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C Pramod
Assoc. Professor, Dept. Pharmaceutical Sciences, CPAS, Puthuppally Campus, Kottayam, Kerala, India

Dr. Sudhakaran Nair
Professor and Research Guide, Mahatma Gandhi University, Kottayam, Kerala, India

Dr. Jyoti Harindran
Principal and Head of Research Department, Department, Pharmaceutical Sciences, CPAS, Cheruvandoor Campus, Kottayam, Kerala, India

Dr. Ratheesh
Professor and Head of the Department of Biochemistry, St Thomas College, Pala, Kerala, India

Corresponding Author:
Dr. Sudhakaran Nair
Professor and Research Guide, Mahatma Gandhi University, Kottayam, Kerala, India

Anti-inflammatory effects of *Lablab purpureus* Linn in polyphenolic *fraction* from methanolic leaf extract on experimental animal model

C Pramod, Dr. Sudhakaran Nair, Dr. Jyoti Harindran and Dr. Ratheesh

Abstract
Natural products have been a source of leads for the development of the most effective drugs currently available for the treatment of a variety of human diseases. Biodiversity will continue to provide novel leads for drug development, but major obstacles like, the large-scale procurement of sufficient source material for bulk production, the potency and inherent toxicity of many natural products resulting in narrow therapeutic indices, as well as significant problems associated with the development of suitable vehicles and dosing schedules for the administration of the drugs to patients limit efficient progress and result in decreased interest in the pharmaceutical sector. Demand for medicinal plant is increasing in both developed and developing countries. An abundance of ethnomedical information on plant uses can be found in scientific literature but has not yet been compiled into a usable form. Collection of ethnomedical information especially in the developing countries remains primarily an academic endeavour of little interest to most industrial groups.
Allergy and inflammation are the one of the immune dysfunctions, which is a serious health problem worldwide. Substances that cause allergy are called allergens, such as pollen, food, dust mites, cosmetics, mould spores and animal hairs. Inflammation may due to the release of mediators during ejection of these allergens. A large number of phytochemicals from the diet have been demonstrated to exhibit antiinflammatory activities by interfering with multiple signalling pathways. The polyphenolic fraction of the methanolic extract of *Lablab purpureus* Linn was subjected to various antiinflammatory studies (Chronic). We proved that it have promising antiinflammatory (>0.005) activities when compared to the control.

Keywords: MEL methanolic extract of *Lablab purpureus* L, (PPF) Polyphenolic fraction

1. Introduction
Arthritis represents one of the most prevalent chronic diseases. Osteoarthritis and rheumatoid arthritis are two most common types of arthritis characterized by joint inflammation including immune cell infiltration, synovial hyperplasia, pain and swelling [1]. Rheumatoid arthritis (RA) is a chronic, inflammatory condition of unknown etiology, affecting approximately 1% of the general population. It is a progressive, disabling, chronic multisystem disease that is characterized by pain, swelling and stiffness of the synovial joints [2]. Although spontaneous remission can occur; it often progresses to a chronic state associated with significant functional disability and substantial morbidity [3].
The animal model of rheumatoid arthritis can be established by adjuvant-induced arthritis (AA) to mirror their pathologies [4]. Complete Freund's adjuvant (CFA) is a well used adjuvant to induce arthritis because CFA is composed of killed mycobacteria, which contain various pathogen-associated molecular patterns including toll-like receptor 2 [5, 6].
In AA, local injection of adjuvant stimulates antigen presentation by dendritic cells, leading to T cell autoimmunity and the secretion of pro-inflammatory factors by macrophages [7]. Pro-inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, nitric oxide (NO) and prostaglandin E2 (PGE2) could further aggravate systemic inflammation.
Progressive systemic TNF-α, IL-1β, IL-6 and NO increase the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and decrease the activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in tissues.
These consequent events further aggravate protein and lipid oxidation, and injury tissues. Thus, oxidative damage of the vital organs, particularly the liver, is considered as secondary complications of arthritis [8].

Moreover, the over expression of inducible nitric oxide synthases (iNOS) and cyclooxygenase-2 (COX-2), as synthases of NO and PGE2, has been proven to be related to the progressive inflammation and tissue damage observed in arthritis [9].

