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EVALUATION OF IN VITRO ANTI-ARTHRITIC ACTIVITY OF BOSWELLIA SERRATA AND ALOE BARBADENSIS AGAINST THE DENATURATION OF PROTEIN

Pharmacology

Ms. Pallavi Sitaram Shelke — M Pharm 2nd Year, Pharmacology Department , PDEA's S.G.R.S. College Of Pharmacy , Saswad.

Mrs. Pradnya Nilesh Jagtap* — HOD Of Pharmacology Department , PDEA's S.G.R.S. College Of Pharmacy , Saswad. *Corresponding Author

Ms. Prajakta Rohidas Tanpure — M Pharm 1st Year, Pharmacology Department , PDEA's S.G.R.S. College Of Pharmacy , Saswad.

ABSTRACT

Anti-denaturation effects of plant extracts in heat treated Egg Albumin and Bovine Serum Albumin (BSA), are potential therapeutic parameters for finding anti-inflammatory compounds without the use of animal for preliminary pharmacological screening. Boswellia Serrata [BS] and Aloe Barbadensis [AB] can bring natural compounds with significant anti-arthritic effects. So our aim was to investigate in vitro anti-arthrtic activity of aqueous extract of BS and AB and their combinations by using denaturation of protein methods. The extract at different concentrations was incubated with egg albumin and bovine serum albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Methotrexate was used as the reference standard drug. The present finding exhibited a concentration dependent inhibition of protein (albumin) denaturation by B. Serrata and A. Barbadensis extracts. The effect of Methotrexate was found to be less than extracts. In conclusion it can be concluded that B. Serrata and A. Barbadensis extracts possessed in vitro anti-arthritic effect against the denaturation of protein and B. Serrata was stronger than A. Barbadensis. Also combination of B. Serrata and A. Barbadensis extracts had potent synergistic effect on inhibition of protein (albumin) denaturation.

KEYWORDS

Anti- Denaturation, Boswellia Serrata, Aloe Barbadensis, In- Vitro Anti- Arthritic Property.

INTRODUCTION:

Rheumatoid arthritis is a systemic autoimmune disease that causes chronic inflammation of connective tissue primarily in the joints that involves synovial proliferation and cartilage destruction. The first joint tissue to be affected is synovial membrane which lays the joint cavity [1]. Researchers have found many new mediators viz, TNF- a, IL-2 and enzymes which take part directly or indirectly in perpetuation of RA [2,3,4].

Protein denaturation has been identified as the cause of inflammation in arthritis. Indications are that when living tissues are injured, inflammation results. This is characterized by redness, pain, heat, swelling, as well as loss of function in the affected area. Disruption of the electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure occurs. In addition, a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair, occur, causing the protein to lose its molecular conformation and functions or become denatured [5-8]. It is therefore deduced that, compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents [7].

According to the World Health Organization [WHO], about three-quarters of the world's population relies on traditional medicine for primary healthcare needs and most of this treatment involves use of plant extracts or their active components [9] (Egan, 2002). Arthritic conditions are treated using traditional medicine with considerable success. Although various modern drugs are used to treat these type of disorders their prolonged usage may cause severe side effects .So there is an urge to develop new therapeutic agents with minimum side effects [10].

The present investigation is to scientifically evaluate for in vitro anti-arthritic activity on bovine serum albumin and egg albumin assay suggest about their mechanism for therapeutic activity.

PATHOGENESIS OF RHEUMATOID ARTHRITIS [11,12,13]:

- In response to antigenic exposure in a genetically predisposed individual (HLA-DR), CD4+T-Cell are activated.
- These cells elaborate cytokines like tumour necrosis factor α, interleukin IL-1 and IL-6.
- These cytokines activates endothelial cells, B lymphocytes and macrophages.
- Activated B-cells releases IgM antibody against IgG – this mole cule is termed as rheumatoid factor.
- IgG and IgM immune complexes trigger inflammatory damage to the synovium, small blood vessels and collagen.
- Activated endothelial cell express adhesion molecules which stimulate inflammatory cells.
- Activation of macrophages releases more cytokines which cause damage to joint tissue and vascularisation of cartilage called as pannus formation.

Damage and destruction of bone and cartilage followed by fibrosis and ankylosis result in joint deformities.

