INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR ISSN: 2231-6078
11(2): April- June.: (2022),30-33

Anti Inflammatory of Leaf of the Plant Araucaria Columnaris

Dharmedra Singh Malviya[1], Bheem Singh[2]
TIT College of Pharmacy Education & Research , Bhopal

Research Article

Abstruct
The present work was undertaken with an objective to establish the anti-inflammatory action of leaf extracts of Araucaria columnaris. The inhibition of albumin denaturation and protease actions were used to establish the anti-inflammatory action. The presence of antioxidant property could be responsible for the anti-inflammatory action of the plant. Fractionation and isolation of the components from extracts would be carried out in future to ascertain the responsible phytochemicals for the anti-inflammatory action.

Keywords: Anti-inflammatory, Araucarian Columnaris, Non-steroidal anti-inflammatory drugs, Antiprotease action **,** Steriols and triterpenoids

Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes [1]. Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. There are mainly two types of inflammation which are as follows: Acute inflammation: It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes. Chronic inflammation: It is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. Inflammation is a common clinical conditions and rheumatoid arthiritis (RA) is a chronic debilitating autoimmune disorder [2] that affects about 1% of the population in developed countries [3]. The classic signs of inflammation are local redness, swelling, pain, heat and loss of function [4]. Inflammation is a stereotyped response, inherent to vascularized tissues, which has the objective of reestablishing tissues homeostasis. The inflammatory process has cellular and humoral components, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic systems (complement, kinins and coagulation), respectively. These components work synergistically and simultaneously, causing vascular alterations and leukocyte recruitment to the lesion. Leucocytes (initially neutrophils), begin to phagocytose bacteria and cellular debris, performing a primary clearance of the lesion. The peak of neutrophil recruitment is followed by the arrival of macrophages into the tissue, which phagocytoses the remaining cellular and bacterial residues, including apoptotic neutrophils [5].

At the same time, lymphocytes can be activated in the lymph nodes by antigen-presenting cells (e.g., dendritic cells) from the tissue, initiating the production of antibodies by B cells and the migration of T helper lymphocytes to the inflamed site. Following the course, stromal and parenchymal cells multiply and reconstitute the tissue, whilst most of the remaining macrophages and lymphocytes leave through the lymphatics. Inflammation is essential for the survival of the host, but is accompanied by its classical cardinal signs rubor, calor, tumor and dolor (redness, heat, tumor and pain), which are the main cause of patient discomfort, especially after surgical procedures. This impels health professionals to prescribe anti-inflammatory drugs, a practice that should be restricted to the shortest period possible following the patient's lesion or surgical intervention. The reason for that is the mechanism of action of NSAIDS, which is the inhibition of the enzyme cyclooxygenase (COX) which takes part in the synthesis of pro-inflammatory lipid mediators known as prostanglandins and tromboxanes.

Corresponding Author Mail-Id:
dharmendrasingh9907@gmail.com

Ironically, the same mediators that induce the initial phase and symptoms of inflammation are those who will take part and stimulate the expression of other enzymes that synthesize mediators responsible for the resolution of inflammation, or in other words, its end. For example, prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) induce the expression of the enzyme 15- lipoxygenase (15- LOX) in its active form in leucocytes, which catalyzes a step in the production cascade of a potent pro-resolving mediator named lipoxin A4 [6].

Lipoxin A4 is a member of a group of lipid mediators of resolution that includes resolvins, protectins and the aspirin-triggered analogs of these classes. It does not have immunosuppressive properties, in contrast, it activates specific cellular mechanisms, such as the stimulation of nonphlogistic recruitment of monocytes (that is: without elaborating pro-inflammatory mediators), activation of macrophage phagocytosis of microorganisms and apoptotic cells, increase in phagocyte exit through the lymphatics, expression of antimicrobial molecules and inhibition of further neutrophil and eosinophil infiltration [7]

Treatment of inflammation

Non-steroidal anti-inflammatory drugs (NSAIDs) have been the cornerstone of pain management in patients with osteoarthritis and other painful conditions. In the United States an estimated 5% of all visits to a doctor are related to prescriptions of non-steroidal anti-inflammatory drugs and they are among the most commonly used drugs [8,9] . In 2004, rofecoxib, marketed as a cyclooxygenase-2 (COX 2) selective inhibitor, was withdrawn from the market after the results of a randomised placebo controlled trial [10] showed an increased risk of cardiovascular events associated with the drug. This finding was confirmed in other trials and a cumulative meta-analysis [11] .Since then debate has surrounded the cardiovascular safety of cyclo-oxygenase-2 selective inhibitors, followed by similar concerns about traditional non-steroidal anti-inflammatory drugs [12]. More recently, the US Food and Drug Administration decided against the approval of etoricoxib because of its inadequate risk-benefit profile [13]. Due to the risks associated with these synthetic NSAIDS, a lot of focus has been directed towards the discovery of drugs from natural products.

