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Assessment of *In-Vitro* Anti-Arthritic Potential of Methanolic Leaf Extract of *Ricinus communis*

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Abstract: The use of plants for the treatment of various diseases was recorded in the history. Many conventional medicines originate from plant sources: many decades ago, most of the few effective drugs were plant-based. Examples include quinine (from cinchona bark), digoxin (from foxglove), morphine (from the opium poppy) and aspirin (from willow bark). The discovery and development of new drugs from plant sources continues, with drug companies engaged in large-scale pharmacologic screening of herbs. Arthritis is a condition typically characterized by pain, aching, stiffness and swelling in and around one or more joints. Rheumatoid arthritis (RA) is a disorder in which the body's own immune system starts to attack body tissues. Ricinus communis, the castorbean or castor-oil-plant is a species of flowering plant in the spurge family, Euphorbiaceae. Many authors have reported that the plant has promised Antioxidant activity, Antinociceptive activity, Antiulcer activity, Antiasthmatic activity, Anti-fertility activity, Antihistaminic Activity etc cited in literature review. The present study is aimed to evaluate the methanolic leaf extract of Ricinus communis for acclaimed anti-arthritic activity by using protein denaturation method. Diclofenac sodium is used as a standard drug. From the results of the present study it can be stated that the methanolic leaf extract of Ricinus communis at two different concentrations (250mcg/ml & 500mcg/ml) is capable of inhibiting the denaturation of protein in rheumatic disease.

Keywords: Methanolic leaf extract, anti-arthritic activity, *Ricinus communis*.

INTRODUCTION

Herbal medicines are defined as a plant or plant part or an extract or mixture of these used in herbal treatment.

The pharmacological treatment of many diseases started long ago with the use of herbs. Recently herbal medicine has become the main scientifically based system for the treatment of many diseases. Because of public as well as medical establishments, studies leading to the scientific explanation of plant therapeutic capabilities are allowing this practice to gain increasing credibility and acceptance within the medical community [1].

Rapid changes in lifestyles have occurred with industrialization, urbanization, economic development and market globalization over the past decade have increased the growing epidemic of chronic non communicable diseases including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension, stroke and some types of cancer which are significant causes of disability and pre mature death in developing and newly developing countries, placing additional burdens on already overtaxed national health budget [2].

The use of, and search for, drugs and dietary

supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals which could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants [3].

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine

and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development [4].

Ricinus communis, the castorbean or castoroil-plant is a species of flowering plant in the spurge family, Euphorbiaceae. Many authors have reported that the plant has promised Antioxidant activity, Antinociceptive activity, Antiulcer activity, Antiasthmatic activity, Anti-fertility activity, Antihistaminic Activity etc.

Arthritis is a condition typically characterized by pain, aching, stiffness and swelling in and around one or more joints. There are several diseases where joint pain is primary, and is considered the main feature. Generally when a person has "arthritis" it means that they have one of these diseases, which include: Osteoarthritis, Rheumatoid arthritis, Gout and pseudo-gout etc. The symptoms can develop gradually or suddenly. Certain rheumatic conditions can also involve the immune system and various internal organs of the body. Rheumatoid arthritis (RA) is a disorder in which the body's own immune system starts to attack body tissues.

MATERIALS AND METHODS

The assessment of *in-vitro* anti-arthritic activity was carried out according to Vollala V. Rajesham *et al* [5] method. The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Ricinus communis* extracts (250 and 500 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37° C for 20 min and then heated at 57°C for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin.

Thus, test tubes were divided into the following groups:

Test Control: 0.05 ml of distilled water + 0.45 ml of bovin serum albumin (5%)

Test (250 \mug/ml): 0.05 ml of 250 μ g/ml of extract in water + 0.45 ml of bovin serum albumin (5%)

Product Control for 250μg/ml: 0.05 ml of 250 μg/ml of extract in water + 0.45 ml of distilled water

Test (500 \mug/ml): 0.05 ml of 500 μ g/ml of extract in water + 0.45 ml of bovin serum albumin (5%)

Product Control for 500μg/ml: 0.05 ml of 500 μg/ml of extract in water + 0.45 ml of distilled water

Standard (250 μg/ml): 0.05 ml of 250 μg/ml of standard drug in water + 0.45 ml of bovin serum albumin (5%)

Product Control for Standard (250 μg/ml): 0.05 ml of 250 μg/ml of standard drug in water + 0.45 ml of distilled water

Standard (500 μg/ml): 0.05 ml of 500 μg/ml of standard drug in water + 0.45 ml of bovin serum albumin (5%)

Product Control for Standard (500 \mug/ml): 0.05 ml of 500 μ g/ml of standard drug in water + 0.45 ml of distilled water

The test control represents 100% protein denaturation. The results were compared with diclofenac (250 $\mu g/ml$ & 500 $\mu g/ml$) treated samples.

The percentage inhibition of protein denaturation was calculated as follows.

Percent inhibition = 100- (O.D. of test - O.D. of product control) x 100
O.D. of Control

RESULTS AND DISCUSSION Results

The evaluation of *In vitro* anti-arthritic activity of *Ricinus communis* was done by protein denaturation

method according to Vollala V. Rajesham *et al.* [5]. The evaluation results are mentioned below (table 1 and figure 1).

Table-1: Percentage inhibition of tests and standard

Treatment	Percentage inhibition
Test 250 μg/ml	42.10%
Test 500 μg/ml	48.10%
Standard 250 µg/ml	53.50%
Standard 500 µg/ml	65.09%

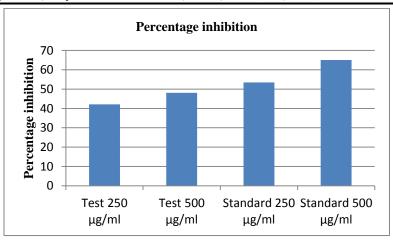


Fig-1: Graphical representation of percentage inhibition in protein denaturation method

Discussion

Arthritis is predominantly a disease of the elderly, but children can also be affected by the disease. More than 70% of individuals in North America affected by arthritis are over the age of 65. Arthritis is an inflammation process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [6].

Rheumatoid arthritis (RA) is a disorder in which the body's own immune system starts to attack body tissues. The attack is not only directed at the joint but too many other parts of the body. In rheumatoid arthritis, most damage occurs to the joint lining and cartilage which eventually results in erosion of two opposing bones. RA often affects joints in the fingers, wrists, knees and elbows, is symmetrical (appears on both sides of the body), and can lead to severe deformity in a few years if not treated.

Most of the investigators have reported that denaturation of the protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins [7]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [8].

Anti-arthritic effect of ethanolic leaf extract of Ricinus communis was studied significantly by using invitro inhibition of protein denaturation model. In vitro protein denaturation test has shown significant activity at various concentrations and its effect was compared with the standard drug diclofenac sodium. The maximum percentage inhibition of protein denaturation was observed as 48.6% at 500 μg/ml and with 250 μg/ml concentration percentage inhibition of protein denaturation it was found to be 42.1%. These test results were compared with the percentage of inhibition of diclofenac sodium as a standard drug. The percentage of inhibition of higher dose of the standard drug (diclofenac sodium) was 65.09% while the lower dose has shown 53.5% inhibition percentage (Table 1 & figure 1).

Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study it can be stated that methanolic extracts of leaves of *Ricinus communis* is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

CONCLUSION

From this study, it can be concluded that methanolic leaf extracts of *Ricinus communis* were assessed for antiarthritic activity and it was found that it has mild potential to inhibit arthritic inflammation. This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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