are believed to play a crucial role in the stabilization of lipid oxidation, which is linked to their antioxidant activity.[23] The concentration-dependent DPPH radical quenching activity was demonstrated by the antioxidant potential of the extracts as revealed by the DPPH assay.

Inflammation is a multifaceted biological response to noxious stimuli and is linked to a plethora of pathophysiological conditions. The denaturation of proteins is a feasible and uncomplicated technique for evaluating the anti-inflammatory capacity. Compounds derived from natural sources that possess the ability to impede protein denaturation hold significant promise as a potential therapeutic agent in the realm of anti-inflammatory drug development.[24] The present study demonstrates that the methanolic extract of *V. altissima* exhibits a similar inhibitory effect on protein denaturation as the standard drug diclofenac sodium. The involvement of proteinases in the pathogenesis of tissue damage during inflammatory responses has been well-established. Notably, the extract of *V. altissima* has been found to exert a significant inhibitory effect on proteinase activity. Previous studies have demonstrated that phytochemicals possess anti-inflammatory properties by inhibiting proteinases.[25]

Exposure of cells to a hypotonic environment increases intracellular fluid, which subsequently causes hemolysis. This renders the cell more susceptible to damage via lipid peroxidation induced by free radicals.[26] The inhibition of inflammatory reactions can be achieved through the suppression of phospholipases release by compounds that stabilize cell membranes. Phytochemical compounds, including flavonoids, tannins, and saponins, have been identified as agents that can stabilize biological membranes.[27] An escalation in the suppression of erythrocyte membrane hemolysis was noted in correlation with the concentration of extracts. The methanolic extracts, which possessed the greatest antioxidant capacity, demonstrated the highest degree of inhibition of membrane hemolysis. This finding is consistent with prior research indicating that compounds with notable antioxidant capacity are also involved in the stabilization of RBC membranes.[14]

In response to a stimulus, macrophages generate multiple proinflammatory factors such as COX, 5-LOX, MLO and NO. The induction of COX and 5-LOX in RAW 264.7 murine macrophage cells following LPS stimulation increases the production of prostaglandins and leukotrienes. The suppression of said enzymes and subsequent decrease in NO levels may serve as a beneficial means of mitigating inflammation. *V. altissima* is a medicinal plant used in traditional medicine to treat skin lesions, wounds, and arthritis in various tribal communities. Numerous reports exist concerning the therapeutic properties of *V. altissima*. The present investigation centers on the anti-inflammatory properties of various *V. altissima* extracts on RAW 264.7 murine macrophage cells that have been induced with LPS. The data obtained from the MTT assay indicates an absence of toxicity even at elevated concentrations of the extracts. The extracts exhibit potential for application in anti-inflammatory research without inducing any adverse reactions.

The expression of cyclooxygenases occurs in response to inflammatory stimuli and is observed in various cell types such as macrophages, leukocytes, fibroblasts, and synovial cells. The COX activity was reduced in a dose-dependent manner upon treatment of all the extracts. The current investigation demonstrates that VAME exhibits a superior capacity to inhibit COX and mitigate inflammation. The inhibition of the arachidonic acid pathway of prostaglandin synthesis led to the acquisition of this by the VAME. Plant extracts that are rich in polyphenols have the ability to hinder the activity of lipoxygenase. This, in turn, disrupts the metabolism of arachidonic acid and acts as a scavenger for reactive oxygen species that are produced during the metabolism of arachidonic acid.[28] The study findings indicate that the extracts possess the ability to inhibit both COX and LOX in LPS-induced RAW 264.7 murine macrophage cells.

The myeloperoxidase (MPO) enzyme is of significant importance in the process of oxidant production by neutrophils. Moreover, it is noteworthy that this enzyme is the most prevalent proinflammatory agent generated in response to an inflammatory stimulus, and its presence is a contributing factor to the pathogenesis of arthritis.[29] The conspicuous characteristic of various anti-inflammatory ailments is the aggregation of neutrophils. The study demonstrated that the extracts of *V. altissima* have the potential to impede inflammation by hindering the increase of neutrophils through the inhibition of MPO, which is an endogenous component of neutrophils.

The nitrite level was evaluated in RAW264.7 murine macrophage cell lines that were stimulated with LPS. Nitric oxide (NO) is a reactive second messenger species and a versatile gaseous molecule that is synthesized from L-arginine by iNOS. It is implicated in a range of physiological and pathophysiological processes, including vascular and neurological functions and cytotoxic functions in activated inflammatory cells.[30] The treatment with LPS results in an elevation of nitrite level activity. On the contrary, the administration of extracts from the *V. altissima* plant exhibited a significant reduction in the levels of nitrite and its derivatives. The findings indicate that the extract has the potential to function as effective anti-inflammatory agents by disrupting the production of NO. The increasing popularity of natural products that exhibit dual anti-inflammatory and antioxidant properties is noteworthy. Numerous plant extracts have been documented to possess antioxidant characteristics and concurrently exhibit anti-inflammatory effects.[31]

Conclusion

V. altissima has been found to exhibit promising anti-inflammatory effects, as demonstrated by its ability to prevent protein denaturation, inhibit proteinase activity, and reduce HRBC-membrane hemolysis. The methanol extract displayed the most significant efficacy level by decreasing inflammation-associated markers in RAW 264.7 macrophage cells stimulated with LPS. *V. altissima* has been identified as a plentiful source of polyphenolics, which may have the potential as a pharmaceutical component for mitigating inflammatory responses.

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