2. Materials and Method

2.1 The fractionation of methanolic extract by Harbone's method [10] 1973.

The methanolic leaf extract of the *Lablab purpureus* L. was homogenized with methanol and water in the ratio of 4:1 and filter. The filter was evaporated to 1/10 volume at 40 ^{0}C and acidified with 2M sulphuric acid and extracted with chloroform.

The organic layer was separated and dried. (Chloroform extract) which may contain terpenoids and Phenolic compounds. The aqueous layer was basified using ammonium hydroxide and adjusted the pH up to 10. Then it was treated with chloroform and methanol evaporated to dryness and the extracted may contain alkaloids.

The residue of the MEL treated with 4:1 methanol-water mixture was subjected to treat with ethyl acetate and filter. The residue which contains fiber and filtrate is evaporated by rotary evaporator in low pressure yielding the dried extract. The extracted may contain neutral compounds (Neutral extract) like fats waxes etc.

2.2 Acute oral toxicity [11, 12].

The intense oral noxiousness study was done according to the principles set by the OECD (Organization for Economic Co-operation and Development) current rules 423 got from the CPCSEA (board of trustees with the end goal of control and supervision of investigates creatures), MSJE under Indian administration.

The animals received humane care, in compliance with the host institutional animal ethics guidelines. All experiments were conducted as per the guidelines of the animal ethics committee CPCSEA (Registration No. 149/CPCSEA) according to Government of India accepted principles for laboratory animals' use and care. The experimental protocol was subjected to the scrutiny of Institutional animal ethical committee (IAEC) of Department of Pharmaceutical Sciences and approval no: 01/PR/UCP/CVR/2011.

2.3 Experimental design for different fractions from MEL on acute inflammation.

The rats were treated with extracts such as different fractions obtained from MEL at different concentrations (5, 10, 20, 50 mg/Kg bwt). After 1 hour of pre-treatment of plant extract and standard drug Diclofenac sodium given intraperitoneal, the inflammatory agent, Lambda carrageenan, 0.1 ml of 1% in normal saline was injected to the rats into the plantar aponeurosis of the right hind paw of the rat producing acute inflammatory edema leading to marked increase in the volume of the limb. The group II received only carrageenan and group I received only saline. The group III, IV and V received fiber, terpenes and polyphenolic and alkaloid fractions respectively. The paw volume was measured at hourly interval for 3 hours by using paw edema meter pethismometer. The percentage edema was calculated by Winter *et al.* [13] method.

2.4 Experimental design for adjuvant-induced arthritis

The right hind paw of animals was immunized by injecting 0.1 mL of complete Freund's adjuvant containing heat-killed mycobacteria in paraffin oil.

The duration of the experiment was 30 days. After overnight fasting, rats were sacrificed by euthanasia. For histological analysis, paw tissues were dissected, fixed in 10% buffered formalin and then decalcified for 7 days in 20% EDTA. The tissues were then processed and embedded in paraffin. Synovial fluid was obtained by injecting 100 µL of normal saline into the knee joints followed by gentle aspiration. Paw tissue and blood were also collected for various biochemical estimations.

2.5 Assay of Cyclo-oxygenase activity

COX activity was assayed by the method of Shimizu[14]. Lysed monocytes and paw tissue isolated from control, standard and test groups were incubated with Tris-HCl buffer (pH 8), 5mM glutathione and 5mM hemoglobin for 1 min at 25 ºC. The reaction was initiated by the addition of 200µM arachidonic acid and terminated after 20 min incubation at 37 ºC by the addition of 10% TCA in 1N HCl. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

2.6 Estimation of thiobarbituric acid reactive substances (TBARS)

1g tissue was homogenized in 9 volumes of 1.15% KCl, centrifuged and took the supernatant. To 0.2ml of tissue homogenate, 0.2ml of 8.1% SDS, 1.5ml of 20% acetic acid at pH 3.5 and 1.5ml of an aqueous solution of TBA. The volume was made up to 4ml with distilled water and then heated in boiling water bath at 95 ºC for 60 minutes using glass ball as condenser. After cooling with tap water, 1ml of distilled water and 5ml of n-butanol: pyridine reagent was added and shaken vigorously. After centrifugation at 4000rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured against blank butanol. Lipid peroxide levels were expressed as mmol of malondialdehyde produced [15].

