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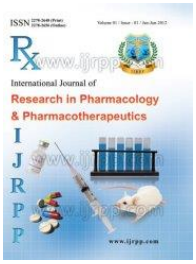

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Comparison of anti- inflammatory activity between conventional NSAIDS and ayurvedic preparations- *Invitro* method

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ABSTRACT

Inflammation and pain are the most common health problems treated with traditional remedies which mainly comprise medicinal plants. A number of natural products are used in the traditional medical systems in many countries. An alternative medicine for the treatment of various diseases is getting more popular. Many medicinal plants provide relief of symptoms comparable to that of obtained from allopathic medicines. Therefore agents of natural origin with very little side effects are required as substitute chemicals therapeutics. The Ayurvedic preparations include Rheumartho and Rheumatil (150µg/ml, 300µg/ml, and 450µg/ml) was investigated for anti-inflammatory effects in Invitro Method. Human red blood cells membrane stabilization method and inhibition of albumin denaturation was adopted for the *in-vitro* anti-inflammatory activity. The ayurvedic drugs were effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that Rheumatil and Rheumartho at concentration 150 µg/ml, 300µg/ml protect significantly ($P<0.01$) the Erythrocyte membrane against lysis induced by heat. It was concluded that Ayurvedic preparations (Rheumartho and Rheumatil) possess anti-inflammatory with lesser side effects and more beneficial effects.

Keywords: Anti-inflammatory and HRBC, Inhibition of albumin denaturation, Ayurvedic preparations.

INTRODUCTION

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

The inflammatory response represents a generalized response to infection or tissue damage and is designed to remove cellular debris, to localize invading 16 organisms and arrest the spread of infection. The inflammatory response is characterized by the following symptoms: Reddening of the localized area, swelling, pain and elevated temperature. Reddening results from capillary dialation that allows more blood to flow to the damaged tissue. Elevated temperature results from capillary dialation which permits increased blood flow through these vessels, with associated high metabolic activities of neutrophils and macrophages. The dialation of blood vessels is accompanied by increased capillary permeability causing swelling as fluid accumulates in the spaces surrounding tissue and cells. Pain in the case of inflammation is due to the lysis of blood cells that trigger the production of bradykinin and prostaglandins. The area of inflammation also becomes walled off as a result of the development of fibrinous clots. The deposition of fibrin isolates the inflamed area, cutting off normal circulation. The fluid in the inflamed area is known as inflammatory exudates, commonly called as pus. These exudates contain dead cells and debris in addition to body fluids. After the expulsion of the exudates, the inflammation may terminate and tissues may return to their normal state (Atlas, 1995). Pain belongs to a basic sensory abnormality associated with inflammation. Pain develops when nerve fiber terminals of polynodal nociceptors become sensitized by mediators of inflammation. The pain producing inflammatory mediators are bradykinin, prostaglandins (PGE1 and PGE2) and leukotrienes, especially LTB4. Pain becomes evoked by the synergistic action of bradykinin and prostaglandins (Antoni, 1991**).**

Acute inflammation is a short-term process which is characterized by the classic signs of inflammation which are: swelling, redness, pain, heat, and 17 loss of function due to the infiltration of the tissues by plasma and leukocytes. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). The first four characteristics have been known since ancient times and are attributed to Celsus. Loss of function was added to the definition of inflammation by Virchow in 1870 (Vidya et al., 2001). The process of acute inflammation is initiated by the blood vessels neighbouring to the injured tissue. It allows the exudation of plasma proteins and leukocytes into the surrounding tissue. The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation since the lymphatic system does not have the capacity to compensate for it, and the increased blood flow to the area causes the red color and increased heat. The blood vessels also get altered permitting the movement of leukocytes through the endothelium and basal membrane constituting the blood vessel. Once in the tissue, the cells migrate along a chemotactic gradient to reach the site of injury, where they can attempt to remove the stimulus and repair the tissue. Inflammatory mediators, act in parallel to propagate and mature the inflammatory responses. These include the complement system, coagulation system and fibrinolysis system. Finally, down-regulation of the inflammatory response terminates acute inflammation. Removal of the injurious stimuli halts the response of the inflammatory mechanisms, which require constant stimulation to propagate the process. Additionally, many inflammatory mediators have short half lives and are quickly degraded in the tissue, helping to quickly cease the inflammatory response once the stimulus is removed.

Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, plasma cells) tissue destruction and attempts at healing, which include angiogenesis and fibrosis (Vidya et al., 2001). Endogenous causes include persistent acute inflammation. Exogenous causes are varied and include bacterial infection, especially by Mycobacterium tuberculosis, prolonged exposure to chemical agents such as silica, tobacco smoke, or autoimmune reactions such as rheumatoid arthritis. In acute inflammation, removal of the stimulus halts the recruitment of monocytes 19 (which become macrophages under appropriate activation) into the inflamed tissue, and existing macrophages exit the tissue via lymphatics. However, in chronically inflamed tissue the stimulus is persistent, and therefore recruitment of monocytes is maintained, existing macrophages are tethered in place, and proliferation of macrophages is stimulated.

In general, NSAIDs are absorbed almost completely from the gastrointestinal tract; tend not to undergo pre-systemic elimination. They are highly bound to plasma albumin. Their t1/2 ranges from 1 to 60 hours. The vast majority of NSAIDs are weakly acidic drugs that localize in the synovial tissue of inflamed joints (Lawrence, Bennett and Brown, 1997).

