Research Article

In vitro Anti-arthritic Activity of *Hemidesmus indicus* Root Extract

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ABSTRACT

Inflammation is a complex biological process and is an initial response to tissue injury.it is mediated by the release of autacoids and usually precedes the development of the immune response. Rheumatoid arthritis is an auto immune disorder which affects the adult population worldwide. Frequently used by patients with RA for pain relief, especially in the weeks or months before the diagnosis is established. Plant based compounds have become increasingly employed in management of Rheumatoid arthritis. *Hemidesmus indicus* belongs to Apocynacea (dogbane family). Common names are Anantamula, Ananthamoola, East Indian sarsaparilla, false sarsaparilla. It is an effective anti-inflammatory, direutic and vulnerary. The aim of the article is to investigate the Anti-arthritic efficacy of *Hemidesmus indicus In vitro*.

Keywords: Inflammation, autacoids, Rheumatoid arthritis, free radical scavenging, protein denaturation.

INTRODUCTION

Rheumatoid arthritis (RA) is an auto immune disorder affecting one third of the population. NSAIDs are analgesics frequently employed in the management of RA[1]. RA Patients frequently use them for pain relief, especially in the weeks or months before the diagnosis is established[7]. NSAIDs do not prevent joint destruction or slow the progression of the disease, so they should rarely, if ever, be used as the sole medication for patients with RA[2]. Medications used to treat RA are divided into nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, synthetic DMARDs, and biologic DMARDs, also called biologic response modifiers or biologics[4].

NSAIDs use causes gastric ulcer as a noted adverse effect hence herbal products are quite safer to use with minimal side effects and also cost effective. *Hemidesmus indicus* is also known as INDIAN SARSAPARILLA which is a species of plant that is found most common in South Asia[11]. Morphology of sarsaparilla is twining, slender, lactiferous and sometimes prostate or semi-erect shrub[10]. It has slender, numerous, thickened stem.[15] The leaves are opposite, has a short petiole, very variable and elliptic-oblong to linear-lanceolate[3]. Its used as beverage and used as ingredient in ayurvedic medicine and unani preparations against diseases of blood, inflammation, diarrhea, respiratory disorders, skin disease, syphilis, fever, bronchitis, asthma, epileptic fits in children etc[5]. It is also known as FALSE SARSAPARILLA. It is administered in the form of powder, infusion or decoction as syrup[6]. Its root contain hexatriacontane, lupeol, its octacosanoate, alpha-amyrin and beta-amyrin, its acetate and sitosterol[8]. The leaves contain tannins, flavonoids, rutin and coumarino[14]. Anti-inflammatory action produce their therapeutic activity through inhibition of cyclooxygenase enzyme (enzyme that produce prostaglandins) COX-1 is constitutive and makes PGs that protect the stomach and kidney[9] The well-known protective activity of aspirin on colon cancer may be through COX-2 action[12].The protein denaturation and protease inhibition causes the Anti-arthritic effect of *Hemidsemus indicus* ethyl acetate extract.[13]

MATERIALS AND METHODS

Plant material

Ethyl acetate extract is obtained from Green Chem Herbal Extracts & Formulations, Bangalore as a gift sample.

Evaluation of *in vitro* anti-arthritic activity

Inhibition of Protein Denaturation method [16,17]

Concentration of test substance: 1000 to 200µg/ml

Standard: Diclofenac sodium

Chemicals Required: Bovine serum albumin,1N HCl, Phosphate buffer (pH 6.3)

Instrument: Incubator, Spectrophotometer - 660nm

The following 4 solutions will be used

1. **Test solution** (0.5ml) consist of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of test solution in various concentration and pH will be adjusted to 6.3 by using a small amount of 1N Hcl .The samples were incubated at 37^{0}C for 20 minutes and heated at 57^{0}C for 3 minutes. After cooling, to the sample add 2.5ml of Phosphate buffer (pH 6.3).

