WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 9, 1489-1495. Research Article ISSN 2277–7105

INVITRO ANTIINFLAMMATORY ACTIVITY OF *AILANTHUS EXCELSA* (ROXB.) LEAVES

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Article Received on
16 March 2024,

Revised on 05 April 2024,
Accepted on 26 April 2024

DOI: 10.20959/wjpr20249-32227

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ABSTRACT

Objective: The study investigated the antiinflammatory activity of hyd roalcohol extract of medicinally and economically useful leaves of *Aila nthus excelsa* (Roxb.) using vitro-based assays: proteinase inhibition, Lipoxygenase Inhibition Assay. **Methods:** Proteinase inhibitory activity of the leaf extracts was performed according to the method of Sakat et al. Lipoxygenase inhibition activity of the extracts of leafy vegetables was assayed according to the method of Wu. **Results:** Proteinase inhibitory activity of the leaf extract was within the range of 20.1–25.8%. The lipoxygenase inhibition was within the range of 3.8–37.0%, showed an improved ability to inhibit lipoxygenase activity. **Conclusions:** With the results of above invitro studies it has been concluded for anti-inflammatory activity.

KEYWORDS: *Ailanthus excelsa*(Roxb.) leaves, invitro, hydroalcohol extract, anti-inflammatory activity.

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folklore medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries.[1] Standardisation of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardisation of herbals and it's formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and

applying suitable standards.[2] High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images.[3] *Ailanthus excelsa (*Roxb.) a plant used in the Indian school/system of medicine for variety of purposes.[4] *Ailanthus excelsa (*Roxb.) belonging to family Simaroubaceae.[5] In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery,especially when there is a blood in stool.[6,7] *Ailanthus excelsa* is a fast growing tree and is extensively cultivated in many parts of India in the vicinity of villages; it is cultivated as an avenue tree for its deep shade and can be used for ant-erosion purposes.[8] The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma.[9,10]

Inflammation is generally referred to as a complex biological response of vascular tissues to harmful stimuli. As well, inflammation is associated with pain, and it involves in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, among others.[11] Inflammation is also described as the body response to inactivate or eliminate the invading stimuli or organisms, to remove the irritants and set the stage for tissue repair, and the process is accelerated by the release of chemical mediators from injured cells or tissues and migrating cells.[12] The migration of leukocytes from the venous systems to the site of damage, and the release of cytokines, are known to play a crucial role in the inflammatory response.[13] These chemicals cause widening of blood capillaries (vasodilation) and the permeability of the capillaries. This will lead to increased blood flow to the injured site.[13] Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli, and is achieved by the progressive movement of plasma and leukocyte-like constituents from the blood, into the injured tissues/locations. Chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation, and is characterized by simultaneous breakdown and healing of the tissue from the inflammatory process.[14] Non-steroidal anti-inflammatory drugs (NSAID) are commonly used for the management of inflammatory conditions. However, these drugs have several adverse side effects, especially gastric irritation, leading to the formation of gastric ulcers. Therefore, the search for natural sources and phytochemicals with anti-inflammatory activity has greatly increased in recent years. Various epidemiological studies provide convincing evidence that natural dietary constituents, such as polyphenols and flavonoids, that humans consume as food, possess many biological activities.[15,16] Further, several epidemiological studies also indicated that the incidence of chronic diseases, such as cancer, cardiovascular

diseases, and inflammation, is inversely correlated with the consumption of fruits and vegetables rich in polyphenols, such as flavonoids.[17]

The study investigated the antiinflammatory activity of hydroalcohol extract of medicinally and economically useful leaves of Ailanthus excelsa (Roxb.) using vitro-based assays: proteinase inhibition, Lipoxygenase Inhibition Assay.

MATERIALS AND METHODS

Plant material

Leaves of *Ailanthus excelsa (*Roxb.) were collected in the Month of August from the agricult ural fields of Tirunelveli district, Tamilnadu. The plant was identified and leaves of *Ailanthus excelsa* were authenticated and confirmed from Dr.V. Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing morphological features (leaf and stem arrangement, flower/inflorescence arrangement, fruit and seed morphology etc.).