Plant Profile [14-16]

Araucaria is a genus of coniferous trees in the Araucariaceae family. Nineteen species in the genus are known to exist, while most of them are used for timber and ornamental purposes Araucaria angustifolia, Araucaria araucana, Araucaria bidwilli, Araucaria cunninghamii and Araucaria heterophylla have been explored for their medicinal properties. Description Araucaria columnaris is a narrow columnar coniferous tree with short, usually horizontal branches that grow in verticils around the slender, upright trunk that leans towards the tip. These branchlets are dressed with small, green, spirally arranged and overlapping leaves which appear as needles when juvenile and grow triangular and scalar when adult. The bark of the tree exfoliates in thin strips and is rough, resinous and grey in color. The tree can grow up to 50-60 meter in height and 9- 15 meter in width.

Chemical constituents and uses

The plants of Araucaria species have been known to possess emollient, antiseptic, antiemetic, wound healing actions. They have been used in toothache, amenorrhoea, respiratory infections and rheumatism. Some phytochemical isolated from various plants of the genus include flavanoids ginkgetin, Bilobetin, II-7-O-methyl-robustaflavone, cupressuflavone,Cabreuvin, Irisolidone, Lignans, Abietanes and terpenes like geraniolene, limonene and Labdane.

Material and Methods

Collection and identification of the plant material

The leaves of Araucaria columnaris were collected from the local places of Bhopal, Madhya Pradesh in the month of February and authenticated at Technocrats Institute of Technology Pharmacy, Bhopal. The voucher specimen was deposited in Department of Pharmacognosy, Technocrats

Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh for future reference.

Preparation of the plant material

The authenticated plant leaves were washed with distilled water, dried under shade and powdered using a blender at low speed. The powdered leaves were stored in air tight container until taken for use.

Extraction of leaves [32]

The powdered leaves were used for the extraction process. 500 g of powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity including benzene, ethyl acetate, and methanol by hot continuous extraction process for about 27 h. The aqueous extraction was carried out by cold maceration process after completion of the solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume to 1/10. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture. The dried extracts were stored in desiccators for further processing.

Preliminary phytochemical screening [33]

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

Alkaloids

Mayer's test: To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test tube.

- Wagner's test: A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube. • Hager's test: A few drops of Hager's reagent were added to few ml of plant extract along the sides of test tube.
- Dragendroff's Test: A few drops of Dragendroff's reagent were added to 1 ml of the each extract.

Glycosides Saponin glycosides

- Froth test: 1 ml solution of the extract in water was placed in a test tube and shaken vigourously.

Anthraquinone glycosides

- Borntrager's test: The extract was boiled with 1.0 ml of dilute sulphuric acid in a test tube for 5 minand filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane and the lower layer (dichlormethane) was separated and shaken with half its volume of dilute ammonia.

Cardiac glycosides

- Kedde's test: The extract was extracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol are added to the above residue. The solution is made alkaline with 20% sodium hydroxide solution.

Keller killiani test (Test for deoxy sugars): The extract was extracted with chloroform and evaporated to dryness. To the residue was added 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. The solution was transferred to a test tube and 0.5 ml of conc.sulphuric acid was added along the wall of the test tube.

Tannins and phenolic compounds

- Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride.
- Ferric chloride test: To the extract was added a freshly prepared solution of ferric chloride.
- Vanillin hydrochloride test: Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.
- Alkaline reagent test: Test solution of the extract was treated with sodium hydroxide solution.

Flavonoids

- Shinoda test: To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.
- Zinc hydrochloride reduction test: To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.
- Alkaline reagent test: To the test solution a few drops of sodium hydroxide solution was added. Later if colour appeared, a few drops of conc. HCl were added to it.

Proteins and amino acids

- Millons test: Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).
- Ninhydrin test: The solution of extract was boiled with 0.2% solution of ninhydrin.