2.7 Estimate the mRNA expression of cytokines

2.7.1 RT-PCR analysis of rat IL-6, TNF- α, iNOS and COX-2

Total RNA was isolated from paw tissues using RNA isolation mini kit (Sigma Aldrich, USA) according to the manufacturer's instructions. Primer pairs for rat IL-6, TNF- α, PGE2, COX-2 and GAPDH were as follows:

Table 2.1: List of forward and reverse primers used in the RT-PCR analysis

Gene	Forward primer	Reverse primer
IL-6	5'CCACTGCCTTCCCTACTTCA3'	5'TGGTCCTTAGCCACTCCTTC3'
TNF-α	5'GTCGTAAACCACCAAGC3'	5'GACTCCAAAGTAGACCTGCCC3'
COX-2	5′ATCTGCCTGCTCTGGTCAATG-3′	5′-CAATCTGGCTGAGGGAACACA-3′
PGE2	5′-CAGCACAGAGGGCTCAAAGC-3′	5′-TCGTCGGCCAGCTCTTTCT-3
GAPDH	5'CCTGCTTCACCACCTTCTTG3'	5'ATCCCATCACCATCTTCCAG3'

RT-PCR was performed in an Eppendorf the rmocycler, using a two-step. RT-PCR kit, where reverse transcription and DNA amplification was done separately. 2µg of total RNA was used as template in the first reaction that included dNTPs, oligo (dT) and reverses transcriptase enzyme. The second reaction included appropriate primers, PCR enzyme, cDNA formed in the first step and dNTPs. The PCR products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide and visualized by transillumination. The images were photographed and the intensities of bands were measured. The amounts of target PCR products were normalized against GAPDH PCR product in the corresponding samples.

2.7.2 Western blot analysis for MMP-2 and MMP-9

Western blots were performed according to published methods [16] (Wang *et al*., 2005) [16]. Briefly, cells were washed twice with PBS and scraped into a lysis buffer. Protein concentrations were analyzed with a Bio-Rad system. Equal amounts of protein (20 µg/lane) for each sample were electrophoresed through a 10% SDS-PAGE gel.

After electrophoresis, the proteins were transferred to nitrocellulose membranes and the blots were subsequently probed with the following MMP-2 and MMP-9 antibodies (1:1000 Sigma Aldrich). For detection, horseradish peroxidase-conjugated secondary antibodies were used (1:2000) followed by enhanced chemiluminescence development. Bands were scanned and semi quantified by densitometry. GAPDH was used as an internal control.

2.8 Histopathological analysis of the paw tissue

A histopathology is a vital tool for the diagnosis of RA, which provides an easy way of determining disease progression at anatomical levels in animals. Animals affected with RA show marked cellular infiltration, synovitis, synovial ulceration, cartilage destruction, and bone damage compared to the normal animals. On the 21st day, one of the animals from each group was sacrificed by cervical dislocation.

The entire tissue was rapidly dissected out and tissue sections (5µm) fixed by immersion at room temperature in 10% formalin solution. For the histological examinations, paraffin-embedded tissue sections of paw tissue were stained with hematoxylin-eosin (H&E). The tissue samples were then examined and photographed under a light microscope for observation of structural abnormality. The severity of paw tissue inflammation was judged by two-independent observers blinded to the experimental protocol.

3. Results

3.1 Preparation of the fraction of methanolic extract

The fractionation of methanolic extract of *Lablab purpureus* Linn by Harbone's method. The methanolic extract was homogenized with 4:1 methanol and water and separated the residue.

This residue was treated with ethyl acetate gave semisolid dark brown coloured fraction (fibre) (7.34% w/w Yield) and filtrate was treated with chloroform fraction to gave dark brown coloured solid mass (Phenolic compounds and terpenes) (11.58% w/w Yield).

