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Research Article

In vitro Anti Arthritic Activity of Grape Seed Ethanolic 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. Grape seed extract commonly known as *vitis vinifera* It contains proanthocyanidins, a polymer of Catechin molecule. It has antioxidant and free radical scavenging, Anti microbial, Anti diabetic, Immuno modulatory, Anti cariogenic, Hepato protective activity.

The aim of the article is to evaluate the anti arthritic activity of grape seed ethanolic extract invitro by protein denaturation method.

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

INTRODUCTION

The immune response occurs when immunologically competent cells are activated in response to foreign organism or antigenic substance liberated during acute or chronic inflammatory response. [1]The outcome of the immune response for the host may be beneficial as when it causes invading organism to be phagocytosed or neutralised.Chronic inflammation results in pain and destruction of bone and cartilage that leads to severe disability and in which systemic changes occur that result in shortening of life .the discovery of COX isoforms(COX-1 and COX-2) led to the concepts that constitutive COX-1 isoform tends to be homeostatic in function,while COX-2 is induced during inflammation and tends to facilate inflammatory response[2-4]. On this basis highly selective COX-2 inhibitors have been developed and marketed on the assumption that such selective inhibitors would be safer than nonselective COX-1 inhibitors but without loss of efficacy.

The theraupeutic strategies are reduction of inflammation with nonsteroidal anti inflammatory drugs(NSAIDs) often results in relief of pain for significant period.the glucocorticoids also have powerful anti inflammatory effects and when first introduced were considered to be ultimate answer to treatment of inflammatory arthritis[5-6]. Another important group of agents are characterised as slow acting anti rheumatic drugs(SAARDs) or disease-modifying antirheumatic drugs(DMARDs).They may slow the bone damage associated with rheumatoid arthritis and are thought to affect more basic inflammatory mechanism than do the NSAIDs.

Basically NSAIDs causes noted side effects like peptic ulcer. Plant based compounds have been used in place of above drug which has very less side effects and also cost effective[7].

Grape seed extract(GSE) known as *Vitis vinifera.L*.These natural product possess various therapeutic uses and pharmacological actions.This is attributed to the presence of proanthocyanins.Various researches have been conducted to assess their medicinal uses across various fields of health sciences. [8]Stilbenoids *Trans*-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as *Botrytis cinerea* and delta-viniferin is another grapevine phytoalexin produced following fungal infection by *Plasmopara viticola* Anthocyanins *Vitis vinifera* red cultivars are rich in anthocyanins that impart their colour to the berries[9-12].

MATERIALS AND METHODS

Plant material

Grape seed extract is obtained from Green Chem Herbal Extracts & Formulations, Bangalore as an gift sample.

Evaluation of invitro anti-arthritic activity[13,14]

Inhibition of Protein Denaturation method

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

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 37^0C for 20 minutes and heated at 57^0C for 3 minutes. After cooling, to the sample add 2.5ml of Phosphate buffer (pH 6.3).

Test control solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% aqueous solution) and 0.05ml of

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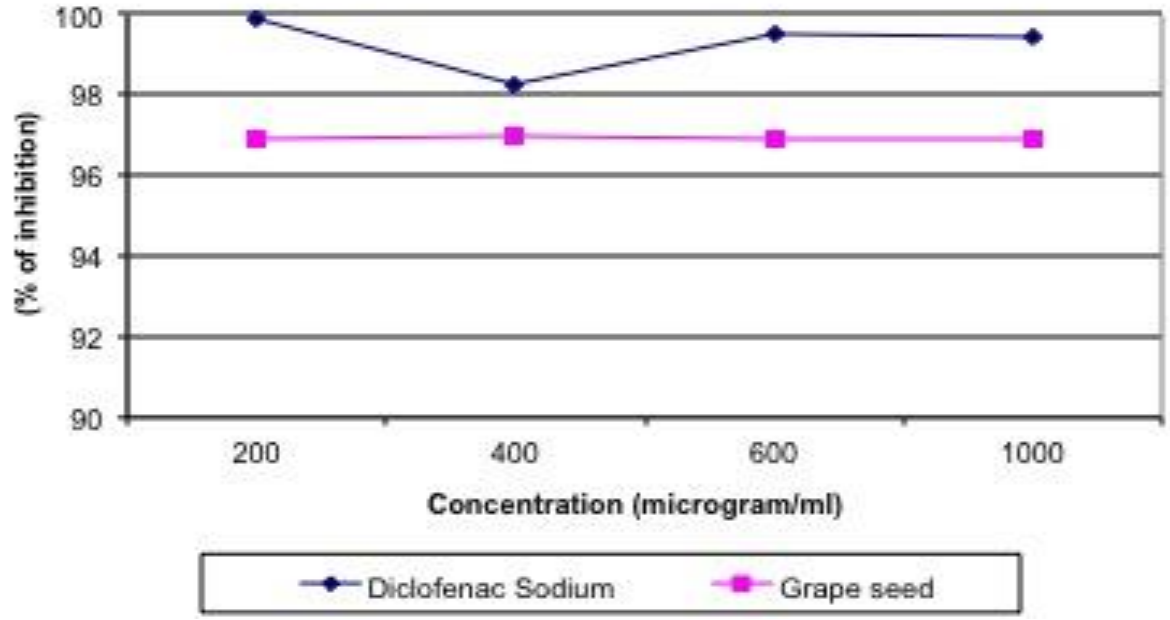

Figure 1:Anti arthritic activity of Grape seed extract

Table 1: *Invitro* Anti-Arthritic Activity Of Grape Seed Extract

S.NO	Concentration µg/ml	% of Inhibition
1	200	96.88
2	400	96.96
3	800	96.88
4	1000	96.92

distilled water and pH will be adjusted to 6.3 by using a small amount of 1N Hcl the samples were incubated at 37^0C for 20 minutes and heated at 57^0C for 3 minutes. After cooling, to the sample add 2.5ml of phosphate buffer (pH 6.3)

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 37^0C for 20 minutes and heated at 57^0C for 3 minutes. After cooling to the sample add 2.5ml to phosphate buffer (pH6.3)

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 37^0C for 20 minutes and heated at 57^0C 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.

Percent 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 will be compared with diclofenac sodium treated sample.

RESULT AND DISCUSSION

Grape seed extract exhibits significant anti arthritic activity. The ethanolic seed of Grape extract shows an inhibitory activity at 200-1000µg/ml by inhibiting denaturation of protein and its effect was compared with standard drug diclofenac sodium. The results are depicted in Table 1. Auto antigen production in rheumatoid arthritis is due to denaturation of protein. From the results of the present study it can be stated that ethanolic extract of *Grape seed* is capable of managing the production of auto antigen and inhibiting the protein denaturation in rheumatoid arthritis.

CONCLUSION

Protein denaturation method *invitro* on leaves of *Acacia catechu* showed the presence of anti-arthritic activity. Hence further research could be targeted on the *in vivo* Anti-inflammatory /Anti-arthritic activity.

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