The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation and Extraction of Plant material

Preparation of Hydroalcohol extract by Soxhlet Extraction Method: The powder of *Ailanthus excelsa* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using Water and ethanol (1:1) proportion. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get Hydroalcohol extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10º C. The Hydroalcohol extract of *Ailanthus excelsa* leaves was subjected to the following investigations

1. Preliminary phytochemical screening.
2. Invitro anti-inflammatory activity

Proteinase Inhibitory Activity Proteinase inhibitory activity of the leaf extracts was performed according to the method of Sakat et al.[18], which is modified by Gunathilake et al.[19] Briefly, the reaction solution (2 mL) consisted of 0.06 mg trypsin, 1 mL of 20 mM Tris-

HCl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract 0.980 mL methanol). The solution was incubated (37 ◦C for 5 min), and then 1 mL of 0.8% (w/v) casein was added, and the mixture was further incubated for an additional 20 min. At the end of incubation, 2 mL of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm against buffer as the blank. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula: % inhibition of denaturation = 100 × (1 − A2/A1 where A1 = absorption of the control sample, and A2 = absorption of the test sample.

Lipoxygenase Inhibition Assay Lipoxygenase inhibition activity of the extracts of leafy vegetables was assayed according to the method of Wu[20], with some modifications as described in Gunathilake et al.[19] Briefly, a mixture of a solution of sodium borate buffer (1 mL, 0.1 M, pH 8.8) and lipoxygenase (10 µL, final concentration 8000 U/mL) was incubated with 10 mL leaf extract in a 1 mL cuvette at room temperature (30 ± 2 ◦C) for 5 min. The reaction was initiated by the addition of 10 µL linoleic acid substrate (10 mmol). The absorbance of the reaction solution was measured at 234 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as the control, and the percentage inhibition of lipoxygenase was calculated using the following equation: % inhibition = 100 × (absorbance of the control−absorbance of the sample)/ absorbance of the control.[21]

RESULTS AND DISCUSSION

Proteinase Inhibitory Activities

Proteinase inhibitory activity of different concentration was studied the inhibition levels were within the range of 20.1–25.8%. Hydroalcohol extract of Leaves have shown significantly higher ($p < 0.05$) proteinase inhibition level.

Lipoxygenase Inhibition Activity

Inhibition levels were within the range of 3.8–37.0%, within the concentrations of 2-100 µg/ml. The results of this study showed that there was significant cooorelation between the studied anti-inflammatory properties.

Proteinases have been associated with arthritic reactions. Neutrophils, in their lysosomal granules, carry many serine proteinases.[22] Proteinases of leukocytes play a significant role in

the development of tissue damage during inflammatory processes. According to Das and Chatterjee[23], a significant level of protection was provided by proteinase inhibitors. Various recent studies have shown that many flavonoids contributed significantly to the antioxidant and anti-inflammatory activities of many plants. Therefore, the presence of bioactives present in these leaves may contribute to their anti-inflammatory activity. Our previous studies have shown that these leafy vegetables are rich in polyphenols, flavonoids, and carotenoids.[24] Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes. Leukotrienes play an important role in several inflammatory diseases, such as arthritis, asthma, cancer, and allergic diseases.[25] The mechanism of anti-inflammation activity may involve a series of events in which the metabolism of arachidonic acid plays an important role.[26] In this process, arachidonic acid is cleaved from the membrane phospholipids upon appropriate stimulation of neutrophils, and can be converted to leukotrienes and prostaglandins through lipoxygenase and cyclooxygenase pathways, respectively.[26] Lipoxygenase catalyzes deoxygenation of polyunsaturated fatty acids to produce cis, trans-conjugated diene hydroperoxides, such as leukotrienes, which are essential mediators in a variety of inflammatory events.[26]

CONCLUSION

In conclusion, results indicate that the hydroalcohol extracts of leaves of *Ailanthus excelsa* possess anti-inflammatory properties at varying levels. Results indicate that these anti-inflammatory activities may be due to the occurrence of bioactive compounds, such as polyphenols, flavonoids, and carotenoids in these leaves.

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