Acidified the aqueous layer of the above fraction and treated with $CHCl_3$ greenish brown semisolid (alkaloids) (9.78% w/w), which was basified and mixed with a mixture of chloroform ad methanol to produced colourless crystalline quaternary alkaloids (9.94% w/w)

3.2 Pharmacological screening

3.2.1 *In vivo* anti-inflammatory screening

3.2.1.1 Acute oral toxicity study

The acute oral toxicity study of methanolic leaf extract of *Lablab purpureus* Linn was studied on female albino *wistar* rats by administering the extract from lower to higher dose and the animals were observed for 24 hours. This study was carried out as per the OECD guidelines 423 of acute toxic class method. The study was initiated with the lowest dose 50mg/Kg and followed by with the highest dose 2000mg/kg by staircase method as per direction from the institutional animal ethical committee to minimise the number of animal. The study found that the 2000mg/kg was safer to the animals

Table 3.2: Showed the anti-inflammatory effect of various fraction of the methanolic extract in carrageenan induced rats at different doses

Sl. no	Methanolic fractions	Dose Mg/Kg bwt	Edema with SEM				% of inhibition (3rd hour)
			0-hour	1-hour	2-hour	3-hour	
1	Fibers	5	1.20±0.014	1.76±0.0047	2.97±0.0039	1.27±0.0076	53
		10	1.50±0.019	1.86±0.0048	1.97±0.0036	1.47±0.0077	58
		20	2.40±0.0036	2.57±0.0025	2.65±0.0052	2.90±0.0025	60
		30	2.50±0.012	2.76±0.0048	2.97±0.0036	1.27±0.0075	62
		50	2.50±0.012	2.76±0.0048	2.97±0.0036	1.27±0.0075	62
2	Polyphenols/Terpenes	5	2.50±0.0073	2.67±0.0068	2.73±0.0022	3.22±0.0021	76
		10	2.52±0.0079	2.68±0.0069	3.03±0.0028	3.32±0.0022	77
		20	2.60±0.0077	2.78±0.0068	3.23±0.0025	3.42±0.0025	82
		30	2.60±0.0077	2.78±0.0068	3.23±0.0025	3.42±0.0025	82
		50	2.60±0.0077	2.78±0.0068	3.23±0.0025	3.42±0.0025	82
3	Alkaloids	5	2.20±0.012	2.16±0.0048	2.07±0.0036	2.97±0.0077	73
		10	2.50±0.012	2.76±0.0048	2.97±0.0036	2.98±0.0071	74
		20	2.50±0.012	2.76±0.0048	2.97±0.0036	3.27±0.0077	77
		30	2.40±0.014	2.66±0.0050	2.57±0.0031	2.95±0.0073	72
		50	2.20±0.012	2.16±0.0048	2.07±0.0036	2.97±0.0077	73
4	Quaternary alkaloids	5	2.30±0.0025	2.26±0.0047	2.13±0.0063	1.20±0.0031	65
		10	1.50±0.0025	1.96±0.0044	1.11±0.0062	1.28±0.0039	67
		20	2.50±0.0025	2.96±0.0044	3.13±0.0063	3.20±0.0036	70
		30	1.20±0.0029	1.06±0.0041	3.13±0.0064	2.40±0.0035	69
		50	2.50±0.0020	2.16±0.0042	2.43±0.0067	1.22±0.0039	68
5	Diclofenac (Standard)	20	2.40±0.0025	2.89±0.0032	3.03±0.005	3.14±0.0044	74

Anti-inflammatory effect of different fractions of PPF at dose of 5, 10, 20, 30, 50 mg/Kg bwt was evaluated in carrageenan paw edema model. MEL fraction of polyphenol/terpenes at dose of 20mg/Kg bwt showed significantly higher edema inhibition compared to other fraction. Hence MEL polyphenolic fraction of at a dose of 20mg/Kg bwt having promising anti-inflammatory activity. Details are reported in the table 3.2.