2. **Test control solution** (0.5ml) consists of 0.45ml of Bovine serum albumin (5% aqueous solution) and 0.05ml of distilled water and pH will be adjusted to 6.3 by using a

small amount of 1N Hcl the samples were incubated at 37^{0}C for 20 minutes and heated at 57^{0}C for 3 minutes. After cooling, to the sample add 2.5ml of phosphate buffer (pH 6.3)

3. **Product control** (0.5ml) consists of 0.45ml of distilled water and 0.05ml of test solution in various concentration and pH will be adjusted to 6.3 by using a small amount of 1N Hcl. The samples were incubated at 37^{0}C for 20 minutes and heated at 57^{0}C for 3 minutes. After cooling to the sample add 2.5ml to phosphate buffer (pH6.3)

4. **Standard solution** (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution and 0.05ml of diclofenac sodium solution in various concentrations and pH will be adjusted to 6.3 by using a small amount of 1N Hcl. The samples were incubated at 37^{0}C for 20 minutes and heated at 57^{0}C for 3 minutes. After cooling, to the sample add 2.5 ml of phosphate buffer (pH6.3)

The percentage inhibition of Protein denaturation will be calculated as follows.

$$\% \text{ Inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

The control represents 100% protein denaturation. The result is compared with diclofenac sodium treated sample.

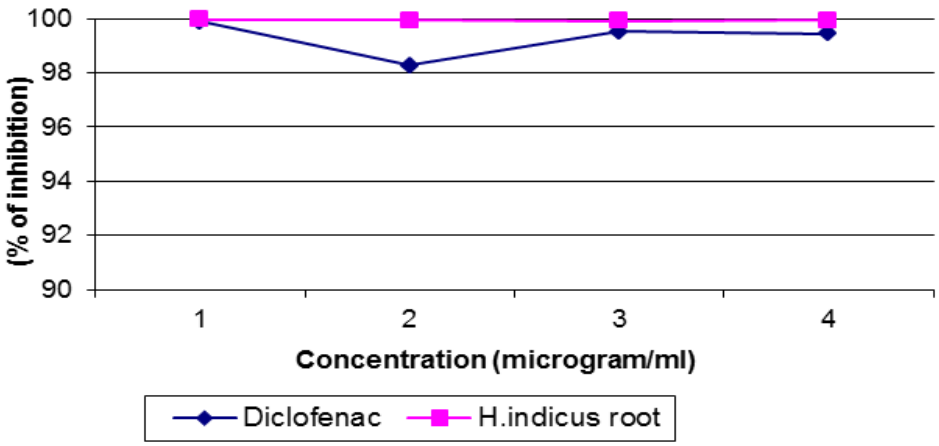

Figure 1: Anti-arthritic activity of *Hemidesmus indicus* root extract

RESULTS AND DISCUSSION

Hemisdesmus indicus exhibits significant anti-arthritic activity. The extract shows an inhibitory activity at 200 µg/ml - 1000 µg/ml by inhibiting denaturation of protein and its effect was compared with standard drug diclofenac sodium. The results are depicted in table 1 and represented in Fig 1. Auto antigen production in rheumatoid arthritis is due to denaturation of protein from the results of the resent study it could be stated that ethanolic extract of *Hemidesmus indicus* is capable of controlling the production of auto antigen and inhibiting the protein denaturation in RA.

Table 1: *In Vitro* anti-arthritic activity of *Hemidesmus indicus* root extract

S.NO	Concentration µg/ml	Diclofenac % of inhibition	*Hemidesmus indicus* root extract % of inhibition
1	200	99.88	99.97
2	400	98.27	99.94
3	800	99.52	99.90
4	1000	99.45	99.94

CONCLUSION

The *in vitro* study by inhibition of protein denaturation method emphasizes the anti-inflammatory/anti-arthritic effect of *Hemidesmus indicus* root extract to that of the standard drug diclofenac sodium. The anti-arthritic activity may be due the presence of chemical profile like flavonoids, phenols, polyphenols and steroids. Further studies are necessary, to identify the active constituent(s) that is responsible for the anti-arthritic efficacy.

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