Steriols and triterpenoids

- Libermann Burchard test: Extract was treated with few drops of acetic anhydride, boiled and cooled.Conc.sulphuric acid was added from the sides of the test tube.
- Salkowski test: The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

Total Phenolic Content [34]

The extraction of phenolic compounds was based on a modified method by Hsu et al. Briefly 5 g dried powder of leaves was mixed with 80 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 100 mL with methanol. The solution was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL) for subsequent analyses.

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na2CO3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of thesamples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Antioxidant activity of Petunia extracts by DPPH radical scavenging assay [34]

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Separately, 1mM solution of DPPH and extract solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the extract solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

Determination of Anti-inflammatory action [35]

Though a number of methods are available for in vitro evaluation of anti inflammatory activity, only two methods were considered for the present work.

Inhibition of albumin denaturation

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution.

The technique of inhibition of albumin denaturation reported by Kumari et al [36] was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume.

The extracts rich in flavanoids were dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control.

The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the extract solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of the extract solution.

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Antiprotease action

Preparation of Tris-HCl buffer

An accurately weighed quantity of 121.44 g of Tris was dissolved in 800 mL of distilled water. The pH of the solution was adjusted to 7.0 by addition of appropriate volume of concentrated HCl and the final volume of the solution was made up to 1 L with distilled water.

The technique of antiprotease action reported by Oyedepo et al [37] and Sakat et al [38] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of protease was calculated by the following formula:

Percentage inhibition = (Abs control –Abs sample) x 100/ Abs control

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.001 ($P<0.001$) vs control .

Result and Discussion

The present work focused on preparing successive solvent extracts of Araucaria columnaris and evaluating its anti-inflammatory action using in vitro models. The results obtained from the investigation are presented in the following sections. Pharmacognostic Study The branchlets of Araucaria columnaris grow in whorls and are having small green, spirally arranged leaves. The leaves appear as scales and are triangular in shape. The leaf bearing branchlets are narrow at the petiole, broaden towards the middle and narrow down towards the apex.

Similar findings about the macroscopic characters of the plant are reported in literature where the branchlets are said to be whorling around the slender stem and leaves appeared as needles when juvenile and scalar and needle shaped on maturing [15]. The transverse section of the leaf exhibits the presence of epidermis with thick cuticle. The mesophyll consists of elongated or columnar cells called as the palisade parenchyma. Irregularly arranged cells with large intercellular space are also present. They are called as the spongy parenchyma (Figure 5.2). Air sacs are clearly visible in the cross section of the leaf.

Extraction Yields

The extraction yield of the leaf using different solvents is presented in Figure 5.3. The extraction abilitiy of different solvents was found in the order: methanol>water>ethylacetate>benzene.

Phytochemical Screening

In order to detect the presence of alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids, a small fraction of all the dried extracts were subjected to the phytochemical screening by resuspending a small amount of each extract suitably into the sterile distilled water/ethanol. All the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table 5.1. The findings of the phytochemical analysis suggest the presence of saponin glycosides, cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The presence of tannins, abietanes [39], and cardiac glycosides in Araucaria columnaris has been previously reported [19].

Total Phenolic content

The benzene, ethylacetate, methanolic and aqueous extract of Araucaria columnaris were evaluated for quantifying the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data (Table 5.2). From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be $y = 0.004x + 0.001$ (Fig 5.4). The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 5.3. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of benzene, ethylacetate, methanolic and aqueous extract of Araucaria columnaris were 6.04±0.79, 8.06±0.44, 61.87±0.17 and 29.35±0.63 GAE mg/g, respectively. The methanolic extracts exhibited highest amount of total polyphenol content compared to all other solvent extracts.

DPPH radicals scavenging activity

DPPH is known to be nitrogen centered free radical that can accept an electron or hydrogen radical leading to the formation of highly a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents and then lose its color stoichiometrically depending on the number of electrons consumed and can be quantified spectrophotometricallty at 517 nm. The results obtained (Table 5.4- 5.7) present the results of DPPH scavenging property of the plant extracts.

The IC50 value of the DPPH scavenging potential for benzene and ethylacetate was found to be more than 250µg/mL whereas for methanol and aqueous extracts was found to be 147.05 and 209.3 µg/mL respectively. A comparative analysis of the results of DPPH scavenging for the different extracts is presented in Figure 5.5

Evaluation of anti-inflammatory activity

The anti inflammatory activity was assessed using the in vitro models and the results of each model were statistically evaluated for significance.