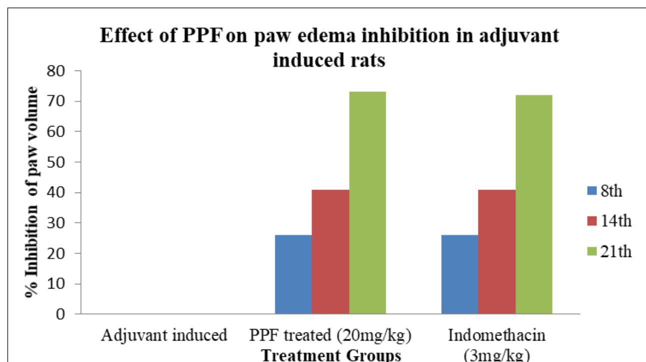


Fig 3.1: showed the effect of PPF on paw edema inhibition in adjuvant induced rats

3.2.2 Effect of PPF on paw edema inhibition in adjuvant induced rats

PPF at a dose of 20 mg/Kg bwt (Fig.3.1) showed 73% inhibitory effect on adjuvant induced chronic model of inflammation which is higher than that of standard drug indomethacin.

The percentage inhibition of paw volume by PPF. Values expressed as average of 6 rats in each groups. a Statistical difference with adjuvant induced group at P ≤0.05.

Table 3.3: showed the effect of, TBARS, and COX on paw tissue

Groups	TBARS (mmol/g)	COX (U/mg protein)
Normal	2.6 ± 0.03	3.9 ± 0.01
AA	6.4 ± 0.01[a]	8.0 ± 0.03[a]
AA+PPF	2.96 ± 0.02[a,b]	4.4 ± 0.04[a,b]
AA+IND	3.2 ±0.03[a,b]	4.6 ± 0.03[a]

The COX activity (Table 3.3) was increased significantly (P≤0.05) in adjuvant induced rats when compared to normal rats. However, PPF treatment significantly reduced COX activity.

Lipid peroxidation indicator TBARS was measured in paw tissue. The concentration of TBARS (Table 3.3) was increased significantly (P≤0.05) in adjuvant induced rats when compared to normal rats. However, PPF treatment significantly (P≤0.05) reduced TBARS concentration.

Table 3.3. Inhibitory effect of PPF on the concentration of plasma, TBARS and COX. Values expressed as average of 6 samples ± SEM in each group. a Statistical difference with control group at P≤0.05. b Statistical difference with adjuvant rats at P≤ 0.05.

3.2.3 Effect of PPF on COX-2 and PGE2 gene expression

The gene-level expression of inflammatory marker genes like COX-2 and PGE2 (Fig. 3.2A) was up-regulated in arthritic rats. PPF administration significantly down regulated the gene expressions on the PPF treated sample as compared to arthritic model.

3.2.4 Effect of PPF on TNF-α and IL-6 gene expression

The up-regulated expression of cytokines like IL-6 and TNF-α (Fig. 3.2B) during arthritic induction was significantly suppressed by PPF administration.

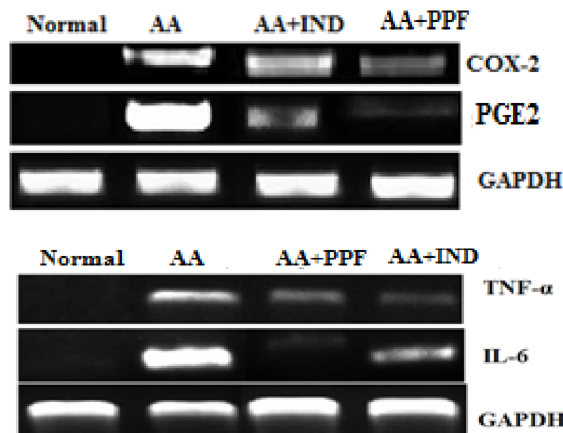


Fig. 3.2 (A): Inhibitory effect of PPF on the expression of the pro-inflammatory cytokines COX-2 and PGE2. Total RNA was isolated and the expression of COX-2 and PGE2 was determined by reverse transcriptase-PCR. GAPDH was used as a control. (B): Inhibitory effect of PPF on the expression of the pro-inflammatory cytokines TNF-α and IL-6. mRNA expression by means of reverse transcriptase PCR of the mRNA isolated from paw tissue by using an RNA isolation kit. For quantification, the mRNA expression data were normalized to the GAPDH signal.

(B): Inhibitory effect of PPF on the expression of the pro-inflammatory cytokines TNF-α and IL-6. MRNA expression by means of reverse transcriptase PCR of the mRNA isolated from paw tissue by using an RNA isolation kit. For quantification, the mRNA expression data were normalized to the GAPDH signal.