Most of the currently used anti inflammatory drugs either steroidal or non steroidal have undesirable side effects ranging from gastrointestinal irritation to cardiovascular effects. So, natural compounds derived from plants or other sources are especially important to be developed into anti inflammatory drugs. More diverse chemical structures are found in plants which would be absolutely specific in inhibiting a particular enzyme. Such compounds may contain chemical groups susceptible to chemical or biological transformation. Their derivatives may exhibit novel properties which may be advantageous in the pharmacological view.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and drugs used in this study were procured from local pharmacy store (Rheumartho and Rheumatil) and from Hyderabad (Vijaya enterprises)

Anti-inflammatory Activity

In-Vitro Anti-inflammatory Activity

The human red blood cell membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAID's for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% Dextrose, 0.8%Sodium citrate, 0.5% Citric acid & 0.42% Nacl) and centrifuged at 3,000 rpm for 20 min. The Packed cells were washed with Isosaline and a 10% suspension was made. Various concentrations of methanolic extract of *Cocculus hirsutus* were prepared (100, 200 mcg/ml) using with CMC and to each concentration 1ml of phosphate buffer, 2ml of hypo saline & 0.5ml of HRBC suspension were added. It is incubated at 37°c for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560nm. Diclofenac (mcg/ml) was used as reference standard and a control was prepared omitting the extracts (Rajendran Vadivu *et al*., 2008).

Inhibition of Albumin Denaturation Activity

The anti-inflammatory activity of Ayurvedic preparations are studied by using inhibition of albumin denaturation technique which was studied according to mizushima et al [10] and sakat et al [11] followed with minor modification the reaction mixture was consist of test extracts and 1% aqueous solution of bovine albumin fraction, ph of the reaction mixture was adjusted using small amount of 1n Hcl. The sample extracts where incubated at 37 degree centigrade for 20 min and then heated to 51 degree centigrade for 20 min after cooling the samples the turbidity was measured at 660nm (u v visible spectrophotometer model 371,elico India ltd) the experiment was performed in triplicate. The percentage inhibition of protein de naturation was calculated as follows; percentage inhibition= (absorbance control- absorbance sample) x 100/ absorbance control

RESULTS AND DISCUSSION

Inhibition of albumin Denaturation

Rheumatil

Protein denaturation is a Process in which protein lose their tertiary structure and secondary structure by application of external stress or compound , such as strong acid or base , a concentrated inorganic salt , an organic solvent or heat . Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of ayurvedic drugs (R/R) to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 93% was observed at 150µg / ml .Dichlofenac sodium a standard anti inflammation drug showed the maximum inhibition 85% at the concentration of 100µg/ml compared with control.

Treatment(S)	**Concentration[µg/ml]**	**Absorbance at 660nm**	% inhibition of protein denaturation
Control	-	0.83±0.04	-
Rheumatil	150µg/ml	0.26±0.02	93
Rheumatil	300µg /ml	0.4±0.3	90
Rheumatil	450µg/ml	0.3±0.4	34
Diclofenac sodium	150µg/ml	0.20±0.01	85

The data represent the Mean ± SEM

INHIBITION OF ALBUMIN DENATURATION

Rheumartho

Protein denaturation is a Process in which protein lose their tertiary structure and secondary structure by application of external stress or compound , such as strong acid or base , a concentrated inorganic salt , an organic solvent or heat . Most biological proteins lose their biological function whene denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of ayurvedic drugs (R/R) to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 74 % was observed at 150µg / ml. Dichlofenac sodium a standard anti inflammation drug showed the maximum inhibition 85% at the concentration of 100µg/ml compared with control.

Treatment(S)	**Concentration[µg/ml]**	**Absorbance at 660nm**	**% inhibition of protein denaturation**
Control	-	0.83±0.04	-
Rheumartho	150µg/ml	0.29±0.02	74
Rheumartho	300µg /ml	0.4±0.1	89
Rheumartho	450µg/ml	0.5±0.1	50
Diclofenac sodium	150µg/ml	0.20±0.01	85

The data represent the Mean ± SEM

HEAT INDUCED HAEMOLYSIS

Rheumatil

The ayurvedic drugs were effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that rheumatil at concentration 150 and 300µg/ml protect significantly ($P<0.01$) the Erythrocyte membrane against lysis induced by heat. Diclofenac sodium 100µg/ml offered a significant ($P<0.001$) protection against damaging effect of heat solution.

TREATMENT(s)	CONCENTRATION (µg/ml)	ABSORBANCE AT 560NM	% INHIBITION OF PROTEIN DENATURATION
Rheumatil	150µg/ml	0.7±0.1	89
Diclofenac sodium	100µg/ml	0.07±0.02	71

The data represent the Mean ± SEM

HEAT INDUCED HAEMOLYSIS

Rheumartho

The ayurvedic drugs were effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that rheumatil at concentration 150/300µg/ml protect significantly (P<0.01) the Erythrocyte membrane against lysis induced by heat. Diclofenac sodium 100µg/ml offered a significant (P<0.001) protection against damaging effect of heat solution.

TREATMENT(s)	CONCENTRATION (µg/ml)	ABSORBANCE AT 560NM	% INHIBITION OF PROTEIN DENATURATION
Rheumartho	150µg/ml	0.6±0.1	93
Diclofenac sodium	100µg/ml	0.07±0.02	71

The data represent the Mean ± SEM

CONCLUSION

The results of the study show that the Ayurvedic preparations possess in vitro anti-inflammatory activity on HRB and albumin denaturation.

Further detailed study on Ayurvedic preparations using different flogestic agents in this area will enable us to understand the mechanism of action underline the above mention activity with lesser side and adverse effects when compared to conventional NSAIDs.

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