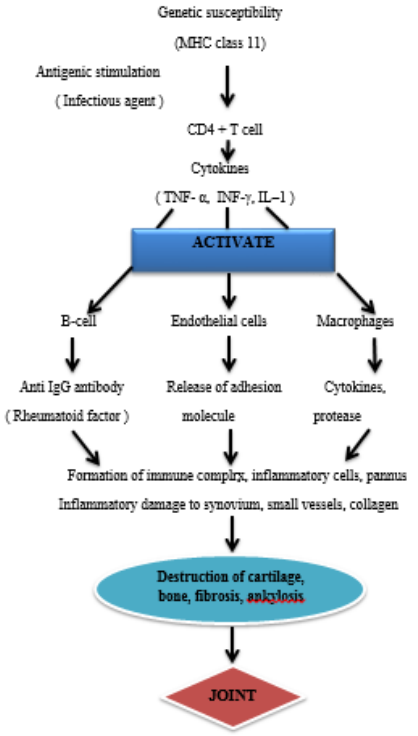


Fig.1: Pathogenesis of RA

MATERIALS AND METHODS :

PLANT MATERIAL

Herbal extract powders of Boswellia serrata and Aloe barbadensis are obtained as an gift sample from Sane Guruji Hospital and Ayurvedic Medical Store , Hadapsar, Pune.

CHEMICALS

Methotrexate (Imutrex) obtained from cipla company. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

ASSESSMENT OF INVITRO ANTI – ARTHRITIC ACTIVITY : PROTEIN DENATURATION BY USING EGG ALBUMIN [14]

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen s egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of aqueous extract of Boswellia serrata & Aloe babadensis (50:50 Ratio) so that final concentrations become 100,200,400,800 and 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in a BOD incubator for 15 min and then heated at 70ºC for 5 min. After cooling, their absorbance was measured at 660 nm. Methotrexate was used as reference standard drug.

FORMULA :

The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage inhibition = (Abs control - Abs sample) X 100/ Abs control

PROTEIN DENATURATION BY USING BOVINE SERUM ALBUMIN [15]

The reaction mixture was consisting of aqueous extract of Boswellia serrata & Aloe babadensis (50:50 Ratio) at different concentrations and 1 % of aqueous solution of bovine albumin. The samples were incubated at 37ºC for 20 min and then heated at 57ºC for 20 min. After cooling the samples, absorbance of turbidity was measured at 660 nm.

FORMULA :

Percentage of inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs control - Abs sample) X 100/ Abs control

RESULT AND DISCUSSION:

The effect of aqueous extract of Boswellia serrata and Aloe barbadensis was evaluated against denaturation of egg albumin and bovine albumin. The results are summarized in table 1 and 2. The Combination of herbal extract exhibited significant anti-arthritic activity at 100- 1000 µg/ml by protein denaturation inhibition. The effect of herbal extract was studied by comparing with the standard methotrexate. The auto antigen production in rheumatoid arthritis is due to denaturation of protein and several studies reveal that protein denaturation is one of the reason for rheumatoid arthritis.

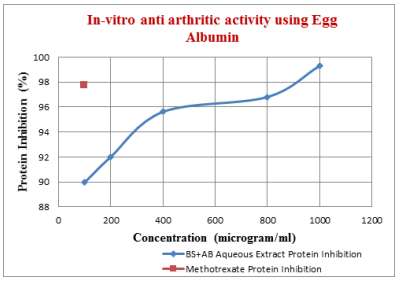
Methotrexate at concentration 100 µg/ml showed less effect compared to BS + AB extract at concentration (1000 µg/ml). The maximum activity is exhibited by the herbal extract at a concentration of 1000µg/ml. From the study conducted, it can be concluded that B. serrata and A. barbadensis combination can be successfully used in the management of arthritis.

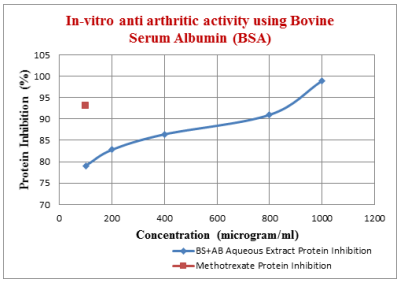
Table 1: Effect of BS+AB extract on protein denaturation (Fresh egg albumin)

Treatment	Concentration (µg/ml)	Absorbance (at 660 nm)	Percentage of Protien inhibition (%)
Control	-	1.525	-
BS + AB aqueous extract	100	0.1530	89.96
	200	0.1218	92.01
	400	0.0667	95.62
	800	0.0486	96.81
	1000	0.0106	99.30
Methotrexate	100	0.0342	97.75

Table 2: Effect of BS+AB extract on protein denaturation (Bovine serum albumin)

Treatment	Concentration (µg/ml)	Absorbance (at 660 nm)	Percentage of Protein inhibition (%)
Control	-	0.567	-
BS + AB aqueous extract	100	0.1186	79.08
	200	0.0975	82.80
	400	0.0770	86.41
	800	0.0512	90.97
	1000	0.0059	98.95
Methotrexate	100	0.0396	93.01





CONCLUSION:

The in vitro anti-arthritic study conducted on the ayurvedic herbal plant combination concluded that the Boswellia serrata and Aloe barbadensis exhibited significant anti- inflammatory activity and hence can be used effectively in the management of arthritis.

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