3.2.5 Western blotting analysis of MMP-2 and MMP9

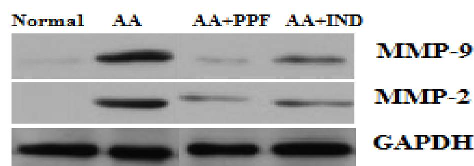


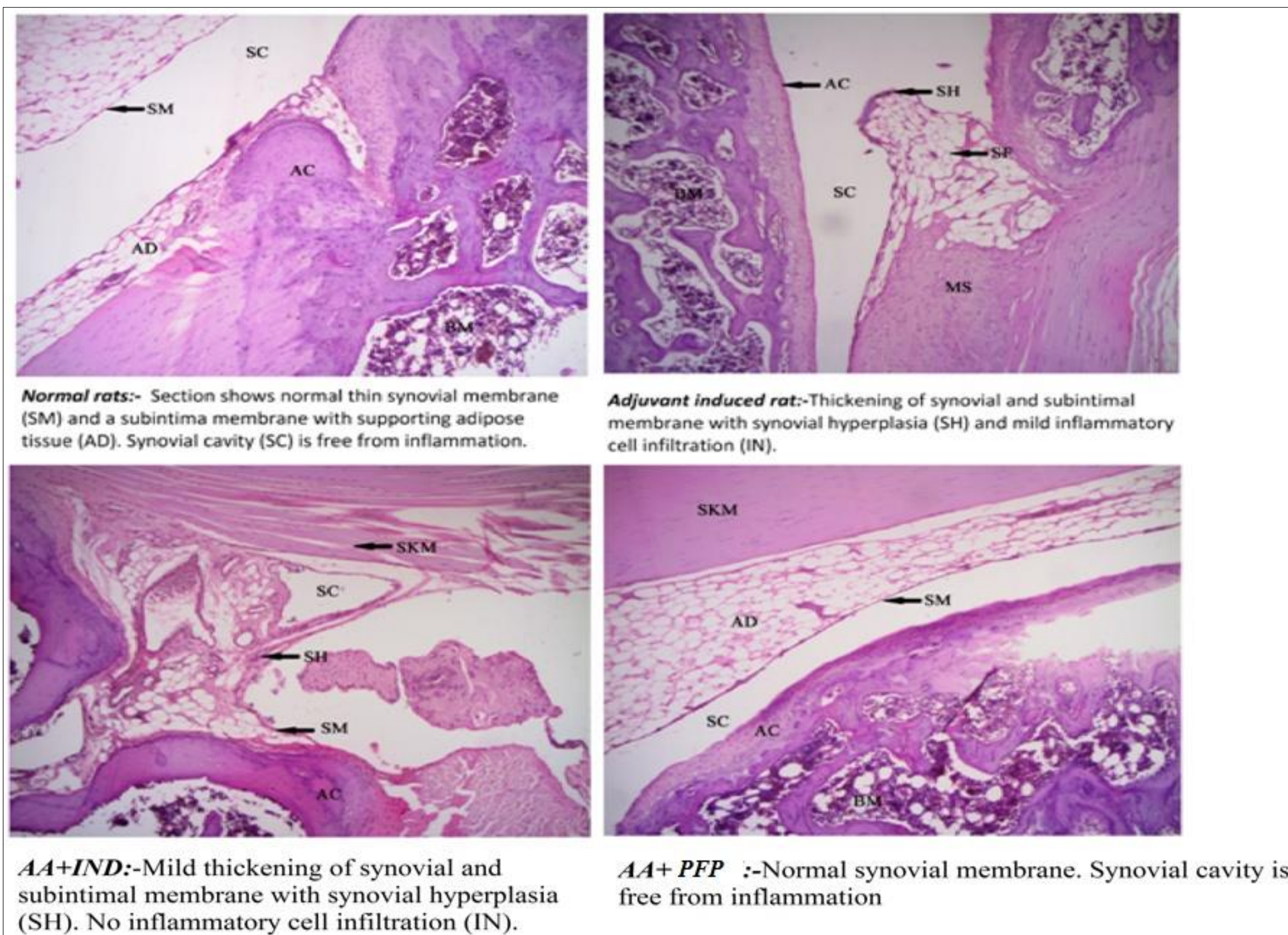
Fig 3.3: Western blotting analysis of MMP-2 and MMP-9