Albumin Denaturation Inhibition

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis [40]. According to Opie [41], tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity. All the concentration levels of methanolic and aqueous extract showed the inhibition of albumin denaturation (Table 5.8, Figure 5.6). The 500 µg/mL aqueous extract had shown the greatest inhibition capacity (70.36±3.899%) whereas the lowest inhibition capacity was exhibited by 100 µg/mL of the methanolic extract (11.52±3.291%). The inhibition protein denaturation by 100 µg/mL solution of standard drug Ibuprofen was found to be 78.73 ± 3.561%.

Antiprotease action

Dysregulated release of mast cell proteases is accepted to have a say in the pathogenesis of a number of inflammatory conditions including asthma, abdominal aortic aneurysm formation, vessel damage in atherosclerosis and hypertension, arthritis, and ischemia/reperfusion injury [42]. Table 5.9 presents the antiprotease activity exhibited by aqueous and methanolic extracts at various test concentrations. The highest inhibition capacity was exhibited by aqueous solution of 500 µg/mL concentration, inhibiting 54.93±3.209% while the 100 µg/mL methanolic solution was able to inhibit only 9.29±3.163% protease activity. Aspirin solution of 100 µg/mL concentration was able to inhibit 65.13±2.547% of protease activity (Figure 5.7).

Conclusion

The present work was undertaken with an objective to establish the anti-inflammatory action of leaf extracts of Araucaria columnaris. The inhibition of albumin denaturation and protease actions were used to establish the anti-inflammatory action. The presence of antioxidant property could be responsible for the anti-inflammatory action of the plant. Fractionation and isolation of the components from extracts would be carried out in future to ascertain the responsible phytochemicals for the anti-inflammatory action

References

1. Kumar S, Bajwa BS, Singh K, Kalia AN. Anti-Inflammatory Activity of Herbal Plants: A Review. International Journal of advances in Pharmacy, Biology and Chemistry. 2013; 2(2): 2277-4688

2. Nadkarni AK. Indian Materia Medica. Popular Prakashan, Bombay. 2006

3. Cardinali PD, Esquifino IA. Circardian disorganization in experimental arthritis. Neuro- Signals. 2003; 12: 267-282.

4. Pervical M. Understanding the natural management of pain and inflammation. Clinical Nutrition insights. 1999, 4: 1-5
5. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annual Reviews in Pathology. 2008; 3: 279-312.
6. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nature Immunology. 2001; 2(7): 612-619.
7. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual antiinflammatory and pro-resolution lipid mediators. Nature Reviews Immunology. 2008; 8(5): 349-361.
8. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nature Medicine. 1999; 5(6): 698-701
9. Dai C, Stafford RS, Alexander GC. National trends in cyclooxygenase-2 inhibitor use since market release: nonselective diffusion of a selectively cost-effective innovation. Archives of Internal Medicine. 2005; 165: 171-177. 10. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. Journal of American Medical Association. 2002; 287:337-344.
11. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. New England Journal Medicine. 2005; 352:1092-1102.
12. Juni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet. 2004; 364: 2021-2029.
13. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. Journal of American Medical Association. 2006; 296:1633- 1644.

Table 1.1 :Absorbance data of gallic acid (at 765 nm)

Concentration ppm	Absorbance at 765 nm
0	0
10	0.049
20	0.101
30	0.145
40	0.179
50	0.241
60	0.289
70	0.328
80	0.387
90	0.421
100	0.478

Table 1.2 :Total phenolic content

Extract	Total phenolic content (GAE mg/g)
Benzene	6.04±0.79
Ethylacetate	8.06±0.44
Methanol	61.87±0.17
Aqueous	29.35±0.63

Conc (µg/mL)	DPPH Scavenging %	
	Extract	Ascorbic acid
50	3.3±0.31	94.9±0.16
100	5.6±0.47	-
150	8.9±0.11	-
200	11.8±0.89	-
250	15.7±0.68	-

Table 1.3: DPPH Scavenging potential of benzene extract

Table 1.4: DPPH Scavenging potential of methanolic extract

Conc (µg/mL)	DPPH Scavenging %	
	Extract	Ascorbic acid
50	13.9±0.33	94.9±0.16
100	28.6±0.84	-
150	55.8±0.96	-
200	68.2±0.66	-
250	87.7±0.39	-