MMP-9 expression is regulated by several cytokines and growth factors, including interleukins, interferons, EGF (Epidermal growth factor), NGF (Nerve growth factor), basic FGF (Fibroblast growth factor), VEGF (Vascular endothelial growth factor), PDGF (Platelet-derived growth factor), TNF-α (Tumor necrosis factor-alpha), TGF-b (Transforming growth factor-b), the extracellular matrix metalloproteinase inducer emmprin and also osteopontin. Many of these stimuli induce the expression and/or activation of c-fos and c-junto-oncogene products, which heterodimerize and bind activator protein-1 (AP-1) sites within of MMP-9 gene promoters. Details may be shown in the (Fig: 3.3)

3.2.6 Histopathological evaluation

One animal from each group was sacrificed by cervical dislocation for the determination of the histopathology of ankle joint. Histopathological change of animals was assessed and tabulated below.

Section 1 denotes normal rat: the showed Section shows normal thin synovial membrane (SM) and a subintimal membrane with supporting adipose tissue (AD). Synovial cavity (SC) is free from inflammation.
Section 2 denotes the adjuvant-induced rat: The section showed thickening of synovial and subintimal membrane with synovial hyperplasia (SH) and mild inflammatory cell infiltration (IN).
Section 3 denotes the adjuvant-induced rat + standard drug Indomethacin: The section showed mild thickening of synovial and subintimal membrane with synovial hyperplasia (SH). No inflammatory cell infiltration (IN).
Section 4 denotes the adjuvant-induced rat + Test (polyphenolic fraction 200mg/Kg): the section showed the normal synovial membrane. The synovial cavity is free from inflammation. Details may be shown in the (Fig: 3.5)



The explanation of the fig 3(5)

Section 1 denotes normal thin synovial membrane(SM) and a subintimal membrane with supporting adipose tissue (AD). Synovial cavity (SC) is free from inflammation.
Section 2 denotes thickening of synovial and subintimal membrane with synovial hyperplasia (SH) and mild inflammatory cell infiltration (IN).
Section 3 denotes the mild thickening of synovial and subintimal membrane with synovial hyperplasia (SH). No inflammatory cell infiltration (IN).
Section 4 denotes the normal synovial membrane. The synovial cavity is free from inflammation.

Discussion

In the case of chronic inflammatory study the MEL undergo fractionation by Harbor method. The various fractions subjected to *in-vitro* and *In-vivo* studies using Raw 264.7 cell and paw edema inhibition in adjuvant-induced rats' model. In this study the polyphenolic fraction showed 82% of edema inhibition, which was greater than other fractions. So we selected 20mg/bwt of polyphenolic fraction PPF for the chronic model.

The present study highlights the capacity of PPF to improve the anti-inflammatory status and antioxidant defences in AA rats. Rheumatoid arthritis is a systemic chronic inflammatory disorder which may produce progressive joint damage. The release of toxic substances in the synovium during joint inflammation may lead to cartilage destruction Darlington *et al*., 2001 [17].

The adjuvant-induced arthritic experimental model has been used extensively for studying immuno-inflammatory processes of arthritic diseases in humans, in particular rheumatoid arthritis (RA), as well as for screening and testing novel anti-arthritic agents Billingham, 1983 [18].

The therapeutic efficacy of the drugs and the degree of inflammation was determined by observing the paw swelling of experimental rats. The edema formation in peri-articular tissues such as ligament and joint capsule leads to soft tissue swelling around ankle joints in arthritic rats.

In the present study, chronic inflammation was induced by Freund's complete adjuvant in rats that developed a chronic swelling in multiple joints, with influence of inflammatory cells, erosion of joint cartilage and bone destruction. The

release of number of inflammatory mediators during chronic inflammation leads to pain, destruction of bone and cartilage that can lead to severe disability. In the present study, administration of 20 mg/kg of PPF showed significant inhibition of paw oedema volume and the inhibition was higher than that of indomethacin treated group.

IL-6 also plays an important role in cartilage and bone degradation during arthritis. IL-6 is also reported to stimulate secretion of PGE2 by synovial cells contributing to joint damage in inflammatory conditions like rheumatoid arthritis Anderson *et al*., 1996 [19]. In the present study APPs were found elevated in arthritic rats.

In the present study, PPF supplementation significantly down regulated the expression of IL-6 in the paw tissue of arthritic rats. PPF supplementation also restored the levels of APPs to near normal values in arthritic rats. Thus decreased IL-6 production correlated with decreased APP production thereby reducing disease severity.

The evaluation of paw swelling is a simple and quick procedure for evaluating the degree of inflammation and the therapeutic efficacy of the drugs. Arthritic rats showed the soft tissue swelling around ankle joints that were considered to be due to oedema of peri-articular tissues such as ligament and joint capsule.

Prostaglandins induce the relaxation of arteriolar smooth muscle cells and increase the blood supply to the tissue. The ability of the drug to reduce oedema formation may thus be related to its inhibitory action on prostaglandin synthesis. COX-1 and COX-2 are two distinct but related enzymes, which is responsible for prostaglandin synthesis.

The COX-1 is a constitutive enzyme, responsible for maintaining normal renal function, gastric mucosa integrity and hemostasis Griswold *et al*., 1996 [20]. During inflammatory conditions, the inducible isoforms COX-2 enhances the production of prostaglandins that are associated with inflammation.

The present study shows that administration of PPF significantly reduces paw edema volume in arthritic rats. Also the activity of total COX and COX-2 expressions was decreased in MEL treated groups. The reduction of paw swelling and decreased expression of COX-2 indicate the immunological protection provided by the PPF. Increased levels of TNF-α were found in sera and synovial fluid of RA patients, suggesting that TNF-α may play a role in the pathology of the disease. Blocking of TNF-α followed by inactivation of T cells, macrophages, and fibroblasts interrupt the inflammatory process Yazdani-Biuki *et al*., 2005 [21].

Production of NO in suitable magnitude serves as a key signaling molecule in various physiological processes. But at the same time NO has been found to cause pathological conditions in chronic inflammation Gross *et al*., 1995 [22]. Our study showed that nitrite production in PPF treated animals was significantly lower as compared to adjuvant treated rats.

This suggests that the reduction of chronic inflammation in arthritic animals might be due to the downregulation of iNOS expression followed by decreased cellular production of NO. Therefore, the inhibitory activity of PPF may be due to their anti-inflammatory properties and ability to counteract NO-induced oxidative damage, which eventually helps in remodelling of cells.

Lipid peroxidation is one of the critical mechanisms of injury associated with RA, Tissue thiobarbituric acid reactive substances (TBARS) analysis is an extensively used method for evaluating lipid peroxidation in tissue. In the present investigation, the increased levels of TBARS were observed in arthritic rats. The elevated levels of TBARS were significantly decreased after the treatment of PPF. PPF protects against free radicals formation and reduces the inflammation. These observations suggested that adjuvant-induced arthritis may be associated with lipid peroxidation and anti-arthritic effect of PPF reduce lipid peroxidation and causing a modulation in the cellular antioxidant defense system.

In the present study, we found that PPF significantly inhibited the production of the pro-inflammatory mediators and cytokines and their mRNA expression.NF-κB plays a critical role in the regulation of cell survival genes and coordinates the expression of pro-inflammatory enzyme and cytokines such as COX-2, IL-6, and TNF-α, and iNOS. Since the expression of these pro-inflammatory mediators is modulated by NF-κB, our findings suggest that the transcriptional inhibition of pro-inflammatory mediator production by PPF maybe occurs via blocking of the NF-κB signaling pathway.

Histopathological analysis of PPF treated ankle joint, strengthen the evidence of resolution of arthritis. PPF (20mg/kg) showed a significant effect when compared to induced arthritis rats. Synovial cytology showed that inflammatory cells were suppressed by PPF and indomethacin administrated rats as compared to an induced arthritis rat.

Conclusion

Our study proved that the polyphenolic fraction (20 mg/Kg) from methanolic extract of *Lablab purpureus* Linn have promising anti-inflammatory activity by various *in vitro* and *in vivo* screening.

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